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FOREWORD

I am pleased to put into the hands of readers Volume-2; Issue-4: July-Aug 2017 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",** an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from crossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief
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Allocative Efficiency of Resource use on Beekeeping in Chitwan District of Nepal

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Abstract*—Agriculture is facing with increasing pollinators decline all over the world affecting the functioning of regulatory and production service of pollination in adverse manner. Study on ways to conserve pollinating agents like bee is crucial in modern intensive agriculture. In this context a study was conducted to estimate the productivity and resource use efficiency of bee keeping in Chitwan district of Nepal. The study used data collected from randomly selected 48 bee keepers using face to face interview technique in the year 2014. Descriptive statistics, gross margin analysis, benefit cost analysis and multiple regression analysis using Cob-Douglas form were employed to achieve study objectives. It was found that farmers were rearing honey bee on an average of about 34 hives per farm with annual productivity of bee products equivalent to 36 Kg honey per hive. Gross margin of beekeeping in the research area was found to be NRs. 3111.55 per hive with undiscounted benefit cost ratio of 1.71. Human labour use, expenditure on sugar, drugs and comb foundation and; migration cost were significantly contributing to the productivity of beekeeping and were required to increase their use by 39%, 34% and 74%, respectively to achieve optimum profit. It was suggested to increase the level of all variable inputs through loan, subsidy and insurance to promote beekeeping enterprise in the study area for ensuring optimum profit to farmers and conservation of the most important agent of pollination.*

***Keywords*—: Allocative efficiency, beekeeping, Chitwan, pollination, production function.**

I. INTRODUCTION

Agriculture provides primary occupation to about 65.6% of total population in Nepal [1]. However, agriculture is only a means of subsistence for the majority and share only 31.4% of national Gross Domestic Product (GDP) to the economy [2]. Agricultural land is degrading by heavy use of chemical fertilizers, pesticides and other forms of pollutant technologies [3]. In addition such agrochemicals has led to decline of beneficial insects, such as crop pollinators and bioagents [4].

In the Hindu Kush Himalayan (HKH) region, evidence of the decline in pollinator numbers has been reported from apple farming in Jumla district of Nepal [5]. An increase in honey hunting and the ruthless hunting of the nests of wild honeybees is contributing to the decline in the population of indigenous honeybees [6]. Evidence of decline in population of *Apis laboriosa* in Kaski district of Nepal was reported in another similar study [7]. [8] reported pollination deficit on mustard in natural condition, and therefore, recommended management of honeybee for higher production and productivity of the crop. Pollinator loss in Chitwan has been attributed to habitat loss resulting from misuse of fertilizers and pesticides, reluctant in beekeeping, deforestation, loss of natural vegetation, increased commercial agriculture, use of high yielding varieties and; many other abiotic and biotic factors [9].

In the context of declining pollinators like honey bee, one of the key approaches available to promote the pollination management practice like beekeeping is the increase in their economic performance at farm level. This study aimed estimation of resource productivity and resource use efficiency of beekeeping in Chitwan district of Nepal. The findings of this research answers some resource use related issues on rearing of honey bee and alert the planners, policy makers and farmers to make necessary adjustments on inputs used in beekeeping for its commercialization which indirectly support to manage problems related with decline of natural pollinators.

II. MATERIALS AND METHODS

2.1 Study site and sampling design

The study was conducted in Chitwan district of Nepal. Six Village Development Committees (VDCs) namely Padampur and Jutpani from Eastern Chitwan; Phulbari and Mangalpur from Central Chitwan; and Meghauli and Sukranagar from Western Chitwan were selected randomly. Two farmers' group formed under Global

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Pollination Project (GPP) with size of twenty five group members in each were randomly selected from each VDC. Thus, a total of 50 farmers from each VDC and 300 farmers in total were the number of farmers selected for a study on different pollinator friendly agricultural practices adopting in the area. This study was part of those study on pollination management practices and beekeeping was found to be adopted by 45 farmers from among those 300 farmers under study. Primary data was collected with the use of semi-structured interview schedule using face to face interview technique in 2013-2014. Data collected from the face to face interview was cross checked with one group discussion in each VDC. Secondary data required for the study were collected from the publications of different governmental and nongovernmental organizations. Collected data were entered in SPSS and analyzed using STATA to have required inferential statistics. The details of different analytical techniques used are presented hereunder in different subsections.

2.2 Cost of production

All variable inputs like human labor, sugar, drugs, comb foundation and migration cost involved in beekeeping were considered and valued at current market prices to calculate cost of production. During cost estimation, both purchased and own farm produced inputs were accounted.

Total variable cost = C_{labor}+ C_{sugar}+C_{drugs}+ C_{comb} + $C_{migration}$

Where,

C_{labor} = Cost on human labor used (NRs./hive),

C_{sugar} = Cost on sugar used (NRs./hive),

C_{drugs} = Cost on drugs (NRs./hive),

C_{comb} = Cost on comb foundation (NRs./hive), and

$C_{migration}$ =Cost on migration of bee hives (NRs./hive)

2.3 Return and margin analysis

Gross return was calculated by multiplying the total volume of product from beekeeping by the average price of the product at harvesting period [10]. Thus gross return was calculated by using following formula:

Gross return (NRs./hive) = Total quantity produced of main and by products (kg/hive) × Price (NRs./kg)

Gross margin calculation was done to have an estimate of the difference between the gross return and variable costs. Gross margin was calculated by using the method as given by [11], using following formula;

Gross Margin (NRs./hive) = Gross return (NRs./hive) - Total variable cost (NRs./hive)

2.4 Benefit cost analysis

Benefit cost ratio is the quick and easiest method to determine the economic performance of a business. It is a relative measure, which is used to compare benefit per unit of cost. Undiscounted benefit cost ratio was estimated as a ratio of gross return and total variable cost. Thus, the benefit cost analysis was carried out by using formula;

$$\text{B/C ratio} = \frac{\text{Gross return (NRs./hive)}}{\text{Total variable cost (NRs./hive)}}$$

2.5 Production function analysis

Cobb-Douglas form of production function in the following form was fitted to examine the resource productivity, efficiency and return to scale.

$Y= aX_1^{b1} X_2^{b2} X_3^{b3}e^u$

Where,

Y = Gross return (NRs./hive),

X_1 = Cost on human Labor (NRs./hive),

X_2 = Cost on sugar, drugs and comb foundation (NRs./hive),

X_3 = Cost of migration (NRs./hive),

e = Base of natural logarithm,

u = Random disturbance term,

a = Constant, and

b1, b2 and b3 represent Coefficients of respective variables.

The Cobb-Douglas production function in the form expressed above was linearised into a logarithmic function with a view of getting a form amenable to practical purposes using OLS technique as expressed below;

$\ln Y= \ln a+b_1\ln X_1+b_2\ln X_2+b_3\ln X_3$

Where,

ln= Natural logarithm, and rest of the other abbreviations are same as previous explanations.

Calculation of Return to Scale (RTS) in beekeeping was obtained by adding coefficients from log linearised Cobb-Douglas production function as follows;

RTS= $\sum b_1$, b_2 and b_3

The sum of b_1 to b_3 from the Cobb-Douglas production function indicates the nature of return to scale.

Return to scale decision rule employed was;

RTS>1: Increasing return to scale

RTS=1: Constant return to scale

RTS<1: Decreasing return to scale

2.6 Resource use efficiency

The allocative efficiency of a resource used was determined by the ratio of Marginal Value Product (MVP) of variable input to the Marginal Factor Cost (MFC) for the input and tested for its equality to one i.e. (MVP/MFC)=1 . Following [12] the efficiency of resource use was calculated as;

r= MVP/MFC

Where,

r= Efficiency ratio,

MVP= Marginal value product of a variable input, and

MFC= Marginal factor cost

Decision rule for resource use efficiency is that a efficiency ratio (r) equal to unity indicates the optimum use of that factor, the ratio more than unity indicates that

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gross return could be increased by using more of the resource and the ratio of less than unity indicates the excess use of resource which should be decreased to minimize the loss [13]. Again, the relative percentage change in MVP of each resource required to obtain optimal resource allocation, i.e. r=1 or MVP= MFC was estimated using the following equation below [14];

D= (1-MFC/MVP) × 100

Or, D= (1-1/r)× 100

Where, D represents absolute value of percentage change in MVP of each resource, and r for efficiency.

III. RESULTS AND DISCUSSION

3.1 Cost, returns and profit from honey beekeeping

Farmers were rearing honey bee on an average of 33.73 hives per farm with productivity of 36 kg/hive honey equivalent (Table 1). It was slightly less compared to 40.71 Kg/hive as found by [15] . In the research area, gross return of beekeeping was estimated to be about NRs. 7,482.2, while total cost of beekeeping per hive was estimated to be about NRs. 4,370.57. Gross margin from beekeeping in the research area found to be NRs. 3,111.55 per hive. It was observed that the overall undiscounted benefit cost ratio of beekeeping in the research area was 1.71which were slightly varied with some previous findings. [16] reported it to be 2.41 and [9] reported it to be 1.81. Such better benefit cost ratio advocates very strongly on the profitable potential of beekeeping in the study area.

3.2 Resource productivity on beekeeping

Estimated values of regression coefficients and related statistics of Cobb-Douglas production function of beekeeping are shown in Table 2. Three explanatory variables namely human labor cost, expenditure on sugar, drug and comb foundation and; migration cost were considered to show their effects on production of honeybee. All of those three variables were significantly contributing to the productivity of beekeeping at 1% level of significance. The regression coefficient for human labor cost was 0.361, which had depicted that with 100% increase in cost on human labor, gross return from beekeeping could be increased by about 36%. Similarly, with the increase in expenditure on sugar, drug and comb foundation by 100%, gross return could be increased by about 31% as its coefficient is 0.306. Likewise, with 100% increase in migration cost, gross return could be increased by about 17% as its coefficient is 0.169.

The coefficient of multiple determination (R^2) of the production function was 0.77 for beekeeping which indicated that about 77% of variations in gross return have been occurred due the explanatory variables, which were included in the model (Table 2). The value of adjusted R square was 0.75 indicating that after taking into account the degree of freedom (df), 75% of the variation in the dependent variable explained by three explanatory variables included in the model.

The measures of the overall significance of the estimated regression was shown through F value. F value was 46.44 and it was significant at 1% level implying that all the explanatory variables included in the model are important for explaining the variation of the productivity of beekeeping. Returns to scale reflect the degree to which a proportional change in the output due to proportionate change in input. The sum of the coefficients of different inputs stood at 0.836 for honey production (Table 2). This indicates that the production function exhibited a decreasing return to scale and implied that if all the inputs specified in the function are increased by 100% income will increase by about 83.6%.

3.3 Resource use efficiency on beekeeping

The estimated MVP and MFC of different inputs used in beekeeping production are presented in Table 3. After the analysis of prices of both inputs and output, it was evident that ratio of MVP to MFC of all the factors of production were positive and greater than one. This revealed that they were being under-utilized and profit could be increased by increasing their level of use. All the inputs human labor, expenditure on sugar, drug and comb foundation and especially, migration cost were underutilized on beekeeping in study area. The adjustment in the MVPs for optimal resource use indicated that for optimal allocation of inputs their level of use should be increased. Human labor was needed to increase by 39% to obtain the optimum profit from beekeeping enterprises. Similarly, expenditure on sugar, drug and comb foundation and; migration cost were required to be increased by 34% and 74%, respectively (Table 3).

IV. CONCLUSIONS

The research conducted to assess the productivity and resource use efficiency of beekeeping revealed that farmers were rearing honey bee on an average of 33.73 hives per farm with productivity of honey equivalent to 36 Kg per hive. Gross margin of beekeeping in the research area found to be NRs. 3111.55 per hive with observed value of undiscounted benefit cost ratio of 1.71. Three explanatory variables namely human labor cost, expenditure on sugar, drug and comb foundation and; migration cost significantly contributed to productivity of honey be at 1% level of significance. Return to scale value of honey beekeeping was 0.836 and reflected the decreasing return to scale. Human labor, expenditure on sugar, drug and comb foundation and especially, migration cost were underutilized on beekeeping in study area. It was suggested to increase the labour use, materials use like sugar, drug and comb foundation and, migration cost by 39%, 34% and 74%, respectively to harvest

optimum profit by farmers. The research findings suggest that there is ample opportunity of promoting beekeeping in study area with the recommended adjustment in resource use to harvest optimum profit. The level of underutilized resources in beekeeping can be promoted through extension, subsidy, insurance and loan facility to the beekeeping enterprises.

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Table.1: Economic statement of beekeeping in Chitwan during 2013-2014

Measuring criteria	Average value
Average number of hives per farm	33.73
Productivity-main product equivalent (Kg/hive)	36
Gross return (Rs./hive)	7,482.12
Total cost (Rs./hive)	4,370.57
Gross margin (Rs./hive)	3,111.55
Benefit cost ratio	1.71

Source: Field survey, 2014

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Table.2: Estimated values of coefficients and related statistics of Cobb-Douglas production function of beekeeping

Factors	Coefficient	Std. Error	t-value	Sig. level
Constant	3.009**	0.777	3.87	0.000
Human labor cost (Rs./hive)	0.361**	0.114	3.17	0.003
Expenditure on sugar drug and comb foundation (Rs./hive)	0.306**	0.306	3.09	0.004
Migration cost (Rs./hive)	0.169**	0.045	3.72	0.001
F-value	46.44**			0.001
R square	0.77			
Adjusted R-square	0.75			
Return to scale	0.836			

Note: **Significant at 1% level of confidence

Source: Field survey, 2014

Table.3: Allocative efficiency of inputs used in beekeeping in Chitwan during 2013-2014

Inputs (Rs./hive)	Geometric mean	MVP	MFC	MVP/ MFC	Efficiency	Adjustment required (%)
Human labor	1,618.86	1.63	1.00	1.637	Under utilized	38.897
Sugar, drugs and comb foundation	1,474.25	1.52	1.00	1.523	Under utilized	34.353
Migration cost	329.71	3.76	1.00	3.762	Under utilized	73.417

Source: Field survey, 2014

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Extraction, chemical composition, use in induced protection and cross-reactive antigens between exopolisaccharides from *Tremella fuciformis* Berk and *Xanthomonas campestris* pv. *citri* (Hasse) Dye

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Abstract *— Exopolysaccharides (PS) are the major components on the surface of bacteria and also produced by fungi. These molecules are important in human health, in order to control diabetes as well as protect plants against attacks of foliage diseases. The objective of the present work was to study the partial chemical structure of the carbohydrate, use in control disease in plants and cross-serological relationship (cross-reactive antigens between isolates from fungi (Tremella fuciformis (Tf) and bacteria (Xanthomonas campestris pv. citri (Xcc)). Tf was developed in culture medium containing sorghum seeds during 20 days, and Xcc in the PDA (potato dextrose agar) medium for an 8 days period. The polysaccharide was removed from the culture medium, precipitated with ethanol, and quantified total sugar. By TLC was observed that 2 isolates presented galactose, glucose, mannose, arabinose and xylose in different proportions. Fucose and ribose was not found in the PS from Xcc but present in Tf. In ELISA, antiserum to Xcc revealed an antigenic homologous reaction with the same bacteria and heterologous with Tf. Barley plants pretreated with PS from Tf and later challenged with conidia from B.sorokiniana, demonstrated protection against the pathogen. Results suggested that PS from Tf presented induction of protection. Both PS (antigens) present an identical epitope demonstrated by reaction in Elisa test. The antibody against Xcc was specific for an epitope and bounded to another antigen due to having similar chemical properties.*

Keywords— Polysaccharides, fungi, bacterial, cross-reactive antigen.

I. INTRODUCTION

Natural biopolymers (polysaccharides - PS) have a potential application in food, cosmetics, pharmaceuticals and oil industries. The macromolecules are water soluble which may be ionic or non-ionic in nature, and which increase the viscosity of the medium in conjunction with different physical and chemical agents. Because of their wide diversity in physical structure and properties, they can change the rheological properties and texture of the products in which they are incorporated into [1]. In recent years, there has been a major emphasis on the search for novel microbial polysaccharides, and a wide variety of microbial strains are reported to produce polysaccharides with varied compositions and useful properties [2].

Tremella fuciformis (family *Tremellaceae*, order *Tremellales*, class *Basidiomycetes*) is probably one of the most beautiful fungi growing in subtropical and tropical areas, or even temperate zones. It was first found in Brazil but has developed to an artificially cultivated species in Taiwan, China and some other countries in Asia [3]. This mushroom is called snow fungus, white jelly mushroom, and have been used as food and folk medicines for centuries in Asian countries [4, 5].

At the moment in Brazil, *Tremella fuciformis* (*Tf*) was grown in medium with sorghum for obtained PS. The polysaccharide was given by gavage for rats when induced by streptozotocin in order to develop type 1 diabetes mellitus (DM1) for a period of 30 days. Results indicate that PS is beneficial in control of DM1 when the level from blood glucose is up to 130mg/dL accomplished

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by reducing cholesterol, triglyceride, GPT, urea and increasing HDL cholesterol [6, 7].

Another important application from PS was with promoted induction of protection against disease in plants. Bach et al. [8] demonstrated that PS from *Tf* in three concentrations, stimulated the ability of plants to compensate the damaging effects of the pathogens (*Bipolaris sorokiniana*) on barley plant (Variety BRS Brau) metabolism. The same protection was observed with xantham gum from *Xanthomonas campestris* pv. *citri* (*Xcc*), *Xanthomonas campestris* pv *campestris* and commercial xantham gum against the same disease on barley and wheat plants [9, 10].

About chemical structure of the PS obtained from dried *Tf*, commercially avaliable in China, and extracted by boiling water, determined that it consists of a linear backbone of (1→3) α-D-mannan with side chains composed of glucuronic acid, xylose and fucose [11, 12]. *Xanthomonas* produced xanthan gum and is an important industrial biopolymer as example food. According to several authors, the PS are antigenic macromolecules and may be related to pathogenicity [13 - 15].

The objective from the present work was to study the partial chemical structure of the carbohydrate and investigate the use under greenhouse conditions as an inducer of protection in barley plants (cultivar Embrapa BRS Elis) against pathogen. Another method for identification PS was study the cross-serological relationship (cross-reactive antigens between isolates from fungi (*Tremella fuciformis Tf*) and bacterial (*Xanthomonas campestris pv. citri Xcc*). This could aid in the possible explanation for its use in control of diseases both for the use in plants such as for humans beings.

II. MATERIALS AND METHODS

Preparation of polysaccharide from Tremella fuciformis

Tremella fuciformis received from Brasmicel, were cultured in potato dextrose agar (PDA) for 8 days, and then transferred to plastic bag containing sorghum seeds. The bags were incubated during 45 days for micelial growth in chamber with controlled temperature (27±1^{0}C) and dark. For production of polysaccharides (PS), a solid medium was made and added 100g of sorghum seeds (Embrapa seed variety 308) that was first cooked in water and after crushed in a blender in 400mL of water and boiled again. The mixture was filtered through sieve, gauze, cotton cloth, completely to 500mL water and supplemented with 0.5g of agar. After boiled, the solution was transferred to bottles and sterilized [8].

Sorghum seeds with mycelium were inoculated to bottle with solid medium and incubated for 10, 20, 30 days in chamber with controlled temperature (25 ± 1^{0}C) and dark. After the period, PS was removed and solution was reduced to half-volume by vacuum evaporation. This reduced volume was, then, treated with cold ethanol (70%) to polysaccharide precipitation. To facilitate the precipitation (ppt), the solution was kept at 4^{o}C for an additional 24 h and then centrifuged at 4000g for 10 min at 18^{o}C, and the precipitates were collected, washed twice with alcohol and then solubilized in water. After, the gum was submitted to dialysis in bags with 10.000 daltons against buffer phosphate pH=7 (0.05mol/L) for retired phenols. Tests were performed to quantify beta glucan and total sugar. Beta-glucan was determined by Lever method [16] involving the beta-glucanase enzyme (Sigma). For standard in test, was used laminarin that said one unit of enzyme can be liberated 1 μM of glucose/min at 37°C [17]. For total sugar the method used was Anthrone [17-19].

The PS was loaded onto a Sepharose CL_4B column (2.4 cm x 100 cm, Pharmacia) and eluted with the same buffer at a flow rate of 2ml/min. The carbohydrate moiety in the PS was monitored by absorbance at 480 nm. A pool fractions from 10, 12 and 14mL was quantify by Anthrone.

Preparation of Suspension of pathogen

The pathogen used was *B.sorokiniana* obtained from infected barley leaves (Fundação Guarapuava- Agraria, Paraná) and grown on plates using potato dextrose agar (PDA). After 10 days of incubation, conidia, removed by brushing the surface of the agar, were suspended in 10 ml of sterile water and filtered through gauze. The concentration of conidia was adjusted to 10^{5}conidia per ml and Tween 20 (poly-oxyethylene sorbitan monolauret, Sigma Chemical Co) was added to give a final concentration of 0.05% [20, 21].

Preparation of barley and treatments

Barley plants (Embrapa Elis – from Foundation Agraria, state of Paraná), were grown in clay pots (15cm diameter, ten seeds) contained red soil fertilized (NPK 10:10:10 and micronutrients) in a greenhouse under a 12h photoperiod (approximately 190IE/m^2/s) for approximately 3 weeks when plants reached the tillering stage (stage 5) [22]. Groups of 10 plants was used in each treatment and replicated three times. The data were submitted to variance analysis. Plants were arranged in a complete randomized block design and the combination of challenger and protector in each treatment. Plants were maintained at room temperature and a 12h photoperiod (7.35Wm-2 of fluorescent light) throughout each treatment unless indicate otherwise [20]. Approximately 10ml of the conidia suspension, PS of *Tf*, or water were used in each treatment. Treatments were: (a) healthy:

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plants were sprayed with water; (b) Inducer: plants were sprayed with PS of *Tf* (conc 2mmol of sugar); (c) pathogen inoculated: plants were pulverized with the conidial suspension of the pathogen; (d) 24 hour Inducer-pathogen: first treated with PS and 24 hours later inoculated with the conidial suspension; (e) 48 hour Inducer-pathogen: as in group d but inoculated with the conidial suspension 48 hours after inducer; (f) 72 hour Inducer-pathogen: as in group d but inoculated with the conidial suspension 72 hours after inducer. During the first 24 hours after inoculation with the pathogen, all plants were kept in the dark at room temperature in a humid chamber (80% relative humidity). After that, plants were transferred to the greenhouse. Protection level was evaluated 7 days after inoculation with the pathogen, based on the number of infected leaves in ten plants [20, 21].

Preparation of polysaccharide and antisera for Xanthomonas campestris pv. citri

Bacterial cells were grown for 8 days on potato dextrose agar (PDA) in Petri disches at 27º C in the dark. The PS was removed from surface with water. One hundred mL of solution were centrifuged (7,000g- 20min), supernatants were reduced to half-volume by vacuum evaporation at 40ºC and precipitated with 75% ethanol. The precipitates were dried under a N_2 stream and dissolved in distillate water. Beta glucan and total sugar was measured as described for *Tremella*.

Antisera for *Xanthomonas campestris* pv.*citri* pathotype A were produced in New Zealand White rabbits (approx. 2kg) in Biological Institute, São Paulo, Brazil in year 2000. Rabbits were immunized with one single 1.0 mL intra-lymph node injection of 900ug of glucose equivalents emulsified with an equal volume of Freund complete adjuvant (Difco). Sera were collected from the marginal ear veins 20 days after the injection [23].

Immunoglobulin (Ig) was precipitated from rabbit sera with 100% sat. ammonium sulfate, repeated twice, and dissolved in 0.85% NaCl solution followed by dialysis against the same solution. Enzyme conjugation was performed by adding 0.5mL of Ig (1 mg/mL) to 0.09 mL of alkaline phosphatase (Boehringer) followed by dyalisis against 2mM sodium phosphate buffer pH 7.4 plus 0.85% NaCl and 0.8mL of 0.25% glutaraldehyde for 1h at 4ºC. Ig and conjugates were diluted 1:2 and antigens (PS) were used in the concentration of 1mmol of glucose equivalents/mL. Normal sera were prepared with the same method as negative control.

Treatment of antigens with sodium periodate

The method was based in Bach and Guzzo [13] when 1mmol of glucose/mL of each PS, as measured by the anthrone test [19] and treated with 0.05mol sodium periodate. Another group of equally treated samples were then reduced with sodium borohydride (Sigma) and subjected to hydrolysis with 0.03mol acetic acid for 1h (Hydrolyzed sample). Control samples were treated with ethylene glycol prior to the periodate treatment. Periodate-treated, hydrolyzed and control samples were used as antigen for serological tests by ELISA test.

Elisa Test

Antigens (PS) from *Xcc* and *Tf* were tested by double antibody sandwich-ELISA [24] and the experimental conditions for the serological reactions and immunoglobulins was based in Bach and Alba [23] and, Bach and Guzzo [13]. ELISA values were obtained by measuring absorbance at 405nm. The concentration of PS from *Tf* and *Xcc* used in work was equivalent to 1mmol of glucose.

Chromatography Thin layer (TLC)

The sugar was analyzed by thin layer chromatography (TLC) carried out on Merck silica gel 60 F254 plates (20 cm x 20 cm). Aliquots of standards solutions of glucose, galactose, mannose, xylose, arabinose, ribose and fucose (10mg/mL of each from Sigma) were applied as spots at the origin on a plate and developed with butanol: acetic acid: water (4:1:1 by vol.) in a pre-saturated chromatography chamber. The thin layer chromatography plates were dried at 60°C and the sugars were visualized at 254nm UV light and after detected by spraying chromatograms with 5% ethanolic sulphuric and heating in the oven at 100°C until clear sugars spots appeared [25, 26]. The CPATLAS program was used to determine the area of spots and relative front from each standard of developed chromatoplate. 10mmol from PS samples were treated with 100uL of beta glucanase (10UI) and 200uL of alfa glucanase (10UI) from Sigma in presence of citrate buffer (50mM, pH=5) and after 10min at 37C, was submitted to boiling for one hour at 100C. Those was separated in TLC.

III. RESULTS

Extraction of polysaccharide and concentration of beta glucan

When isolates of *Tf* and *Xcc* grown in medium, was observed as loose slime secreted by the microorganisms. After precipitation and solubilized in water we have a gum solution. Table 1 compared concentration of sugars present in PS from *Xcc* and from *Tf*. Tf polysaccharide increased until day 20, being after it decreased, most likely due to the increased oxidation, or enzyme presence. Present sugars were Alfa-glucose (millimol) and Beta-glucan.

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Table.1: Concentration of beta-glucan linked-(1,3)(1,6) and total sugars from preparations of samples from Tremella fuciformis (Tf) and Xanthomonas campestris pv. citri (Xcc).

Polysaccharide from	mg Beta-glucan/mL	mmol total sugar
Tf (10days)	85.2 +/- 0.586a*	12.5d
Tf (20days)	153.6 +/- 0.625c	28.0a
Tf (30days)	130.5 +/- 0.615b	27.3c
Xcc	120.8 +/- 0.666d	21.4b

*****Different letters on columns indicate statistically significant differences among groups ($p < 0.01$; ANOVA + Student´s test).

In parallel studies with suspension of *Tf* was observed presence of spores. The appearance in 10 days was ovoid like yeasts. In 20 and 30 days the appearance was type yeast elongated. In work we used for studies *Tf* from 20 days because PS presented white color and from 30 days PS was transformed a brown color.

Gel Filtration of PS

The PS from *Tf* 20 days and from *Xcc* was monitored by a gel filtration in a Sepharose CL_4B column, by which one polysaccharide was eluted as shown in Fig. 1. Peaks from tubes 0-4 included external volum. Pool fractions (tubes 5,6,7) from *Tf* presented 5.12mmol of glucose and from *Xcc* only 2.04mmol of glucose.

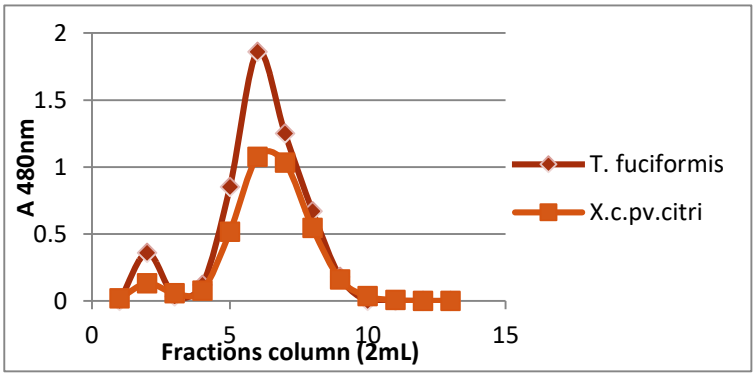

Fig.1: Elution profile of polysaccharides produced from Tremella fuciformis (20days) and Xanthomonas campestris pv. citri in Sepharose CL-4B chromatography.

Thin Layer Chromatography

By TLC was possible to observe that isolates from *Xcc* and *Tf* contain galactose, glucose, mannose, arabinose and xylose in different proportions but similar. Fucose and ribose was not found in the PS from *Xcc* (Table 2).

Table.2: Composition of the PS from Tremella and Xanthomonas obtained by TLC.

Isolates	galactose Rf=0.16	Glucose Rf=0.18	mannose Rf=0.20	arabinose RF=0.21	fucose RF=0.27	xylose RF=0.28	Ribose RF=0.31
T. fuciformis 20days	3.54*	132.5	121.6	3.85	1.56	5.48	2.85
X. c. pv. *citri*	3.85	147.8	125.4	3.15	x	6.48	X

* area of spots in CPATLAS.

Elisa test

Results obtained through ELISA from the combination of PS from *Xcc* and the same antisera have specific reaction. In contrast, negative reactions occurred with normal serum when used in the test. Similarly, antigen from *Tf* reacted specifically in heterologous antigen-antibody combinations and give reaction in Elisa at 0.82. The results can indicated that reaction was similar as observed with antigen from Xcc or that was equivalent to 100% of interaction. To confirm that the serological reaction depends from the polysaccharides antigen, samples could be treated with sodium periodate. Homologous and

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heterologous serological reactions were completely abolished by treating the PS antigens with periodate for antisera against *Xcc*. The carbohydrate contents in all periodate treated samples decreased in comparison to the non-treated samples. Reactions in Elisa test were not recovered in homologous and heterologous reactions (Table 3).

Table.3: Effect of periodate treatment on antigenicity of PS from Xanthomonas and Tremella and contents of carbohydrate antigenic fractions.

Antigens	Treatments	Antibody PS from Xcc*.	Concentration of sugar after treatment of periodate	
			control	treated
Xcc	Control	0.85	1mmol	1mmol
	Treated	0.00	1mmol	0.28mmol
	Hydrolyzed	0.00	1mmol	x
Tf 20days	control	0.82	1mmol	1mmol
	treated	0.00	1mmol	0.18mmol
	hydrolyzed	0.00	1mmol	x

* Absorbance (A405) Elisa Test

Induction of protection

Barley plants when treated with PS from *Tf* presented protection ranged from 70 to 85% as compareted with infected leaves (Table 4).

Table.4: Percentage of protection in barley leaves against B.sorokiniana by an PS from Tremella fuciformis at 2mmol of sugar.

Treatments	% protection*
tremella Control	x
tremella 24h	70b
tremella 48h	80b
tremella 72h	85b
healthy	x
infected	0a

See Materials and Methods for a description of each treatment. * mean percentage of protection from total of 10 plants per treatment and three repetitions. ** a different letter in collums indicates a statistically significantly difference from the infected plants ($P<0.05$)

IV. DISCUSSION

Polysaccharides have the highest capacity for carrying biological information and differ greatly in their chemical composition, molecular weight, conformation, glycosidic linkage, and degree of branching, etc [27]. Weintraub [28] working with immunology said that the surface polysaccharide confers protection against the disease. The immunological properties of PS from bacterial can be used in vaccines, for study cross-reactive antigens, serogrouping or serotyping systems and others. PS from *Xcc* and *Tf* were used and demonstrated protection in barley plants against infection by *Bipolaris sorokiniana* [8-10, 13]. So the objective was to evaluate the presence of crossed antigens.

The chemical composition and efficiency of extraction processes of polysaccharides in fruiting bodies from *T. fuciformis* were not completely clear because there are different methods. In Brazil, it is difficult to find fruiting bodies, and work in labor is possible when grown in medium. Many solid medium were used, but the best was made with sorghum seeds (variety 308-Embrapa).

Polysaccharides from *Tf* in this work, after precipitation with alcohol, presented white color that can be transformed in brown color in some days because enzyme

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peroxidase was presented and it acted in compound. After washing, precipitate and dialysis, the compound became white colored that can be kept in freeze for several years [7, 8]. With PS from *Xcc*, the color after precipitation, was white.

The concentration of beta glucan from *Tf* demonstrated that increased until day 20, being after it decreased, most likely due to the increased oxidation and correlated with the color from precipitated after 20 days. About the content of sugars *Tf* present more beta glucan and total sugars when compared with *Xcc* (Table 1). The results with production of polysaccharides with spores its important and the appearance are according with those observed by Chen & Hou [29]; Cho et al. [30] and Seviour et al.[31]. Chen & Hou [29] said that spores were broadly ellipsoid with 7-9 x 6-7um, smooth and hyaline. According to Cho et al.[30] and Seviour et al.[31], exopolysaccharide production from *Tf* revealed that the morphological form grows in mainly three different yeast-like forms: ovoid, elongated, and double yeast forms. Cho et al.[29] said that it is noteworthy to mention that the increased population of elongated yeast probably contributed to an increased PS production. So, in results (Table 1) we have formation of elongated form in *Tf* in 20 days that coincide with a higher glucose concentration.

Gel permeation chromatography of the polysaccharides on a Sepharose CL-4B column yielded two major peaks: a narrower peak in fractions (tubes 0-4) and another broad peak present in tubes 5-6 corresponded from 10 to 12mL. In this separation can be see that have the same peaks for *Xcc* and *Tf* that correspond to 5.12mmol of glucose for *Tf* and 2.04mmol for *Xcc*.

By TLC was observed that PS from *Xcc* and *Tf* presented in polymers the sugars as: glactose, glucose, mannose, arabinose and xylose. The results with *Tf* in part are in accord with several authors that estimated ratio of mannose, fucose, xylose, and glucuronic acid is 9:1:4:3 making glucoronic acid accounted for 17.6% of the polysaccharides [32-34]. Khondkar [34] was isolated PS from liquid cultures of nine *Tremella* species grown in Malt-yeast extract media for 6 days at 27ºC, and the results demonstrated that the polysaccharides in aqueous solution consisted of the following monomeric sugars: fucose, ribose, xylose, arabinose, mannose, galactose, glucose and glucuronic acid. The backbones of the polysaccharide structures consisted of α-(1→3)-links while the side chains were β-linked. In results (Table 2) from this work have not observed glucuronic acid.

Bach and Guzzo [13] worked with PS from *Xanthomonas campestris* pv. *citri* and *Xanthomonas campestris* pv. *manihotis* and observed the sugar compositions in isolates that suggesting that antigenic determinants may depend on the sequence of sugars, linkages, branching and stereochemistry of PS. Lozano and Sequeira [35] said that PS coats the outer membrane and seems to play a role in the specific recognition mechanism between the bacterial or fungal cell and the host cell walls. The PS can also prevent the interaction to host cell wall.

For determinated serological relationships among the preparations of PS, preliminary assays showed that Ouchterlony double-diffusion technique detect a weak cross-reactive antigens between *Xcc* and *Tf*. This indicates that these substances occur in low concentration, both in bacterial and fungi. For more contributions was made by sensitive serological techniques like ELISA.

Table 3 demonstrated that have a clear indication that cross-reactive antigens must be involved in this case. This assumption is corroborated by the evidence that antiserum to *Xcc* reacted with PS from *Xcc* (homologous reaction) and also with PS from *Tf* (heterologous reaction). To confirm that serological reaction depends from the polysaccharides antigen, samples was treated with sodium periodate. Sodium periodate oxidation eliminated all serological reactions suggesting that, perhaps, periodate-susceptible 1,2; 1,4; 1,3; 1,6 linked non-terminal residues or non-reducing terminal units that could be present in antigenic sites. It can be assumed that all antigenic determinants did not recover serological activity after reduction with sodium borohydride and mild acidic hydrolysis, suggesting that perhaps the periodate degradation led to splitting of the polysaccharide chain. Apart from this, it can be concluded that PS have antigenic determinants of *Xanthomonas* and *Tremella* and with an identical epitope. The antibody against Xcc was specific for an epitope and bounded to another antigen due to having similar chemical properties.

The results presented in Table 4 indicate that protection was conferred to barley plants when the inducer (PS) was applied in the plant, and after 72h from the treatments the protection was higher. This same effect was observed by Bach et al [20], Castro and Bach [21] in work with other elicitors.

V. CONCLUSION

The use of polysaccharides in agriculture aimed at disease control on plants has been important as a alternative control in biotechnology. The results suggest that PS from Tremella can be induce protection against disease in barley plants. In study from PS in the *Xanthomonas-Tremella*, interaction there is a "key" cross-reactive antigens. The hypothesis is that the molecular structures of polysaccharides should be similar and have the same common properties and both can be used for the same aim: the plant protection against diseases or as a medicinal usage.

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Ethnobotany Study of Medicinal Plants Used in the Treatment of Respiratory Diseases in the Middle Region of Oum Rbai

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Abstract**—** *The ethnobotanical study carried out in the region of Oum Rbia (Morocco) made it possible to identify the medicinal plants used by the local population and to collect the maximum information on this use.*

A survey of 1360 people from the region's population noted that 170 people use medicinal plants against respiratory diseases. Women accounted for 55.3% of the workforce versus 44.7% for men; Married people 70% against 28% for singles. The illiteracy rate is high (34.1%).

The leaves are the most widely used part of the plant. Infusion and decoction are the most commonly used methods for preparing traditional remedies.

The most widely used species in the treatment of respiratory diseases are: Origanun glandulosum, Eucalyptus globulus, Nigella sativa, Mentha pulegium, Lavandula stoechas, Zingiber officinale, Ammodaucus leucotrichus, Ficus carica. In addition, some species have toxicity either because of the ignorance of the necessary dose or because the people treated are affected by other diseases.

Thus, the survey made it possible to inventory 66 medicinal species which are divided into 36 plant families; Lamiaceae (21.2%), Myrtaceae (10.6%), Apiaceae (8.8%), Amaryllydaceae (7.7%) and Zingiberaceae (7.1%).

These results resulted in a catalog of medicinal plants used in the study area to treat respiratory diseases. It is a local know-how that must be considered as a heritage to be preserved and developed.

Keywords— Oum Rbia, Ethnobotany survey, respiratory diseases and medicinal plant.

I. INTRODUCTION

The respiratory system (nasal passages, bronchi and lungs) can be the subject of several diseases: acute infections such as pneumonia and bronchitis, or chronic conditions such as asthma and COPD. Thus, respiratory diseases, regardless of the age of the patients, represent approximately 5.5 million medical consultations per year (Ministry of Public Health, 2001). In the majority of cases (85%), these patients have acute respiratory diseases, mainly angina or acute bronchitis. The remaining cases (15%), have a chronic respiratory disease or a suspicion of pulmonary tuberculosis (Ministry of Public Health, 2001). Among chronic respiratory diseases, asthma occupies the first place and pulmonary tuberculosis accounts for only 4 to 5% of cases (Ministry of Public Health, 2001).

The World Health Organization estimates that traditional medicine covers the primary health care needs of 80% of the population in developing countries (Vines, 2004). Plants still play a very important role in the medical traditions and life of the inhabitants of the Maghreb, but the rules of their use sometimes lack rigor and do not take into account the new demands of modern therapy (Bellakhdar, 2006).

Morocco, whose geographical location has a flora rich in diversity, has about 4200 species of which only a hundred are currently exploited according to El Meskaoui (2008).

In order to preserve the natural heritage of the Middle Oum Rbai region, we carried out an ethnobotanical study collecting the knowledge, attitudes and practices of the local population concerning all the plants used in the Treatment of respiratory diseases.

II. MATERIAL AND METHOD

1. The study area:

Oum Rabia means the provinces of Khouribga, Fkih Ben Saleh and Kasbat Tadla. It is linked to the Beni Mellal-Khénifra region following the territorial division of 2015 (Fig. 1).

The Beni Mellal-Khénifra region is limited to the west by the regions of Casablanca Settat and Marrakech-Safi, to the south by the Draa Tafilalet region, to the east by the

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eastern region and to the north by the regions of Fez Meknes and Rabat Sale Kenitra.

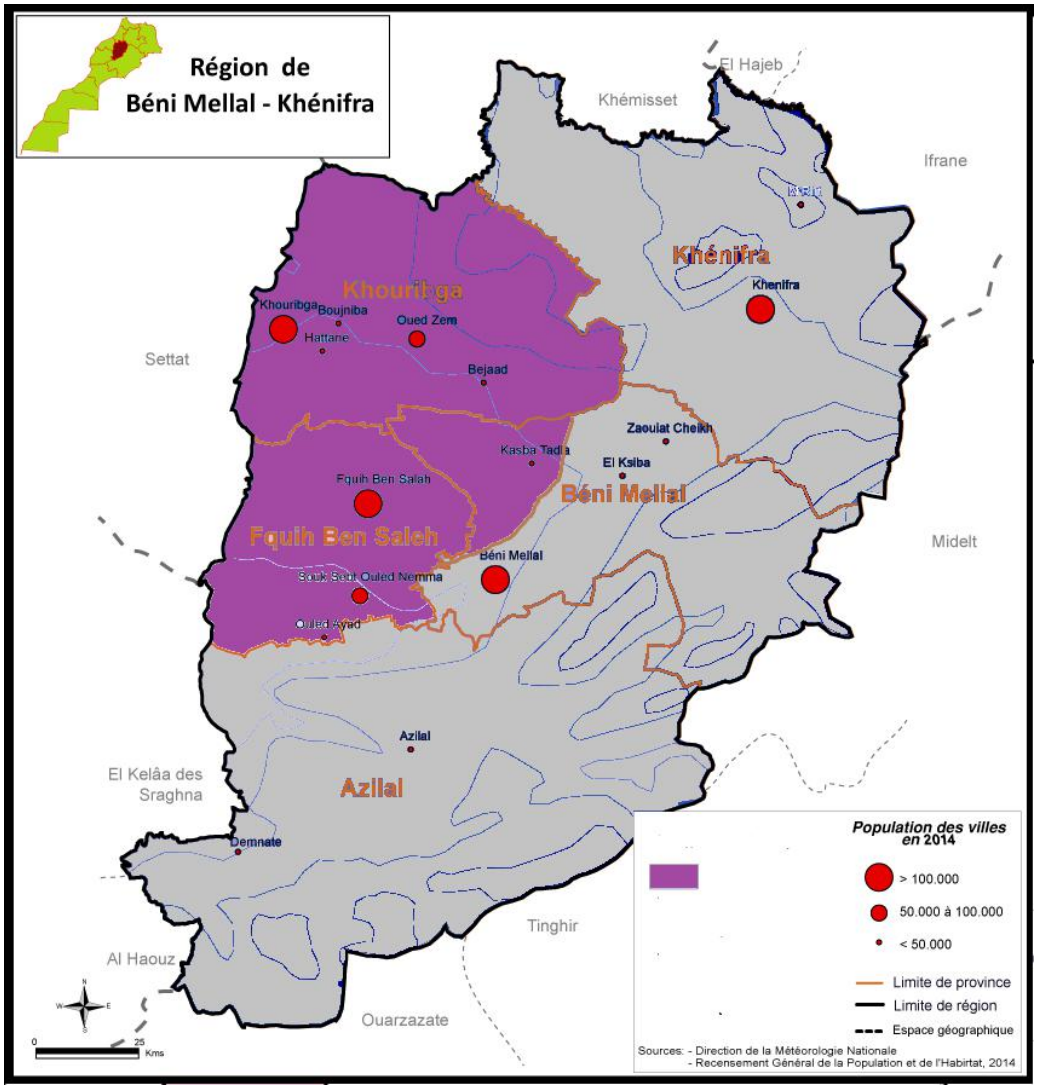

Fig.1: Map of the study area (General Monograph of the Beni Mellal-Khénifra Region, 2015)

2. The methodology:

An ethnobotanical survey was conducted in the Oum Rbia region between 2010 and 2015 and was based on stratified random sampling; A pre-established questionnaire based on information on the profile of the informant (Age, intellectual level, family situation ...) and on the other hand on the use of plants in traditional medicine (vernacular name of the plant, part used, dose used, method of preparation, disease treated, etc.). Plants are collected in the wild or obtained in traditional healers.

The local name of the plant is given by the interviewees or by the traditional healers and the scientific name is determined in the laboratory. Determination of species was carried out thanks to the New flora of Algeria and the southern desert regions Quézel & Santana (1962, 1963), volumes I and II; The Practical Flora of Morocco Fennane et al. (1999, 2007), Volume 1 and 2; Catalog of vascular plants in Northern Morocco, including identification keys, by Valdés et al (2002), volumes I and II; And the Vascular Flora of Morocco: inventory and chorology Fennane and Ibn Tattou (2005). Books such as: Medicinal plants of Morocco Sijelmassi (1993); The traditional Moroccan pharmacopoeia Bellakhdar (1997); And Moroccan medicinal and aromatic plants Hmamouchi (2001) have also been used for the recognition of medicinal plants.

III. RESULTS

The survey of 1360 people in the study area identified 170 people who use herbal medicines against respiratory diseases.

- **The use of medicinal plants according to the survey:**

The calculations of the following proportions are made on the basis of the number of people who use medicinal plants against the ailments of 170 people.

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1) **The informant by sex**: The analysis of Figure 2 shows that women predominate in the use of medicinal plants in herbal medicine for respiratory diseases with 55% compared with 45% for men.

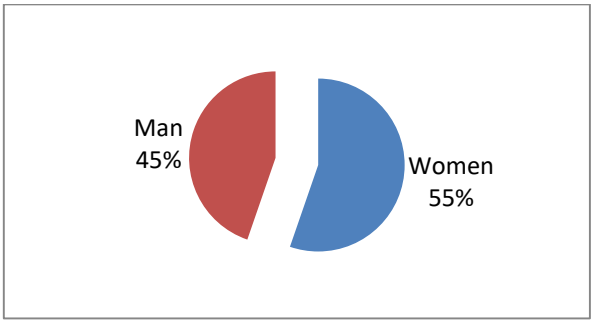

Fig.2: Use of Medicinal Plants by Sex in the Oum Rbai Region

2) **The informant according to age**: Analysis of the results obtained shows that 54% of the respondents belong to the age group] 30-50], 21% are over 50 years old, 18% are part of the age group] 20-30] and 7% are aged less than 20 years (Figure 3).

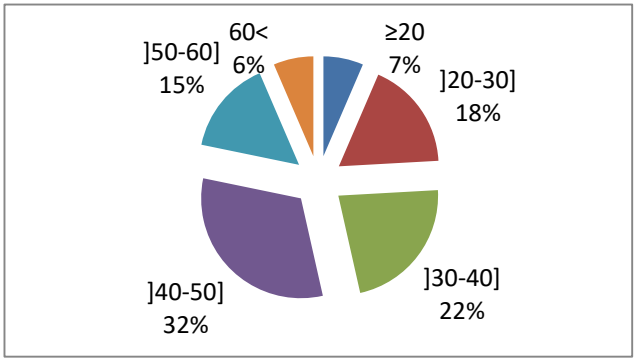

Fig.3: Use of Medicinal Plants by Age in the Oum Rbai Region

3) **The informant according to the intellectual level:** the results show that the illiterate persons represent 34% of the workforce, followed by secondary education with 27%, primary school with 17%, while the college level has 12 % And academics 10%.

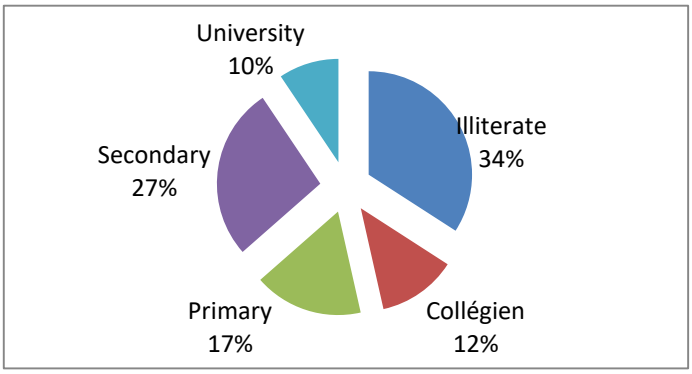

Fig.4: Use of Medicinal Plants by Study Level in the Oum Rbai Region

4) **Profile of the informant according to the family situation:** married people predominate with 70%, against married couples who represent 28% and widowers 2% (Figure 4).

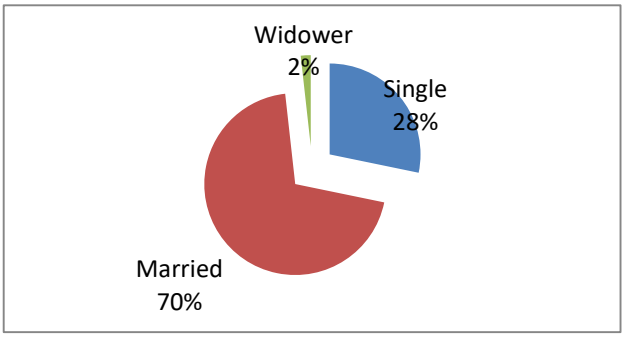

Fig.5: Use of Medicinal Plants by Family Status in the Oum Rbai Region

➢ **The use of medicinal plants according to the part used:** Phytotherapy for the treatment of respiratory disorders is based first on the leaves (70 citations), then on the seed (34 citations), and finally on the rhizome and the whole plant with 18 citations for each of the two categories. For other parts of the plant, the number of citations is much lower (Figure 6).

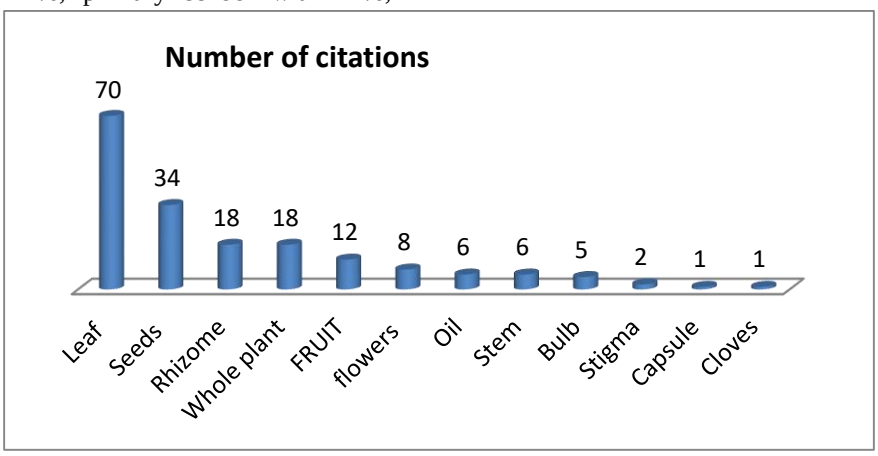

Fig.6: Plant parts used in herbal medicine in the Oum Rbai Region

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- **The Flora analysis:** The floristic analysis shows that *Origanum glandulosum* is the most used species against respiratory diseases in the region of Oum Rbai followed by *Eucalyptus globulus*, *Nigella sativa*, *Mentha pulegium*, *Lavandula stoechas*, *Zingiber officinale*, *Ammodaucus leucotrinarum* and *Ficus carica* (figure7).

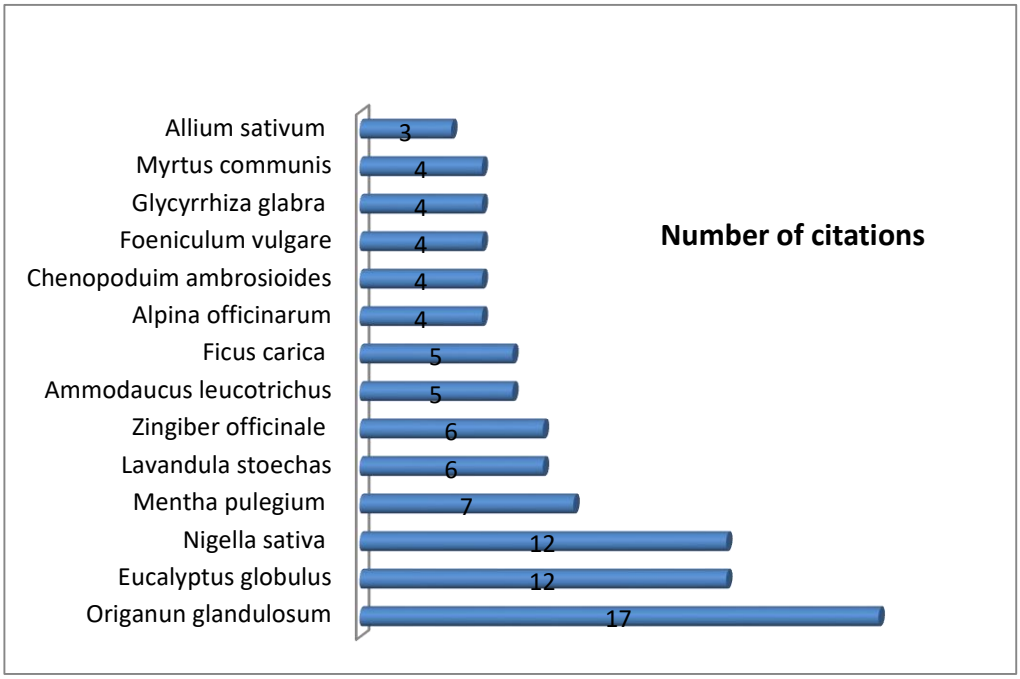

Fig.7: Medicinal plants most commonly used to treat respiratory diseases in the Oum Rbai region

The analysis of the results also shows that the Lamiaceae family is dominant with 21.2% of the resected species, Myrtaceae in second class with 10.6%, Apiaceae with 8.8%, Amaryllidaceae and Zingiberaceae with 7.1% each.

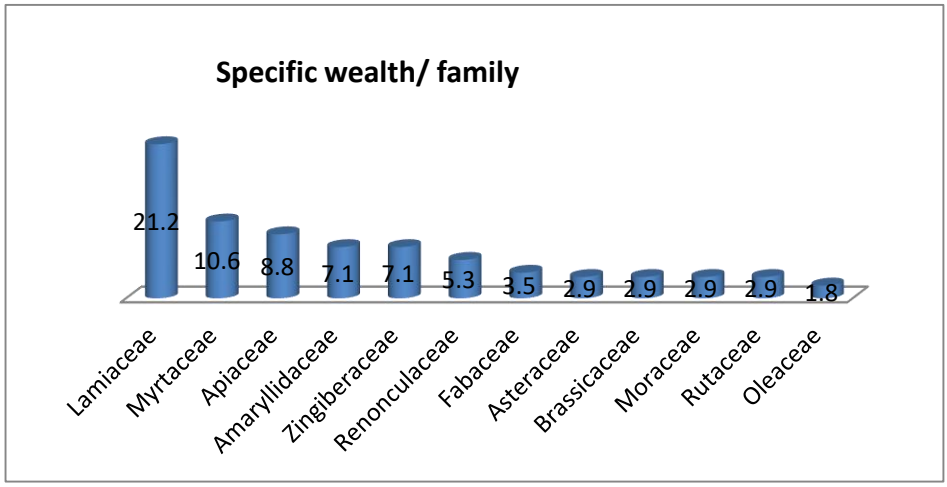

Fig.8: Specific Wealth of Families Represented to Treat Respiratory Diseases in the Oum Rbai Region

- **Respiratory diseases most frequently treated by plants:** According to the results obtained, respiratory diseases most frequently treated by medicinal plants in the region of Oum Rbia are: influenza first with 47 citations, cooling with 44 citations, 39 citations concern respiratory diseases in general Because the inquiry does not specify the exact disease, but it uses the plant for all that concerns the respiratory apparatus, the cough (19 citations).

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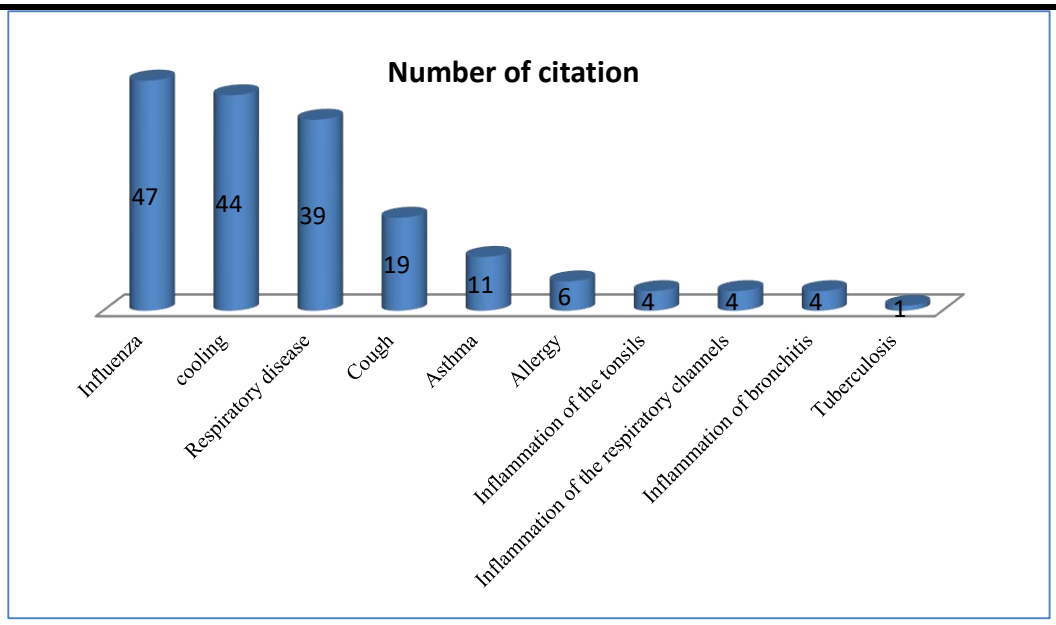

Fig.9: Respiratory diseases treated by plants in the Oum Rbai region

IV. DISCUSSION

Analysis of the results obtained from 170 people using medicinal plants against respiratory diseases shows that women predominate for the use of medicinal plants with 55% against 45% for men. These results are consistent with those obtained by Hmamouchi (1999), Mehdioui (2008) and El Hilah (2015). 54% of respondents belong to the age group] 30-50], 21% are older than 50 years, 18% are in the age group] 20-30] and 7% are less than 20 years. This can be explained by the company's return to the use of traditional herbal medicine with a good transmission of the popular knowledge of the elderly towards the young (Hseini, 2008). Illiterates represent 34% of the workforce, followed by secondary schooling with 27%, primary education 17%, college 12% and university 10%. A renewed interest in medicinal plants is noticed; People are beginning to become aware of the side effects of pharmaceutical treatments based on chemical molecules. The married population predominates with 70%, against the singles who represent 28% and the widowed 2%. These results are related to the responsibility of brides towards the needs of the family. This is consistent with the results obtained by other studies at the national level (Hseini, 2008, Benkhnigue, 2010, El Hilah, 2015 ...). Phytotherapy for the treatment of respiratory diseases relies primarily on the leaves, seed, rhizome and whole plant, for the other parts of the plant the number of citations is much lower.

Plant analysis shows that *Origanum glandulosum* is the most widely used species against respiratory diseases in the Oum Rbai region followed by *Eucalyptus globulus, Nigella sativa, Mentha pulegium, Lavandula stoechas, Zingiber officinale, Ammodaucus leucotrinarum* and *Ficus carica*. The most common families are Lamiaceae, Myrtaceae, Apiaceae, Amaryllidaceae and Zingiberaceae. Lamiaceae also rank first in the study carried out in the Central Plateau of Morocco by El Hilah in 2016.

The respiratory diseases most frequently treated by medicinal plants in the region of Oum Rbia are flu, cooling, coughing. As for asthma, allergy and more serious lung diseases, the population uses less herbal medicine.

V. CONCLUSION

Surveys of 1360 people in the region have identified 170 people who use herbal medicines against respiratory diseases. The diseases most commonly used by herbal medicine are influenza, chills and coughs; People have less recourse to this mode of medication when conditions are more serious, such as asthma and tuberculosis. Thus, the survey made it possible to inventory 66 medicinal species which are divided into 36 plant families. . Women are predominant with 55.3% against 44.7% for men. Traditional medicine is more practiced by married people with 70% against 28% for singles. The illiteracy rate is high at 34.1%. As for the plant, the leaves are the most used part. Infusion and decoction are the means of preparing the most used remedies. The species most commonly used in the treatment of respiratory diseases are: *Origanun glandulosum, Eucalyptus globulus, Nigella sativa, Mentha pulegium, Lavandula stoechas, Zingiber officinale, Ammodaucus leucotrichus, Ficus carica.* In addition, some species have toxicity either because of the ignorance of the necessary dose or because some people have other diseases. The results show a relative importance of the following families: Lamiaceae (21.2%),

Myrtaceae (10.6%), Apiaceae (8.8%), Amaryllydaceae (7.7%) and Zingiberaceae (7.1%). These results resulted in a catalog of medicinal plants used in the region to treat respiratory diseases. The catalog of 66 species testifies to a local know-how of great value, it is a heritage that must be preserved and managed in the sustainability.

Table.1: List of medicinal plants used in the treatment of respiratory diseases in the Middle Oum Rbia region

Family	**Vernacular name**	**Scientific name**	**Used part**	**Preparation**	**Administration mode**	**Disease**
Amaryllidaceae	Thoum	*Allium sativum*	Bulb	Decoction	Oral	Respiratory, cooling
	Lamkhenza	*Chenopoduim ambrosioides*	Leaf	Infusion, Juice	Oral, fumigation, massage	Influenza, tonsillitis, respiratory
	Selk	*Beta vulgaris/serpetual spinach*	Leaf, whole plant	Decoction	Oral	Respiratory
	Sabra	*Agave americana*	Leaf	Decoction	Oral	Asthma
	Pinard/sabanikh	*Spinacia olearea*	Leaf	Decoction	Oral	Respiratory
	Basla hamra	*Allium cepa*	Seed	Powder	Oral	Allergy
Apiaceae	Krafess	*Apium graveolens*	Leaf, oil	Infusion	Oral	Calming bronchitis
	Nafeaa/basbass	*Foeniculum vulgare*	Seed, oil	Decoction , powder	Oral, massage	Influenza in babies and allergy
	Camoun souffi	*Ammodaucus leucotrichus*	Seed	Infusion	Oral	Influenza, cough
	Kazbour lbir	*Coriandrum sativum*	Whole plant, seed	Infusion	Oral, Rinsing	Inflammation of respiratory ducts, cold
	Habat hlawa	*Pinpinella anisum*	Seed	powder	Oral	Allergy
	Maadnouss	*Petroose linum sativum*	Aerial part	Decoction	Oral	Respiratory
	Camoun	*Cuminum cyminum*	Seed	tisane	Oral	Respiratory
Asphodelaceae	Sebbar	*Aloe socotrina* l	Leaf	Tisane	Oral	Influenza
	Blalouz/ joudour barwk	*Asphodelus microcarpus*	Rhizome	Powder	Goute	Asthma
Asteraceae	Taskra	*Echinops spinosus*	Stem	Decoction	Oral	cooling
	Buagad /hindiba barri	*Cichorium intybus*	Leaf	Infusion	Oral	Respiratory
	Tarhella/oum karman	*Inula helenium*	Leaf, flowers, rhizome	Infusion	Oral	cough
	Chih	*Artemisia herba-alba asso*	Leaf	Infusion	Oral	Respiratory
Berberidaceae	Bosman	*Berberis vulgaris*	Fruit, stem bark	Decoction	Oral	Bronchial Inflammation

Borraginaceae	Lessan laard	*Borrago officinalis*	Leaf, Seed	Infusion	Oral	Cough, Influenza
Brassicaceae	Hab rchad	*Lipiduim sativum*	Seed	Powder	Oral	Tonsillitis, Influenza
	Fijl elhessan	*Armoracia rusticana*	Rhizome	crude	Oral	Influenza
Cactaceae	Karmouss hindi	*Opuntia ficus indica*	Flowers	Infusion	Oral	Cooling
Caprifoliaceae	Borwabez	*Sambucus canadensis*	Leaf, Flowers	Infusion	Rinsing	Bronchial Inflammation
Caryophyllaceae	Wijan/bilsan	*Sambucus nigra*	Whole plant	Decoction	Oral, Rinsing	Respiratory
Champigions	Terfass	*Terfezia leonis*	Bulb	Decoction	Oral	Cooling
Colchicaceae	Temrat legrab/lessan jmel	*Androcymbium gramineum*	Leaf	Infusion	Rinsing	Bronchial Inflammation
Euphorbiaceae	Takaout	*Euphorbia resinifera*	Leaf	Powder	Oral	Asthma, Influenza, cough
Fabaceae	Kharoub timarin hindi	*Ceratonia siliqua*	Seed	juice	Oral	Asthma, cough
	Arkssouss	*Glycyrrhiza glabra*	Rhizome	Powder	Oral	cooling
Gentianaceae	Tamrat alakrab/hchechat laakreb	*Centaurium spicatum*	Whole plant	Decoction	Oral	Inflammation of respiratory tract
Hydrangeaceae	Taililote/taylulut	*Capparis spinosa*	Seed	Cooked	Oral	Cooling
Illiciaceae	Badyana	*Illicium verum*	Capsule	crude	Oral	Allergie
Iridaceae	Zaafran	*Crocus sativus*	Stigma	Decoction	Oral	Cooling, Respiratory
Juncaceae	Smar	*Juncus maritimus*	Seed, rhizome	Powder	Oral	Asthma
Lamiaceae	Zaatar	*Origanun glandulosum*	Leaf	Infusion	Oral	Influenza, Cooling
	El khouzama/halhal	*Lavandula stoechas*	Leaf	Infusion	Oral	Cooling, Influenza, allergy, cough
	Flio	*Mentha pulegium*	Leaf	Infusion	Oral	Cooling
	Miriwt	*Marrubium vulgrave*	Leaf	Infusion	Oral	Cooling
	Jaaidiya	*Teucrium fruticans*	Aerial part	Infusion	Oral	Influenza
	Merdedouch	*Origanum majorana*	Whole plant	Infusion	Oral	Cooling
Liliaceae	Korrat	*Allium porrum*	Bulb	Decoction	Oral	Asthma in children
Malvaceae	Khebiza	*Malva sylvestris*	Leaf	Decoction	Oral	cough
Moraceae	Karmouss	*Ficus carica*	Fruit	Decoction	Oral	Respiratory

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Myristicaceae	Elgouza sahraouiya	*Myristica fragrans*	Seed	Powder	Oral	Influenza
Myrtaceae	Caliptus	*Eucalyptus globulus*	Leaf	Infusion, Decoction	Oral, fumigation	Influenza, cooling
	Krenfel	*Eugenia caryophyllata*	Cloves	Infusion	Oral	Antiseptic Respiratory
	Rayhan	*Myrtus communis*	Leaf	Infusion	Oral	Cooling, cough, Influenza
Oleaceae	Zaytoun	*Olea europaea*	Huile	Tisane	Oral, massage	Influenza, cough
Papilionaceae	Lhalba	*Trigonella foenum graecum*	Seed	Crude, tisane	Oral	Respiratory
Pinaceae	Tayda	*Pinus halepensis*	Leaf	Decoction	Oral	Respiratory
Plantaginaceae	Massassa	*Plantago major*	Leaf	Juice	Oral	Asthma, tuberculosis
	Zerktouna	*Plantago psyllium*	Whole plant	Infusion	Oral	Asthma
Poaceae	Lakbal/draa	*Zea mays*	Oil	Oil	Oral, massage	Asthma
Portulagaceae	Rejla	*Portulaca oleracea*	Aerial part	Decoction	Oral	Cooling
Ranunculaceae	Saneuj/haba sawda	*Nigella sativa*	Seed	Powder	Oral	Cooling , allergy, respiratory
Rutaceae	Fijl ajmal	*Ruta graveolens*	Leaf, Flowers	Juice	Oral	Inflammation of respiratory canals
	Lemon	*Citrus sinensis*	Fruit	Infusion	Oral	Influenza, tonsillitis
	Hamed	*Citrus limon*	Fruit	Juice	Oral	Respiratory
	Zanboua	*Citrus bigaradia*	Fruit	Juice	Oral	Cough
Valerianaceae	Sanbel	*Valeriana j atamansi*	Leaf	Powder	Oral	Asthma
Verbinaceae	Louiza	*Verbena officinalis*	Leaf	Infusion	Oral	Respiratory
Violaceae	Banafsaj	*Viola tricolor*	Leaf, Flowers	Infusion	Oral	Influenza
Zingiberaceae	Khedenjal	*Alpina officinarum*	Rhizome	Infusion	Oral	Cooling
	Skenjbir	*Zingiber officinale*	Rhizome	Infusion	Oral	Cough, Influenza

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Study on Assessment of Physico chemical properties of Industrial wastes

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Abstract— *The physicochemical parameters of wastewater collected from five sampling sites were investigated. These parameters were analyzed by standard methods. The pH of the waste water varied from 4.7 to7.66, while the waste water conductivity ranges from 1205.3 to 7130.17 µScm^{-1}. The maximum total dissolved solid was 8100mg/l.and the maximum biological oxygen demand was 2763.35 mg/l. The chemical oxygen demand of the selected samplesites varied widely (772.56–3105.13 mg/l), the nitrate content was found to be maximum in sample W5 (166.00mg/l), and the sulfate content was found to be high in samples W1 andW5 (500and 4875mg/l). The chloride and sulphied contents were maximum at samples of W3 and W5 their concentrations were8543.45 and 10.7mg/l respectively. The physic chemical parameters studied in this work were varied between the samples and almost all parameters studied were higher compared with the permissible limit prescribed by the United States Environmental Protection Agency and World Health Organization.*

Keywords— Wastewater, Physicochemical, conductivity, Biological oxygen demand, Chemical oxygen demand, TDS, Chloride, Sulphied.

I. INTRODUCTION

The pollutants from the wastewater are harmful to the public health and the environment, and they are toxic to the aquatic organisms as well. The wastewater treatment helps to remove contaminants from water to decrease pollutant load [1]. Water pollution occurs through natural processes in certain cases, but most of the pollutions caused by human activities [2]. The used water of a community is called wastewater or sewage. The waste waters are not treated before being discharged into waterways, which causes serious pollution in the particular environment [3]. There are three major categories of pollutants that cause pollution in water. The first category includes disease-causing agents such as viruses, protozoa, parasitic worms, and bacteria, which enter sewage systems and untreated waste. Because of the abundance of these microbes, wastewater acts as the common source of transmission for diseases such as dysentery, cholera, and typhoid. The second category of water pollutants includes oxygen demanding waste, which includes the biodegradable matter such as plant residues and animal manure, which are added to the water naturally or by human beings. In natural process, this biological waste uses oxygen present in the waste water and thereby results in oxygen depletion. Once all the oxygen has been depleted, bacteria are able to take control of the sewage, by making the water polluted. The third category of water pollutants includes water soluble inorganic pollutants such ascaustics, salts, acids, and toxic metals. Another kind of water pollutants includes ammonium salts, nitrates, phosphates, and so on. The pollutants such as nitrates and phosphates are the important nutrients, andthis favor thegrowth of algae and thereby results in eutrophication [4 -6]. Studies on the water quality were carried out by various researchers on various effluents. Earlier studies revealed that anthropogenic activities strongly affect the water quality. This was a result of cumulative effects not only from upstream development but also from inadequate wastewater treatment facilities [7]. The waste water quality can be measured by analyzing the variations of total suspended solids, total phosphorous, chemical oxygen demand (COD), copper, iron, nickel, nitrogen, lead, zinc, and so on [8-10].

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Wastewater is any water that has been adversely affected in quality by anthropogenic influences. It comprises liquid waste discharged by domestic residences, commercial properties, industries, and/or agriculture and can encompass a wide range of potential contaminants and concentrations [11]. The contamination and quality of irrigation water are of the main concern especially in the regions with limited water resources [12]. Characterization of wastewater and activated sludge has been used for control and optimization of existing processes and development of new processes. The most possible sources of water, soil, and plant pollutions are sewage sludge and residues of industries and intensive fertilization [13]. The importance of testing a waste characterization in this study is to identify the composition of the waste so actions can be taken to reduce the amount of trash discarded [14]. The waste water discharged from various domestic and industrial sources has been characterized by various researchers [11, 13]. Urban environmental management is one of the important issues as the urbanization trend continues globally. The under-management of municipal wastewater in many southern urban areas is a major challenge. Management of wastewater in metropolitan cities is a very difficult task. The unsafe disposal of wastewater results in water pollution as well as terrestrial pollution. It causes various health problems that are epidemics due to the processing of the contaminated water [15, 16]. This wastewater eutrophicates the water bodies, causing the mortality of aquatic biological resources. Hence, the role of treatment plants is in the sustainable use of wastewater as they make the water usable for various purposes [17]. The major objective of the present study was to characterize the wastewater discharged from different industries in Kombolcha and Debreberhan town, Ethiopia. A study of this kind will improve our knowledge on the quality of wastewater being discarded into the environment due to various anthropogenic activities.

II. MATERIALS AND METHODS

For the present study, effluent samples were collected from different industries in Ethiopia. The effluent samples were collected from the outlet of the process. The effluent was collected in polythene containers of two liters capacity and were brought to the laboratory with due care and was stored at 20^{o}C for further analysis. Chemicals used for the analysis of spent liquor were analytical grade reagents. The physical and chemical characteristics of industrial effluents parameters viz. pH, total alkalinity, total acidity, COD, BOD5, total solids (TS), total dissolved (TDS), totalsuspended solids (TSS), chlorides and sulfides were analyzed as standard method of APHA (1998)[18].

Table.1: Location of sampling site

Location/site	Sample
Waksu Textile	W1
Kombolcha tannery	W2
Debreberhan tannery	W3
Hayek Tannery	W4
Debreberhan Ethanol	W5

III. RESULTS AND DISCUSSION

The values of the physico-chemical parameters observed in the present study may serve as an indicator of the fertility or pollution level of the study area. The experimental data on physico-chemical properties of wastewater samples collected from different indusial area of Kombolcha and Debreberhanare shown in Figures1–10.

Table.2: Physico Chemical characterization of industrial waste water

Parameter	*W1*	*W2*	*W3*	*W4*	*W5*
PH	*5.2*	*6.77*	*7.66*	*5.7*	*4.7*
EC	*1205.3*	*4287.8*	*4156.7*	*5863.2*	*7130,17*
TDS	*1370*	*4300*	*4270*	*6840*	*8100*
BOD	*878.2*	*1267.32*	*1196.12*	*2321.01*	*2765.35*
Alkaline	*2500*	*2000*	*4500*	*4100*	*2501.3*
COD	*772.56*	*1287.65*	*1246.03*	*2461.54*	*3105.13*
Cl^-	*3403.2*	*886.25*	*8543.45*	*5388.4*	*3010.33*
S^{2-}	*6.3*	*0.135*	*0.475*	*2.235*	*10.7*
NO_3-N	*48.5*	*11.00*	*33.00*	*34.00*	*166.00*
SO_4^{2-}	*500*	*250*	*375*	*425*	*4875*
SS	*700.02*	*600*	*1050*	*1550*	*6500*

Determination of pH

The pH of all five samples was measured immediately after its collection using a pH meter. The pH of the water sample collected from different sites was ranging from 4.7 to 8.2 and the result was shown in table 2.and Figure 1. The pH of the water is known to influence the availability of micronutrients as well as trace metals [19]. It is well known that the pH is an important parameter in evaluating the acid–base balance of water. A pH value of 7 is neutral; a pH less than 7 is acidic, and a pH greater than7 represents base saturation or alkaline. The principal component regulating

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ion pH in natural waters is the carbonate, which comprises CO_2, H_2CO_3, and HCO_3 [20].

Electrical conductivity

Water conductivity is mainly attributed to the dissolved ions liberated from the decomposed plant matter [21] and input of inorganic and organic wastes [22]. EC values are noted to be different for various samples, ranging from 1205.3-7130.17 μScm^{-1}, and the result was shown in Figure 2. EC depends on the dissolved solids in the discharged water. The EC being the measure of dissolved solid in solution implies that sampleW5 had more dissolved solids than other sample sites. High EC values indicate the presence of high amount of dissolved inorganic substances in ionized form. The fluctuations in EC in any particular location depend on the fluctuation in TDS and salinity [23].

Determination of total dissolved solids

The amount of TDS in this study varies from 1370 to 8100 mg/l, and the result was shown in Figure 3. In the waste water, TDS are composed mainly of bicarbonates, chlorides, carbonates, phosphates, and nitrates of calcium, magnesium, sodium, and potassium; manganese; salt; and other particles [24]. The higher values of TDS may be due to the discharge of waste from effluents from various small-scale industries in these towns. Kataria et al. [25] reported that increase in the value of TDS indicated pollution by extraneous sources.

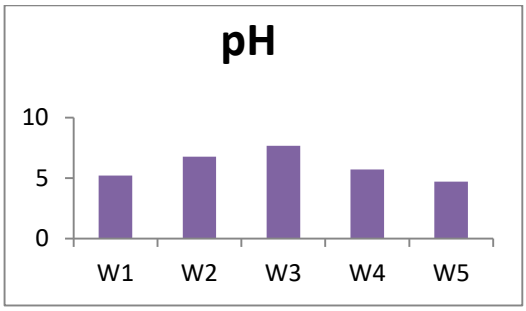

Fig.1: PH of industries wastewater

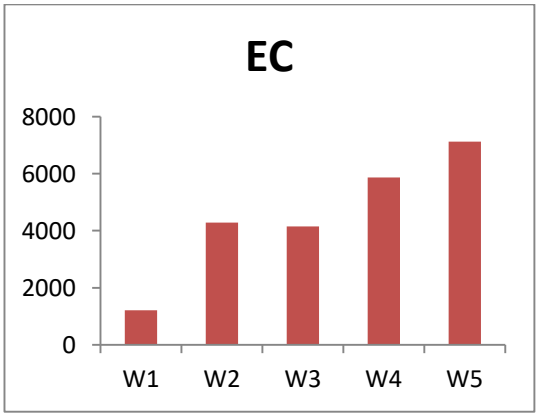

Fig.2 EC of industries waste water

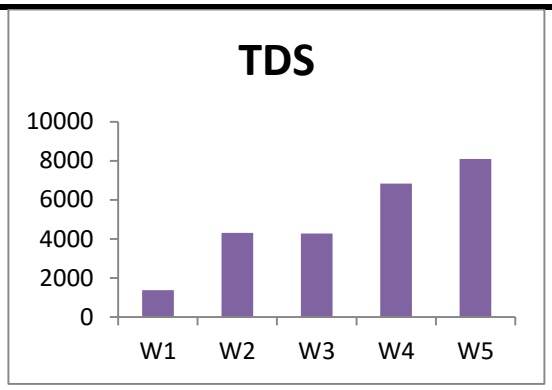

Fig.3: TDS of industries waste water

Determination of Biological oxygen demand

BOD showed the minimum value of 878.2 mg/l and the maximum value of 2765.35 mg/l. The registered BOD value was high in the present study (Fig. 4). BOD increases due to biodegradation of organic materials that exerts oxygen tension in water body [26]. Increases in BOD can be due to heavy discharge of industrial wastewater, animal and crop wastes, and domestic sewage. BOD value has been widely adopted as a measure of pollution in the particular environment. It is one of the most common measures of organic pollutant in water. It indicates the amount of organic matter present in water. Sources of BOD in aquatic environment include leaves and dead plants, woody debris, animals, animal manure, industrial effluents, wastewater treatment plants, feedlots, and food- processing plants and urban storm water runoff [27].

Determination of Chemical oxygen demand

COD showed the minimum value of 772.56 mg/l and the maximum value of 3105.3 mg/l (Fig. 5). All organic compounds with few exceptions can be oxidized by the action of strong oxidizing agents under acidic condition. The COD determination is a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant. While determining COD, oxygen demand value is useful in specifying toxic condition and presence of biologically resistant substances. The COD and BOD values are a measure of the relative oxygen - depletion Dissolve oxygen. The DO content of the wastewater collected from different sources decreases with increase COD and BOD. BOD directly influences the amount of DO in rivers and streams. The greater the BOD and COD the more rapidly oxygen is depleted in the waste water. This means that less oxygen is available to higher forms of aquatic life. The effect of a waste contaminant. Both have been widely adopted as a measure of pollution effect. COD is also one of the most common measures of pollutant organic material in water. COD is similar in function to BOD, in which both

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measure the amount of organic compounds in waste water [27].

Determination of Nitrate

The nitrate content of wastewater samples varies from 11.00 to 166.00mg/l, and the result was shown in Figure 6. Nitrate content is an important parameter to estimate organic pollution in a particular environment, and it represents the highest oxidized form of nitrogen. Nitrate is one of the very common contaminants in ground water and surface water. Nitrate occurs naturally in source water as a result of decaying plants. However, there are other manmade sources of nitrate that can increase its presence in source waters to dangerous levels. Agricultural sources of nitrates include livestock waste matter and chemical fertilizers. The presence of nitrates in the water samples is suggestive of some bacterial action and bacterial growth [28].

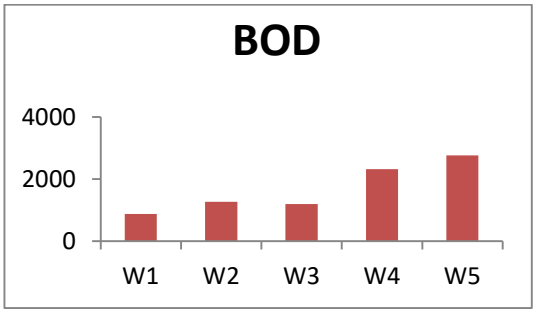

Fig.4: BOD of industries wastewater

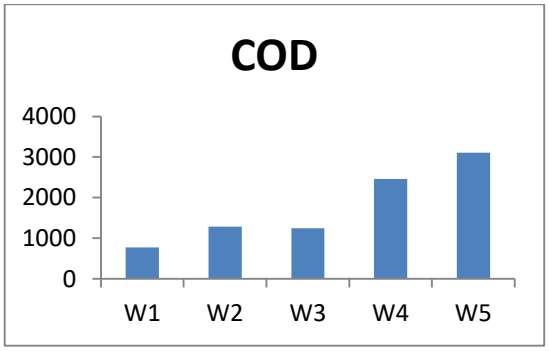

Fig.5: COD of industries waste water

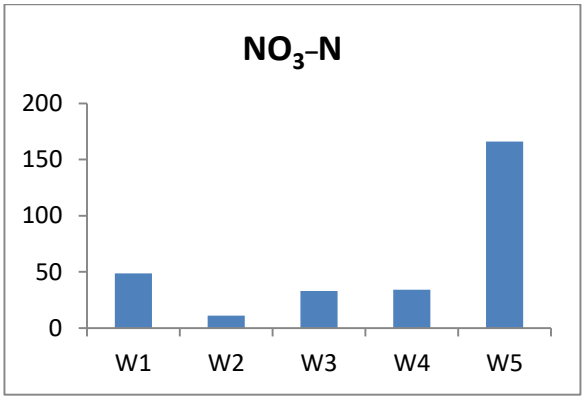

Fig.6: Nitrogen in Nitrate of industries waste

Determination of Sulfate

The sulfate content of wastewater varies from 600to 6500mg/l, and the result was shown in Figure 7. Sulfates are considered toxic to plants or animals at beyond normal concentrations. Sulfates are formed due to the decomposition of various sulfur- containing substances present in water bodies. The sulfate ions ($SO4^{-2}$) occur naturally in most water supplies and hence are also present in wastewater. In human beings, small concentrations cause a temporary laxative effect [29]. Sulfate occurs naturally in water as a result of leaching from gypsum and other common minerals [30].

Determination of Chloride

Thechloride content of wastewater samples varies from 886.25 to 8543.45mg/l and the result was shown in table 2. Figure 9.And the levels exceed the permissible chloride level of 1000 mg/L of effluent discharge into inland surface waters. The chloride content in water sample gives an idea of the salinity of water sample.

Determination of Sulfide

Sulfides are particularly objectionable because hydrogen sulfide will be liberated if they are exposed to a low pH environmental, and if they are discharged into stream containing iron, black precipitates will be formed. Sulfides may be toxic to stream organisms or to organisms employed in biological treatment systems. The results of present study revealed that sulfide level from industrial wastes were given in Table 1 and figure 10. W1, W3, W4and W5 exceeds the permissible sulfide level of 2 mg/ L. of effluent discharge into inland surface waters [31].

Determination of Alkalinity

Alkalinity of water is its acid neutralizing capacity. It is the sum of all the bases. The alkalinity of natural water is due to the salt of carbonates, bicarbonates, borates silicates and phosphates along with hydroxyl ions in the Free State. However, the major portion of the alkalinity is due to hydroxides, carbonates and bicarbonates. The results of present study revealed that alkalinity level from each industrial waste are given in Table 1 and figure 11.

Determination of Total Suspended Solids (TSS)

The results of present study revealed that TSS level from different industrial processes are given in Fig. 6 and it exceeds the permissible TSS level of (20-200) mg/ l. These suspended impurities cause turbidity in the receiving streams. The composition of solids present in industrial effluent mainly depends upon the nature and quality of raw material processed in the industries. High level of total suspended solids present in the industrial effluent could be attributed to their accumulation during the processing of

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finished proses. Presence of total suspended solids in water leads to turbidity resulting in poor photosynthetic activity in the aquatic system [32] and clogging of gills and respiratory surfaces of fishes [33].

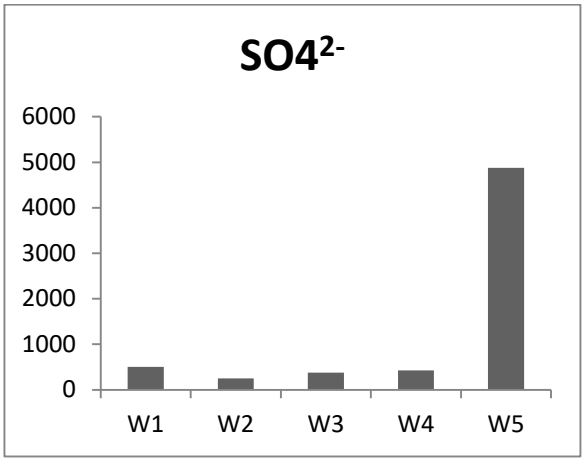

Fig.7: Sulphate of industries wastewater

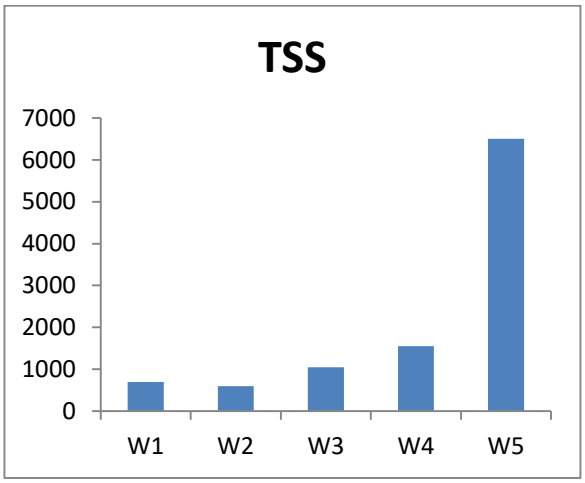

Fig.8: Total suspended solid of industries wastewater

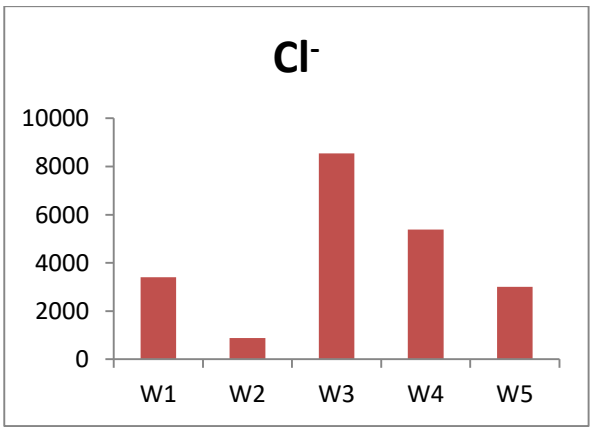

Fig.9: Chloride of industries wastewater Fig.10: Sulphied of industries waste water

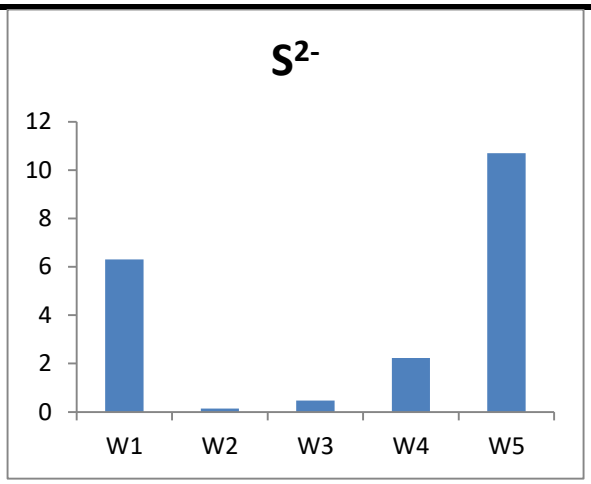

Fig.10: Sulphied of industries waste water

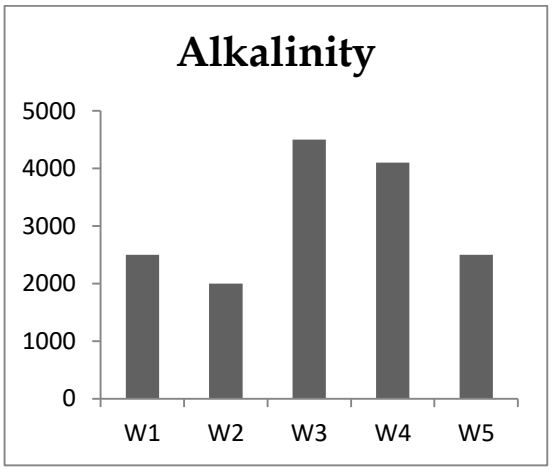

Fig.11: Alkalinity of industries wastewater

IV. CONCLUSION

From the result of physico-chemical analysis of industrial effluents, it has been concluded that PH, EC, TDS, Chlorides, Sulphate, sulphied, Nitrate,alkalinity, TSS, BOD and, COD are very high in concentration compared to the standards prescribed by WHO and EPA. Such effluent should not be discharged in to the nearby water body or soil without treatment. They are unfit for irrigation. The high level pollution of the industrial effluents cause's environmental problems which will affect plant, animal and human life [30].

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Effect of sulphur supplementation on micronutrients, fatty acids and sulphur use efficiency of soybean seeds

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***Abstract*—** *The present study was aimed at finding the influence of different sources and doses of sulphur fertilizers on micronutrient status and oil composition in soybean seeds. Soybean is the major source of edible vegetable oils and high protein seed supplements in the world. Sulphur deficiency causes soybean protein quality to decline and also decreases nitrogen-use efficiency of fertilizers. Soybean is a good source of nutrients which could further be amended with biofortification and use of fertilizers, to meet the nutrient deficiencies. Various limiting factors affect the yield of soybean crop by affecting the yield potential. Sufficient sulphur deficiency is one such limiting factor and have become common all over due to intensive crop systems and higher yielding varieties. Micronutrients play an important role in quality and quantity of soybean yield. Sulphur fertilizers viz gypsum and single super phosphate (SSP) were used at three different doses. Soil analysis have been done to evaluate the fertility status of soil prior to the experiment. Different treatments of sulphur supplementation had significant effect on seed micronutrient accumulation, nitrogen sulphur ratio and fatty acid profile. Sulphur supplementation increased zinc and iron content in mature soybean seeds, however, copper and manganese were found to be least effective. Sulphur supplementation with gypsum @ 20 kgha^{-1} increased plant height and pods per plant. Increase of oleic acid coincided with the decrease of linoleic acid with sulphur supplementation during both the cropping seasons of study.*

***Keywords*—fatty acids, gypsum, micronutrient, soybean, single super phosphate.**

I. INTRODUCTION

Soybean has a great potential as a source of important nutrients and nutraceuticals of implication to human health. Soybean contains a high nutritional value due to the high concentration of oil (18-25%) and protein (38-50%) and is a popular food all over the world (Tidke *et al* .,2015). Soybean is the major source of edible vegetable oils and of high protein seed supplements in the world. Sulphur deficiency causes soybean protein quality to decline (Gayler and Sykes 1985) and also decreases nitrogen-use efficiency of fertilizers (Ceccoti 1996). Various limiting factors affect the yield of a particular crop by affecting the yield potential. One such limiting factor is sufficient nutrient supply (Sahu *et al.,* 2017). Sulphur deficiencies have become common all over due to intensive crop systems and higher yielding varieties.

The agronomic productivity of soybean plants is dependent upon their capacity to partition a significant proportion of assimilates to the seeds, and the economic value of the crop is directly related to the seed composition (Sebastia *et al.,* 2005). But the current practice of applying large amounts of nitrogen fertilizers to crops without considering sulphur requirements is becoming a concern for crop quality (Tea *et al.,* 2007). Sulphur plays a very important role in various plant growth and developmental processes being the constituent of sulphur containing amino acids methionine (21% S) and cysteine (27% S), and other metabolites such as glutathione (Devi *et al.,* 2012). The sulphur requirement by plants varies with the developmental stage and with species whereas its concentration in plants varies between 0.1 and 1.5% of dry weight. Even if sulphur is only 3% to 5% as abundant as nitrogen in plants, it plays essential roles in various important mechanisms such as Fe/S clusters in enzymes, vitamin cofactors, GSH in redox homeostasis, and detoxification of xenobiotics (Anjum *et al.,* 2011). Oilseeds not only respond to applied sulphur, but their requirement for sulphur is also the highest among other crops, thereby attributing a role for the nutrient in oil biosynthesis (Ahmed *et al.,* 2007)

Micronutrients have the potential to contribute in maximizing yields. Nutrients evaluated in the studies presented here include Fe, Cu, Mn and Zn. Soybean also contains ~5% minerals. It is relatively rich in K, P, Ca, Mg and Fe. Soil conditions must be taken in consideration when evaluating micronutrients. Organic matter plays an

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essential role and is the main source of most micronutrients, especially for Zn and Cu. Soil pH influences the bioavailability of micronutrients in the soil. Availability of B, Cu, Fe, and Zn tends to decrease as pH increase. Soil texture can also affect the availability of micronutrients; coarse texture soils have the tendency to be low on B concentration. Soils with poor aeration are more likely to have Fe, Zn and Mn deficiencies.

Soybean oil makes up nearly 60% of the world's oil seed production and is by far the world's dominant vegetable oil (http://www.soystats.com) which has also been employed as source of bio-diesel fuels (Graham and Vance 2003). The fatty acid composition in oilseeds is an important consideration for breeding programs (Daun 1998). Five fatty acids make up nearly the entire oil portion of soybean seed. Soybean oil averages 12% palmitic acid (16:0), 4% stearic acid (18:0), 23% oleic acid (18:1), 53% linoleic acid (18:2), and 8% linolenic acid (18:3) (Wilson 2004). The 16:0 and 18:0 fractions are saturated fatty acids and constitute 15% of the soybean oil. The remainder of the oil (about 85%) is made up of unsaturated fatty acids. Soybean lines are currently being developed to express modified fatty acid profile thus increasing the potential uses of oil (Spencer *et al.*, 2003). Sulphur interactions with nitrogen nutrients are directly related to the alteration of physiological and biochemical responses of crops, and thus required to be studied in depth.

II. MATERIALS AND METHODS

Soybean var. SL525 was raised in the experimental fields of Pulses Section of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana by recommended packages and practices. The experiment was laid out in Randomized Block design (RBD) with three replications. The field and the plots were of sizes 21.7 m × 17.4 m and 5 m × 2.7 m respectively. Each plot comprised of 6 rows which were 45 cm apart. The spacing between the blocks was 1.2 m. Two different sulphur sources i.e. Gypsum and Single Super Phosphate (SSP) were used at three different dose rates respectively. There were seven treatments including control 0, 10 kg S ha^{-1}, 20 kg S ha^{-1}, 30 kg S ha^{-1} through gypsum and 10 kg S ha^{-1} , 20 kg S ha^{-1}, 30 kg S ha^{-1} through SSP. The soil of each plot was uniformly fertilized with urea as a nitrogen source and rock phosphate as phosphorus source. In calculating the amount of phosphorus, its content in SSP was reduced from the rock phosphate. Fertilizers were applied at the time of final land preparation as basal dose. The composite soil samples from 0-15 cm and 15-30 cm profile layers were collected before sowing from randomly selected sites from experimental area and analyzed for initial soil fertility status and other soil characteristics**.**

Plant height (cm) was measured from the base of the main stem to the tip of the youngest leaf using measuring tape at maturity. The number of pods per plant was taken by counting all pods in the tagged plants, and the average number of pods per plant was determined.

The micronutrients were determined from 1:2; soil-extractant ratio using DTPA-TEA (Diethylene triamine penta acetic acid-triethanolamine) buffer (0.005 M DTPA+ 0.001 M $CaCl_2$ + 0.1M TEA, pH 7.3) as per method proposed by Lindsay and Norvell (1978) and concentration of these micronutrients was measured on an atomic absorption spectrophotometer (AAS). Water extractable sulphate was determined by Tabatabai (1974).

N: S ratio was determined by estimating the total nitrogen content by Microkjeldahl method (McKenzie and Wallace 1954) and total sulphur content (Chesnin and Yien 1950) by wet digestion with nitric acid-perchloric acid mixture.

Fatty acids were analyzed by forming their ethyl esters (Uppstrom and Johansson 1978). The ethyl esters prepared were identified and estimated as relative percentage by gas liquid chromatography (GLC). The esters thus prepared were analysed using M/s Nucon Engineers AIMIL Gas chromatograph (solid state) model: 57 or equipped with a flame ionization detector fitted with a 6' x 1/8" stainless steel column, packed with 6% BDS (Butane diol succinate) on 100-120 mesh chromosorb HP. The conditions for the separation were as follows: Oven temperature : 190-200^oC ; Injector and flame ionization detector temperature: 240-250^oC; Hydrogen flow: 40 ml min^{-1} ; Nitrogen pressure: 2.5 kg sq^{-1} inch ; Air flow : 300-400 ml min^{-1}. The sample (0.2 µl) was injected into the GLC by means of a 10 µl 'Hamilton' syringe. Tentative identification of the peaks was done by comparison of their retention time with those of standard fatty acyl esters. The relative percentage of different fatty acids was analysed using Nuchrom software.

III. RESULTS AND DISCUSSIONS

Plant height showed insignificant variation under the influence of different treatments of gypsum and SSP as sulphur source. Pods per plant is an important component of yield which did not reveal any significant differences among various treatments of sulphur and in comparison to control. However, number of pods per plant increased to 47.5 with gypsum @ 20 kg S ha^{-1}, in comparison to control (42.9). With SSP as sulphur source, number of pods per plant increased to 46.7 with the dose rate of 30 kg S ha^{-1}. Increase in plant height and other yield attributes such as pods per plant, 1000 seed weight indicated the positive effect of sulphur nutrition on vegetative growth because of the availability of more photoassimilates.

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Table.1: Soil characteristics of the experimental site

Soil Characteristics	2011		2012		Methods used
	Depth		Depth		
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	
Soil texture	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	
pH	7.60	7.50	7.70	8.00	1:2 soil : water suspension (Jackson 1967)
Electrical Conductivity (mmoles cm^{-1}) at 25°C	0.10	0.06	0.15	0.10	Solubridge conductivity meter (1:2 soil : water suspension) (Jackson 1967)
Organic carbon (%)	0.60 (High)	0.48 (Medium)	0.51 (Medium)	0.36 (Low)	Walkley and Black's rapid titration method (Walkley and Black 1934)
Available Phosphorus (kg/acre)	11.4 (High)	11.4 (High)	14.3 (High)	11.8 (High)	0.5 N sodium bicarbonate extractable P by Olsen's method (Olsen *et al* 1954)
Potassium (kg/acre)	138 (High)	105 (High)	30 (Low)	72 (High)	Ammonium acetate extraction method (Piper 1966)
Sulphur (%)	0.20	0.08	0.22	0.10	Williams amd Steinbergs (1959).
Nitrogen (%)	0.23	0.19	0.26	0.21	McKenzie and Wallace (1954)
Zinc (kg $acre^{-1}$)	1.38	1.28	1.56	1.04	Lindsay and Norvell (1978)
Iron (kg $acre^{-1}$)	3.76	4.0	6.94	4.88	Lindsay and Norvell (1978)
Manganese (kg $acre^{-1}$)	7.14	7.74	9.28	8.54	Lindsay and Norvell (1978)
Copper (kg $acre^{-1}$)	0.32	0.44	0.40	0.34	Lindsay and Norvell (1978)

Mohanti *et al* (2004) recorded highest plant height with 30 kg S ha-1 in soybean. Similarly, Ravi *et al* (2008) reported significant increase in height of safflower with sulphur application @ 30 kg S ha-1. Nasren and Farid (2006) recorded highest number of pods per plant with 60 kg S ha-1 followed by 40 kg S ha-1 in soybean. Application of sulphur @ 40 kg ha-1 enhanced plant height, branches, pod per plant and 1000 seed weight in green gram (Sharma and Singh 1979) whereas application @ 60 kg S ha-1 produced higher pod length, seed per pod and 1000 seed weight in black gram (Singh and Aggrawal 1998).

Nitrogen and sulphur assimilation get restrained in plants with the deficiency of either of the nutrient. Nitrogen content in seeds was not significantly influenced by different treatments of sulphur fertilization. The highest nitrogen content was observed in control soybean seeds, where no sulphur was applied. Nitrogen content decreased to minimum value with gypsum @ 20 kg S ha^{-1}. Significant variations in sulphur content was observed in mature soybean seeds under the influence of sulphur fertilization. With gypsum, highest sulphur content was observed in seeds treated @ 20 kg S ha^{-1}. Similarly, with SSP, maximum sulphur content was observed @ 20 kg S ha^{-1} in soybean seeds. N:S ratio was highest in control seeds (49.56), and decreased with sulphur supplementation in comparison to control. With gypsum and SSP both @ 20 kg S ha^{-1}, N:S ratio reduced to 24.2 and 28.38 respectively (Table 2).

Table.2: Effect of different levels and sources of sulphur on physiological parameters and sulphur use efficiency in soybean seeds

TREATMENT Amount of sulphur added to soil (kg ha^{-1})	Plant Height	Pods per plant	Water extractable sulphate	N:S Ratio	Fetilizer sulphur use efficiency
Control	55.3±2.08	42.9±0.70	0.88±0.03	49.56	-

Gypsum	**10**	55.0±3.93	46.8±3.43	0.85±0.02	30.00	8.72
	20	56.3±2.51	47.5±5.20	0.79±0.01	24.22	12.75
	30	55.6±1.52	46.4±1.36	0.77±0.02	26.81	15.11
SSP	**10**	57.5±3.0	42.6±3.70	0.81±0.01	31.90	7.17
	20	59.4±4.93	46.2±2.80	0.77±0.01	28.38	12.63
	30	58.4±1.40	46.7±1.61	0.73±0.02	34.86	10.32
Overall mean		56.78	45.58	0.80	32.24	11.11
Critical difference (p<0.05)		NS	NS	0.033		

*Data is represented as mean ± S.D of three replications

Sulphur fertilization affected nitrogen assimilation as indicated by decreased N:S ratio which is an indicator of quality of legumes (Eppendorfer 1971) and decrease in this ratio suggested more uptake of sulphur. Increased sulphur uptake had increased nitrogen utilization assisting in synthesis of certain biochemicals in the seed. Soybean seed have intrinsic biochemical ability to synthesize high amount of protein when sufficient raw material is available. Kumar *et al* (2013) also reported decrease in N:S ratio with sulphur and nitrogen treatments in mungbean seeds although higher effect was observed to be with sulphur fertilizers. In cowpea, N:S ratio decreased with the increasing dose of sulphate fertilizers (Evans *et al*., 2006). Minimum N:S ratio was recorded with application of 40 kg S ha^{-1} over control treatment in soybean seeds (Najar *et al.*, 2011). On the contrary, N:S ratio increased in soybean under sulphur stress (Sexton *et al*., 1998). Sharma and Gupta (1992) reported that the application of 60 kg S ha^{-1} caused significant increase in sulphur and nitrogen content whereas Fazili *et al* (2010) reported increase in sulphur content in mustard seeds with 40 kg S ha^{-1}.Significant variation was observed in content of water extractable sulphate in soybean seeds under the influence of sulphur supplementation (Table 2). The amount of water extractable sulphate was reduced with the different treatments of sulphur in the form of gypsum and SSP during both the cropping seasons in dose dependent manner. Sulphur application affected crop yield through the effect on S-use efficiency and its components (uptake efficiency and utilization efficiency). Data on fertilizer sulphur use efficiency (FSUE) revealed that gypsum showed FSUE in the range of 8.72 to 15.11, highest @30 kg ha^{-1} (Table 2). Comparatively, SSP showed lesser FSUE upto 7.11 with 10 kg ha^{-1}. Highest FSUE (15.11) was recorded with gypsum applied @ 30 kg S ha^{-1}. SSP also showed highest FSUE (12.63) with 20 kg S ha^{-1} . In the present study, gypsum was found to be efficient fertilizer in terms of sulphur use as compared to SSP. In addition to the sulphur, calcium present in gypsum might have created a favourable environment for efficient sulphur utilization, thereby leading to higher yield and higher sulphur-use efficiency and its components. Najar *et al* (2011) reported highest sulphur use efficiency with 10 kg S ha^{-1} in soybean whereas Sriramachandrasekharan (2012) reported highest FSUE @ 50 kg S ha^{-1} applied as gypsum in radish.

Soybean is also a good source of micronutrients which could further be amended with biofortification and use of fertilizers, to meet the nutrient deficiencies. Micronutrients play an important role in quality and quantity of soybean yield. Different treatments of sulphur supplementation had significant effect on seed micronutrient accumulation. Sulphur supplementation increased zinc and iron content in mature soybean seeds, however, copper and manganese were found to be least effective (Table 3). Both gypsum and SSP @ 10 kg S ha^{-1} increased Zn content upto 62 and 60 mg kg^{-1} respectively, in comparison to control seeds (39 mg kg^{-1}) where no sulphur was applied. But, with the increase in sulphur doses, Zn content showed a decreasing trend and was minimum with both the fertilizers when applied @ 30 kg S ha^{-1}. Iron concentration was higher in soybean seeds under sulphur nutrition, as compared to control seeds (58 mg kg^{-1}). Maximum iron concentration (90 mg kg^{-1}) was observed with gypsum applied @10 kg S ha^{-1}, and it decreased to 63.5 and 69.5 mg kg^{-1} with increase in sulphur dose upto 20 and 30 kg S ha^{-1} respectively. Similar changes in iron concentration was observed when SSP was applied at different levels.

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Table.3: Effect of different levels and sources of sulphur on micronutrients (mgkg^{-1}) in soybean seeds

TREATMENT Amount of S added to soil (kg ha^{-1})		Zinc (Zn)	Copper (Cu)	Iron (Fe)	Manganese (Mn)
Control		39	9	58	22.5
Gypsum	**10**	62	10.5	90	22.5
	20	42	10	63.5	21
	30	35	10	69.5	21.5
SSP	**10**	60	9	79.5	25
	20	41	6.5	64.5	19.5
	30	40.5	6	71.5	23
Overall Mean		45.64	8.71	70.92	22.14

Gypsum applied at different dose rates resulted in higher copper concentration in soybean seeds as compared to control whereas application of various levels of SSP showed reverse trend. Manganese concentration was not affected by application of different doses of gypsum but SSP @ 10 kg S ha^{-1} increased manganese concentration as compared to control. The results are in agreement with the previous studies on micronutrient concentration in soybean where significant increase in their concentrations with soil fertilizer application have been reported (Jha and Chandel 1987, Rhoads 1984). Nutrients gets partitioned according to their mobile ability. Optimum metal homeostasis is achieved by the plant through precise regulation of transport, distribution and remobilization of elements, which is controlled by source and sink signals. Variations in micronutrient concentration by sulphur application might be due to changes in any of the processes involved in the nutrient partitioning.

Fatty acid composition of seed lipid is an important determinant of oil quality. Soybean oil is highly demanding worldwide in terms of total fat supplies of world (Soya and Oilseed Bluebook 2010), because of high content of polyunsaturated fatty acids essential for human nutrition (Emken 1995). They are precursors of prostaglandins and hormones that play an important activity in the regulation of physiological and biochemical functions of human body. The relative content of fatty acids influences the physical and chemical characteristics of the oil, thus the suitability of the oil for a particular use. Fatty acid composition of soybean seeds as affected by sulphur supplementation is presented in Table 4. Different treatments of sulphur supplementation exhibited non-significant differences for palmitic acid during both the years. Seeds treated with gypsum @ 30 kg S ha^{-1} and SSP @ 10 kg S ha^{-1} registered maximum palmitic acid content upto 14.54 and 14.04%, as compared to control (13.72%). Similar results were found during second year of study. Maximum palmitic acid content recorded was 13.90 and 14.33% with gypsum @ 30 kg S ha^{-1} and SSP @ 10 kg S ha^{-1} respectively, which was statistically similar to palmitic acid in control seeds. Stearic acid was found to be higher (4.47%) in treatment with gypsum @ 10 kg S ha^{-1}, as compared to control (3.57%) during first year of study. Gypsum @ 10 and 20 kg S ha^{-1} and SSP @ 30 kg S ha^{-1} significantly increased stearic acid content in soybean seeds, with maximum content of 4.34% obtained with gypsum @ 10 kg S ha^{-1}.

In 2011, oleic acid was significantly reduced with sulphur supply @ 30 kg S ha^{-1} with gypsum, upto 30.29%, as compared to control (32.06%). Gypsum @ 10 and 20 kg S ha^{-1} did not reveal any significant differences in oleic acid content. With SSP, higher value of oleic acid was registered upto 32.27% and 32.13% with 10 kg S ha^{-1} and 30 kg S ha^{-1}, respectively, although the results were found to be non-significant. However, during second year of study, oleic acid increased significantly with all the treatments of sulphur supplementation except with SSP @ 30 kg S ha^{-1}, where its content significantly decreased. Maximum content of oleic acid (29.59%) was registered with gypsum @ 20 kg S ha^{-1}, and the lowest content (27.05%) was registered in control seeds, where no sulphur was applied. With SSP, maximum oleic acid content (28.95%) was recorded @ 20 kg S ha^{-1}.

Linoleic acid was found to be unaffected with sulphur supplementation with all the treatments except SSP @ 20 kg S ha^{-1}, where its content significantly increased to 47.51 % as compared to control (46.70%) during first cropping season. However, during second cropping year, linoleic acid significantly decreased with gypsum and SSP @ 10 kg S ha^{-1} and 20 kg S ha^{-1} respectively.

Linolenic acid increased significantly with all the treatments of sulphur supplementation as compared to control during first cropping season. Although, during second year, insignificant variations in linolenic acid content was observed. In 2011, maximum linolenic acid content registered was 4.79 and 4.78% with both the fertilizers @ 10 kg S ha^{-1}. In year 2012, maximum linolenic acid recorded was 5.33 and 5.21% with gypsum @ 20 kg S ha^{-1} and SSP @ 30 kg S ha^{-1} respectively, but found to be non-significantly affected as compared to

control (4.81%).

In present study, very narrow differences in fatty acids content were observed under the influence of sulphur fertilization.

Table.4: Effect of different treatments of sulphur on fatty acid composition (relative percentage) in soybean seeds.

	TREATMENT Amount of S added to soil (kg ha^{-1})		Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Unsaturation (%)	Oleic: Linoleic Ratio
2011	Control		13.72 ± 0.21	3.57 ± 0.21	32.06 ± 1.01	46.70 ± 1.75	3.94 ± 0.45	82.40	0.68
	Gypsum	10	13.78 ± 0.77	4.47 ± 0.07	31.05 ± 1.59	46.43 ± 0.50	4.79 ± 0.24	82.75	0.67
		20	13.98 ± 0.88	3.77 ± 0.35	31.26 ± 1.42	46.55 ± 1.21	4.43 ± 0.31	82.24	0.66
		30	14.54 ± 0.98	3.63 ± 0.18	30.29 ± 1.10	46.88 ± 0.26	4.66 ± 0.02	81.83	0.64
	SSP	10	14.04 ± 1.29	3.10 ± 0.44	32.27 ± 1.02	45.79 ± 0.06	4.78 ± 0.11	82.85	0.70
		20	13.12 ± 0.50	3.75 ± 0.36	30.96 ± 1.20	47.51 ± 0.99	4.65 ± 0.66	83.12	0.72
		30	13.02 ± 0.60	4.07 ± 0.21	32.13 ± 1.23	46.29 ± 1.18	4.48 ± 0.33	82.91	0.69
	Overall Mean		13.74	3.76	31.43	46.59	4.53	82.58	0.68
	Critical difference (P<0.05)		NS	0.51	1.05	0.77	0.38		
2012	Control		13.17 ± 0.62	3.63 ± 0.10	27.05 ± 1.18	51.33 ± 1.55	4.81 ± 0.31	83.19	0.52
	Gypsum	10	12.92 ± 0.06	4.34 ± 0.08	28.39 ± 1.81	49.56 ± 1.14	4.78 ± 0.97	83.14	0.57
		20	13.60 ± 0.64	4.08 ± 0.36	29.59 ±0.41	47.38 ± 0.65	5.33 ± 0.40	82.31	0.62
		30	13.90 ± 0.13	3.52 ± 0.02	28.38 ± 1.59	49.88 ± 1.38	4.30 ± 0.58	82.56	0.56
	SSP	10	14.33 ± 0.79	3.07 ± 0.41	29.17 ± 0.57	48.81 ± 0.86	4.61 ± 0.08	82.59	0.59
		20	13.31 ± 0.16	3.58 ± 0.24	28.95 ± 0.28	49.22 ± 0.69	4.93 ± 0.59	83.10	0.58
		30	13.89 ± 0.27	3.99 ± 0.60	28.04 ± 1.35	48.85 ± 1.66	5.21 ± 0.72	82.11	0.57
	Overall Mean		13.58	3.74	28.51	49.29	4.85	82.71	0.57
	Critical difference (P<0.05)		NS	0.34	1.23	1.75	NS		

In the earlier studies reported in literature, changes in fatty acid profile of soybean seeds with sulphur fertilization has been reported when applied in higher doses i.e. more than 80 kg S ha^{-1}. Response of oleic acid to sulphur supply during both the cropping seasons was found to be inconsistent, and is supported by the findings of Ahmed and Abdin (2000), who reported non-significant differences among sulphur levels for oleic acid content in rapessed. Differences in the composition of fatty acid in seed oil can be due to environmental conditions also (Boschin *et al.*, 2007). Fatty acid composition of soybean oil changes considerably with maturity along with seed oil deposition (Graef *et al.*, 1985, Ishikawa *et al* 2001). Triacylglycerols, palmitic

acid, linolenic acid tend to decrease with maturity whereas linoleic acid increases. Oleic acid tends to increase to a maximum and then decline slightly. Linolenic acid was significantly affected by sulphur supplementation during first cropping season as compared to the second season. Interaction of sulphur with climatic conditions at the time of seed development might have influenced the fatty acid composition and shown variations in their relative proportions due to certain environmental factors and nutrient availability. Cazzato *et al* (2012) reported increase in monounsaturated and polyunsaturated fatty acids in lupin seeds with 30 kg S ha^{-1} and the improvement in lupin seed composition through the increase in oleic and linolenic acids whereas Howell and Collins (1957) observed very little effect of nitrogen, phosphorus and sulphur on fatty acid profile of soybeans. Correlation studies revealed significant inverse relationship between oleic acid and linoleic acid of r = -0.880 (2011) and r = -0.639 (2012) at $p<0.05$. Increase of oleic acid coincided with the decrease of linoleic acid during both the cropping seasons. This might be due to the effect of sulphur nutrition on ω-6-destaurase activity which converts oleic to linoleic acid. This supported the hypothesis of sequential desaturation of formation of unsaturated fatty acids in soybean oil. Inverse relationship of oleic and linoleic has also been reported by Flagella *et al* (2002) in safflower.

IV. CONCLUSION

Sulphur application affected crop yield through the effect on sulphur use efficiency, uptake efficiency and utilization efficiency. Sulphur fertilization affected nitrogen assimilation as indicated by decreased N:S ratio. This ratio depicts the quality of legumes as decrease in this ratio suggested more uptake of sulphur. Increased sulphur uptake had increased nitrogen utilization. Nutrients gets partitioned according to their mobile ability. Optimum metal homeostasis is achieved by the plant through precise regulation of transport, distribution and remobilization of elements, which is controlled by source and sink signals. Variations in micronutrient concentration by sulphur application might be due to changes in any of the processes involved in the nutrient partitioning. Fatty acid composition of soybean oil changes considerably with maturity along with seed oil deposition. Interaction of sulphur with climatic conditions at the time of seed development might have influenced the fatty acid composition and shown variations in their relative proportions due to certain environmental factors and nutrient availability. Increase of oleic acid coincided with the decrease of linoleic acid during both the cropping seasons. This might be due to the effect of sulphur nutrition on ω-6-destaurase activity which converts oleic to linoleic acid. This supported the hypothesis of sequential desturation of formation of unsaturated fatty acids in soybean oil.

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Evaluation of the Activity of Insecticides Plants in the Far North Region of Cameroon

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Abstract**—** *This study proposes to assess the activity of insecticide plants in the far North region of Cameroon. The leaves or bark of four local plants (Azadirachtaindica, Boswelliadalzeilii, Khayasenegalensis and Ocimumcanum) were harvested, dried and powdered for the formulation of insecticidal chopsticks at different doses. Toxicity tests have been conducted on adult culicidae mosquitoes by fumigation. They reveal low levels of mortality after 15 minutes of exposure to the smoke of the chopsticks. Remanence due to chopsticks smoke leads to high rates of mortality after 6 and 24 hours of exposure. Mortality rates increase with the dose of each vegetable powder. Lethal doses were calculated 6 hours after exposure for each plant powder. Those of the leaves of Azadirachtaindica proved to be the most efficient thus with the lowest LD50 value of 36.14%. These vegetable powders can be used as natural insecticides instead of chemical insecticides.*

Keywords— Insecticides, Culicidae adult, fumigation, mortality, remanence rate, lethal dose.

I. INTRODUCTION

Insects form an important group of animal in ecosystem. Some are useful (sources of food, pollinators and Entomophagous) others are harmful (insect pests of plants or vectors of diseases). Insects can also be vectors of diseases to man it the case of mosquitoes and many others. Mosquitoes are insects of the order diptera. The family of *culicidae* are the most important, includes three species mainly pathogenic for man: the anopheles, aedes and culex. Anopheles are scare in temperate zone and they are undoutable especially in tropical countries thus are vectors of malaria (Slip et *al*., 2006). It's a parasitic disease of waterborne origin which continues to pose a problem to public health (Brahimand et *al*., 2006).

In anti-mosquito campaign fight, the active ingredients of the insecticides used belong to the organophosphates, pyrethroids and carbamates synthesis (Brahimand et *al*., 2006). These preparations, although they are proved to be very effective on mosquitoes culicidae, have several drawbacks. Indeed, in addition to their high cost, they can be at the origin of various environmental problems. The significant accumulation of active ingredients of insecticides of synthesis in aquatic and terrestrial ecosystems cause a problem of pollution (Barbouche et *al*., 2001). Moreover, the active substances of the products used have a broad spectrum of action and does not eliminate non-target organisms. All these disadvantages added is also a big problem of development of resistance to chemical insecticides, by insect treated (Georghiou et *al*., 1975; Sasmalet *al*., 1977).

To ensure a better response, while maximally preserving the environment, new alternatives are more encouraged. Natural substances that present a broad spectrum of Pharmacology action can be used as alternative insecticides for replacement, the use of extracts of plants as insecticides is known is known since. According to Jacobson (1989), more than 2000 plant species possess an insecticidal activity have already been identified. In fact nicotine, pyrethrum and rotenone are already known as control agents against insects (Crosby, 1966). In parts of black Africa, tobacco mixed in water leaves were used to combat mosquitoes.

In Cameroon, the work of Saotoing (2005), confirm the insecticidal effect of the essential oil extracted from the leaves of *Ocimum Canum* (Basil of small leaves) and several other local plants on adult mosquitoes. To follow this idea of local Herbal insect control, we propose to assess the activity of insecticides on some plants in the far North region of Cameroon.

To achieve this, mosquito larvae will be harvested and breed for adult mosquitoes, and then insecticides chopsticks will be formulated based on plant powders of the following plants: leaves of *Ocimum canum* and *Azadirachta indica*, bark of *Khaya senegalensis*, the leaves and the bark of *Boswellia dalzielii*. Finally, we will assess the insecticidal activity of these chopsticks on adult mosquitoes by fumigation.

II. MATERIALS AND METHODS

Presentations of the study area

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The chief town of the far - North Region of Cameroon (Maroua) is our main study area. It is located at an altitude of 400 meters, in the savannah region. We note characteristic presence of plants such as *Anogeissus leocarpus* on soft and non-cleared soil and *Boswellia dalzielii* on stony soil and *Balanites aegyptiaca* (Barbouche et *al*., 2001).

Harvest and breeding of *Culicidae*

1 - Larva specimens.

The larvae were harvested in the town of Maroua for the months of June and July 2011. Several stagnant (gite) water was systematically examined to have a diversity of *culicidae*.

The larvae are marked by their horizontal position on the surface of the cottage. They are collected from their roosts using a ladle and introduced in boxes with water from the cottage to be transported to the laboratory of the Institute of agricultural research for development where they will be breed. Once in the laboratory larvae are transferred into a plastic basin where they are kept in natural conditions for breeding with water of different deposit.

2 - Breeding of *Culicidae*

Larvae collected directly from deposits are transferred in small basins with a few pinches of nutritional powder (powder shrimp + biscuits) for 2 to 3 days each morning. Every day the water from the basins is replaced by spring water. Replacement of source water is to prevent pollution resulting from the presence of powder nutrient. Two to three days after the introduction of the larvae in the basins, larvae have been transformed into nymphs. Then nymphs removed from basins are transferred to plastic glasses using a micropipette. Glasses containing nymphs were placed inside the cages made of canvas's mosquito net of about 8200 cm^3 in volume. In each cage, is placed a plate containing moistened cotton of sweet juice made from sucrose 10%, providing food for adult mosquitoes. At the emergence within the cages, adult mosquitoes can feed before being submitted to toxicity tests.

3. Preparation of plant material

The plant material is made of leaves of (*Ocimum canum*, *Azadirachta indica*) and bark of *Khaya senegalensis*, leaves and bark of *Boswellia dalzielii.*

3.1 - Harvesting and drying of the leaves and bark

Green leaves (*Ocimum canum* as well as of *Azadirachta indica*) and the bark of *Khaya senegalensis* and *Boswellia dalzielii* are collected and introduced in a bag. They are then dried in the shade in order to keep the active ingredient they contain. Indeed, the active ingredient very often volatile evaporates in the presence of heat (Brey, 2005). Every day, we pass hand to expose all sheets at room temperature in order to avoid that those below are either not well dried. Similarly, to ensure complete drying of the bark, they are returned from time to time to expose both sides at room temperature. Bark drying requires about ten days. As for the leaves, after 5 to 6 days of drying, they can crumble by friction: they are then ready for the preparation of plant powder.

3.2.-Preparation of the vegetable powder

The dried leaves of the selected plants are powdered. The barks are powdered with a scraper in aluminum.

Obtained plant powders constitute base product for the manufacture of many types of insecticides which formulation varies depending on the type of use or even the type of target insect.

3.3.-Formulation of the insecticide strips

Insecticides are formulated from plant powders (bark or leaves). For each insecticide formulated, vegetable powder is the active ingredient. In order to vary the percentage of active ingredient, a witness powder was used. The choice of the indicator must meet a number of conditions: it must not present insecticidal activity, it must allow the plant material to form a paste, it should burn enough.

For each vegetable powder, five different percentages in plant material sticks are formulated. These percentages are: 15%, 30%, 45%, 60% and 75%. For each percentage dry matter (vegetable powder +witness) Mtotal mass was set at 4 grams to 3ml of water approximately. The masses of vegetable powder and powder witness are measured using an electronic scale of brand Lutron having the features GM-300, 300.00 g 0, 01 g.

For a baguette containing x % of plant material, a mass M of plant material was measured as:

$$M = \frac{M_{Totale} \times x}{100} = \frac{4x}{100}$$

For the conduct of the sinkers, the following steps have been respected:

- On the balance and make sure using the water level that it is positioned horizontally on the bench. The balance then mark 0.00 g
- Place a sheet of paper considered here as the tare weight on the scale. The balance will then mark the mass of the sheet of paper.
- Press on the "tare" button on balance to cancel the mass of the sheet of paper and bring balance to 0.00 g
- Gradually collect plant material using the spatula and place on the sheet of paper asked on the scale until it mark the mass M corresponding to the dose x % reporting.
- Complete plant material located on the balance by the witness powder until it marks the mass Mtotal= 4.00 g

The corresponding indicator MT mass is obtained by making the following difference:

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$$M_{Témoin} = M_{totale} - M = 4 - M$$

For each percentage, the measured masses of plant material and witness are introduced into a Petri dish and homogenized box. Using a syringe, 3 ml of water is added to this mixture and homogenized again.

The resulting mixture is introduced into a cylindrical mould. Out of the mold, we obtain a wet cylindrical chopsticks. Each chopsticksis label. A label of chopsticks bears the name of the plant, its extract to be used and the corresponding percentage of plant material. After a two (02) days of drying in the shade, the chopsticks are ready to be inflamed and produce smoke for toxicity testing.

4 - Toxicity tests

To increase the credibility of toxicity tests, preliminary experiments were conducted to determine the optimal witness powder and the average lifespan of the *culicidae* in breeding conditions of the laboratory.

4.1-Preliminary experiments

4.1.1. – Choice of the witness powder

Chopsticks have been realized with two different witness powders namely corn powder and powder of the tubers of cassava (starch). The objective is to determine which of the two powders has the following advantages:

- Insecticide effect near zero
- Capacity of adhesion with vegetable powder with water
- Slow consumption.

4.1.2-Choosing the age of adult *culicidae* subjected to toxicity test

At the adult State, the *culicidae* can still live one to several weeks in their natural environment. The life expectancy of the adult *culicidae* was evaluated. This evaluation was conducted in the breeding conditions of the laboratory. We note that 3 to 4 days after emergence in adulthood, mosquitoes die. Considering this reality of laboratory, we opted to perform tests on the *culicidae* between the first and the second day after their emergence in adulthood.

4.2.–Putting into evidence the insecticide effect of the chopsticks on the adult *culicidae*.

Toxicity tests are performed on the adult *culicidae* by the method of fumigation. This method is to ignite an insecticide stick near studied insects. The flaming sticks burn producing smoke whose effect on insects will translate its insecticide activity or not.

Insecticide strips made are inflamed and placed on a support, and introduced in the cages each containing 50 mosquitoes.

Each cage of mosquito is exposed to smoke from insecticide stick for 15 minutes.

The "knock-down" effect is observed and appreciated on mosquitoes after three minutes for each dose and for each plant powder.

The fifteenth minute of exposure, the insecticide chopsticks are removed from the cage and first killed mosquito count is done. Six hours and 24 hours later, a second count and a third count are performed.

The different results are carefully noted for later use in the calculation of the rates of mortality and the determination of the LD50.

5 - Statistical analysis

5.1-Calculation of the rate of mortality of adult mosquitoes

It is important to check the initial population of the living adult mosquitoes before any experience. In fact, it can happen that mosquitoes are already dead before the start of the experiment, or that all nymphs caged did not emerge in adulthood. This check allows you to redefine the adult mosquito population initially fixed at 50 per cage. We can then calculate the rate of mortality T according to formula:

$$T = \frac{Numbers\ of\ mosquitos'\ death\ during\ experience}{Numbers\ of\ life\ mosquitoes\ before\ experiment} \text{X } 100$$

5.2.-determination of the lethal dose 50 (DL50)

For each vegetable powder, the dose that led to a 50% mortality of adult mosquitoes is the DL50. It is represented by the regression equation expressing the rate of mortality as a function of different doses for each plant powder.

$y = ax + b$

y: Rate of mortality (in %)

x: Consider dosage of plant mater (in %)

The determination of LD50 values enable to make a comparative study of the effectiveness of the studied plant powders, indeed the most effective plant powder is the one that has the lowest LD50 that is those presenting insecticide effect at lower dose.

III. RESULTS AND DISCUSSIONS

At the end of the work in the laboratory, we manufactured insecticides chopsticks made from powders of some plants in the region of the far North Cameroon. These tubs were made at different doses. To assess the insecticidal activity of these sticks, we harvested and breed mosquito larvae for adult mosquitoes on which we carried out fumigation toxicity tests.

1-Insecticides tub and control tub

We observe that the two powders have no insecticidal effect, adhesion of starch in the presence of water is satisfactory compared to corn powder, corn powder

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consumption is faster, which reduces the time of exposure to mosquitoes.

At the end of this experience, cassava powder stands as the effective witness for toxicity tests.

For each selected vegetable powder, five different percentages in plant material sticks are formulated. These percentages are: 15%, 30%, 45%, 60% and 75%. For each percentage, we set the mass M_{Total} of dry matter (vegetable powder + witness) at 4 grams for 3 ml of water approximately.

Table 1 shows the masses of vegetable matter and control powder by dose of tub.

Table.1: Masses of vegetable matter and powder control by dose of tub

Doses / Masses	15%	30%	45%	60%	75%	Témoin
Mass of powder plants *M (g)*	0,6	1,2	1,8	2,4	3,0	0,0
Mass of control of powder $M_{control}$ *(g)*	3,4	2,8	2,2	1,6	1,0	4,0
Total Mass M_{Total} *(g)*	4,0	4,0	4,0	4,0	4,0	4,0

The insecticide tub obtained after drying in the shade is compact and fragile depending on dosages of powder used for each plant. Chopsticks at low doses are less fragile than those with high doses. Chopsticks color also varies with dose: low dose of plant powder, the tub seems clearer.

2-Evaluation of insecticidal activity of chopsticks.

2.1-Test using the control tub

The witness test is performed using the control tub incorporated mass 0% vegetable powder and 100% of starch powder. The action smoke from the control tub causes agitation of mosquitoes without all times knock them out.

2.1.1-Variation in the rate of mortality for 15 minutes of exposure

The variation of mortality 15 minutes after exposure of adult mosquitoes at different doses of five plant powders is represented in figure 1.

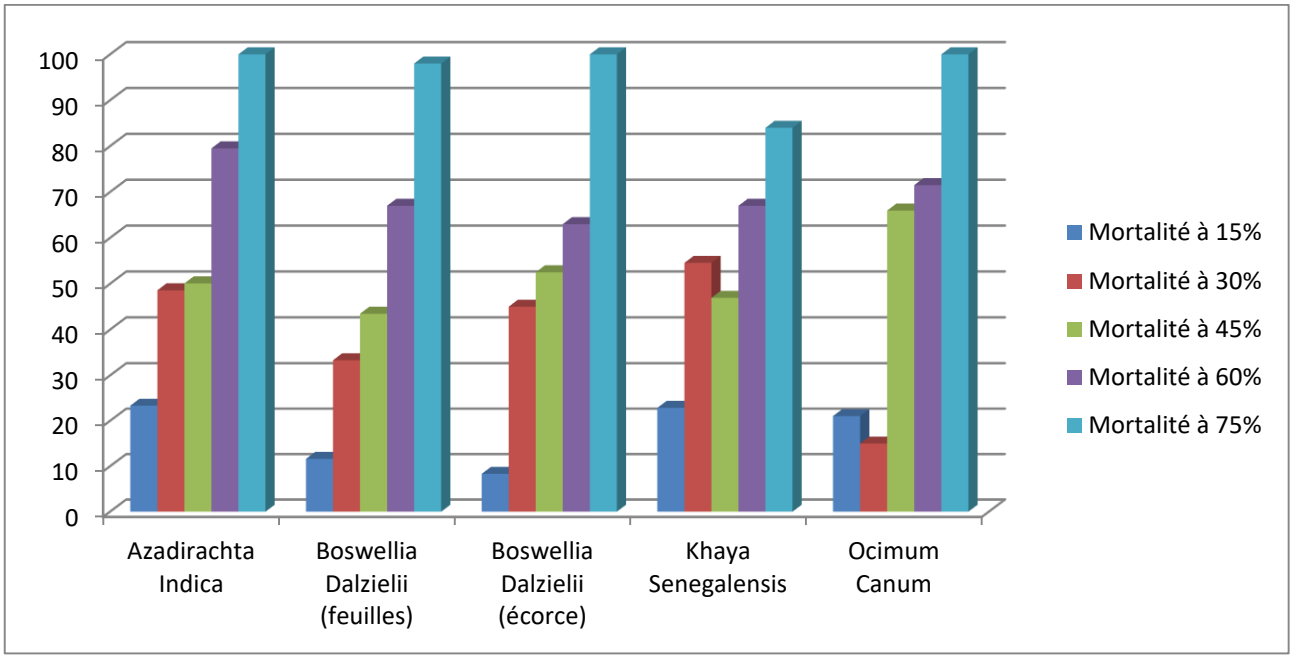

Fig.1: Mortality of mosquitoes depending on the dose of the plant powder for four species of plant after 15 minutes of exposure

The figure 1shows that after exposing adult mosquitoes at different doses of five plant powders for 15 minutes, the actual insecticidal effect is observed. The mortality rate is quite low and increases with the dose for each vegetable powder (table 2).

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Table.2: Mortality of mosquitoes depending on the dose of the powder plant for four plant species after 15 minutes of exposure

Doses / Plantes	15%	30%	45%	60%	75%	Témoin
Azadirachta indica (leaves)	8,57 ± 3,10	13,33 ± 3,77	18,18 ± 2,68	20,5 ± 2,46	28 ± 2,56	0
Boswellia dalzielii (leaves)	4,65 ± 2,43	13,33 ± 8,88	15,22 ± 2,50	16,27 ± 2,35	17,36 ± 2,24	0
Boswellia dalzielii (bark)	5,87 ± 6,50	14,01	17,50 ± 2,93	17,5 ± 2,29	18 ± 2,36	0
Khaya senegalensis (bark)	5,71 ± 3,02	9,18 ± 10,74	18,37 ± 2,41	19,01 ± 2,28	22 ± 2,20	0
Ocimum canum (leaves)	10,00 ± 2,75	13,15 ± 2,97	19,15 ± 2,53	21 ± 2,32	23,40 ± 2,40	0

At lower doses 15-30% *Ocimum canum* presents higher mortality rates of 10 and 13, 15% respectively. For high doses 60% and 75%, *Azadirachta indica* is the insecticide with the mortality rate of 20.5 and 28% respectively. In all of these results, a first classification of toxic efficacy on tested vegetable powders is highlighted, thus the most toxic plant powders are those of the leaves of *Azadirachta indica* and *Ocimum canum*. The least toxic of the leaves is *Boswellia dalzielii*.

2.1.2-Variation in rate of mortality 6 hours after exposure to mosquitoes

The variation of mortality 6 hours after exposure of adult mosquitoes at different doses of five plant powders is represented in figure 2.

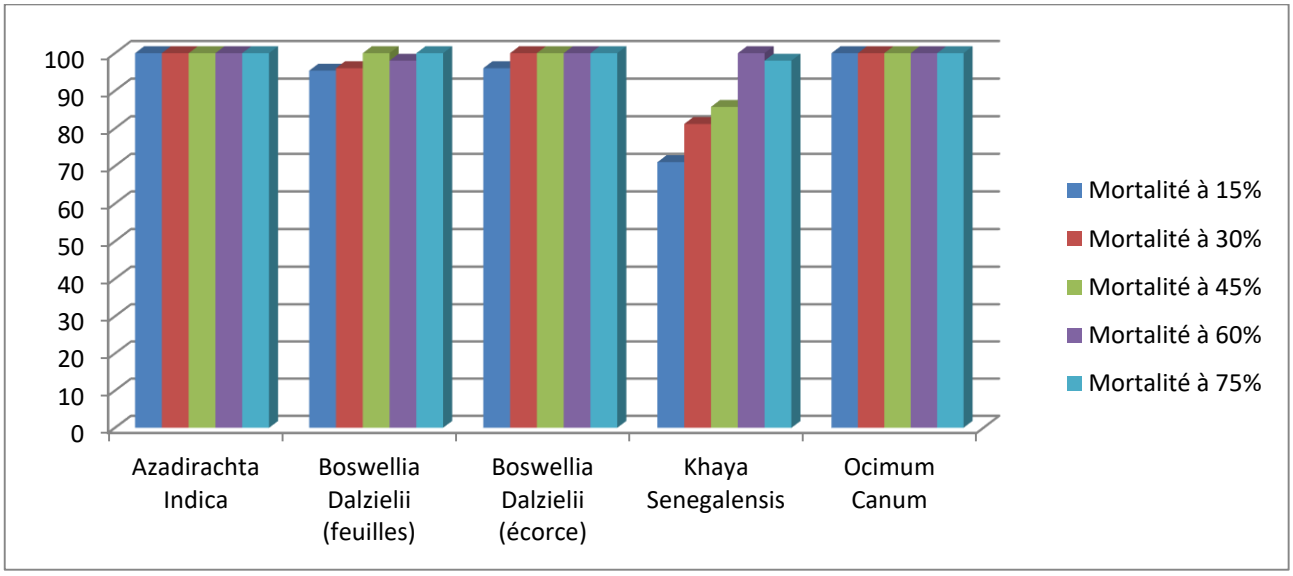

Fig.2: Mortality of mosquitoes depending on the dose of the plant powder for four species of plant 6 hours after exposure

The figure 2 shows that six hours after the exposure of the mosquitoes, the insecticide effect is accentuated. The mortality rate also increases with the dose for each plant powder.

At low doses 15-30% bark of *Khaya senegalensis* have the highest mortality rates 22.86 and 54.55% respectively. For high doses 60% and 75%, *Azadirachta indica* is the highest insecticide with the mortality rate of 79.51 and 100%, respectively. In all of these results a second identical classification to the first classification (figure 2).

On effectiveness of toxic plant powders tested is highlighted, thus the most toxic plant powders are those of the leaves of *Azadirachta indica* and *Ocimum canum*. The less toxic is leaves *Boswellia dalzielii*.

2.1.3-Variation in the rate of mortality 24 hours after exposure

The variation in mortality rate 24 hours after exposure of adult mosquitoes at different doses of five plant powders is represented in figure 3.

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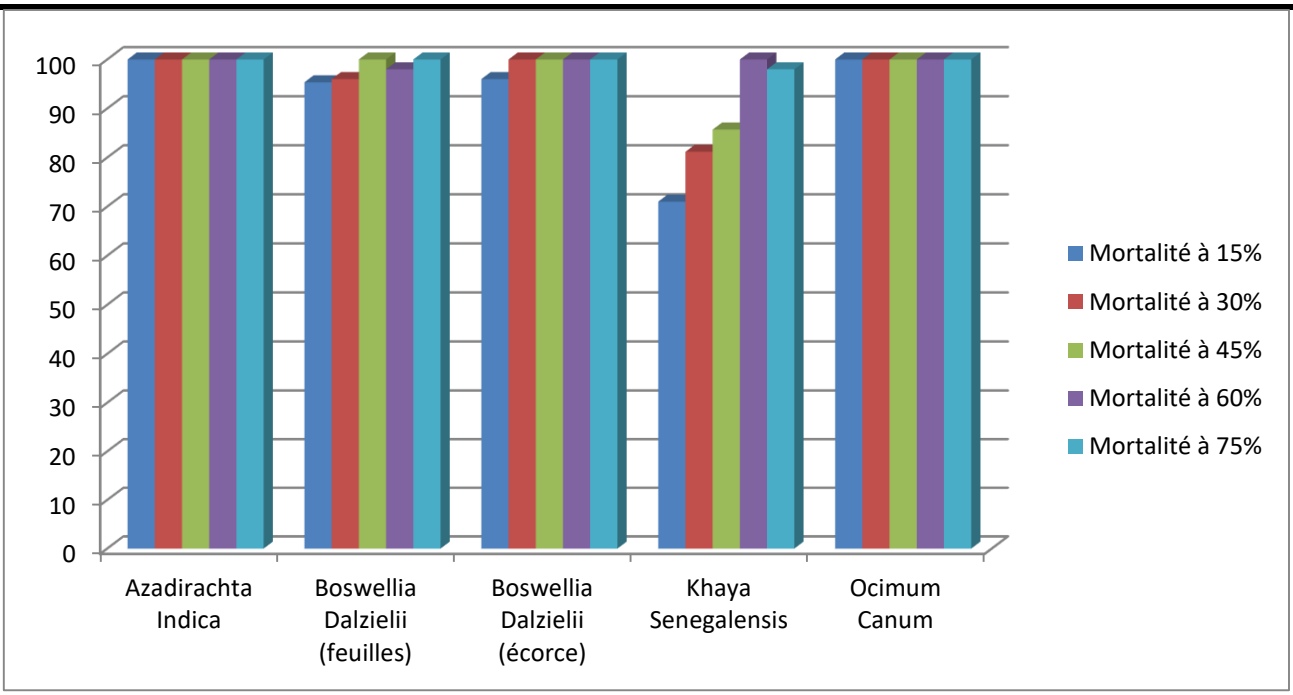

Fig.3: Mortality of mosquitoes depending on the dose of the plant powder for four species of plant after 24 hours of exposure.

The figure 3 shows that the mortality rate is high among all vegetable powders and varies depending on the dose.

In plant powders of *Azadirachta indica* with *Ocimum canum*, mortality is capped at 100% from the 15% dose. In all of these results a classification of effectiveness of toxic powders tested is highlighted, so the most toxic powders are those of the leaves with mortality rates highest namely 10 and 13, 15% respectively of *Azadirachta indica* with *Ocimum canum* and least toxic is the leaves of. *Boswellia dalzielii*.

2.2-Assessment of the LD50 (lethal dose 50)

The determination of the lethal dose of each vegetable powder that causes 50% of mortality in adult mosquitoes was made from the regression line representing (expressed as a percentage) mortality rates according to the doses of these powders.

Table 3 represents the regression equation, correlation coefficient, and LD50 values obtained for different plant powders.

Table.3: Different LD50 of powder plant use on adult mosquitoes after 6 hours of exposure

Plantes	Equation de régression	Coefficient de corrélation linéaire	DL50
Azachdirachta indica (leaves)	*y = 1,293x + 3,246*	0,99540468	36,1470268%
Boswellia dalzielii (leaves)	*y = 1,269x - 5,351*	0,98587165	43,6161351%
Boswellia dalzielii (bark)	*y = 1,279x - 3,160*	0,97512005	41,5615553%
Khaya senegalensis (bark)	*y = 1,038x + 6,979*	0,95905135	41,4555917%
Ocimum Canum (leaves)	*y = 1,338x - 4,580*	0,95890812	40,8003588%

The analysis of the different results shows that 6 hours after exposure, the powder of the leaves of *Azadirachta indica* has proved to be more effective with an LD50 of 36.14%, followed by powder of leaves of *Ocimum canum* and barks of *Khaya senegalensis* respectively with LD50 of 40.80% and 41.45% (table 3).

All plant powders used *Boswellia dalzielii* sheets proved to be less efficient compared to others, because its LD50 is highest at 43.61% (table 3).

In sum, the toxic effects of each plant on adult mosquitoes depend on the plant, the dose and duration of exposure.

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3-Discussion

The LD50 for adult mosquitoes showed that among the 5 plant powders tested, two had proved interesting in terms of toxicity, namely those of *Azadirachta indica* and *Ocimuin canum*. They are in fact the lowest LD50. These results are in agreement with those of Saotoing (2005), who after studying the insecticide effect of six essential oils of plants present in the three northern regions of Cameroon, got the lowest lethal concentration 50 (LC50 = 11. 9 mg/m2) in *Ocimum canum*. Similarly, Francis et *al*., (2009) in Littoral Cameroon, class essential oil of *Ocinium canum* before essential oils of *Ocimum gratissimum* and *Thymus vulgaris*in terms of insecticidal efficiency on adult mosquitoes. These different results are obtained with very low LD50 and LC50 for ours. Indeed, the essential oils extracted from the leaves, seeds or bark of plants have high concentrations of active ingredient for plant powders. It is sufficient therefore to extract a low concentration of essential oil to get satisfactory results.

Mortality rates observed in the species *Ocimum canum* and *Khaya senegalensis* 6 hours after exposure of mosquitoes at 15% and 30% dose are 21.05% and 54.55% respectively while for same plants at doses 30% and 45% for there was a decrease of mortality rate respectively 15% and 46.94%. These same irregularities are also observed 24 hours after exposure of species of *Boswellia dalzielii* (leaves) and *Khaya senegalensis* where we observed only at 45% and 60% doses, the rate of mortality are declining compared to the doses 60% and 75% respectively. These irregularities could be explained by the non-homogeneity made sticks. Indeed these tubs are made from a mixture in which its homogeneity could be perfect. Thus, for a tub at a given dose, it may submitted by location of doses more or less above the desired dose.

Furthermore, the results obtained with the same sticks for 15 minutes of exposure do not exhibit these irregularities. This leads us to introduce time as a second possible cause of these irregularities. The time factor is simultaneously the duration of the experiment and the lifetime of adult mosquitoes. Indeed, toxicity tests are carried out on adult mosquitoes from one to two days of age, these tests last 24 hours; this gives us the possibility of having adult mosquitoes of 3 days of age at the end of the experiment. However the estimated useful life of the adult mosquitoes bred in the conditions of the experiment is 3 to 4 days. It could therefore have an interference between the mortality of mosquitoes due to the insecticide effect and the mortality of insect due to their short lifespan.

IV. CONCLUSION

The use of insecticides of synthesis, more regulated for the protection of the environment, is the origin for most numerous cases of insects' resistance. In this context, the use of natural molecules (ecological and economic interest) to insecticide properties is of lesser toxicity to humans turns out be an alternative approach to the use of synthetic insecticides.

In this study, we proceeded to the formulation of the insecticide tubs on bases of four plants one found in the Far North region of Cameroon and assessing their insecticidal potential on adult mosquitoes of said region. All samples showed an interesting insecticidal activity. These natural fuel shave as advantages: availability, abundance, their efficiency, their low cost and above all less toxic to man and the environment.

The vulgarization of the virtues of these plant species in medium-term have an intermediate solution in preventing malaria control, but also in improving the production of certain foodstuffs which are major concerns for the people of the far North region of Cameroon.

We are considering as a result of the present work to clarify the nature of compounds chemicals responsible for insecticidal activity observed in the studied plants. This might be possible through a chromatographic study of essential oils extracted from leaves, bark and seeds of these plants.

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Environmental Impacts of the liquid waste from Assalaya Sugar Factory in Rabek Locality, White Nile State, Sudan.

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Abstract— *The study aimed to assess the environmental health impacts of the liquid waste from Assalaya Sugar Factory, the efficiency of the existing Assalaya effluent treatment plant, the dilution factors available in the White Nile to gather with wastewater environmental impacts. A descriptive cross-sectional focused on the Factory and its neighborhoods. Four hundred and thirty two out of 3931 households were statistically determined as the sample size, the individual samples were picked using multi-stage stratified method 432 households selected as sample size. Data were collected by using structured questionnaires, field observations, laboratory analysis and interviewing the concerned and affected persons. The effluent load discharged from the factory into the Al - jassir canal at the White Nile was analyzed for BOD, COD, pH, PO_4, TDS, TSS, Turbidity, Color, and flow rate.The Data were processed by using the Statistical Package for Social Science (SPSS) version 16, Chi-square test, test associations and office excel 2007. The study showed that Eighty one percent of the households used the surplus irrigation canal as a source for water supply. 64% of the respondents suffered from diarrhea, vomiting and allergic diseases, the rather low rate of water consumption and the bad quality of water consumed were reflected adversely on hygiene and consequently increased water related diseases. The study concludes that always or sometime 49.5% of the water collectors were children and used animals and plastic containers for water collection and transportation. The conducted laboratory water analysis revealed that the average concentrations of PO4, COD and BOD of the raw wastewater produced by Assalaya Sugar Factory were 4260, 3800 and 1500 mg/l, respectively, these values were above the WHO recommended concentrations for the disposed treated effluent (2, 250 and 30 mg/L respectively). As to physical analysis; the turbidity on the average was higher (540 NTU) and the color was (854 TCU) also high.*

Keywords—Environmental Impacts, Liquid waste, Assalaya Sugar Factory, SPSS.

I. INTRODUCTION

The environment is external to individual human host it can be divided into physical, biological, social and cultural, all of which can influence populations, health status [WHO, 1995]. The relationship of man with the environment is necessarily symbiotic; the equilibrium between the two must be maintained at all costs. Unfortunately, on account of the various activities of man, the composition and complex nature of environment get changed. Such activities include industrial, construction, transportation, etc. These activities, although desirable for human development and welfare, lead to generation and release of objectionable materials into the environment, thus turning it foul, and make our life miserable [UNDP 1994].

Urbanization and industrial growth complicate the problem because:

1- Natural resources were considered as free goods, but population growth puts a strain on these resources.
2- Environment is a sink where all the waste produced by man is assimilated.
3- These two facts reflect the importance of efficient utilization of resources and the minimization of both the quantity and toxicity of waste as important first stage interventions in environmental management, sound management of remaining waste will help to

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protect the local and global environment [Tilak, K.S., Janardhana Rao, N.H. And Lakshmi, J. 1991].

Industry and environment:

Modern man and the complexity of his activities, especially in the fields of industry and technology produced substances that are foreign to the natural components. These substances affect the air or water or land or biological components. Macro and microorganisms have suffered from either some level of contamination that rose to dangerous pollution. Industrial processes produce liquid, solid and gaseous wastes which have negative effects on the environment and people; acid rain is an example, it results from emissions of sulfur dioxides and nitrogen oxides from chimneys and exhaust pipe. These wastes are generated either during processing, or at the end of the production process. At a closer range this atmospheric pollution can also increase respiratory diseases. Liquid wastes when disposed in water bodies without treatment greatly affect the aquatic organism [Roghaia, A.A. 1989].

Waste and global issues:

There are a number of aspects associated with waste, which have global implications. These include the effect on the ozone layer from CFCs, which remain in the old refrigeration equipment, and the greenhouse gases such as carbon dioxide and methane have been estimated to make a contribution seven to ten times greater than the same volume of carbon dioxide to the greenhouse effect. Rio conference objectives for waste management, as stated in the 1992 Earth summit Agenda:

- To minimize waste.
- To maximize environmentally sound waste re-use and recycling.
- To promote environmentally sound waste disposal techniques.

Local authorities should undertake recycling simply because of the local and global benefits, such as the recovery of CFCs, or the collection of waste automobile oil and diesel from garages which, if not managed properly, pollute water courses. The European Union has agreed a "proximity principle", which for waste requires that materials are handled and treated as close as possible to their point of origination, and not "exported to regions where waste management practices may be cheaper[Park, K., 1997]

Regional effect:

According to the fact that, all neighboring countries, share some geographical or geological structures (i.e. Rivers, lakes, aquifers, air and forests), then there is an interaction in all aspects. So certain problem can arise, such as pollution (heat transfer and radiation), carbon dioxide buildup and greenhouse effect, and acid rain [Alexander P. Economopoulos, 1993]

Local effects:

The seed of pollution germinates within the local level, then expands to affect the neighboring countries to regional and global levels, some problems, such as smoking or malodorous industries, are local and can readily be controlled; the trouble is easily located and can usually be corrected by better methods of combustion or waste disposal. Industrialization and concentration of population in selected pockets of the country bring, in their wake, large quantities of industrial and sewage wastes which find their way into either the air or natural water bodies. Various gaseous emissions may be noxious and toxic or in the case of oxides being the source of acid rain. The wastes discharged directly to receiving water bodies where they impair water quality and affect aquatic life; threaten living organisms either in their health or their lives or their diversity [Idris, E., 1983].

Pollution:

Definition:

Pollution is defined as, the introduction by man of waste matter or surplus energy into the environment which directly or indirectly cause damage to man and his environment other than himself, his household, those in his employment and those with whom he has direct trading. While biologists define pollution as undesirable changes in the physical, chemical, or biological characteristics of air, water, land that can harmfully affect the health, survival activities of human or our living organisms. Therefore, pollution may either be man-made such as insecticides, pesticides or could be a substantial rise in matter which has already been in the environment such as ozone or destroying some natural component. Pollutants are introduced into the environment in significant amounts in the form of sewage wastes, accidental discharges or as by product of a manufacturing process or other human activity [Dix, H. M., 1981].

Water pollution:

Water pollution is defined by Dix (1981) as a natural change in the quality of water, which renders it unusable or dangerous with regards to food, human, animal health, industry, agriculture, and fishing or leisure pursuits. Also water pollution is defined by W.H.O in terms of:-

1- Its nature:

- Physical: temperature, suspended matter, color, ect.
- Microbiological: microorganisms such as bacteria, viruses, and protozoa.

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- Chemical: mineral pollution (salts, heavy metals) or organic pollution (pesticide, hydrocarbons, solvent).

2- Its Origin:

Urban: Community wastewater, rain water, refuses tips.

Industrial: Liquid and solid waste from industrial activities (refineries, paper mills), storage of products (hydrocarbons, industrial wastes.) or extraction of raw materials (mines, quarries).

Agricultural: farming practices (fertilizers, plant protection products), slurry spreading the food industry (slaughter houses).

1- Its distribution in time:

Permanent: infiltration from leaching of waste discharges.

Accidental: broken pipes, overturned tanks.

Seasonal: plant protection products, highway deicing products.

2- Its distribution in space:

- Diffuse of agricultural origin, on-site sanitation.
- Localized: storage facilities, industries, and urban waste.
- Linear: highways, railways, rivers and watercourses.

The ecological and economical damages:

Damages caused by the untreated oil spill from industry and power generation unit to the sea, may result in mortality in birds and contamination of shore lines, resulting in severe biological effects on near shore organisms. Also may be fouling of vessels, nets and harbor facilities, requiring expensive clean up. The impact of oil on the open ocean environment is more difficult to assess, but it's likely that the spill will have some effects on fisheries and in general, on organisms present in the ocean surface waters. [Dix, H. M., 1981]

Sources of water pollution:

The major sources of water pollution can be classified as municipal, industrial, and agricultural. Municipal water pollution consists of wastewater from homes and commercial establishments for many years, the main goal of treating municipal. Wastewater was simply to reduce its content of suspended solids, oxygen-demanding materials, dissolved inorganic compounds, and harmful bacteria. In recent years, however, more stress has been placed on improving means of disposal of the solid residues from the municipal treatment processes. The basic methods of treating municipal wastewater fall into three stages: -

- Primary treatment, including grit removal, screening, grinding, and sedimentation.
- Secondary treatment, which entails oxidation of dissolved organic matter by means of using biologically active sludge, which is then filtered off.
- Tertiary treatment, in which advanced biological methods of nitrogen removal and chemical and physical methods, such as granular filtration and activated carbon absorption are employed.

The handling and disposal of solid residues can account for 25 to 50 percent of the capital and operational costs of a treatment plant. The characteristics of industrial waste waters can differ considerably both within and among industries. The impact of industrial discharges depends not only on their collective characteristics, such as biochemical oxygen demand and the amount of suspended solids, but also on their content of specific inorganic and organic substances. There are three options available in controlling industrial wastewater. Control can take place at the point of generation in the plant; Wastewater can be prorated for discharge to municipal treatment sources; or wastewater can be treated completely at the plant and either reused or discharged directly into receiving waters [World Bank. 1995]. Comprising over 70% of the Earth's surface, water is undoubtedly the most precious natural resource that exists on our planet. Without the seemingly invaluable compound comprised of hydrogen and oxygen, life on Earth would be non-existent, it is essential for everything on our planet to grow and prosper. Although we as humans recognize this fact, we disregard it by polluting our rivers, lakes, and oceans. Subsequently, we are slowly but surely harming our planet to the point where organisms are dying at a very alarming rate. In addition to innocent organisms dying off, our drinking water has become greatly affected as is our ability to use water for. In order to combat water pollution, we must understand the problems and become part of the solution [Mackenzie, 1996]. Water quality is closely linked to water use and to the state of economic development. In industrialized countries, bacterial contamination of surface water caused serious health problems in major cities. By the turn of the century, cities in Europe and North America began building sewer networks or domestic wastes downstream of water intakes [Mac Donnell, LJ 1996]. Development of these sewage networks and waste treatment facilities in urban areas has expanded tremendously in the past two decades. However, the rapid growth of the urban population (especially in Latin America and Asia) has outpaced the ability of governments to expand sewage and water infrastructure [Brassard, PG 1996].

Industry in Sudan:

Industry in the Sudan started with the turn of last century, when the expansion of cotton production was followed by the construction of ginning factories, industry for import

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substitution started as recently as 1960. Sugar industry began at El Guneid in 1961, at El Girba in 1963 and at Asslaya in 1976. Then a tannery in Khartoum was established; followed by five dispersed food- processing plants. Before World War II, the British governor's policy in Sudan was to export agro industrial products. However, 1956, the year of political independence, could be taken at the gate for real industrialization in Sudan.

Assalaya Sugar factory

The main responsibility of the factory is to process cane into sugar at the highest possible yield and the lowest possible cost. The factory is designed to crush 6500 tons of cane daily to produce 110,000 tons of sugar. Due to raw materials, major technical, and manufacturing defects the factory did not reach the design capacity. The factory has no proper system for wastewater disposal. Twelve samples of river water and wastewater are to be collected and tested for the following parameters {Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Phosphate content (Po4), PH. Total dissolved solid (TDS), Total suspend solid (TSS), Turbidity, Color and Conductivity}.

Cane Cultivation Area:

The area planned for cane cultivation of is 33282 acres, but only 28648 acres are actually handled, out of which four thousand acres proved to have too much sodium salt, which resulted in low cane yields. It is planned to increase the area to match the design capacity of the factory by adding another 8500acresconsidered as a first stage. The crop is cultivated by sprouts and harvested at the age of 10-16 months.

Irrigation:

Water is pumped every 10 days by a series of pumping stations comprised of 16 pumps, {five pumps (30 m^3/ second) located in the first station, four pumps (24 m^3/ second) in the second station, four pumps (22 m^3/ second) in the third station and three pumps (15 m^3/ second) in the fourth station}. The distance between a first station and second station is 6 km, second station to third station is 4 km, third station to fourth station is 8 Km. The actual requirement to operate the pumps was calculated to be nine megawatts and the daily water discharges to be 90000 m^3.

Quantity of waste products from the Factory:

The quantity, of molasses is estimated to be:-

3.5% of crushed cane weight, which is equivalent to 35000 tons per season of operation of 170 days. 3.25% of cane weight, which is equivalent to 32500 tons during the operation period. 4.5% of Bagasse weight from cane, which is estimated at 45000 tons during the operation period. Water consumed for wash and boil which is estimated to 27000m^3/h. [Ministry of industry & Ministry of finance 1995]. The traditional handcraft industries, such as wood products, leather and ivory; have been in existence since ancient times. Their success depends on small capital and the use of the local raw materials. The development of cotton ginning can be marked as a second stage of industry, in many ways. Thus the government policy has been to promote industrial and agricultural expansion. The first step was the approval of the Enterprises Act of 1956, which was issued to encourage the local and foreign capital investments in industry. This raised the current prices of products at the time from 1% in 1955/56 to 9.4% in 1970/71, and employment jumped from 900 to 4000 during the same period in 1960 [Boon, C. J., 1990]. The contribution of the industrial sector increased to 7.6%, and in 1973 increased to 15%. However, it fell to 8% in 1981 and went further down to 5% in 1985.Industry plays a relatively small role in the economy of the Sudan and accounts for less than twelve percent of GDP, ten percent of employment and less than one percent of exports. Manufacturing employment is about 2000,000 people. The sector suffers from infrastructure bottlenecks and shortages of trained manpower, raw materials and the foreign exchange needed for importing essential intermediate inputs [Elhaj, K. O. (1984)]. This situation agreed with the fact that says industrial development is given a high priority in most developing nations because such as development creates employment and generates revenue that is badly needed. Most of the industries in the Sudan are found in the urban areas except industries that are attracted to their source of raw material; Khartoum, Khartoum North, Omdurman, Port Sudan, Maringan & Gadida El Thawra industrial areas are examples of industrial complexes in urban areas, whereas sugar factories, textile, jute and cement factories are better examples of those attracted to their source of raw material throughout Sudan [Shakkak, N. B. 1985].

Objectives:

- Determine the pollution loads from the Factory, which are disposed in an Aljassir canal on the White Nile by using various parameters (e.g. BOD, COD, pH, PO4, TDS. TSS, Turbidity, color, conductivity and the effluent flow rate).
- Evaluate the existing industrial effluent treatment and disposal system installed by the Assalaya Sugar Factory and determine efficiency.
- Evaluate the effluent pollution problem caused by Assalaya Sugar Factory in the White Nile in general and in Aljassir canal particularly.

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II. MATERIALS AND METHODS

Study Design and Setting

To achieve this work the following descriptive cross- entail, a study was conducted in order to evaluate the Environmental Health Impact of Assalaya Sugar Factory (ASF) in Rabek. Questioning about industrial liquid waste from the plant was studied, with special emphasis on liquid waste discharged from the Assalaya Sugar Factory.

Sampling Methods:

1) A Questionnaire to households in the residential area, according to sample size selection (. 432 out of 3931 HH.)

2) Laboratory analysis for Assalaya Sugar factory effluents (both chemical & physical analysis), across the treatment plant, Aljassir Channel and the White Nile.

Sample Size:

1) The total numbers of households are 3931 where 432 households were selected as sample size from the study population by using the following sample formula

$$n = deff * \frac{Z^2.S2}{d^2}$$ [Murray Speigal – 2004 – Statistics – Schuam Senes]

Where:-

Z = the standard normal variable corresponding to 95% confidence interval (Z = 1.96).

S = Standard deviation. (S $\hat{}$ 3.1622777).

d = Margin of error. (d $\hat{}$ 0.4219).

n = Represent the required size of the sample.

deff = (design effect = 2).

Sample size result:-

$$n = deff * \frac{Z^2.S^2}{d^2} = 2 * \frac{(1.96)^2 * (3.162)^2}{(0.4219)^2} = 2 * \frac{(3.8416)* (10)}{(0.1779)} = 431.8 = 432 \text{ HH}$$

Accordingly a sample size of 432 HH was attained.

Where:

The sub sample size (nh) = $\frac{Nh * n}{N}$

h = Central Sector, Western Sector, Eastern sector.

N h_1 Total of household on one sector

n = (432) a sample size of HH.

N = (3931) total of household sectors

$n_1 = \frac{N_1 * 432}{N}$

The sub sample size result: -(n1= 122, n2 = 220, n3 = 90)

2)Sites of sample collection: to Laboratory analysis for Assalaya Sugar factory effluents (both chemical & physical analysis), across the treatment plant, Aljassir Channel and the White Nile.

The samples were collected from twelve sites randomly in the production season and Non production season

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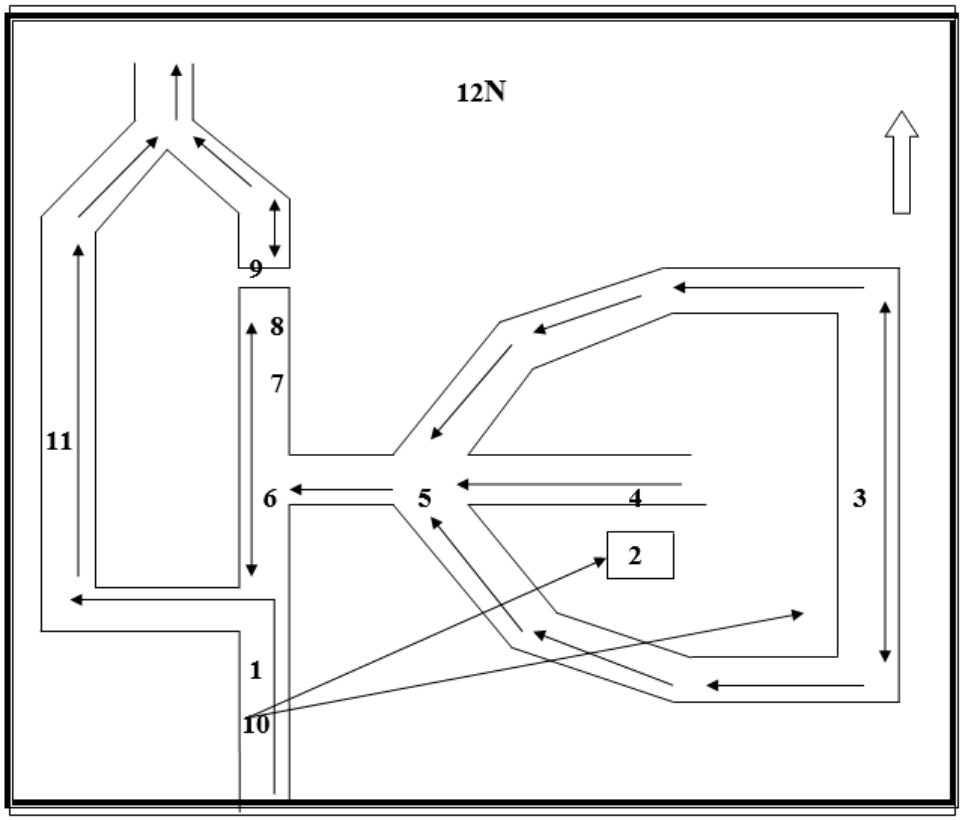

Fig: Locations of water and wastewater sampling in an Assalaya Sugar factory, Al-Jassir channel and White Nile

Data Collection: The data it was divided to tow section, section one was collected by using a structured questionnaire to households in the residential area according to sample size selection (. 432 out of 3931 HH.) and section tow by using Laboratory analysis for Assalaya Sugar factory effluents (both chemical & physical analysis), across the treatment plant, Aljassir Channel and the White Nile.

Data analysis: Data were analyzed by using SPSS version 16, and the chi - square test was carried out with 95% confidence level to find associations between the different variables. P-values less than 0.05 were considered statistically significant.

III. RESULTS AND DISCUSSION

Table.1: PO4 – (Phosphate) concentration

Po4 mg/L		Po4 mg/L (1)	Po4 mg/L (2)	Po4 mg/L (3)
Total	Mean	9.7917	8.6676	2.3167
	Std. Deviation	1.39828	2.96758	1.41860
	N	12	12	12
	Minimum	30.00	.15	30.00
	Maximum	4260.00	1.03	470.00
	Variance	1.955	8.807	2.012

Table.2: Concentration of (Chemical Oxygen Demand)

COD mg/L		COD mg/L (1)	COD mg/L (2)	COD mg/L (3)
	Mean	2.0031	1.8362	1.0385
	Std. Deviation	2.71724	3.17653	1.97526

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Total	N	12	12	12
	Minimum	8.00	1.12	6.67
	Maximum	3000.00	1000.00	3800.00
	Variance	7.383	1.009	3.902

Table.3: Biochemical Oxygen Demand (BOD) Concentration

BOD mg/L		BOD mg/L (1)	BOD mg/L (2)	BOD mg/L (3)
Total	Mean	3.9075	7.3890	7.3786
	Std. Deviation	8.87088	1.38637	1.37207
	N	12	12	12
	Minimum	3.00	4.30	4.30
	Maximum	600.00	250.00	1500.00
	Variance	7.869	1.922	1.883

Table.4: Concentration of turbidity (NTU)

Turbidity (NTU)		Turbidity (NTU) (1)	Turbidity (NTU) (2)	Turbidity (NTU) (3)
Total	Mean	129.5000	143.3333	51.7500
	Std. Deviation	1.60855	2.01239	93.05924
	N	12	12	12
	Minimum	16.00	10.00	2.00
	Maximum	470.00	540.00	336.00
	Variance	2.587	4.050	8660.023

Table.5: Degree of color

Color (Tcu)		Color (Tcu) (1)	Color (Tcu) (2)	Color (Tcu) (3)
Total	Mean	1.6642	2.1233	2.3417
	Std. Deviation	2.27330	2.25340	2.66283
	N	12	12	12
	Minimum	18.00	10.00	20.00
	Maximum	854.00	640.00	743.00
	Variance	5.168	5.078	7.091

Table.6: Source of used water , 2013

Source of used water	No	%
Main canals	80	18.5
Surplus irrigation water	350	81.0
Others	2	0.5
Total	432	100

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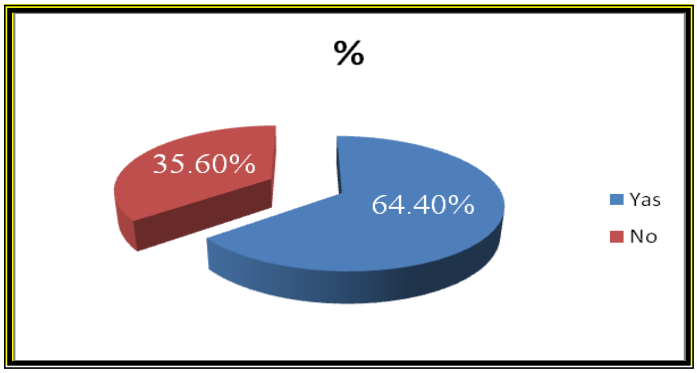

Fig.1: Percentages of the diseased

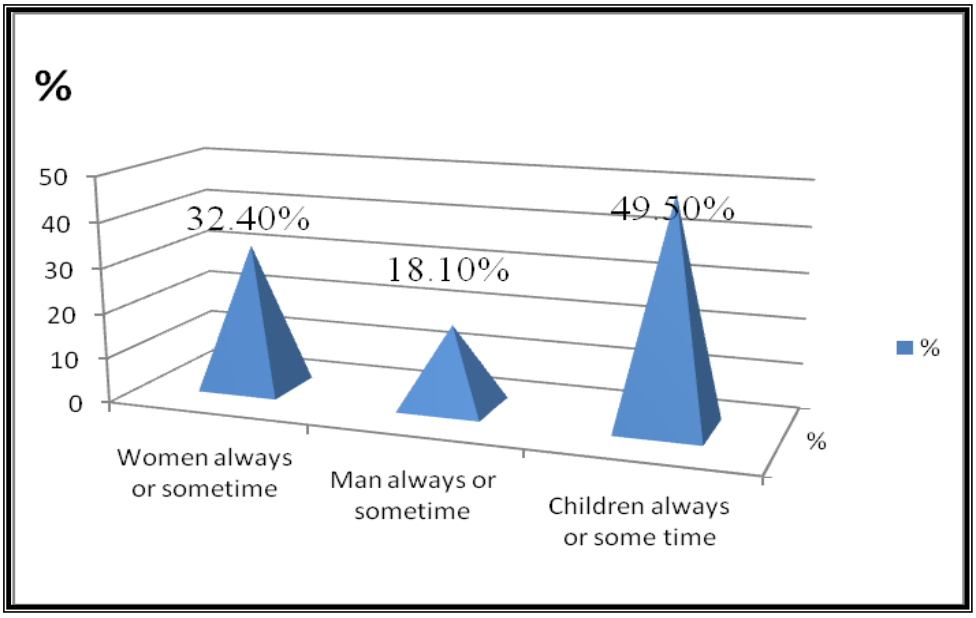

Fig.2: Water collectors

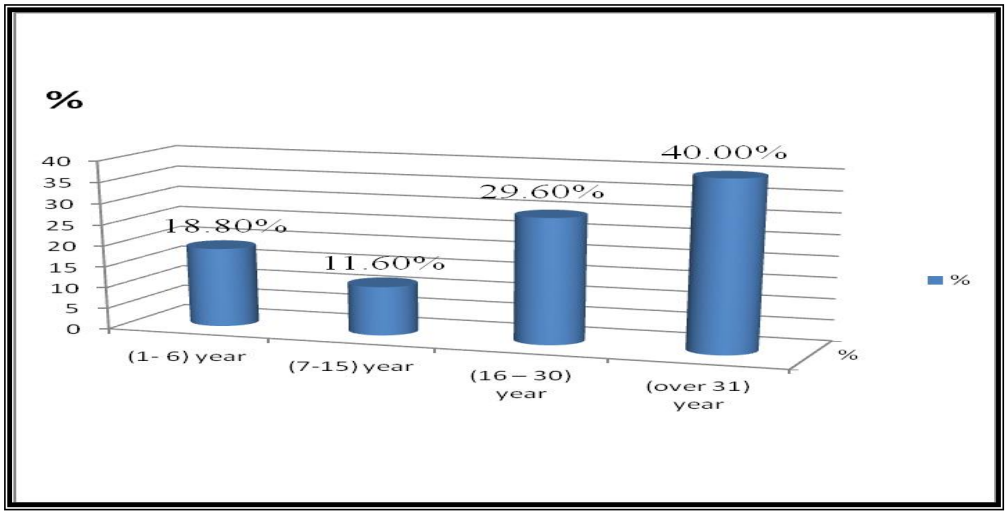

Fig.3: Age distribution of people affected

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This study was conducted to evaluate the Environmental Health Impact of the liquid waste of Assalaya Sugar Factory in Rabek locality.

Regarding water and waste analysis the PO_4 concentration was 4260 mg/L which is higher than the recommended concentration by the WHO 2 mg/L, in (Industrial sewage and irrigation effluent) {*Table 1*}, comparison by the phosphorus (as orthophosphate) is the limiting nutrient in freshwater and aquatic system. The natural total phosphorus is generally less than 0.03 mg/l, whereas the natural levels of orthophosphate usually range from 0.005 to 0.05 mg/l. No guideline values suggested by WHO for drinking water, however the EPA water quality criteria state that phosphates should not exceed 0.05 mg/l if streams discharge into lakes or reservoirs, 0.25 mg/l within a lake or reservoir and 0.1 mg/l in streams or flowing waters [WHO, 2006].Also the COD concentration was 3800 mg/L which is higher than the permissible level according to the WHO standard 150 mg/L *{Table 2},*. BOD concentration was 1500 mg/L which is higher than the WHO recommended concentration 30 mg/L *{Table 3}*. Comparison by the Chemical oxygen demand (COD) does not differentiate between biologically available and inert organic matter, and it is a measure of the total quantity of oxygen required to oxidize all organic material into carbon dioxide and water. COD values are always greater than BOD values, but COD measurements can be made in a few hours while BOD measurements take five days. If effluent with high BOD levels is discharged into a stream or river, it will accelerate bacterial growth in the river and consume the oxygen levels in the river. The oxygen may diminish to levels that are lethal to most fish and many aquatic insects. As the river re-aerates due to atmospheric mixing and as algal photosynthesis adds oxygen to the water, the oxygen levels will slowly increase downstream. The drop and rise in DO levels downstream from a source of BOD are called the DO sag curve [Sudanese Standardsand Metrology Organization SSMO2007]. The Self-purification aspects of rivers were given strong consideration when BOD standards were established for these water bodies. The waters having a BOD of less than 1 mg/l can be relatively un impacted by humans and primary candidates for conservation. About 31.4%, 29.9% and 13.8% of drinking water sources in Japan, have BOD values less than 1 mg/l, 2 mg/l and 3 mg/l, respectively. If BOD exceeds 3 mg/l, it affects coagulation and rapid sand-filtration processes, conventional water treatment plants, requiring expensive advanced water treatment. Therefore, BOD standards are set at 2 and 3 mg/l, respectively, for glass 2 and 3 waters. For class {I} fisheries, BOD is set at less than 1 mg/l, since oligosaprobic fishes such as salmon and smelt require water with a BOD Less than 2 mg/l. For class {II} fisheries, BOD is set at less than 2 mg/l, since mesoprobic fish such as carp require water with a BOD Less than 3 mg/l. For class {III} fisheries, BOD is set at less than 3 mg/l, since class {III} fisheries require water with a BOD Less than 5 mg/l. For class E, conservation of environment, BOD is set at less than 10mg/l to prevent odor caused by the anaerobic decomposition of organic matter [WHO, (2006]. As to the physics analysis; the turbidity concentration was 540 NTU and color was 854 TCU, which are higher than the permissible levels for drinking water according to SSMO standards (5 NTU, 15 TCU) respectively.*{Table 4,5},*. Comparisons by the turbidities of 10 NTU or less represent very clear waters; 50 NTU is cloudy; and 100- 500 or greater is very cloudy to muddy. Some fish species may become stressed at prolonged exposures of 25 NTUs or greater. Furthermore, Barnes (1998) recommended that to maintain native fish populations in Georgia Piedmont Rivers and streams that random monthly values should never exceed 100 NTU; that no more than 5 percent of the samples should exceed 50 NTU; and no more than 20% should exceed 25 NTU. Similarly, average TSS concentrations in the range of 25-80 mg/L represent moderate water quality. An average concentration of 25 mg/L has been suggested as an indicator of unimpaired stream water quality. High turbidity levels affect fish feeding and growth; the ability of almonds to find and capture food is impaired at turbidities from 25 to 70 NTU. Gill function in some fish may also be impaired after 5 to 10 days of exposure to a turbidity level of 25 NTU. Turbidities of less than 10 describe very clear waters. Turbidity units are supposed to correspond to TSS concentrations, but this correlation is only approximate. Turbidity in a stream will fluctuate before; during and after storm flow give general criteria for all waters, which include narrative standards for turbidity: “All waters shall be free from material related to municipal, industrial or other discharges which produce turbidity, color, odor or other objectionable conditions which interfere with legitimate water uses,” [Sudanese Standards and Metrology Organization SSMO 2007].The study explained also the community responses and the surveyed study area revealed that there wasn’t disposal system of (Industrial sewage and irrigation sewage) in an Assalaya Sugar factory and disposed directly on the Al – jassir channel. Comparison by

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the All sugar factories were found to be releasing factory wastewater directly into the Blue and White Nile without pre-treatment. This wastewater contains an elevated biological oxygen demand (BOD), which can reach 800-3,000 ppm. The resulting pollution of river water is suspected to be the leading cause of frequent fish kills, particularly in the Blue and White Nile. It should be noted that the Kenana factory is in the process of constructing a wastewater treatment plant to address this problem. Others have yet to follow suit [UNEP teams. 2010]. Regarding knowledge about the problems related to the (81%) of householders used the source of water from surplus irrigation*{Table 6}* the study also revealed that the effluent pollution from Asslaya Sugar Factory within the survey showed that the (64.4%) of householders were the affected by diarrhea or vomiting or allergic disease as the result using of main canals for drinking, *{Figure1}* , and water collectors there were children, the outcome of the study shows that effect of used surplus irrigation water for drinking in (49.5%) of householders, *{Figure 2}*The study indicated that 40% of the affected among the population were over 31 year of age, *{Figure 3}* comparison by the all the pathogens discussed in the previous section have the potential to reach the field. From the time of excretion, the potential for all pathogens to cause infection to usually decline due to their death or loss of infectivity. The ability of an excreted organism to survive outside the human body is referred to as its persistence. For all the organisms, survival is highly dependent on temperature with greatly increased persistence at lower temperatures. Also the first exposure of excreted pathogenic organisms outside the body is usually water. This blend with fresh water is often referred to as sewage. This sewage is then subjected to treatment prior to discharge, used directly for crop production or discharged to a watercourse where indirect use then occurs downstream. There are many studies on the survival or persistence of excreted organisms in water and sewage [FAO – Swedish Defence research agency 2004]. The surface water streams are also affected by industrial effluents and organics. Most of the treated industrial effluents are disinfected with chlorine which reach the receiving bodies and react with organic compounds to form chlorinated organics. The presence of these compounds in the water can cause cancer. Nitrates and nitrites are common inorganic pollutants that are released from fertilizer industries and excess nitrite levels are fatal to infants (blue disease) and also lead to eutrophication of water bodies. The major pollutant from the cement and thermal power industries is particulate matter that causes diseases. Some people are likely to experience pneumoconiosis (respiratory allergies, asthma and lung diseases) [Mohamed, A.A., 1999]. The differences in pressure can cause contaminants to be drawn or forced into the distribution system. Contamination introduced due to backflow into the distribution system then flow freely into other customer connections. The following conditions must be present for contamination to occur through cross-connections:

• A cross-connection exists between the potable water distribution system and a non-potable source.

• The pressure in the distribution system either becomes negative or the pressure of a contaminated source exceeds the pressure inside the system.

• The cross-connection is not protected, or the connection is protected and the mechanism failed, allowing the backflow incident. The extent of contamination in the distribution system depends, in part, on the location of the cross-connection, the concentration of the contaminant entering the distribution system and the magnitude and duration of the pressure difference causing the backflow [Survey of State and Public Water System Cross-Connection Control Programs. Washington. U, S.A 2000]. The health hazards associated with direct and indirect wastewater use are of two kinds: the rural health and safety problem for those working on the land or living on or near the land where the water is being used, and the risk that contaminated products from the wastewater use area may subsequently infect humans or animals through consumption or handling of the foodstuff or through secondary human contamination by consuming foodstuffs from animals that used the area [FAO, 2004].

Wastewater Treatment Plants:

Assalaya Sugar factory is located North Rabak town (300 Km South of Khartoum), White Nile State, Sudan. Rabak is bordered on the west by the White Nile River. The Assalaya Sugar factory started production in 1979 – 1978 with a design capacity of 6,500 Tone of the cane / Day and with an annual production of 110,000 tons/year of refined sugar.

The installed wastewater treatment system for the factory is a biological treatment system. It consists mainly of three stages:

Anaerobic Pond. The effluent of the mill house and process are passed through screens and grease traps, and then drained by gravity into an anaerobic pond. The anaerobic pond is deep, allowing the anaerobic degradation of the organic matters to take place through microbiological growth.

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Facultative pond: The effluent of the anaerobic pond is drained by gravity into the facultative pond. Facultative has longer retention time, less depth and large surface area necessary for algal growth and consequent aerobic action.

Maturation Pond: The effluent of the facultative pond is then drained into a shallow Maturation Pond to allow for further polishing and aerobic action [Elhassan, B.M and Rabih A, 2012].

The effluent from the Maturation Pond is discharged (via a lift station) into the cane field irrigation system.

Table: Assalaya WWTP design features.

Pond	Depth m		Area m^2	Volume m^3	
	Total	Water		Total	Water
Anaerobic	5.84	4.0	9672	56484	38668
Facultative	3.65	1.5	33825	123462	50737
Maturation	3.89	0.8	33345	129712	26676

White Nile flow next to the factory (Malakal & Khartoum) * 10^6 M^3 /d

Month	Nov.	Dec.	Jan.	Feb.	M.	AP.	May
Malakal	78	73	58	65	78	61	411
Khartoum	99	79	54	45	41	42	46
Average	88.5	76	56	55	59.5	51.5	43.5
Assalaya W.W	5976M^3 /d						
Dilution factor	14809	12718	4371	9203	9956	8618	7279

Dilution factor available in the White Nile during the production season

$$= \frac{\text{River flow rate}}{\text{Flow of factory waste water}} = \frac{QR.\ F}{QF.\ F}$$

$$\text{Dilution Nov.} = \frac{88.5 * 10^6\ m^3/d}{5976 m^3/d} = 14809$$

$$\text{Dilution Dec.} = \frac{76 * 10^6\ m^3/d}{5976 m^3/d} = 12717$$

$$\text{Dilution Jan.} = \frac{56 * 10^6\ m^3/d}{5976 m^3/d} = 9370$$

$$\text{Dilution Feb.} = \frac{55 * 10^6\ m^3/d}{5976 m^3/d} = 9203$$

$$\text{Dilution M.} = \frac{59.5 * 10^6\ m^3/d}{5976 m^3/d} = 9956$$

$$\text{Dilution Ap.} = \frac{51.5 * 10^6\ m^3/d}{5976 m^3/d} = 8617$$

$$\text{Dilution May} = \frac{43.5 * 10^6\ m^3/d}{5976 m^3/d} = 7279$$

The average dilution factor across the production season is = 10278

So if we exclude the interactions between the contaminants in the waste water and the high concentration at the disposal point and the low flow stagnation within the Al - jassir canal. The immediate effect and accumulative effect on the aquatic life

The available dilution factor in the river is sufficient to reduce the degree of contaminants in the White Nile to an acceptable level [Abdeen Mohamed Ali Salih, Mohamed Ahmed Adem khadam, 2001].

IV. CONCLUSION

The discharges of industrial sewage and irrigation effluent are highly polluted in terms of Phosphate content, Turbidity, Chemical Oxygen Demand, Biochemical Oxygen Demand and Color.

Household members around the Assalaya Sugar Factory use water for drinking from the main canal without treatment, and sometimes they withdraw surplus irrigation water directly.

The study reflected strong correlation between the Industrial sewage and irrigation effluent disposal and the spread of water related diseases.

The study recommended Assalaya Factory should redesign and reconstruct its present wastewater treatment plant because of its inefficiency and should adopt the cleaner

production principle through waste recycling and reuse, thus minimizing the quantity of the generated waste and consequently its impact on the environment.

The study recommends:

- Assalaya Factory should redesign and reconstruct its present wastewater treatment plant because of its present inefficiency.
- The surplus irrigation water should be reused in irrigation and not be disposed into the Al - jassir canal.

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Assessment of Physicochemical parameters and Water Quality Index of Vishwamitri River, Gujarat, India

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Abstract— *Development and industrialisation exert pressure on the riverine system deteriorating the serenity of the rivers. The present study was carried out in Small River flowing through Vadodara city viz., Vishwamitri River. The study revealed better water quality before its entry into the urban area. Despite of presence of STPs, there is poor water quality affecting the aquatic life and ecology. The paper throws light on pollution aspect and need to develop decentralised treatment system to tackle the river pollution problem.*

Keywords- BOD, COD, DO, Vishwamitri River, Water Quality Index.

I. INTRODUCTION

The rivers are the important sources of surface water and India is a blessed country and rightly referred to as *Land of Rivers*" because of numerous rivers and lakes crisscrossing the terrain and landscape. Apart from source of fresh water, rivers play major role in assimilation or transportation of municipal and industrial waste water. Riverine sediments play an important role as pollutant accumulator and often reflect the history of the river pollution (Jain, 2004). Sediments act as both carriers and sinks for contaminants in aquatic environments (Mishra and Dinesh 1991). Studies have shown that domestic and industrial sewage, agricultural wastes have polluted almost all of Indian rivers (Pani 1986). Most of these rivers have turned into sewage carrying drains. This poses a serious health problem to millions of people who continue to depend on this polluted water from the rivers. Major rivers present in Gujarat includes Sabaramti, Naramada, Mahi, Tapti and Purna. Apart from these there are small rivers running across the landscape. Most of these rivers receive industrial and domestic sewage before draining into the sea. However, monitoring of river pollution is done in the major rivers and smaller rivers are not monitored. Rivers like Sabarmati, Vishwamitri, Tapi and Aji rivers are loaded with tons of industrial pollution, sewage and garbage every day. The paper deals with one such river Vishwamitri river passing through Vadodara city. Rivers Present study deals with the aim to evaluate the key stresses responsible for deteriorating the water quality and to undertake the comparative study of upstream and downstream of river.

The present paper uses the WQI index to express the quality of water and is the major indices used to assess the pollution and one of the effective ways to create awareness among the public. Quality of water is defined in terms of its physical, chemical, and biological parameters (Almeida, 2007). Water quality index allows for a general analysis of water quality on many levels that affect a stream's ability to host life and whether the overall quality of water bodies poses a potential threat to various uses of water (Akkaraboyina and Raju 2012).

II. STUDY AREA

The Vishwamitri River is a seasonal river, which flows east to west between the Mahi and Narmada rivers in Gujarat, India. It originates in the hills of Pavagadh and flows west through the city of Vadodara and joins with the Dhadhar River and Khanpur River and empties into the Gulf of Khambat, near Khanpur village. A total of ten stations is selected , out of which station 1 to 6 represent outside the City limits and categoriesd as upstream whereas station 7 to 10 represents the location within the city.

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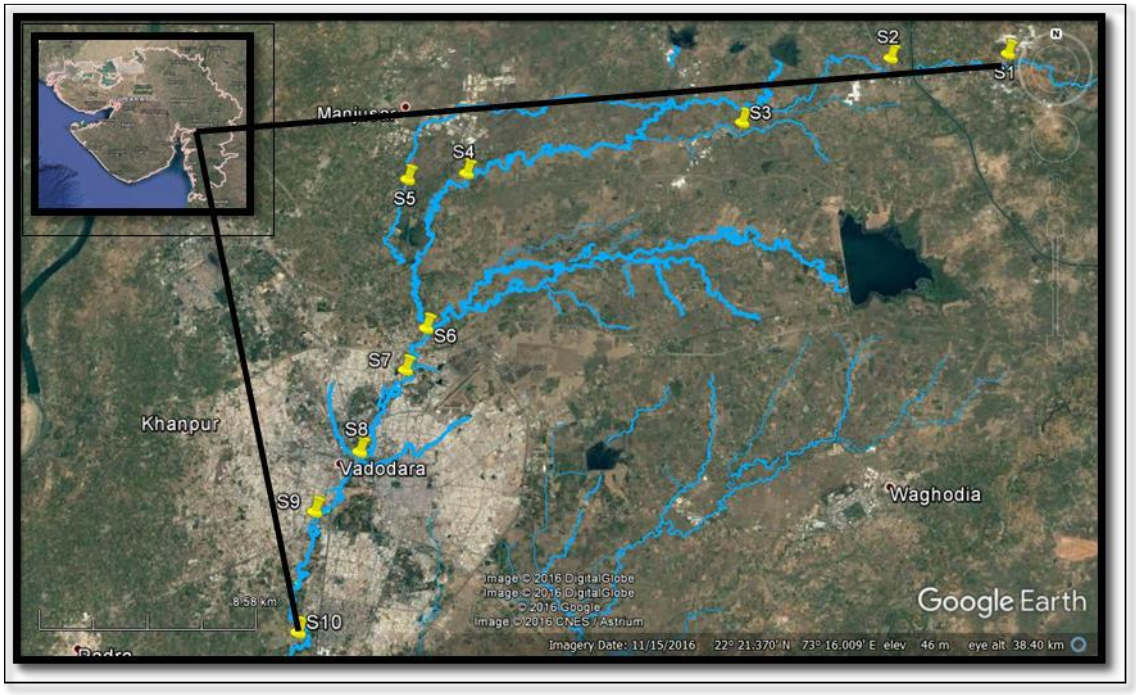

Fig.1: Sampling points of study area

III. MATERIALS AND METHODOLOGY

The study is carried out between Decembers to March 2017. The physical parameters like temperature, pH, and conductivity is measured directly in the field using respective instruments. Dissolved oxygen was fixed in the field and around 2 litres of water collected for further analysis. A standard method was adopted for the water quality assessment (APHA). Chemical Oxygen Demand was estimated by Open Reflux method. Nitrite (NO_2-N) was determined by Colorimetric method and Nitrates was estimated by Cadmium reduction method. Total phosphate is estimated by Ascorbic acid method. Silicate was estimated by Colorimetric method. Turbid metric method was used for the estimation of Sulphates. Statistical analysis carried out using the Statistical package SPSS (Version 20) and PAST (Version 7).

In this study the WQI was calculated by using the standards of drinking water quality recommended by BIS (1993) and ICMR (1975). The weighted arithmetic index method has been used for the calculation of WQI. Total 10 parameters (pH, Conductivity, TDS, TSS, DO, BOD, Hardness, Nitrate, Flouride and Sulphate) were selected for calculating the WQI.

Further quality rating or sub index (qn) was calculated using the following expression-

Qn = 100 x [Vn-Vo] / [Sn-Vo]

Where, qn = Quality rating for the nth water quality parameter.

Vn = Estimated value of the n^{th} parameter at a given sampling station.

Sn = Standard permissible value of the nth parameter.

Vo = Ideal value of nth parameter in a pure water.

Unit weight was calculated by a value inversely proportional to the recommended standard values

Sn of the corresponding parameters.

Wn = K/ Sn

Where, Wn = Unit weight for the nth parameter.

Sn = Standard value for nth parameter.

K = Constant for proportionality

The overall Water Quality Index (WQI) was calculated by aggregating the quality rating with the unit weight linearly.

WQI = Σqn Wn/ΣWn

IV. RESULTS AND DISCUSSIONS

Water temperature ranged from 19^0C to 24^0C. The water temperature is in accordance with the winter season, when the sampling is done.

The pH values in present study ranged from 6.8 (Station 3) to 8.3 (station 8). Similar pH range was reported in previous study (Deshkar *et al.*, 2014). Thus, there is no change in the pH level of the water.

Conductivity showed positive correlation with TDS, TSS, BOD, Total phosphate and Sulphate (significant at 0.01 level), as conductivity is the sum of anions, cations, dissolved ions, sulphates, carbonates, bicarbonates, chlorides and others. The hierarchical cluster analysis carried out for Conductivity (Fig.2) for different stations reflected two major clusters. Cluster A included Stations 1 to 7 with low conductivity values (290 to 660 µs) and cluster B with station 8, 9 and 10 showed higher conductivity (800 to 1340 µs). This shows that there is

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deterioration of water downstream of the river after it enters the city limits. The addition of industrial effluents and other domestic discharges has contributed to presence of higher ions (Nair *et.al* 1989 and Sugunan, 1989).

The chloride concentration in the present study increases as one moves downstream from station 3 (20.99 mg/l) to station 10 (109.96 mg/l). The presence of higher chlorides in the residential and commercial area of the river is reported (Hunt *et al.*, 2012). Further, the relatively high concentration of Total nitrogen, Phosphates and Total phosphorus ions affect the conductivity which in turn influence the concentration of chlorinity.

The Total dissolved solid (TDS) varied from 290 mg/L (Station 3) to 810 mg/L (station 9) and the concentrations are within the prescribed limits of CPCB (1500 mg/l). The discharge from nearby areas at station 8 and 9 has contributed to higher TDS. At upstream stations there is less human influence into the river and water is comparatively clean compared to downstream stations. The chlrodies has also contributed to the TDS levels (Taylor, 1984).

Total suspended solid (TSS) of water depend on suspended particle of soil, silt and is directly related to the turbidity of water. The highest TSS value was recorded at station 9 (410 mg/L) and low recorded at station 3 (110 mg/l). The present result was higher compared to the previous study in the same river (Deshkar *et al.*, 2014). The increased TSS values over the time period shows increase in discharge of untreated sewage into the river.

The Total hardness ranged between 104 mg/L (station 2) to 280 mg/L (station 8). The values increased from upstream to downstream. The higher values at the downstream stations may be due to the discharge of untreated sewage and effluents. Similar values were observed in Parna River (Pandey *et.al*, 2000).

The Dissolved oxygen (DO) showed marked difference in the riverine stretch. In the upstream stations DO ranged from 6.0 mg/l (station 1 and 3) to 11.3 mg/l (station 5). On the other hand DO was absent in the stations 6 to 10. The low concentration of DO in the fresh water aquatic system indicates presence of high organic load (Yayyntas *et al.*, 2007). The direct discharge of untreated sewage into the river has lead to anaerobic conditions. In the present study the cluster analysis has shown three clusters A, B and C. Cluster A includes the stations 1, 3, 4 and 6, while Cluster B includes 7, 8, 9 and 10 and cluster C includes station 3 and 5. Incidentally cluster C is distantly related with cluster A and B by more than 75%. At these stations there is presence of higher DO, which is attributed to photosynthetic activity of the aquatic vegetation that release oxygen. The minimum of 3 mg/L dissolved oxygen is necessary for healthy fish and other aquatic life (Clair. *et al.*, 2003). Thus, the water quality within the city limits is not suitable for aquatic life.

In the present study the concentrations of Biological oxygen demand (BOD) ranged from 3 mg/L (Station 1) to 92 mg/L (Station 10). Station 10 being the last station sampled showed the presence of high organic load because of cumulative effect. The untreated sewage disposal into the river may lead to the bacterial growth and consume the dissolved oxygen in the river resulting in oxygen depleted zone (Kulshrestha and Sharma, 2006; Kumar and Chopra, 2012).

The negative correlation observed between DO and BOD, both are inversely related as higher BOD represents oxygen depletion zone and demand represents more oxygen to degrade the organic pollutants present in the system. Chemical oxygen demand showed high significant positive relations with BOD, Nutrients and TDS but negative relation with DO. This indicates presence of industrial discharges that have influenced the water quality.

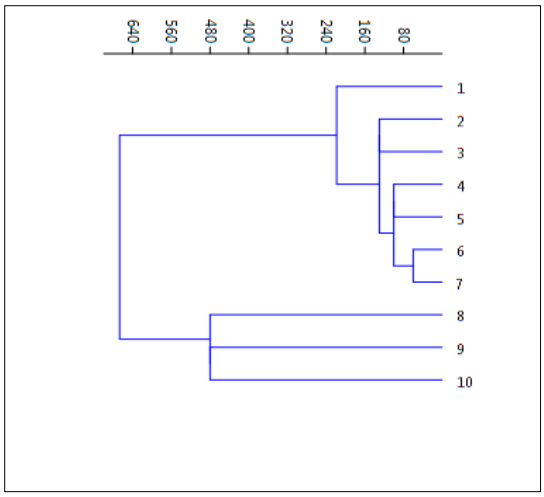

Fig.2: Euclidian cluster analysis of conductivity

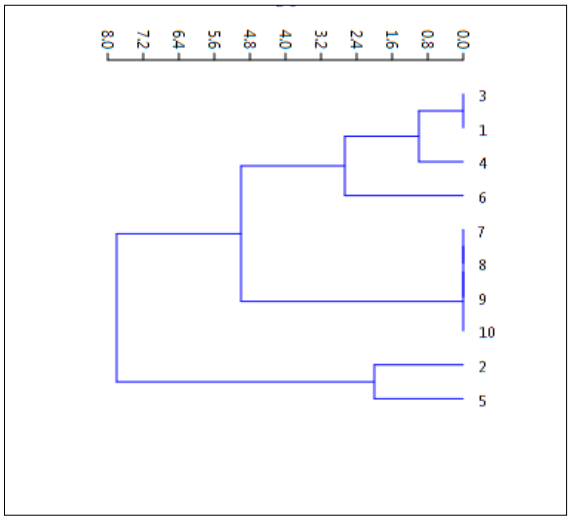

Fig.3: Euclidian cluster analysis of DO

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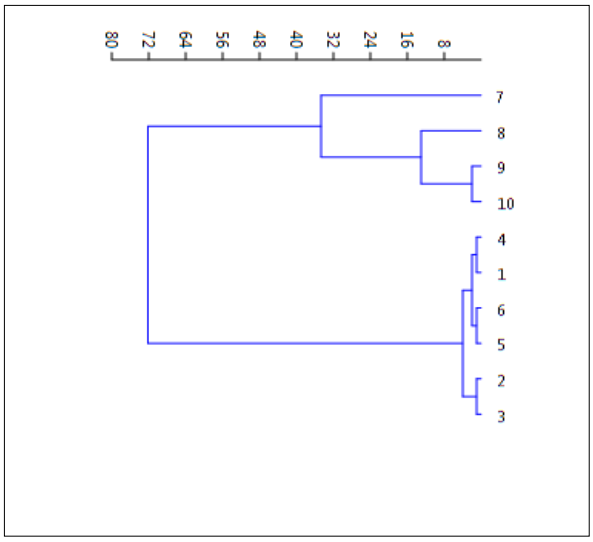

Fig.4: Euclidian cluster analysis of BOD

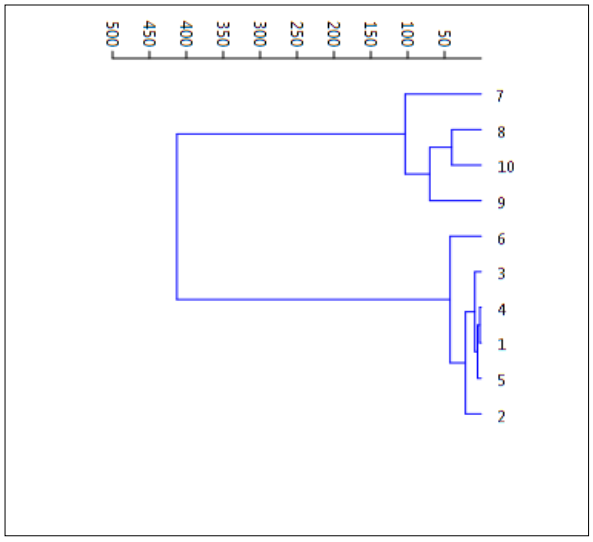

Fig.5: Euclidian cluster analysis of COD

The values of Chemical oxygen demand (COD) ranged from 40 mg/L (station 3) to 520 mg/L (station 7). The higher COD value was recorded at downstream stations because of direct discharge of industrial effluents and improper functioning of STPs. Further, the values of COD at downstream stations were above the permissible limits prescribed by CPCB (250 mg/L). The figure 5 cluster analysis depicts two clusters A and B; Cluster A includes stations 1 to 6 with similar water quality, while cluster B includes 7 to 10. The COD values were noted higher compared to the earlier study (Deshkar *et al.*, 2014) indicative of increase in pollution load because of expanding population.

The BOD to COD ratio differed in the riverine stretch. The BOD: COD value was more than 0.5 indicative of need for biological treatment of the water. On the other hand the BOD: COD ration was 0.1 at station 7 and 8, indicates presence of toxic waste and there is need for tertiary treatment and stabilization. Thus, the water quality of the river in the urban limits is highly polluted.

In the present study the Nitrate concentration ranged from 0.006 mg/L to 0.263mg/L. The maximum concentration recorded at station 7 (0.263 mg/L) and minimum at station 1(0.006 mg/L). The agricultural runoff, nitrate rich fertilizers and animal faeces into the river may lead to the higher values of nitrate (Tank *et. al*, 2013). The concentrations are within the CPCB prescribed limits.

The values of Nitrite ranged from 0.013 mg/L (station 6) to 0.291mg/L (station 7). The presence of low nitrite value is indicative of conversion of nitrite to stable nitrate by microbial activity. This conversion has exerted pressure on the DO levels. Total nitrogen showed positive correlation with Nitrates, Nitrites as these are related. The increase in Total nitrogen value at downstream stations is recorded because of discharges from industrial effluent.

The values of Total phosphate ranged from 0.060 mg/L (station 2) to 0.800 mg/l (station 8). The major source of phosphates is domestic sewage and hence the value increases as one moves downstream of the riverine stretch. The values of Sulphate was reported high at station 10 (375 mg/L) and low at station 3 (135 mg/L).

Table.1: Results of water quality parameters

PARAMETERS	CPCB standards	UNITS	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
pH	5.5-9.0		7.03	8.1	8.3	8.2	8.03	8.1	7.2	6.8	8.2	7.2
Temperature	-	ºC	20	24	22	23	20	23	20	23	20	24
Conductivity	-	µs	660	420	290	460	560	490	430	1340	800	1280
TDS	1500	mg/L	560	460	290	400	480	420	600	800	810	780
TSS	100	mg/L	240	148	110	158	168	138	248	340	410	345
DO	4	mg/L	6	9.3	6	5	11.3	3	0	0	0	0

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BOD	30	mg/L	3	8	9	4	5	6	5.2	78	90	92
COD	250	mg/L	51	45	40	70	68	65	520	420	370	460
Chloride	-	mg/L	39.9	37.9	20.9	33.9	71.9	34.9	87.9	107.9	979	109.9
Total hardness	-	mg/L	150	104	148	130	133	140	190	280	200	190
Calcium hardness	-	mg/L	110	76	91	100	72	80	108	100	124	104
Nitrate	-	mg/L	0.006	0.016	0.042	0.013	0.008	0.003	0.263	0.014	0.035	0.200
Nitrite	10	mg/L	0.034	0.021	0.057	0.016	0.035	0.013	0.291	0.023	0.02	0.096
Total nitrogen	-	mg/L	0.41	0.392	0.329	0.3	0.446	0.372	0.715	1.874	1.976	2.013
Total phosphate	-	mg/L	0.200	0.060	0.0470	0.076	0.060	0.033	0.093	0.800	0.500	0.760
Sulphate	400	mg/L	142	172	135	175	216	204	191	265	314	375
WQI= ΣWnqn/ΣWn			68	75	99.1	88.5	116.5	78	320.51	543.18	581.52	593.4

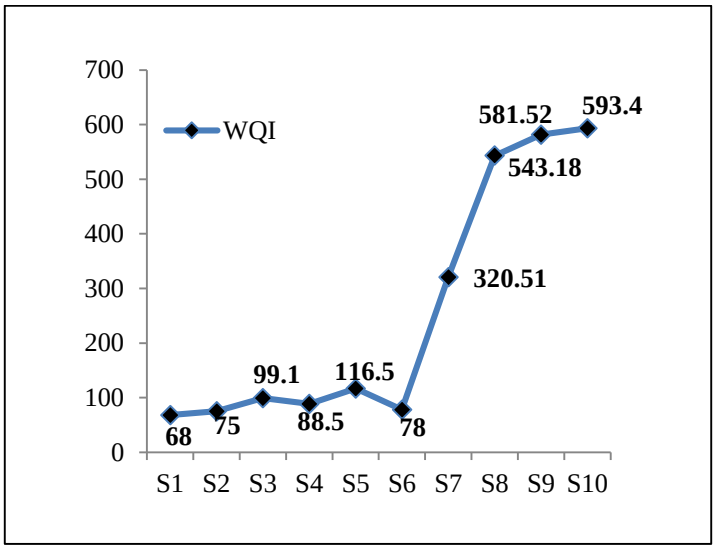

Fig.6: Graph of WQI

As per the WQI rating, the upstream stations (1 to 6) are categorized as C and D grade indicating that the water can be used for irrigation. However, the downstream stations are categorised as grade E indicative of presence of high pollution load and the water cannot be used for any purpose. Despite of presence of six STPs in Vadodara city, there is inefficiency in the functioning of the STPs leading to presence of high pollution in the river. The river pollution is a foremost issue with not only major river of India but also the minor rivers. As the urbanisation increases the problem of pollution is bound to increase and there is need to decentralise the sewage collection. Perhaps new technology of treatment has to be designed so as to reduce the pollution load of the rivers. In coming years, this would be an important issue for minor rivers as well.

V. CONCLUSION

From the present study it is concluded that the water quality of Vishwamitri River showed good quality and low pollution prior to its entry into the Vadodara city. The discharge of untreated sewage and dumping of solid waste in or on the bank of the river have contributed to poor water quality in the downstream locations. There is discrepancy in the functioning of the STPs and untreated sewage finds its way into the river system disturbing the ecology and aquatic life.

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Breastfeeding Practices of Postnatal Mothers: Exclusivity, Frequency and Duration

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***Abstract*—** *Mothers who perceive breastfeeding to be healthier, easier and more convenient breastfeed longer than those who perceive that breastfeeding is restrictive, inconvenient and uncomfortable. This study focused on the breastfeeding practices of postnatal mothers with regard to exclusivity, frequency and duration. It was a cross-sectional research design covering the three levels of health care institutions in the South-East Zone of Nigeria. Convenient sampling method was used to select 299 postnatal mothers who visited infant welfare clinics along with their infants. Three research questions and one null hypothesis guided the study. The instruments used for data collection were questionnaire on patterns of breastfeeding by postnatal mothers (QPBF) and checklist on health status of infants with varied breastfeeding patterns (CHSIVBP). Frequency distribution and percentages were used to answer the research questions while chi-square test was used in testing the null hypothesis at 0.05 level of significance. The result showed that most of the postnatal mothers practiced EBF for a short period, majority breastfed their infants on demand day and night, and majority also reported that their infants suckle the breast for more than 20minutes. Also breastfeeding patterns of the postnatal mothers was found to differ significantly across the three levels of health care institutions. Childbearing mothers need to be motivated on the need to practice EBF for six month postpartum.*

Keywords—*Breastfeeding, Duration, Exclusivity, Frequency, Postnatal Mothers.*

I. INTRODUCTION

Breast milk is nature's most precious gift to the newborn, an equivalent of which is yet to be innovated by the scientific community despite tremendous advances in science and technology (Faridi, 2008). Scientific research studies have clearly proved that breastfeeding provides the most suitable nutrition for infants and protects them against infections, allergies and asthma (WHO, 2001). Other documented benefits of breastfeeding to the mother include emotional wellbeing, economic benefits, spacing of pregnancies, protection against breast cancer and reduced incidence of type 1 diabetes mellitus (Sadauskaite-kuehne et al, 2004; WHO, 2001).

In the context of Millennium Development Goal 4, scientific evidences have highlighted initiation of breastfeeding immediately after birth without squeezing out colostrum and exclusive breastfeeding for the first six months as the key to tackle infant nutrition and also survival of infants (WHO and UNICEF, 2003). Studies on accelerating child survival published in the Breastfeeding Promotion Network of India (BPN) Lancet clearly established that universalization of early initiation of breastfeeding within half an hour after birth has tremendous potential in reducing 31% of neonatal deaths which is about 10% of total child deaths (Gupta, 2007). WHO(2001) warned that early introduction of supplementary feeding usually has a negative effect on the return to exclusive breastfeeding. Piwoz et al (1994) observed that supplements may not be given daily but they are unlikely to be withdrawn once they are introduced. According to Wilmoth and Elder (1995), supplemental feeding exposes infants to foreign contaminants and infection at a very vulnerable stage of life. Brown, Dewey and Allen (1998) added that this may likely explain the higher infant mortality rate of partially bottle-fed infants compared with exclusively breastfed infants. Researchers have shown that exclusive breastfeeding is associated with increased weight gain among babies of normal birth weight (Scarlett et al, 1996). Despite this observed benefit, studies have also shown that early introduction of infant formula and other foods have remained a problem among postnatal mothers (Almroth and Latham, 1982). Hence this study intends to determine the breastfeeding patterns of postnatal mothers with regard to exclusivity, duration and the frequency of breastfeeding.

Research Questions.

- To what extent do postnatal mothers practice exclusive breastfeeding?

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- How frequent do postnatal mothers breastfeed their infants?
- How long do postnatal mothers allow their infants to suckle the breast during each feed?

Hypothesis.

- Breastfeeding patterns of postnatal mothers do not significantly differ across the levels of health care institutions.

II. MATERIALS AND METHODS

Design and sampling.

This study was a cross-sectional research design. A convenient sample of 299 postnatal mothers who visited infant welfare clinics along with their infants in three levels of health care institutions (two health centres, two General Hospitals and two teaching hospitals) were used for the study. Ethical approval was obtained for the study and informed consent was obtained from the mothers. Inclusion criteria for the study were all healthy postnatal mothers irrespective of parity who were breastfeeding their infants, and all infants born at term aged 0-12months who were breastfed irrespective of the pattern of breastfeeding. Exclusion criteria were preterm babies and babies with any other underlying disorder (organic and non –organic) and mothers with medical disorders that could interfere with breastfeeding. Also mothers who indicated not to participate were excluded from the study, and also their infants were not used. The mothers were approached by the researchers at the time of their visits to the infant welfare clinics along with their infants. Interview method was adopted by the researchers to obtain data from the respondents at that time as well. Confidentiality was ensured by not including names of the respondents in the data collection.

Instrument.

Two instruments (Questionnaire and Checklist) were used among the mother-infant pair for data collection. Questionnaire on patterns of breastfeeding by postnatal mothers (QPBF) was used to obtain data on characteristics of the postnatal mothers. Section A of the instrument elicited information on the demographic characteristics of the respondents (e.g age, marital status, educational level, parity and employment status, etc). Section B of the questionnaire elicited information on the breastfeeding patterns adopted by the postnatal mothers (eg. time of commencement of breastfeeding, duration of exclusive breastfeeding, time of commencement of partial breastfeeding, frequency of breastfeeding, additional feeds with breastfeeding, etc). The responses to section B of QPBF were scored on a 4-point scale ranging from 1 point for poor pattern of breastfeeding, 2 points for fair pattern of breastfeeding, 3 points for good pattern of breastfeeding and 4 points for normal/ideal breastfeeding pattern. Checklist on the health status of infants with varied breastfeeding patterns (CHSIVBP) was developed for the study by the researchers to obtain information on the responses of the infants to the breastfeeding patterns adopted by their mothers. These data were obtained confidentially from the medical records of the infants, and included such information as the infants birth weight, age, weight gain pattern, height, nutritional status, vulnerability to infection, etc. The instruments (QPBF) and CHSIVBP) were tested for reliability, and a test-retest reliability coefficient of 0.72 and 0.75 respectively were obtained over a one month interval.

Data Analysis.

Standard descriptive statistics of means and standard deviation were used to summarize the variables. Frequencies and percentages were used to answer the research questions while Chi-square test was adopted in testing the null hypothesis at 0.05 level of significance. SPSS version 21 was used for the data analysis.

Table.1. Descriptive statistics of the measured variables

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	N	Mean	Std. Deviation
Age of Mother	297	27.5926	5.81171
Level of health institution	299	1.9967	.81717
Level of Health institution 2	299	1.9967	.81717
MS	299	1.0301	.17115
Edu	299	3.3344	.60909
Parity	299	1.5886	.49291
Employment Status	299	1.3746	.48483
Family Type	299	1.0100	.09983
Religion	299	1.0100	.09983
Place of Residence	299	1.3378	.47375
Time of Commencing Breastfeeding	281	3.7331	.70958
EBF Duration	197	2.6447	1.17179
Commencement of Partial BF	241	2.0415	1.26423
Breastfeeding Frequency	296	3.6892	.71641
Breast Sucking Duration	299	3.0602	.94641
Additional Food	230	2.7522	1.44003
Breastfeeding Pattern	299	2.6210	.70112
Sex of Infant	299	1.5619	.49699
Birth Weight	296	3.3274	.49365
Present Weight	89	5.3719	2.14391
Weight Gain Pattern	288	1.0556	.22946
Height Pattern	298	1.0470	.22728
Nutritional Status	299	1.0635	.24435
General Body System	299	1.1271	.36255
Vulnerability of the Infant to infection	299	1.1204	.32598
Thriving of Infant	299	1.0870	.28224
Health Status	299	1.0797	.24013

Table 1 shows the descriptive statistics of the measured variables. The mean age of the postnatal mothers was 27.5926 with standard deviation (SD) of 5.81171, mean for the levels of health care institutions 1.9967 with SD of 0.81717; for marital status (MS) of the mothers, the mean was 1.0301 with SD of 0.17115, mean for educational level of the mothers 3.3344 with SD 0.60909, mean for parity of the mothers was 1.5886 with SD of 0.49291; mean for employment status of the mothers was 1.3746 with SD of 0.48483. Family type of the mothers had mean score of 1.0100 with SD of 0.09983; religion had mean score of 1.0100 with SD 0.09983, place of residence of the mothers had mean of 1.3378 with SD 0.47375. For time of commencement of breastfeeding the mean was 3.7331 with SD 0.70958; mean for exclusive breastfeeding (EBF) duration 2.6447 with SD 1.17179; mean for time of commencement of partial BF was 2.0415 with SD of 1.26423; breastfeeding frequency had mean of 3.6892 with SD of 0.71641; breast suckling duration had mean of 3.0602 with SD 0.94641; mean for additional food was 2.7522 with SD 1.44003; for breastfeeding patterns the mean was 2.6210 with SD 0.70112, mean for sex of the

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infants was 1.5619 with SD of 0.49699; birth weight of the infants had mean of 3.3274 with SD of 0.49365; mean of the present weights of the infants at time of data collection was 5.3719 with SD of 2.14391. weight-gain pattern of the infants had mean of 1.0556 with SD of 0.22946; mean height for the infants was 1.0470 with SD 0.22728; mean for the infants nutritional status 1.0635 with SD 0.24435; for infants' general body system the mean was 1.1271 with SD of 0.36255. For vulnerability of the infants to infection, the mean was 1.1204 with SD 0.32598; mean for thriving of the infants was 1.0870 with SD of 0.28224 while the health status of the infants had mean of 1.0797 with SD of 0.24013.

Table.2: Extent of practice of Exclusive breastfeeding (EBF) by postnatal mothers

Variable	EBF Duration	N	percent	Valid %
	.00	2	0.7	1.0
	One month	44	14.7	22.3
	Two months	38	12.7	19.3
Exclusive	Three months	51	17.1	25.9
Breastfeeding	4-6 months	62	20.7	31.5
(EBF)	Total	197	65.9	100.00
	Missing system	102	34.1	
	Total	299	100.00	

Table 2 shows that out of 197 postnatal mothers, 44(22.3 valid %) exclusively breastfed their infants for one month, 38(19.3 valid %) exclusively breastfed their infants for two months, 51(25.9 valid %) exclusively breastfed for three months while 62(31.5 valid %) breastfed their infants exclusively for 4 to 6 months.

Table.3: Frequency of breastfeeding by postnatal mothers

Variable	Breastfeeding frequency	N	%	Valid %
	Every 4 hours	9	3.0	3.0
	Every 2 hours	17	5.7	5.7
	On demand at day time only	31	10.4	10.5
Breastfeeding	On demand day and night	239	79.9	80.7
	Total	296	99.0	100.0
	Missing system	3	1.0	
	Total	299	100.0	

Above table 3 shows that out of 296 postnatal mothers, 9(3.0 valid %) breastfed their infants every 4 hours, 17(5.7 valid %) breastfed their infants every 2 hours, 31(10.5 valid %) breastfed their infants on demand at day time only, while 239(80.7 valid %) breastfed their infants on demand at both day and night.

Table.4: Duration of suckling by the infants of postnatal mothers

Duration of suckling	Frequency	Valid %
<10 minutes	22	7.4
11-15minutes	59	19.7
16-20 minutes	97	32.4
>20minutes	121	40.5
Total	299	100.0

Table 4 shows that out of 299 post natal mothers 22 (7.4%) indicated that their infants suckle the breast for less than 10 minutes during each feeding, the infants of 59 (19.7%) postnatal mothers suckle the breast for 11to 15 minutes; the duration of

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suckling for the infants of 97 (32.4%) mothers was 16- 20 minutes while the suckling period for the infants of 121 (40.5%) mothers was more than 20 minutes.

Table.5: Chi- Square test comparison of the breast feeding patterns of post natal mothers across the primary , secondary, and tertiary health institutions

Variable	Levels of Health Institutions	N	Mean Rank	df	X^2-cal	p-value	Level of significance
Breastfeeding pattern	Primary	100	153.05	2	2.681	0.262	0.05
	Secondary	100	138.87				
	Tertiary	99	158.16				
	Total	299					

In Table 5, the X^2 of 2.681 was more than the p-value of 0.262 at 0.05 level of significance. Therefore the null hypothesis is rejected. The breastfeeding patterns of post natal mothers differ significantly across the primary, secondary, and tertiary health care institutions

III. DISCUSSION

Finding from the study indicate that post natal mothers practice exclusive breast feeding (EBF) but most of them (133=67.5 valid%) did not extend the practice up to four to six months age of the infants as stipulated by WHO & UNICEF (2003) (table2). Some (22.3%) practiced EBF for only one month, some (19.3%) for two months, while 25.9% practiced EBF for three months. Studies have shown that mothers find it difficult to adhere to the expert recommendation for continued and exclusive breastfeeding (Whalen and Cramton, 2010). WHO (2003) reported that only 35% of infants worldwide were exclusively breastfed during the first six months of life. These reports as well as the findings of this study imply that EBF is ignored by most mothers.

Finding from the study indicate that few postnatal mothers had scheduled time of breastfeeding their infants. 3% fed their infants at 4 hourly intervals while 5.7% fed their infants on 2 hourly schedule, 10.5% fed their infants on demand at day time only, but majority (80.7%) breastfed their infants on demand day and night (table 3). This result is similar to the findings in some previous studies. Kurzewski and Gradner (2005) reported that majority of mothers feed their babies when they are hungry and/or crying (that is, demand feeding), few babies are fed on schedule, some babies are breastfed on 3 to 6 occasions per day, some mothers breastfeed more than 11 times per day; and that overnight, 68% of babies are fed less than four times, 25% breastfed five to six times and a few (5%) feed more than seven times. However, Subbiah and Jeganathan (2012) reported that some mothers said they avoid breastfeeding baby at night because it causes colic to the baby. It is important to note that whatever frequency of breastfeeding one adopts, experts (WHO, 2003; Pilliteri, 1999) have advised that infants should be breastfed on demand.

The result of the study showed that for majority of the postnatal mothers (40:5%), their infants suckled the breast for more than 20 minutes while in few mothers (7.4%), the duration of suckling for their infants was <10 minutes (table 4). Pilliteri (1999) stated that often, newborns that are being breastfed drop off to sleep during the first few feedings, and that mothers should stimulate and awake such babies by stroking the baby's back, tickling the bottom of the baby's foot or by changing the baby's position during feeding. kurzewski and Gardner (2005) in their study found out that the duration of suckling was mostly 16-20 minutes, less than 10 minutes for some, 11 to 15 minutes, and more than 20 minutes for some infants. Suckling promote milk production and milk flow. According to Pilliteri (1999) the primary method for relieving engorgement is emptying the breasts of milk by having the infants suck more than previously, or at least continuing to suck as much as before. Research suggests that nipple soreness is not related to the length of time the infants is at the breast but to improper positioning or improper removal of the infant from the breast (Littleton and Engebretson, 2007)

Finally, findings from the study indicate that the breastfeeding patterns of the postnatal mothers differ significantly (X^2=2.681; p-value = 0.262) across the primary, secondary and tertiary health care institutions (table 5). Previous studies similarly have shown that hospital practices such as early breastfeeding initiation, infant rooming-in and providing breastfeeding only have helped to improve breastfeeding initiation, duration and exclusivity (Dennis, 2002; Murray, Rickketts & Dellaport, 2007; Dieteritch et al, 2013). Nkala & Msuya (2011) have

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also noted that health system practices influence breastfeeding in different areas in developing countries. Also it is noteworthy that primary, secondary and tertiary health care institutions differ in their scope of responsibilities. The primary level focuses on preventive care while the secondary and tertiary levels focus on diagnoses and referrals. These differences in the scope of functions could contribute to the significant difference in the breastfeeding patterns of the postnatal mothers across the three levels of health care institutions.

IV. CONCLUSIONS

The study indicate shortfall in the duration of EBF, high rate of demand feeding at both day and night as well as significant difference in the breastfeeding patterns of postnatal mothers across the primary, secondary and tertiary health institutions.

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Isolation and Identification of Fungi from fast food restaurants in Langa Bazar

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Abstract**—** *A total of (218) samples from Eleven different foods were processed between October 2016 and February 2017 which include (Tomato, Chicken meat, red meat, falafel, potato, bread, eggplant, cabbage, celery, cucumber and onion). Samples were collected from 4 different fast food restaurants inoculated on Potato dextrose agar and Sabouraud Dextrose Agar. Isolated fungus identified morphologically and microscopically in accordance with standard procedures. Results showed that six fungal genera were associated with the selected fast food restaurants. The isolated fungal genera were Aspergillus sp., Alternaria sp., Mucor sp., Rhizopus sp., Saccharomyces sp., Brettanomyces sp. The number of total colonies in October were 236 and in February were 119 and the number of colonies were higher when cultured on Potato dextrose agar than Sabouraud Dextrose Agar. There was variation in the pattern of occurrence of the fungus in fast foods Aspergillus sp. appears to be the most pathogenic fungi that present in the food samples.*

Keywords— Fungus, Fast food restaurants, Food spoilage.

I. INTRODUCTION

Food spoilage is any change in the appearance, smell, or taste of a food product that makes it unacceptable to the consumer. Spoiled food may still be safe to eat, but is generally regarded as unpalatable and will not be purchased or readily consumed. Food spoilage causes losses to producers, distributors, and consumers in the form of reduced quality and quantity and higher prices (1). Another case of spoilage could be that the nutrients (e.g. vitamin content) in the food have deteriorated to the point that the food product no longer meets its declared nutritional value. The time it takes for a food product to reach one of these spoilage conditions is generally termed the product's shelf-life potential food spoilage microorganisms include bacteria, fungi (mold and yeast), viruses, and parasites.(2).

Action by microorganisms is a common means of food spoilage and the most common cause of foodborne illness. Microbial spoilage is a major concern for so-called perishable foods such as fresh fruits, vegetables, meats, poultry, fish, bakery products, milk, and juices. Meat and dairy products, with their high nutritional value and the presence of easily metabolized carbohydrates, fats, and proteins provide ideal environments for microbial spoilage (3).

A wide variety of fungi, including species of *Rhizopus*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Botrytis* spoil foods. Since fungi grow readily in acidic as well as low-moisture environments, fruits and breads are more likely to be spoiled by fungi than by bacteria. *AspergillusFlavus* infects peanuts and other grains, producing aflatoxin, a potent carcinogen monitored by the food and drug Administration (4).

The factors that affect microbial growth in food and constantly the association that develop also determine the nature of spoilage and any health risks posed for convenience they can be divided into four groups Intrinsic factors Intrinsic factors of a food include nutrients, growth factors, and inhibitors or (antimicrobials), water activity, pH, and oxidation–reduction potential. The influence of each factor on growth in a food system the factors are present together and exert effects on microbial growth in combination, either favorably or adversely (5,6). , Extrinsic factors Which include environmental factors like temperature, Relative humidity and gases, Implicate factors Include Specific growth, Synergism, Antagonism and commensalism competition among the microorganism that cause changes of physical and chemical structure of the food and spoiled the food (7,8,9).and Processing factors Include the tools with slaughtering and cutting the water which use in washing, vehicles which are used in transporting, and retail market (8).

Food-borne illness by fungi:

A food-borne illness occurs when a person becomes ill after eating or drinking contaminated foods or beverages. Nothing that we eat or drink is completely pure, and many

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microorganisms live in the foods and beverages that we consume. Microorganisms or microbes are tiny organisms such as bacteria, Fungi, viruses, and parasites that exist in our bodies as well as in plants, animals, food, water, air, and soil. Many of these microbes do us no harm, and some "friendly" ones are necessary for a healthy body, but other microbes can cause infections that lead to serious, or even fatal, illness. Additionally, there are harmful chemicals and other substances in food that can cause a food-borne illness (10).

Fungi include mold and yeasts which are more adaptable to various conditions than other microorganisms. Have high tolerance for acidic condition and more often responsible for food spoilage than for food-borne illness (11).

II. MATERIAL AND METHODS

Media preparation:

Two media can be used, Potato Dextrose Agar (PDA) and Sabourated Dextrose Agar (SDA). They were prepared according to the manufacturer's instructions 39gm (PDA) powder in 1 L of distilled water for PDA medium. And 62gm (SDA) powder in 1L distilled water for SDA medium , then sterilized and used for fungal cultivation.

Collection of samples and Isolation:

A total of 218 sample from 11 different foods were obtained from 4 restaurant three times a day respectively (9:30 AM, 12:00 PM, 4:30 PM) during the months of October 2016- February 2017. The food samples were taken from restaurant in sterile plastic bags according to Cheesbrough 1984 Direct plate used for culturing the Individual yeast and mold species (figure 2.1) The samples transferred with sterile forceps into Petri dish contain sterilized SDA Saboured dextrose agar and PDA Potato Dextrose Agar and the plates were incubated at 25°C for 5-7 days(12)

Fig. Sampling and Culturing of fungi on SDA and PDA.

Identification:

Fungal isolates were transferred to sterilized plates for purification and identification. The grown fungi were placed on a slide, stained with gram stain for yeast identification and lacto phenol cotton blue to detect fungal structures covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (12).

Macroscopic and microscopic observations were carried out on the cultures. The physical characteristics of the mycelia such as the colour and structure were noted as well as the microscopic characteristics (13,14).

III. RESULTS

3.1.Variation of colony numbers in Fungus during October:

After collecting the samples and culturing we obtained the colonies and after examining the colony under microscope we obtained some fungal genera that associated with food spoilage. Figure 3.1.showspercentage of colonies isolated from fast food restaurants during October (22/10/2016).

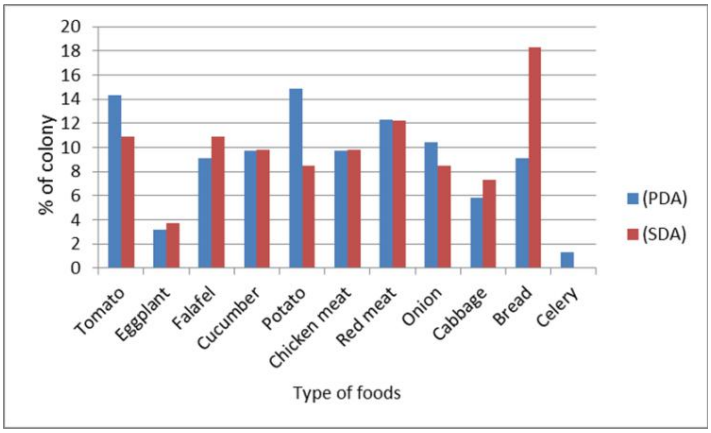

Fig.3.1: Percentage of total colony isolated in food during October in (PDA, SDA).

3.1.2. Variation of colony of Fungus number during February:

The number of colony decreased when sample collected and examined by cultivation to obtain colony.figure 3.2 shows the percentage of colonies isolated from fast food restaurant during February (14/2/2017).

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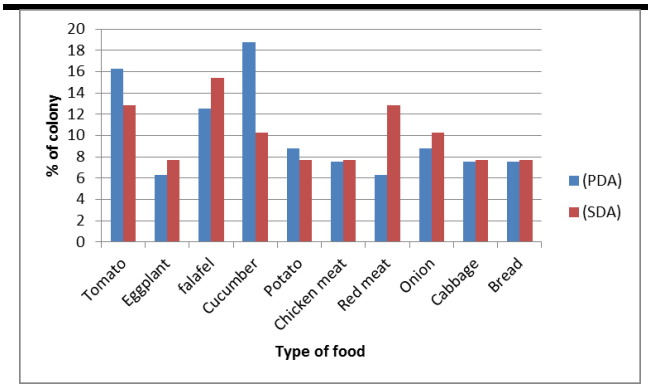

Fig: Percentage of total colony isolated in food during February in (PDA, SDA) medium

3.2. Detection of Fungus In different foods:

From a total of (218) collected samples and 11 different foods from different fast food restaurants a total of 6 genera (mold and yeast)
were isolated as shown in the table 3.1

Table.3.1: Occurrence of fungal genera in different food sample.

No.	Food type	Fungal genera
1	Tomato	Alternaria sp.
2	Eggplant	Mucorsp.
3	Falafel	Saccharomyces sp.
4	Cucumber	Aspergillus sp.
5	Potato	Mucor sp.
6	Chicken meat	Aspergillus sp.
7	Red meat	Aspergillus sp.
8	Onion	Aspergillussp.
9	Cabbage	Brettanomycessp.
10	Bread	Rhizopus sp.
11	Celery	Alternariasp.

The major problem associated with fast food is the frequent incidence of contamination .Due to the nature of these foods and their methods of preparation involving extensive handling. The results of the present study revealed that different molds were isolated from 11 different food samples namely eggplant, falafel, chicken meat, red meat, celery, cabbage, potato, tomato, bread and cucumber collected from four locations fast food restaurant in Langa Bazar. The isolated molds were identified as food-borne. The molds include *Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Rhizopus* sp. and *Saccharomyces* sp. and *Brettanomyces* sp. Several studies have reported similar molds from different snacks and fast food contamination from water, air, storage distribution facilities, environment and human activities researchers (15,16,17) .Yassin and his coworkers isolated same fungal strains from restaurant and meat shops in al Samawa (18).

3.3. Cultural and Microscopic properties of Fungal isolates:

Microscopically examination for isolates from Cucumber, Red meat, Chicken meat and onion showed that Conidiophores and septate, unbranched hyphae. With a swollen apex (vesicle) .Phialides born on the metulae (biseriate). Conidia in dry chains forming compact columns (columnar) that can be identified as *Aspergillus* sp. Colonies on PDA agar at 25ºC within 7 days, and consisting of a dense felt of green conidiophores. (Figure 3.3)

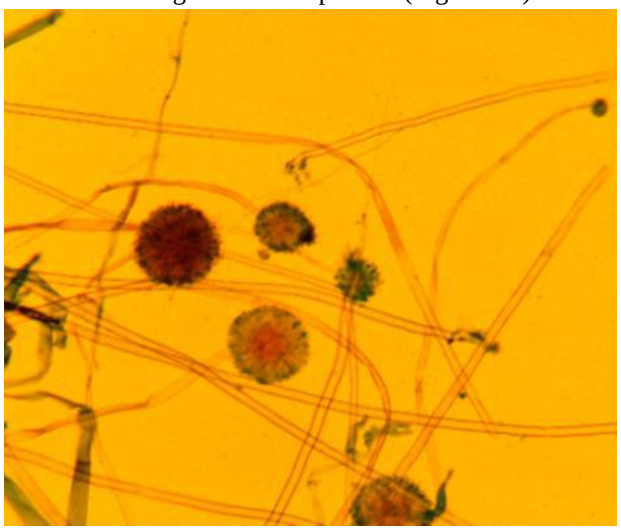

Fig.3.3::Microscopic examination of Aspergillus sp.

Microscopically examination for isolates from falafel .showed that cells quite large, globules to sub globes, reproduce by budding as showed in figure 3.4.and the colonies on PDA agar at 25 ºC within 7 days, and whitish mucous texture that can identified as Saccharomyces sp.

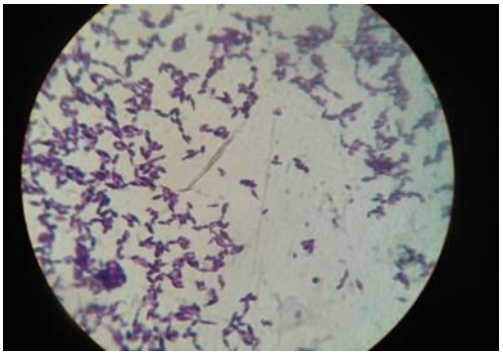

Fig.3.4: Microscopic examination of Saccharomyces sp.

Isolates from cabbage under microscope appears that the cells are olive to cylindrical shaped and Colonies on PDA agar at 25 ºC appear as white small soft cotton textures as

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showed in figure 3.5. and the isolates identified as *Brettanomyces* sp.

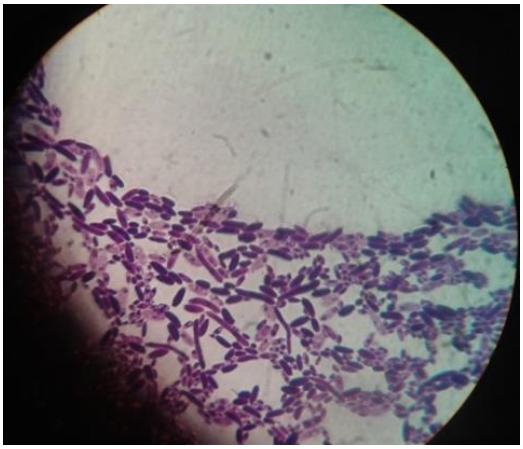

Fig.3.6: Shows microscopic examination of Brettanomyces sp.

Microscopic features that was used to help in identification of isolates from Bread showed that sporangiospores, sub globes, and irregular in shape with Apophysis and having stolone and Rhizoids and colonies on PDA, was whitish becoming grayish-brown. Figure 3.6. And can be identified as Rhizopus sp.

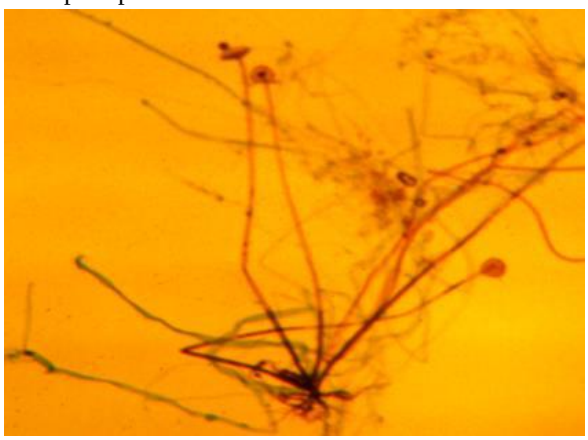

Fig.3.5: Microscopic examination of Rhizopus sp.

When isolate from Eggplant and Potato examined under microscope hayphae was non septate, sporangiophores simple at first, later slightly sympodially branched, with sporangium at terminal ends without apophysis and colonies on SDA at 25^{o}C, resemble white-to-gray cotton candy, darkening with time. Fegure 3.7. The isolates can be identified as *Mucor* sp.

Fig.3.7: Microscopic examination of Mucorsp

Microscopically appearance of isolates from tomato and celery showed that septate branched pale brown to olive brown with secondary conidiophores, conidia in long branched chains. Colonies on PDA at 25^{o}C for 7 days, olivaceous-black or greenish black surrounded with white cotton hyphae and is identified as*Alternaria* sp. (figure 3.8.).

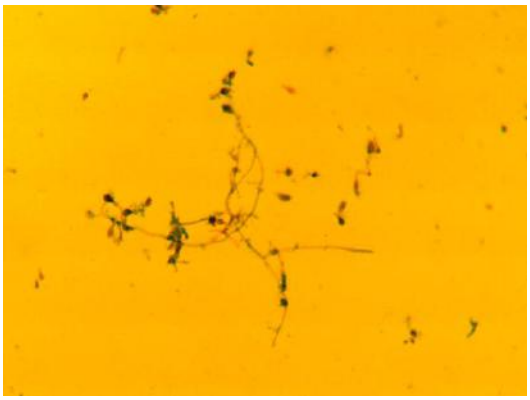

Fig.3.8: Microscopic examination of Alternaria sp.

As showed in figure 3.9: There was no growth of fungus on plate contain PDA that incubate in 25 ^{o}C for 7 days (used as control).

Fig.3.9: PDA medium used as control.

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After collecting the samples and after subculturing them on PDA and SDA the number of colonies that obtained were (96) colonies at 9:30 AM (81) colonies at 12:00 PM and at 4:30 PM (59) colonies as showed in table 3.1 the number of colonies at 9:30 AM were more than other times (12:00 PM and at 4:30 PM) the reason for this result is using previously made foods that is not stored properly at 9:30 AM and using of newly made foods in the noon and after noon.

The number of total colonies in October were 236 and in February were 119 and this return to the fact that the tempreature in month October is higher than in month february and so making and storing the foods in high tempreature and in an inappropriate condition that help to increase number of fungal colony during October.

The number of colonies were higher when cultured on PDA than SDA in spiteof using antibiotic in both PDA and SDA to prevent the growth of bacteria and other microrganism the reason for the result may be due to that PDA is a general purpose media for molds and yeasts. Potato dextrose agar (PDA) may be slightly superior to Sabouraud dextrose agar (SDA) for growing molds since SDA was originally formulated to detect fungi associated with skin infection (19).

Aspergillus sp. was the most spoilage fungi isolated from most type of foods and this result are resemble to the experiments that done by Easa 2010 which isolated different species of *Aspergillus* from traditional fast foods (20)

As it appear in our result we obtained the growth of different fungi and this may be due to not wearing special fastfood uniform during woking or not using appropriate methods and means to keep the equipments and the surrounding clean also another cause is food processing was in an open place where it is close to sidewalk pedestrians and cars. Also it may be due to cutting the vegitables and meats on the same cutting board or wrapping a broken knife handle with plastic covers and using of expired (sauce, mayonnaise, amba, ketchup).

IV. CONCLUSIONS

Several types of fungi were isolated from fast food restaurant which cause food spoilage. The number of colonies isolated from fast food restaurants was higher during October because of increase of temperature and number of shoppers. Some food samples showed high number of Colonies during early morning. The result showed there are some pathogenic fungi present in fast food restaurants like (Aspergillus sp., Alternaria sp., Mucor sp., Rhizopus sp., Saccharomyces sp., Brettanomyces sp.) and the number of colonies on PDA was more than SDA.Our suggestion for fast food restaurant isto keep the foods away from contamination and spoilage and this will be by commitment with health rules that deal with restaurants cleanly and proceeding regular tests for restaurants workers and to Study presence or absence t of mycotoxin in the food products.

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Status of large mammals: case study of gorilla (*Gorilla gorilla diehi*), chimpanzee (*Pan troglodytes ellioti*) and buffalo (*Syncerus caffer*), Menchum South, NW Cameroon

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***Abstract*—** *This study entitled status of large mammals: case study of gorilla, chimpanzee and buffalo, was carried out in the Black Bush Area of Waindow (BBAW), Menchum South, NW Cameroon from January-March, 2014. The general objective was to determine the status of large mammal's species and to investigate the presence of gorilla, chimpanzees and buffalo reported by the indigenous people in order to contribute to the conservation of these species in the region. The recce-survey method was used for species inventory whereby direct and indirect observations of bio-indicators of these species and human signs were recorded. From the result, the encounter rates of buffalo and chimpanzee were 0.35 and 0.26 signs per km respectively with no gorilla sign observed. Spatial distribution maps revealed great concentration of these species in the northern zone. Hunting recorded the highest encounter rate of 0.42 signs per km of anthropogenic activities. As such, one could deduce that the pressures exacerbated on these animals due to human activities and encroachment by Nigeria traders, and grazers placed the remaining species under intense threat of disappearing within the study areas. It is therefore necessary to intensify conservation efforts so as to urgently address these species concerns.*

***Keywords*—** *status, conservation, Gorilla, chimpanzee, buffalo, BBAW.*

I. INTRODUCTION

Despite the global increase in the number of protected areas, it is greatly remarked that biological values are not delimited by protected areas since all species do not have the same habitat requirements as well as do not have the same range boundaries (Milligan, 2009). As such, Non-protected areas retain value and are therefore of prime importance for wildlife conservation activities due to the presence of large charismatic mammal species such as gorilla, chimpanzee, buffalo and their role in the connectivity between protected areas (Bennett, 1998). Moreover, despite the impressive commitment to conserve biodiversity, many large mammal species in protected areas are constantly declining due to the conversion of wildlife friendly habitat for agriculture (Jones et al., 2007 and Newmark, 2008), as well as the irreversible disappearance of non protected areas that act as connective corridors to protected areas (Dobson et al., 1999). To this effect the sustainable management of large mammals outside protected areas should therefore be considered as an aspect for wildlife conservation (Halladay and Gilmour, 1995). A raft of studies is showing that Africa is losing species from many national parks despite the fortification of biodiversity conservation (Western et al., 2009). The underlying causes are due to deficiencies in boundary design, loss of many connective corridors, inadequate protection and ecological management of large mammals in non protected areas (Western et al., 2009).

Cameroon is part of the Congo Basin and harbours a wide range of biological resources. It is the fourth most biodiversified country in Africa after the Democratic Republic of Congo, Tanzania and Madagascar (UNDP, 2001). With 409 mammal species, of which 14 are endemic; 165 reptile species; 916 bird species, of which 8 are endemic while about 150 are migratory; 9000 plant species, of which 156 are endemic; 200 amphibian species, of which 63 are endemic; and about 1500 Butterfly species (UNDP, 2001). This have boast the domain of wildlife conservation in which Cameroon has developed a network of protected areas which covers a surface area of about 8138800 hectares and 17 National Parks, all of which covers about 19.2% of the national territory. Other protected areas are grouped into the following categories; 6 wildlife reserves, 1

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wildlife sanctuary, 3 Zoological Gardens, 46 hunting concessions and 22 community hunting zones (MINFOF, 2010). However, this success seems to have had a limited impact in terms of countering the decline in biodiversity. Indeed, most of these species are considered threatened with high extinction rate. This problem is further exacerbated by the fact that most large mammals species lives outside protected areas. For example, recent data shows that gorillas, chimpanzees and buffalos are more populous in hunting zones and some concessions than in protected areas (MINFOF, 2010). This highlights the need to recognize non protected areas in which these species are found. Although it is generally assumed that protected areas offers the best type of legal protection for wildlife conservations, the implication is that in countries which lack financial resources and infrastructures, large mammals species will be inadequately protected in protected areas thus the need to protect them even more in non protected areas (William et al., 1990).

For instance, Law no 94/01 of 20 January 1994 (also refer as Forestry code), to lay down forestry, wildlife and fishery regulations and its subsequent Implementation Decree. Section 11 of the law stipulates that: "the genetic resources of the national heritage shall belong to the State of Cameroon". No person shall use them for scientific, commercial or cultural purposes without prior authorization". This is the case of the non protected area of Waindow, in which the profitable exploitation of gorillas, chimpanzees and buffalos by local residents is considered as a valuable option to reconcile development and to improve standard of living without taking into consideration the sustainable management of these species. Thus a greater call for concern in which the sustainable use of these species within the study area should be considered as a vital issue for conservation objectives. However, the application of such a concept in communal lands outside protected areas has to meet two main requirements to ensure a sustainable implementation. First, it must rely on the support of local communities, through their active involvement in wildlife management operations and hence decision making (Hulme and Taylor, 2000). Second, it requires precise and regular information on wildlife abundance and trends to ensure that management schemes are adaptive and allow for a sustainable use of wildlife populations (Kremen et al., 1994).

The main problem that calls the attention of this research is decrease in the population of large mammal's species in the BBAW. This decrease is driven by habitat loss resulting from human activities such as; illegal logging, poor agricultural practices, encroachment in critical corridors, poaching due to high demand for bush meat, traditional medicines, for festivals and rituals couples with poor governance (Tsi and Chuo, 2016). Habitat loss in this area is cause by habitat fragmentation and degradation resulting from poor agricultural practices such as forest fires outbreaks due to slash-and-burn agriculture. Logging companies are almost absent in the region but wood for house construction and for artisanal wood processing is rampant as local engine saws are often used to cut down trees (Picture 16: Appendix 3). These pressures are further exacerbated due to encroachment of grazers from Nigeria and from other parts of the country who still carried out transhumant and at times cut down certain trees species to feed their cattle especially during draught (Chuo, 2014). Equally the destruction of many biases especially the hot spring at Itiaku by cattle is a serious threat to connective routes of large mammals species in this area.

Poaching remains a principal threat to various large mammals' populations in this area. The availability of many short guns of one to five ranks, the present of many different kinds of cartridges for instant cartridge 66, 32g (Picture 9: Appendix 3) used to kill duikers, monkeys and other small mammals, cartridge BB, 34g (Picture 10: Appendix 3) used to kill bush buck, olive baboons and other medium size mammal, and cartridge 99, 36g (Picture 11: Appendix 3) specialized to kill leopard, hyena, gorillas, chimpanzees, buffalos and other large mammals (Chuo, 2014). Equally the high availability of snare wires arranged in inches of 3, 6-8, and 15-20 (Picture 12, 13 and 14: Appendix 3) to trap animals of sizes duikers, bush buck and buffalo respectively sold at relatively cheap prices through black marked business in the area have intensify hunting. The presence of many domestic dogs greatly encourages group hunting especially during the dry seasons. The wide use of "gamaline and arata bomb" through food substances to poison these large mammals is a serious threat (Chuo, 2014). In these areas, large mammals such as gorillas, chimpanzees and buffalos are highly demanded for their hands, legs, heads, skulls, skins and/or the whole animals for use during traditional rituals and festivals. They are equally hunted for food, medicinal purposes and commerce. Primate capture either for pets (Picture 8: Appendix 3) or kept in preparation for sacrifices to shrine is a predominant aspect highly practice by the natives of these areas (Tsi and Chuo, 2016). The relative isolation of this area, inaccessibility and indigenous brutality, has led to poor governance making it difficult to provide information on the abundance, distribution and threats to large mammals population. This serious weakness has given free liberty for poachers to do as they want thus the continued decline of large mammal species in this area.

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II. MATERIAL AND METHODS

2.1. The location of the study area

The Black bush area of Waindow is located between latitude 6° N and 7° N and longitude 9°E and 10°E and is situated in Menchum South Constituency, North West Region of Cameroon. It has an altitude of about 900m to 2140m above sea level in the mountains and about 200m to 600m in the valleys and a surface area of 97,667 ha. It is situated toward the western boundary of the region which stretches along the international border between Cameroon and eastern Nigeria. The main rivers that flow through this area are the rivers Ivin, Menchum, and Kimbi. All of these join the Kasina-la, which flows into Kasina-la State, Nigeria. The figure 1 shows the map allocation of the BBAW in Menchum Division, North West Region of Cameroon.

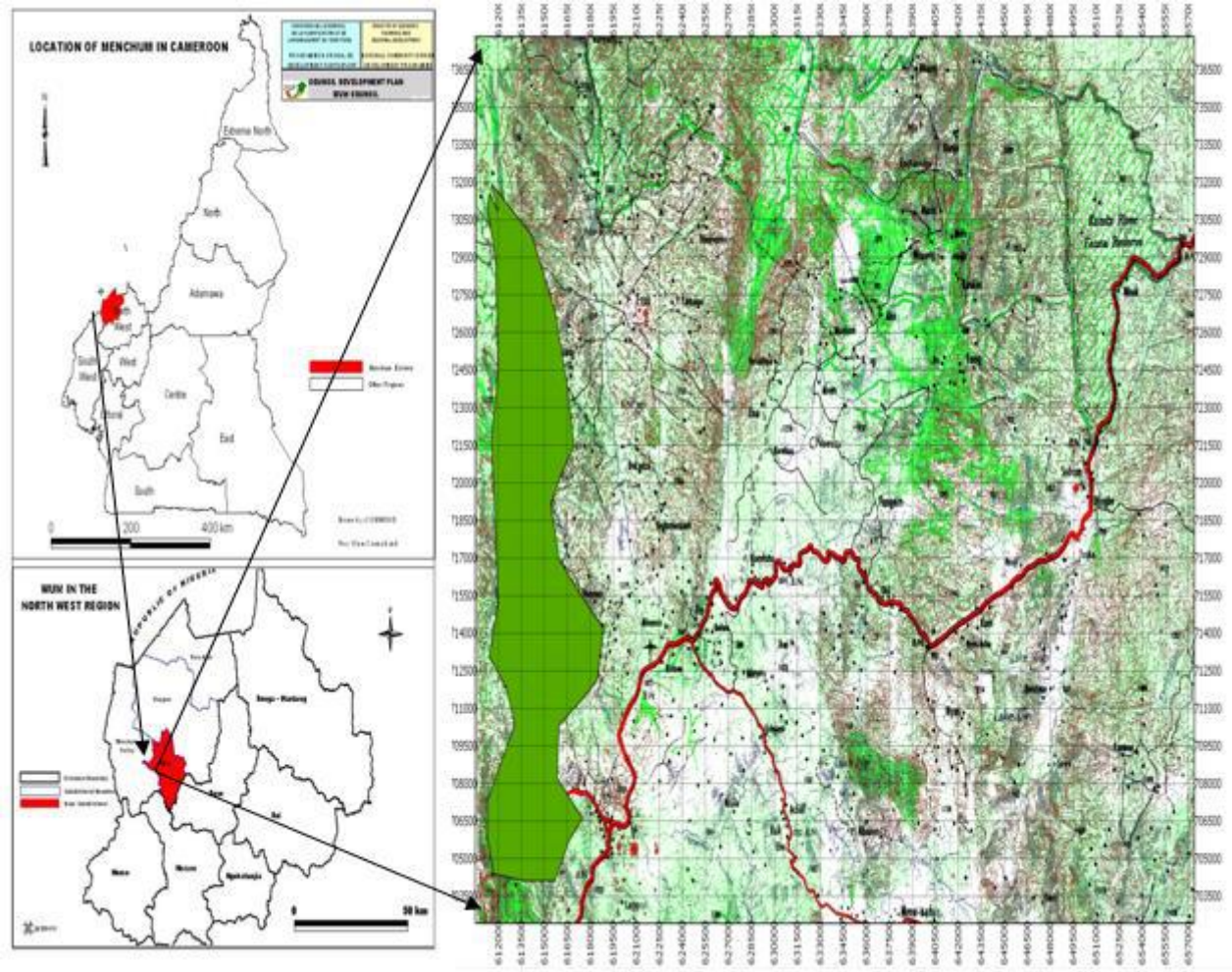

Fig.1: Map of Cameroon showing location of the BBAW in Menchum Division

This area constitutes a significant portion of the Bamenda highlands montane forests. It harbours important large mammal species such as Gorilla, Chimpanzee, Buffalo, Olive baboon, Drills, Putty-nosed monkey, White monkey, Mona monkey, Red eared guenon, Bush pig, Bush buck, Duikers, Leopard, Hyena, Hippopotamus and others some of which are classified by IUCN Red data book list as threatened species and by the Cameroon Wildlife law as critical endangered species (MINFOF, 20010). This area possessed wide variety of birds' species most of which are highly endanger in the IUCN red list of endangered species. Among these birds species a few of them include Banner manna's turaco, pied crow, gray headed sparrow, swallow, collared sunbird, spectacle weaver, owls, hawk, barn owl, osprey, scaly francolin, giant king fisher, and martial eagle (COMINSUD, 2011), which are clear indicators proving the high biodiversity richness of the study area. This area equally contains trees species such as Sapelli, Iroko, Obeche, Pygeum, and Mahogany. Some important plant species such as *Ficus spp* are harvested and used in traditional medicine especially by those who do not have access to modern health facilities. BBAW also provide many non timber products such as very rich honey, djansang, bush mango,

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biter cola, bush pepper, fire wood, charcoal and other which are of great benefit to the local community (COMINSUD, 2011).

2.2. Data collection

Data collection to estimate the abundance and distribution of large mammal's species with respect to human activities was undertaken using the recce survey method (White and Edward, 2000). The zone was subdivided into quadrates of 1.5 km x 1.5km. Inside each sampled quadrate, a recce of 1500m long was established. Thirty eight (38) recces of 1.5km long oriented in a random manner were walked within the study area making a total effort of 57km recce as shown on the sampling plan (Figure 2: Appendix 1). Recces walk, cross major drainage features (shrub savannah', 'open low shrubs' and 'open to closed woody vegetation or thickets') in order to sample a representative proportion of all vegetation types. The exact positions of signs observed in the field were determined by the use of a GPS GARMIN 10 *Euon*. A digital camera was used to take photographs of animal and human signs while and a Binocular of mark Nikon were use to observe or view animal away from the recce.

Recces were walk by a team of five persons, consisting of a leader, observers, one field assistants, one laborer and one local hunter. The leader was responsible for reading the bearing, searching for animal signs and recording data. The observer focused on the ground, in search for signs such as dung, foot prints and tracks as well as looking upward for detecting direct observation of animals and other signs (such as nests). The hunter was responsible for identifying tracks and dung of different animal species in cases where identification was difficult. The field assistant remains on the correct compass bearing check for terrestrial signs of animal presence and concentrates attention in the trees, looking for nests and primates while the labourer was responsible for opening the bush ahead along the compass bearing with the help of a machete. Data on all large mammal sightings, vocalizations and signs (dung, nest, tracks, carcasses, furs, footprints and food remains) were recorded on a data collecting sheet. All human signs, village sites (used or disused), cutlass cut, regularly used human trails, honey extraction, snare line (active or abandoned), gun shots, camp sites (active or abandoned), fire places, current or past agricultural activity, bark striping for medicine, sites where nuts have been cracked open, used batteries, shotgun shells, cigarette packets, hunting, fishing, logging, and fruits gathering along transect were also recorded on a data collecting sheet.

2.3. Data analysis

Field data sheets were decoded and information entered into Microsoft excel. The observations were grouped according to the different mammal species and type of anthropogenic activity. Relative densities were calculated manually since the number of indices encountered did not attain 60 for all species to use DISTANCE programme. The Encounter rate (ER) = Total number of objects or signs observed divided by the length (L) of transect (in kilometer). **ER= N /L**

Where: **N =** Number of objects/signs observed

Lt = Length of recce (Km)

This permitted us to estimate the relative abundance of animal population and signs of anthropogenic activities. The GPS points of chimpanzees, buffalos and other large mammal's species indicators and human activities recorded per quadrant were exported to Arc View computer program 3.3 and geo-referenced to produce different spatial distribution maps. The classes of encounter rate were then defined in order to group similar quadrates and represent zones of different concentrations. Different color bands and corresponding color intensities were used to represent different encounter rates on the distribution maps. This permitted the definition of important zones for mammal's species like (chimpanzee, buffalo) in order to determine management strategies for their conservation. Regression analyses were carried out to test the relationship between the encounter rate of large mammals and anthropogenic activities. Encounter rates of these two variables were exported to SPSS to produce fitted Regression line.

III. RESULTS

3.1. Relative abundance and spatial distribution of species

Following recce-surveys method, 14 large mammals' species were recorded in the study site resulting from indirect observations (dung, voices, and nest) and direct sighting (Table 1: Appendix 2). The family of cercopithecinae has more species richness (*Cercopithecus nictitans Cercopithecus aethiops, Erythrobus patas*, *Papio Anubis*, and *Cercopithecus mona)* follow by the family of Bovidae with four different species (*Cephalophus monticola, Tragelaphus scriptus Syncerus caffer, and Cephalophus dorsalis*). This result contrasts those of (Wum council, 2012) in the Wum Municipality, North West Region –Cameroon in which 29 large mammal species were reported to inhabit this area.

3.1.1. The indices of species identify in the BBAW

Walking along human and buffalo trails, many indices were indirectly or directly identify. For the 14 large mammals' species observed in the study area, sums of

138 indirect signs were identified and 31 large mammals were directly observed (Table 2: Appendix 2). Generally, large mammal species were identified through eight (8) biological indices (dung, foot prints, tracks, food remains, resting or nursing sites, carcass, nest and voices). Thirty one (31) large mammals were seen directly either single or in group of 2 to 8. The most observable biological indices were dung (34) followed by footprints (32) and then tracks (30). For indices registered by individual large mammal species, buffalos had the highest field observed indices (40) followed by blue duikers (22) and chimpanzees (20) (Table 2: Appendix 2).

3.1.2. Encountered rate of sighted of species in the BBAW

The sighting of gorilla, chimpanzee and buffalo was carried out along side other large mammal's species in which a total of 31 animals were counted from 8 sighted different species and group into 2 main families *Bovidae* and *Cercopithecinae* (Table 3: Appendix 2). The Vervet monkey had the highest encounter rate of 0.14 sighting per km whereas Buffalo and Blue Duiker had the least encounter rate of 0.04 sighting per km. No direct observation of Gorilla or Chimpanzee was recorded. From the results, two groups of placenta mammals, the herbivorous placenta mammals and tree climber placenta mammals were identified (Table 3: Appendix 2). This result contrasts those of (Fonkwo et al., 2011) in the Bakosi landscape area South West Region of Cameroon who sighted 5 species, three of which were herbivores and two primate species.

3.1.3. Encountered rate of indirect signs of the species in the BBAW

A total of fourteen (14) species of large mammals signs were recorded belonging to eight (8) families (Table 4: Appendix 2). In terms of species richness, the family of Cercopithecinae recorded the highest species number of five (5) species follow by the family of Bovidae with four (4) species. Result stipulate that buffalos have the highest relative abundance with an encountered rate of 0.35 signs per km follow by chimpanzees with an encountered rate 0.26 signs per km. No Gorilla *(Gorilla gorilla)* sign were observed. Blue Duiker *(Cephalophus monticola)*, Putty-nosed monkeys (*Cercopithecus nictitans)* and Leopard *(Pamthera pardus)* were the least abundant species with each having an encounter rate of 0.02 signs per km. The overall mean encounter rate of indirect indices of these species was estimated to be 0.2 signs per km (Table 3: Appendix 2). Comparing the overall Relative Density of these species (0.2 signs per km) with that of (Nkemnyi et al., 2012) 0.64 signs per km, indicate that, one would identify less than one large mammal signs for every kilometer walked in the study area. According to the result, the study area have high number of species richness fourteen (14) grouped into eight (8) families as compare to that (PSMNR-SWP, 2008) in the Nguti Council Forest, South West Province –Cameroon who registered eight (8) species grouped four (4) families.

3.1.4. The Spatial Distribution of Chimpanzee and Buffalos Species

Generally, habitat requirements such as river courses, salt licks, vegetation, resting sites and less anthropogenic activities are some of the factors which affect the abundance of species distribution in an area (Tsi et al., 2006). The spatial distribution map of Chimpanzee species, which is the only species found in the family of Pongidea whose signs were identified due to the absence of gorilla signs, shows that the relative density of Chimpanzees are high in the Northern section ($0.41 < ER < 0.60$) and average in the northwest of the study area ($0.21 < ER < 0.40$). Low relative density of Chimpanzees ($0.01 < ER < 0.20$) were equally observed in the northwest and southeast zones of the study area (Figure 3: Appendix 1). Equally, the relative density of Buffalos was high in the northwest, northeast and southeast sections of the study area ($0.11 < ER < 0.20$). Low relative density of Buffalos ($0.01 < ER < 0.10$) were equally observed in the northwest and southeast of the study area (Figure 4: Appendix 1).

3.2. Encounter rate and spatial distribution of anthropogenic

The continuous increase in population follow by poverty increment, have led to the excessive exploitation of forest and non-timber products such as forest trees for timber and household items, hunting for bush meat, rituals and festivals and land for crops cultivation and animal rearing. As such the harvesting of these natural resources leaves signs which were recorded to assess the impact of anthropogenic activities in relation to large mammals in the BBAW. All signs of anthropogenic activities recorded in the study area were grouped into six main activities in a decreasing order: all activities associated to hunting (wire traps, hunting camps, hunters tracks, hunting stones and gun shells cartridge) registered the highest encountered rate of 0.42 signs per km. Follow by faming activities (farmer's camps, corn farm, cocoa farm, cocoyam farm, groundnuts farm, palm tree plantation) with an encountered rate of 0.25. Grazing, logging, bush fire and construction recorded 0.12, 0.09, 0.07 and 0.05 signs per km respectively (Figure 5: Appendix 1). Similar results were obtained by (Fonkwo et al., 2011) in the Muanenguba proposed Integral Ecological Reserve where hunting signs were the most frequent. The analysis

suggests a total mean encounter rate of 0.24 signs per km (that is about one signs per km). This result contrasts those of (Nkemnyi, 2011) in the Lebialem-Mone Forest Landscape, Cameroon, where a mean encounter rate was above 0.5 signs per km of all human activities recorded.

Generally, anthropogenic activities were reducing as observers move toward the northern directions of the BBAW with a corresponding increase in large mammals sighting. That is human activities in the study area, were generally decreasing from south to north as large mammals sightings abundance increase. Equally recce with the fewest large mammals signs have the greatest recorded human activity. The signs of anthropogenic activities were high in the southern section of the study area ($0.31 < ER < 0.40$), few in the northwest ($0.11 < ER < 0.20$) and equally low in some areas of the Northern and southern ($0.01 < ER < 0.10$) sections of the study area (Figure 6: Appendix 2).

3.3. Effects of anthropogenic activities on the distribution of species

In order to show the effect of anthropogenic activities on the distribution of large mammals, using the encounter rates of large mammals' species and human activities, the coefficient of determination R^2 was calculated. Result shows that there is a significant relationship ($R^2 = 0.773$) between the encounter rate of large mammals and anthropogenic activities with (F (d.f. = 1, 4) =13.640, P = 0.012). This means that, the effect of anthropogenic activities on the distribution of large mammal's species in the study site is high since 77.3% of variation in the encounter rate of large mammals was provoked by anthropogenic activities. Reason suggested for this may be increases in hunting techniques, a shift from food crops to cash crops farming and population growth.

IV. DISCUSION

All the large mammals' species that were encountered in the study site resulting from indirect observations (dung, voices, and nest) and direct sighting following recce-surveys method were classified into seven different families. Base on the family observation, it is evidence of it diversified habitat, the availability of different food preferences which enable each species to maintain a particular ecological niche. The low number or absence of other mammal's signs could be that these mammals scarcely visit these areas. Signs may have been degraded or wash away by late rains or destroyed by other animal movement due to seasonal variation. Absence of other animal's carcass could mean no germ or disease attacked them or they were not attacked by carnivores. The presence of thick vegetation probably makes it difficult to see these signs couples with the fact that the study was focus on the identification of particular species such as gorilla, chimpanzee and buffalo signs. The low number of non- sighted species could be due to the fact that in some cases movements were done in the wind ward direction in which these high sensitive mammals easily hide themselves or escape from the slightest noise or odour. Moreover, most of the animals could be nocturnal or rare in this area.

Considering the fact that, the dung, tracts, feeding remains or any other index of a species surveyed within a particular area at a precise time may have been produced by the same mammal, in other to avoid double counting of species, only one type of index that is peculiar to a particular species was used to obtain the encountered rate of that species in the study area. The high encounter rate of buffalos and chimpanzees could be due to suitable habitat such as shrub savanna with many gallery forests which habours different variety of fruits, grass, resting sites as well as many streams and rivers which are necessities for animal's diversity. The low encounter rates of the other species could have been due to intense hunting activities especially for traditional rituals and festivals as well as for food. For instance many hunters were encountered in several occasions with fresh or dried meat of different mammal's species in their hunting camps and in the nearby villages. The absence of gorilla signs can either be due to limited time of sighting or due to the roughness of the relief that makes further penetration difficult as well as the fact that they are rare in this area.

Understanding the distribution of large mammals species, permits researchers to locate areas of high biological diversity by targeting specific areas for protection or areas to allow improved management (Tsi et al., 2006).With the exception of gorilla in which no sign was identified, others large mammal's signs were generally located in the study site at different encounter rate. This varies may be as a result of different preferences for resources and the level of human disturbance. For instance, in less disturbed areas, the probabilities of encountering large mammal's signs were highest. Area in which large mammal's species were less encountered could be areas experiencing secondary growth due to corridor loss, bias destruction, hunting and logging. Equally areas covered with high agricultural practices and grassy vegetation gave the lowest encounter rate. Probably because of habitat fragmentation and degradation due to agricultural expansion, interlinked movements of pastoralist's especially wild bush burning to counter pasture maturation. Generally, habitat requirements such as river courses, salt licks, vegetation, resting sites and less anthropogenic activities are one of the factors which affect the abundance of species distribution in an area (Tsi

et al., 2006). The concentration of chimpanzee, buffalo and other large mammals in the northern area of the study site is suggested to be due to the availability of many tree fruits, a wild range of habitat, many streams and rivers, availability of diverse shrubs and grass species which provide enough water, and resting site and permit them to hide from poachers. While their low observation in other areas is suggested to be due to expansion of agricultural land, intense hunting and competition for pasture land with cattle grazers.

The harvesting of these natural resources, leaves signs which were recorded to assess the impact of anthropogenic activities in relation to large mammals in the study site.The high overall mean encounter rate of hunting (0.42 signs per km) in this study site may be due to the fact that hunting is carried out through the year. The availability of hunting equipment at very cheap prices as well as the high cultural value attached to hunting (such as birth and death ceremonies, rituals practices as well as chieftaincy tittles) suggests the high encounter rate of hunting. Agricultural activities amongst these anthropogenic activities gave a mean encounter rate of 0.25 signs per km. This activity is one of the major occupations of the indigenous people and the main causes of habitat fragmentation and degradation within the study area. Most of these activities are carried out along valleys through bush fallowing method. Reasons suggested for this could be that most of the area even though savannah, are very hilly with the exception of some plains. As such the steep rough slopes makes it difficult to cultivate and are equally infertile as most of the top fertile soils are easily wash away during heavy rains and dump in the valleys most of which are forested. The strenuous topography had make the local people believed that areas cover by forest are very fertile when cleared and burnt. Grazing is also a common anthropogenic activities mostly carried out by Mbororos whose main occupation is cattle rearing. Being a wide savanna and the possibility of having many sources of water from streams and rivers and all year round pasture for the survival of cattle especially during the dry season are suggested facts encouraging pastoralist in carrying out effective transhumance. Surveys inventory equally identified logging areas most of which are carried out by the local communities for construction and for making furniture. Some of the timber is being sold at the regional and to local Nigeria traders. Uncontrolled rampant bush fire were identified and are mostly cause by grazers to allow shoot out new pastures to feed their cattle and by hunters to chase out animals from thick bushes.

The spatial distribution of anthropogenic activities, show that, anthropogenic activities were reducing as observers move toward the northern directions of the study site with a corresponding increase in large mammals sighting. That is human activities in the study area, were generally decreasing from south to north as large mammals sightings abundance increase. Despite the steep rough topography and the inaccessibility of the study site, the distance from the village to the northern part as well as the strenuous means of transportation of bush meat which is mostly carried on head, there is high intensity of hunting activities in the northern site. This is due to the availability of an illegal market in Iso and Beleng made up of mostly Nigerian trader. These traders supply hunting materials such as, short guns of one to five ranks. The present of many different kinds of cartridges for instant cartridge 66, 32g used to kill duikers, monkeys and other small mammals, cartridge BB, 34g used to kill bush buck, olive baboons and other medium size mammal, and cartridge 99, 36g specialized to kill leopard, hyena, gorillas, chimpanzees, buffalos and other large mammals. Equally the high availability of snare wires arranged in inches of 3, 6-8, and 15-20 to trap animals of sizes duikers, bush buck and buffalo respectively sold at relatively cheap prices through black marked business. They equally encourage continuous increase in bush meat prices due to competition, thus intensifying hunting. The presence of many domestic dogs greatly encourages group hunting especially during the dry seasons. The wide use of "gamaline and arata bomb" through food substances to poison these large mammals is a serious threat. In these areas, large mammals such as gorillas, chimpanzees and buffalos are highly demanded for their hands, legs, heads, skulls, skins and/or the whole animals for use during traditional rituals and festivals. They are equally hunted for food, medicinal purposes and commerce. Primate capture either for pets or kept in preparation for sacrifices to shrine is a predominant aspect highly practice by the natives of these areas. The high concentration of agricultural activities in the southern region of the studying area is probably because of its gentle slopping topography, and accessibility to farms. Furthermore, it can be due to it closeness to the villages and the increasing human population. Grazing dominate the central region of the black bush area of Waindow simply because it the site in which the local elites demarcated for pastoralist thereby separating the agricultural land from the grazing land.

V. CONCLUSION

This study, reveal the presence of 14 large mammal's species with no gorilla sign recorded in the areas surveyed. Buffalos and Chimpanzees were the most abundant species with the highest encounter rate of 0.35 and 0.26 signs per km respectively. The overall mean encounter rate of large mammal's species in the study

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area was estimated to be 0.2 signs per km. In other words, one would identify less than one mammal signs for every kilometer walked in study site. Therefore, the study area even though poor in species number and species richness contains important flagship species such as chimpanzee and buffalo. Large mammals were spatially distributed in almost all the parts of the study area. The relative densities of Buffalos and Chimpanzees species were high in the northern region. Anthropogenic activities include intensive hunting, poor farming method, over grazing, illegal logging and wild bush fire. Among these activities hunting appears to be the most prevalent activity because of its high encounter rate of 0.42 signs per km. The anthropogenic activities shows an average effect on the abundance and distribution of large mammals species when plotted together with the encounter rate of large mammals through regression analyses (R^2 =0.773). Furthermore, the overall area has been neglected due to its relative isolation and has lead to increasing encroachment by Nigerian's traders and grazers thus high level of threat to the remaining chimpanzees and buffalos in the study site. As such, conservation attention is an immediate priority for the survival of these fast declining species. That is, it is very necessary to rapidly employ conservation strategies that can help to urgently conserve the remaining chimpanzees, buffalos and other species in the study area.

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APPENDIXES

Appendix 1: Figures

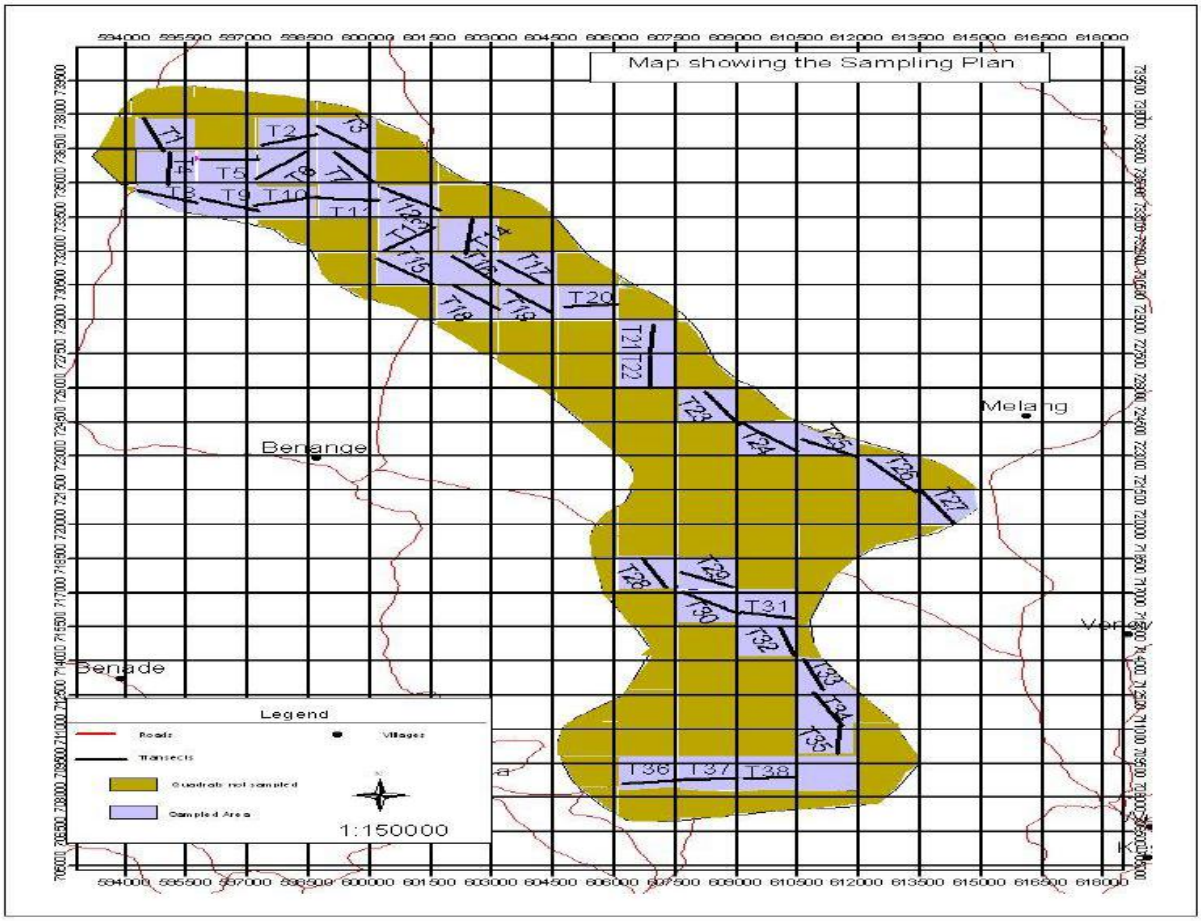

Fig.2: GIS Map showing representation of recces for animal inventory in the study area

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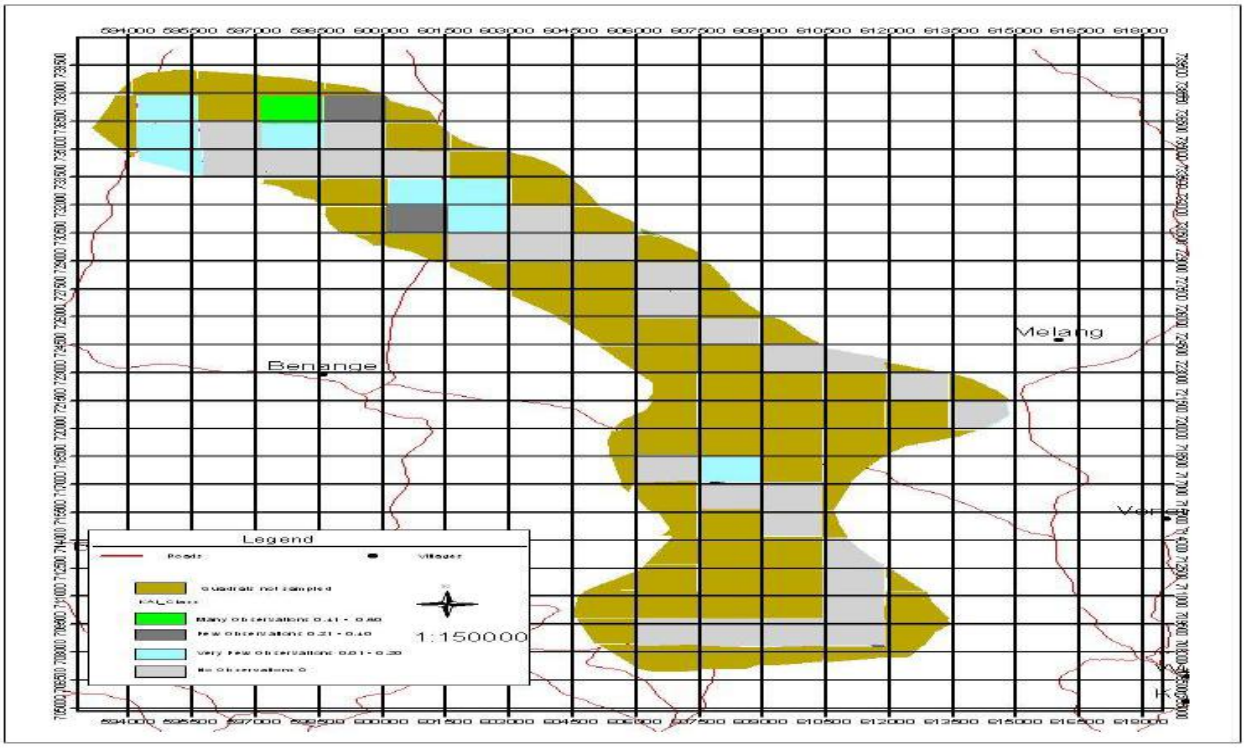

Fig.3: Spatial distribution of Chimpanzee (Pan troglodytes ellioti) in the study site

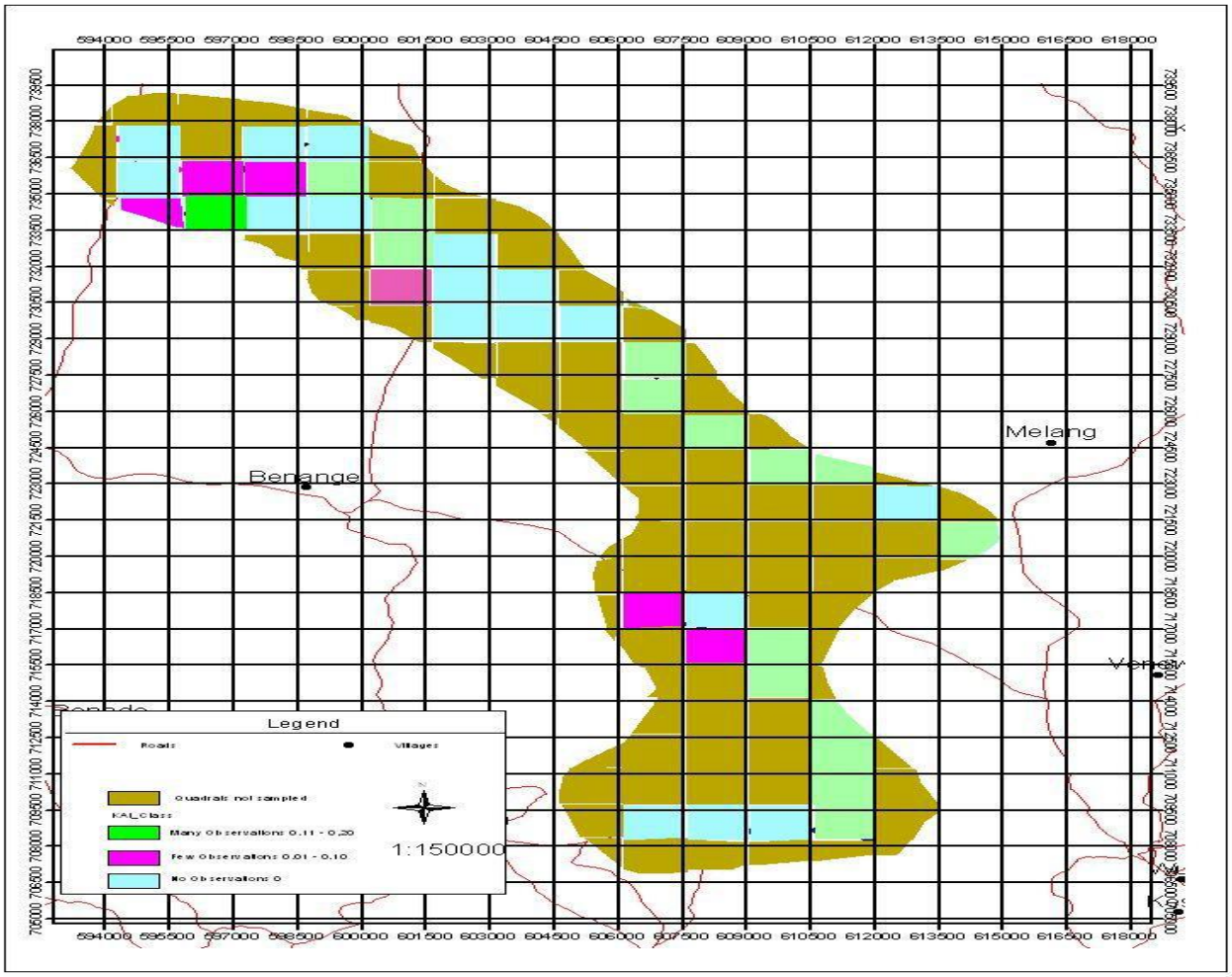

Fig.4: Spatial distribution of Buffalo (Syncerus caffer) in the study site

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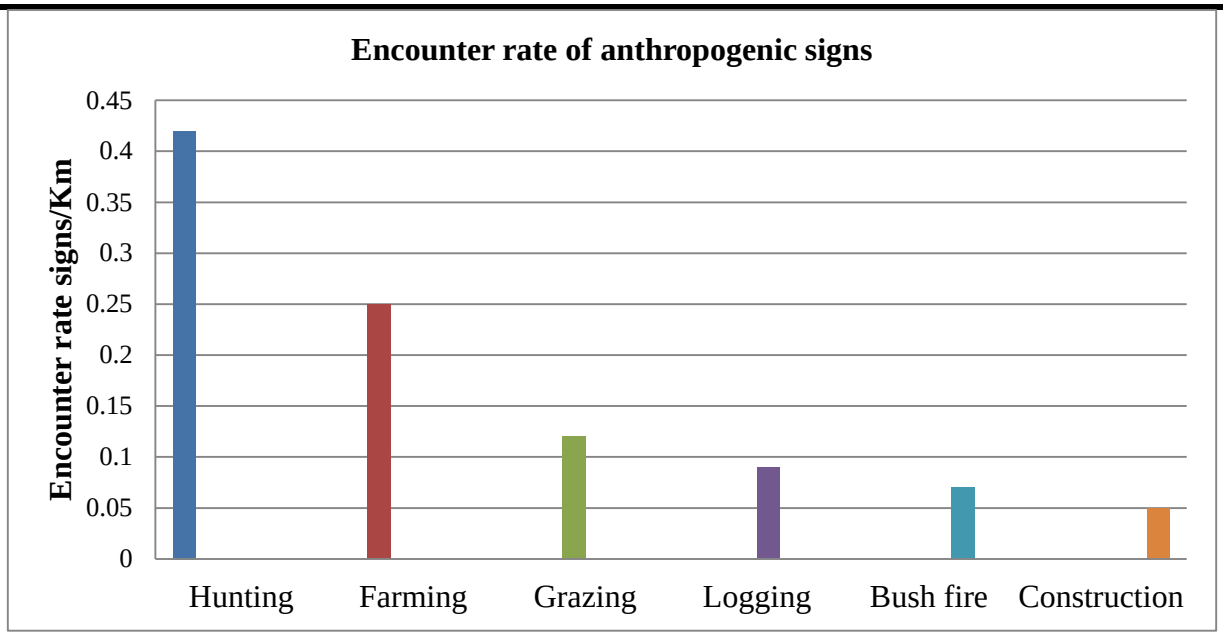

Fig.1: Relative abundance of anthropogenic activities in the study site

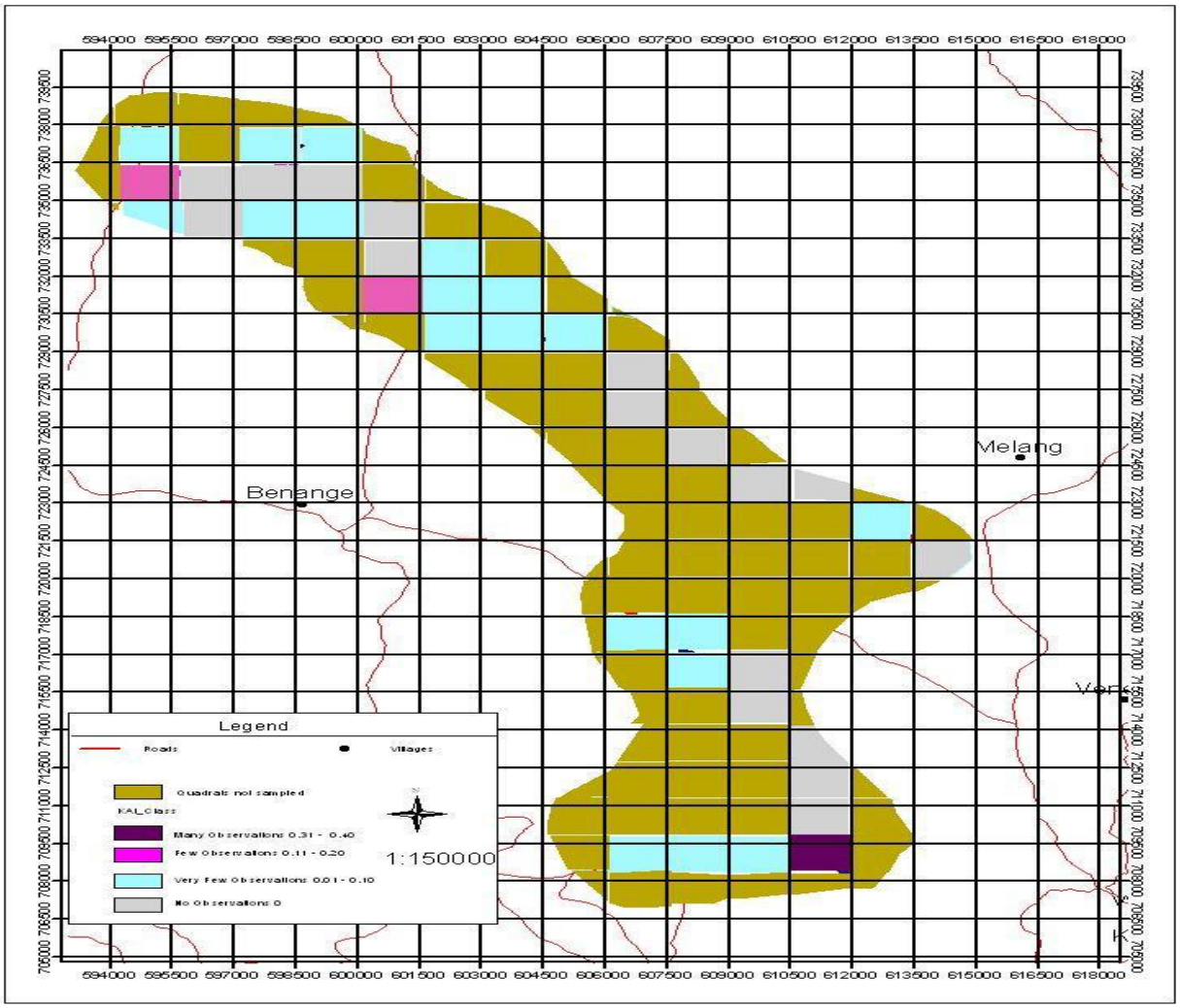

Fig.6: Spatial distribution of anthropogenic activities in the study site

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Appendix 2: Tables

Table.1: Large mammal species recorded in the BBAW

Family	Common Name	Scientific Name
Bovidae	Blue duiker	*Cephalophus monticola*
	Bushbuck	*Tragelaphus scriptus*
	Red duikers	*Cephalophus dorsalis*
	Buffalo	*Syncerus caffer*
	Defassa waterbuck	*Kobus defassa*
Feilidae	Leopard	*Pamthera pardus*
Suidae	Red river hog	*Potamochoerus porcus*
Canidae	Red fox	*Vulpes vulpes*
Viverredae	Africa civet	*Vuverra civetta*
Cercopithecinae	Putty-nose monkey	*Cercopithecus nictitans*
	Vevet monkey	*Cercopithecus aethiops*
	Patas Monkeys	*Erythrobus patas*
	Olive Baboon	*Papio anubis*
	Mona Monkey	*Cercopithecus mona*
Pongidae	Chimpanzee	*Pan troglodytes*

Table.2: Indices of large mammal species identified in the BBAW

Species	IO								DO	Total
	D	FP	T	FR	RN	N	C	V		
Blue duiker	1	3	2	/	/	/	/	/	2	8
Bush buck	3	4	7	/	/	/	/	/	1	15
Red duiker	2	9	6	1	/	/	1	/	3	22
Buffalo	20	8	9	/	1	/	/	/	2	40
Chimpanzee	/	/	3	/	/	15	/	2	/	20
Leopard	/	/	/	/	/	/	1	/	/	1
Red fox	3	2	/	/	/	/	/	/	/	5
Red River Hog	2	4	/	5	1	/	/	/	/	12
Africa Civet	3	2	/	/	/	/	/	/	/	5
Olive Baboon	/	/	/	1	/	/	/	2	4	7
Patas Monkeys	/	/	2	1	/	/	/	/	/	3
Mona Monkey	/	/	/	2	/	/	/	2	5	9
Putty nosed monkeys	/	/	1	1	/	/	/	1	6	9
Vervet monkey	/	/	/	4	/	/	/	1	8	13
Total	34	32	30	15	2	15	2	8	31	169

Legend: Indirect observation (IO), Direct observation (DO), dung (D), foot prints (FP), tracks (T), food remains (FR), resting or nursing sites (RN), nest (N), carcass (C), voices (V), and /= no observation

Table.3: Encounter rate of large mammal species sighted in the BBAW

Common Name	Family	Scientific Name	SP	TDC km)	ER
Buffalo	Bovidae	*Syncerus caffer*	2	57	0.04
Bush Buck	Bovidae	*Tragelahus scriptus*	1	57	0.02
Red Duiker	Bovidae	*Cephalophus dorsalis*	3	57	0.05
Blue Duiker	Bovidae	*Tragelahus scriptus*	2	57	0.04
Olive Baboon	Cercopithecinae	*Papio anubis*	4	57	0.07
Mona Monkey	Cercopithecidae	*Cercopithecus mona*	5	57	0.09

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Putty-nosed monkeys	Cercopithecidae	*Cercopithecus nictitans*	6	57	0.11
Vervet monkey	Cercopithecidae	*Cercopithecus aethiops*	8	57	0.14

Legend: encountered rate (ER), total distance covered (TDC), species population (SP**)**

Table.4: Encounter rate of indirect signs of large mammal species

Common Name	**Family**	**Scientific Name**	**TNI**	**TDC** (km)	**ER**
Buffalo	Bovidae	*Syncerus caffer*	20	57	0.35
Bush Buck	Bovidae	*Tragelahus scriptus*	3	57	0.05
Blue Duiker	Bovidae	*Cephalophus monticola*	1	57	0.02
Red Duiker	Bovidae	*Cephalophus dorsalis*	2	57	0.04
Leopard	Felidae	*Pamthera pardus*	1	57	0.02
Red fox	Canidae	*Vulpes vulpes*	3	57	0.05
Red river hog	Suidae	*Potamochoerus porcus*	2	57	0.04
Africa civet	Viverredae	*Viverra civetta*	3	57	0.05
Gorilla	pongidae	*Gorilla goirlla*	/	57	/
Chimpanzee	Pongidae	*Pan troglodytes*	15	57	0.26
Olive Baboon	Cercopithecinae	*Papio anubis*	2	57	0.04
Patas Monkeys	Cercopithecidae	*Erythrobus patas*	2	57	0.04
Mona Monkey	Cercopithecidae	*Cercopithecus mona*	2	57	0.04
Putty-nosed monkeys	Cercopithecidae	*Cercopithecus nictitans*	1	57	0.02
Vervet monkey	Cercopithecidae	*Cercopithecus aethiops*	4	57	0.07
Min					**0.350**
Max					**.020.**
Mean					**2**

Legend: encountered rate (ER), total number of indices (TNI), total distance covered (TDC), when 0.1< ER < 0.5= low observation, < ER > 0.5 =High observation, and / = no observation

Appendix 3: Field pictures

Picture 1: Chimpanzee nests

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Picture 2: Buffalo dung

Picture 3: Duiker Carcass

Picture 4: Patas monkey skin

Picture 5: A Rotten Duiker

Picture 6: Roasted Duiker

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Picture 7: Roosted Olive Baboon and Bush Buck ready for market

Picture 8: Killed python

Picture 9: A young capture Velvet Monkey

Picture 10: Cartridges (66-32g)

Picture 11: Cartridges (BB-34g)

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Picture 12: Cartridges (OO-38g)

Picture 13: Wire snares

Picture 14: Duiker trap

Picture 15: Buffalo trap

Picture 16: Hunter's sleeping rocks

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Picture 17: Logging

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In vitro mutagenesis of *Cymbidium* La bell "Anna Belle" by γ-rays irradiation and oligochitosan interaction

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***Abstract*—** *The optimum media for multiplication of protocorm like bodies (PLBs) and shoot buds of Cymbidium La bell "Anna Belle" were studied in order to prepare the in vitro samples for irradiation. The values of LD_{50} (lethal dose of 50% samples) of PLBs, shoot buds and plantlets of tested Cymbidium after cultivation of 4 months were also determined about 35.0, 41.0 and 83.1 Gy, respectively. The addition of oligochitosan played as an very important trigger for promotion on the generation of shoot bud from PLBs after irradiation. The in vitro variations have been generated by γ-rays irradiation of PLBs with doses in range of 20 - 50 Gy. The highest mutant frequency (3.83‰) of C. La bell was found by the irradiation of PLB samples at 30 Gy. The different properties of obtained in vitro variations compared to wild types were found to be chlorophyll, short leaves, long leaves, and violet pericardium variations. The genetic relationships among generated variant lines in M_1V_4 and wild type were analyzed using RAPD techniques.*

Keywords*— *Cymbidium, in vitro propagation, irradiation, mutation, oligochitosan.

I. INTRODUCTION

Cymbidium is a genus of 50 species from Asia and they have attractive values in both art and commercialization [1]. *Cymbidium* La bell "Anna Belle" was imported to Vietnam several years ago and showed many good properties such as large number of flower shoots, big flower size, long life in vase, ease of plantation and high yield. Since *Cymbidium* is a very popular and favorite potted orchid in Asia, there have been several studies carried out for *in vitro* propagation of this genus using different methods. Brown el al. [2] studied on *in vitro* propagation of this orchid by seed and shoot tip culture method, while Nayak et al. [3,4] used shoot-tip, PLBs and thin cross section for cultivation. Other methods for micropropagation using callus [5,6] and embryo [7] were also established.

In addition, γ-rays irradiation technique in combination with tissue culture method had proven to be useful for mutation breeding and this technique has contributed towards improvements in agricultural crops and ornamental plants. According to the report of the joint FAO/IAEA programme for nuclear techniques in agriculture, there have been 3100 officially released mutant varieties from 170 different plant species in more than 60 countries. Among the mutant varieties, about 90% of these mutant varieties were generated by using radiation [8]. Several new flower varieties with high commercial value, such as chrysanthemum [9-12], anthurium [13], *Curcuma alismatifolia* [14], lily [15,16], etc. have been generated by γ-rays.

So far, most of cultivated *Cymbidium* varieties have been induced by crossing and the number of mutant orchids generated by radiation techniques is very few. For these reasons, the study aimed to optimize *in vitro* propagation conditions and generate *in vitro* mutagenesis of *Cymbidium* La bell "Anna Belle" by irradiation method combined with tissue culture techniques.

II. MATERIALS AND METHODS

Plant materials and chemicals

The orchid used in the present experiment namely *Cymbidium* La bell "Anna Belle" was supplied by Lang Biang Farm Ltd. This orchid grew in pots at an elevation of about 1500 m above sea level with an average temperature of about 18 ^oC and a range between 10 to 30 ^oC. N_6-benzyladenine (BA), indol-3-butyric acid (IBA), α-napthylacetic acid (NAA), thidiazuron (TDZ) and three kinds of medium namely Murashige and Skoog's (MS), Vacine & Went (VW) and Knudson C (KC) were supplied by Sigma-Aldrich Co. (St. Louis, Missouri USA). Oligochitosan with a molecular weight of about 16 kDa was prepared by the irradiation method as described previously [17].

Initial explant preparation

The new growths were taken from cymbidium pots at Lang Biang Farm, rinsed with tape water, and freed of dead, damaged, or excessively hard external tissues and parts. The shoots were then immersed in a mix of household bleach-distilled water (1:1; v/v). Excision of

explants was performed under a dissecting microscope on an open laboratory bench washed with 95% ethanol. External leaves and leaf primordial were removed to expose the shoot tips. Full-strength KC medium supplemented with coconut water (CW) (10%), sucrose (20 g/l) and activated charcoal (1 g/l) were used as the basic medium.

Protocorm like body proliferation

Protocorm like bodies (PLBs) with the size about 4 mm were cultured in MS, VW and KC media containing 10% CW, sucrose (20 g/l), 1 g/l charcoal and supplemented with BA or BA in combination with TDZ or NAA for PLBs multiplication. The number of PLBs, shoot buds and plantlets were determined after incubating in 30 days.

Shoot bud proliferation

For investigating the proliferation of shoot buds, PLBs with the size about 4 mm were cultured in Erlenmeyer flasks containing basic medium supplemented with BA, NAA or TDZ. The number of shoot buds per clump was determined after 6 weeks culture.

***In vitro* plantlet regeneration**

Individual shoot buds with 5 cm high and 3 expanded leaves detached from 6-week old shoot bud clumps were cultured in 250 ml Erlenmeyer flasks containing MS, VW and KC media with or without supplementation of 0.1 mg/l NAA and 10% CW. The shoot height and root length of *in vitro* plantlets were determined after culturing for 6 weeks.

Determination of LD_{50}

The radiosensitive tests for *in vitro* samples of the tested *Cymbidium* were established by irradiating PLBs, shoot buds and plantlets with γ-rays from a Co-60 source at various doses (five hundred of samples were applied for each dose) with a dose rate of 0.2 Gy/s. The survival rate of irradiated samples was determined after cultivation of 4 months for calculating the optimal dose for radiosensitivity, *i.e.* LD_{50} (lethal dosage of 50% irradiated samples) [14,18].

Variation induction

To generate *in vitro* variation, a thousand of PLB samples were irradiated by γ-rays at doses of 10, 20, 30, 40 and 50 Gy. The irradiated PLBs were then cultured in KC medium supplemented with 10% CW, sucrose (20 g/l), 1 g/l charcoal and 50 mg/l oligochitosan to generate the shoot bud. The culture medium was changed every 2 month for 12 months and the individual shoot buds with 3 - 4 expanded leaves generated from irradiated samples was detached for screening.

RAPD analysis

Total genomic DNA was extracted from frozen young leaves following the modified CTAB (cetyl trimethyl ammonium bromide) procedure described by Li et al. [19]. 100 mg frozen leaf tissue were ground to powder in liquid nitrogen using a mortar and pestle. The powder was transferred into 10 ml centrifuge tubes and was mixed with 3 ml of preheated (65 °C) 2X CTAB extraction buffer (100 mM Tris-HC1 (pH 8.0), 1.4 M NaC1, 20 mM EDTA (ethylene diamine tetra acetic acid), 2% CTAB, 2% (w/v) P-mercaptoethanol, 1% (w/v) polyvinylpyrrolidone (PVP40)). The mixture in the tubes was incubated at 65 °C for 30 min, then cooled and mixed with 500 µl tris-phenol, and held at 65°C for 15 min. The tubes were gently inverted upside down for several times and centrifuged at 4°C and 14,500 x g for 15 min. The supernatant was transferred to a 10 ml centrifuge tube and the same volume of chloroform was added. Then, incubation and centrifugation were repeated once. The supernatant was mixed with 0.7 volume of isopropanol, and then held at -20 °C for 30 min. The aqueous phase was discarded while the pellets were washed with 70% ethanol twice and absolute alcohol twice, then transferred to a 1.5 ml centrifuge tube, and dried at room temperature. The dried pellets were suspended in 600 µl HS-TE (10 mM Tris-HC1 (pH 8.0), 1 M NaCl, 0.1 mM EDTA (pH 8.0)), and then an equal volume of phenol/chloroform was added and centrifuged. The last procedure was repeated using chloroform replacing phenol/chloroform and centrifuged. The supernatant was uniformly mixed with 0.7 volume of isopropanol, and then held at -20 °C for 1 h. The mixture was centrifuged and the pellets were washed with 70% ethanol and absolute alcohol, respectively, and then dried at room temperature. The dried pellets were precipitated in 100 µl TE [100 mM Tris-HC1 (pH 8.0); 1 mM EDTA (pH 8.0)]. RNA was removed by RNaseA (100 µg ml^{-1}) for 1 h at 37 °C. The yield of DNA per gram of leaf tissue extracted was measured using a BioPhotometer (Eppendorf) spectrophotometer a 260 nm. The DNA purity was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm and the quality was evaluated by 0.8% agarose gel (0.5 µg/ml EB) electrophoresis. Working solutions of the DNA were prepared by diluting the stocks at 10 ng/µl in sterile distilled water.

The RAPD reactions were performed following the procedures described by Li, et al. [19]. A total volume of 25 µl containing: 25 ng of template DNA, 0.2 µM of 20 decamer oligonucleotide primers (OPA and OPD from 01 to 10) (Sigma), 1.0 U Taq DNA polymerase (Takara, Japan), 0.2 mM each *dNTP* (Sigma, Molecular biological grade), 2 mM $MgCl_2$, 10 mM Tris-HC1 (pH 8.3), 50 mM KC1 (pH 8.3), and sterilized water. DNA amplification was carried out in Mastercycler gradient (Eppendorf) and the thermal cycling programme was as follows: An initial denaturation cycle of 3 min at 94ºC, followed by 40 cycles comprising of 1 min at 94ºC, 1.5 min at 37ºC, and 2.5 min at 72°C; 72°C for 5 min was used for the final extension. The amplification products were separated by electrophoresis in 1.5% agars gels in 0.5X TBE at 150 V.

Each amplification reaction was repeated at least twice. RAPD polymorphic bands were visualized and photographed using ultraviolet illumination, and were scored as present (1) or absent (0). The data were analyzed using the SIMQUAL (similarity for qualitative data) routine to generate Dice similarity coefficients and these similarity coefficients were used to construct dendrograms using the NTSYSpc (ver.2.10) program.

Incubational conditions and statistical analysis

The pH of media was adjusted to 5.8 by KOH and HCl before autoclaving at 121 ^{o}C and 105 kpa for 15 min. Cultures were incubated in a cultural room at 25 ± 1 ^{o}C under 16-h photoperiod provided by fluorescent lamp at 45 µmol m^{-2} s^{-1} photosynthetic photon density flux. All experiments were repeated three times. Data were statistically analyzed by variance analysis (ANOVA) and means were compared using the least significant difference (LSD) at 5% probability level and standard deviation Duncan's multiple range test [20].

III. RESULTS

PLB and shoot bud multiplication

Protocorm like bodies (PLBs) with the diameter about 4 mm and shoot buds with 3 cm high and 2 expanded leaves were used for PLBs and shoot buds multiplication, respectively. The results in Table 1 showed that the supplementation with 2.0 mg/l BA displayed the best effect on the formation of PLBs of *C* La bell (10.29 PLBs per samples). In addition to, the supplementation of BA (2.0 mg/l) combined with 0.1 - 0.2 mg/l TDZ or NAA stimulated the formation of PLBs.

On the other hand, the results from Table 2 showed that the highest shoot proliferation rate (8.25 - 8.29 shoot buds per sample) was obtained by the supplementation of BA at 0.2 mg/l. For shoot bud proliferation of mentioned *Cymbidium*, number of shoot buds generated from PLB cultured on MS or KC media after 6 weeks incubation was higher than that of sample culture on VW medium.

Plantlet regeneration

The shoot buds with 5 cm high and 3 expanded leaves were used as starting materials for plantlet regeneration. The results from Table 3 showed that the supplementation of NAA (0.1 mg/l) and coconut water (10%) stimulated the growth of *Cymbidium* plantlets incubated on all of three media (MS, KC and VW) and the best suitable media were found to be MS and VW.

Effect of γ-rays on the growth of *in vitro* samples

To investigate the LD_{50} (the lethal dose of 50% samples) of *in vitro Cymbidium* samples, the PLBs with the diameter about 4 mm, shoot buds with 3 cm high and 2 expanded leaves or plantlets with good development were used for irradiation. The results from Fig. 1 indicated that LD_{50} of PLBs and shoot buds of *C.* La bell after cultivation of 4 months were 35.0 and 41.0 Gy, respectively, while the LD_{50} value of plantlets was determined about 83.1 Gy after cultivation 4 months in a greenhouse.

Induction and screening *in vitro* variation lines with oligochitosan association

A thousand PLBs were irradiated at the dose range of 20 - 50 Gy and then cultured on basic medium containing 0.2 mg/l BA with or without supplementation of oligochitosan. It can be seen from Table 4 that, the survival samples after irradiation developed very low in the medium without supplementation of oligochitosan and the number of generated shoot bud was quite low (see Fig. 2) even for samples irradiated at a low dose (10 Gy). In contract, the irradiated PLBs cultured in medium supplemented with oligochitosan generated a large number of shoot bud after 12 month even for samples irradiated at 50 Gy.

The results from Table 4 also showed that the variation was not induce from sample irradiated at 10 Gy, while the total frequencies of *in vitro* variation of *C.* La bell generated from the samples irradiated at 20, 30, 40 and 50 Gy were found about 1.60, 3.83, 3.15 and 2.27‰, respectively. The highest variation frequency of *C.* La bell was obtained by the treatment of 30 Gy. The variations of mentioned *Cymbidium* orchids generated by γ-rays irradiation mainly belong to four types as follow (see Fig. 3): Chlorophyll variations, short leaves variations (leaves become shorter), long leaves variations (the leaves become longer and bigger) and violet pericardium variations (the color of pericardium become violet).

RAPD analysis

Among the screened variations, 3 stable variant lines screened in M_1V_4 (CLB-20Gy-1.1: Large leave variation, CLB-20Gy-2.1: Short leave variation and CLB-20Gy-3.1: Chlorophyll variation) were analyzed by RADP technique using 20 decamer oligonucleotide primers (OPA and OPA from 01 to 10). 7 primers (OPA 01, OPA 10, OPD 02, OPD 05, OPD 06, OPD 07 and OPD 08) were found to generate polymorphic amplified fragments with an average polymorphic ratio approximately 86.8% (Table 5). The scored applified data are presented in Fig. 5 and similarly matrix in Table 6. It can be seen that the genetic relationships of wild type (CLB-ctrl) and variation lines namley CLB-20Gy-1.1 CLB-20Gy-2.1 CLB-20Gy-3.1 were found at 80, 46.7 and 60%, respectively, while the genetic relationship among mentioned variation lines was calculated from 53.3 to 66.7%. The phylogenetic tree (Fig 5) indicated that the group of mutants is different from the other one with only the control.

IV. DISCUSSION

Optimization of *in vitro* cultural condition

6-benzylaminopurine (BA) is a good plant hormone for PLBs multiplication. In this study, BA displayed the best effect on the formation of PLBs of *C.* La bell (10.29 PLBs per samples and the obtained results are in good agreement with that of Najak et al. [4]. The addition of 2.0 mg/l BA combined with NAA or TDZ at concentrations of 0.1 and 0.2 mg/l displayed a stronger effect on the PLB multiplication compared with that of a single addition of BA. This medium is very important for preparation of a large number of PLBs of this variety for irradiation.

In addition to, BA, TDZ and NAA have been widely used for proliferation of many kinds of plant *in vitro*. The results from Table 2 showed that the supplementation of the mentioned plant hormones increased the shoot proliferation rate of the tested orchid. It can be seen that BA had a higher effect on stimulation of shoot proliferation rate compared to those of TDZ and NAA, specially, the highest stimulation effect (8.25 - 8.29 shoot buds per clump) was found by the supplementation of BA at 0.2 mg/l and the obtained results are in good agreement with results on *C aliofolium* (L.) Sw. of Najak et al. [3]. On the other hands, for shoot bud proliferation of mentioned *Cymbidium*, the usage of MS and KC mediums were found to be better than that of VW. Therefore, the MS medium supplemented with 0.2 mgl BA was selected as the best suitable medium for shoot bud generation of irradiated samples.

The plantlet regeneration is very important step in tissue culture technique to induce the *in vitro* plantlets. In present study, the plantlets were induce for two purposes, first was induce the materials for irradiation and the second was induce plantlets from the shoot clusters of irradiated samples. It was found that the supplementation of NAA (0.1 mg/l) and 10% coconut water (CW) stimulated the growth of *Cymbidium* plantlets incubated on three kinds of mediums (MS, KC and VW) and the best suitable mediums were found to be MS and VW mediums. The supplementation with CW (10%) and NAA (0.1 mg/l) was optimum for plantlet regeneration of tested *Cymbidium* variety.

Thus, the completed process of *in vitro* propagation including initial explant preparation, PLB multiplication, shoot tip generation and plantlet regeneration of *Cymbidium* La bell "Anna Belle" has been built up. This process was used for preparation samples for irradiation and cultivation of the survival samples collected after irradiation.

Generation of variation lines by γ-rays irradiation

Among thousands of mutant varieties have been generated by radiation methods, ornamental and decorative plants occupied about 25% [8,21]. γ-rays reported as useful mutagens for plant mutation breeding and it was employed to develop 64% of the radiation-induced mutant varieties [9,10-16,21]. Kozlowska-kalisz [22] reported that, a dose of 20 Gy inhibited growth of samples and 70 Gy was the lethal dose of PLBs of the *Cymbidium* orchid. For generation of mutation of plants by γ-rays irradiation, the investigation of the radiosensitivity test, *i.e.* lethal dose of 50% samples (LD_{50}) is important for plant mutation breeding studies to focusing the effective doses at which frequency the mutants will be high obtained [9,13,22-25]. In this study, five hundred of *in vitro* samples (PLBs, shoot buds or plantlets) of *C.* La bell were applied for each irradiation dose. The results from Fig. 1 indicated that LD_{50} of PLBs and shoot bud after cultivation of 4 months were 35.0 and 41.0 Gy respectively, while the LD_{50} value of plantlets was determined about 83.1 Gy after cultivation 4 months in a greenhouse.

After obtaining the LD_{50}, A thousand of PLBs of mentioned orchid were applied for γ-rays irradiation at doses of 10, 20, 30, 40 and 50 Gy for mutant generation. Samples after irradiation were then cultured on basic media containing 0.2 mg/l BA with or without supplementation of oligochitosan. It can be seen from Table 4 that, the survival samples after irradiation developed very low in the medium without supplementation of oligochitosan and the number of generated shoot bud was quite low (see Fig. 2) even for samples irradiated at a low dose (10 Gy). There is no variation is found from irradiated samples incubated in medium without oligochitosan supplementation. The reason may due to the number of generated shoot bud is small. In contract, the irradiated PLBs cultured in medium supplemented with oligochitosan generated a large number of shoot bud after 12 month even for samples irradiated at 50 Gy. Oligochitosan has been proved to have several novel features such as growth promotion for *in vitro* flower plant samples and increase of the survival rate of plantlets acclimatized in a greenhouse [17], induction of antibiotic phytoalexins to prevent infection from fungal diseases [26,27], reduction of the damage caused by toxic elements (zinc, vanadium etc.) [28] and enhancement of the seed germination rates [29]. Recently, we also found that oligochitosan promoted the generation of shoot bud from PLBs of slipper orchids (*Paphiopedilum callosum* and *Paphiopedilum delenatii*) after irradiation by 320 MeV $^{12}C^{6+}$ ion-beams accelerated with an AVF cyclotron [30].

γ-rays irradiation can induce mutants in numerous plants such as chrysanthemum [12,13,25,31]. Mutation breeding by application of *in vitro* techniques in combination with gamma rays irradiation method has been successful with several flower plants such as chrysanthemum [32,33] and slipper orchids [30], and so on. Even so, the use of radiation for mutation breeding of *Cymbidium* orchids is still limited.

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The results from Table 4 also showed that the variation was not induce from sample irradiated at 10 Gy, while the total frequencies of *in vitro* variation of *C.* La bell generated from the samples irradiated at 20, 30, 40 and 50 Gy were found about 1.60, 3.83, 3.15 and 2.27‰, respectively. The highest variation frequency of *C.* La bell was obtained by the treatment of 30 Gy. The variations of mentioned *Cymbidium* orchids generated by γ-rays irradiation mainly belong to four types as follow (see Fig. 3): Chlorophyll variation, short leaves mutants (leaves become shorter), long leaves variation (the leaves become longer and bigger) and violet pericardium variation (the color of pericardium become violet). Among the screened variations, the variations with the color change of pericardium into violet color (violet pericardium variations) and the variations changes in leaf size (the leaves become longer or shorter) were found from samples irradiated from 20 to 50 Gy, while the chlorophyll variations were only found by the samples irradiated at lower doses (20 – 40 Gy). In previous report [30], the *in vitro* mutant types of *P. callosum* and *P. delenatii* such as chlorophyll mutants and leaf size mutants were generated by ionization irradiation of PLBs.

RAPD analysis

The usage of random amplified polymorphic DNA (RAPD) markers to detect the genetic relationships of orchid has been reported and it was found that the RAPD is a convenient technique for detecting the difference in DNA among *Cymbidium* cultivars [19,34]. Puchooa et al. [13] also used RAPD technique for the analysis of the change in DNA of mutant anthurium generated by gamma radiation. In addition, changes in DNA caused by chemical agent treatment of mutant *Senna occidentalis* [35] and mutant *Helianthus annuus* [36] resulted in genetic variation were successfully detected using RAPD technique. On the other hand, Shin et al. [37] also used the same technique for analyzing the genetic relationship between mutant sweet potato and wild type plant. In our previous report, the chande in DNA of *P. delenatii* and *P. callosum* mutant lines induced by ion beams was also successful analyzed by RADP using ODP decamer oligonucleotide primers. In present study, we also used RAPD with 7 selected ODP and OPA primers for analyzing the genetic relationships of wild type of tested *Cymbidium* variety and 3 selected mutant lines induced from samples irradiated at 30 Gy (CLB-30Gy-1.1, CLB-30Gy-2.1 and CLB-30Gy-3.1). All of the 7 selected primers induced polymorphic amplified fragments with a high polymorphic ratio (86.8%). The low genetic relationships between 3 variation lines (CLB-30Gy-1.1, CLB-30Gy-2.1 and CLB-30Gy-3.1) and of wild type (CLB-ctrl) indicated the changes in DNA of mutant lines by ion-beams. Our results are in agreement with the findings of Puchooa et al. [13] and Mostafa [36]. Since genetic relationships among 3 mutant lines and of wild type were rather low (46.7 – 80.0%), the phylogenetic tree grouped the mutants and the control into different clusters. The mutant lines were scored in one cluster, while the control was scored in the others. Thus, changes in DNA induced by γ-rays irradiation and resultant genetic variation in *C.* La bell "Anna Belle" mutant lines can be rapidly detected by RAPD analysis. Our results are in agreement with our previous results [30] and those of previous studies using RAPD for analyzing the changes in DNA of mutant plants [13,35-37].

V. CONCLUSIONS

The optimum conditions for PLBs multiplication, shoot bud proliferation and plantlet regeneration were found to be KC medium supplementation 2.0 mg/l BA in combination with 0.1 mgl NAA, KC medium supplementation 2.0 mg/l BA, and MS medium supplementation with 0.1 mg/l NAA, respectively. The values of LD_{50} of PLBs, shoot buds and plantlets of *C* La bell "Anna Belle" after cultivation of 4 months have been determined. To generate the mutants of the mentioned orchid by γ-rays irradiation, PLBs were suitable samples for irradiation and the supplementation of oligochitosan was very important for generating the shoot buds from irradiated sample of this Cymbidium. γ-rays irradiation at doses from 20-50 Gy induced *in vitro* mutants of Cymbidium orchid and the suitable dose was found at 30 Gy. The screened mutant lines provide promising materials for developing new mutant varieties for *C* La bell "Anna Belle".

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Table.1: Effect of phytohormone onto the PLB multiplication rate of C. La bell "Anna Belle"

Plant hormone, mg/l			PLBs per explant[x]
BA	NAA	TDZ	
0	0	0	4.04[a]
0.5	0	0	6.70[b]
1.0	0	0	8.15[c]
2.0	0	0	10.29[e]
3.0	0	0	9.45[d]
5.0	0	0	9.11[d]
10	0	0	9.36[d]
2,0	0.1	0	12.12[g]
2,0	0.2	0	11.58[f]
2,0	0.3	0	10.55[e]
2,0	0.5	0	9.80[d]
2,0	1.0	0	9.12[d]
2,0	0	0.1	11.15[f]
2,0	0	0.2	11.08[e]
2,0	0	0.3	10.48[e]
2,0	0	0.5	10.14[e]
2,0	0	1.0	9.42[d]

[x] Mean values followed by the same letter within a column are not statistically different according to a Duncan's multiple range test at $P < 0.05$.

Table.2: Effect of plant hormone on shoot bud proliferation for C. La bell "Anna Belle"

Plant hormone	Conc., mg/l	Shoot bud proliferation		
		MS	VW	KC
Control		4.40[a]	4.68[c]	4.70[b]
BA	0.1	5.51[b]	5.69[c]	5.70[e]
	0.2	8.25[c]	8.07[e]	8.29[f]
	0.3	6.13[b]	5.39[d]	6.18[d]
	0.5	4.18[a]	4.40[b]	5.07[c]
TDZ	0.1	4.16[a]	4.15[b]	4.33[a]
	0.2	3.84[a]	4.35[b]	4.24[a]
	0.3	3.79[a]	4.24[b]	4.02[a]
	0.5	3.41[a]	3.55[a]	3.78[a]

NAA	0.1	4.81[a]	4.88[c]	4.90[b]
	0.2	4.72[a]	4.63[c]	4.73[b]
	0.3	4.13[a]	4.75[c]	4.77[b]
	0.5	3.92 [a]	4.19[b]	4.23[a]

Mean values followed by the same letter within a column are not statistically different according to a Duncan's multiple range test at $P < 0.05$.

Table.3: Plantlet generation of C. La bell "Anna Belle"

Medium	NAA, mg/l	CW, %	Shoot hight, cm	Root length, cm
MS	0	0	7.77 ± 0.11	1.8 ± 0.13
	0.1	0	8.02 ± 0.11	2.4 ± 0.10
	0.1	10	8.49 ± 0.10	3.3 ± 0.11
KC	0	0	7.34 ± 0.14	1.9 ± 0.10
	0.1	0	7.60 ± 0.17	2.5 ± 0.12
	0.1	10	8.14 ± 0.09	3.0 ± 0.11
VW	0	0	7.51 ± 0.22	1.9 ± 0.13
	0.1	0	8.10 ± 0.19	2.1 ± 0.10
	0.1	10	8.43 ± 0.20	3.1 ± 0.11

Table.4: The in vitro variation types of C. La bell "Anna Belle" generated by γ-rays

Dose, Gy	OC concentration, mg/l	Number of shoot bud generation	Type variation	Number of variation	Frequency, ‰
10	0	1520	-	0	0
	50	20000	-	0	0
20	0	1041	-	0	0
	50	19128	*- Chlorophyll variation*	*3*	*0.15*
			- Short leaves variation	*7*	*0.36*
			- Long leaves variation	*19*	*0.99*
			- Violet pericardium variation	*2*	*0.10*
			Total	21	1.60
30	0	478	-	0	0
	50	15952	*- Chlorophyll variation*	*2*	*0.13*
			- Short leaves variation	*38*	*2.39*
			- Long leaves variation	*20*	*1.25*
			- Violet pericardium variation	*1*	*0.06*
			Total	61	3.83
40	0	231	-	0	0
	50	16142	*- Chlorophyll variation*	*3*	*0.17*
			- Short leave variation	*29*	*1.60*
			- Long leave variation	*21*	*1.16*
			- Violet pericardium variation	*4*	*0.22*
			Total	57	3.15
50	0	129	-	0	0
	100	3965	*- Short leave variation*	*2*	*0.50*
			- Long leave variation	*4*	*1.01*
			- Violet pericardium variation	*3*	*0.76*
			Total	9	2.27

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Table.5: The sequences of 7 selected RAPD primers and the amplification results on C. La bell "Anna Belle"

Primer No.	Primer code	Sequences (5' → 3')	Total amplified fragments (a)	Polymorphic amplified fragments (b)	Polymorphic ratio (b/a x 100), %
1	OPA 01	CAGGCCCTTC	7	5	71.4
2	OPA 10	GTGATCGCAG	12	12	100
3	OPD 02	GGACCCAACC	10	9	90.0
4	OPD 05	TGAGCGGACA	11	10	90.9
5	OPD 06	ACCTGAACGG	11	7	63.6
6	OPD 07	TTGGCACGGG	13	13	100
7	OPD 08	GTGTGCCCCA	12	11	91,7
Sum			64	67	
Mean			10.7	9.6	86.8

Table.6: Percent of similarity matrix from wild type C. La bell "Anna Belle" and 3 selected variation lines

	CLB-ctrl	CLB-30Gy-1.1	CLB-30Gy-2.1	CLB-30Gy-3.1
CLB-ctrl	100			
CLB-30Gy-1.1	80.0	100.0		
CLB-30Gy-2.1	46.7	60.0	100.0	
CLB-30Gy-3.1	60.0	66.7	53.3	100

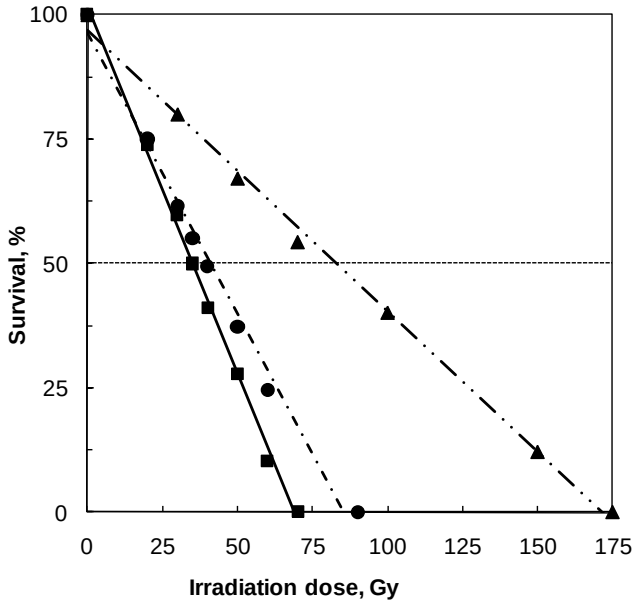

Fig.1: Survival ratio of Cymbidium La bell "Anna Belle" samples irradiated by γ-rays after cultivation of 4 months. (■): PLBs, (●): shoots buds and (▲): plantlets

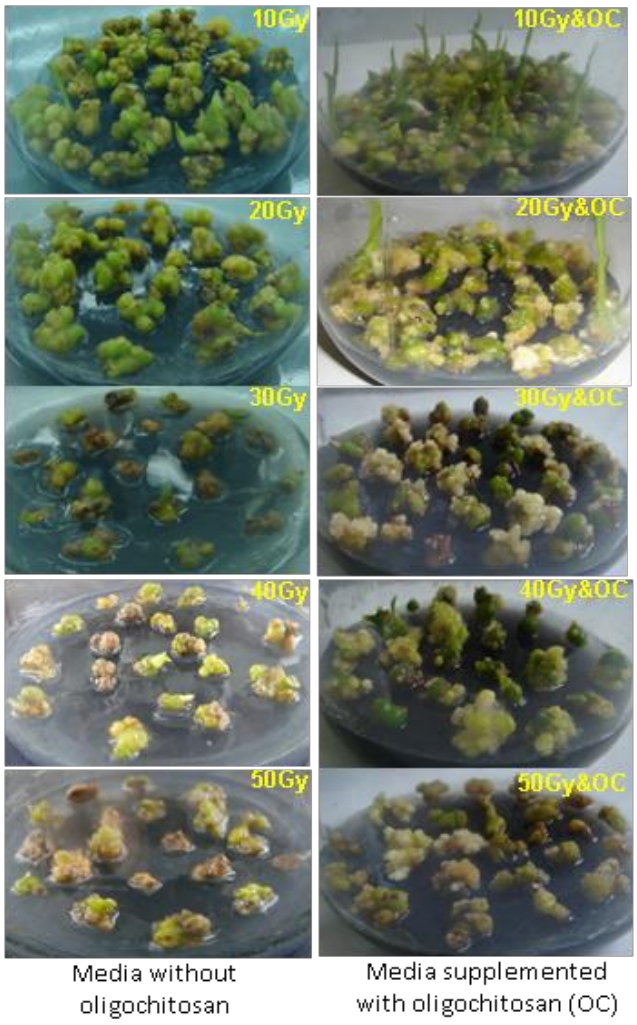

Fig.2: The development of PLBs after irradiation at various doses and cultured on media supplemented with and without oligochitosan

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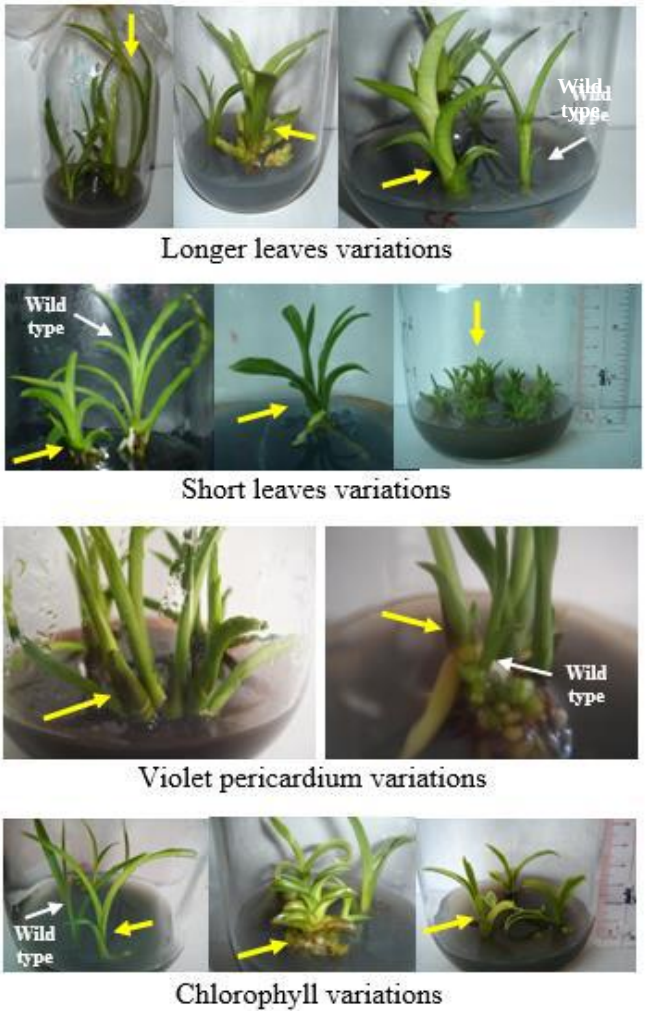

Fig.3: The in vitro variation types of Cymbidium La bell "Anna Belle" generated by γ-rays irradiation

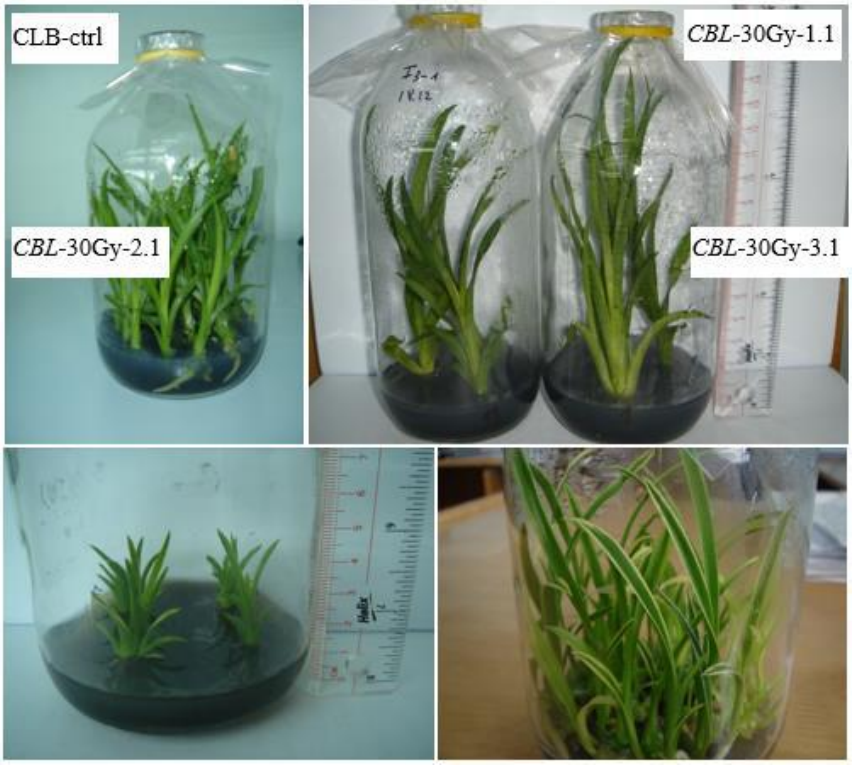

Fig.4: The stable variant lines of Cymbidium La bell "Anna Belle" generated by γ-rays irradiation at 30 Gy. CLB-ctrl: Wide type, CLB-20Gy-1.1: Large leave variation, CLB-20Gy-2.1: Short leave variation, and CLB-20Gy-3.1: Chlorophyll variation

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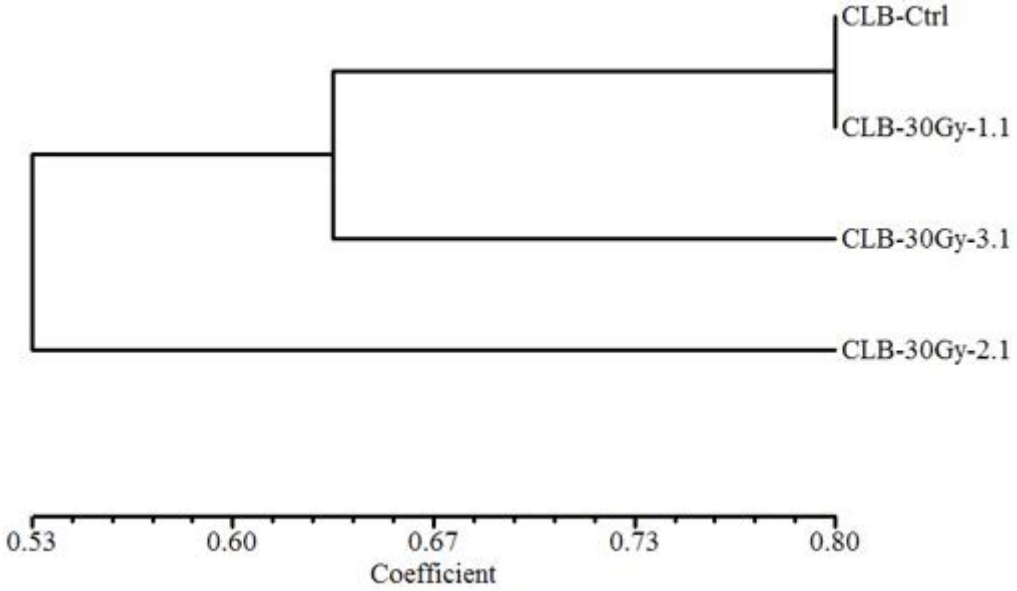

Fig.5: Dendrogram of the genetic relationship between the wild type and 3 selected variation lines of C. La bell "Anna Belle"

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The Parity Rate of Indoor-Resting Adult Female *Anopheles* and *Culex* Mosquitoes and Their Implication in Disease Transmission in Nnamdi Azikiwe University Female Hostels Awka, South Eastern Nigeria

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***Abstract*—** *A study on the parity rate of indoor-resting Anopheles and Culex mosquitoes and their implication in disease transmission was carried out in Nnamdi Azikiwe University female hostel between June and July 2016. The mosquitoes were sampled weekly from 24 randomly selected rooms using pyrethrum knock-down collection (P.K.C). A total of 516 mosquitoes comprising of 4 species: Anopheles gambiae, Anopheles funestus, Culex quinquefasciatus and Culex annulioris, were collected during the study period. The mosquitoes were examined for their abdominal gradings/gonotrophic stages and dissected for parity determination. Culex quinquefasciatus (61.43%) constituted the most abundant species followed by Anopheles gambiae (30.04%) and Anopheles funestus (7.56%) and the least being Culex annulioris (0.97%). Results showed that majority of the vector species were fed and parous and variations among the parity rates of the 4 species was significant (P <0.05). The high rate of the fed and parous mosquito species is of utmost concern in the hostel environment and therefore control measures aimed at eliminating the breeding sites and reducing its contact with the students should be embraced and practiced so as to minimize disease transmission among the students.*

***Keywords*— Anopheles, female hostel, indoor-resting, mosquitoes, parity.**

I. INTRODUCTION

Mosquito-borne diseases are major health problems in Nigeria as in other parts of sub-saharan Africa. Apart from malaria, other mosquito-borne diseases have also accounted for huge economic loss, social disgrace, low productivity, absenteeism, sleeplessness among others in many parts of the country (Anosike *et al*., 2003). Malaria and lymphatic filariasis are mosquito-borne diseases that account for the largest global burden of mortality and morbidity in the world's poorest countries (WHO, 2010). More than half of the world's population is at risk of at least one of the diseases. Malaria is caused by *Plasmodium* parasite and transmitted by *Anopheles* mosquitoes. According to the latest estimates from WHO (2015), there were an estimated 438, 000 malaria deaths worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). It is highly endemic in Nigeria with about 97% of people at risk of the disease (PMI, 2013). Lymphatic filariasis (LF) which is one of the most debilitating neglected tropical disease (N.T.D) in the world is caused by the parasitic worms:*Wuchereria Bancrofti, Brugia malayi and Brugia timori* and is transmitted by *Anopheles, Culex, Aedes* and *Mansoni* mosquitoes (WHO, 2010). The disease is endemic in 81 countries with an estimated 120 million people infected and 40 million people with clinical manifestations including lymphoedema (elephantiasis) of the limbs and urogenital disorders, especially hydrocoele in men (WHO, 2010). Nigeria bears the highest burden of lymphatic filariasis in Africa, with an estimated 80 to 120 million people at risk (Hotez *et al*., 2012). It is common to find malaria and lymphatic filariasis in the same human population and sharing the same mosquito vectors (Burkot *et al*., 1990). It is therefore,

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common to find co-infections of malaria and lymphatic filariasis parasites in a single mosquito vector in these areas. The diseases have been observed to co-exist in some parts of Nigeria, such as New Bussa, Niger State (Awolola *et al*., 2006). These diseases cause high death toll on both human and animal populations and lead to poor socio-economic development of many countries.

The transmission of mosquito-borne diseases to human by their vectors is achieved through blood-feeding (Kuno, 1995; Lehane, 2005; Scott and Takken, 2012). The mosquito's reproductive cycle starting with the blood-meal and ending with egg-laying, also called the gonotrophic cycle (GC), is continous during the life of the female and is temperature-dependent (Pant *et al*., 1973; Saifur, 2012). During their bloodmeals, females can ingest the disease-causing pathogen that will disseminate into the mosquitos' body by passing first into the midgut, then crossing the intestinal barrier, amplifying into the haemocoele and eventually reaching the ovaries and salivary glands. The percentage of females that have already deposited their eggs, called the parous females, increases as the age of the mosquito population increases, concomitantly with the transmission risks. Many studies have attempted to estimate the survival of mosquitoes directly through capture-mark-release-capture (Fouque *et al*., 2006; Maciel *et al*., 2007; Harrington *et al*., 2008) or indirectly with the parity rates assuming a direct relationship between parity rates and survival (Davidson, 1954; Garret-Jones and Grab, 1994; David *et al*., 2012). The first method although more accurate are not available for routine surveillance. On the contrary, the estimation of parity rates from field collection is more feasible hence the aim of this study.

II. MATERIALS AND METHODS

2.1 Study Area

The study was conducted from June to July 2016 in Stella Okoli and Dora Akunyili Female hostels of Nnamdi Azikiwe University in Awka, Anambra state. Nnamdi Azikiwe is located in Awka, Awka-south L.G.A, Anambra state. It is a semi-urban area with geographical co-ordinates of 6.25^0N and 7.12^0E (Unizik portal.edu.ng). The school is located in the tropical rainforest zone, although it has derived savanna vegetation. The daily temperature ranges from 27^0C-30^0C between June and December but rises to 32^0C-34^0C between January and April, with the last few months of the dry season marked by intense heat. It has a relative humidity of 70% reaching 80% during rainy season and an annual rainfall of about 2000mm (Iloeje, 2001). The hostels are made up of 98 rooms each with 4 students in each room, is surrounded by tall trees, relatively tall grasses and shrubs. There are ground collections of stagnant water due to rainfall in the rainy season and the side gutters are heavily filled with stagnant waste water from student activities. The collections of water in the environment therefore serve as breeding sites for mosquitoes.

2.2 Selection of Rooms for Survey

A cross-sectional design was used to conduct this study. The two female hostels are made up of 196 rooms (98 in each). Twelve rooms were randomly selected from each of the hostels total of 196 rooms; 98 for each hostel, 12 rooms for sampling adult mosquitoes. Rooms selected for study were the ones where people slept prior to Pyrethrum knock-down collection (PKC).

2.3 Collection of Mosquitoes

Pyrethrum knock-down collection was employed in collecting the indoor-resting adult mosquitoes. This was conducted thrice in a week in the early morning hours between 6:00 and 8:30am. Prior to the collections, edible and fragile materials were evacuated from the rooms. The floor is covered with a large white sheet. Thereafter, windows and doors were closed and eaves were stuffed to prevent escapees. The room is sprayed with commercial pyrethrum-based aerosol (Raid) on every corner of the rooms in a clockwise direction until the rooms were filled with the insecticidal mist. After 15 minutes, the room was opened and the sheet was carefully picked up at the corners and taken outside. The knocked-down mosquitoes were picked with the aid of forceps and transferred into labelled petri dishes lined with moist cotton wool and filter paper for preservation. The collections were identified in the Department of Parasitology and Entomology Laboratory.

2.4 Identification and Grading of Mosquito Species

The collections were identified using gross morphological keys described by Gillet (1972). Sexing of the mosquitoes was done and only the female mosquitoes were retained. The gonotrophic stages/abdominal grading of the species were examined according to the external appearance of the stomach contents as described by WHO (1994). They were classified as unfed, fed, half-gravid and gravid.

2.5 Dissection of Mosquito Species

The unfed and fed mosquitoes were dissected using entomological dissecting pins under a stereo microscope. The wings and legs of each mosquito were removed while the remaining part was dissected by placing it on a clean grease-free slide containing a drop of normal saline which was substituted for distilled water. The ovarian tracheoles was then observed to determine if the female was parous or nulliparous (Detinova, 1962). The uncoiled ovarian

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tracheoles are referred to as nulliparous while the coiled as parous.

2.6 Statistical Analysis

Analysis of variance was used to test the significant difference in the densities of the weekly-collected mosquito species. It was also used to test for significant differences in the parity rates of the different species.

III. RESULTS

A total of 516 female mosquitoes comprising of 4 species were collected during the study period. They were; *Anopheles gambiae*, *Anopheles funestus*, *Culex quinquefasciatus* and *Culex annulioris* (Table 1). Out of the 4 species, *Culex quinquefasciatus*, 317 (61.43%), was the highest collected species followed by *Anopheles gambiae* (s.l), with a total number of 155 (30.04%). *Culex annulioris* was the least with a total number of 5 (0.97%). There was no significant difference in the density of the 4 mosquito species (p>0.05). The table 2 showing the abdominal grading of the various mosquito species reveals 217(42.05%) mosquitoes fed, 140 (27.13%) half-gravid, 81 (15.70%) unfed and 78 (15.12%) gravid. The fed mosquitoes are higher than the unfed mosquitoes. Fed and unfed mosquitoes were seen in *An. gambiae*,*An. funestus* and *Culex quinquefasciatus* but *Culex annulioris* were only fed, 4 (80.00%) and gravid, 1 (20.00%). Variations in the abdominal grading/gonotrophic stages of the mosquitoes was not significant (P>0.05). The number of parous mosqutioes is higher than nulliparous mosquitoes. *Culex quinquefasciatus* has the highest number of parous 108 (53.2%) and nulliparous mosquitoes 46(54.8%) while *Culex annulioris* has the least 1(0.5%) and 3(3.6%) respectively. It has also been observed that the total number of parous and nulliparous mosquitoes decreased down the weeks. Variations in the parity rates of the different species were significant ($p < 0.05$).

Table.1: Species composition of adult female mosquitoes collected during the studies

Weekly collection	Anopheles gambiae (%)	Anopheles funestus (%)	Culex quinquefasciatus (%)	Culex annulioris (%)	Total (%)
Week one	66 (42.58)	21 (53.85)	149 (47.00)	4 (80.00)	240 (46.51)
Week two	47 (30.32)	15 (38.46)	102 (38.18)	0 -	164 (31.78)
Week three	42 (27.09)	3 (7.69)	66 (20.82)	1 (20.00)	112 (21.71)
Total	155 (30.04)	39 (7.56)	317 (61.43)	5 (0.97)	516

Fcal =0.28 <Ftab = 4.35 (P>0.05)

Table 2: Gonotrophic stages of the adult female mosquito species

MOSQUITO Species	Anopheles gambiae (%)	Anopheles funestus (%)	Culex quinquefasciatus (%)	Culex annulioris (%)	Total
Unfed	20 (12.90)	7 (17.95)	54 (17.03)	0 -	81 (15.70)
Fed	83 (53.55)	18 (46.15)	112 (35.33)	4 (80.00)	217 (42.05)
Half-gravid	34 (21.94)	10 (25.64)	96 (30.28)	0 -	140 (27.13)
Gravid	18 (11.61)	4 (10.26)	55 (17.35)	1 (20.00)	78 (15.12)
Total	155(30.04)	39(7.56)	317(61.43)	5(96.9)	516

Fcal =2.42 < Ftab = 3.49 (P>0.05)

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Table.3: Percentage of parous and nulliparous adult female mosquito species collected

Weekly collection	*Anopheles gambiae* (%)		*Anopheles funestus* (%)		*Culex quinquefasciatus* (%)		*Culex annulioris* (%)		Total no. of parous mosquitoes (%)	Total no. of nulliparous mosquitoes (%)
	P	NP	P	NP	P	NP	P	NP		
Week one	29 (37.7)	10 (52.6)	11 (73.3)	4 (66.7)	46 (42.6)	21 (45.7)	1 (100.0)	2 (66.7)	87 (42.9)	37 (44.0)
Week two	24 (31.2)	4 (21.1)	6 (75.0)	2 (33.3)	44 (40.7)	19 (41.3)	0 -	0 -	74 (36.4)	25 (29.8)
Week three	24 (31.2)	5 (26.3)	1 (100.0)	0 -	18 (16.7)	6 (13.0)	0 -	1 (33.3)	43 (21.2)	12 (14.3)
Total	77 (38.0)	19 (22.6)	18 (8.9)	6 (7.1)	108 (53.2)	46 (54.8)	1 (0.5)	3 (3.6)	203	84

*P = Parous and NP = Nulliparous*Fcal =3.5> Ftab = 3.50 (P<0.05)

IV. DISCUSSION

Mosquito-borne diseases still remain a major public health problem in Nigeria and their transmission is becoming frequent on daily basis due to widespread of mosquitoes as a result of increasing breeding sites and conducive environments. The presence of the four species of mosquitoes found in the study is an indication that the climatic and environmental condition of the female hostel is conducive to support their breeding, development and survival. The relatively high density collected during the study period indicates the preponderance of breeding sites around the area and this implicates the observed lapses in sanitary conditions; aiding the preponderance of stagnant water bodies around the hostel.

The most abundant mosquito species collected was *Culex quinquefasciatus*. This could be most likely explained by the presence of large blocked drainages with very dirty stagnant water, ground collections of dirty water, soak-away pits among others which serve as their breeding grounds. This result is in agreement with the study of Adeleke *et al*. (2010) who recorded*Culex quinquefasciatus* to be the second predominant species of mosquitoes occurring in Abeokuta. Also, Okorie *et al*., (2011), recorded *Culex* species (98%) as the most abundant of all the mosquito species in Ibadan. The environment however, may not have favoured *C. annulioris*, that may be the reason why the species has the least occurrence.

On the other hand, a good number of *Anopheles* species were collected. Although the density is quite reduced compared to the results of Onyido *et al*. (2008) who recorded 69.45% of *Anopheles gambiae* and 15.27% of *Anopheles funestus* in indoor collection in the same. The reduction of the number may be as a result of government sponsored intervention programmes that have taken place to control mosquitoes for some years now, example is indoor residual spraying (IRS) and sharing of insecticide treated mosquitoes nets. No matter the number, it is still a source of threat to the students staying in the hostels.

The need for blood by adult female mosquitoes to develop their eggs is one of the reasons that they have become successful vectors of tropical diseases. Results from the study shows that much more mosquitoes were bloodfed. Thus, a greater percentage of the mosquitoes have had contact with human host during bloodfeeding and as such, there could be a greater tendency of the infected mosquitoes to have transmitted parasites like *Plasmodium* and/or filarial

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worms that cause malaria and filariasis respectively which are the two important human tropical infections that are endemic in Africa (Ejezie and Akpan, 1992). The proportion of *Anopheles gambiae* and *Culex quinquefasciatus* that were fed were higher than the other *A. funestus* and *C. annulioris*. This was not surprising as both species are anthropophilic. This finding was observed by Ebenezer *et al*. (2013) during a similar study in some parts of Bayelsa state and by Adeleke *et al*. (2010) in Abeokuta. The high percentage of bloodfed mosquitoes clearly shows that the two genera; *Anopheles* and *Culex*, are not just endophagic but also endophilic as they were caught while resting in their hidden corners.

Consequently, there was high rate of parous females which is in accordance with the recorded 75% of parous mosquitoes collected indoors by Uttah *et al*. (2013) at Ekorinim area of Calabar, Cross River state and contrasted with Adeleke *et al*.(2010) who recorded higher percentage of nulliparous mosquitoes which he explained could be as a result of high productivity of their breeding sites. The present result suggest that majority of the mosquitoes were able to obtain a bloodmeal and complete at least one or more gonotrophic cycles and thus indicates high survival rate and high vectorial capacity of disease transmission as only the parous flies could transmit diseases. Also the majority of the mosquitoes being parous indicate that there are older populations of them which might be as a result of failure or reduced application of vector control measures and interventions in the hostel and its environs. It may also be those who have resisted and survived the methods of intervention.

Parity among mosquitoes may have a bearing on disease circulation. This is because mosquitoes that have already acquired pathogens while feeding, have increase tendency of transmitting these pathogens especially parasites when they seek a fresh bloodmeal. According to Hardy *et al.* (1983), mosquito feeding behaviour under different ecological conditions may contribute to its longevity and consequently influence the transmission of mosquito-borne diseases. Thus, the more frequent a mosquito bloodfeed, the more likely will it transmit diseases.

V. CONCLUSION

The relative high density of mosquitoes encountered in this study is source of threat to the students and therefore is of public health concern. The high percentage of fed and parous mosquitoes also encountered exposes the students to suffering mosquito-borne diseases being transmitted by them which they have little or no natural resistance to and consequently affects their academics for a reasonable period of time. Strong emphasis should be laid on the importance personal protection and environmental hygiene in the University environment and hostel management should make it a policy which every student should abide by.

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Comparison between Manual and Automatic Identification of Diatoms of Merja Fouarate (Morocco)

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Abstract— *The objective of this work is to compare the results of the classical identification of diatoms by optical microscope to the automatic identification of the diatoms. For the first method, we determined the diatoms using an optical microscope and the identification keys.*

Concerning the second method, we relied on the processing and analysis of the images to automatically recognize a diatom. Our aim is to compare the results of the manual identification of diatoms with the results of the automatic identification of the same sample. The purpose of this comparison is to verify whether the automaticanalysis can give acceptable results in order to replace the manual determination. We used the Image j software for the development of our program and based on the notion of points of interest and the freeman code.

The results of the determination of the diatoms by optical microscope which lasted more than one month did not exceed 92% (for 104 species of diatoms 96 species were identified), whereasthe automatic identificationrequires only a few seconds, with much better results (97%).

Keywords— Diatoms, current, identification by microscope, automatic identification, digital image processing, Fouarate, Kenitra, Morocco.

I. INTRODUCTION

Rich in thousands of species (Mollo and Noury, 2013), diatoms are diversified into several groups, genera and species, and have been the subject of extensive discussions and divergent opinions concerning the taxonomy and nomenclature (Tolomio, 2011). This diversification presents morphological variations making the identification of diatoms by optical microscope tedious (J. Prigiel and Coste, 1993; Chahboune et *al.*, 2015; Siddour *et al.*, 2007; Manoylov. 2014) and heavy for the uninitiated. Automatic image recognition provides a valuable help both for the identification of diatoms (Chahboune *et al.*, 2015), and especially as a teaching tool to help in identification for new diatomists.

Our study aimed to use two identification methods of the diatoms of the Merja Fouarat, one is the classical method which is based on the identification key using the optical microscope, the other is the automatic method which relies on image analysis and interpretation techniques. The comparison of the two methods allowed us to define to what extent the automatic identification method can meet the challenges encountered in order to identify the diatoms precisely and in a very short time.

II. MATERIALS AND METHODS

1- Characteristics of the study sites

Located on the plateau of Mamora, between the pli-quaternary clay plain of the Rharb and the granitic, paleozoic , western schisto-sandstone Meseta, the Merja Fouarat is the site of a water table located in the sand and limestone sand of the Plio-Villafranchien (Combe, 1975), with Mediterranean climate and pronounced oceanic influence (sub-humid low temperature, temperate in winter).

The MerjaFouarat is fed by:

- The inflows of Oued Fouarat representing the first important valley of the Mamora plateau (Thauvin, 1966) with a source located at Ras-El-Aîn, about 10 km south of the Merja;
- Abundant and close rainfalls lead to the exfiltration of natural water from the groundwater (Nassali *et al.*, 2002), accompanied by short duration floods;
- Wastewater from peripheral neighborhoods.

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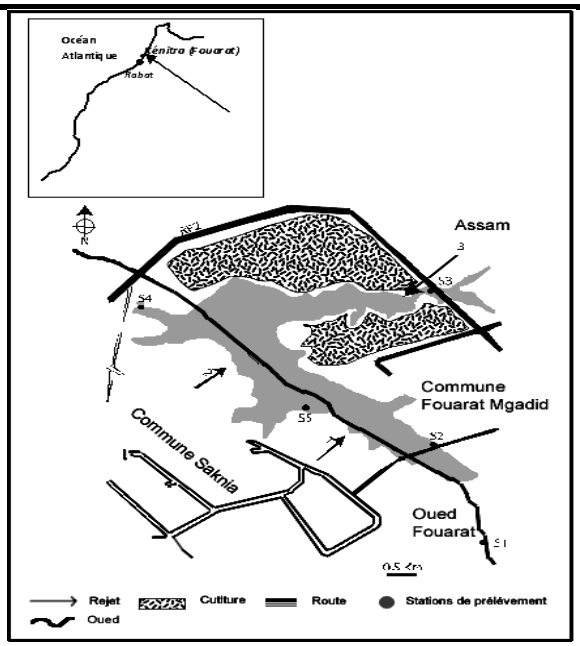

Fig.1: Location of study sites

Caption: S1: Reference station: (Oued Fouarat)
S2, S3, S4 and S5: Stations along the Merja Fouarat

2- Sampling

At the five stations, three diatom sampling campaigns were performed. The diatoms were removed by scratching natural substrates using a nylon bristle brush. The samples thus obtained were immediately fixed in formalin (10%). After a few days of decantation, the diatoms were treated with nitric acid on a hot-plate then mounted with naphrax between the blade and the lamella.

3- Identification of diatoms

The identification of diatoms by optical microscope was carried out with the help of the work of Germain, 1981; Krammer and Langbertalot, 1986, 1988, 1991a and b). The optical microscopic counts of at least 450 frustules were performed on permanent preparations. The numbers of each taxon were transformed into relative abundance (Chahboune *et al.*, 2011). The automatic identification consisted of a classification from the same sample. This identification relied on the software Image j (Chahboune *et al.*, 2015), a digital image processing application developed at the National Institute of Health (Wayne, 1997).

III. RESULTS AND DISCUSSION

The identification of the diatoms by microscope required an enormous effort both in terms of learning, which lasted five months in order to acquire the skills of a diatomist, and in terms of the determination of the diatoms which lasted longer than a month with a result approaching 92%, for 96 diatom species out of 104. 8 species (*Navicula* sp 1, *Navicula* sp2, *Navicula* sp3, *Nitzschia* sp1, *Nitzschia* sp2, *Gomphonema* sp1, *Gomphonema* sp2, *Surerella*) were difficult to classify. Indeed, the particularities of some potentially more complex species are difficult to solve (Tolomio, 2011; Ector and DašaHlúbiková, 2010; Lavoie, 2008; Coste and Ector, 2000).

The automatic identification in turn required a pre-processing phase of the images and the results were around 97%. The automatic classification work confirms our approach:

- ADIAC teams have achieved good classification rates above 96% on a much larger sample, even surpassing the results of human experts. (Jalba *et al.*, 2004).
- The work of (Siddour *et al.*, 2007) which consisted in characterizing the contour of the diatoms according to a vector of the Fourier descriptors from a database of diatom images, obtained a good classification rate of 97%. As for (Claudon, 2007), he presented a classification method based also on the windowed Fourier transform, but taking into account the internal structures and the ornamentations of the diatoms. His analysis on a reference sample was approaching 100%.

Therefore, we find that the automatic classification is effective. However, the automatic recognition of diatoms is a long term project because its success requires that the image database be continuously supplied. Clearly, the system we have developed can only identify the diatoms listed. If a diatom does not exist in the database, it will not be recognized. Further work should help fuel the image database.

IV. CONCLUSION

We can infer that automation has a double contribution: it allows a rapid identification with great success results, and especially allows the novice to avoid a tedious learning.

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Operational parameters affecting the removal and recycling of direct blue industrial dye from wastewater using bleached oil mill waste as alternative adsorbent material

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***Abstract*—** *In this work the ability of "bleached" oil mill solid waste to reduce the dyestuff content in industrial textile wastewater was studied. Bleaching treatment consists in a preliminary oil mill solid waste management with NaOH and $NaClO_2$ for obtaining cellulosic materials, mainly removing lignin from the waste surface. Thus, a novel bioadsorbent from agricultural residues, named bleached olive pomace (OP), was presented. Direct Blue 78 was studied as a model azoic dye. Experiments were planned to study the effect of different initial conditions on the adsorption processes: oil mill waste amount as grains and as a fine powder (OP_P), solution temperature values, initial dye concentration, pH values and electrolytes influence. The results showed that the adsorption process using bleached oil mill waste determined an excellent degree of water color reduction, reaching the best work conditions when pH 2 and OP_P were used. The presence of electrostatic interactions was also suggested. The adsorption appeared to be influenced by temperature values showing an endothermic character. Interestingly, to confirm the role of ionic interactions between dye and sorbent at pH 2, fashionable results were obtained. The adsorption process was verified also at pH 6 with 100% of dye removal in presence of both NaCl and Na_2SO_4 avoiding the aforementioned strong acid conditions. A very important aspect of this work is the recycle of both the dye and the adsorbent, with particular attention to the dye reuse for coloring cotton fabric.*

***Keywords*— Adsorbent recycle, adsorption, bleaching process, Olive pomace, textile dyes.**

I. INTRODUCTION

In this generation, environmental questions such as water contamination are becoming progressively important.[1] As reported recently by Ertugay *et al.*[2] more than 25% of the total world population suffers from health and hygienic problems related to pollutant inflowing water.[2] Indeed, with the human development and improvement of technologies, large amounts of wastes are discharged every day into water. The composition of these pollutants encompasses a variety of contaminants as heavy metals, dyes, and/or other undesirable chemical compounds. The problem get worst and becomes more serious when dyes from textile industries flow into the water.[1,3] The nature of dyestuffs is very large and they are usually classified in accordance with the dyeing processes: some interact by ionic bonds or form covalent bonds or interact by electrostatic forces, and others interact by hydrophobic forces. It is worth to mention that among these, azo dyes are the dominant class among commercial dyes and belong to the category of direct dyes, largely used to color cellulose fibers mainly interacting through hydrophobic forces.[4] Nonetheless, the release of azo dyes into environment is of great concern, due to both their highly visible color in water, and their toxicity, mutagenicity and carcinogenicity, it still continues to find new applications in high-technology areas.[5,6] Although the amount of discharged dyes in water is not exactly known, recently Pirkarami *et al.*[7] reported that more than 5000 tons of dyeing materials are drained into the environment every year, affecting the human life and the global ecosystem.[7] Indeed, dyes are stable to light and heat, they have a high organic content, and the most part of them have complex aromatic structures not biodegradable.[8] As a result, the removal of synthetic dyes from industrial effluents is a great actual challenge. In this field the Authors of this paper have years of experience presenting innovative methods and materials with great performances in

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decoloring wastewater.[9-12] Overall, for these purposes, physical adsorption is presented as a simple method with several advantages: ease of operations, simplicity of design, high efficiency and low cost applications in water decoloration processes. Among adsorbent materials for wastewater treatment, the agricultural wastes are very interesting sorbent materials requiring little processing, but showing good adsorption capacity, selectivity, low cost, free availability and easy regeneration. However, as reported by Sayğili *et al.*[8], the application of untreated agricultural or plant wastes as adsorbents can also bring several problems and then, with the aim of improving their performance, the wastes are modified with different reagents and methods.[8] Regarding to the adsorbent used in this paper, *i.e.* oil mill solid residue (olive pomace), a chemical treatment by means of acids, bases or H_2O_2 are generally proposed in literature.[13] As alternative, the thermal pretreatments is also suggested, with the most part of papers related to the achievement of active carbon from oil mill solid waste. In this context, as recently evidenced by ourselves[9], a very deep survey of papers related to the olive pomace use in dye removal from water showed as the number of publications was not high, being the most part of them focused on the heavy metal removal or requiring strong pre-treatment conditions.[9] In the same paper, we presented the performance of the olive pomace in removing disperse dyes from wastewater, by previously adsorbent treatment in hot water at 100°C, showing also the recycle of both the adsorbent material and dyes.[9]

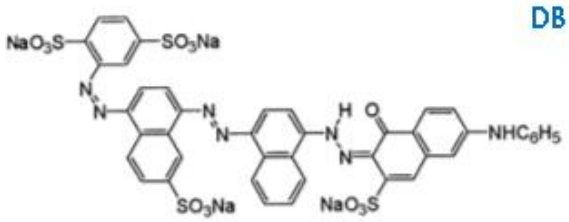
In this article, we show the best conditions adoptable to eliminate another class of dyes, direct dyes, from water enabling also the possibility to recover and reuse the dye itself. For the first time, the olive pomace was treated with NaOH and $NaClO_2$/acetic acid (bleaching process) to gradually remove lignin and holocellulose, in turn restituting mainly cellulose characterized by a high affinity with azo dyes.[14] Indeed, this procedure is usually used to obtain cellulose material from natural plant fibers.[15] As for the adopted model dye, Direct Blue 78 (DB) received by Colorprint Fashion, a Spanish textile industry, was used to reduce the contamination of colored water used for dyeing processes. Overall, effluent treatments aiming to reduce the amount of dyes having a structure similar to DB, are known in literature, as for example degradation methods through Fenton's oxidation process.[2]

The Direct Blue 78 photocatalytic degradation using TiO_2 nanoparticles immobilized on recycled wool-based nonwoven material, was presented by Markovic *et al*.[16] The DB 71 adsorption from aqueous solution onto pistachio hull waste as a low-cost adsorbent was also presented by Biglari *et al.*[17] Activated carbon and poly pyrrole polymer composite prepared from *Thevetia Peruviana* were presented as materials to adsorb DB 71. [18] Without doubt the list is not complete for describing the plethora of studies in literature about the azoic blue dye removal or its degradation in aqueous environment, however in the present paper we highlight the use of a handy material exhibiting very high capabilities in sequestering the direct dye within few minutes in appropriate conditions. Moreover the recycle of DB 78 by means of desorption was obtained, enabling the possibility to color again cotton fibers, with

3 an environmental friendly green cycle and an alternative use of oil mill waste. As a whole the environmental disorder was reduced.

II. MATERIALS AND METHODS

2.1 Chemicals. All the chemicals used were of analytical grade and samples were prepared using double distilled water. Direct Blue 78 (chemical formula: $C_{42}H_{25}N_7Na_4O_{13}S_4$, MW: 1055.1 g×mol^{-1}) reported in Scheme 1, was received by Colorprint Fashion, S.L and used without further purification. Dye stock solutions with a concentration of 1.0×10^{-4} M were prepared and dilutions were carried out with double distilled water in order to obtain different dye concentrations namely 5×10^{-5} M and 1.0×10^{-5} M. The pH of the various aqueous solutions was adjusted using concentrated HCl and NaOH solutions. NaOH and HCl were purchased from Sigma-Aldrich (Milan, Italy). The same commercial source was also adopted for the following chemicals: Acetic acid (99,9 %), $NaClO_2$, NaCl and Na_2SO_4.



Scheme.1: Chemical structure of Direct Blue 78.

2.2 Preparation of the biosorbent. The biosorbent material was the solid waste of oil mill named Olive Pomace (OP), obtained during the oil production. OP was obtained from a local oil mill settled in Bari, south of Italy. OP was treated exploiting a procedure generally used to bleach natural fibers for removing impurities, lignin and holocellulose.[14,15] Several experimental conditions were studied, however among these the following procedure was proposed in this paper:

a) Alkaline treatment**:** 26.00 g of OP were boiled, for 2 h, in 140 mL NaOH 3M with continuous magnetic stirring.

b) Bleaching: The OP was subsequently boiled, for 2h, in 70 mL of $NaClO_2$ 1,7% w/w and 70 mL of Acetic buffer, 0.5M.

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The experiments were performed both using OP as obtained after the treatment and sieved (OP_P) obtaining a fine powder.

2.3 Experimental procedures. The experiments were conducted in 10 mL glass beakers containing known concentrations of dye solutions. The effects of the biosorbent and dye dosages on dye removal from wastewater, were assessed changing the amount of OP/OP_P, from 0.10 to 1.00 g, at 5×10^{-5} M and 1×10^{-5} M of dye concentration. The effect of both temperature and salt concentration were also evaluated in the range 25 ÷ 70°C and 5×10^{-3} M ÷ 1M, respectively. The mixtures were stirred at 140 rpm for different contact times using a digitally controlled magnetic stirrer. The adsorption process was studied following the DB absorption spectrum evolution at 600 nm when the solution was in contact with the pomace. In accordance with papers reported in literature[9] the adsorption capacity q_t (mg×g^{-1}) at time *t* of dye, was inferred by applying the following equation (1):

$$q_t = \frac{C_0 - C_t}{W} \times V \qquad \text{Equation 1}$$

where *V* represents the adopted total volume of solution (herein 10 mL), *W* is the weight of the dry adsorbent material (g), C_0 and C_t represent the initial concentration and the concentration at time *t* of the dye (mg×L^{-1}).

2.4 Desorption studies. The DB desorption studies from pomace surface were carried out by using a basic solution at pH 12. The olive pomace was loaded with dye's initial concentration (5×10^{-5} M in 10 mL at pH 2) for 15 minutes. The dye loaded pomace samples were separated from the initial dye solutions, then washed with distilled water for the removal of unadsorbed dye and placed in contact with 10 mL of a 0.01 M of NaOH solution. The efficiency of desorption was calculated by using the following equation: where m_d is the amount of dye desorbed and m_a is the amount of dye adsorbed.

$$E\,\% = \frac{m_d}{m_a} \times 100 \qquad \text{Equation 2}$$

The same Equation was used to calculate also the efficiency of adsorbed dye. In that case m_d is the amount of dye adsorbed and m_a is the initial amount of dye.

2.5 Dyeing experiments. The dyeing experiments were performed dyeing cotton pieces (1cm×1cm) for 60 minutes at 95°C in presence of increasing amounts of sodium sulfate to promote the dye exhaustion, that is the process of dye transferring from the water to cotton fibers.

2.6 Visible and FTIR-ATR spectroscopic measurements. Visible absorption spectra were recorded using a Varian CARY 5 UV-Vis-NIR spectrophotometer (Varian Inc., now Agilent Technologies Inc., Santa Clara, CA, USA). FTIR-ATR spectra were recorded within the 600–4000 cm^{-1} range using an Fourier Transform Infrared spectrometer 670-IR (Varian Inc., now Agilent Technologies Inc., Santa Clara, CA, USA), whose resolution was set to 4 cm^{-1}. 32 scans were summed for each acquisition.

2.7 Scanning Electron Microscopy (SEM). In the case of SEM analysis, an electron microscope FESEM-EDX Carl Zeiss Sigma 300 VP was used. The samples were fixed on aluminum stubs and then sputtered with graphite by the use of a Sputter Quorum Q150.

III. RESULTS AND DISCUSSIONS

Fig. 1 (panels A and B) reports the SEM images of the adsorbent material. The morphology investigation shows the presence of irregular domains that conferred to OP the important features to be a material having a high porous character. In detail, Fig. 1A and Fig. 1B show OP before and after the adopted bleaching process, respectively. Interestingly, as it can be seen directly from Fig. 1B, the surface of the biosorbent occurred not affected by the bleaching process, maintaining the porous surface already observed in Figure 1A, which can be better appreciated in Fig. 1C. Indeed, changing the magnification ratio, the presence of cavities and irregular islands are better evidenced in that figure enabling OP to host dye molecules from aqueous solutions.

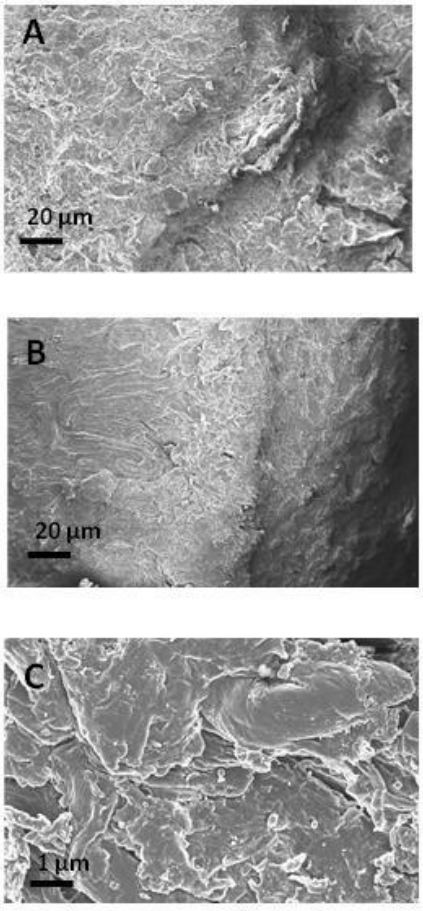

Fig. 1: SEM images of olive pomace before (A) and after the treatments with a scale bar of 20 µm (B) and 1 µm (C).

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OP was recently presented in literature by ourselves for the removal and recover of a disperse dye from wastewater, using OP simply washed with hot water in order to remove impurities.[9] Using the OP treated in the same way for removing direct azo dyes (data not shown), it is possible to obtain the dye removal, but not its recovery. This led us to change the treatment procedure of biosorbent, obtaining also a quick dye removal using suitable conditions of work. In detail, the OP was subjected to an alkaline treatment followed by a bleaching procedure. The changes induced by these new processes can be appreciated observing the FTIR-ATR spectra reported in Fig. 2A and Fig. 2B. Before the treatment (Fig. 2A), OP showed typical bands indicating the presence of lignin, cellulose and cellulose-like structures as main components.[9]

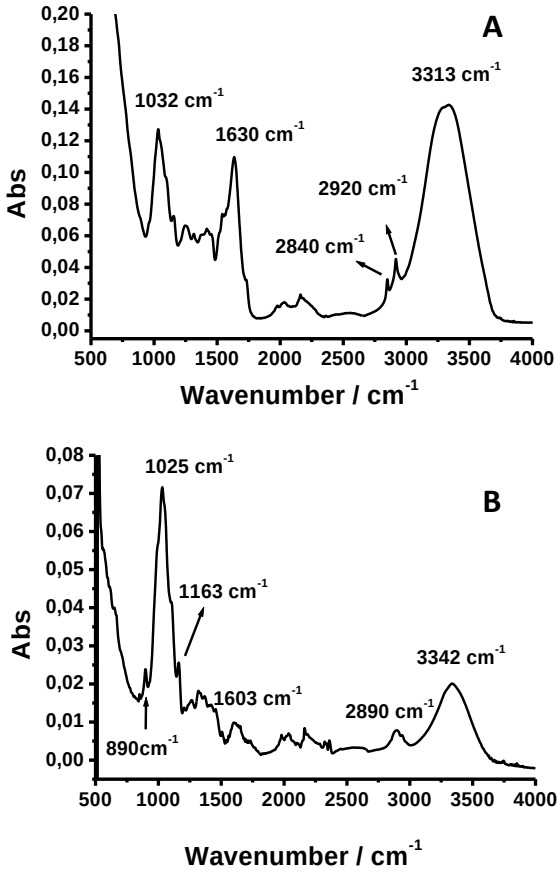

Fig. 2: Comparison between detailed views (wavenumbers range: 500-4000 cm-1) of FTIR–ATR spectra of olive pomace before (A) and after the treatments (B).

Along with bands at 2920 cm^{-1} and 2840 cm^{-1} evidencing the presence of lignin and carbohydrates, signals in the region 1520-1540 cm^{-1} indicated the presence of esters in the lignin structure. The bending vibrations of aliphatic -CH were also observed at 1366–1320 cm^{-1}. Bands at 1540 cm^{-1} and 1630 cm^{-1} suggested the presence of amino and carboxyl groups, respectively.

Not surprisingly, an intense band was detected at 3313 cm^{-1} and ascribable to the hydroxyl and amino group stretching. The broad bands at 1160–1000 cm^{-1} represented the characteristic C-O-C and OH vibrations of polysaccharides and, among them, of cellulose. When the bleaching process was applied to OP, the variation on the material surface, ascribable to changes of natural polysaccharides, modified the corresponding FTIR-ATR spectrum (Fig. 2B). Indeed, after the treatment, significant variations were observed in the fingerprint region affecting mainly the signals of lignin. In particular, the bands at about 1630 cm^{-1} and 1032 cm^{-1} (Fig. 2A) changed their ratio with the latter signal that moved to 1025 cm^{-1} (Fig. 2B). Along with these variations the signals detected at 3313 cm^{-1} before the treatment (Fig. 2A) shifted at about 3342 cm^{-1} indicating the main presence of O–H stretching modes of wood fibers (Fig. 2B).[19-23] Moreover, as indicated by Kondo, this signal can be ascribed to a peculiar intramolecular hydrogen bond in cellulose structure.[24] As suggested by Poletto *et al.*[25] the band at about 2890 cm^{-1} (Fig. 2B) indicated the stretching vibration mode of methyl and methylene groups present in the spectra of all of the fiber components, but mainly in the spectrum of cellulose.[25] Bands detected at 1600 cm^{-1} and below this region, when the treated sample is considered (Fig. 2B), were assigned to C–H and C–O deformation, i.e. bending or stretching vibrations of carbohydrates. Not surprisingly, the deformation or stretching vibrations, observed at 1163 and 1025 cm^{-1}, confirm the presence of C–O–C and C–O groups.[25] Overall results suggested that lignin was the main component removed during the treatment, with the remaining adsorbent material largely composed by cellulose and cellulose-like structures with traces of lignin.[9,14,15]

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Fig. 3: Camera pictures of OP as appear before (A) and after the treatments (B). Treated OP after the adsorption of DB (C). The amount of pomace and dye were settled at 0.25 g and 5×10-5 M in 10 mL, respectively. The reuse of both the dye and adsorbed material is also depicted.

The removal of lignin was also confirmed by observing Fig. 3A and Fig. 3B that show as the color of the adsorbent changed after the treatments: olive pomace grains altered their color from dark brown to yellow, when the bleaching process was performed (Fig. 3B). The removal of lignin could be considered as the main factor inducing these chromatic changes. As well as to SEM images (Fig. 1), these camera pictures offer a macroscopic view of the pomace aspect, confirming the irregular character of the material offering active sites to host dye molecules. The dye used in this paper, as an example of direct ionic azo dyes, was Direct Blue 78 (DB78, See Scheme 1). The dye was removed from water with an excellent performance by means of adsorption process on treated OP surface. However, particular conditions of work must be adopted and will presented in the next section of the manuscript. As it can be seen macroscopically, by observing the OP camera pictures in Fig. 3C, the OP grains in contact with DB78 solutions changed their color from yellow to blue, indicating the uptake of dye molecules from wastewater. The dye recovery was also obtained offering the possibility to open a green virtuous cycle in which both the adsorbent and the dye can be re-used.

3.1 Adsorption experiments.

3.1.1 Effect of pH. As a first step of this study, 1.00 g of OP as grains was placed in wastewater containing DB dye and the UV-Vis absorption spectra were collected each 30 minutes monitoring the DB78 absorption maximum at 600 nm (Fig. 4).

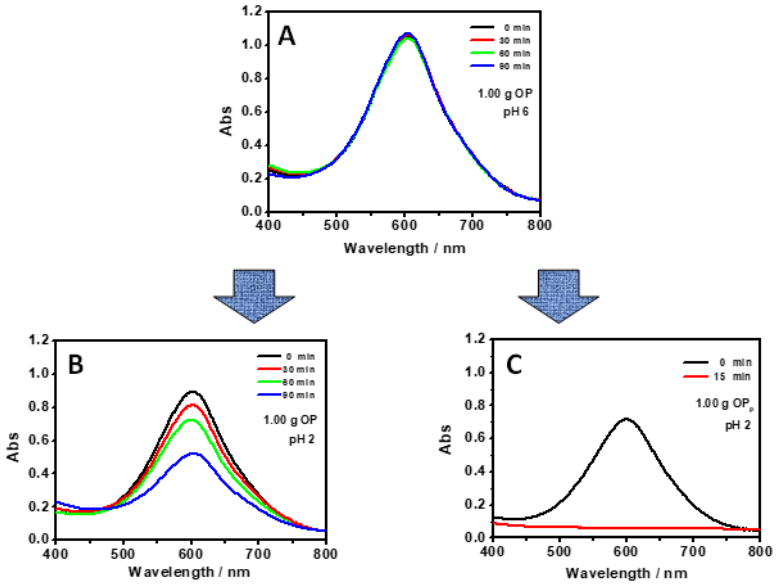

Fig. 4: Visible absorption spectra of DB78 dye obtained at several contact time when the dye solution was in contact with 1.00 g of olive pomace as a grains, pH 6 ***(A)*** *and pH 2* ***(B)*** *and powdered, pH 2* ***(C)****. The dye concentration was settled at* 5×10^{-5}*M in 10 mL.*

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Before the measurements, the sample at pH 6 was centrifugated and the surnatant was subject to the spectroscopic analysis. The absence of results (Fig. 4a) induced us to change the solution pH. Interestingly, when the pH was decreased to pH 2, important results were obtained with the 50% of dye molecules removed from water in 90 minutes, if OP was used as grains (Fig. 4B). Interestingly, by using the same amount of pomace, but crushed in fine powder, extraordinary results were observed: only 15 minutes were necessary to completely remove the dye from the solution (Fig. 4C). The efficiency of 100 % was thus presented. For that reason, the study was focused on the pomace reduced in fine powder (OP_P) as biosorbent and the effect of solution pH values was evaluated in the range 2-12. The amount of pomace and dye were settled at 0.25 g and 5×10^{-5} M in 10 mL, respectively.

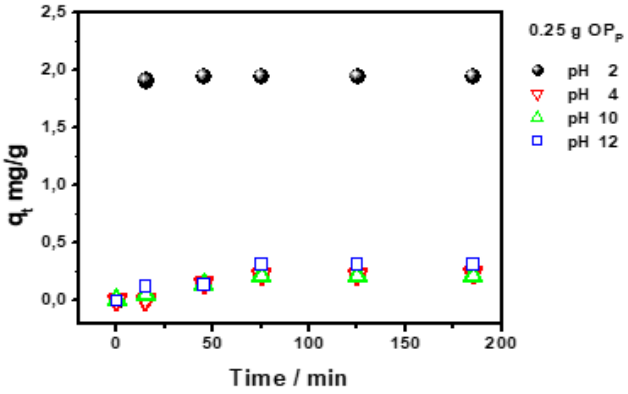

Fig. 5: Effect of solution pH values, ranging from 2 to 12, on the adsorption capacity qt (mg×g-1) of DB78 removal from aqueous (5×10-5 M) solutions, at fixed OPP amount (0.25 g).

In order to better evidences the effect of various pHs affecting the DB78 removal from water, the q_t values (using (1)) were calculated and compared in Fig. 5. Clearly, it is evident that the dye removal from wastewater was obtained only at pH 2, with slight and not important variations at the other pH values ranging from 4 to 12. To better explain this finding, it is noteworthy that DB78 is a ionic negatively charged dye that in aqueous solution carries a net negative charge due to the presence of sulfonate groups. The UV-Vis absorption spectra of DB78 reported in Fig. 4 suggested as the dye was almost insensitive to the acidity of the solution. The typical absorption band, located at about 550-600 nm, attributable to the characteristic chromophoric part of the dyes comprising the azo-groups (–N=N–) interacting with the adjacent aromatic moieties, was detected. The lack of spectral modifications in the wavelength position of the dye aqueous solutions at different pHs, suggested the $\pi\rightarrow\pi^*$ nature of that band. While, the change in the signal intensity could be attributed to the protonation of the central secondary amino group ($pK_a< 4$). The sulfonate groups, can be considered deprotonated in the adopted range of pH values, due to their $pK_a< 2$.[26,27]

These results were in excellent agreement with the considerations reported by Abbott *et al.*[5] about the role of pH values on the pH-dependent equilibrium forms of DB78.[5] Consequently, in our condition, as well described by Sayğili *et al.*[8] in their studies related to the chemical modification of a cellulose-based material to improve its adsorption capacity for anionic dyes, at the increasing of the dye solution pH, the adsorptions of the dye decreased due to the electrostatic repulsion between the negatively charged pomace surface and DB78 charges.[8] Moreover, in presence of OH^- ions in excess, these efficiently competed with dye anions for the adsorption sites onto the sorbent surface. While, Safa *et al.*[29] described that, lowering the pH values, the concentration of H^+ ions increased, positively charging the surface of the biomass. In that condition electrostatic interactions between positively charged biomass surface and negatively charged dye molecules could be taken in account.[29] The nature of electrostatic interactions was confirmed when OP_P after the adsorption of DB78 dye (at 0.25 g of pomace and 5×10^{-5} M of DB78 in 10 mL) was placed in contact with a basic solution containing NaOH (0.01 M), at pH 12. Preliminary the OP_P loaded with DB78 was carefully washed with water in order remove unadsorbed dye molecules and then was placed in a solution at pH 12. By using (2), the efficiency of the dye desorption was evaluated: the 70% of the adsorbed dye was recovered after 1 h (see Table 1, the first cycle of adsorption/desorption).

Table 1: Experiments of adsorption/desorption of DB78 at concentration of 5×10^{-5} M (Volume: 10 mL), at a fixed amount of OP_P (0.25g), on the contact time enough to obtain the reported uptake or the recovery of dye molecules from aqueous solutions at pH 12 (Volume: 10 mL).

CYCLES		Efficiency (%)	Time (min)
1	ADSORPTION	100	15
	DESORPTION	70	60
2	ADSORPTION	100	30
	DESORPTION	80	60
3	ADSORPTION	100	30
	DESORPTION	90	60

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The obtained results suggested that the adsorbed dye cannot be desorbed by simple water, but it is necessary the use of a strong base, as NaOH, indicating also that the attachment of the dye onto the adsorbent occurred through electrostatic interaction.[8]

The excellent performance of the presented material was highlighted by the possibility of re-use of both the adsorbent and dye, for others cycles of adsorption/desorption. Table 1 reports the obtained results after 3 consecutive cycles. From the data in Table 1, it is possible to observe as the DB78 adsorption maintained almost unaltered the performance obtaining the complete removal of the dye from water within 30 minutes. While, the recovery of dye was unfortunately not complete, although efficiencies of 80% and 90% were obtained in the first 60 minutes for the 2nd and 3rd cycle, respectively. Further, this paper acquire a great importance considering the real re-use of desorbed DB778 in dying cotton fibers, however this aspect will be discussed at the end of the paper.

3.1.2 Effect of salts. To better understand the mechanism of DB78 molecule adsorption on OP_P, the process was further studied changing the ionic strength of the solutions. It is worth to mention that textile dyeing processes are generally performed using large amount of salts[9]; as a results this evaluation becomes important for industrial applications. Surprising results were obtained.

Since an amount of 0.25 g of OP_P resulted to be so efficient in removing the dye to use 15 min for a 100% removal, these experiments were carried out with the amount of OP_P lowered at 0.10 g in order to appreciate the spectral variations. Indeed, if the same experimental conditions were adopted (this aspect is discussed later in the paper), decreasing the weight of adsorbent, the efficiency of dye uptake from water decreased, enabling to follow the absorption spectrum of DB78 at several contact time. Overall, adopting pH 2 as pH of work, 5×10^{-3} M as dye concentration in 10 mL and 0.10 g of pomace, Fig. 6A shows the obtained results.

Starting from the results related to NaCl, by adding an appropriate amount of salt, such as 5×10^{-2} M, 7×10^{-2} M and 1×10^{-1} M, in the mixture of dye and pomace, the adsorption process was improved. On the other hand, increasing or decreasing the amount of salt around these values, slight variations were obtained (see also Fig. S1A to better appreciate variations). A similar trend was obtained also change the nature of salt, *i.e.* Na_2SO_4. As an example, Fig. S1A and Fig. S1B report the effect on the adsorption process when Na_2SO_4 was used at concertation of 1×10^{-1} M and 1 M. From these results, along with the role of the electrolyte concentration on the adsorption process, the effect of salts having different nature was also evidenced. The process resulted to be more sensitive by using Na_2SO_4. A high ionic strength was induced under these experimental conditions.

Overall, several reasons can be considered to characterize the behavior observed in presence of salts: (i) the increase of ionic strength induced the compression of the diffuse double layer on the electrostatic attraction and consequently contributed to the adsorption process. Further, the osmotic pressure of solution increased with salt concentration and consequently a concentration polarization layer will be built up by the salt;[30] (ii) the addition of salt rendered the dye molecules more hydrophobic favoring the adsorption process. Indeed, in the presence of counter-ions, the charges associated to DB78 molecules were screened inducing the presence of novel interactions affecting the adsorption process.[4]

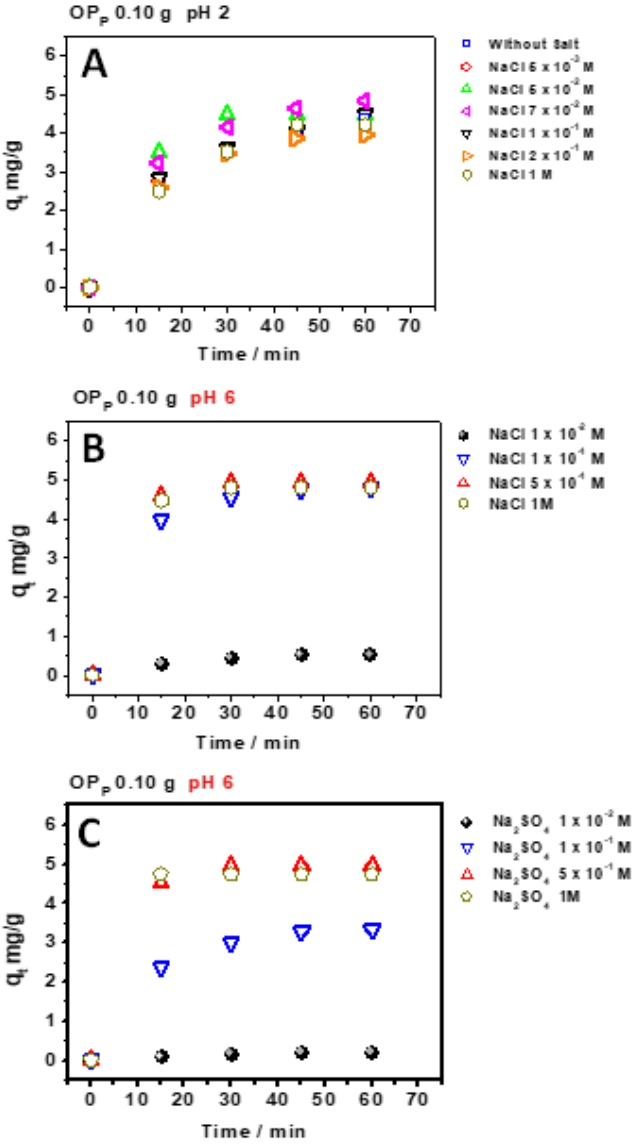

*Fig. 6: The effect of salts on the adsorption capacity q_t ($mg\times g^{-1}$) for DB removal from aqueous solutions (5×10^{-5} M DB78, 0.10 g OP_P in 10 mL) at pH 2 evaluating the concentration of NaCl in the range 5×10^{-3} M – 1 M **(A)**; at pH 6 evaluating the concentration of NaCl **(B)** and Na_2SO_4 **(C)** in the range 1×10^{-2} M – 1M.*

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Accordingly, both the charges of the pomace and DB78 were reduced inducing specific dipole–dipole and hydrogen bonding interactions as well as non-specific induction and dispersion interactions; hydrophobic interactions may also be important.[5] Miyamoto *et al.*[32] suggested that direct ionic dyes interrupt hydrophobic stacking between cellulose polymer forming hydrogen bonds. Moreover, the planes of glucose rings interacted with the dye aromatic moieties, and the sulfonate groups of the dye molecules interacted with the cellulose hydroxyl groups. In addition, the CH groups of glucose rings and aromatic moieties of dyes (e.g., naphthalene and biphenyl moieties) interacted weakly.[32] As reported by Porter, the cellulose substrate was considered as heterogeneous material with several accessible regions in which dye molecules can be hosted.[33]

Surprisingly, if on one hand by using OP_P in neutral medium the adsorption did not take place, on the other hand the use of electrolytes as NaCl and Na_2SO_4 favor the adsorption process also at pH 6. Fig. 6B and Fig. 6C report the obtained results. In these conditions a high amount of salt was necessary to remove the dye from water with a reverse effect about the nature of used salts. The efficiency of the adsorption process occurred improved by using NaCl. Indeed, by comparing 0.1 M as salt concentration, excellent results were obtained quickly when the adsorption process was studied in presence of NaCl (Fig. 6B and Fig. 6C) than with Na_2SO_4. The differences observed between NaCl and Na_2SO_4 could be ascribed to different effects in screening the charges of DB78 dye and adsorbent. However by comparing Fig. 6A, Fig. 6B and Fig. 6C, the efficiency of the adsorption process appeared overall improved when the electrolytes were added in DB solution at pH 6. So, along with the differences obtained changing the salt and pH, these results suggested that the ionic strength alone cannot be considered to obtain a comprehensive description of the adsorption process. The presence of more important hydrophobic interactions should be considered under these experimental conditions. The thickness of the electric double layer surrounding the dye molecule decreased as the concentration of electrolytes increased allowing the dye molecules to move closer to each other favoring hydrophobic interactions.[34] Not surprisingly, when the release of dye was studied under these conditions, at pH 12, its recovery was not obtained suggesting that the nature of interactions was changed. The results discussed so far suggested as the best work condition was obtained at pH 2 with also the dye recover. Therefore, from now on, the attention was focused on the adsorption process performed at pH 2 evaluating the effect of the adsorbent dosage and dye concentration on the adsorption process.

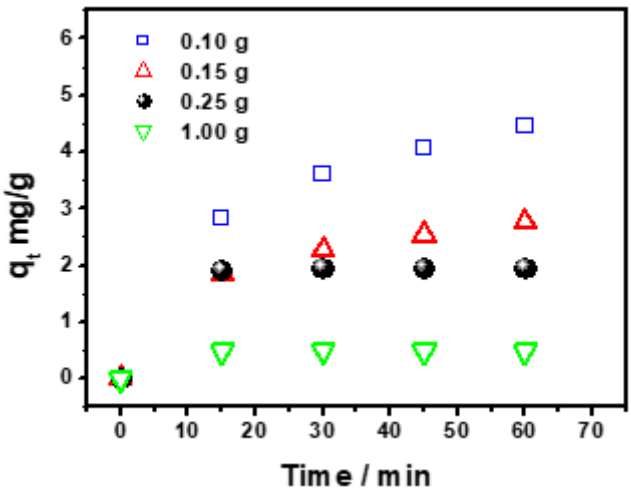

Fig. 7 : Effect of OPP amount (in grams for 10 mL of dye solution) on the adsorption capacity qt (mg×g-1) of DB78 dye removal (5×10-5M) from aqueous solution at pH 2.

3.1.3 Adsorbent dosage and dye concentration. The amount of OP_P was changed from 0.10 g to 1.00 g fixing the concentration of DB78 at 5×10^{-5} M in 10 mL. The q_t values arisen from these experiments were calculated using (1) and are reported in Figure 7. At first glance, observing the time necessary to reach the plateau region, point in which theoretically the dye could be completely adsorbed, increasing the amount of OP_P increased the removed percentage of dye from water. This behavior was imputed to the presence of more free active sites, able to host dye molecules.[9] On the other hand, when an high amount of OP_P was used the active sites were not saturated. These results were confirmed when the amount of dye was decreased.[9] Adopting 0.10 g as fixed amount of OP_P and 1×10^{-5} M as dye concentration, 15 minutes were enough to completely adsorb DB78 from water (Fig. S2), against 90 minutes necessary to remove it from a concentrated solution, under the same experimental condition. Lowering the concentrations, all dye molecules interacted with the binding sites of the biosorbent. On the other hand, the adsorbent is characterized by a limited number of binding sites, which become saturated at a certain concentration. At higher concentrations, the great part of dye molecules are left in the solution due to the saturation of binding sites, observing a decreased dye removal percentage.

3.1.4 Effect of Temperature. As reported by several papers, studying the adsorption processes, temperature values could be an important factor in affecting the removal of dyes from water.[9] Indeed, in accordance with the thermodynamic nature of process, that can be endothermic or exothermic, changing the temperature can positively or negatively influence the kinetic of adsorption.[35,36] For such purpose, the temperature was changed from 25°C to 70°C by using the lowest amount of pomace, *i.e.* 0.10 g, fixing the concentration of dye at 5 ×

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10^{-5} M. The obtained results are reported in Figure S3 and are related to the q_t values obtained under these conditions. The temperature played an important key role during the adsorption process. Passing from 25°C to 70°C the contact time necessary to remove DB78 from water changed from 90 minutes to 15 minutes, obtained an improvement in the process efficiency similar to that observed using 0.25 g of pomace at room temperature.

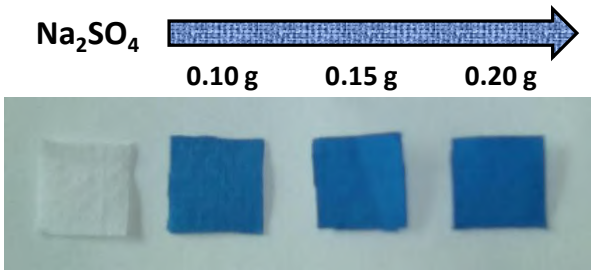

Fig. 8: Camera pictures of dyeing experiments related to desorbed DB78 (5 ×10-5 M in 10 mL) on pieces of a cotton textiles with increasing concentrations of sodium sulphate (10, 15 and 20 g/L). The experiments were performed at 95°C for 60 minutes.

Several factor could considered to justify this behavior: (i) increasing the temperature values, the mobility of dye molecules increased; (ii) or as well described by Akkaya *et al*.[8], increasing the temperature there is also an increase of the number of active sites available for adsorption on the material surface along with the desolvatation of the adsorbing species and the decrease in the thickness of the boundary layer surrounding the adsorbent, so that the mass transfer resistance of adsorbate in the boundary layer decreased; (iii) last but not least, the swelling effect of the internal structure of the pomace, enabling large dye molecules to penetrate into the structure in higher quantities, that could not be excluded.[9] Indeed, as documented in our recent paper related to disperse dye removal, this effect was very important. This observation is extremely important. Interestingly, during the dyeing process of textiles, hot water is used and the generated wastewater is discharged at considerable high temperatures. These results with the extraordinary effect of salts in dye solution, auxiliary agents used to dyeing the textiles, offer the concrete possibility to use bleached olive pomace for the removal and recover of dye from wastewater. For these purpose, experiments were performed using the desorbed dye from olive pomace to color pieces of textiles.

3.1.5 Dyeing experiments of cotton fibers. The colored solutions obtained after the desorption experiments, were used to dye cotton fibers. White cotton fiber having a superficial area of 2.25 cm^2, were placed in contact with 10 mL of desorbed DB78 solution for 60 minutes at high temperature (95°C) in presence of increasing amounts of sodium sulfate (10, 15 and 20 g/L), as auxiliary agent to color the fibers as suggested by Colorprint Fashion. The effect of salt is clear evident in the camera pictures reported in the Fig. 8. As expected, the adsorption of dyestuff increased increasing the sodium sulfate concentration and it could be ascribed to processes affecting the dyeing method, *i.e.* the neutralization of negative charge of cotton fibers by sodium ions during the dyeing.

IV. CONCLUSIONS

In this paper, the operational parameters affecting the adsorption of a commercial direct dye (Direct Blue 78) from wastewater were evaluated, proposing also an alternative use of oil mill waste (named olive pomace, OP) and obtaining excellent results both in the removal and recycle of dye and adsorbent. The biosorbent was modified through previous treatments with NaOH and $NaClO_2$ for removing impurities and lignin from the surface of the substrate. A cellulosic material was thus obtained. These findings were confirmed using FTIR-ATR spectroscopy, evidencing the main bands of cellulose and cellulose-like structures, arisen after the treatment. A bleached adsorbent was obtained. Experiments were performed both using OP as grains and in powder (OP_P). The latter exhibited extraordinary performance removing in 15 minutes the DB78 dye from water using only 0.25 g of the biosorbent. However, such a behavior was obtained settling the pH of dye solution at 2 units. The presence of electrostatic interactions inducing the adsorption process was thus evidenced, since at pH>2 there was no dye adsorption. The use of electrolytes as NaCl and Na_2SO_4 further confirm these findings. More specifically the latter salt increased the percentage of dye removal if compared with experiments performed in the presence of NaCl. Overall the influence of ionic strength was taken into account, showing as the process was affected by appropriate amount of salts. These results were better emphasized when the recycle of the dye was studied. Indeed, adopting pH 12 as medium of work, DB78 molecules were desorbed from OP_P enabling the recycle of both the dye and biosrbent. As an example three cycles of adsorption/desorption were performed showing as the material exhibited the same efficiencies in dye removal with the not complete desorption of dye molecules. A mean value of 80% was obtained for each cycle. Interestingly, when experiments were performed at pH 6 in presence of the listed salts, the adsorption occurred, suggesting a change in the type of adsorbate/adsorbent interactions, favoring hydrophobic interactions instead of electrostatic ones. In that condition the adsorption occurred with a greater efficiency if compared with results obtained at pH 2, however the desorption of dye was not obtained. Starting from these considerations, the attention was focused on experiments performed at pH 2 studying the effect of the biosorbent amount and dye concentration. Both the experiments

involving the change of OP_P weight and dye concentration evidenced the role of free active sites hosting DB78 molecules. Indeed, increasing the amount of OP_P the efficiency in the adsorption process increased. Accordingly, the same results were obtained decreasing the amount of dye. The adsorption process was also influenced by temperature exhibiting an endothermic character. The increase of temperature values induced an increase of the process efficiency.

Thus, the comprehensive investigation about the parameters affecting the adsorption process involving DB78 and OP_P, carefully studied in this paper, evidenced as without salt and in acid conditions, the recycle of dye is possible. Whereas, the presence of salt in neutral medium, although improves the efficiency of the dye removal process, prevents the dye recover. In conclusion, the reuse of dye adsorbed results possible at pH 2 using different amount of Na_2SO_4, auxiliary agent generally used during the industrial dying process.

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Supporting Material

Operational parameters affecting the removal and recycling of direct blue industrial dye from wastewater proposing bleached oil mill waste as alternative adsorbent material

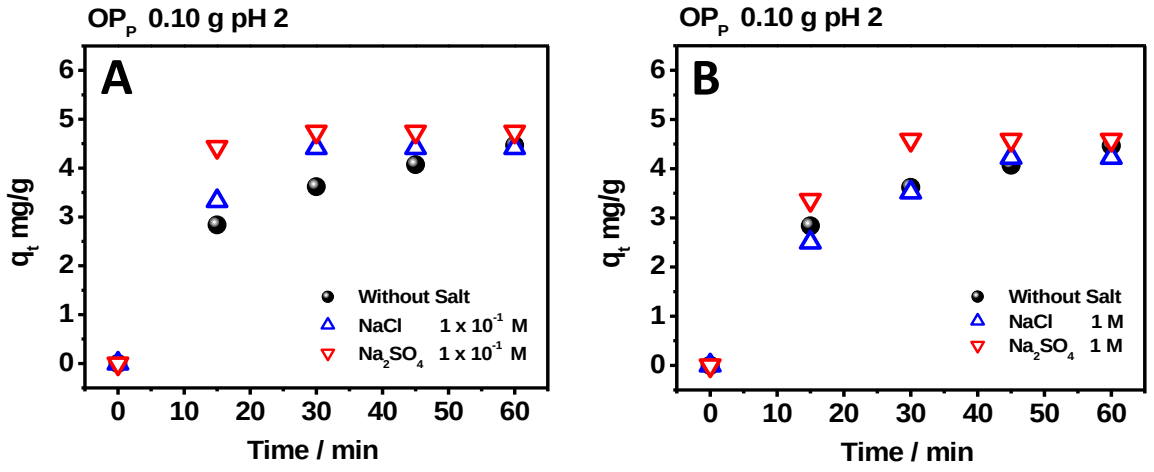

Fig.S1: The effect of salts on the adsorption capacity q_t (mg g^{-1}) for DB removal from aqueous solutions (5 $\times 10^{-5}$ M DB, 0.10 g OP_P in 10 mL) at pH 2 comparing the efficiency between NaCl and Na_2SO_4 at 1$\times 10^{-2}$ M and 1M.

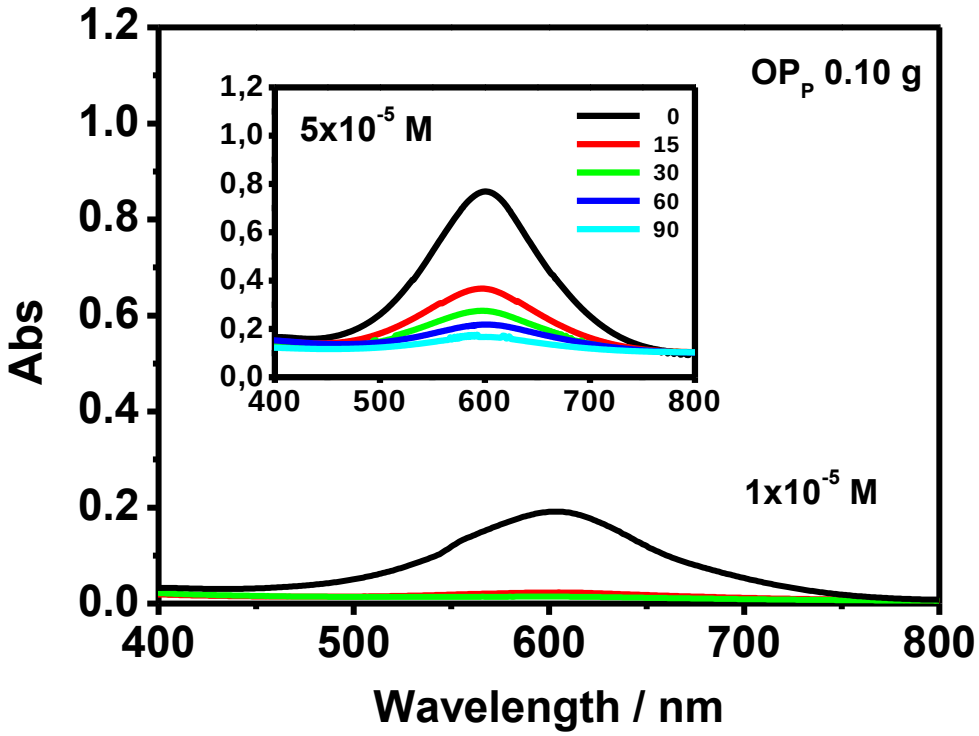

Fig.S2: Effect of DB dye concertation (5$\times 10^{-5}$M and 1$\times 10^{-5}$M, in 10 mL) on the adsorption capacity q_t (mg g^{-1}) using 0.10 g of OP_P at pH 2.

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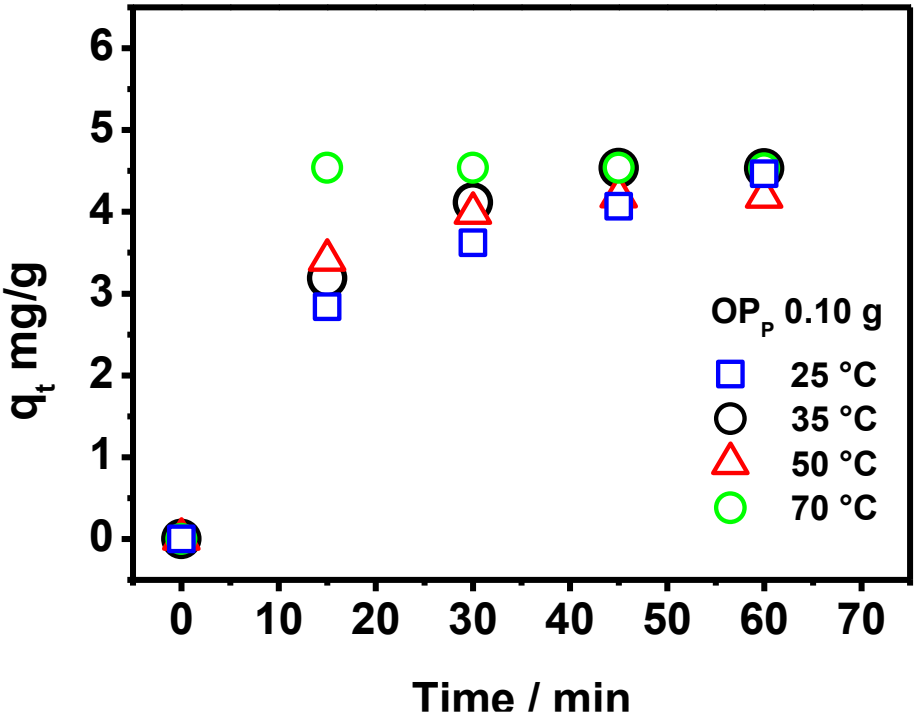

Fig.S3: Effect of temperature values ranging from 2°C to 70°C on the adsorption capacity q_t (mg g^{-1}) of DB dye removal (5×10^{-5}M) from aqueous solution at pH 2; 0.10 g of OP_P were used.

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Comparative Study of Zootechnical Performances and Survival Rates in Rainbow Trout Subjected to Two Foods with Different Formulation

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Abstract— *Considering its economic and halieutic interest, the rainbow trout (Oncorhynchus mykiss, Walbaum, on 1792) is one of the species the most appreciated in the world, in particular for the sports fishing. To compare the effects of two food of different formulation, (the one premises(place) used by the center of salmon farming and the other one imported) on some biological parameters of the trout rainbow, an experimental study was realized between 1st Mars and June 15th, 2016 in the National Center of Hydrobiology and Fish farming of Azrou on 2000 fish fry stemming from the same prize of eggs and restarted randomly in 4 rectangular ponds fed with fresh water and fed four times by days during 107 days. The obtained results show good that the best performances of growth in length and in weight, the survival rate and feed efficiency are attributed to the imported food.*

Keyword— Rainbow trout, local food, imported food, feed efficiency, growth, rate of survival.

I. INTRODUCTION

Brown trout (Oncorhynchus mykiss, Walbaum, 1792) was introduct in Morocco since 1925 from France and North America (Mouslih, 1996; Abba *et al*., 2013). The objectives of this introduce are to promote the sport and comercial fishing in Morocco. The breeding of this salmonid is carried out in the structures belonging to the National Center of Hydrobiology and Fish Farming (CNHP) in the Middle Atlas, specifically in the province of Ifrane.

The objective of this study is compare the zoological performance, survival rate and growing of alevins in relation to the type of local feed used in the center. In this investigation we also performed the comparison between the conversion indices.

II. MATERIALS AND METHODS

1-Description of the breeding room

This experimental study was conducted in a room at the National Center of Hydrobiology and Fish farming, in specific rectangular troughs in parallel with a suitable volume of 0.16 m3, and circular tanks fed by taps, The water comes from the source with a flow rate of 0.97 m3 / h. Grids were placed downstream of each trough in order to avoid the exit of the fry. The troughs and tanks are equipped with a diffuser aeration system to maintain the dissolved oxygen concentration close to saturation.

2- Measures of water quality indicators

Ecological parameters influence the life cycle of fish, especially during the incubation and nursery period (Huet, 1970, Piper *et al*., 1982, MENVIQ 1990, Jalaber and Forestier, 2010). In situ measurements of water quality indicators for the nursery period (pH, dissolved oxygen, temperature, and electrical conductivity) were made by portable devices (Thermo orion 810 oximeter, IP67 pH meter, Conductimeter- salinometer-thermometer type Jenco 3250) during the experimental period spread over 4 months.

3- Biological materials and parameters

Digestibility and nutrient balance are more important in breeding fish, and any deficiency or imbalance in food

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may lead to malformations and high mortality rates (Hilton and Slinger, 1981).

Feed control in breeding fish is an important step in the quality of the flesh of fish (Kolditz *et al*., 2008; Aba, 2013) compared to that of fish caught in the fishery. 2000 alevins of rainbow trout from the same batch of eggs were randomly distributed in four rectangular tanks with 500 alevins in each tank (A7, B7, A8 and B8). The alevins were manually fed by two different food, one imported for the alevins of the tank A7 and B7 and the other for the alevins of the tanks A8 and B8 (Tab.1). The daily ration was divided into four meals distributed from 9 am to 5 pm, seven days a week for 107 days. Each week 3 batches of 10 fish per trough were anesthetized by a biological anesthetic (clove) after 24 hrs in order to carry out the weight measurements to calculate the quantities of food required for all the biomass present in the tanks. The two foods tested are pellets with a diameter of 2 mm. In addition to the qualitative and quantitative differences, the structure of the granules of the imported food is homogeneous, while the local food has granules with a heterogeneous structure.

Table.1: Composition of the two experimented food

Constituents	Imported food	Local food
Crude Proteine	47%	48%
Crude fat	18%	22%
Crude fiber	1,33%	2,2%
Crude ash	8,75%	8,3%
total phosphorus	1,32%	0,8%
Calcium	0,80%	------
Sodium	0,62%	------

3-1 Feed rate:

The quantities of food distributed weekly were calculated and weighed according to the alevins density in each tank and the water temperature, which is measured daily throughout the experimental period.

The rate of feeding is calculated according to the following formula: (Total weight * Feeding rate) / 100

3-2 Growth parameters

Growth is the simplest criterion to be apprehended in the nursery stage. It is expressed by the evolution of the total length and the total weight of fish, estimated from a sample generally composed of 33 individuals, 33 alevins were taken at random in every tank. In order to take measurements of the growth parameters without stressing the fry, the latter are anesthetized by a natural anesthetic which is the clove (Syzygium aromaticum) thanks to its composition in eugenol. The total length (Lt) corresponding to the length of the fish from the tip of the muzzle to the end of the longest radius of the caudal fin is determined by an ichthyometer graduated in cm. The total weight (Pt) is determined using an electronic balance (Brehm type B30) with an accuracy of 0.1 g.

3-3 Zootechnical Parameters

- Weight gain% (W .G):

It allows evaluating the weight growth of the fish during a given time (Goubier, 1975). It is calculated from the following relation: W.G% = (Final average weight (g) - Initial average weight (g))

- Individual daily growth (I.D.G):

This parameter allows us to estimate the daily weight gain of farmed fish (DGA-IGA, 2008). It is determined from the following relation: IDG (g / d) = (Final weight (g) - Initial weight (g)) / Breeding time (Days)

- Specific growth rate (SGR)

Growth rate is a term used in aquaculture to estimate the production of farmed fish after a certain period; it is given by the formula (Goubier, 1975).

SGR (%/ Day) = ([ln (final weight) - ln (initial weight)] x 100) / Duration of the experiment in days

- Survival rate (SR):

The survival rate is calculated from the total number of fish at the end of the experiment and the number at the beginning of the breeding, according to the relationship below:

SR (%) = (Number of final fish x 100) / Initial fish number

III. RESULTS AND DISCUSSION

1. Water Quality Parameters

The water quality parameters at the station of the salmonids of the National Center for Hydrobiology and Fish Culture are shown in the table below

Table.2: Average values of water quality parameters in the nursery

Physicochemical parameters of water	T°C	O2 dissolved mg/ L	pH
Average values	14	6,9	7

The temperature of the water acts at several levels of the fish life cycle, impacting metabolism and incubation of eggs (Treasurer, 1983, Diamond, 1985, Gillet, 1991, Mallet 1999, Morin, 2012). The results obtained for the temperature of the water at the level of the various troughs and the rearing tanks are around 14 ° C, the same temperatures were recorded in 2011 (Abba, 2011). The results obtained for the temperature of the water at the level of the various tanks are around 14 ° C, the same temperatures were recorded in 2011 (Abba, 2011). The average dissolved oxygen content in the tanks is 6.90 mg / l. In addition, various tanks are equipped with a diffuser aeration system allowing the concentration of dissolved oxygen close to saturation to be maintained. For Hydrogen potential (pH), it is very close to neutrality (pH

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= 7). According to MENVIQ, (1990), Painchaud, (1997) and Morin, (2012), the physicochemical parameters of the waters at the salmonids breeding station meet the criteria for good water quality of the salmonids since the pH is between 6, 5 and 9, the temperature of the water is almost stable at 14 ° C. and between 10 and 15 ° C. and a concentration of dissolved oxygen of more than 6 mg / l.

2-1 Biological parameters

2-2 Growth of weight

The comparative growth curves show that the alevins weights in the different treatments evolve in the same direction during the first five weeks of the study (Fig. 1). After 14 weeks of feeding, the final weight of the alevins by the imported feed is very high compared to the weight of the fry fed with a local feed (up to 30% higher than the initial weight). According to (Philippart and Melard, 1987) growth is a complex biological process that involves many factors whose role and contribution must be known. In our case, the difference in growth resides in the type of feed received by each batch of alevins since the ecological conditions are the same (T °, pH and oxygen rate), so the growth seems to be the result of Increased feed intake in fry fed the imported food, poor digestion (Fig. 5) for alevins fed with local food, and a slight increase in weight.

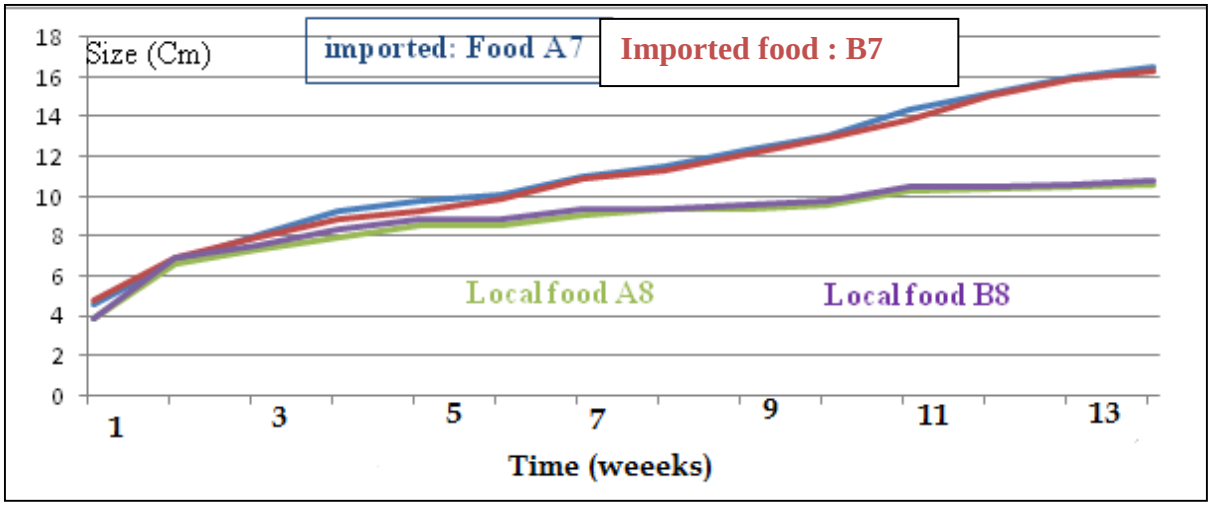

Fig.2: Evolution of the size averages alevins during the trial period

Photo.1: Difference in size between two trout alevins
(Top fingerlings fed by imported food, bottom alevins fed by local food)

2-4 Survival rate

The study revealed a very high mortality rate of fry (747) in troughs fed by local food throughout the study period (74.7%), compared with only 2 for fry fed imported food (0 , 2%) during the same test period (Fig. 4). The final number of fry in each trough is shown in Table 2. The results obtained can be explained by a poor digestion of the local food as shown by the dissection of the alevins after their mortality (Photo 1) since all the alevins of the troughs are subjected to the same environmental conditions. This bad digestion can be due to the high percentage of rate of lipid in the local food, because the food(supply) intended for the spawn and for the alevins have to contain lower levels of lipids with regard to(compared with) those of the food for fishes in phase of swelling (FAO, on 2017), either in a change of the quality of the food (oxidation under the influence of the light) due to the type of packaging (transparent plastic bags), against a packaging with plastic bags polyethylene opaque for the imported food.

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Table.2: Final number of alevins in every tank

Tanks	initial Number	Final Number
A7	500	498
B7	500	500
A8	500	176
B8	500	77

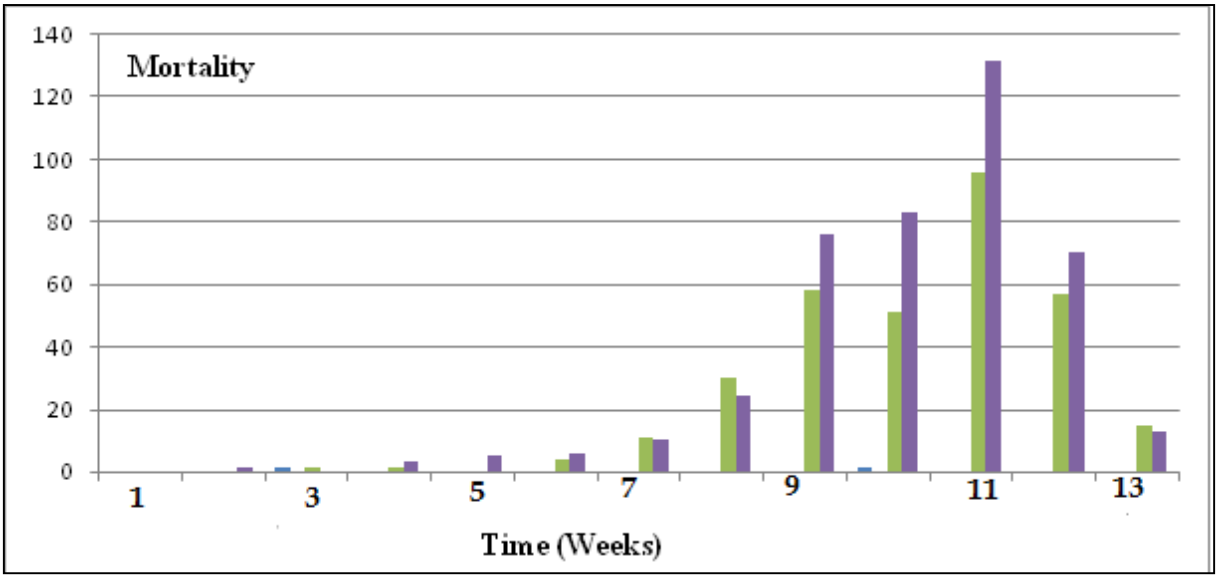

Fig.4: Mortality rate of alevins during the period of study (Local food (A8) (B8))

Fig.5: Dissection of the died alevin showing the bad digestion of the local food

2-5 Zootechnical Parameters

This experimental test shows that the performance of the zootechnical parameters varies significantly between the two diets (Tab.3). The highest values in terms of weight gain were obtained with the imported diet. For daily individual growth, the mean for fry fed imported food is 0.44 compared to only 0.065 for those fed on the local food. For the specific growth rate, it is 2.76% for fingerlings with imported diets; this growth is due to a high ingestion of the food by the fry. These results corroborate and explain well the mortality rate recorded in the other fry or the survival rate which is followed by 99.8% for the fry fed by the imported food compared with only 25.3% for the others.

Table.3: Results of the performances of the zootechnic parameters obtained during the experimental try

Indices	Tank 7		Tank 8	
	A	B	A	B
Weight Initial (g)	2,5	2,66	2,57	2,59
Weight Final (g)	51,3	48,7	9,04	9,52

Weight gain (g)	48,8	46,04	6,47	6,93
Individual Daily Growth (IDG) (g/j)	0,45	0,43	0,06	0,07
Specific growth rate (SGR) (% pc/j)	2,82%	2,71%	1,17%	1,21%
Survival rate (%)	99,6%	100%	35,2%	15,4%

IV. CONCLUSION

Fish culture, especially that of salmonids, requires the mastery of basic physicochemical parameters, namely temperature, dissolved oxygen, and the potential Hydrogen of the environment, as well as nutrition adapted to each stage of development of the cycle of life. The combination of these two parameters (ecological and food) is the key to all fish production. At the experimental station, key factors such as temperature, oxygen concentration and pH are very favorable. The results obtained (survival rate and growth in weight and size) of the alevins fed by the imported food are very satisfactory (auges A7 and B7), whereas the results obtained in the tanks A8 and B8 (Low growth, very high mortality High, etc.) are attributed to the type of local feed received by the fry during the same trial period. An analysis of this latter diet can answer the various hypotheses raised and which are the causes of poor results obtained. Similarly, this diet can also be a topic of study for later stages (pre-magnification and magnification).

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Lindane and Endosulfan Sulfate Isomers in Crassostrea virginica (Gmelin, 1791) Oyster Populations in Lagoon Systems from Central Gulf of Mexico

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***Abstract*—** *The aim of this study was to determine Lindane and Endosulfan Sulfate isomers in Crassostrea virginica oyster populations (Gmelin, 1791) in the Mandinga and Alvarado lagoon systems located in the central Gulf of Mexico. Samples were taken from the main oyster banks of each lagoon system, during the three representative seasons of the region, wet, dry and north winds. By means of free diving, 30 commercial size oysters (7 ± 3 cm) were collected in four oyster banks or stations of the Mandinga lagoon system, totaling 360 organisms, while in the Alvarado lagoon system there were a total of 90 oysters during the annual cycle. Concentration of lindane and endosulfan sulfate isotopes in C. virginica was performed with a gas chromatograph (Thermo Electron Model Trace GC Ultra 115V, Thermo Fisher Scientific Inc©, Monterrey, Nuevo León, México) with an Electron capture detector. Results showed that in the Alvarado Lagoon system mean concentrations of C. virginica oysters for lindane pesticide were 4.11 ± 3.83 ng·g^{-1}, whereas for the Mandinga lagoon system, were 8.69 ± 5.15 ng·g^{-1}. Endosulfan sulfate showed the highest average concentration in the Mandinga lagoon system with 24.68 ± 1.20 ng ·g^{-1}. In addition, the endosulfan sulfate presents differences in its spatial distribution; high concentration levels in the Mandinga lagoon system whereas the lindane heterogeneity at all sampling points in both lagoons. Values of concentrations and relationships between compounds suggest recent contributions that could correspond to the excessive fluctuations of water discharged into the lagoon caused by the atypical rains of the year of sampling. It was concluded that endosulfan sulfate and lindane show concentration in all the points of sampling in both lagoons.*

***Keywords*— coastal lagoons, Crassostrea virginica, endosulfan sulfate, lindane, toxicity.**

I. INTRODUCTION

Oyster *Crassostrea virginica* (Gmelin, 1791) is distributed from the Gulf of St. Lawrence, Canada to the coasts of Brazil and Argentina [1-3]. In Mexico, this fishery is mainly exploited in the coastal lagoons of the Gulf of Mexico. In 2014, the state of Veracruz recorded the highest oyster production, a result of the cooperative work of Tamiahua, Pueblo Viejo, Mandinga and Alvarado lagoons [4]. However, organisms that inhabit the Mandinga and Alvarado lagoon systems, located in the central area of the Gulf of Mexico, are subject to constant anthropogenic nature stress, which in turn has caused pollution issues in these important systems.

The Mandinga lagoon system is influenced by the Jamapa river [5], while the Alvarado lagoon system receives it from the Papaloapan River and Blanco River. These three rivers discharge in their slopes what they in turn receive as an impact of industrial and agricultural activities, which end up deposited in the aforementioned lagoon systems. Agricultural crops are part of the process of pesticide mobility, due to their retention properties, which modify the process of exchange of volatile substances between soil and air, which ultimately reach the lagoon systems. In agriculture, products with active substances known as organochlorine pesticides are applied [6,7], among the most used today is lindane, which is characterized by being long-lived and persistent. Organic matter is an important factor in the adsorption of lindane;

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an increase in organic matter increases persistence, while increasing solubility results in increased mobility of lindane. From the degradation of hexachlorocyclohexane (HCH) results in (α-HCH, β-HCH and γ-HCH) from these isomers γ-HCH is known as lindane [8].

Another important pesticide is endosulfan, a toxic compound that has been widely used in Mexico. Endosulfan has been found to produce neurotoxic, hematotoxic and nephrotoxic effects in mammals and is highly toxic to aquatic organisms. Endosulfan is an organochlorine pesticide consisting of a mixture of the isomers endosulfan (alpha) and endosulfan (beta). The National Institute of Ecology (INE) in Mexico, highlights in its "Diagnosis on the endosulfan status in Mexico" conducted in 2011, that endosulfan belongs to the group of cyclodienes and is chemically similar to aldrin, chlordane and heptachlor. Its main metabolite is endosulfan sulfate, it can be found in the environment by photolysis of endosulfan or as a result of its oxidation by microorganisms. Lindane and endosulfan sulfate are persistent, toxic, and bioaccumulative isomers that can travel great distances. As a consequence, they are proposed to be included in the Stockholm Convention [9,10]. The *C. virginica* oyster is a benthic organism which due to its development, feeding and reproduction system is in contact with different compounds that bioaccumulate and biomagnify in trophic chains until reaching human consumption [11-14], and is used as a biomarker [15]. The objective of this study was to determine the concentration of Lindane Isomers and Endosulfan Sulfate in oyster populations *Crassostrea virginica* (Gmelin,1791) in lagoon systems from central Gulf of Mexico to determine if there are potential risks to public health due to their consumption.

II. MATERIALS AND METHODS

This study was developed in the Mandinga lagoon system located between 19° 00'-19°06'N and 96°02'-96°06'W while the Alvarado lagoon system is between 18°44'-18°52'N, 95°44'-95°57'W. Both systems are located in the central zone of the Gulf of Mexico, (Fig. 1). It is shown, in both lagoon systems, the main oyster banks where the sampling work was carried out. This banks were chosen for their production capacity.

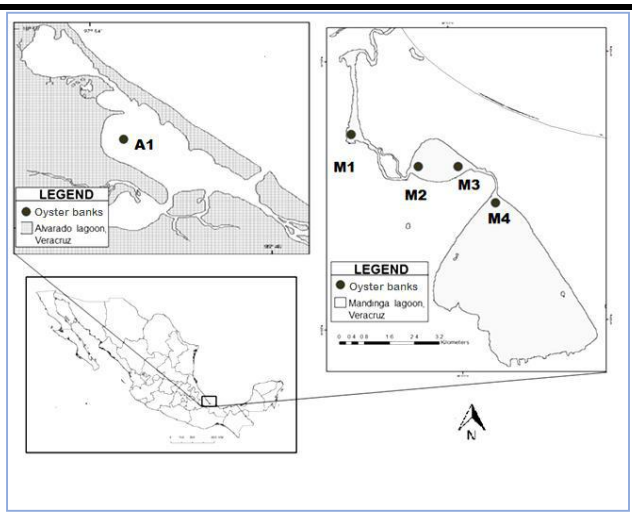

Fig.1: Oyster banks located in the Mandinga and Alvarado lagoon systems in the central area of the Gulf of Mexico.

Those works were carried out in three representative seasons of the region, wet, dry and north winds. By means of free diving, 30 commercial size oysters (7 ± 3 cm) were collected in the Mandinga lagoon system in four oyster banks or stations, totaling 360 organisms, while in the Alvarado lagoon system there were a total of 90 oysters. Organisms were removed and cleaned at each sampling station. They were transported in accordance with NOM-109-SSA1-1994 (2003) [16], to the Research and Aquatic Resources Laboratory (LIRA) of the Technological Institute of Boca del Río (ITBOCA) to carry out the cleaning and conservation process of the oyster samples. Oysters were cleaned with running water to remove excess sludge and adhered particles. Subsequently, the oyster muscle tissue was extracted with the use of a de-sheller, as well as the removal of the intervalvar liquid. Once this soft tissue was removed, it was deposited in previously labeled Ziploc® sealed bags. Immediately, these were placed in a deep freezer in order to continue the drying process. Frozen samples were dehydrated in a Thermo Savant Moduly OD-114 lyophilizer for 72 hours at -49 °C and a pressure of $36x10^{-3}$ mbar. After lyophilization, samples were ground in an Osterizer blender until a fine particle size. Subsequently, these samples were stored inside a desiccator with silica gel, to avoid possible absorption of moisture as well as samples fungal contamination. The analytical material used in the study was prepared following the analytical protocol for pesticide residues described by Waliszewski *et al*. (2008) [17]. The glassware used for this process was washed with 10% phosphate free neutral soap, then by potassium dichromate and rinsed with potable water. Thereafter, it was rinsed with distilled water Milli-Q grade, petroleum ether and acetone to eliminate all residues of phosphates and fats. Subsequently it was drained and dried with

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airflow. Once dried, they were stored in Ziploc® sealed bags and then in properly labeled containers with lid. All solvents and reagents used were analytical grade, to avoid any cross-contamination of the samples.

The purity of the petroleum ether used to wash the glassware was evaluated periodically using gas chromatography. In order to analyze the pesticides lindane and endosulfan sulfate in oyster samples, it was used hexane (Backer) with a boiling temperature range of 40-50 °C and sodium sulfate powder (Backer) previously activated and purified In a forced air oven (Riossa CF-102) at a temperature of 650 °C for 16 h, and with sulfuric acid (Merck) with a purity of 95 to 97%. For quality control, the chromatograph readings for organochlorine pesticides and their isomers were adjusted to follow the calibration of a 5-point curve from a linear regression. Reference samples were used to prepare the calibration curve using a ChemStation HP 3398A equipment, (ChemService, Inc., West Chester, Pennsylvania 19381, USA). In order to guarantee a recovery of 93%, fortification tests were carried out at different concentrations.

Concentration of lindane and endosulfan sulfate in oyster samples was performed following Murphy's technique (1972) [18], and modified by Waliszewski *et al*. (2008) [17]. The process started by weighing 10 g of lyophilized and milled sample for each season and placed in a Teflon beaker with 20 ml of acetone and 20 ml of hexane as solvents. The volume of this solution was divided into two parts; one was used for lipid extraction and the other to determine the concentration of organochlorine pesticides. The valves are placed on the lid of the vessel prior to incorporation of the sample and solvents, the lid must be gently adjusted to the bottom, the closed vessels are placed in the holder with a torque meter calibrated at 60 pounds of pressure, until the adjustment of the vessel in the same is achieved. Lipid extraction is performed by the modified pesticide method Green Chem of Murphy (1972) [18]. The temperature of the microwave oven CEM Model MARS-X was programmed at 110 °C and a pressure of 200 psi, with 10 minutes of maintenance and 10 minutes of cooling. After the process, they were placed under a fume hood for the control of toxic vapors. While there, the beakers with the samples were opened, then 15 ml of hexane and 15 ml of acetone were added and filtered through sterile Whatman No.4 filter paper. The filtered volume is placed in 250 ml flat bottom flasks and immediately a further washing of the material contained in the filter paper was performed, adding an additional 15 ml of hexane and 15 ml of acetone. The extracts collected in the flasks were allowed to cool for 15 minutes. Each flask was then placed in a roto-evaporator at a water bath temperature of 45 °C and 150 revolutions, until a 40 ml evaporate was obtained. After obtaining 40 ml, samples were allowed to stand and cooled for 30 minutes. Subsequently the residual material was weighed on a digital scale to determine gravimetrically the total lipids in each sample.

To continue the analysis of the pesticides lindane and endosulfan sulfate in oysters, 10 ml of the original solution was placed into a 50 ml tube with Bakelite stopper. Then, 1 ml of concentrated sulfuric acid was slowly added, not exceeding 4ml, and agitated vigorously for 1 min to precipitate the fat, after which the solution was left to rest for 15 minutes to separate phases. The supernatant was filtered in a funnel with No.4 filter paper onto a 8.0g layer of anhydrous sodium sulfate previously activated at a temperature of 100 °C. The filtrate obtained was placed in a 50 ml flat bottom flask, where 10 ml of ethyl ether was added to wash the sulphate and extract the remains of fat.

The sample was again placed in a roto-evaporator until approximately 1 ml of purified sample was obtained. The resulting extract was transferred via a Pasteur micro-pipette into a vial (Reacti-vial, Pierce®). Such vial and its respective replicas were placed in a glass bottle to protect it and kept in the freezer at 4 °C until its reading. Concentration of the organochlorine pesticides lindane and endosulfan sulfate in *C. virginica* oyster was performed with a gas chromatograph (Thermo Electron Model Trace GC Ultra 115V, Thermo Fisher Scientific Inc©, Monterrey, Nuevo León, México) with an Electron capture detector. Pesticide separation was performed on a 30m x 0.32mm x 0.25µm chromatographic column with 14% cyanopropylphenyl polysiloxane (Thermo Fisher Scientific Inc© Belleford, PA, USA). Where ultrapure nitrogen (Praxair-Mexico) was used as the entrainment gas at a flow rate of 2.5 ml/min. Operating temperatures were as follow: detector 300 °C, injector 250 °C and column 160 at 280 °C (4 °C/min). The injection volume was 1 µl in splitless mode.

III. RESULTS AND DISCUSSION

Mean concentrations of pesticides for each isomer, in ostion banks analyzed at the Mandinga (M) and Alvarado (A) lagoon systems located in the central Gulf of Mexico, are shown in Table 1.

Table.1. Pesticide isomers' mean concentration in ng·g^{-1} in oyster banks of the Mandinga (M) and Alvarado (A) lagoon systems.

Oyster banks	Lindane	Endosulfan sulfate
M1	7.88 ± 4.04	24.68 ± 1.20
M2	7.89 ± 4.02	11.38 ± 5.58
M3	8.05 ± 4.06	14.88 ± 11.50
M4	8.69 ± 5.15	19.32 ± 1.41
A1	4.11 ± 3.83	12.29 ± 2.84

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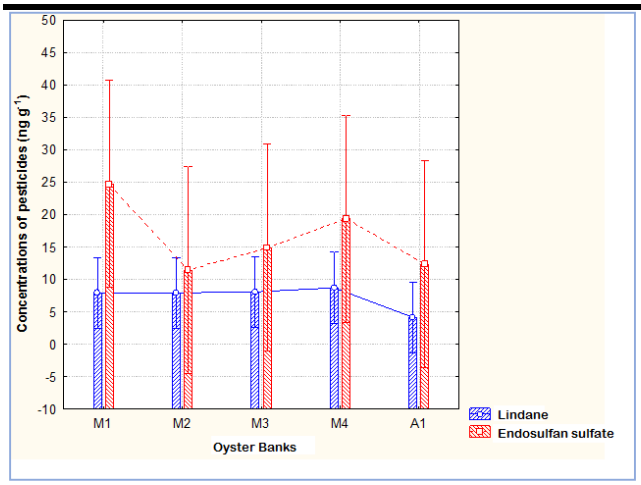

Fig. 2: Mean concentrations of the organochlorine pesticides lindane and endosulfan sulfate in oyster banks of the Mandinga (M) and Alvarado (A) lagoons.

Fig. 2, shows that mean concentrations of lindane in *C. virginica* oysters from the Mandinga lagoon do not show significant difference between banks and seasons, whereas it occurs in oysters from the Alvarado lagoon system. On the other hand, endosulfan sulfate fluctuates significantly between banks and seasons, presenting lower average concentration in the Mandinga bank 2.

The analysis of principal components by multiple factorial with respect to the contribution of the variables showed significant differences between endosulfan sulfate and lindane, which were notorious when they positioned at opposite poles of the axes (Figure 3).

Quantitatively, figures 4 and 5 show that the maximum concentration of lindane and endosulfan sulfate found in *C. virginica* oysters was during the north winds season. Mandinga bank 4, reports 12.62 $ng \cdot g^{-1}$ lindane and 39.35 $ng \cdot g^{-1}$ endosulfan sulfate, it was probably due to the disturbance caused by the wind in the sediments and the marine currents of the estuary which are pushed towards inland [19]. *C. virginica* oysters from Alvarado presented their highest concentration of lindane 7.60 $ng \cdot g^{-1}$ in the dry season, however during the north winds season no concentrations of this pesticide were found in *C. virginica* samples.

Lindane is not found naturally in the environment. The entry of lindane into the environment occurs during its formulation and its use as a pesticide. Bioconcentration in microorganisms, invertebrates, fish, birds and in humans takes place rapidly. Biotransformation and elimination also occur fast when exposure is eliminated, according to WHO (1991) [20]. Lindane is not toxic to bacteria, algae and protozoa [8], but is highly toxic to some fish and aquatic invertebrates. It has been detected in different mammals and birds in the Arctic [8]. Even at lower concentrations it has significant effects, due to this, they were related to the order of biological organization; for example from major to minor: effect on physiological activity> functional and structural alterations of molecules> cellular toxicity> mortality of organisms [21].

The presence of lindane has been detected in surface and drinking water, as well as in industrial and domestic effluents from Europe and the United States. Lindane was found in rainwater in Tokyo (29 - 398 ng/l) and soils in Ukraine contained lindane levels of 0.1-5 mg/kg [20] (WHO, 1991). On the other hand, in the lake Ologe in Nigeria, lindane was predominant in fish samples during rainy season [22].

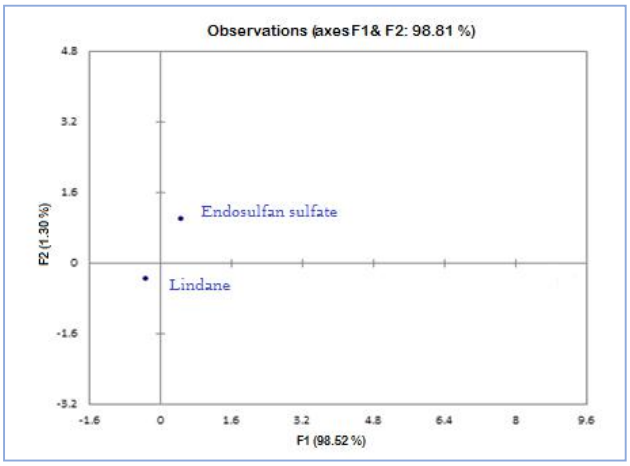

Fig. 3: Location of organochlorine pesticides, lindane and endosulfan sulfate in the axes.

Endosulfan sulfate has been classified by the United States Environmental Protection Agency (US EPA, 2002) [23] as highly toxic for marine and freshwater fish. Values of US EPA (2006) [23] for the protection of aquatic life are 0.22 $\mu g \cdot L^{-1}$. Crustaceans are particularly sensitive to endosulfan. It is known that this pesticide is highly toxic to many lobster and shrimp species, with average lethal concentrations (LC_{50}) close to 1 $\mu g \cdot L^{-1}$ [24].

Molluscs have been selected as monitoring species for their sessile and sedimentary lifestyle, and their ability to integrate environmental contamination in time and space [25]. For this ability to accumulate contaminants *C. virginica* has been used to conduct research to determine the concentration of organochlorine pesticides as endosulfan isomers and its metabolites in organisms from various lagoon systems where this bivalve lives. However, although there are precedents of a gradual increase in the concentration of this pesticide, there is a lack of consecutive monitoring in these lagoon systems. Likewise, Liu *et al.*, (2010) [26] indicated that few data are available to compare due to the scarce information on the concentration of endosulfan in fish and mollusc species.

In compiled investigations on the presence of endosulfan in *C. virginica*, it has been noted that the reported concentrations for β-endosulfan were higher than the

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concentration reported in various investigations compared to the alpha isomer and endosulfan sulfate [27]. This is in agreement with Berntssen *et al*. (2008) [28] about the relatively high concentrations of β-endosulfan, contributing to affirm that this isomer is more persistent. In contrast Wang *et al*., (2014) [29] indicated that biota such as fish and cultures can absorb α-endosulfan much easier than β-endosulfan, they also noted that in these samples the highest concentrations obtained were endosulfan sulfate followed by α and β endosulfan.

In many cases they are also difficult to remove by organisms because they are poorly soluble in water and tend to accumulate in fatty tissues. When organisms are eaten by others, then lindane accumulates in greater proportions in the final stages of the trophic chain [30,23]. So, a pesticide that is in very low concentrations in a forest or lake, without showing any risk, ends up being in concentrations tens or hundreds of times higher in the fatty tissues of the organisms like birds of prey and predatory fish or mammals that are located at the top of the trophic chain [31].

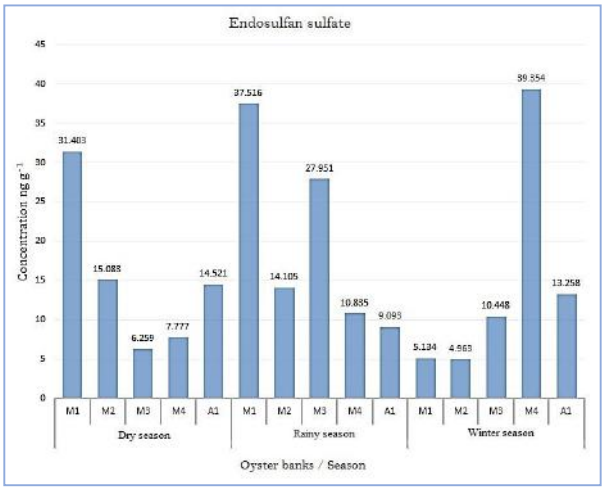

Fig. 4: Concentration of endosulfan sulfate during sampling periods, in the Mandinga and Alvarado lagoon systems, in Veracruz, Mexico.

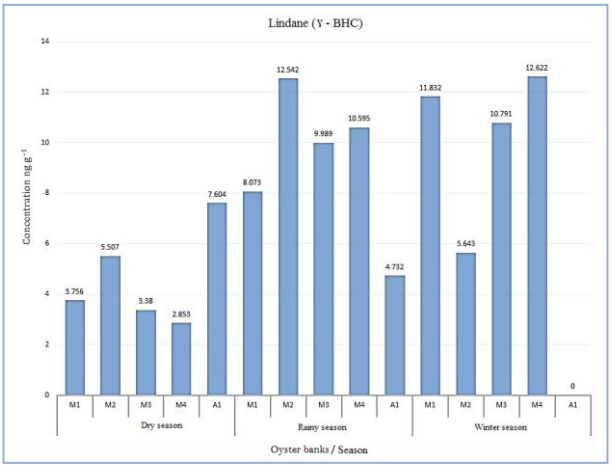

Fig. 5: Lindane concentration during sampling periods, in the Mandinga and Alvarado lagoon systems, Veracruz, Mexico.

IV. CONCLUSION

Organisms sampling was carried out in 2010. A year marked by an important meteorological event with maximum sustained winds of 190 km/h with gusts of 235 km/h, leaving rains in high zones registering maximums of 210 to 355 mm [32] that caused strong currents in the Jamapa river basin. Hence the *C. virginica* oyster of the Mandinga Lagoon System presented evidence of contamination with pesticides: endosulfan sulfate and lindane. At sampling point 4, endosulfan sulfate and lindane were recorded at concentrations that exceed the sediment quality criteria, studies report that these concentrations cause damage to benthic organisms. The concentration of the pesticides endosulfan sulfate and lindane registered in the Mandinga lagoon system is at levels that represent a risk to the benthic community and a potential risk for human consumption.

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Insecticidal activities of diketopiperazines of *Nomuraea rileyi* entomopathogenic fungus

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***Abstract*—** Entomopathogenic fungi are fungal organisms extensively used in various parts of the world as biopesticides against insect pests that cause important economic damage. Various secondary metabolites produced by these fungi have many potential biological activities. The present study was undertaken to evaluate the insecticidal activity of extracts and pure compounds from *Nomuraea rileyi* (Farlow) Samson entomopathogenic fungi against *Spodoptera frugiperda* Smith (Lepidoptera), *Ceratitis capitata* Wiedemann (Diptera) and *Tribolium castaneum* Herbst (Coleoptera), three insect pests that generate serious economic losses in the northwest of Argentina. Diketopiperazines were extracted from the culture free supernatant of the media with ethyl acetate. Antifeedant properties were detected in all extracts under dietary choice conditions (300 ug/ g of diet). The maximum antifeedant activity was noted in cycles (Pro-Val) (86.02) and cycle (Pro-Phe) (73.47), while the rest of the extracts and metabolites exhibited varying degrees of moderate or less toxic effects. The maximum oviposition deterrence against *C. capitata* (55.86%) was recorded with cycle (Pro-Phe) at a 50 µm/cm^2 dose. Culture medium extracts supplemented with insect remains and all pure compounds showed repellent action against *T. castaneum*. The main repellency was observed in phenylacetic acid and cycle (Pro-Val) with RI values of 42 and 41% respectively. The present study would suggest the possible utilization of entomopathogenic fungal metabolites as an effective agent for controlling insect pests that cause important economic losses.

Keywords*— *antifeedant, entomopathogenic fungi, insect pests, oviposition deterrence, repellency.

I. INTRODUCTION

In the last four decades, many research groups have concentrated their efforts in the search for bioactive products derived from natural sources that represent an alternative to conventional insecticides. The results of these investigations constitute new tools for pest control that differ fundamentally from conventional chemical control in that they are more ecosystem friendly.

Among the biological controllers are predatory insects and parasitoids, as well as insect pathogens, including viruses and different genera and species of protozoa, bacteria, fungi and nematodes (Asaff et al., 2002).

Entomopathogenic fungi (EF) are the main biological agents used in integrated pest management systems. To date, more than 750 species of entomopathogenic fungi belonging to almost 100 genera have been identified, most of which are classified among the Zigomicota (entomoptera), Deuteromicota (hyphomycetes) and Ascomicota (Roberts, 1989; Hegedus and Khachatourians, 1995; Khachatourians, 1996). However, only 10 of them have been or are currently being used in commercial or experimental insect control formulations (Lacey et al., 2001).

Although entomopathogenic fungi have many advantages they are highly sensitive a varying climatic conditions like extreme temperatures, drought and ultraviolet light. They need more demanding storage conditions than inorganic molecules to prevent pathogenicity loss. In general, biological insecticides do not kill instantly, but they achieve good levels of control between one and three weeks after application, depending on the pest and the environment. Hence, researchers have begun to look produced metabolites for fungus to control pests and avoid climatic dependance. A review of the literature in recent decades shows that a considerable number of low

molecular weight secondary metabolites isolated from insect pathogens have proved to have insecticidal activity (Gilliespie and Claydon, 1989). Many secondary metabolites produced by entomopathogenic fungi are common to several of them and have been detected in most cases by their in vitro production, which is significantly affected by the conditions and composition of the culture medium (Khachatourians, 1996).

Nomuraea rileyi (Farlow) Samson has been reported as a pathogen of more than 30 species of lepidopteran larvae, especially when they are in humid weather conditions (Devi et al., 2003). It has been mainly isolated from dead insects and cultivated soils, being very closely associated with important phytophagous species, such as *Spodoptera frugiperda* (J.E. Smith) in maize fields (Wyckhuys and O'Neil, 2006). Although the potentiality of the fungus as a biological control agent is recognized, it has not yet been widely used as a mycoinsecticide (Edelstein et al., 2004) because it is highly sensitive to nutritional and environmental conditions as compared to other entomopathogenic fungi and this characteristic limits its mass production. Previous studies have reported that the fungus *Nomuraea rileyi* produces active metabolites against insects (Ignoff et al., 1976; Wasti and Hartmann, 1978; Kucera and Sansinakova, 1968; Mohamed and Nelson, 1984; Ye et al., 1993), some metabolites showed toxic activity against larvae *of Heliohis zea*, *H. virescens* (Mohamed and Nelson, 1994) and Bombyxmori (Ye et al, 1993). Additionally, it is known that the addition of insect derived material in the broth culture could trigger the biosynthesis of bioactive compounds by EF (Lee et al., 2005; Kikuchi et al., 2004; Cartagena et al., 2014).

Spodoptera frugiperda (Lepidoptera), is a polyphagous species with a wide geographic distribution, from Argentina and Chile, to the south of the United States. Its preferential host is maize, causing important losses and putting at risk the productivity of the same. In Argentina it was declared a national pest in 1988 (Murua et al., 2003). Among crops attacked are sorghum, alfalfa, cotton, rice, soybean, peanut, tomato, pepper, poroto, onion, sunflower, cabbage, cauliflower, etc. It is the main pest of the northwest and northeast of Argentina (Willink et al., 1990).

The Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) is a key pest that is distributed worldwide and attacks more than 250 species of fruits and vegetables (Morales et al., 2004), causing large economic losses.

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) is one of the most widespread and destructive pests of stored products, feeding on different stored grain, and grain (Padín et al., 2013). In Argentine, it is one key pest of stored grain in the ports (Stefanazzi et al., 2011). T. castaneum known as ‘‘the red flour beetles’’ attack stored grain products causing deterioration, especially loss of quality and weight during storage. In addition, they may cause an allergic response (Kumar et al., 2008).

The objective of the present work was to evaluate the insecticidal activity of extracts and pure compounds isolated from cultivations of *Nomurae rileyi*, entomopathogenic fungus, with and without the addition of insect-derived material against *Spodoptera frugiperda* (Smith) (Lepidoptera), *Ceratitis capitata* (Wiedemann) (Diptera) and *Tribolium castañeum* (Herbst) (Coleoptera), insect plagues that generate serious economic losses in the Argentine northwest.

II. MATERIALS AND METHODS

2.1 *General*

NMR spectra were recorded on a Bruker AC spectrometer operating at 300 MHz for 1H and 125 MHz for 13 C with TMS as internal standard in $CDCl_3$. The mass spectra were recorded on a THERMO POLARIS Q (EIMS). For HPLC separation of mixtures, Waters equipment was used. Detection was accomplished by the use of refractive index detector. Column Phenomenex Luna C8 (5 µm, 10 mm i.d. x 250 mm), was used. Retention time was measured from the solvent peak.

2.2 *Culture of entomopathogenic fungi*

Nomuraea rileyi strain ARSEF 1972 were isolated from *Spodoptera frugiperda* [Lepidoptera: Noctuidae], in Bahia (Brazil) in 1985. The strains were assigned to Dr. Mario Arena, by Professor Richard A. Humber, ARSEF Director (ARS Collection of Entomopathogenic Fungal Cultures), New York (USA). *N. rileyi* was maintained to Sabouraud-maltose agar supplemented with 1% yeast extract (SMAY) and was incubated in an oven at 25±2 °C for 14 -15 days until they developed dense sporulation. The spores were resuspended with sterile water containing 0.05 % Tween 80. The desired spore concentration were determined using an improved Neubauer chamber and adjusted to 8.7 10^8 spores/Ml.

2.3 *Experimental culture media*

Three experimental culture media were developed:

Medium A: Sabouraud-maltose fortified with 1% yeast extract (SMY) + 3 % (v/v) of the suspension spores of *N. rileyi* ARSEF 1972.

Medium B: SMY + 1 % (w/v) of *S. frugiperda* cuticles (powder) + 3 % (v/v) of the suspension spores of *N. rileyi* ARSEF 1972.

Medium C: SMY + 1 % (w/v) of *S. frugiperda* cuticles (powder)

All culture media (A, B and C) were cultivated for 15 days at 25 °C at 180 rpm on a rotating shaker.

2.4 *Preparation of extracts from different culture media*

After the incubation period, the biomass and insoluble residues were separated to the supernatants by filtration. The filtrate media obtained from the different culture media was carried out three liquid-liquid extractions with ethyl acetate (AcOEt) in equal parts. To the biomass and insoluble residues were made solid-liquid extractions with AcOEt and then with methanol (MeOH). The organic phases obtained were then evaporated on a rotary evaporator under reduced pressure. The weight and yield of the dry extracts were determined for each case. Fig. 1 shows the experimental design of the extracts obtained.

2.5 *Isolation, purification and structural elucidation of fungal metabolites*

The ethyl acetate extract from the supernatant of Medium B (4,5 g) was subjected to silica gel CC (70–230 Mesh) with $CHCl_3$ and increasing amounts of EtOAc (0–100%) and finally MeOH, as eluents, to give 9 fractions of 10 mL each. The fractions III, IV, V and VI were selected for their activity to continue the isolation.

Fr. III (148.7 mg), which eluted with a mixture of $CHCl_3$–EtOAc (85:15), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 65:35, 1.5 mL min^{-1}) to give compound **1** (58.1 mg, Rt 4 min).

Fr. IV (147.1 mg), which eluted with a mixture of $CHCl_3$–EtOAc (80:20), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 2:1, 1.5 mL min^{-1}) to give compounds **2** (10.5 mg, Rt 3 min) and **4** (9.6 mg, Rt 11 min).

Fr. V (146.1 mg), which eluted with a mixture of $CHCl_3$–EtOAc (50:50), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 45:55, 1.5 mL min^{-1}) to give compound **3** (18.2 mg, Rt 3 min).

Fr. VI (132.2 mg), which eluted with a mixture of $CHCl_3$–EtOAc (0:100), were combined and submitted to HPLC (Column Phenomenex Ultremex C8, MeOH–H_2O 50:50, 1.5 mL min^{-1}) to give compound **5** (5.6 mg, Rt 8 min).

The structures these compounds were completely elucidated by using extensive spectroscopic methods and by comparison with data previously reported in the literature (Adamczeski et al., 1995; Pedras et al., 2005; Huang et al., 2010; Yan et al,. 2004; Wang et al., 2010).

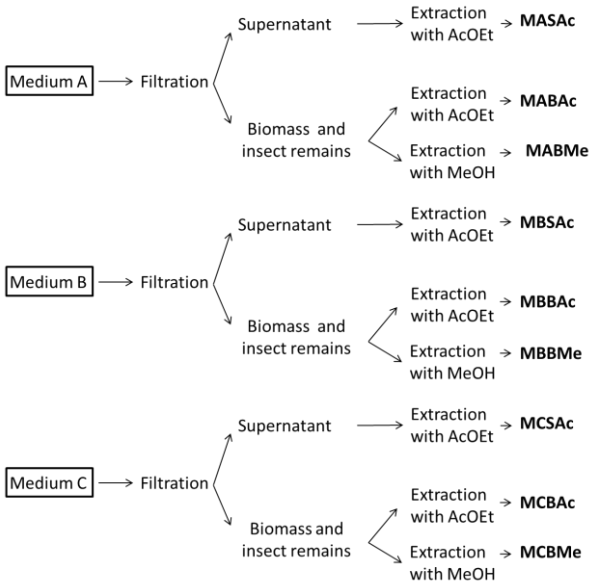

Fig.1: *Ethyl acetate extract from the medium supernatant without insect remains* ***(MASAc)****; Ethyl acetate extract from the medium biomass without insect remains* ***(MABAc)****; Methanolic extract from the medium biomass without insect remains* ***(MABMe)****; Ethyl acetate extract from the medium supernatant supplemented with insect remains* ***(MBSAc)****; Ethyl acetate extract from the medium biomass supplemented with insect remains* ***(MBBAc)****; Methanolic extract from the medium biomass supplemented with insect remains* ***(MBBMe)****, Ethyl acetate extract from the medium supernatant of insect remains* ***(MCSAc)****; Ethyl acetate extract from the medium biomass of insect remains* ***(MCBAc)****; Methanolic extract from the medium biomass of insect remains* ***(MCBMe)****.*

2.6 *Insect rearing*

S. frugiperda larvae were obtained from our laboratory population. The larval diet consisted of a mixture of yeast (3 g), bean boiled and milled (250 g), wheat germ (12.5 g), agar agar (12.5 g), ascorbic acid (1.5 g), methyl p-hydroxybenzoate (1.5 g), formaldehyde (4 mL of a 38% water solution), and water (500 mL).

The colony of *C. capitata* used in the bioassays derived from the laboratory of the Experimental Agroindustrial "Obispo Colombres" station. It was initiated with pupae of oranges infested obtained in northwestern Argentina. Adults were fed with a solution prepared with water and a mixture of sugar and hydrolyzed protein ratio (3: 1) diet. The brood chamber is maintained at 24 ° C ± 2 ° C, 60 ± 10% relative humidity and a photoperiod of 12L: 2D.

T. castaneum was collected from flour contaminated by the insect. They were identified taxonomically, through entomological keys and raised in the laboratory under controlled conditions (25 ± 2) ° C and (65 ± 5) HR in brood chamber and fed a diet of self-rising flour.

2.7 *Antifeedant test against S. frugiperda (Choice test and No Choice test)*

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The antifeedant activity was tested based on the methods described by Vera et al., (2008). A portion of artificial diet was mixed with acetone and, after solvent removal *in vacuo*, this portion was employed as control diet. Another portion was mixed with an acetone solution of each treatment, at 300 and 150 µg/ g of diet for extracts and pure respectively. In the no choice test, the same amount of control and treated diets were placed in a different glass tube with a larva inside. Larvae were allowed to eat and, when 50 % of control diet had been eaten, control and treated diets were removed from the tubes and weighted accurately. The experiment was carried out in 20 replicates. To evaluate the feeding behavior a "Feeding election index" was calculated as FEI = (1 – T/C) 100, where C and T represent the amounts eaten of control and treated diets, respectively.

2.8 *Insecticidal bioassay against S. frugiperda*

Insecticidal bioassay against *S. frugiperda* was investigated based on the methods described by Sosa et al., 2017. The duration (days) of the larval period, percentage the larval and pupal mortality and the number of malformed adults were registered at 300 µg/ g of diet for all the extracts.

2.9 *Effects of extracts on food consumption and utilization*

Ten days after the beginning of the experiment, the larval weight and diet eating were determined again, in order to record the relative consumption rate (RCR), relative growth rate (RGR), efficiency of conversion of ingested food (ECI), were calculated as follows (Farrar et al, 1989; Haouas et al., 2010).

2.10 *Oviposition-Deterrent Activity against C. capitata*

Oviposition-Deterrent Activity against *C. capitata* was investigated based on the method described by Socolsky et al., 2008. Artificial fruits (oviposition substrates) were prepared. The surface of the wrapped cylinder was pricked with a needle and treated with an acetone or methanol solution of the sample to be tested. An amount of 50 µg of extracts/cm^2 and 25 µg pure compounds/cm^2 were deposited, respectivily. The inhibition of oviposition index (OI) was calculated: OI = [(1 − T/C) × 100]

2.12 *Bioassay food preference and repellency against T. castaneum*

This test was adopted according to the method previously described by Cartagena et al., 2014. The concentrations of extracts and pure compounds were 250 and 125 µg per g of diet, respectively. After 24 hours, count of the individuals present was performed on both diets (T and C) and food preference was assessed by calculating the food Preference Index (PI):

PI= (% ITD - % ICD) / (% ID + %ICD)

Where

% ITD = % insects in the treated diet

% ICD = % insects in the control diet

For PI value between - 1.00 and - 0.10 states that the substance is repellent; PI between - 0.10 and 0.10 + neutral substance is between 0.10 and + 1.00 + substance is attractant (Procopio et al., 2003; Stefanazzi et al., 2006).

The repellency is determined by the Repellency index (RI), where positive values indicate RI repellency and negative values, atractancia (Pascual Villalobos, 1998).

(C-T)/(C+T) x100

Where **C =** Insect in the control diet and **T =** Insect in the treated diet

2.13 *Statistical analysis*

The results are reported as mean ± SEM. The differences in the mean values were evaluated by analysis of variance (ANOVA). The Tukey test was used for all pair wise multiple comparisons of groups. In all statistical analysis, $P > 0.05$ was considered not significant.

III. RESULTS AND DISCUSSION

3.1 *Isolated metabolites*

The use of fungal biological control agents is a rapidly developing field and is increasingly adopted and accepted worldwide in the management of agricultural pests (Jaronski, 2010; Hajek and Delalibera, 2010). In most of the works carried out for the biological control of pests, concentrations of conidia of entomopathogenic fungi are used. There are few bibliographic records of fungal extracts used as insecticides.

N. rileyi, another potential entomopathogenic fungus. It has been shown that many insect species belonging to Lepidoptera, including *S. litura* and some belonging to Coleoptera, are susceptible to *N. rileyi* (Ignoffo, 1981). The host specificity of *N. rileyi* and its ecofriendly nature encourage its use in insect pest management. Wasti and Hartmann, 1978 and Mohamed and Nelson, 1984 have reported the toxic effects of mycelial extracts on *Lymantria dispar*, *Heliothis zea* and *H. virescens* larvae. Onofre in 2002 presented the insecticidal effect of *N. rileyi* on *A. gemmatalis* after ingestion. However, most studies to date have focused on the activity of *N. rileyi* as an insecticide and its use as a control agent, but very little is known about the metabolites responsible for this effect. Prompiboon in 2008 and Supakdamrongkul in 2010 were the first to isolate and identify *N. rileyi* metabolites that were active against *S. litura*.

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In this work, after exhaustive chemical processing of the MBSAc extract (5.0387 g) with a yield of 414.71 mg/L, it was possible to isolate five pure compounds: phenylacetic Acid (**1**), 1-Phenylbuten-2,3-diol (**2**), cycle (Pro-Val) (**3**), cycle (Pro-Leu) (**4**) and cycle (Pro-Phe) (**5**). Compounds 3, 4 and 5 belong to the diketopiperazine family (DKPs) and were reported for the first time in *N. rileyi* (Fig. 2).

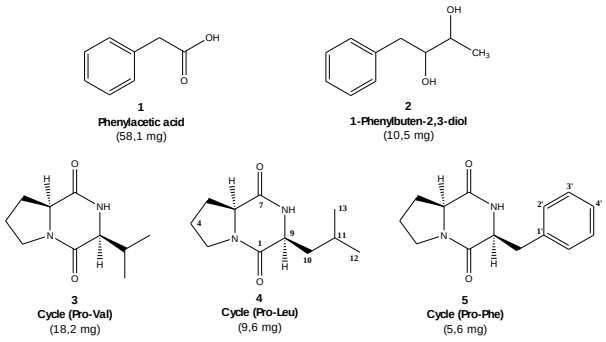

Fig.2: *Compounds isolated from ethyl acetate extract from the medium supernatant supplemented with insect remains* ***(MBSAc)***

Diketopiperazines are naturally occurring cyclic dipeptides, many of which show a wide range of antimicrobial, antiviral, antitumor and immunosuppressive activities (Prasad, 1995). They have been isolated from yeasts, lichens, fungi, bacteria and marine sponges (Mitova et al., 2005) and their skeleton is generated by the cyclization of two L-α-amino acids, so that most naturally occurring DKPs have a cis configuration (Bull et al., 1998).

Santamarina, 2002 and Gimenez, 2000 carried out tests against *Oncopeltus fasciatus* with extracts obtained from different strains, such as *Aspegillus*, *Penicillum*, *Fusarium*, *Alternaria* and *Trichoderma harzianum*, obtaining positive results. *Beauveria bassiana*, is one of the most studied entomopathogenic fungi. It produces metabolites such as bassianin, beauvericin, bassionolide, beauveroiolide, bassacridin, oosporein, and tenellin (Jeffs and Khachatourians, 1997; Quesada-Moraga and Vey, 2004). This would indicate that the production of active metabolites is involved in the antagonistic effect of these agents.

3.2 *Antifeedant activity*

In the Choice Test all the extracts tested (300 µg/g of diet) presented an anti-alimentary effect with FEI values above 42%. When the insects were fed one diet, only extracts supplemented with insect showed intake inhibition but it was significantly lower. However, compounds **2**-**5** isolated from the MBSAc extract were active in both tests (150 µg/g of diet). Diketopiperazines **3** and **5** were the most active in both tests (FEI_{CH} = 86 and FEI_{NCH} 81%) and (FEI_{CH} = 73 and FEI_{NCH} 75%), respectively. Compound **1** was the only one that stimulated ingestion in both tests (Table1).

A higher antifeedant index normally indicates a decreased rate of feeding. An antifeedant is a chemical that inhibits feeding without killing the insect directly, but causing it to die through starvation when it remains near the treated foliage (Yasui et al., 1998; Pavunraj et al., 2012). Antifeedants offer first line of crop protection against notorious insects. Any substance that reduces food consumption by an insect can be considered an antifeedant or feeding deterrent (Isman, 2002). In general, antifeedants have profound adverse effects on insect feeding behavior (Hummelbrunner and Isman, 2001). Antifeedants can be described as allomone substances which inhibit feeding and do not kill the insect pests directly, but rather limit their developmental potential considerably and act as a phagodeterrent or phagorepellent over test as well as permanent insect pests feeding on the plant (Lakshmanan et al., 2012).

3.3 *Insecticidal bioassay against S. frugiperda*

As shown in Table 2, the extracts tested at 300 µg / g showed no significant differences in the physiological indices (RCR, RGR, ECI) with respect to the control.

The test was continued by analyzing lethal and sublethal effects produced by the substances until the insects reached their last stage Table 2. The larval period was not altered by the extracts tested. The AcOEt extracts from the biomass and the supernatants of both culture conditions showed a larval mortality between 10 and 20%. Malformation of adult insects was observed. The MABMe extract showed the moderate percentage of malformation (40%), which would result in a reduction of the viable and fertile population of the insect.

3.4 *Oviposition Deterrent test*

The extracts from the fungus-insect medium were the most active, showing moderate to strong action with deterrence values higher than 50% at 50 µg / cm^2. The highest percentage belonged to MBBAc (OII 79%). Other authors have reported a reduction in the oviposition rate of *C. capitata* in fruits treated with commercial formulations containing conidia of *Beauveria bassiana* (Falchi et al., 2015). They also evaluated the efficacy of the commercial bioinsecticide "Naturalis" based on the *B. bassiana* ATCC 74040 strain against *C. capitata* (Wiedemann) fruit oviposition. In laboratory conditions, females of the Mediterranean fly preferred to place 5 to 3 times more eggs in untreated fruits than in fruits treated with co-formulants of the bioinsecticide (Ortu et al., 2009). Isolates of *Metarhizium anisopliae* and *B. bassiana* were active against adults and pupae of *C.*

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capitata (Lacey et al., 2001; Ortu et al., 2009; Ekesi et al., 2002; 2005; Dimbi et al., 2003; Konstantopoulou and Mazomenos, 2005) while *Paecilomyces fumosoroseus* (Wize) has been shown to reduce fertility and fecundity of the Mediterranean fruit fly (Castillo et al., 2000). However, since there are no reports of insecticidal activity of *N. rileyi* on dipterans, our results would be the first to report on the activity of *N. rileyi* extracts in the inhibition of *C. capitata* oviposition. All compounds tested showed a greater or lesser effect on the inhibition of oviposition of *C. capitata* at the concentration of 25 µg / cm^2 with percentages ranging from 16 to 70%, the most active being 1-phenylbuten-2,3-diol compound (2), followed by 5 and 1. This activity could be attributed to the presence of an aromatic ring, a structural similarity that these compounds possess.

3.5 *Bioassay food preference and repellency against T. castaneum*

When analyzing the results obtained in *T. castaneum*, the medium-insect extracts of *Nomuraea* were found to be repellent. The methanolic extract of the MBBMe biomass proved to be the most active, while the extracts from the control-fungus medium proved to be attractive (Table 4). The activity of *Paecilomyces fumosoroseus* on adults and larvae of *T. confusum* and *B. bassiana* against *Capnodis tenebrionis* (Coleopterus) are reported in previous works (Michalaki et al., 2007; Marannino et al., 2006).

Most accounts of insecticidal activity of *N. rileyi* are mainly on Lepidoptera. However, Ignoffo, 1981 reported its activity on two *Hypera punctata* and *Leptinotarsa decemlineata* beetles. Cartagena, 2014 shows that the addition of *T. castaneum* components (2% w / v) in a culture of the fungus *Aspergillus parasiticus* MOR3 induces the production of insect repellent substances mentioned above. This would suggest the specific biosynthesis of fungal metabolites in order to control the insect. In this work, all compounds tested showed repellent action, the most active being compounds 1 and 3 with RI values of 42 and 41% and PI values of -0.43 and -0.42 respectively, with no mortality at 125 µg / g diet in any of the compounds tested (Table 4).

There is little information on the insecticidal activity of DKPs. Only Cycloechinulin, was reported to be effective in the control of coleoptera and lepidoptera such as *H. zea* and *Carpophilus hemipterus* (de Guzman et al., 1993). Three DKPs isolated from *Eurotium cristatum* with the isopentyl group substituted by indole residues, showed cytotoxic activity against *Artemia salina* (Chinese Patent No: CN102675293 2012; Chinese Pat-ent No: CN102669110, 2012). Recent studies revealed that Cycle (Trp-Phe) exhibited dose-dependent anti-alimentary, larvicidal and pupicidal activity against *H. armigera*. In addition, the purified compound prolonged the larval and pupal period when compared to the untreated control (Sathya et al., 2016).

IV. CONCLUSIONS

Our results are the first account of insecticidal activity of *N rileyi* extracts on three species of insect pests. In addition, isolated dikepiperazines are reported for the first time in this fungus. Although there are papers on the activity of some DPKs on lepidopterans and beetles, no publications were found on the insecticidal activities of the three DPKs studied in this work. Hence, this would be the first study to publish the insecticidal activity of these compounds, besides being the first report on the insecticidal activity of DPKs in Diptera, specifically *Ceratitis capitata*.

These results would be promising for the development of control agents for *S. frugiperda*, *C. capitata* and *T. castañeum*, pests that generate serious economic losses in the northwest of Argentina. In turn, these results show the potential of diketopiperazines as leading structures for the synthesis of new, more potent and environmentally friendly insecticides.

Conflict of interest

The authors declare that there are no conflicts of interest and they have no actual or potential competing financial interests

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Table.1: Antifeedant activity of extracts and pure compounds of culture from N. rileyi ARSEF 1972 on S. frugiperda

		FEEDING DETERRENCE INDEX	
		FEI_{CH} Choice test (%)	FEI_{NCH} No choice test (%)
Extracts (300 µg/g)	MASAc	73.92 ± 7.91a	-7.41 ± 0.71a
	MABAc	69.92 ± 11.73a	-21.73 ± 2.15b
	MABMe	63.51 ± 12.60a	-34,32 ± 8,63c
	MBSAc	61.70 ± 17.11a	21.41 ± 4.22d
	MBBAc	75.28 ± 13.56a	22.30 ± 4.93d
	MBBMe	42.43 ± 17.56b	10.13 ± 3.81e
Compounds (150 µg/g)	1	-24,39 ± 4,04a	-20,04 ± 5,24a
	2	58,91 ± 8,58b	65,64 ± 8,02b
	3	86,02 ± 7,92c	81,66 ± 6,61b
	4	49,85±5,79b	42,74±6,78c
	5	73,47±7,57c	75,21±6,91b

Feeding election index ± SEM (n = 20). * The values in a column with the same letter do not present significant differences (P> 0.05, Tukey Multiple Range Test).* FEI (%) = Feeding election index = [(1 − T/C) × 100], where C and T represent the amount of control and treated diets, respectively, consumed during the test.

Table.2: Effect of N. rileyi ARSEF 1972 culture extracts, incorporated in an artificial diet, on larval development of S. frugiperda larvae, pupae and adult insects.

	PHYSIOLOGICAL INDEX			LETHAL AND SUB-LETHAL EFFECTS				
Extracts (300 µg/g)	RCR (mg/mg/days)*	RGR (mg/mg/days*	ECI (%)*	Larvae period (days)*	Pupae period (days)*	Larvae Mortality (%)	Pupae Mortality (%)	Malformation Adults (%)
Control	**0.51±0.06a**	**0.25±0.03a**	**21.11±2.91a**	**15.41±1.41a**	**11.15±1.21a**	**5**	**-**	**5**
MASAc	0.59±0.05b	0.18±0.01b	31.63±3.72b	15.13±2.61a	10.31±1.32a	11	-	23
MABAc	0.57±0.06b	0.18±0.01b	32.23±3.51b	14.11±1.31a	10.62±1.61a	20	-	14
MABMe	0.52±0.08a	0.19±0.01b	31.14±4.40b	15.92±1.13a	10.42±1.44a	-	17	40
MBSAc	0.59±0.04b	0.23±0.02c	37.40±4.51b	13.32±1.02a	11.73±1.24a	20	5	20
MBBAc	0.49±0.04a	0.22±0.01c	32.21±6.43b	14.51±1.33a	11.61±1.24a	10	25	20
MBBMe	0.58±0.04b	0.23±0.01c	37.11±3.15b	13.72±1.22a	11.62±1.23a	5	-	35

ECI: Efficiency of conversion of ingested food; **RGR**: Relative growth rate. **RCR**: Relative consumption rate.
*Values in a column with the same letter do not show significant differences (P> 0.05, according to Tukey's Multiple Range Test). The values in the columns represent the mean ± SEM (n = 20).

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Table.3: *Effect of culture extracts and compounds from N. rileyi ARSEF 1972 on the oviposition behavior of C. capitata*

		OVIPOSITION INHIBITION		
		Number of Eggs Laid on the Treated Fruit*	Number of Eggs Laid on the Control Fruit*	OII (%)*
Extracts (50 µg/cm^2)	MASAc	207.33 ± 12.06c	501.33 ± 20.13c	58.63 ± 2.17a
	MABAc	252.00 ± 28.00d	437.33 ± 22.03 d	42.26 ± 7.47c
	MABMe	148.00 ± 7.21e	404.00 ± 14.42d	63.30 ± 2.92a
	MBSAc	98.00 ± 9.17a	269.33 ± 34.02a	63.14 ± 6.29a
	MBBAc	45.00 ± 4.24b	216.00 ± 0.00b	79.17 ± 1.96b
	MBBMe	113.33 ± 6.11a	320.00 ± 53.81a	64.11 ± 4.51a
Compounds (25 µg/cm^2)	1	117.33± 8.33a	208.00± 22.27a	43.43± 2.79a
	2	61.00± 1.41b	248.00± 5.66b	75.39± 1.13b
	3	180.00± 8.00c	214.67± 8.33a	16.13± 2.70d
	4	174.67±6.11c	248.00± 6.93b	29.57± 1.64c
	5	56.67± 3.06b	189.33± 8.33a	70.05± 1.60e

The values in the columns represent the mean ± SEM (n=3).*Values in a column with the same letter do not show significant differences (P> 0.05, according to Tukey's Multiple Range Test). OI (%) = Inhibition of oviposition index = [(1 − T/C) × 100], where C and T represent the amount of eggs in the treated and control oviposition substrate respectively.

Table.4: *Effect of the extracts and compounds of N. rileyi ARSEF 1972 on the behavior of T. castaneum*

		FOOD PREFERENCE AND REPELLENCY INDICES		
		RI (%)*[a]	PI (%)*[b]	
Extracts (250 µg/g)	MASAc	-27,56 ±2.61d	0.28±0.02d	attractive
	MABAc	-26.39 ±1.96d	0.26±0.03d	attractive
	MABMe	-27.16 ±4.01d	0.27±0.04d	attractive
	MBSAc	30.00 ±7.07a	-0.30±0.07a	repellency
	MBBAc	17.75 ±4.90b	-0.18±0.05a	repellency
	MBBMc	50.35 ±5.93c	-0.50±0.06c	repellency
Compounds (125 µg/g)	1	42.50±3.49a	-0.43±0.04a	repellency
	2	32.50±3.50b	-0.33±0.04b	repellency
	3	41.79±2.54a	-0.42±0.02a	repellency
	4	27.50±3.54b	-0.28±0.04b	repellency
	5	17.50±3.54c	-0.18±0.04c	repellency

The values in the columns represent the mean ± SEM (n=3).*Values in a column with the same letter do not show significant differences (P> 0.05, according to Tukey's Multiple Range Test). [a]Positive values indicate repellency. [b]PI values between -1.00 and -0.10 indicate that the extract is repellent; between -0.10 and +0.10 the extract is neutral and if the PI is between +0.10 and +1.00 the extract is attractive.

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Effect of Knives Type on Some Operational Characteristics fora Locally Assembly Motorized Vibration Cutter Used for Date Palm Fronds Pruning

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Abstract— *The experiment was conducted to evaluate the effects of pruning cutting knives for locally assembly motorized vibration cutter on some operational characteristic used for date palm fronds. An implement was fabricated to cut the fronds around the date palm tree trunk. Three types of knife included A,B and Cwas used in this study .One frond cutting time, One palm frond cutting time, cutting level,productivity, noise level, vibration and efficiency was measured in this experiment . Complete block design with three replications was used in this study. Least significant differences (L.S.D) under 0.05 level was used to compeer the mean of treatment.*

The results showed thatB type gave a lower time in cutting one frond stood 3.11 sec. A type got lowest time of cutting three rows of fronds stood 1.74 min, also gave less differences in surface cutting level andlevel of noising stood 5.66 mm and 78.04 (db) respectively.B type knife got less vibration stood 5.25 m.sec^{-2}. Also it gave the higher amount of productivitystood 8.80 palm /h. A type gave a high efficiency, it got 78.76%.Using manufacturing equipment for cutting date palm frond was successfully done.

Keywords— Knives type, cutting time, fronds cutting, vibration,productivity, noise, cutting level, and efficiency.

I. INTRODUCTION

Date palm (*phoenix dactylifera* L.) can be consider as one of many oldest trees which human has derived benefit and it has been cultivated since long ancient time [11], also considered as oldest known fruit and only desert plant defiantly domesticate in its harsh environments [15].Date palm tree has been cultivated for at least 5000 years in north Africa and the middle east [4].date palm tree has along trunk, with about 30m height and lives about 100 years [10]. Many operations that need to performed during several times of the year to maintain thecrown of the date palm [6].Therefore,Cutting the dry and dead fronds is one of important date palm crown maintenance because, they hinder the worker to climb the palm trunk to do essential operations like pollination and harvesting [1] .Frond base is the strongest part of palm frond irrespective of moisture content and maturity [3]. Numerous of traditional tool are used to cut the palm fronds like knives and saws tools [12], these tools has many disadvantages ,generally, need more energy for cutting,this energy mainly comes from worker and this effort can be reduce by sharpness the tool edge and man self-skill, therefore, the worker should be strong enough to maintain his energy all day, generally workers cannot be able to maintain his energy for the whole day and normally stop when they fell tired [8].So, tools and Implements are playing a significant role to reduce effort ,cost and operations time, alsoFrond analysis could provide the basic parametric requirements of the blade for efficient cutting [14] . Farmers looking for tools and equipment used motorized power to cut the palm frond in leastof effort and cost.

Pruning is an important agricultural operation to remove date palm tree leaves; leaf bases and the fiber also remove spines and high offshoot [1]. Many factors are affecting in cutting plant material like physical properties of plant material, react against the cutting tool, method of cutting, cutting angle and speed of cutting also sharpness and shape of cutting tool [13].Development of a more effective, efficient and ergonomic cutting mechanism for palm frond cutting is important in facing futureglobal competition [2].

Chisel, sickle and curved knife are widely used as cutting tools in many countries and still effective because there's no new tools have surpassed them [8] .Thus, if there's a mechanical equipment that requires less power for date palm pruning would be able to work longer and increase of productivity, this study was conducted.

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II. MATIRIALSAND METHODS

The experiment was carrying out for testing a local assembly manufacturing palm fronds motorized cutter with three types of locally forged knives A,B and C were used in this experiment.Knives were forged by usinghigh temperature ovenat**790 C**° for 30 min after that doing water cooling to give them good hardness, then, tested the knives hardness by using Rockwell test, it is stood 65 HR.Time of one frond cutting, three rows of frond time cutting, level of surface cutting,field productivity, hand tool vibration, equipment noise and field efficiency was measured in the experiment. Randomized complete block design (CRBD) with three replications was used in this study, lest significant different (L.S.D) and 0.05 levels were used to compare the mean of treatment.

The motorized vibration date palm fronds cutter and three types of knives was manufactured and assembled at the mechanical workshop in the department of agricultural machines and equipment, college of agriculture, university of Baghdad. The equipment consists of many main parts.

2.1-Components of equipment, (Fig.1).

The motorized vibrator palm frond cutter consists of the essential following parts:-

1- Gasolineengine has 2 hp, two stroke engine, and 3000-5000 rpm.

2-Flexible shaft connection, it consists of rubber tube and rotary flexible shaft 0.5 cm rectangle section.

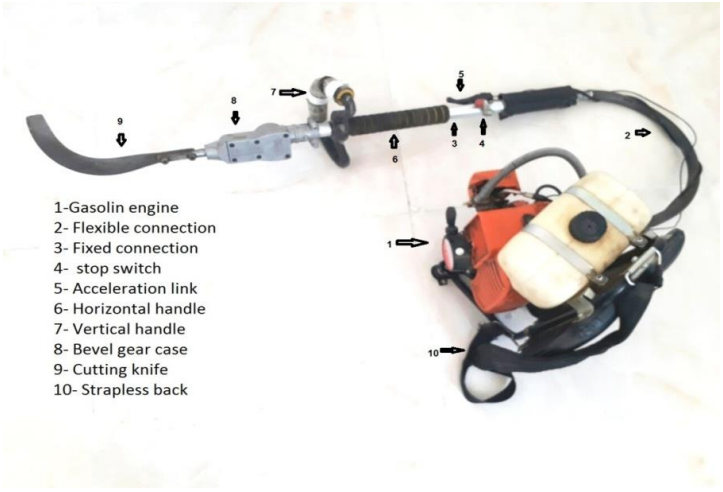


Fig.1: Motorized vibration date palm fronds cutter

3-Fixed connection ,consist of rotor circle section shaft with radius of 6.8 mm inside of aluminum pore with 25mm diameter.

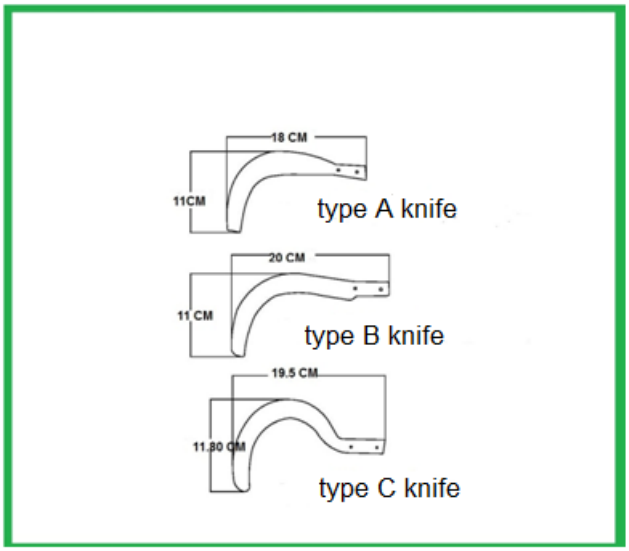
4- Gear case, used to convey the rotary motion to vibration motion.

5- Cutting tools, it is considers as a rigged curved knives include:-

A-Type A knife is a classic knife has length 18 cm, width 11cm and 1mm thickness in the front.

B- Type B knife with open end has length 20 cm, width 11cm and thickness 1 mm.

C- Type C shape knife, it has length 19.5 cm, width, 11.80 cm and 1 mm thickness (Fig.2)



2.2- Studied Properties

2.2.1.Time of cutting one frond

Cutting time measured by using stop watch when used equipment to cutthe fronds in date palm field,(frond. Sec)**.**

2.2.2Time of cutting three rows frond.

Time measured by using same method in 2.2.1.

2.2.3 Differences of cutting surface level .

Was measured the different in cutting surface level (mm).

2.2.4- Productivity

It was measured by calculate the total time of cutting including the lost time for rest and moving in the field also the time of refuel the engine and maintenance in one palm per hour (palm.h).

2.2.5 –Noise

Measured by using noise meter (db)

2.2.5- Vibration

Measured by using vibration meter (m.sec^{-2}).

2.2.7 Field efficiency

Field efficiency was measured by using the following question which proposetby[7]

$Fe=P_p/P_t$ x100

Whereas: **Fe**= Field Efficiency % ,**P_p**= Practical productivity , Palm/ h

P_t= Theoretical productivity Palm/ h

III. RESULTS AND DISCUTION

3.1 Cutting for one frond time,(Sec).

Table 1 shows the effect of knife type on time for one front cutting , A and B type of knives showed no significant different in the time of cutting one frond stood 3.11 and 3.22 secrespectively compared with C type witch got 3.34sec .

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3.2 cutting for one date palm time,(min).

Table 1 shows the effects of knife typeon time of cutting three rows of date palm tree,the results showed no significant different between A and B type, they got 1.74 and 1.76 min respectively, but there's a significant different with C type ,it got 4.04 min.

3- Variation in surface cutting level, (mm**).**

The result in table 1 showed the effects of type of knife in cutting leveling. Type A showed the superiority in the variation of cutting level stood 5.66 mm compared with B and C type which they got 5.81 and 5.92 mm respectively.

3.4Productivity (palm/h)

The relationship of type of cutting knife to the one date palm frondscutting productivity is shown in table 1 ,the Btype got highest field productivity amounted 8.80 palm/h, compared with A and C type witch they got 8.77 and 6.09 palm/h respectively.

Table.1: The effect of the cutting angle on the time of frond cutting, one palm fronds cutting time, cutting surface leveling and productivity

Knife type	One frond cutting time sec	One palm fronds cutting time /min	Cutting surface leveling/mm	Productivity Palm/h
A	3.11	1.74	5.66	8.77
B	3.22	1.76	5.81	8.80
C	4.34	4.04	5.92	6.09
LSD	0.11	0.10	0.11	0.13

3.5- Vibration level,($m.sec^{-2}$).3.

The results in table (2) showed the effect of type of knife in level of vibration that translate to the worker hand ,the effect of B type showed the superiority to get lower vibration stood 5.25 $m.sec^{-2}$ compared with B and C which they got 5.36,5.27 respectively.

3.6- Noise ,(db).

Table 2 showed the result of noise level test of an equipment .A type got less amount of noising level stood 78.04db compared with B and C type which they got 78.48 and 82.44db respectively .the reason for that may be due the less power required to cut fronds .

3.7- Field productivity efficiency, (%).

Table 2 shows no different between A and B type of cutting knife on the field efficiency witch they got highest amount stood 87.76 and 87.36 %,but the C type got a lowest efficiency stood 60.90 % compared ,the result showed changethe knife type from A and B to C type led to decrease to field efficiency , the reason due to the increase of cutting force which lead to increase cutting time.

Table.2: The effect of the cutting angle on the nice level, vibration level, and cutting efficiency.

Knife type	Vibration level $m.sec^{-2}$	Noise db	cutting efficiency%
A	5.36	78.04	87.76
B	5.25	78.48	87.36
C	5.27	82.44	60.90
LSD	0.19	0.93	1.747

IV. CONCLUSION AND RECOMMENDATION

Using the locally assembling equipment for cutting date palm fronds with three types of cutting knives is successfully done .due to the above results it is clear that A and B knife types got the lower time in one frond cutting, cutting one date palm . Type B knife got highest productivity compared with others type.But they don't have different etween all types in level of vibration incutting operation. on the other hand , C type got a lowest amount of field efficiency, therefore using A and B type of cutting knives to cut date palm fronds which gave the best results was recommend .

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Inventaireet caractérisation des bas-fonds dans le bassin versant de l'Oti au Bénin à l'aide des images Landsat et ASTER DEM

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Résumé— *Ces dernières décennies, l'exploitation des bas-fonds a considérablement augmenté en nombre et superficie en raison de la fertilité de leurs sols et de leur caractère hydromorphe. Le présent article vise à analyser le potentiel en bas-fonds au sud du bassin versant de l'Oti. L'approche méthodologique repose sur la méthode semi-automatique qui a pris en compte les indices de végétation (NDVI, NDWI, TWI) et les paramètres (pente et accumulation d'eau) générés à partir des images Landsat OLI et ASTER DEM. En outre la caractérisation des bas-fonds inventoriés a été basée sur la délimitation phytogéographique, sur les données biophysiques et socio-économiques du bassin. L'analyse multicritère réalisée a permis d'estimer la superficie des bas-fonds identifiés à 359 894,92 ha soit 44,79 % de la superficie totale de la partie sud du bassin versant de l'Oti. Environ 28698 bas-fonds aménageables couvrant une superficie de 53 588,06 ha ont été inventoriés et représentent environ 6,67 % de la superficie totale. Aussi, il est noté que 6,33 % de la superficie des bas-fonds inventoriés par la méthode semi-automatique, soit 42,52 % du potentiel en bas-fonds aménageables sont connus par les autorités locales des Communes des départements de l'Atacora et de la Donga. Au total, trois types de bas-fonds ont été ressortis après caractérisation. Il s'agit des bas-fonds des massifs atacoriens (BFMA), les bas-fonds de la pénéplaine ondulée (BFPO) et les bas-fonds de la plaine gourma (BFPG). L'exploitation rationnelle de ces bas-fonds est devenue inévitable compte tenu des nécessités actuelles et pour les perspectives agricoles futures.*

Mots clés— Bassin versant de l'Oti, bas-fond, cartographie, caractérisation.

Abstract— *These last decades, the exploitation of the inlands valleys increased considerably in number and surface because of the fertility of their soils and their hydromorphycharacter. The present article aims to analyze the potential in inlands valleys in the south of the Otibasin pouring. The methodological approach rests on the semiautomatic method that took in account the indications of vegetation (NDVI, NDWI, TWI) and the parameters (slope and accumulation of water) generated from the pictures Landsat OLI and ASTER DEMS. Besides the characterization of the inland valley inventoried has been based on the cutoff phytogeographical, on the biophysical and socioeconomic data of the basin. The achieved analysis multi criteria permitted to estimate the surface of the inlands valleys identified to 359 894,92 ha is 44,79% of the total surface of the south part of the Oti basin pouring. About 28698 flexible inlands valley covering a surface of 53 588,06 ha have been inventoried and represent about 6,67% of the total surface. Also, it is noted that 6,33% of the surface of the inlands valley inventoried by the semiautomatic method, either 42,52% of the potential in flexible inlands valleys are known by the local authorities of the Townships of the departments of the Atacora and the Donga. To the total, three types of inlands valleys came out again after characterization. It is about the inlands valleys of the massive atacoriens (BFMA), the inlands valleys of the wavy peneplain (BFPO) and the inlands valleys of the gourmaplain (BFPG). The exploitation rational of these shallows became unavoidable considering the present necessities and for the future agricultural perspectives.*

Keywords— Oti basin pouring, inland valley, inventory, cartography, characterization.

I. INTRODUCTION

Ces dernières décennies, l'exploitation des bas-fonds a considérablement augmenté en nombre et en superficie, en raison de la fertilité de leurs sols et de leur caractère hydromorphe. Cesbas-fonds constituent alorsdes surfaces de très grand intérêt dans ce environnement marqué par la

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variabilité climatiques et les mutations des modes d'utilisation des terres agricoles (Souberou et *al.,* 2016).

Dans les pays en développement,il est noté un déplacement du front des activités agricoles de plus en plus vers les milieux hydromorphes (plaines inondables, bas-fonds, vallées), selon Mahaman et Windmeijer (1995). Ainsi, les écosystèmes des bas-fonds se sont révélés comme un ensemble de ressources dont la mise en valeur devient une nécessité impérieuse pour le développement, l'intensification et la diversification de la production agricole (Oloukoi, 2005). Ils sont doncun enjeu pour le développement durable de l'agriculture notamment pour un pays comme le Bénin.

La mise en valeur des bas-fonds revêt un intérêt important et est devenu un enjeu majeur du développement agricole afin de réduire les contraintes hydriques (Souberou et *al.,*2016). Or, la mise en valeur des bas-fonds exige une connaissance du potentiel disponible à travers leur spatialisation. Maisle niveau de connaissance du potentiel en bas-fonds du Béninen particulier du sud du bassin versant de l'Oti, malgré les inventaires nationaux par approche de terrain des bas-fonds, est faible. C'est en ce sens que leur inventaire par une autre méthode d'inventaire et leur caractérisation sont l'une des priorités de cette décennie afin de disposer d'une source d'information systématique sur les écosystèmes de bas-fonds.

A cet égard, les images de la télédétection, par le caractère homogène, synoptique et répétitif des observations, constituent une source d'informations particulièrement bien adaptée (Lebaut et Manceau, 2015). De nombreux travaux ont utilisés ces données et le Système d'Information Géographique dans l'identification des zones humides, la caractérisation de leurs différents habitats, et ceci à différentes échelles et analyses (Houhoulls et Hills, 2000 ; Memoris, 2011 ; Rapinel, 2012 ; Souberou, 2013, Thenkabail, 2013). La méthode semi-automatique a été appliquée dans la plupart de ces travaux qui ont recours aux images satellitaires de différentes résolutions (moyennes, hautes) et ceux-ci en fonction des objectifs des études.

La spatialisation des zones humides en utilisant les informations spatiales et spectrales reste un aspect très peu abordé dans les études déjà réalisées au Bénin.La plupart de ces travaux sont réalisés par des projets /programmes et timidement par des recherches scientifiques. C'est le cas de Chabi et *al.*,2010 et de Souberou et *al.,* 2016 qui ont fait respectivement la cartographique des bas-fonds au centre et au nord-ouestdu Bénin en utilisant une approche multidimensionnelle combinant les données de la télédétection, leSystème Information Géographique et les informations issues des investigations de terrain. Ces auteurs pensent que les résultats issus de la validation de cette méthode d'identification des bas-fonds est fiable que la technique d'inventaire de terrain. L'objectif de la présente étude est de cartographier le potentiel en bas-fonds au sud du bassin versant de l'Oti. Il s'agira d'inventorier les bas-fonds pour en donner un aperçu exhaustif et de les caractériser en utilisant la délimitation phytogéographique et les données biophysiques.

II. CADRE GEOGRAPHIQUE DE L'ETUDE

Le champ d'étude est une portion du bassin hydrographique de la volta (BHV) qui s'étend sur le territoire de la République du Bénin. Cette portionest précisément située dans le bassin versant de l'Oti (WHYCOS, 2006). Situé au nord-ouest du Bénin, il est à cheval sur les départements de l'Atacora et de la Donga et occupe 47,20 % de sa superficie totale du bassin versant de l'Oti au Bénin (figure 1). Elle est comprise entre 09°19'6'' et 10°54'8'' de latitude nord d'une part et 0°45'34'' et 1°41'48'' de longitude est d'autre part. Administrativement, on y retrouve entièrement les Communes de Boukoumbé, de Cobli, de Ouaké et une partie des Communes de Bassila, de Copargo, de Djougou, de Kouandé, de Matéri, de Natitingou, de Tanguiéta et de Toucountouna.

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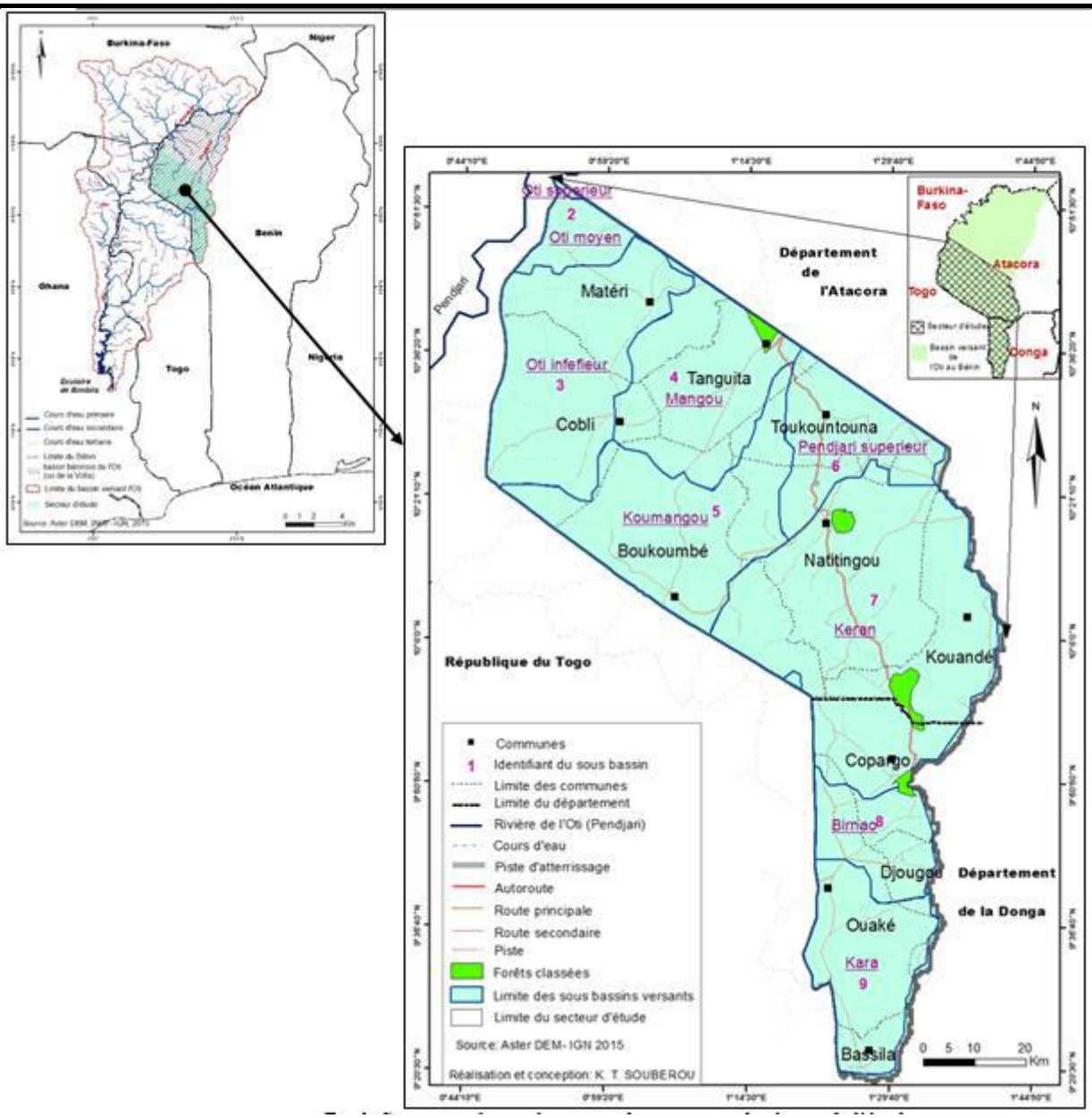

Fig.1: Situation géographique et administrative du champ de l'étude

La géologie bâtie sur le socle sédimentaire précambrien de la volta (OBEMINES, 1989) est constituée de formations cristallines et sédimentaires d'origine plus récentes présentant une orientation générale NNE – SSW. La géomorphologie fortement liée à la structure géologique révèle du fait de l'orographie la présence d'un nombre important de zones humides. Trois grandes unités morphologiques caractérisent la partie. Il s'agit de la chaîne de l'Atacora, de la pénéplaine et de la plaine de Gourma. Le relief est accidenté avec des altitudes s'échelonnant entre 118 et 667 m (Modèle Numérique d'Altitude du milieu d'étude, ASTER DEM, 2007).

Le climat est de types soudanien et guinéo-soudanien qui s'intègrent dans celui de l'Afrique de l'Ouest (Adjanohoun et *al.*, 1989). Il est caractérisé par deux grandes saisons : une saison pluvieuse (mai à octobre) et une saison sèche (novembre à avril). Les précipitations annuelles se situent entre 967 et 1255 mm. Le nombre de jours pluvieux au nord-ouest varie de 60 à 70 jours (Ouorou Barre, 2014).La température moyenne est d'environ 27, 35° C avec les variations de 17° C à 36° C avec une amplitude thermique annuelle de l'ordre de 18°C (Idiéti, 2012 ; Ouorou Barre, 2014). Au regard de ces facteurs climatiques surtout des hauteurs de pluies, le champ de recherche est bien arrosée et le réseau hydrographique est dense. Neuf sous bassins versants sont identifiés dans la partie sud du bassin versant de l'Oti au Bénin. Il s'agit des unités de gestion des ressources en eau (Birnao, Kara, Kéran, Koumangou, Mangou, Oti inférieur, Oti moyen, Oti supérieur et Pendjari) drainées par les affluents de la rivière Pendjari (qui devient Oti au Togo) et puis de quelques autres affluents de l'Oti (Kéran

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et Kara) qui prennent leurs sources au pied de la chaine de l'Atacora.

Trois (03) grands ensembles édaphiques sont rencontrés dans le secteur d'étude. Il s'agit des sols minéraux bruts, des sols ferrugineux tropicaux et des sols ferralitiques (Ouorou Barre, 2014). Par contre, le couvert végétal est marqué par la présence d'une gamme variée de formations végétales notamment denses, galeries, claires, boisées, arborées, arbustives et des mosaïques de cultures et jachères. Cette végétation est en dégradation croissante suite aux pressions agricoles et humaines (Traitement de l'image Landsat OLI TIRS, 2015).

La population, estimée à 723 762 habitants selon le RGPH 4 (INSAE, 2014) présente une diversité socio-culturelle constituée de 32 ethnies qui cohabitent et œuvrent pour le développement du bassin versant de l'Oti au Bénin.Cette forte hétérogénéité sociale de la population est toujours en quête des terres fertiles pour l'agriculture (principale activité). Elles interviennent toutes dans la valorisation des bas-fonds constituant un palliatif aux variabilités climatiques.Il est noté que le nombre de ménages agricoles est passé respectivement de 38041 en 1992 (RGPH2), à 63781 en 2002 (RGPH3) et à 93272 en 2013 (RGPH4). Ainsi, l'effectif des ménages agricoles a connu une forte augmentation ces deux dernières décennies (1992 à 2013) due à l'agrandissement des ménages et par conséquent une multiplication de la main d'œuvre familiale.

III. DONNEES ET METHODES D'ETUDE

3.1 Données

Les données suivantes ont été utilisées dans le présent travail :

- une scène d'image satellite Landsat OLI TIRS ortho rectifiées respectivement du 16décembre2015 avec une résolution de 30 m et une image satellite ASTER DEM de 2000, améliorée le 26 juin 2009 avec une résolution de 30 m (ces données sont acquises à partir des sites web de l'université de Maryland dans le cadre du projet Global Land CoverFacility (GLCF, http://glcfapp.glcf.umd.edu:8080/esdi/) et United States Géological Survey (USGS, http://earthexplorer.usgs.gov/);
- une carte topographique, feuilles de Sansanné-Mango, de Natitingou et de Djougou au 1/200 000 ;
- la carte générale du Bénin au 1/600 000 (IGN France, 1960 ; IGN France et IGN Benin, 2000) montrant les limites des communes du Bénin ;
- la carte des subdivisionsphytogéographiques du Bénin à l'échelle de 1/600 000
- la carte géologique à l'échelle de 1/600 000 qui date des années 1976 :
- les cartes des unités pédologiques des années 1960 (les feuilles de Djougou et Natitingou au 1/200 000) couvrant le secteur d'étude;
- les statistiques climatologiques (précipitations, températures) obtenues à l'Agence pour la Sécurité de la Navigation Aérienne en Afrique et à Madagascar ;
- des points de géolocalisation par GPS (Global Positioning System) des bas-fonds exploités dans le secteur d'étude ;
- les types d'aménagements hydroagricoles mis en place dans les bas-fonds ont été appréhendés à partir des observations directes sur le terrain.

3.2 Traitement des données et analyse des résultats

La méthode de traitementdes données comporte des étapes successives qui ont conduire à l'inventaire des bas-fonds au sud du bassin versant de l'Oti et à leur caractérisation. L'approche d'inventaire des bas-fonds repose essentiellement sur l'exploitation des images LandsatOLI TIRS (2015), ASTER DEM (2000) et de la carte topographique.

3.2.1 Génération des indices et des paramètres d'identification des bas-fonds

L'étude d'identification des bas-fonds par la méthode semi-automatique implique la définition d'un certain nombre d'indices calculés à partir des bandes (2, 3 et 4) de l'image OLI TIRSet la détermination du niveau d'ondulation et les zones potentielles d'accumulation d'eau au sud du bassin versant de l'Oti à partir de l'image ASTER DEM. Elle s'est basée sur la combinaison et la superposition des critères établis à partir du NDVI (NormalizedDifferenceVegetation Index), du NDWI(NormalizedDifference Water Index), du TWI (Tasseled-cap Wetness Index), de la pente et des zones d'accumulation d'eau. Le tableau 1 présente l'étendue et les valeurs retenues pour les indices et paramètres considérés comme critères d'identification.

Tableau.I: Indices et paramètres d'identification des bas-fonds

	Indices et paramètres	Etendue	Valeur retenue
1	Normalized Différence Vegetation Index(NDVI) Rouse et al., 1974	-1 à +1	-0,045 à 0,373

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2	Normalized Difference Water Index (NDWI) McFeeters, 1996	-1 à +1	-0,15 à 0
3	Tassseled-cap Wetness Index (TWI) Cicone, 1984 Crist	0 à 100 %	0 à 30
4	Pente	0 à 100 %	inférieur ou égal à 2 %
5	Accumulation d'eau	7 à 1728	7 à 583,80

Source :*Souberou et al., 2013 et Thenkabail 2013*

La classification des indices de végétation est basée sur les résultats des travaux de Davranche (2008), de Djaufack (2011), de Leroux (2012), Souberou (2013) et Thenkabail (2013) pour ressortir les valeurs qui ont permis l'identification des zones de végétation des bas-fonds par rapport aux unités d'occupation du sol. Ensuite, les pentes obtenues ont été reclassifiée en tenant compte des normes établies pour le Diagnostic Rapide de Pré-Aménagement (DIARPA), (Legoupil et*al.,* 2000 ; Jamin*et al.,* 2002 ; Chabi et *al.,* 2010), qui considère un aménagement de bas-fond techniquement et économiquement viable si les pentes sont inférieures ou égales à 2 %. Enfin, les zones d'accumulation d'eau obtenues ont été classifiéessur la base d'une interprétation de la plage des valeurs du plus petit au plus grand (CETE Nord-Picardie, 2009).

3.2.2 Extraction des bas-fonds et validation des résultats

Les opérations booléennes du logiciel Arc GIS ont été utilisées pour superposer des informations précédemment obtenues en format vecteur. Le résultat issu de l'intersection des critères d'identification, a servi à ressortir les bas-fonds aménageables dont la superficie est inférieure ou égale à 25 hectares selon le Diagnostic Rapide de Pré-Aménagement (Jamin, et *al.,* 2002).

Le résultat d'inventaire obtenu a été croisé avec une carte de référence des zones de bas-fonds réalisée à partir de relevés de terrain (levés GPS). Cette superposition des points des bas-fonds échantillonnés sur les bas-fonds inventoriés a permis de valider l'approche d'inventaire utilisée dans cette étude et de vérifier l'efficacité à partir de l'estimation du taux de zones humides de bas-fonds correctement détectées.

Les bas-fonds trackés ont permis de calculer le taux de conformité. Ce taux de conformité a permis d'évaluer quantitativement la qualité des résultats d'inventaire par approche automatique par rapport aux bas-fonds digitalisés sur le terrain (Kindjinou, 2013). Ce taux indique le niveau de fiabilité de l'approche utilisée. Il est calculé par la formule **T = n x 100 / N** (**n** est le nombre de pixels inclus «bas-fond digitalisé» obtenu après superposition des pixels des bas-fonds trackés sur ceux dérivés du traitement numérique ; et **N** est le nombre total de pixels de chacun des bas-fonds digitalisés) et permet d'évaluer la conformité en pourcentage.

La figure2illustre la procédure d'identification des bas-fonds au sud du bassin versant de l'Oti par traitement des images satellitales et des données de terrain.

3.2.3 Caractérisation des bas-fonds identifiés

La caractérisation des bas-fonds inventoriés s'est basée sur (i) la délimitation phytogéographique qui décompose la zone d'étude en deux (zone soudanienne et zone guinéo-soudanienne selon la classification d'Adjanohoun et *al.,* (1989) et de Houinato et *al.,* (2000) respectant la trilogie (climat-végétation-flore) et (ii) des caractères morphologiques (niveau d'ondulation) générés à partir de l'image ASTER DEM pour classer les bas-fonds au sud du bassin versant de l'Oti. Les informations issues des cartes hydrologique, géologique, pédologique et les données de terrain ont permis de faire une description et une analyse comparative qui sont fondamentales dans l'identification des types d'aménagements hydroagricoles appropriés des bas-fonds selon CBF (1995).

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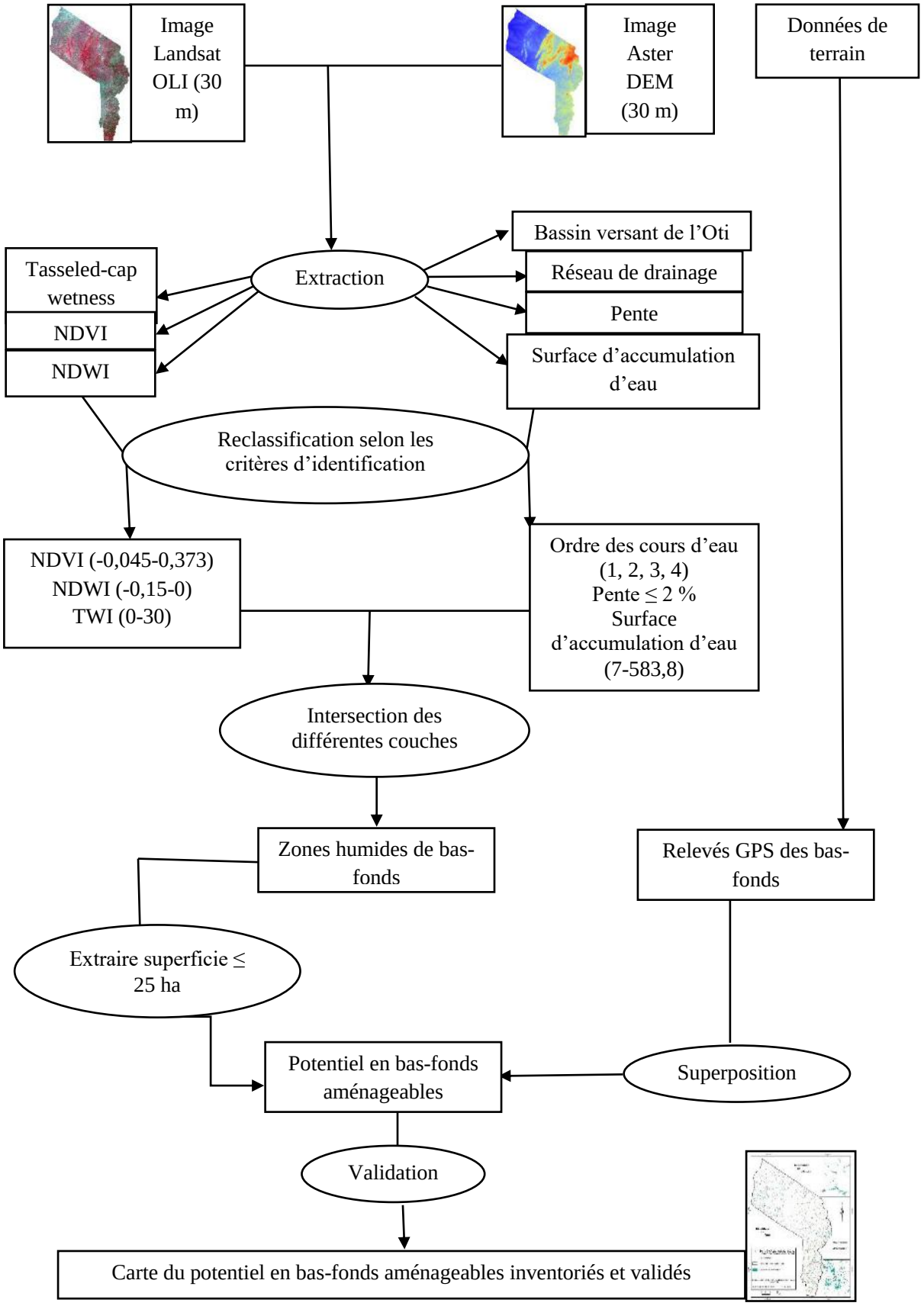

Fig.2 : Approche supervisée d'inventaire des bas-fonds aménageables

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IV. RESULTATS

La démarche méthodologique développée a permis de faire une analyse des indices et des paramètres ayant servi à ressortir le potentiel en bas-fonds au sud du bassin versant de l'Oti

4.1 Analyse des indices et paramètres d'identification des bas-fonds

4.1.1 Indices de végétation

Les indices de végétation calculés à partir de l'image Landsat OLI TIRS ont permis de décrire l'activité chlorophyllienne et de suivre la végétation des bas-fonds en période sèche dans le secteur d'étude. Elles ont permis de faire une discrimination des différents couverts végétaux à travers les valeurs estimées et de montrer les endroits fortement humides. De même, ces indices ont permis de mettre en évidence la présence des surfaces d'eau libre.

Considérant le NDVI par exemple, une forte réflectance de la végétation (forêt dense, forêt claire) est notée dans les endroits qui abritent un relief peu accidenté ou accidenté (0,373-0,791). Une dominance de la formation savane est montrée par la valeur comprise entre -0,045 et 0,373 avec un degré important d'humidité au sol (figure 3).

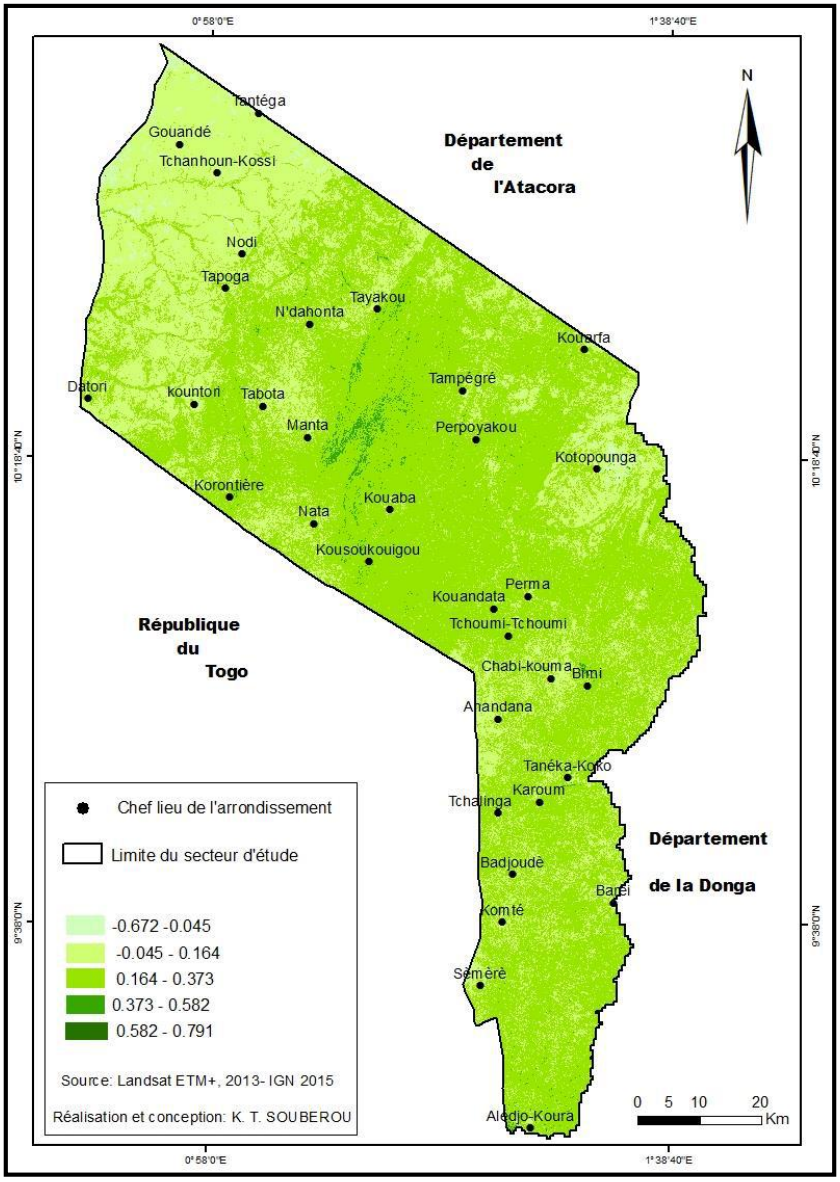

Fig.3: Indice de végétation normalisé de décembre 2015 de la zone d'étude

La valeur de l'indice de végétation au sud du bassin de l'Oti oscille entre -0,672 et 0,791. La valeur du NDVI comprise entre -0,045 et 0,373 indique la réflectance d'une zone de savane, végétation caractéristique des bas-fonds. Par contre, le calcul du NDWI a permis d'estimer la teneur en eau du feuillage du couvert végétal, qui est compris entre -0,15 et 0 pour les bas-fonds, et ceci en saison sèche.

4.1.2 Niveau d'ondulation du relief

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Les pentes du secteur d'étude sont extraites du Modèle Numérique de Terrain (MNT) qui montre les différentes facettes topographiques (endroits élevés ou bas) ainsi que les grands axes de drainage (sens de l'écoulement) des eaux au sud du bassin versant de l'Oti. Elles varient de 0 à 100 % et indiquent la présence des ondulations de terrain qui contribuent à la mise en place des bas-fonds. La pente des bas-fonds est faible et doit être inférieure ou égale à 2 % (Legoupil et *al.*,2000 ; Jamin et *al.*, 2002 ; Chabi et *al.*, 2010 ; Rapport PASA, 2009-2011 ; Souberou, 2013) pour faciliter la mise en place des ouvrages d'aménagements hydroagricoles, techniquement intéressants et économiquement rentables.

Une reclassification des pentes a permis de dégager celles inférieures ou égales à 2 % (figure 4)

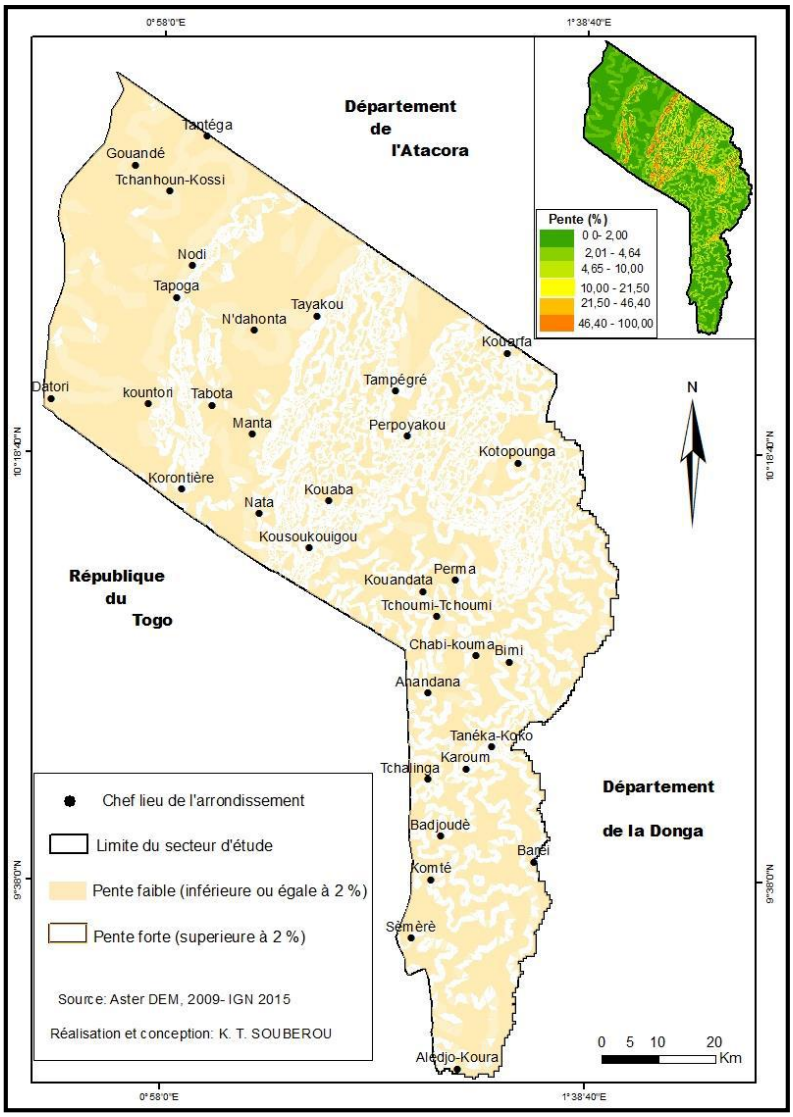

Fig.4: Reclassification des pentes au sud du bassin versant de l'Oti

La reclassification des pentes a permis de distinguer une large plaine au nord-ouest, c'est la plaine de Gourma riche en bas-fonds.

4.1.3 Zones d'accumulation d'eau

Les zones d'accumulation d'eau sont générées à partir de la carte de la direction des flux (écoulement) et considérées comme des surfaces sur lesquelles l'eau stagne pendant un moment avant toute infiltration ou écoulement vers les cours d'eau temporaires (drains). La valeur des surfaces d'accumulation d'eau varie de 7 à 1728 et indique le degré de réception des eaux provenant des versants proches par la surface (figure 5).

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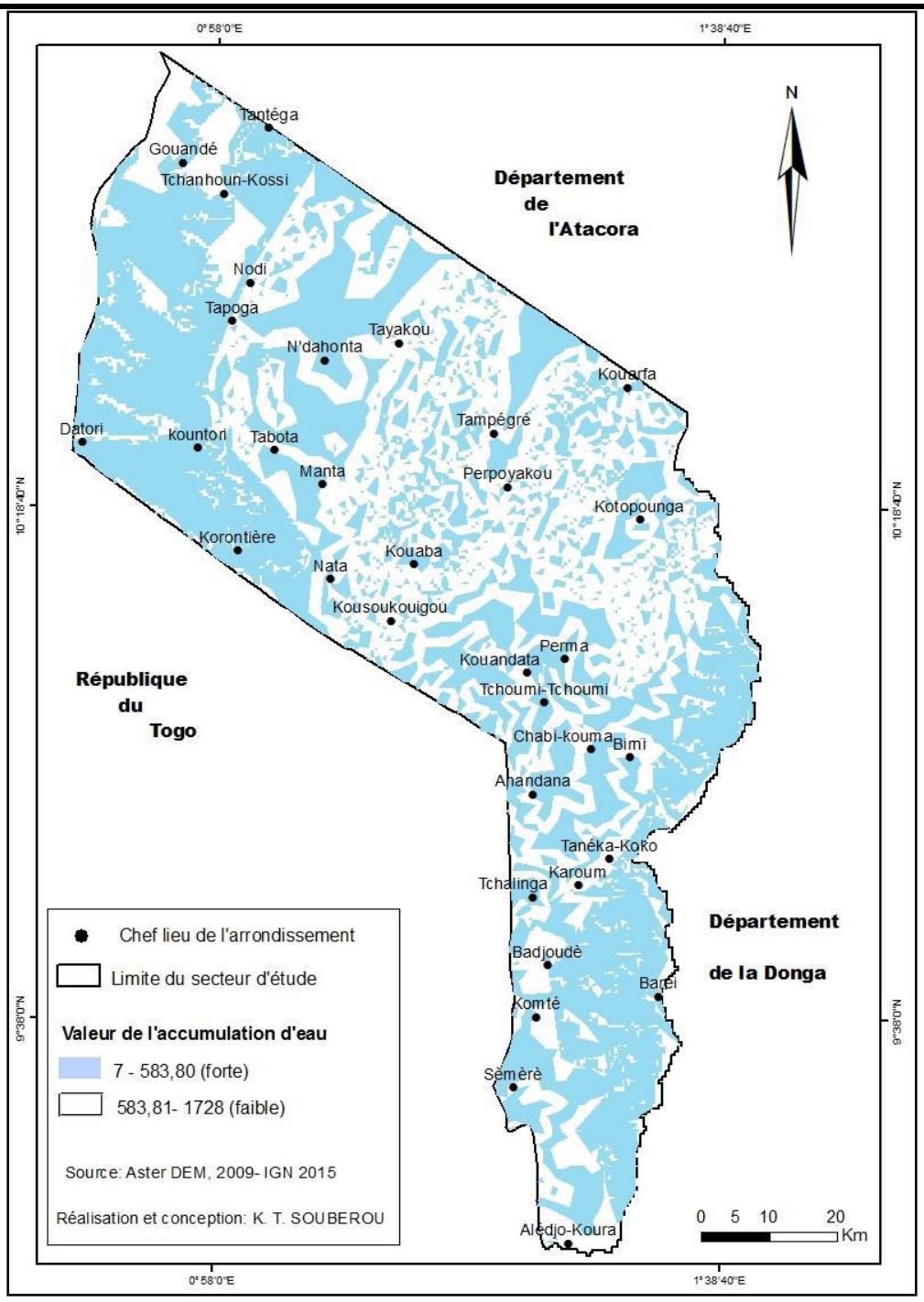

Fig.5: Surfaces de forte accumulation d'eau au sud du bassin versant de l'Oti

La valeur allouée à chaque surface de forte accumulation d'eau au sud du bassin versant de l'Oti détermine si l'accumulation de l'eau est faible ou forte. Elle constitue un indicateur dans la réalisation des plans d'aménagement en vue d'une maîtrise totale de la dynamique de l'eau. Les surfaces de la zone d'étude susceptibles de disposer d'une quantité importante d'eau (forte accumulation) en saison sèche ont été extraites.Aurousseau et Squividant (1995) soulignent que ce paramètre est performant pour modéliser les zones humides ou hydromorphes(bas-fond fond de vallée, cours d'eau, mare, etc.) et que le potentiel de saturation augmente avec sa valeur.

4.2 Zones potentielles en bas-fonds sur la base des requêtes spatiales

L'analyse multicritère des indices et paramètres de sélection des zones de bas-fonds préalablement établi à travers une superposition booléenne (intersection, union) a permis de ressortir le potentiel en bas-fonds au sud du bassin versant de l'Oti (figure 6). L'ordre du réseau de drainage hydrographique a été pris en compte.

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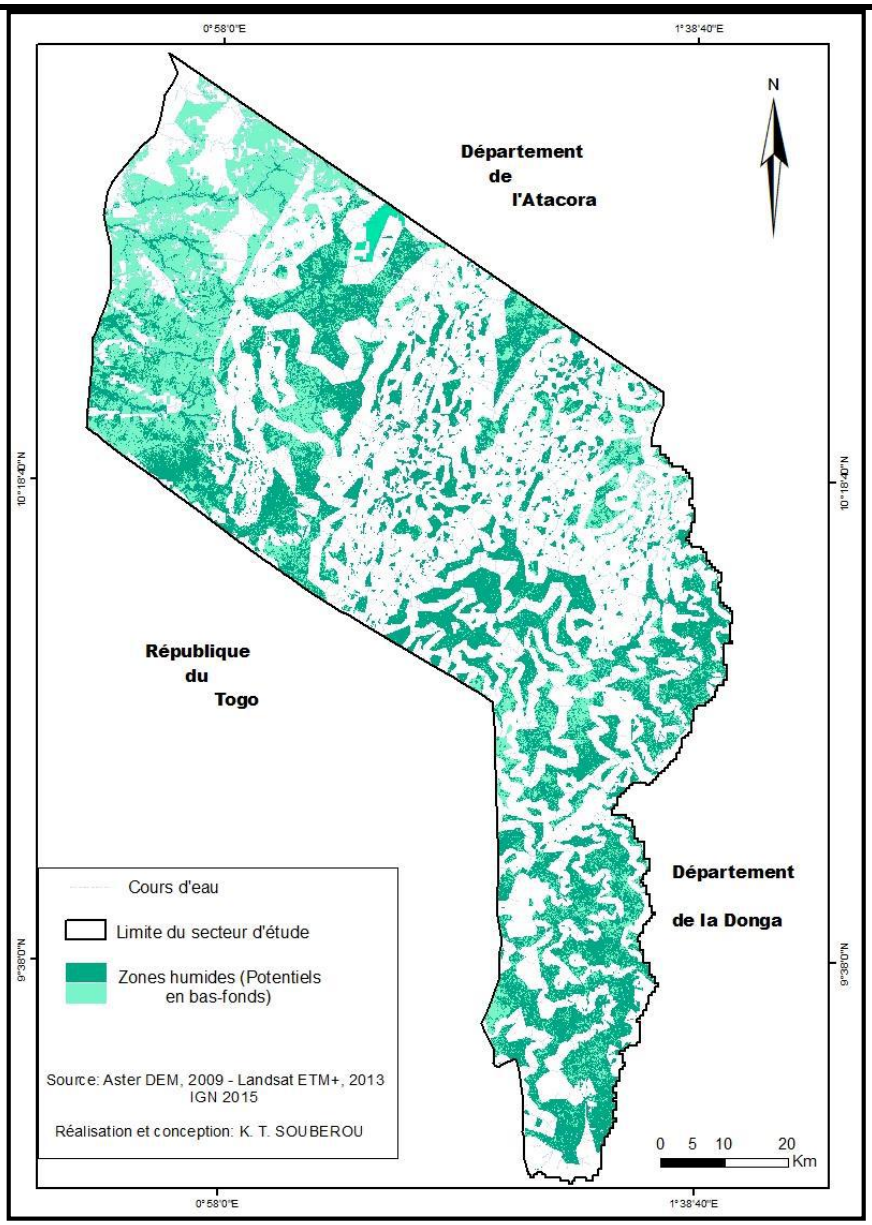

Fig.6: Zones potentielles de bas-fonds au sud du bassin versant de l'Oti

Lazone d'étude est pourvue en bas-fonds couvrant une superficie de 359 894,92 hectares (3 598,95 km²) soit 44,79 % de la superficie totale. Il découle de l'examen de cette carte que le sud du bassin versant de l'Oti dispose de grandes étendues humides.

4.3 Potentiel en bas-fonds aménageables

4.3.1 Statistiques des bas-fonds aménageables

La sélection des bas-fonds aménageables est basée sur le concept tel que défini par le Diagnostic Rapide de pré-aménagement (DIARPA) comme zones dépressionnaires situées en amont du réseau hydrographique et dont la superficie est inférieure ou égale à 25 ha (Legoupil et *al.*, 2000 ; Jamin et al., 2002 ; Chabi et *al.*, 2010 ; Souberou, 2013). La figure 7 présente les résultats après la requête spatiale.

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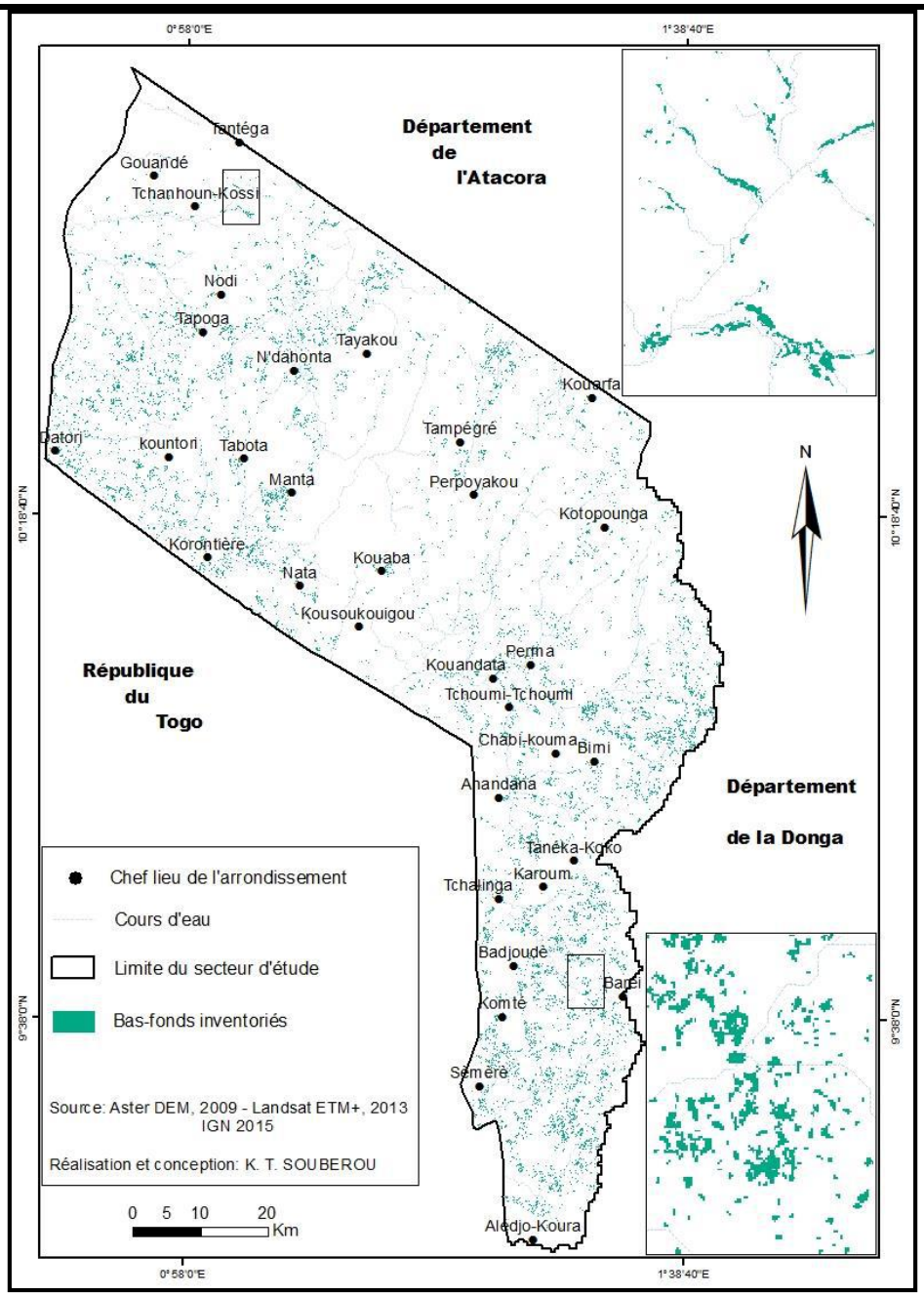

Fig.7: Bas-fonds aménageables inventoriés en aval du bassin versant de l'Oti au Bénin

Le sud du bassin versant de l'Oti est pourvu de 28698 bas-fonds facilement aménageables ayant une superficie inférieure ou égale à 25 ha (figure 7). Ils couvrent une superficie de 53 588,06 hectares (535,88 km² ~ 536 km²), soit 14,89 % de la superficie totale des zones humides du secteur d'étude. Ce potentiel de bas-fonds aménageables représente environ 6,67 % de la superficie totale de la zone d'étude.

4.3.2 Validation des bas-fonds inventoriés par analyse multicritère

Les résultats issus de l'approche de cartographie des bas-fonds ont été validés par superposition des relevés GPS (points) des bas-fonds exploités (aménagés ou non) pris sur le terrain sur ceux inventoriés par traitement d'images satellitales (figure 8). Suite à une requête d'intersection des deux couches, 271 sur les 326 points projetés correspondent aux bas-fonds aménageables inventoriés, soit un taux de conformité de 83,13 %. La proportion élevée de bas-fonds inventoriés et validés a permis de confirmer les critères d'inventaire par traitement numérique des images (indices et paramètres d'indentification dans cette étude).

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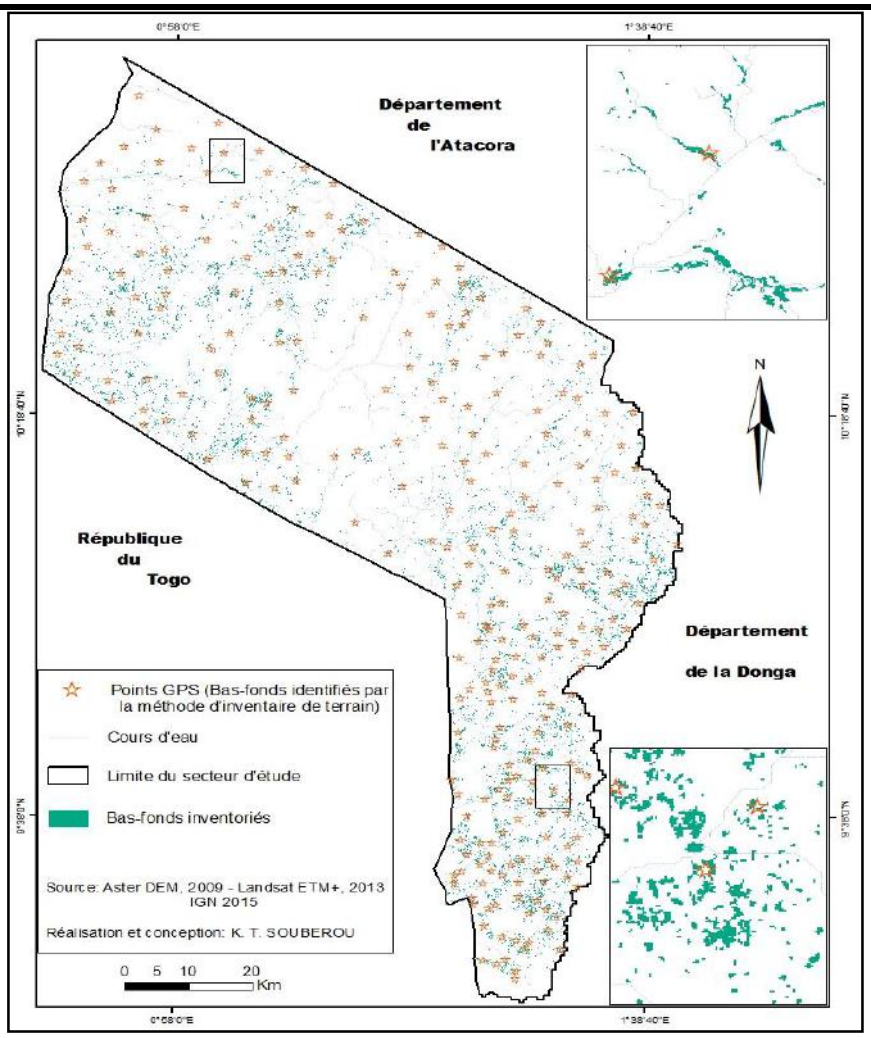

Fig.8: Validation des bas-fonds inventoriés par les relevés GPS collectés sur le terrain

Les points GPS des bas-fonds relevés sur le terrain se sont bien superposés aux bas-fonds inventoriés par méthode de traitement des images satellitales (figure 8).

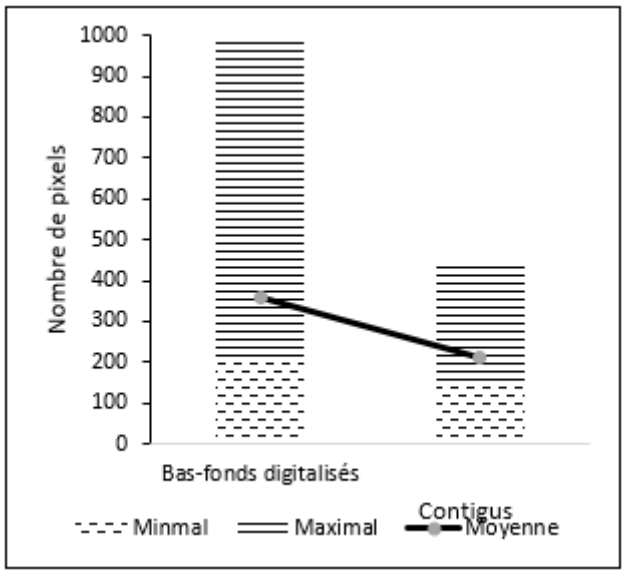

Fig.9: Distribution des pixels des soixante-quatorze bas-fonds trackés

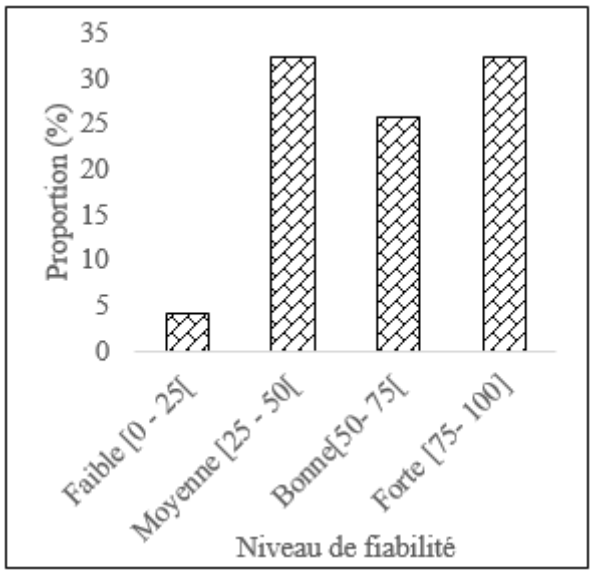

Fig.10: Niveau de fiabilité des taux de conformité par bas-fonds digitalisés

La deuxième méthode de validation des bas-fonds inventoriés par calcul du taux de conformité, a consistée à

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la rastérisation des bas-fonds trackés et des bas-fonds inventoriés (figure 9) en tenant compte de la résolution spatiale de 30 m (pas pour la grille de sortie). Les deux couches rasters (bas-fonds digitalisés et inventoriés) ont permis de regrouper les pixels contigus (figure 10) afin de vérifier l'exactitude des bas-fonds inventoriés par méthode de traitement.

L'analyse comparative de la figure 9révèle que :

- le nombre de pixels des bas-fonds trackés varie entre 214 à 778 avec une moyenne de 357 et un écart type de 157 ;
- les pixels contigus, varient de 152 à 282 avec une moyenne de 210 pixels contigus et un écart type de 22 ;
- le coefficient de corrélation entre le nombre de pixels des bas-fonds explorés sur le terrain (N) et le nombre de pixels contigus (n) est de 0,5868 (58,68 %) pour l'ensemble des bas-fonds trackés. Ce taux a permis d'affirmer que le niveau de fiabilité des résultats est bon et que les bas-fonds pris individuellement donnent des résultats satisfaisants (figure 10).

De l'analyse de la figure 10, il ressort que 4,05 % des bas-fonds digitalisés ont une faible fiabilité que 32,43 % ont une forte fiabilité et que la plupart des bas-fonds inventoriés ont une moyenne fiabilité.

4.3.3 Situation actuelle des bas-fonds inventoriés par la méthode de terrain

Dans le cadre de la mise en place d'un atlas des bas-fonds des départements de l'Atacora, du Couffo, de la Donga et du Mono, l'Organisation Non Gouvernementale Internationale "Protos" et la Coopération Technique Belge ont conjugué leurs efforts pour géo référencer la plupart des bas-fonds au cours de la période 2015-2016 à l'aide des outils de Akvo flow ainsi qu'à leur caractérisation. La base de données extraite de la plateforme a permis de cartographier les bas-fonds. Le potentiel en bas-fonds inventoriés dans les départements de l'Atacora et de la Donga est estimé à 36 264 ha (figure 11).

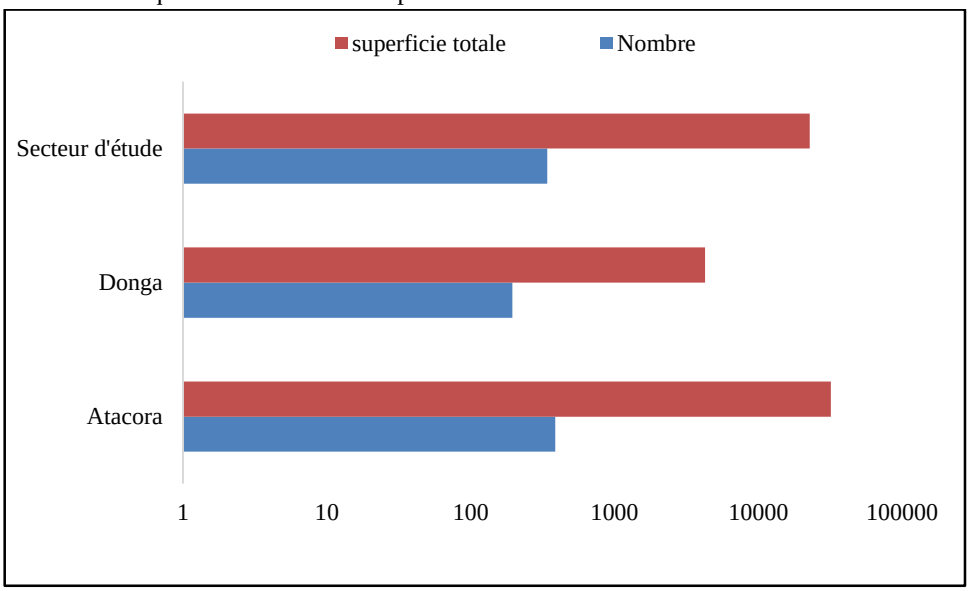

Fig.11: Potentiel en bas-fonds inventoriés par la méthode d'inventaire de terrain

Le potentiel en bas-fonds par département et au sud du bassin versant de l'Oti (nombre de bas-fonds ainsi que leur superficie) est présenté au figure 11. Il en ressort que le secteur d'étude est pourvu en bas-fonds occupant une superficie de 22 786 ha soit 62,83 % la superficie totale des bas-fonds inventoriés dans le département l'Atacora et la Donga. Ce pourcentage représente 6,33 % de la superficie du potentiel en bas-fonds inventorié par la méthode semi-automatique, soit 42,52 % du potentiel en bas-fonds aménageables.

4.4 Caractéristiques des bas-fonds identifiés

Sur la base du découpage phytogéographique, de la topographie et de la géologie trois typesde bas-fonds ont été ressortisà savoir les bas-fonds des massifs atacoriens (BFMA), les bas-fonds de la pénéplaine ondulée (BFPO) et les bas-fonds de la plaine gourma (BFPG).

- les bas-fonds des massifs atacoriens (BFMA) se trouvent dans des vallées étroites, situés à des altitudes comprises entre 407 et 667 m où les versants sont raides à pente forte et convexe. D'une superficie de 83879,09 ha (figure 12), ils présentent des pentes transversales et longitudinales inférieures ou égales à 4 %, se trouvent sur des roches dures telles que les quartzites micaschistes et se caractérisent par deux types de sols (sols minéraux et sols ferrugineux tropicaux). Le réseau hydrographique est dense. Ils reçoivent une pluviométrie moyenne annuelle de1215 mm et une température moyenne annuelle de l'ordre de 28,6°C.

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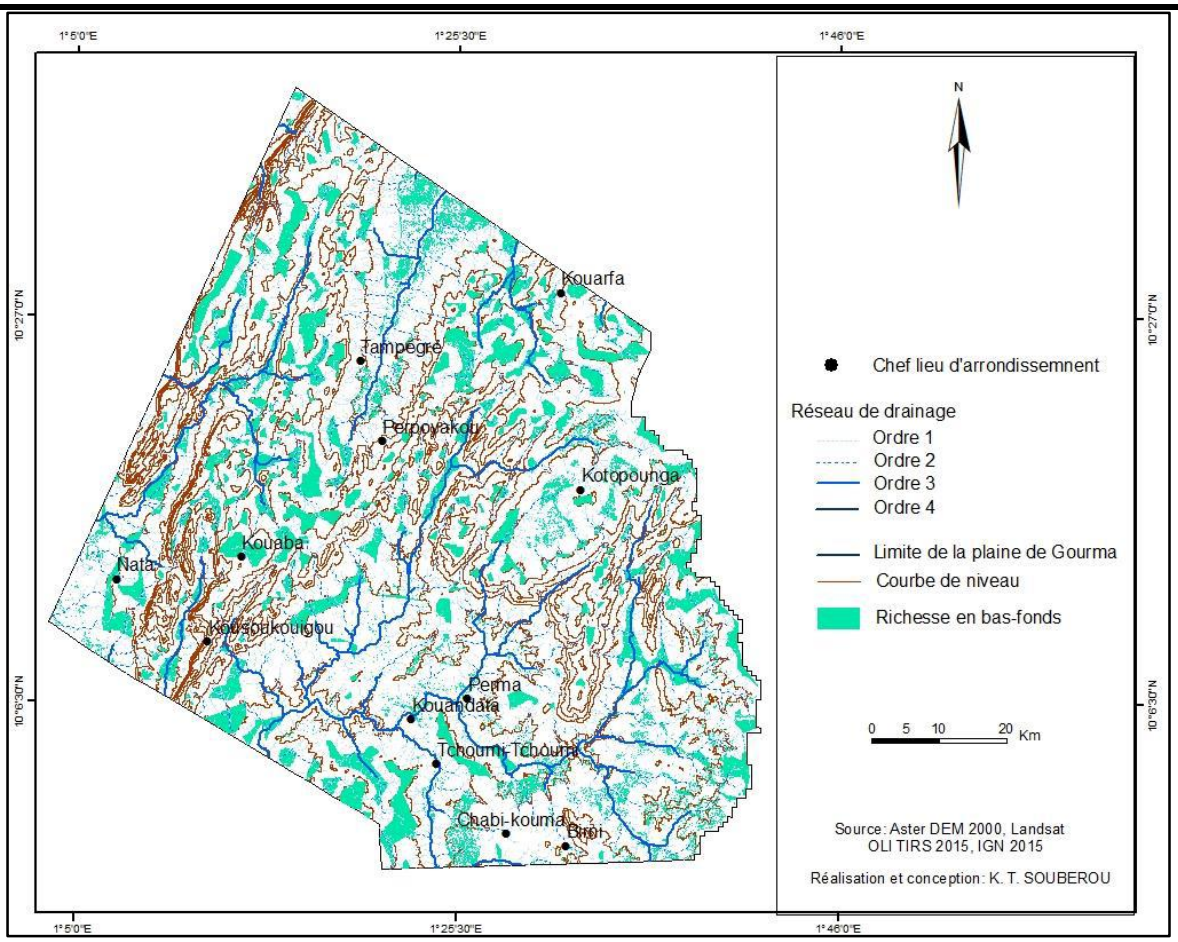

Fig.12: Bas-fonds des massifs atacoriens (BFMA)

- les bas-fonds de la pénéplaine ondulée (BFPO) quant à eux, sont localisés dans des vallées situées à des altitudes comprises 295 entre 406 m, se caractérisent par des versants modérément raides et concaves et des fonds de vallées intermédiaires et peu profonds. Les formations géologiques sont quartzites micaschistes, gneiss à muscovites, orthgneiss à biotite (gneiss à biotite et à amphibole), roche basique et granites syntectoniquesCalco-alcalins. Le secteur de la pénéplaine ondulée renferme 29, 45 % des bas-fonds du bassin versant de l'Oti (figure 13) sous une pluviométrie de 1100 à 1300 mm. La topographie et le fonctionnement hydrologique (liés aux divers affluents) caractérisent chaque bas-fond.

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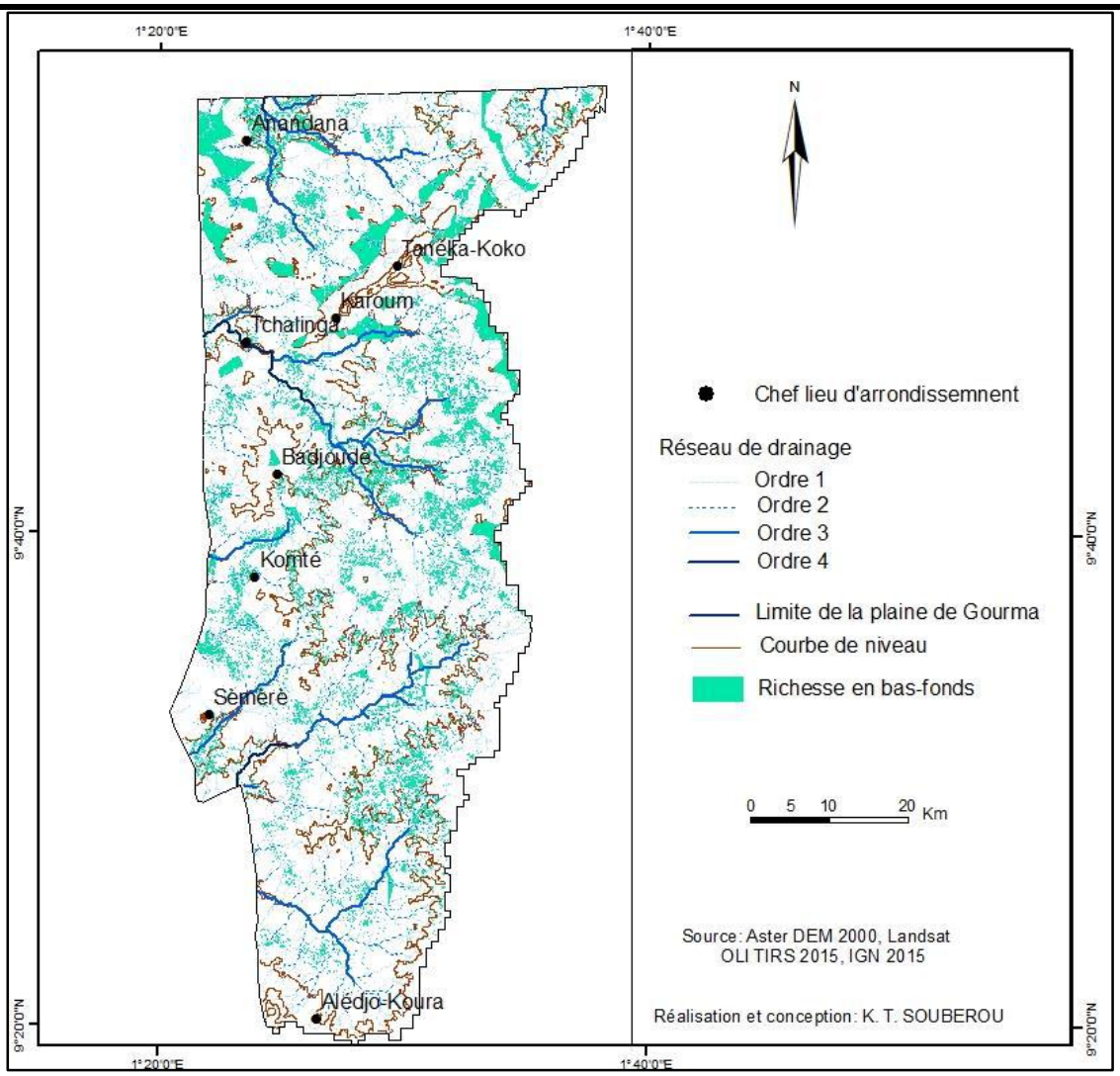

Fig.13 : Bas-fonds de la pénéplaine ondulée (BFPO)

- les bas-fonds de la plaine gourma (BFPG) se trouvent dans des vallées larges situées à des altitudes comprises entre 118 et 294 m où les versants sont doux à pente faible et concave et des fonds de vallées larges. Le régime pluviométrique est monomodal avec une pluviosité atteignant parfois 1300mm. C'est le secteur des bas-fonds plats, larges et de grandes superficies (figure 14). Ils présentent des pentes transversales et longitudinales inférieures ou égales à 2 % et se trouvent sur des formations relativement tendres telles que les schistes de l'Oti, grès de Bombouaka, grès, grès-quartzites, schistes séricitoschistes, schistes et micaschistes. Les caractéristiques des sols sont très diversifiées comme dans les bas-fonds de la pénéplaine ondulée et se résument aux sols minéraux lithiques et aux sols ferrugineux tropicaux. Le régime d'écoulement y est saisonnier et irrégulier.

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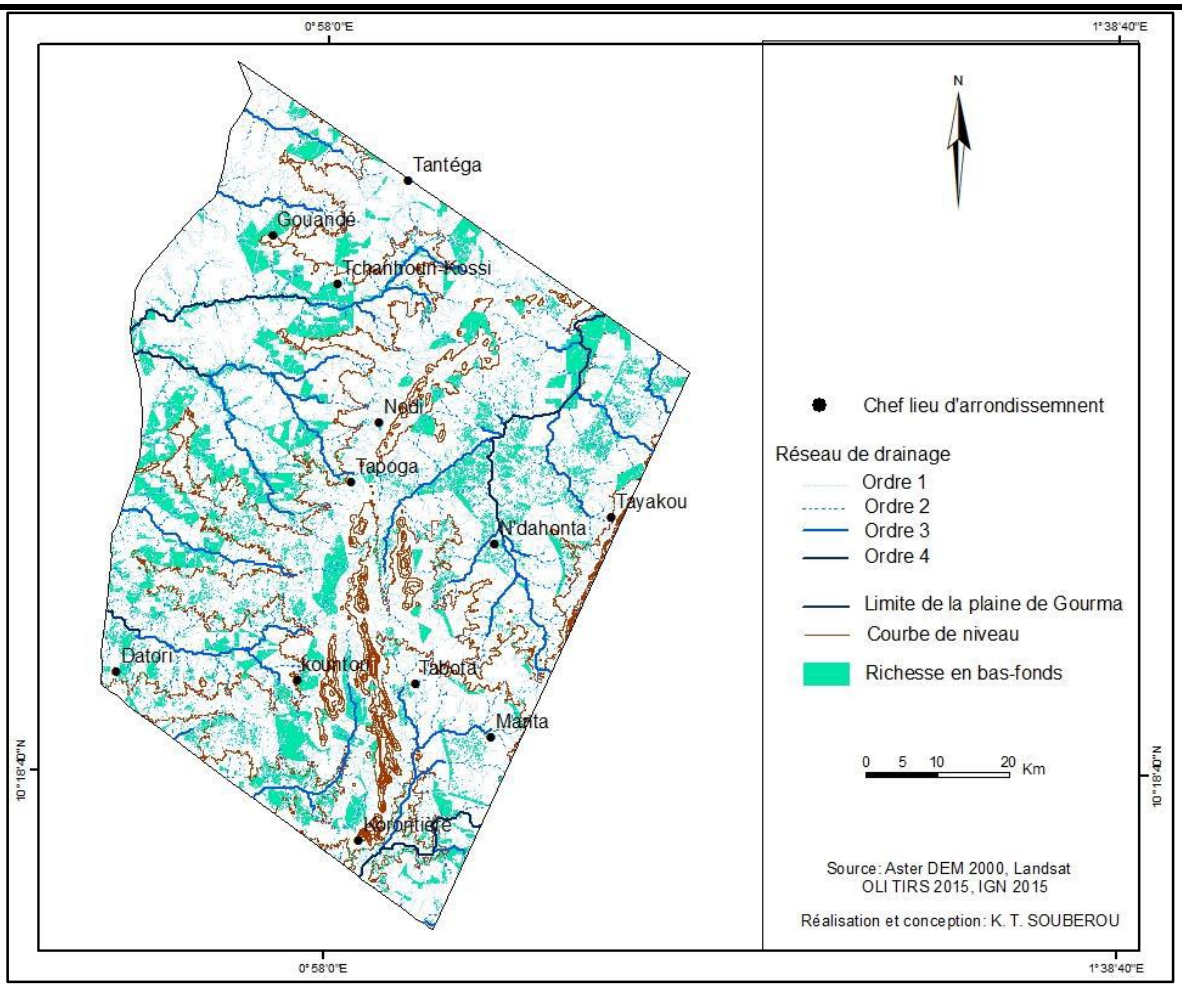

Fig.15: bas-fonds de la plaine gourma (BFPG)

Ces différents bas-fonds identifiés sont liés aux axes de drainage et fonctionnent plus en saison pluvieuse qu'en période sèche. Cette classification basée sur les caractères phytogéographique, topographique et géologique est présentée en annexe avec plus de détails. Le code assigné à chaque bas-fond est fonction de son identifiant et le nom du village dans lequel il se localise dans l'ordre alphabétique respectivement pour l'arrondissement, la Commune et le département auxquels il appartient.

L'exploitation de ces bas-fonds revêt un intérêt important pour l'agriculture essentiellement de type pluvial au sud du bassin versant de l'Oti. Si le potentiel agricole des bas-fonds identifiés dépend bien du milieu, l'usage qu'en font les producteurs n'en est pas le produit direct des résultats de cette étude. Les modes d'exploitation sont diversifiés, évoluent dans le temps et tentent de répondre au mieux aux besoins de la reproduction économique et sociale familiale. Ces bas-fonds qui peuvent moyennant des aménagements hydroagricoles, porter des cultures permanentes et intensives. Raison pour laquelle, ces bas-fonds ont été généralement exploités les quatre derrières décennies parce qu'ils constituent une réponse à certains phénomènes physiques et à la pression foncière croissanteau sud du bassin de l'Oti au Bénin.

V. DISCUSSION

L'absence d'une base référentielle sur les bas-fonds rend difficile le choix des bas-fonds à aménager et répétitives l'identification ainsi que les études de caractérisation des bas-fonds (pré-diagnostic). Comme l'ont montré Clément et al. (2008), l'élaboration de tout programme de mise en valeur de bas-fonds nécessite une bonne connaissance de leurs localisations, de leurs caractéristiques morphologiques et hydrologiques, de leurs statuts fonciers et de leur utilisation actuelle. Malheureusement, le potentiel en bas-fonds de tous les Départements du Bénin demeure encore très peu connu malgré les informations disponibles grâce aux efforts des projets et structures de développement intervenant sur la problématique des bas-fonds. Ce constat a valu une plus grande implication des structures de recherche avec les partenaires techniques pour la mise en place d'un Atlas des bas-fonds au nord-ouest du Bénin en 2015-2016, toujours à partir de la méthode de terrain. La superficie totale des bas-fonds recensés est estimée à 46 264 ha pour les départements de

l'Atacora-Donga contre respectivement 78 000 ha (Cellule Bas-Fonds, 2011) et 56 000 ha selon les statistiques du CBF/DGR (2002). La liste jusque-là n'est pas exhaustive vue la richesse en bas-fonds du champ de l'étude. La mise en évidence du potentiel en bas-fonds à partir des données de terrain possède des limites au niveau de la méthodologie pour le recensement effectif et la caractérisation de ces zones. Elle nécessite un travail complémentaire de prospection de bas-fonds potentiels à partir des données satéllitalesqui permettra d'obtenir une cartographie plus précise en vue d'une prise de décision et de gestion efficiente, intégrée et durable de ces agroécosystèmes.

L'inventaire des bas-fonds à partir des données de la télédétection a fait l'objet de plusieurs travaux de recherches au cours de ces deux dernières années. L'un des objectifs de cette recherche est de ressortir la richesse en bas-fonds disponibles à partir des images issues de la télédétection et sur la base des critères bien définis en suivant la méthode de traitement automatique élaborée et assistée par l'ordinateur.

L'approche d'inventaire par télédétection et SIG utilisée dans cette étude au sud du bassin versant de l'Oti, s'est révélée plus rapide, moins fastidieuse et efficace pour ressortir le potentiel en bas-fonds et par ricochet aménageables que l'approche d'inventaire par relevé de terrain. Cette analyse confirme les conclusions tirées par Chabi et *al.* (2010) dans une étude d'inventaire des bas-fonds au centre du Bénin. L'estimation du potentiel en bas-fonds aménageables a pris en compte trois critères d'identification à savoir la pente, le NDVI et la superficie inférieure ou égale à 25 ha dans le cadre des travaux de recherche de Chabi et *al.* (2010). En plus de ces trois critères, Souberou (2013) a ajouté le critère de zones d'accumulation d'eau dans le bassin versant pour spatialiser le potentiel en bas-fonds de la Commune de Matéri au nord-ouest du Bénin. L'ajout de ce quatrième critère d'identification des bas-fonds à la méthode d'inventaire a permis de rendre la technique plus efficace. Les résultats de cette analyse concordent avec ceux de Hubert-Moy et *al.* (2006). Ceux-ci soutiennent que les conditions géomorphologiques et hydrologiques se positionnent comme les deux aspects les plus déterminants dans la répartition des zones humides. Ce critère complété dérivé du modèle numérique de terrain (MNT) constitue le produit de base sur lequel Kindjinou (2013) a appliqué l'algorithme de caractérisation des bas-fonds de Linsoussi (2012) afin d'extraire le réseau hydrographique et de détecter les zones potentielles de bas-fonds au Togo. Cette technique est similaire, mais le critère NDVI n'a pas été pris en compte dans son étude, alors que la végétation est un facteur primordial dans l'identification des bas-fonds.

Dans la présente étude, ces critères (pente, NDVI, zones d'accumulation d'eau,) ont été complétés par deux indices de végétation Normalized Différence Water Index (NDWI) et : Tasseled-cap Wetness Index (TWI) pour parfaire la méthode d'identification du potentiel en bas-fonds développée en 2013 par Souberou et *al.* (2016). Le choix de ces critères confirme ceux utilisés par Thenkabail (2013) dans l'étude de caractérisation des zones humides de bas-fonds en Afrique (inlandvalleywetland of Africa) en utilisant la télédétection notamment au Ghana, au Benin, au Mali, en Côte d'Ivoire, au Mozambique, au Zimbabwe et au Botswana . Ces critères ont permis de faire une esquisse de ces zones par la méthode semi-automatique utilisée.

Cette méthode d'identification explorée dans ce travail pour la réalisation de la carte de potentialité en bas-fonds a permis de répertorier tous les bas-fonds au sud du bassin versant de l'Oti (359 894,92 ha) et d'en ressortir ceux facilement aménageables dont la superficie inférieure ou égale à 25 ha (53 588,06). Ces bas-fonds identifiés peuvent déjà faire l'objet d'une mise en valeur car ils présentent des caractéristiques hydrologiques et morphologiques qui correspondent aux critères de mise en valeur proposés par le DIARPA (Windmeijer et*al.*,2002 cités par Chabi et *al.*, 2010).

A la lumière des résultats obtenus, il paraît évident que l'usage de l'imagerie de Landsat (ETM+, OLI TIRS) combiné à l'image ASTER DEM peut être indiqué pour les études d'identification des zones humides de bas-fonds. Cette analyse corrobore celle de Thenkabail (2013) qui a utilisé des images issues de la télédétection telle que Landsat ETM+, SRTM, Ikonos et Modisdans l'identification des zones humides des grands bassins hydrographiques de l'Afrique. Cette méthode complétée est facile à mettre en œuvre d'un point de vue informatique et offre une multitude d'outils d'aide à l'interprétation des données. Elle a permis d'obtenir des résultats satisfaisants et s'est montrée plus rapide que l'approche classique qui consiste à aller directement sur le terrain pour faire des levés directs et plus systématique et qui nécessiterait plus de temps, de coût et de moyen surtout si l'espace à couvrir est plus étendu. La cartographie des bas-fonds en utilisant des données de la télédétection apparaît comme un atout majeur pour la sélection des bas-fonds propice pour la mise en place des systèmes rizicoles. Ce résultat confirme ceux de Gumma et *al.* (2009) qui ont montré que les données issues de la télédétection et les outils et techniques SIG fournissent une bonne plate-forme pour générer, intégrer, traiter et analyser les informations.

L'inventaire du potentiel en bas-fonds par la télédétection et le Système d'Information Géographique bien que adéquat et fiable, nécessite l'utilisation des images de très

grande résolution pour une validation exacte des zones potentielles en bas-fonds en dehors des points de géolocalisation. La carte de localisation géographique de ces bas-fonds est un instrument d'aide et intervient en amont de toutes interventions des acteurs de développement (nationaux ou internationaux) pour la réalisation des ouvrages d'aménagement hydro-agricoles afin de contribuer à leur mise en valeur dans ce contexte de changements climatiques.

Au sud du bassin versant de l'Oti, la caractérisation des bas-fonds identifiés a permis de mettre en évidence trois types (bas-fonds des massifs atacoriens, de la pénéplaine ondulée et de la plaine gourma) en fonction de la localisation dans la zone phytogéographique, de la topographie et de la géologie. Ces bas-fonds importants pour la production agricole, sont exploités par la mise en place des systèmes de cultures diversifiées

VI. CONCLUSION

L'inventaire des bas-fonds par la méthode semi-automatique a combiné les indices de végétation (NDVI, NDWI, TWI) et les paramètres (pentes et accumulation d'eau). L'analyse de ces critères d'identification a permis de montrer le potentiel en bas-fonds et facilement aménageables au sud du bassin versant de l'Oti. Ce potentiel de bas-fonds aménageables représente environ 6,67 % de la superficie totale de la zone d'étude. Les points GPS prise sur le terrain ont permis de valider les bas-fonds inventoriés. A la suite de la superposition des deux couches, 271 sur les 326 points projetés correspondent aux bas-fonds aménageables inventoriés, soit un taux de conformité de 83,13 %. Et d'autre part, le coefficient de corrélation entre le nombre de pixels des bas-fonds trackés et le nombre de pixels contigus est 58,68 %. Il a permis vérifier l'exactitude des bas-fonds inventoriés par la méthode de traitement d'image et d'affirmer que le niveau de fiabilité des résultats est bon. Au cours de la période 2015-2016, l'Organisation Non Gouvernementale Internationale "Protos" et la Coopération Technique Belge ont inventorié le potentiel en bas-fonds des départements de l'Atacora et de la Donga, au total, 342 bas-fonds couvrant une superficie de à 36 264 ha sont identifiés au sud du bassin versant de l'Oti. Ce potentiel inventorié par la méthode de terrain est estimé à 6,33 % de la superficie des bas-fonds recensés par traitement des images satellitales, soit 42,52 % du potentiel en bas-fonds aménageables. Cet inventaire reste donc incomplet car la liste des bas-fonds n'a pas été exhaustive. Si l'on considère, les données issues de la télédétection, il est possible d'identifier les bas-fonds de tout le bassin de l'Oti (Volta) au Bénin et de caractériser plus finement les autres zones humides (mares, marais, etc.) en utilisant des images de meilleures résolutions spatiales et spectrales comme cela a été déjà exploré dans plusieurs pays avec des démarches relativement complexes. Il faut alors retenir qu'une meilleure connaissance des bas-fonds (inventaire plus exhaustif) exige la disponibilité des données de très bonnes factures et doit s'appuyer sur une bonne méthodologie pour la constitution d'un répertoire fiable qui ressortira les caractéristiques des bas-fonds en vue d'une mise en valeur efficiente afin deservir d'outil d'aide à la décision pour tout acteur voulant intervenir dans les bas-fonds au sud du bassin versant de l'Oti.

Les résultats mis en exergue dans cet article sont probants pour la résolution utilisée. La caractérisation des bas-fonds inventoriés par traitement numérique a permis de les classer en trois types à partir de la zone phytogéographique dans laquelle ils se retrouvent, du critère de la topographie et de la géologie. Il s'agit des bas-fonds des massifs atacoriens (BFMA), les bas-fonds de la pénéplaine ondulée (BFPO) et les bas-fonds de la plaine gourma (BFPG). L'exploitation de ces bas-fonds, ces trois dernières décennies, est devenue de plus en plus inévitable, car ils occupent une place de choix dans les systèmes de production (rizicole, maïsiculture, etc.) au sud du bassin versant de l'Oti au Benin notamment dans ce contexte de changements climatiques

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ANNEXE

Tableau.II : Typologie des bas-fonds au sud bassin béninois de l'Oti

Zone phytogéographique 1		**Soudanienne (ZPS)**	
Climat	Saisons	une saison sèche et une saison pluvieuse	
Pluviométrie	Régime	monomodal	
	Moyenne annuelle	927 - 1255 mm	
Température moyenne annuelle		18,45 (minimal) -34,58 (maximale)	
Végétation	NDVI	(-) 0,672-0,791	
	Unités d'occupation du sol	Forêt claire et savane boisée, forêt galerie, savane arborée et arbustive et mosaïque de culture et jachère	
Types de vallées		Versants raides à pente forte et convexes et fonds de vallées étroites parfois engorgés	Versants à pente faible (douce) et concave et fonds de vallées larges
Types de Bas-fonds (BF)	**Identifiant**	**1 : BFMA**	**2 : BFPG**
	Code	Le code du bas-fond est donné en tenant du nom du village dans lequel il se localise dans l'ordre alphabétique respectivement pour l'arrondissement, commune et département	
		1 (identifiant) 2 (Département)…(Commune)…(Village)… (bas-fond)	2 (identifiant) 2 (Département)… (Commune)… (Village)… (bas-fond)

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	Superficie	83579,09 ha					170305,91 ha		
Topographie	Unité morphologique	des massifs atacorien (UMMA)					de la plaine de Gourma (UMPG)		
	Altitudes	406,63 -667 m					118-294,55 m		
	Pente	comprise entre 0 et 4 %, prise en compte des bas-fonds où les pentes transversales et longitudinales sont inférieures ou égale à 2 %					Inférieure ou égale à 2 %		
Géologie (Socle sédimentaire précambrien de la volta)		Quartzites micaschistes					Schistes de l'oti, grès de Bombouaka, Grès, grès quartzites, schistes Séricitoschistes, schistes et micaschistes		
Réseau de drainage	Ordre des cours d'eau	1, 2, 3 et 4 selon la classification et hiérarchisation de Strahter (1952) Le régime d'écoulement est saisonnier et irrégulier.							
	Zones d'accumulation d'eau	Valeur comprise entre 7 à 1728							
Pédologie	Types de sols	Sols minéraux bruts	Sols ferrugineux tropicaux : sols sesquioxydes de fer et manganèse				Sols minéraux bruts	Sols ferrugineux tropicaux : Sols sesquioxydes de fer et manganèse	
	Caractéristique	Peu évolués lithiques : sur quartzite et miscaschiteatacorien	Peu lessivés : sur quartzite et miscaschite atacoriens, sur dugneiss à muscovite,	Hydromophes lessivés sans concrection : sur quartzite atacoriens et sur miscascchiste granité	Hydromorphes : sur matériau colluvia-sableux et sablo-argileux, sur roche basique	Hydromorphes lessivés à concrécation : sur matériau kaoliniqueissus de quartzite et micaschite	Peu évolués hydromophes: sur matériau alluvial finement sableux et sur alluvio-colluvial limono-argileux	Hydromorphes lessivés sans concrécation : sur micaschites et schites quartzeux de Buem	Hydromorphes lessivés à concrécation : sur schiste quartzeux de Buem, sur schiste en plaquette, sur matériau colluvial issu du jaspe
		Limono-sableuse avec 16,13 % d'argile, 31,62	limono-argilo-sableuse en surface sur argile, en profondeur avec 28,8 % de sable à la surface, 45,58 % d'argile et 22,16 % de limon				Limono-sableuse, avec 16,13 % d'argile, 31,62 % de limon et 54,39 % de	limono-sableuse à limono-argilo-sableuse, avec 18,20 % d'argile, 22,45 % de limon et 56,96 % de sable	

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		% de limon et 54,39 % de sable			
Type d'aménagements hydroagricoles mis en place		Confession des digues de protection (Cordons pierreux) perpendiculaires au sens d'écoulements de l'eau; Confection des diguettes isohypses (principales et secondaires) en terre compactés enherbés ou en pierres sèches de rétention, équipés d'ouvrages de vidanges ou d'un chenal central; Implantation de bandes enherbées de vétivers (réduction du ruissellement)			
Principales cultures		Riz, mais, sorgho, mil, mil, igname, manioc, fonio et les cultures de contre saison			
Zone phytogéographique 2		**Guinéo-soudanien (ZPGS)**			
Climat	Saisons	une saison sèche et une saison pluvieuse			
Pluvio métrie	Régime	monomodal			
	moyenne annuelle	927 -1255 mm			
Température moyenne annuelle		20,12 -31,41			
Végétation	NDVI	(-) 0,672-0,791			
	Unités d'occupation du sol	Forêt claire et savane boisée, forêt galerie, savane arborée et arbustive et mosaïque de cultures et jachères			
Types de vallées		Versants modérément raides et concaves et fonds de vallées intermédiaires et peu profonds			
	Identifiant	**3 : BFPO**			
Types de Bas-fonds (BF)	Code	3 (identifiant) 7 (Département)…(Commune)…(Village)…(bas-fond)			
	Superficie	106009,92 ha			
Topographie	Unité morphologique	de la pénéplaine ondulée (UMPO)			
	Altitudes	294,56-406,62 m			
	Pente	comprise entre 0 et 4 %, prise en compte des bas-fonds ayant des pentes transversales et longitudinales inférieures ou égales à 2 %			
Géologie (Socle sédimentaire précambrien de la volta)		Quartzites micaschistes ; gneiss à muscovites constitué de roches claires : orthgneiss à biotite (gneiss à biotite et à amphibole) ; roche basique ; granites syntectoniquesCalco-alcalins ;			
Réseau de drainage	Ordre des cours d'eau	1, 2, 3 et 4 selon la classification et hiérarchisation de Strahter (1952) Le régime d'écoulement est saisonnier et irrégulier			

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<table>
<tr><td></td><td>Zones d'accumulation d'eau</td><td colspan="4">Valeur comprise entre 7 à 1728</td></tr>
<tr><td rowspan="3">Pédologie</td><td>Types de sols</td><td colspan="4">Sols ferrugineux tropicaux : sols sesquioxydes de fer et manganèse</td></tr>
<tr><td rowspan="2">Caractéristique</td><td>Peu lessivés: sur gness à muscovite, sur granito-gneiss à biotite, sur roche basique, sur matériau kaolinique et sur matériau issu de gneiss à deux miscaschites</td><td>Hydromorphes : sur gneiss à ferro-magnésiens, sur gneiss à muscovite et micas, sur Schites en plaquette, sur matériau colluvial-sableux et sablo-argileux, sur roche basique</td><td>Hydromorphes lessivés sans concrécation : sur micaschites et schites quartzeux de Buem, sur micaschites granité</td><td>Hydromorphes lessivés à concrécation : sur matériau kaolinique issus de quartzite et micaschite</td></tr>
<tr><td>De texture limon sable sur limon-argilo-sableux à argilo-sableux, composé de 11,03 % d'argile, 29,90 % de limon et 58,1 % de sable</td><td colspan="3">limono-argilo-sableuse en surface sur argile, en profondeur. L'analyse granulométrique montre qu'il s'agit d'un sol à texture argileuse pure avec 28,8 % de sable à la surface, 45,58 % d'argile et 22,16 % de limon</td></tr>
<tr><td colspan="2">Type d'aménagements hydroagricoles mis place</td><td colspan="4">Confession des digues de protection (Cordons pierreux) perpendiculaires au sens d'écoulements de l'eau;
Confection des diguettes isohypses (principales et secondaires) en terre compactés enherbés ou en pierres sèche de rétention, équipés d'ouvrages de vidanges ou d'un chenal central;
Implantation de bandes enherbées de vétivers (réduction du ruissellement)</td></tr>
<tr><td colspan="2">Principales cultures</td><td colspan="4">Riz, mais, sorgho, mil, mil, igname, manioc, arachide et les cultures de contre saison</td></tr>
</table>

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Bioremediation of Chlorpyrifos Contaminated Soil by Microorganism

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Abstract— *India is agricultural based country where 70% of the population survives on it. In order to increase the production of field various pesticides are used. Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphate pesticide which is widely used as insecticide for crop protection. But due to its persistent nature into the environment, it is leading to various hazards including neurotoxic effects, cardiovascular diseases and respiratory diseases. Bioremediation is a technology to eliminate chlorpyrifos efficiently from the environment. In bioremediation of chlorpyrifos the potential degradative microorganisms possess opd (organophosphate degrading) gene which hydrolyses the chlorpyrifos and utilizes it as a sole carbon source.Thus the present review discusses about how through bioremediation the pesticide chlorpyrifos can be degraded using potential soil microorganisms.*

Keywords— Pesticides, Organophosphate, Chlorpyrifos, opd gene, Bioremediation.

I. IMPACT OF MODERN AGRICULTURE ON ENVIRONMENT

Until about four decades agoin agricultural systems, crop yields depended totally on internal resources like recycling of organic matter, biological control mechanisms and rainfall patterns. Agricultural yields were modest, but stable. To safeguard the production, variety of crops were grown in space and time in a field as insurance against pest outbreaks or severe weather. Inputs of nitrogen were gained by rotating major field crops with legumes. In turn rotations suppressed insects, weeds and diseases by effectively breaking the life cycles of these pests. In these types of farming systems the link between agriculture and ecology was quite strong and signs of environmental degradation were seldom evident. But as agricultural modernization progressed, the ecology-farming linkage was often broken as ecological principles were ignored or overridden. In fact, several agricultural scientists have arrived at a general consensus that modern agriculture confronts an environmental crisis. Evidence has accumulated showing that whereas the present capital- and technology intensive farming systems have been extremely productive and competitive; they also bring a variety of economic, environmental and social problems (Altieri and Rosset, 1995).

The very nature of the agricultural structure and prevailing policies in a capitalist setting have led to an environmental crisis by favouring large farm size, specialized production, crop monocultures and mechanization. Today as more and more farmers are integrated into international economies, the biological imperative of diversity disappears due to the use of many kinds of pesticides and synthetic fertilizers, and specialized farms are rewarded by economies of scale. In turn, lack of rotations and diversification take away key self-regulating mechanisms, turning monocultures into highly vulnerable agro-ecosystems dependent on high chemical inputs. Also, fields that in the past contained many different crops, or a single crop with a high degree of genetic variability, are now entirely devoted to a genetically uniform single crop. The specialization of farms has led to the image that agriculture is a modern miracle of food production. However, excessive reliance on farm specialization has negatively impacted the environment (Altieri and Rosset, 1995).

II. USE OF PESTICIDES IN CROP PROTECTION

Pesticides are those substances which are used to control, destroy, repel or attract pests in order to minimise their detrimental effects. Pests are those organisms like weeds, insects, bacteria, fungi, viruses and animals which adversely affect our way of life. Pests can reduce the quality and quantity of food produced by lowering production and destroying stored produce; they can harm our animals (like fleas, worms and diseases); they compete with humans for food and affect the health, welfare and way of life of people; they can destroy buildings (termites) and are a major cause of land degradation (noxious weeds, rabbits, feral pigs, etc). Pest activity greatly increases the costs of farming. Pesticides therefore are used in many situations such as livestock farming, cropping, horticulture, forestry, home gardening, homes, hospitals, kitchens, road-sides, recreational and industrial areas (Jayashree and Vasudevan, 2007).

Pesticides may be derived from inorganic sources (copper, sulphur), natural organic sources (plants) or be organic compounds synthesised in a laboratory.Many of the

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earliest pesticides were either inorganic products or derived from plants, for example burning sulphur to control insects and mites. Other early insecticides included hellebore to control body lice, nicotine to control aphids, and pyrithrin to control a wide variety of insects. Lead arsenate was first used in 1892 as an orchard spray while about the same time it was accidentally discovered that a mixture of lime and copper sulphate controlled downy mildew, a serious fungal disease of grapes. It is still one of the most widely used fungicides. Many of these early chemicals had disadvantages. They were often highly toxic, were very persistent, posing a threat to the environment (Jayashree and Vasudevan, 2007).

III. USE OF CHLORPYRIFOS IN CROP PROTECTION

Chlorpyrifos is a broad-spectrum insecticide. It is a type of organophosphorus insecticide. Chemically, it is *O,O-diethyl-O-(3,5,6-trichloro-2- pyridinol) phosphorothioate*. It is used in field protection of corn, cotton, peaches, apple etc. Termites and insects are susceptible to chlorpyrifos[3]. While originally used primarily to kill mosquitoes, Chlorpyrifos is effective in controlling cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, and well as on lawns and ornamental plants. It is also registered for use in domestic dwellings, farm buildings, storage bins, and commercial establishments. Chlorpyrifos acts on pests primarily as a contact poison, with some action as a stomach poison. It is available as granules, wettable powder, dustable powder, and emulsifiableconcentrate (Mallick *et al.*, 1999).

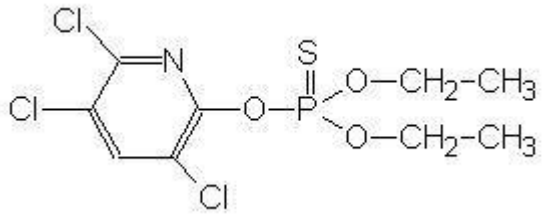

Fig.1. Chemical structure of Chlorpyrifos

IV. PROPERTIES OF CHLORPYRIFOS

The physical and chemical properties of a pesticide plays significant role indetermining its environmental fate and transport. The physical and chemicalproperties of chlorpyrifos are as mentioned below (Ajaz *et al.*, 2005):

Chemical Name - O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate
Molecular Formula - $C_9H_{11}Cl_3NO_3PS$
Rel. Molecular Mass - 350.62
Density - 1.38 g/cc at 46°C
Boiling point - Decomposes before boiling. Thermal decomposition occurs between 160-180°C
Melting point - 41.5 – 44°C

V. HAZARDS OF CHLORPYRIFOS

Chlorpyrifos is moderately toxic to humans. It primarily affects the nervous system through inhibition of cholinesterase, an enzyme required for proper nerve functioning. Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality (Eaton, 2008). Transport of chlorpyrifos to human results in neural disorders, inhibition of DNA synthesis, interference with gene transcription, altered function of neurotrophicsignaling cascade and synaptic function (Lakshmi *et al.*, 2009).

VI. SOIL PERSISTENCY AND ENVIRONMENT FATE OF CHLORPYRIFOS

Chlorpyrifos is moderately persistent in soils. The half-life of chlorpyrifos in soil is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions. Chlorpyrifos was less persistent in the soils with a higher pH. Soil half-life was not affected by soil texture or organic matter content. In anaerobic soils, the half-life was 15 days in loam and 58 days in clay soil. Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. Chlorpyrifos adsorbs strongly to soil particles and it is not readily soluble in water. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater (Ajaz *et al.*, 2005).

TCP*(3,5,6-trichloro-2-pyridinol)*is the principle metabolite of chlorpyrifos, adsorbs weakly to soil particles and appears to be moderately mobile and persistent in soils. The US EPA considers that there is insufficient data to fully assess the environmental fate of Chlorpyrifos. Chlorpyrifos is tightly adsorbed by soil and not expected to leach significantly. Volatilization from soil surface will contribute to loss. Depending on soil type, microbial metabolism of Chlorpyrifos may have a half-life of up to 279 days. Higher soil temperatures, lower organic content and lower acidity increases degradation of chlorpyrifos. Chlorpyrifos inhibits nitrification and nitrogen fixation marginally, many bacterial strains were unable to degrade

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it but some microorganismscan use chlorpyrifos as their only source of carbon and nitrogen (Ajaz *et al.*, 2005).

VII. FATE IN HUMANS AND ANIMALS

Chlorpyrifos is readily absorbed into the bloodstream through the gastrointestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure. In humans, chlorpyrifos and its principal metabolites are eliminated rapidly. Chlorpyrifos is eliminated primarily through the kidneys. It is detoxified quickly in rats, dogs, and other animals. Chlorpyrifos is moderately to very highly toxic to birds. Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Aquatic and general agricultural uses of chlorpyrifos pose a serious hazard to wildlife and honeybees (Cho *et al.*, 2004).

VIII. BIODEGRADATION OF CHLORPYRIFOS BY MICROORGANISM

Availability of different pesticides in field provides exposure of several different kinds of microorganisms to pesticides. Most of the organisms die under toxic effect of pesticides but few of them evolve in different ways and use pesticide compounds in metabolism. Several reports are available indicating degradation of different pesticides when they are available in nature in excess (Horvath, 1972, Hussain *et al.*, 2007 and Lakshmi *et al.*, 2009). Successful removal of chlorpyrifos by the addition of bacteria (bioaugmentation) had been reported (Singh *et al*., 2004).

Degradation strategies exhibited by microbes include: co metabolize the biotransformation of a molecule coincidental to the normal metabolic functions of the microbe; catabolism- the utilization of the molecule as a nutritive or energy source; and extracellular enzymes (phosphatases, amidases and laccases) – secreted into the soil, which can act on the molecule as a substrate. Three basic types of reactions can occur: degradation, conjugation, and rearrangements, and all of which can be microbially mediated. Complete degradation of a chemical in the soil to carbon dioxide and water involves many different types of reactions. Microorganisms are key players in determining the environmental fate of novel compounds because they can be used as carbon and energy sources by microorganisms (Singh, 2008).

1. Biodegradation of chlorpyrifos by Bacteria

Bacteria use natural organics such as proteins, carbohydrates, and many others as carbon and energy sources. Many of the xenobiotic compounds of environmental concern are naturally occurring relatives of these organics. For other xenobiotics, repeated exposure has resulted in the adaptation and evolution of bacteria capable of metabolizing these man-made compounds (Zhang *et al.*, 2005). Microbial degradation of organophosphate pesticides like chlorpyrifos is of particular interest because of the high mammalian toxicity of such compounds and their widespread and extensive use.

Chlorpyrifos has been shown to be degraded co-metabolically in liquid media by bacteria [14]. *Pseudomonas aeruginosa* is the most common Gram negative bacterium found in soil. Isolates of this bacterium have been found to have potential to degrade chlorpyrifos (Fulekar and Geetha, 2008). Enhanced degradation of chlorpyrifos by *Enterobacter* strain B-14 was reported (Singh *et al*., 2004). Yang *et al*., (2005), isolated *Alkaligenes faecalis* DSP3, which is also capable of degrading chlorpyrifos and results in the formation of by product 3, 5, 6-trichloro-2-pyridinol (TCP) (Rani, *et. al*., 2008). A chlorpyrifos degrading *Flavobacterium sp.* is reported by Jilani and Khan (2004).

A few chlorpyrifos-degrading bacteria, including *Enterobacter* strain B-14, *Stenotrophomonas* sp. YC-1, and *Sphingomonas* sp. Dsp-2, have been studied. The metabolism of chlorpyrifos by microorganism in soil has been reported by many scientists. Chlorpyrifos gets oxidized to exon analogue [O,Odiethyl-O- (3,5,6-trichloro-2-pyridinyl) phosphate, III] of insecticide and finally into 3,5,6-trichloropyridinol (II) (Mukherjee *et. al*., 2004). Various *opd* (organophosphate degrading) genes have been isolated from different microorganisms from different geographical regions, which have been shown to hydrolyze chlorpyrifos (Hussain *et al*., 2007).

Chlorpyrifos has been shown to be degraded co metabolically in liquid media by bacteria, and various *opd* genes have been isolated from different microorganisms from different geographical regions, some of which have been shown to hydrolyze chlorpyrifos. Chlorpyrifos has been reported to be degraded co metabolically in liquid media by *Flavobacterium* sp. and also by an *Escherichia coli* clone with an *opd* gene (Singh *et. al.*, 2004). Enhanced degradation of chlorpyrifos by *Enterobacter* strain B-14 was reported by Singh *et al*., (2004). Six chlorpyrifos-degrading bacteria were isolated using chlorpyrifos as the sole carbon source by an enrichment procedure (Rani, *et. al*., 2008).

Chlorpyrifos hydrolysis was greatly accelerated under low moisture conditions, both in acidic and alkaline soils (Ajaz, *et al.*, 2005). *Arthrobacter* sp. strain B-5 hydrolyzed chlorpyrifos at rates dependent on the substrate. Chlorpyrifos (10 mg/L) was completely degraded in the mineral salts medium by *Flavobacterium* sp. ATCC 27551 for 24 h and by *Arthrobacter* sp. for 48 h, respectively.The

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rapid degradation of chlorpyrifos, added to a mineral salts medium as a sole carbon source or applied to the soil, by the *Flavobacterium* sp. ATCC 27551 (isolated from diazinon-retreated rice fields) and the *Arthrobacter* sp. (isolated from a flooded soil retreated with methyl parathion) was reported (Xu, 2007).

The degradation of chlorpyrifos was reported in mineral salt medium by an *Arthrobacter* species that was initially isolated from methyl parathion-enriched soil (Mallick *et al*., 1999). Both *Flavobacterium* sp. ATCC 27551 and *Arthrobacter* sp. effected very rapid degradation of chlorpyrifos, added to the mineral salts medium as a sole carbon source (Mallick *et al*., 1999). Pseudomonas *aeruginosa*, *Bacillus cereus*, *Klebsiella sp.*, and S*erratiamarscecens* obtained from consortia showed 84, 84, 81, and 80% degradation of chlorpyrifos (50 mg/L) in liquid medium after 20 days and 92, 60, 56, and 37% degradation of chlorpyrifos (50 mg/L) in soil after 30 days. Some recent reports indicate bacterial degradation of chlorpyrifos by *Flavobacterium*sp. ATCC 27551 and *Arthrobacter* sp., isolated from contaminated sources, which degrade chlorpyrifosco-metabolically, and *Enterobacter* strain B-14, *Alcaligenes faecalis,* and *Klebsiella sp.*, which degrade and utilize chlorpyrifos as sole carbon source (Jilani S. and Khan, 2004). *Bacillus sp*. And *Micrococcus sp.* possess potential to degrade chlorpyrifos (Getzin, 1981; Gomez *et al*., 2007).

2. Biodegradation by Fungi

Chlorpyrifos has also been reported to be effectively degraded by two soil fungi, *Trichoderma viride* and *Aspergillus niger* [16]. Several chlorpyrifos-degrading fungi, such as *Phanerochaete chrysosporium, Aspergillus terreus,* and *Verticillium* sp. DSP have also been reported [17]. *Verticillium* sp. and *Brassica chinensis* are reported for degradation of chlorpyrifos in culture medium ranging from 1 to 100 mg/L. Methods of *in-situ* bioremediation of *Verticillium* sp. are also developed and achieved good results (Arisoy, 1998; Trejo and Quintero, 2000; Bhalerao and Puranik, 2007). Fungal degradation of chlorpyrifos was reported by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil (Xu, 2007).

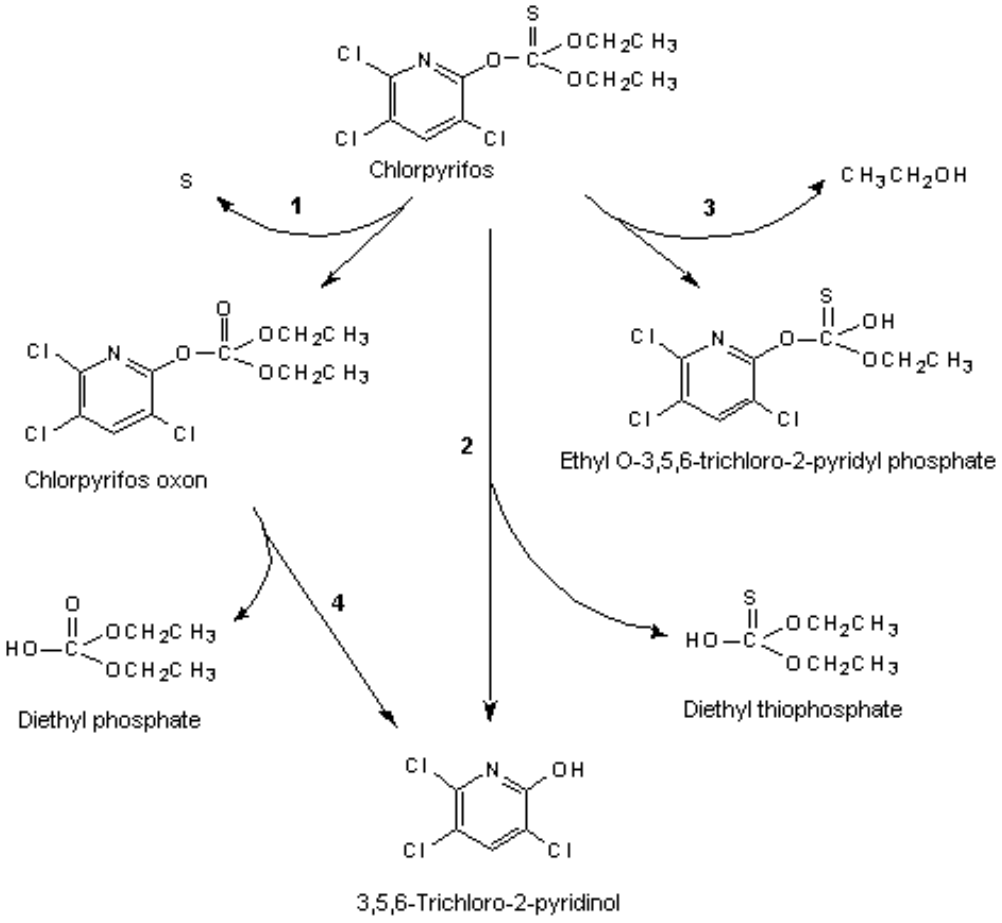

Fig.2: Pathway of Biodegradation of chlorpyrifos by microorganism (Smith *et al.*, 1967).

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A fungal strain capable of utilizing chlorpyrifosas sole carbon and energy sources from soil and degradation of chlorpyrifos in pure cultures and on vegetables by this fungal strain and its cell-free extract is also reported. This strain was identified as an unknown species of *Verticillium.* It opens a new research direction for development of novel bioremediation process .The chlorinated pyridinyl ring of chlorpyrifos undergoes cleavage during biodegradation by *P. chrysosporium*. But the degradation of chlorpyrifos proves more efficient by mixed populations than by pure cultures of fungi. Mixed population of fungi, such as *Alternaria alternata*, *Cephalosporium* sp., *Cladosporium cladosporioides*, *Cladorrhinum brunnescens*, *Fusarium* sp., *Rhizoctonia solani, and Trichoderma viride*, reveal the degradation of chlorpyrifos in liquid culture more efficiently (Singh *et al*., 2004).

IX. ENZYMATIC ACTIVITY IN CHLORPYRIFOS BIODEGRADATION

Organophosphorus hydrolase is one of the important hydrolytic enzymes in detoxification technology that hydrolyze chlorpyrifos pesticides containing P–O, P–F and P–S bonds. The OPH enzymes, including O-Phenylenediamine Dihydrochloride (OPD), Methyl Parathion Hydrolase (MPH) Mevalonate Pyrophosphate Decarboxylase (MPD) etc., were identified for the hydrolysis for chlorpyrifos (Meysami and Baheri, 2003). The degradation of chlorpyrifos induced Organophosphorous Phosphatase (OPP) production and concentration were 28 times higher in the extracellular than inside the cells. The chlorpyrifos degradation efficiency for *L. fermentum*, *L. lactis* and *E. coli* were reported to 70 per cent, 61 per cent and 16 per cent with 3,5,6-trichloro-2-pyridinol (TCP), chlorpyrifos-oxon and diethyl-phosphatase end products respectively. Purification and characterization of a novelchlorpyrifos hydrolase from the fungi *Cladosporium cladosporioides* Hu-01 was done (Bhagobaty *et al.*, 2007).

X. GENES INVOLVED IN CHLORPYRIFOS BIODEGRADATION

The organophosphate-degrading *opd, mpd* genes were isolated from species that were capable to degrade chlorpyrifos. Most of them were plasmid based or located on the chromosome. The *opd* gene from *Agrobacterium radiobacter* was located on the chromosome. Identification of a novel phosphortriesterase enzyme from the coding of gene differs from organophosphate degradative gene (*opd*) in*Enterobacter* strain (Yang et al., 2005).

XI. GENETIC ENGINEERING IN CHLORPYRIFOS BIODEGRADATION

The genetically engineered bacteria with high detoxification potential were developed by gene engineering and enzyme engineering. Xu *et al.,* (2007), reported optimum pH of 7 and inoculum volume of 50 ml/kg on chlorpyrifos residues degradation by mutagenic bacteria DX1 in soil. Kapoor and Rajagopal (2011), studied the degradation of organophosphate pesticides by recombinant organophosphorus hydrolase (Dhanya, 2014).

Caoa *et al*., (2013), cloned a novel 6012 bp gene cluster from TCP-degrading strain P2 responsible for dehalogenation of 3,5,6-trichloro-2-pyridinol (TCP). The gene cluster consisted a monooxygenase gene (*tcpA1*), a flavinreductase gene (*tcpB1*), *tcpR1*, *orf1* and *orf2*. *TcpA1* and *TcpB1* worked together to catalyze the dehalogenation of three chlorine of TCP, and generated a more readily biodegradable product of 3, 6-dihydroxypyridine-2,5-dione. Cloned gene clusters from *Ralstonia sp.* T6 involved in 3,5,6-trichloro-2- pyridinol degradation. The *tcpRXA* genes constitute a gene cluster consisting FADH2- dependent monooxygenase gene*tcpA*, LysR family transcriptional regulator (*TcpR*) and flavinreductase (*TcpX*). T6-ΔtcpA-com, the complementation strain for the mutant strain T6- ΔtcpA, recovered the ability to degrade TCP, and the strain *E. coli* DH10B-tcpRXA, which expressed the tcpRXA gene cluster, had the ability to transform TCP to the green intermediate metabolite 3, 6-dihydroxy pyridine- 2,5-dione (DHPD) (Dhanya, 2014).

The cloning of *mpd gene* from chlorpyrifos degrading bacterial strains to *Escherichia coli* helps in developing its biodegradation capability. Wang *et al.,* (2002), cloned *Escherichia coli* with *opd gene* that degradechlorpyrifos co-metabolically. Yang *et al.,*(2005), cloned the *mpd* gene from chlorpyrifos– degrading bacterium *Stenotrophomonas* isolated using chlorpyrifos as the sole source of carbon by enrichment method that degraded 100 mg/l of chlorpyrifos within 24 hour to DETP and TCP. The thermostability and acidic stability of MPH have been improved bysite-directed mutation. Yang *et al.,* (2005), engineered *P. putida* JS444 with altered specificity of MPH enhance thedegradation of chlorpyrifos (Dhanya, 2014).

XII. CONCLUSION

The organophosphate pesticide chlorpyrifos used against pests not only protects crops but also causes havoc in the environment by its accumulation. Bioremediation is emerging as a beneficial tool in order to create pesticide free environment. The potential microorganisms have the ability to degrade pesticide to the fullest. But still there

needs more research to be done in order to bring the technique into field practice and make it more efficient. For large scale culture of such bacterial isolates to be used for bioremediation purpose, it is essential to determine the optimum growth conditions like temperature and pH. These isolated strains of bacteria are highly adapted to existing environment conditions and thus could be effectively utilized for bioremediation and metabolic detoxification of chlorpyrifos.

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Study of the bryological flora at the archaeological site of Chellah, Morocco

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Abstract*—The Chellah archaeological site in Rabat, listed as a cultural asset since 2012 on UNESCO's World Heritage List, is subject to significant biodeterioration. The aim of this study is to identify the bryophytes that have an important impact on the destruction of the substrate. For this purpose, three prospectionswere carried out in autumn 2014, spring 2015 and winter 2016. The systematic sampling carried out allowed us to identify 20 species of bryophytes belonging to 10 botanical families, of which 4are dominant with 13 species equivalent to 65% of the total. The four families are Pottiaceae, Brachyceciaceae, Funariaceae and Bryaceae; they belong to the class of Muscinae. The liverworts are represented by only 6 species, representing 30% of the total population.*

Among the 20 species inventoried, 3 are newly observed in the region of Rabat: Entosthodon pulchellus (H. Philib.) Brugués; Dydimodon Fallax Hedw. and Trichostomum crispulum Bruch.

When bryophytes settle on substrates, a preliminary soilis initiated to the detriment of the quality of materials and their durability.

Keywords*—*Biodeterioration, bryophytes, historical site, Chellah, Morocco.

I. INTRODUCTION

Like other historical sites in Rabat such as the Hassan tower or the Kasbah of the Udayas, the Chellah archaeological site, a melting pot of several civilizations (Phoenician, Carthaginian, Roman and finally Islamic)is one of the highlights of the capital's history.

The site of Chellah, alsoknown as Chellah necropolisdue to its transformation into a royal necropolis by the Marinids sultans, has undergone several archaeological excavations since 1917 thatallowed uncovering the different occupations of the site (Basset H. & Levi-Provençal E., 1929, Basset H. & Terrasse H., 1932, Boube J., 1966).

Regarding the state of conservation of sites, a preliminary diagnosis by Benharbit M. in 2017, identified the main factors degrading the historical monuments of Rabat, including Chellah. These are climatic, dynamic, biological and anthropic factors. Among the biological factors, Benharbit M. (2017), cite lichens, plant roots and bird droppings. Nettah et al. (2015) worked more particularly on the lichens installed on the construction stones of Chellah. They identified six encrusting species, known as important agents of biodeterioration of building materials: *Aspicilia calcarea*, *Verrucaria nigrescens*, *Toninia aromatica*, *Verrucaria calciseda* (syn.*Baglietoa calciseda*), *Placidum squamulosum* and *Lecania spadicea*. With regard to weeds, 91 species have been recorded in Chellah; their presence on buildings generally indicates the presence of moisture in the joints of materials (Taleb et al., 2005). However, no investigation has yet been carried out on bryophytes, but it is agreed that these plants are pioneer plants that initiate plant succession by participating in pedogenesis; their installation on old masonry participates in their biodeterioration. The aim of this study is thus to draw up a list of bryophytes species that are in the necropolis of Chellah and to see up to what extent they take part in the biodeterioration of the substrates.

II. MATERIAL AND METHOD

The archaeological site of Chellah is located within Rabat at 4 km from the Atlantic coast; itoverhangs the Bouregreg valley and occupies an intramural area of about 7 hectares.

Carthaginian had occupied the site in the 12th century BC and Phoenician in the 6th century BC. Later, Romans settled there and founded a city,mentioned by the Greek

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astrologer and astronomer Ptolemy under the name of Sala, and a river port serving as a Mediterranean counter.

The site was then deserted and given up before being again occupied by the Marinids sultans who built there a necropolis named Al-Ribat Al Mubarak. An inscription in kufic script on the front gate indicates that the work was completed in 739 after hijri(AD 1339).

The Marinids wall, which encircles the site, currently contains characteristic vestiges of the Roman city, including in particular the capitol, the forum, the thermal baths, a nymphaeum and a triumphal arch. From the Marinids occupation, there remain a mosque, a madrasah (Islamic school), a mausoleum, halls for ablutions and several funerary rooms (Fig. 1).

The site, state-owned property, is protected since november 19, 1920 by the royal decree that defines as national historical monument all the complex of Chellah.

Since 2012, Chellah is part of the sites of Rabat inscribed in the list of World Heritage of UNESCO as cultural asset.

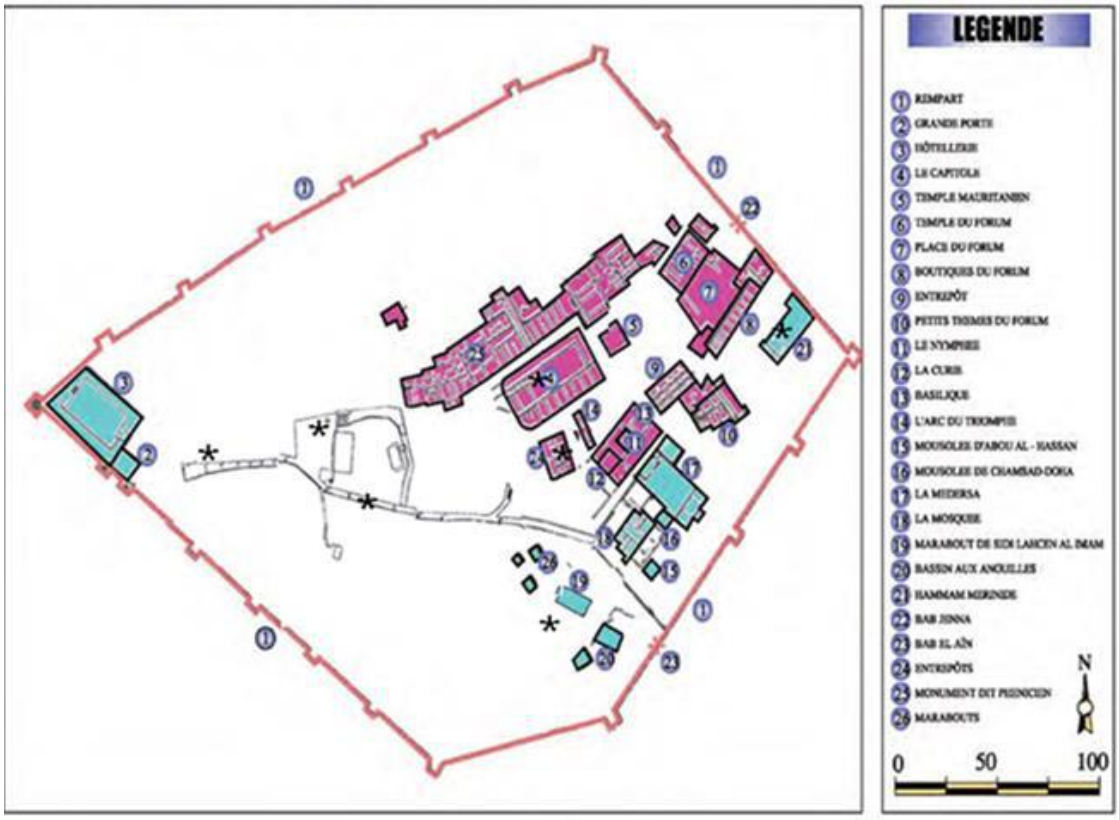

Fig.1:Location of the studied samples in the site of Chellah, Rabat

III. METHODOLOGY

We carried out prospections in the Chellah archaeological site during three seasons: autumn 2014, spring 2015 and winter 2016. These prospections allowed us to follow the biological cycle of the encountered bryophytes, in particular their sporulation. Further investigations were concentrated on the spring season, which is the most favorable period for observing the species with the sporophyte that is necessary for identification. The sampling is systematic and the harvest is therefore carried out at each encountered bryophyte population taking care not to pick up the entire population in order to preserve the species.

We sorted and identified the collected samples in the laboratory. The bryophytes are reviviscent plants that preserve very well after dehydration. Therefore, they are moisturized progressively for the identification.

Species recognition was done using the following identification keys: Augier (1966), Boulay (1884 and 1904), Casas et al., 2006, Coudreuse (2005), Pierrot and 2004). For each sample, morphological and histological characteristics were studied.

IV. RESULTS AND DISCUSSION

Based on the three prospections carried out in Chellah (fall 2014, spring 2015 and winter 2016), 20 species have been inventoried relating to 18 genera, 10 families and 8 orders and belonging to two classes: Muscinae and liverworts.

Muscinae are dominant with 14 species (70%) grouped in 5 orders: Bryales, Fissidentales, Funariales, Pottiales and

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Hypnales. On the other hand, liverwortsare represented by only 6 species (30%) grouped in 3 orders: Marchantiales, fossombroniales and sphaerocarpales.

Over the 10 encountered families, 4 clearly dominate the site (Figures 2 and 3): Potiaceae with 5 genera and 6 species, Brachytheciaceae with 3 genera and 3 species, Funariaceae and Bryaceae with 2 genera and 2 species each. These families alone account for 13 species, or 65% of the total. The other 6 families contribute only by 35%.

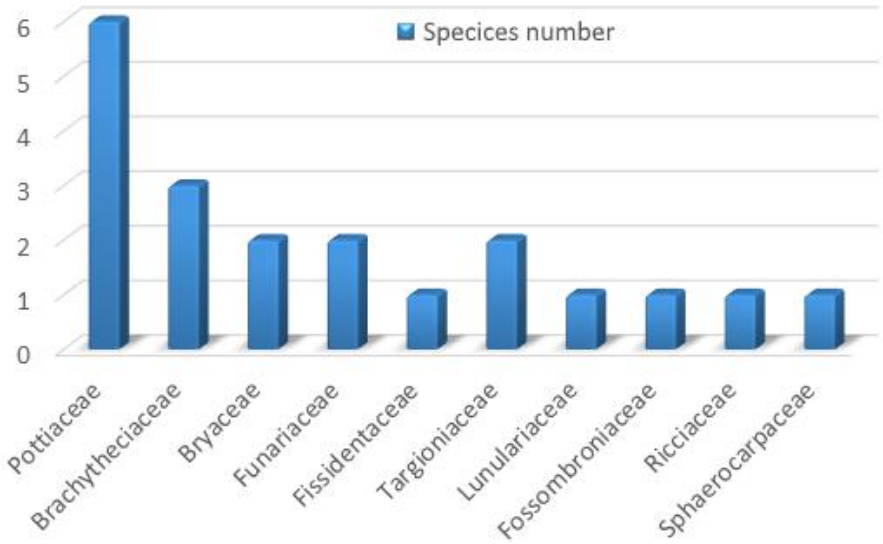

Fig.2: Specific richness per family

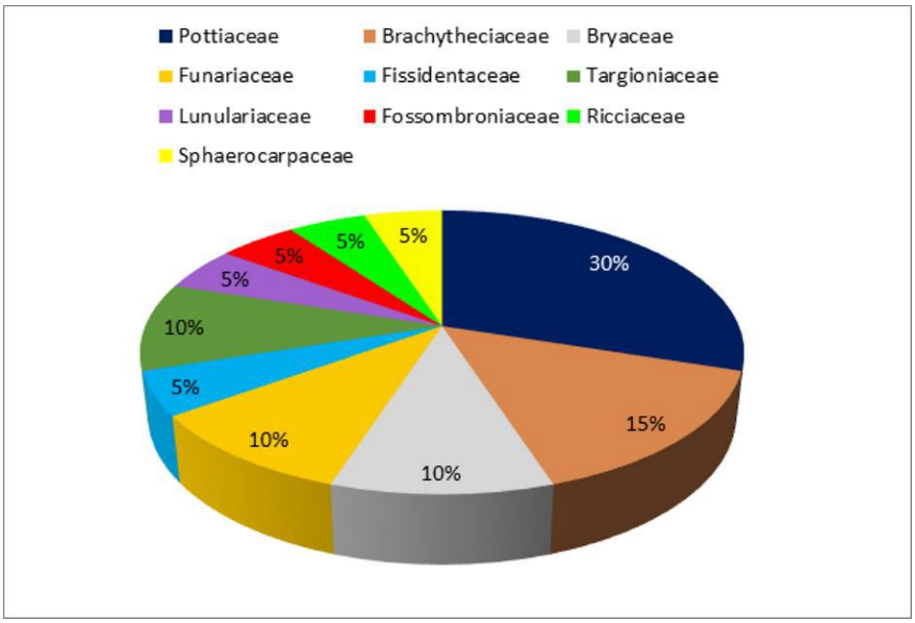

Fig.3: Spectrum of encountered families

Among the 20 inventoried species, 3 are newly described in Rabat region: *Entosthodon pulchellus* (H. Philib.) Brugués; *Didymodon fallax* (Hedw.) R.H. Zander and *Trichostomum crispulum* Bruch. They are illustrated in figures 4 to 6.

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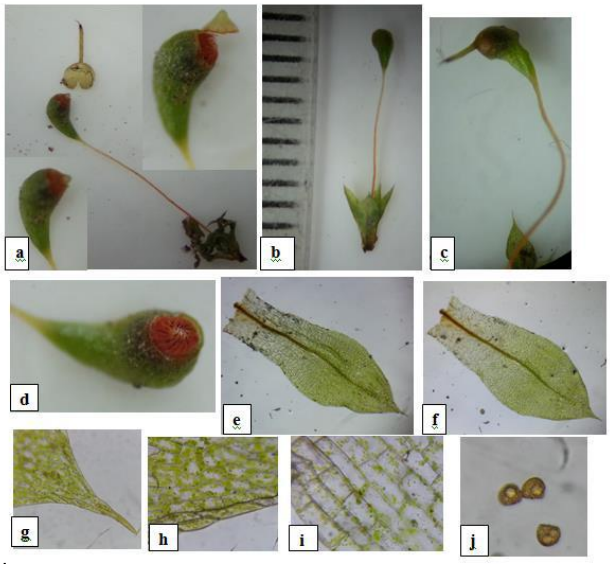

Fig.4.*a, b and c: individual of Entosthodon pulchellus x10; d: capsule showing a double peristome x40; e and f: microscopic observations of the leaves x40; g, h and i: respectively microscopic observations of the apical, median and basal cells of the leaves x400; j: microscopic observation of spores x400.*

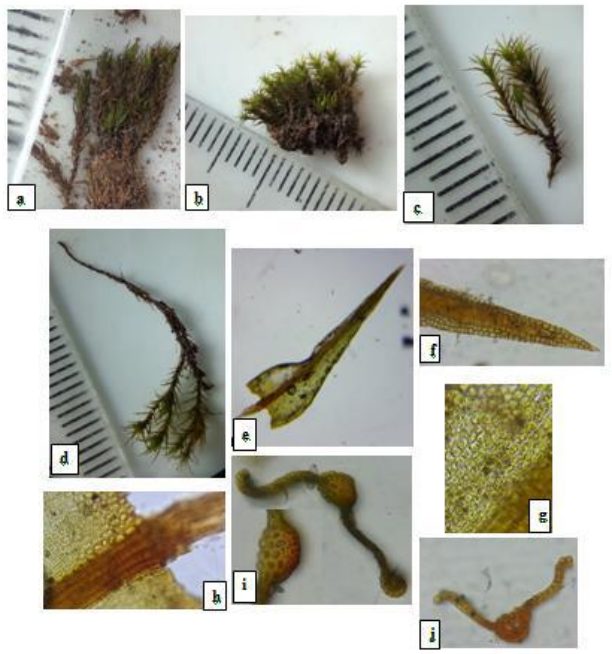

Fig.5.a: observation of a dry tuft of Didymodon fallax x10; b, c, d: observations of leafy stems in a wet state x15; e: observation of a leaf x40; f, g h: respectively observations of the apical, median and basal cells x400; i: observation of the cross sections of a leaf x400.

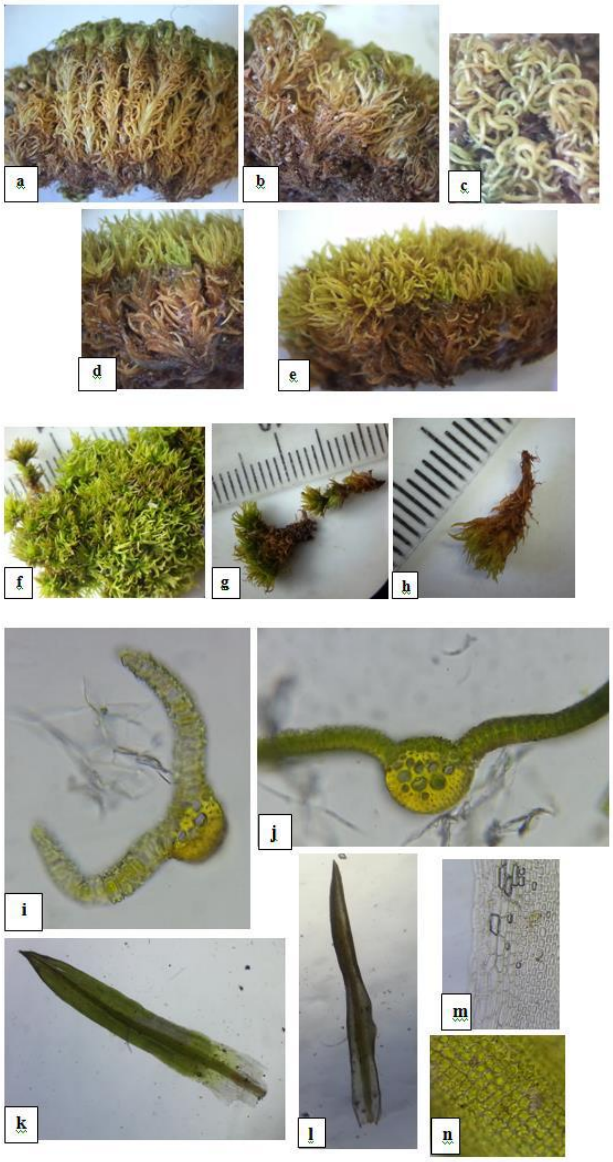

Fig.6: a, b and c: Trichostomum crispulum plant in a dry state x15; d: plant in a wet state x15, e: x10, f,g and h: x 5; i and j: observation of cross sections of a leaf x400; k and l: observation of leaves x40; m and n: basilar cell observations with the margin (m) and apical cells (n) x 400

4.1. Phyllum of Muscinae (Bryophyta)

This phyllum contains 14 species that will be presented per family.

4.1.1. Family of Brachytheciaceae

Rhynchostegium megapolitanum (Bland. ex F. Weber & D. Mohr) Schimp. (*Brachythecium cardotii* Winter; *Eurhynchium megappolitanum* (Bland ex F. Weber & D. Mohr) Milde;*Hypnum confertum* var. *megapolitanum* (Blandow ex. H. Weber & D. Mohr) Hampe;*Hypnum megapolitanum* Blandow ex F. Weber & D. Mohr;*Hypnum megapolitanum* var. *meridionale* Schimp.;*Hypnum megapolitanum* var. *septentrionale* Boulay;*Rhynchostegium megapolitanum* f. *julaceum*

Brizi;*Rhynchostegium megapolitanum* var. *julaceum* (Brizi) Latzel;*Rhynchostegium megapolitanum* subsp. *meridionale* (Schimp.) Giacom.;*Rhynchostegium megapolitanum* var. *meridionale* Schimp.)

In Morocco, the brachytheciaceae was reported on limestone substrates (Braun-Blanquet, 1954, Jelenc, 1955a, 1955b, 1967, Braun-Blanquet, 1954, cited by Ros et al., 1999). Elsewhere, it was reported on gravel and coastal sand by Augier (1966), and on sandy soil, or on old walls according to Boulay (1884). Smith, (2004) described it as a calcic species. In the studiedsite, it was harvested on clay-sandy soil.

Rhynchostegium megapolitanum (Bland, ex F. Weber &D. Mohr) Schimp. is a lying stemmed moss harvested in a vegetative state, with unequally spaced branches of irregular length. The horizontal stem, spread out, carries branches of different thickness. The leaves are spread, drawn up, sharp, dentateand slightly concave with a relatively sharp and long pointed tip and with a simple or bifid vein that extends to about the middle of the leaf blade. The cells of the leaf blade measure 8/1 and have membranes of the same thickness.

Rhynchostegiella curviseta (Brid.) Limpr. (*Eurhynchium curvisetum* (Brid.) Delogne;*Hypnum curvisetum*Brid.;*Hypnum rigidulum* Bruch;*Rhynchostegiella curviseta* var. *laeviseta* (W.E. Nicholson & Dixon) Podp.; *Rhynchostegiella letourneuxii* (Besch.) Broth.;*Rhynchostegium curvisetum* (Brid.) Schimp.;*Rhynchostegium curvisetum* var. *fastigiatum* Bott.; *Rhynchostegium letourneuxii* Besch.; *Rhynchostegium curvisetum* Schimp.).

It has been reported on limestone rocks of streams and on wet walls (Hamada et al., 2002); Which is in agreement with Augier (1966) who also observed it on calcareous stones. Smith (2004) noticed it on rocks, wet stones, top of trees, walls, streams, rivers, canals, lakes, and shady shores. At Chellah, it was harvested on limestone rocks.

Rhynchostegiella curviseta (Brid.) Limpr. is a Pleurocarpus moss with a creeping stem, irregularly ramified by more or less drawn up branches from 5 to 10 mm length and unequally spaced. The drawn up leaves,lanceolate, narrow, and gradually narrowed leaves in the upper half are weakly toothed and have a vein that reaches the middle of the leafblade. The middle limb cells are more than 10 fold longer than broad. The silk is red, rough and slightly curved in S. The actinomorphic capsule is more or less inclined.

Scorpiurium circinatum(Bruch) M. Fleisch. & Loeske (*Hypnum circinatum*Brid.;*Scorpiurium circinatum* (Brid.) Fleisch. & Loeske;*Eurhynchium circinatum* (Brid.) Schimp.;*Alsia circinata* (Brid.) Kindb. illeg. hom.;*Hypnum leskea* Grev.;*Hypnum mediterraneum* Sendtn.;*Hypnum strigosum* var. *circinnatum* Brid.;*Rhynchostegium circinatum* (Bruch) De Not.; *Scorpiurium circinatum* var. *ruderale* (Brizi) M. Fleisch. & Loeske;*Thamnium cossyrense* var. *melitense* Bott.).

It has been described as a terricole by Ros et al. (2001); It was observed on rocks and tree trunks, at an altitude ranging from 1500 to 2000 m a.s.l. (Maire & Werner, 1934; Jelenc,1955a, 1967 ; Ros et al, 1990; Rauh, 1952, cited by Ros et al., 1999); like on vertical limestones and in overhang, sometimes under very shady conditions (Jiménez et al., 2002b). It has also been encountered on sand or on sunny rocks of nature rather limestone (Augier, 1966); Smith, (2004) cites very diversified substrates and insists on the fact that these substrates are not exclusively limestone. It was collected on ground and on the sunny limestone rocks of the studied site.

Scorpiurium circinatum (Bruch) M. Fleisch. & Loeske is a Pleurocarp moss with a reeded stem with twigs in a dry state not reaching 1 mm wide, arched at their end. It occurs in thick tufts of about 2 cm, olive green and faded on the surface. The leaves are oval, about 1 mm in length, revolved only at the base, and denticulate in the upper part of the limb. The middle limb cells are relatively short (3 to 4 out of 1). It was harvested in a vegetative state.

4.1.2. Family of Bryaceae

Ptychostomum capillare (Hedw.)Holyoak & N. Pedersen(*Bryum capillare* Hedw.;*Bryum aschersonii* Müll. Hal.;*Bryum capillare* var. *platyloma* (Schwägr.) Schimp.;*Bryum capillare* var. *rufifolium* (Dixon) Podp.; *Bryum capillare* var. *meridionale* Schimp.;*Bryum capillare* var. *macrocarpum* Huebener; *Bryum capillare* var. *tectorum* Warnst.; *Bryum capillare* var. triste (De Not.) Limpr.;*Bryum capillare* var. *ustulatum* G. Roth.;*Bryum capillare* var. *majus* Bruch & Schimp.illeg. nom. incl. var. *prior*.;*Bryum capillare* subsp. *meridionale* (Schimp.) Podp.;*Bryum cochlearifolium* (Brid.) Hartm.;*Bryum rufifolium* (Dixon) Demaret & R. Wilczek;*Bryum torquescens* var. *gracile* Besch.;*Bryum validicostatum* Cardot & Dixon; *Mnium capillare* (Hedw.) With.).

In Morocco, this species was found on siliceous substrates (High-Atlas) (Werner, 1932; Gattefossé, 1932); on granite (Zaïan) (Gattefossé, 1931-1932, cited by Gattefossé & Werner, 1932) and on granitic and porphyritic rocks (Mayor, 1924). It has been reported on sandstones in Tangier; on sands in the Mamora forest near Rabat and on quartzites near Benslimane (Maire & Werner, 1934). Outside Morocco, (Ros et al., 2000) observed it on the edges and cracks of granitic and quartzitic rocks and on peats between 2100 and 3300 m. It was collected on the shady limestone rocks of the studied site.

Plychostomum capillare (Hedw.) Holyoak & N. Pedersen, is a 4 cmAcrocarpus moss with a circular to

slightly polygonal stem. The twig has a helical shape. Leaves are spatulate, twisted in the dry state, drawn up and spread apart in the wet state. They are cogged at the apex with an excurrent vein, margined on most of their length and mainly on the upper part of the limb where two rows of longer elongated cells are observed, the other cells are less elongated (2-3 of 1 at least), of hexagonal-losangic shape, having tips at the ends of their major axis. The middle of the limb has a large and loose areolation with cells of 2 to 3 on 1 in general. The sporophyte is well developed with an orange smooth silk of 2.5 cm and a rust-colored capsule of 4 mm, hanging with a double peristome.

Bryum radiculosum Brid (*Bryum duriaei* Schimp. ex Besch.;*Bryum murorum* (Schimp.) Berk; *Bryum eurythrocarpum* var. *murorum* Schimp;*Bryum atrovirens* var. *radiculosum* (Brid.) Wijk & Margad.; *Bryum erythrocarpum* var. *limbatum*Berth.;*Bryum erythrocarpum* var. *murale* Wils. ex Hunt. err. pro B. erytrocarpum var. *murorum* Schimp.; *Bryum erythrocarpum* var. *radiculosum* (Brid.) Bruch & Schimp.; *Bryum murale* Wilson ex Hunt. illeg.hom.).

In Morocco, it was observed on land in the Oued El Abid gorges at Bin ElOuidane (Mayor & Werner, 1934, Mayor and Werner, 1932, quoted by Gattefossé & Werner, 1932); (Jelenc, 1967, Jelenc, 1955b, cited by Ros et al., 1999); on the edges of the granitic rocks, between 2210 and 2400 m (Ros et al., 2000). It was first described in the Anti Atlas by Cano et al.in 2002. Elsewhere, it was reported on stony soils or on the wall mortar by Augier (1966) and Smith (2004) who considered it as a calcareous species. On the site of Chellah, it was harvested on limestone rocks.

Bryum radiculosum Brid is a yellowish-green acrocarpus moss, dioecious in compact tufts 4-10 mm high, with well-differentiated stem, oval-lanceolate leaves of about 2 mm, acuminate, denticulate at apex, the brown-russet-red veinis excurrent, the limb is smooth with a narrow and tightly bounded area around the middle (cells measure 6/1 at least). This plant was harvested in a vegetative state during the three prospections.

4.1.3. Family of Fissidentaceae

Fissidens bryoides Hedw (*Dicranum bryoides* (Hedw.) Sw. (*Fissidens arcticus* Bryhn, *hypnum bryoides* Linn.).

It was seen on the walls of Rabat (Braun-Blanquet & Maire, 1924), on non-calcareous shallow ground by Augier (1966), on slopes, along the paths, on wet stones, Boulay (1884). Smith (2004), found it on neutral to acid soils or more rarely on wood or stones in open areas. At Chellah, it was harvested on concrete and wet soil.

Fissidens bryoides Hedw (*Dicranum bryoides* (Hedw.) Sw.,is a small acrocarpus moss of 1 cm of length, with a short, drawn up and simple stemfixed on the substrate by fine and dense rhizoids. The leaves are spread out, inserted over two rows on both sides of the stem. They are oblong to lanceolate, acute, with a dorsal blade (sheath) shorter than the limb measuring about 1/3 of the length of this one and margined byvery narrow cells that are lengthened and hyaline. The cells of the limb are isodiametric and short, square almost round. The vein is simple, excurrente in a small mucron. (5 mm). The silk is short (5 mm), red, the capsule is ellipsoidal, drawn up and green with a simple peristome made up by red teeth divided up to the middle.

4.1.4. Family of Funariaceae

Funariella curviseta (Schwägr.) Sérgio (*Funaria curviseta* (Schwägr.) Milde, *Gymnostomum curvisetum*Schwägr.,*Physcomitrium curvisetum* (Schwägr.) Bruch & Schimp., *Entosthodon curvisetus* (Schwägr.) Müll. Hal.)

It was reported on limestone (Braun-Blanquet, 1954, Jelenc, 1955a, 1967, Braun-Blanquet, 1954, quoted by Ros et al., 1999); In the cracks and crevices of limestone rocks on wet sites with maritime influence. It was collected between the cracks of the limestone rocks of the studied site.

Funariella curviseta (Schwägr.) Sérgio, is a small acrocarpic moss of 5 mm of height, forming grass between the cracks of limestone rocks, with toothed leaves in the upper part, acuminate and without clear margin. The isolation is smooth in the upper part of the limb, is more or less translucent with rectangular cells and a vein finishing close to the top. The silk is about 2 mm long. The mature capsule is brown, symmetrical, pyriform, sloping at pendulum before dehiscence and darwn up at maturity with reticulated spores of 20 µm in diameter. The capis well developed, swollen and has a long beak. The peristome is absent. It forms a stand in association with *Targionia hypophylla* in the studied site.

****Entosthodon pulchellus*** (H. Philib.) Brugués (*Funaria pulchella* H. Philib.)

It has been reported on the cracks of granitic rocks at 2100 m a.s.l. (Ros et al., 2000). It was observed on basic soils in meadow and between rocks, and referred to as calcicole by Smith, (2004). It was picked up on rocks and limestone groundsof the studiedsite.

Entosthodon pulchellus (H. Philib.) Brugués is an 8 to 12 mm acrocarpus moss, forming loose turf on moist soil, the apical leaves, being larger than the others, formoval and whole rosettes. The limb is more or less translucent and terminated in a long filiform point; theareolation is smooth in the upper part, comprises rectangular cells more or less elongated from 2 to 3 in 1 at least. The vein does not reach the top of the limb. The silk is rectilinear; the capsule is oblique, and the urn is smooth. The spores are finely papillose and are about 20 µm in diameter.

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4.1.5. Family of Pottiaceae

Barbula unguiculata Hedw. (*Barbula apiculata* Hedw.; *Barbula fastigiata* Schultz; *Barbula gattefossei* P. de la Varde; *Barbula microcarpa* Schultz; *Barbula obtusifolia* Schultz illeg.hom.;*Barbula unguiculata* var. *apiculata* (Hedw.) Bruch & Schimp.; *Barbula unguiculataf. apiculata* (Hedw.) Mönk.; *Barbula unguiculataf. cuspidata* (Schultz) Mönk.; *Barbula unguiculata* var. *cuspidata* (Schultz) Brid.; *Barbula unguiculata* var. *fastigiata* (Schultz) Huebener; *Barbula unguiculata* var. *latifolia* Bréb.; *Barbula unguiculata* var. *microcarpa* (Schultz) Huebener; *Barbula unguiculata* var. *minus* Hillier; *Barbula unguiculataf. obtusifolia* Mönk.; *Barbula unguiculata* var. *robusta* Lindb.; *Bryum unguiculatum* (Hedw.) With.;*Dialytrichia canariensis* Bryhn;*Streblotrichum unguiculatum* (Hedw.) Loeske.;*Tortula unguiculata* (Hedw.) P. Beauv. illeg. nom.)

It was seen on the edges of granitic rocks, on quartzic soils near streams (Ros et al., 2000); as well as on the bases and trunks of *Quercus rotundifolia* (Draper et al., 2006). It was described as a messicole by Augier (1966), which described it as a pioneer plant on clay soil and limestone sand. It was seen on very diversified substrates by Boulay, (1884), who observed it particularly on disturbed soils or on landfills. It was collected on limestone rocks and on clay-sandy soil of the studied site.

Barbula unguiculata Hedw. is an acrocarpus moss of 1.5 to 2 cm of length. The leafed stem is about 1 cm of length. The basal leaves are revolved and measure 1.5 to 3 mm, with an excurrente vein with two stereidsbands. The leaves are helically and strongly twisted in a dry state. The top of the limb is flat and obtuse and has numerous C-shaped papillae. The sporophyte is made up of a red silk and a 1.5 mm capsule. The spores are smooth, yellowish green and 10 to 12 µm in diameter. The teeth of the peristome describe 2 to 3 spiral turns.

****Didymodon fallax*** (Hedw.) R.H. Zander (*Barbula acuminata*Hedw.;*Barbula adriatica* Baumgartner;*Barbula brevicaulis* Schwägr.; *Barbula brevifolia* (Dicks. ex With.) Brid.;*Barbula fallaxf. brevicaulis* (Schwägr.) Podp.;*Barbula fallax* var. *brevicaulis* (Schwägr.) Huebener; *Barbula fallax* var. *brevifolia* (Dicks. ex With.) Schultz; *Didymodon fallax* var. *adriatica* (Baumgartner) Düll; *Didymodon fallax* var. *brevifolius* (Dicks. ex With.) Ochyra; *Tortula fallax* (Hedw.) Schrad. ex Turner;*Barbula Fallax* Hedw).

It has been seen on fresh rocks in a sterile state (Jahandiez, 1923); at the bottom of limestone rocks, on damp ground; on porphyritic rocks (Maire & Werner, 1934); on limestone (Braun-Blanquet, 1954); Ros et al., 2001 describe it as a terricole species; and according to Jiménez et al., (2002b), it would develop on cracks of rock and on sandy soils. Smith, (2004) noticed that it can be present on clay-rich soils and on sand dunes at altitudes ranging from 0 to 490 m. It was collected on clay-sandy soil of the studied site.

Didymodon fallax (Hedw.) R.H. Zander is an acrocarpusmoss harvested in a vegetative state from 1 to 3 cm in height; It is rust-colored. The stem is ramified, with a differentiated axial beam. Partially revolved leaves are acute and lanceolate 2-3 mm in length, spread-arched, more or less squarrose, in the wet state. The top of the limb is plane and acute with conical papillae that make areolation quite distinct. The base of the limb shows some rectangular and elongated cells whereas others are a little longer than the cells of the higher half of the limb, with thick membranes.

Timmiella barbuloides (Brid.) Mönk (*Barbula cirrhata* Arn.; *Timmiella barbula* Limpr illeg. nom.; *Timmiella barbula* var. *minor* Schimp. ex Luisier; *Trichostomum Barbula* Schwaegr. illeg. nom.; *Trichostomum barbuloides* Brid.).

It develops on limestone rocks (Mayor, 1924) and (Augier, 1966); at the bottom of walls and maritime cliffs of Rabat, as well as on travertines at Taza (Braun-Blanquet and Maire, 1924). It has been reported on siliceous groundo wet places (Maire & Werner, 1934); in tufa caves at Zalagh Mount near Fez and in Taza Gorges (Braun-Blanquet, 1954, Jelenc, 1955a, 1955b, 1967, Ros et al., 1990, Braun-Blanquet, ., 1999).; on clay soil, at the bottom of rocks and walls and in ravines according to Boulay, 1884. At Chellah, it was collected on limestone rocks.

Timmiella barbuloides (Brid.) Mönk, is a 3cm acrocarpus moss. The tuft is of a dark green shining in the dry state. The stem, of 5 mm, is not ramified. The sheathing leaves, involute, of hooked form in the dry state, carry on the upper face high projections each formed by a cell. The limb is largely bistratified with a veinincluding two bands of stereids. The capsule presents a rudimentary ring with a very little twisted peristome with 32 long and fine teeth.

Tortula marginata (Bruch & Schimp.) Spruce (*Barbula marginata* Bruch & Schimp.; *Desmatodon meridionalis* Luisier; *Tortula limbata* auct.).

It has been described as calcicole by Augier (1966) and has been observed on limestone rocks (Ahayoun et al., 2007). Boulay, 1884, observed it on the walls, the rocks and in the shaded and little covered places. Smith (2004),noticed it on wet and shaded basic rocks as well as on the walls of natural and artificial habitats. It was taken from the limestone rocks at Chellah.

Tortula marginata (Bruch & Schimp.) Spruce is a 10 mm acrocarpusmoss. It organizes small stands with lichen in the studied site. The leafed stem is about 2 mm high. The leaves are very little revolved, lingulate and narrow from

2 to 3 mm lenght with an excurrente vein in a rather long point. They are margined on almost all their contour by two strata of cells. The latter are more elongated and narrower than those of the rest of the limb. The areolation is papillose and opaque in the upper part of the limb, where the cells are quadrangular, almost square; it is smooth and translucent in the lower part of the limb, where the cells are rectangular and elongated. The capsule, of about 2 mm, is without cover and contains yellowish smooth spores of 10 µm in diameter.

Tortula muralis Hedw. (*Barbula heribaudii*Corb.;*Barbula muralis* (Hedw.) Crom.;*Barbula muralis* var. *incana* Bruch & Schimp.;*Barbula muralis* var. *obcordata* Schimp.; *Barbula muralis* var. *rupestris* Schultz; *Bryum murale* (Hedw.) With.;*Syntrichia muralis* (Hedw.) Raab;*Tortula aestiva* var. *vulcanicola* Schiffn.;*Tortula muralisf. incana* (Bruch & Schimp.) Sapjegin;*Tortula muralis* var. *incana* (Bruch & Schimp.) Wilson;*Tortula muralisf. obcordata* (Schimp.) Mönk.;*Tortula muralis* var. *obcordata* (Schimp.) Limpr.;*Tortula muralis f. rupestris* (A. Chev.) Sapjegin;*Tortula muralis* var. *rupestris* A. Chev.)

It has been reported on granite (Gattefossé, 1931-1932, quoted by Gattefossé & Werner, 1932); On the Roman ruins of Volubilis (Braun-Blanquet, 1954, Jelenc, 1955a, 1955b, 1967, Braun-Blanquet, 1954, cited by Ros et al., 1999). Cano et al. (2002) described it for the first time in the Anti Atlas on bare soils. Its presence was also noted on the banks of the BouRegregriver; on granite; onlimestone rocks; as well as on limestone slopes (Ahayoun et al., 2007). Boulay, (1884) and Smith (2004) cite very diverse substrates such as walls, roof tiles and cracks in rocks. Smith, (2004) argues that *Tortula muralis* tends to prefer basic substrates and is found more rarely on acidic substrates and is more common in urban areas where it is able to resist atmospheric pollution. At Chellah, it was harvested on limestone rocks.

Tortula muralis Hedw is an acrocarpus moss about 2cm. The leaves are more or less drawn up-isolated in the wet state, contracted and twisted in the dry state, with a strong vein all along the leaf, showing in cross-section a single band of stereids, dorsal, excurrent in one hair smooth or slightly dentil, often flexuous hyaline, long; formed hyaline point, partly at least, by the vein itself. The limb is very papillose at the top, and revolute up to near the summit. The cells of the upper part of the leaf are furnished with manypapillae, they are square-subaronded, very chlorophyllian, elongated towards the middle. The basal cells are hyaline, rectangular and longer. The drawn up cylindrical capsule takes on a blackish color at maturity with a long beak opercula (¼length of the capsule), and a cupped headdress. The peristome has completely divided teeth up to the basilar membrane; it then appears formed of 32 bristles, twisted in whorl with 1-3 turns. The spores are spherical with a smooth wall about 10 µm in diameter.

Tortula muralis var. ***aestiva*** Brid. ex Hedw.(*Barbula aestiva* (Brid. ex Hedw.) Schultz;*Barbula muralis* var. *aestiva* (Brid. ex Hedw.) Röhl.;*Tortula aestiva* (Brid. ex Hedw.) P. Beauv.;*Tortula muralis* subsp. *aestiva* (Brid ex Hedw.) Meyl.).

It is a bright green plant with the same characteristics as *Tortula muralis* Hedw except in the shape of the leaf, which is tightly lingulate, spatulate with an excurrente vein in a yellowish green tip.

Trichostomum crispulum Bruch. (*Mollia crispula* (Bruch) Lindb.,*Trichostomum brevifolium* Sendtn. ex Müll. Hal.;*Trichostomum crispulum* var. *acuminatum* Meyl.; *Trichostomum crispulum* var. *angustifolium* Bruch & Schimp.;*Trichostomum crispulum* subsp. *brevifolium* (Sendtn. ex Müll. Hal.) Giacom.;*Trichostomum crispulum* var. *brevifolium* (Sendtn. ex Müll. Hal.) Bruch & Schimp.; *Trichostomum crispulum* var. *cucullatum* (Cardot ex G. Roth) Podp.; *Trichostomum crispulum* var. *elatum* Schimp.; *Trichostomum crispulumf. longifolium* Bouvet;*Trichostomum crispulum* var. *pseudoweisia* Schimp.; *Trichostomum crispulum* var. *viridulum* (Bruch) Dixon; *Trichostomum hammerschmidii* Loeske & H.K.G. Paul; *Trichostomum viridulum* Bruch)

It develops on the ground and in the cracks at the base of moist granitic rocks (Ros et al., 2000). It has been described as a pioneer on limestone rocks, on gravelly soil and on coastal dunes by Augier (1966). Boulay, (1884), notes its presence on the sandy or marly soil of the hills, in the hollows of the rocks, on the old walls and on the fixed sands of the old dunes. According to Smith 2004, it is found in shaded or exposed basic habitats, on the ground, rocks, rocky banks, cliffs, old wall mortar, and sandy dunes. It was collected on limestone rocks of the studied site.

Trichostomum crispulum Bruch. is an acrocarpusmoss, harvested in a vegetative state, light green to yellowish on surface, brown inside. The leafed stem is 1 to 1.5 cm long with mucronate acute leaves at the apex and strongly tight in the dry state; they are lanceolate from 3 to 4 mm, very papillose-opaque in the upper 2/3 of the limb. The limbis a little curved at the edges, and terminates in small cap, more or less mucronate, with a vein that is not very excurrente; it has two bands of stereids. Cells in the upper limb have thin membranes and generally square cellular contours becoming rectangular and thick membrane towards the lower part.

4.2. Phyllum of liverworts (Marchantiophyta)

4.2.1. Family of Lunulariaceae

Lunularia cruciata (L.) Lindb. (*Dichominum cruciatum* (L.) Trevis.; *Lunularia cruciata* (L.) Dum.;*Marchantia*

cruciata L.;*Lunularia dillenii* Le Jol.; *Lunularia michelii* Le Jol.; *Lunularia vulgaris* Raddi illeg.nom.; *Lunularia vulgaris* Mich.; *Marchantia dillenii* Le Jolis; *Preissia cucullata*Nees & Mont.; *Staurophora pulchella*Willd. illeg.nom.).

In Morocco, it has been reported on walls, hollows of rocks, embankments, banks of wadis (Corbiere, 19132, Corbiere, 1923a, quoted by Gattefossé & Werner, 1932); on wet schistose and sandy rocks in the lower valley of Ourika (Mayor, 1924); on old walls in Rabat (Braun-Blanquet & Maire, 1924). It was also observed outside the immediate contact of warm water from the thermal spring of Lalla Aïa (Gattefossé, 1932); on siliceous substrates of Djebel Guedrouz (Werner, 1932, Gattefossé, 1932, Gattefossé & Werner, 1932); and on limestone rocks (Jovet-Ast, 1956c). Augier (1966), Coudreuse et al. (2005) and Boulay (1984) insist on the wet character of the substrate on which it is observed. It was collectedon rocks and shaded and wet grounds of Chellah.

Lunularia cruciata (L.) Lindb. is a hepatic with thallus of 1.5 to 4 cm in length and 0.6 to 1.4 cm in width, dark green in color with crescent-shaped propagule baskets. The thallus is clearly differentiated,intwo parts: that on the dorsal side is formed of a very green assimilative tissue, and that on the ventral side consists of a parenchyma little or not chlorophyllian, being used for retention or conduction of substances. The assimilative tissue is formed by chambers filled with chlorophyllous bristles (sometimes very short), and regularly drawn up on their floor, opening by pores.

4.2.2. Family of Targioniaceae

Targionia hypophylla L. (*Targionia michelii* Corda;*Targionia convoluta* Lindenb. & Gottsche;*Targionia bifurca* Nees & Mont.;*Targionia germanica* Corda inval. nom.; *Targionia hypophylla* var. *fimbriata* (Müll. Frib.) Müll. Frib.).

In Morocco, it has been reported on fresh rocks (Braun-Blanquet & Wilczek, 1923); Schistose rocks (Braun-Blanquet & Maire, 1924); on the siliceous substrates of Djebel Guedrouz in Haut-Atlas, at 1850 m a.s.l. (Werner, 1932); as well as along cracks on limestone rocks at Adar-Ouaman in the Anti-Atlas (Mayor & Werner, 1934). This messicole species according to Augier, 1966, has been described as a pioneer on argillaceoussoil and calcareous sand. According to Boulay, 1984, it is found on clay-sandy soil, on old walls owing to cracks and on limestone rocks. In Chellah, this species was collected on limerubble stone masonry or clay-sandy soil.

Targionia hypophylla L. is a hepatic with a dark green thallus of 10 to 15 mm long and 4 mm wide. The thallus is clearly differentiated, in two parts: that on the dorsal side contains a very green assimilative tissue, and that on the ventral side consists of a parenchyma little or not chlorophyllian. The assimilative tissue is formed by chambers filled with chlorophyllous bristles regularly drawn up on their floor, closed or opening by pores. The ventral scales are large, and violet. The stomata are surrounded by 2 to 3 circles of cells very different from the epidermal cells. The capsule located on the ventral surface of the thallus is contained in a bivalve envelope. It contains elaters with 2-3 whorls and spores from 50 to 60 µmof diameter.

Targionia lorbeeriana Müller Frib. (*Targionia hypophylla* var. *lorbeeriana* (Müll. Frib.) Aleffi & Schumacker inval.nom.).

In Morocco, it has been reported on calcareous ground and rocks, siliceous ground, granite rocks (Jovet-Ast, 1956c). This species was harvested on limestone rocks in the studied site.

Targionia lorbeeriana Müller Frib., is a hepatic with glaucous thallus with some 4 mm wide lobes. Thallus is clearly differentiated, on a transverse section, into two parts like the previous species. The oval stomata are surrounded by 1 to 2 rings of about 7 large reniform cells. The capsule located on the ventral surface of the thallus is protected by a bivalve envelope. The spores are about 80 µm in diameter. It organizes dense stands on limestone rocks and between their cracks.

4.2.2. Family of Ricciaceae

Riccia crystallina L. emend. Raddi (*Riccia crystallina* subsp. *austrigena*Schiffn.,*Riccia crystallina* var. *major* Lindb., *Riccia crystallina* var. *vulgaris* Lindenb.)

In Morocco, it has been reported on mud (Corbiere, 1913a, Corbiere, 1913b, quoted by Gattefossé & Werner, 1932); on calcareous red ground, on ways (Jovet-Ast, 1955, Jelenc, 1955a, 1967, Jovet-Ast, 1965, quoted by Ros et al., 1999);on mud of dried marshes (Augier, 1966, Boulay, 1984, Hamada et al., 2002).

Riccia crystallina L. emend. Raddi, is a hepatic with radial sling thallus, with obtuse lobes, 1 to 4 mm wide, light green above, pale in lower part. The assimilative tissue is made of lacunary parenchyma. The thallus is one to two times dichotomous. The spores of 70 µm diameter on average carry on their convex face a network of alveoli. It is met with *Sphaerocarpos michelii* forming a same stand.

4.2.3. Family of Fossombroniaceae

Fossombronia angulosa (Dicks.) Raddi (*Jungermannia angulosa* Dicks.).

In Morocco, it has been reported on different substrates: on siliceous soil, on siliceous soil at the edge of a stream; on soil covering rocks of granite, on fresh ground in the shade, on shady rocks partially covered with soil; or on siliceous red soil at the edge of a runoff (Jovet-Ast, 1956c). According to Augier 1966, this species has been reported on non-calcareous wet soil, on the slopes of

hedges, paths and in rather dry places. It is cosmopolitan in the Mediterranean region according to Boulay, 1984. In Chellah, it is found between the rocks of the studied site.

Fossombronia angulosa (Dicks.) Raddi, is a hepatic with leaves of 1-3/0.5 cm with a creeping stem bearing succubus leaves and purple rhizoids. The silk is short with a round capsule containing spores of about 40 µm. The spore has a hemispherical face and a pyramidal face.

4.2.4. Family of Sphaerocarpaceae

Sphaerocarpos michelii Bellardi (*Sphaerocarpos terrestris* (Micheli) J.E.Sm.).

It has been reported on soil covering a granite rock (Jovet-Ast, 1956c); on sandy soil and on the granitic rock above the hot spring of Oulmès (Ahayoun et al., 2007). Augier, (1966) describes it as a messicoleand indicates its presence on clay soil. It was harvested on the clay-sandy soil of the studied site.

*Sphaerocarpos michelii*Bellardi, is a dioecious hepatic with light green thallus with unistratified margins. Antheridia and archegones are located on the dorsal side of the thalli, each is surrounded by an involucre with the pyriform male and the globular female, tightened in the upper part and presenting a small opening. The dehiscence of the capsule is irregular. The spinous spores are united in tetrads of dark brown color measuring approximately 100 µm in diameter.

4.3. Bryophytes and Biodeterioration

Bryophytes are reviviscent plants that settle as soon as the substrate is moistened (fig. 7 and 8); they can occupy very poor environments in organic matter. The samples were taken at different locations in Chellah site (Fig. 1). The obtained results show that bryophyte colonize both the moistenedsoil, the concrete of steps, the zellij, the terracotta brick made up essentially of calcarenite dating from antique and Islamic times (fig. 9, 10 and 11). This rock was extracted from the quarries of Sidi Bou-Knadel, about ten kilometers to the north of Salé,from plio-quaternary age and belonging to the dune cords that extend along the Atlantic coast between El Jadida and Larache. It is a compact, yellow-beige detritic sedimentary rock made up of two detrital fractions: a bioclastic fraction (shell fragments) and a terrigenous fraction composed of millimetric quartz grains bound by a limestone cement.

According to the bioreceptivity criteria described by Guillite (1995), Salé calcarenite offers a set of characteristics favorable to biocolonization. Indeed, the surface quality of Salé calcarenite has a large roughness allowing the anchoring of rhizoids. This surface roughness is also associated with a high porosity of about 24% and an estimated capillarity of 12.37 (El Amrani El Hassani & El Azhari, 2009), which is conducive to retention of water and favorable for the installation and growth of bryophytes.

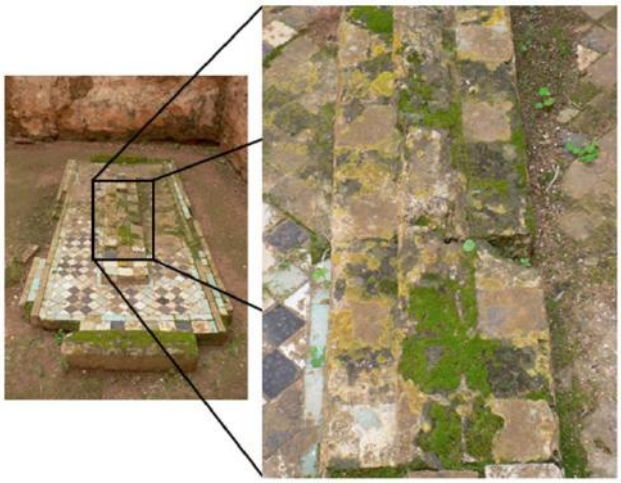

Fig.7: Development of bryophytes on zellij tiles of a tomb in Chellah necropolis contributing to the destruction of the enamel

Fig.8: Terracotta brick wall of the gate covered with bryophytes that maintain a high degree of moisture on the masonry

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Fig.9: Colonization of the base of a calcarenite wall in Chellah by bryophytes thanks to the capillary rise that favors a high degree of moisture

Fig.10: Vertical edge of the calcarenite basin of ablution in Chellah covered by bryophytes

Fig.11: Total recovery by bryophytes of an architectonic element and loss of legibility

Once the supports have been colonized, the bryophytes cause initially a chromatic alteration and thus an aesthetic biodeterioration (Deruelle, 1991; Ortega-Calvo et al., 1991; Warscheid & Braams, 2000; Gaylarde et al., 2003; Allsopp et al., 2004).

The bryophyte binding system will develop in the pores of material, exerting pressures inside the structure that may modify the porosity of the support and in the long term to makematerial to burst (Warscheid & Braams, 2000; Gaylarde et al., 2003). This is called physical biodeterioration.

Other chemical processes are involved: the dissolution of limestone substrate minerals by acidic secretions produced by plants as well as the assimilation of the substrate as a nutrient (Warscheid & Braams, 2000). This is referred to as a chemical biodeterioration.

V. CONCLUSION

The archaeological site of Chellah has been subject to few scientific researches in botanical matter, in particular with regard to the bryophytes. Therefore, our objective consists in describing and identifying the species of bryophytes present in this site and then to establish a list of taxaof these species.

The harvest of the bryophytes allowed us to identify 20 species distributed as follows:

- Fourteen species belonging to the Moss class divided into 5 orders and families and 13 genera, the most widely represented order being the Pottiales with *Fissidens bryoides*, the most widespread species in the site;
- Six species belonging to the Hepatic class including one leaf hepatic. They are distributed in 3 orders, 5 families and genera, with *Lunularia cruciata*, the most encountered species.

The colonization of archaeological materials by bryophytes leads to their aesthetic, physical and chemical biodeterioration due to the acid secretions produced by these plants. All these processes have an important role in pedogenesis and thus in the initiation of plant succession. This fact, without gravity in the natural environment, becomes a source of issues when it concerns materials used in historical monuments whose durability is then threatened.

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Proximate and heavy metals composition of Plantain (*Musa paradisiaca* L.) fruits harvested from some solid waste dumps in Uyo Metropolis, Nigeria

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***Abstract*—** *Plantain thrives well in waste dumpsites. These wastes usually contaminate the soil with heavy metals which become absorbed by the plants. The risk of heavy metal toxicity in humans is an issue of serious concern globally. Proximate composition of plantain fruits harvested from three randomly selected waste dumpsites in Uyo metropolis, Nigeria were determined using AOAC standard analytical techniques while their heavy metals (Pb, Cr, Ni, Cu, Co, Cd and As) concentrations and that of their rhizosphere soil were analysed with Unicam Atomic Absorption Spectrophotometer. Proximate analyses results revealed that carbohydrate content was higher in the fruits harvested from the control uncontaminated soil sites (91.61%) than in the fruits harvested from dumpsite soils (87.23%; 87.89%; 88.00%). Dumpsite soils had higher heavy metals concentrations than the control soil. Lead (Pb) was the highest occurring heavy metal in all the dumpsite soils. Fruits harvested from the dumpsite soils had higher heavy metals concentrations than those from the control soil. Pb was the only heavy metal whose concentration in the plantain fruits was higher than the WHO/FAO permissible limit. This work has established that the selected dumpsite soils have been contaminated with heavy metals which have been absorbed by the plantain cultivated there. Cultivation and consumption of plantain from these dumpsite soils should be discouraged.*

***Keywords*— Dumpsites soils, Heavy metals, Plantain fruits, Proximate composition.**

I. INTRODUCTION

Plantain (*Musa paradisiaca* L.) is a tree-like herb belonging to the Musaceae family. With its high starchy fruits, plantain fruit serves as a staple crop in most parts of the tropics including Nigeria. Plantain fruits have high fibre contents which make it a diet for lowering of blood cholesterol and relieving of constipation thereby putting colon cancer at bay (Okareh, 2015). Plantain has a high demand for organic matter and thrives luxuriantly in waste dumpsites where they produce healthy bunches of fruits.

Solid waste dumpsites are common features in most urban cities in Nigeria as much waste is generated by their teeming human population. Due to scarcity of arable lands in the urban areas, plantain is cultivated in strategic locations where all sorts of solid waste materials are dumped. Leachates from these dumpsites contribute heavy metals to the soil (Ukpong *et al.*, 2013) which according to Ideria *et al*. (2010) constitute the commonest occurring group of solid waste dumpsite soil contaminants. Plantain growing in such dumpsites absorbs these heavy metals along with other nutrients and accumulates them in its fruits. Studies have revealed higher levels of heavy metals such as lead, cadmium, nickel, cobalt, arsenic and chromium in waste dumpsite soils than in soil some distances away from them (Ukpong *et al*., 2013; Amos-Tautua *et al.*, 2014; Olufunmilayo *et al.*, 2014, Tanee and Eshalomi-Mario, 2015). Higher concentrations of these heavy metals have also been detected in fruits and vegetables harvested from waste dumpsites (Imasuen Omorogieva, 2013; Cortez and Ching, 2014; Tanee and Eshalomi-Mario, 2015).

Heavy metals according to Kibria *et al*. (2010) are elements with an atomic weight greater than 20 and are characterised by similar atomic electronic configurations in the outer orbitals. They are mostly harmful chemicals as they are usually persistent, toxic and bio-accumulative in the environment while some of them are also endocrine disrupting as well as carcinogenic (Kibria *et al.* 2016a; Kibria *et al.* 2016b). Some heavy metals such as iron, nickel, zinc, copper are known to be essential and beneficial for the growth and development of plants and animals

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including humans at low concentrations (Cortez and Ching, 2014; Tanee and Eshalomi-Mario, 2015; Balkhair and Ashraf, 2016). Other heavy metals such as lead, cadmium and mercury are highly toxic even at low concentrations (Cortez and Ching, 2014).

The issue of heavy metals contamination of soil and subsequent uptake and accumulation in food crops is fast becoming a major health concern as their presence pose serious health hazards to plants, animals and humans (Rim-Rukeh, 2012). The danger posed by the presence of heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb) and nickel (Ni) is as a result of their bioaccumulation in the environment with time (Helmenstine, 2014). Prolonged intake of heavy metals contaminated food is implicated with cancer, nervous system disorders, cardiovascular diseases, renal problems, destruction of liver, lungs and kidneys. The risk involved in consuming crops harvested from waste dumpsites calls for serious investigation into the concentration of heavy metals in such food items. This research was therefore carried out to investigate the heavy metals (Pb, Cr, Ni, Cu, Co, Cd and As) concentrations of both the dumpsite soils and their plantain fruits as well as the possible effect on the proximate composition of such fruits. Findings from this work are expected to give growers of plantain on dumpsite soils insight as to the risk of heavy metals accumulation in the fruits and the consequent health hazards to their consumers.

II. MATERIALS AND METHODS

Sampling Site

Three dumpsites cultivated with plantain bearing mature unripe bunches of fruits located within Uyo metropolis were randomly selected. Fruits for control were harvested from another location without any waste dumpsite within the same metropolis. The geographical coordinates of Uyo is 5° 3' 0" North and 7° 56' 0" (maplandia.com, 2016). The experimental sites as determined using a Global positioning system (GPS) to geo-reference the sampling positions were respectively designated as: Site A located between latitude 5°1'56.935"N and longitude 7°55'46.714"E; Site B located between latitude 5°1'21.640"N and longitude 7°54'47.592"E; Site C located between latitude 5°0'35.8812"N and longitude 7°55'5.4048"E and the Control site located between latitude 4°59'7.224"N and longitude 7°55'11.244"E.

Collection of Plant and Soil Samples.

Rhizosphere soil samples were collected in triplicates from each site at 15-20 cm rooting zone using soil auger. The plantain fruits were also collected in triplicates from plants whose rhizosphere soil samples were collected. The soil and plant samples were appropriately labeled and taken to the laboratory for proximate and heavy metal analyses.

Preparation of the plantain fruit samples.

Each batch of fruits from each dumpsite was separately washed with distilled water and peeled. They were then cut into thin slices and air dried at room temperature before being transferred to an oven (Gallenkamp) to dry at 80°C. The dried slices were then ground into powder using an electric blender (Model: GND-280 AUTOSHARP) and stored in an airtight container until when needed for analysis.

Proximate composition analysis of plantain fruits

The proximate nutrient composition of the plantain fruits was determined using the standard methods of analysis of Association of Official Analytical Chemists (AOAC, 2000) for moisture, dry matter, crude protein, lipid, ash and fibre. Moisture content was determined by drying in a (Gallenkamp) oven at 105°C to constant dry weight and then calculating the percentage difference between the initial and final weights. The crude protein of the samples was determined using micro-Kjeldahl method. Percentage lipid content was determined using petroleum ether (B.P. 60°C - 80°C) extraction by reflux Soxhlet method and the percentage oil calculated as:

$$\% \text{ Oil} = \frac{\text{Weight of oil} \times 100}{\text{Weight of dry sample}}$$

Ash content was determined by dry ashing in a muffle furnace at 550°C until grayish white ash was obtained and calculating the percentage difference between the sample initial weight and the ash weight. Crude fibre was determined using acid-base digestion with 1.25% H_2SO_4 (w/v) and 1.25% NaOH (w/v) solutions. Carbohydrate content was obtained following Onwuka (2005) method as the difference:

% Carbohydrate = 100 - (% crude protein + % crude fibre + % ash + % crude fat).

Determination of heavy metals contents in the plant samples

The powdered plant samples were ashed in a muffle furnace and digested after the procedure of Adefemi *et al.* (2012). 2g of each ashed plant sample was digested in 15ml of HNO_3 at 80ºC until a transparent solution was obtained. The solution was allowed to cool and then filtered through Whatman filter paper (no. 42) into 100ml volumetric flask and made to mark with distilled water. Each filtrate was

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stored in separately labeled sample bottle ready for the determination of Pb, Cr, Ni, Cu, Co, Cd, As and Fe concentrations using Unicam 939 atomic absorption spectrophotometer (AAS). The AAS was calibrated with appropriate standard solutions for each element and the samples filtrate were aspirated in turns into it to determine the heavy metals concentration.

Determination of heavy metals contents in the soil samples.

Determination of heavy metals concentration of the soil samples was carried out using the method of Cortez and Ching (2014). Each soil sample was oven-dried at 105°C, ground with mortar and pestle and sieved through a 2 mm sieve. About 0.5 g of each sample was weighed into a porcelain crucible and ignited at 450°C in a furnace to destroy organic matter, then digested twice with 10 ml of a mixture of 1:1 mixture of concentrated HNO_3 and HF in a 100 ml polyprylene beaker and placed over a water bath for evaporation till dryness. The residue was dissolved in 20 ml of 2M HNO_3 and diluted to mark in 100 ml volumetric flask. The digest was then used for heavy metals (Pb, Cr, Ni, Cu, Co, Cd and As) concentrations determination using Unicam 939 model of atomic absorption spectrophotometer (AAS).

Heavy metals concentration data from the soil and fruits were used in calculating the accumulation factor as the ratio of heavy metal concentration in the plantain fruit to the heavy metal concentration in their corresponding rhizosphere soil for samples from each site (Li *et al.,* 2012):

$$AF = \frac{\text{Heavy metal concentration in the food crops edible parts}}{\text{Heavy metal concentration in the soil}}$$

All data generated in triplicates were subjected to analysis of variance using SPSS for windows version 19. Means were separated using Duncan's multiple range test.

III. RESULTS

The proximate composition of plantain fruits harvested from the different dumpsites in Uyo metropolis under investigation and that of the control is as presented in Table 1. Carbohydrate (91.61%) content was significantly (P = 0.05) higher in control site fruits than in dumpsites fruits. Crude protein on the other hand was significantly (P = 0.05) lowest (3.76%) in control site fruits. Crude lipid was significantly higher (1.10%) in fruits from dumpsite A than other sites.

Table.1: Proximate composition of plantain fruits (%)

Parameters	Site A	Site B	Site C	Control
Moisture content	*51.28b	53.27a	54.00a	51.00b
Dry matter	48.72a	46.73a	46.00a	49.00a
Crude protein	7.88a	7.77a	7.69a	3.76b
Crude fat	1.10a	0.070c	0.88b	0.93a
Crude fibre	0.30a	0.22b	0.26ab	3.04a
Ash content	3.50a	3.30a	2.28b	3.04a
Carbohydrate	87.23b	88.00b	87.89b	91.61a

*Means of three replicates. Mean within each row followed by different letters are significantly different at P = 0.05 according to Duncan's multiple range test.

Dumpsite soil heavy metals analyses as presented in Table 2 showed that the control site soil had significantly (P = 0.05) lower heavy metal contents for Pb, Cr, Ni, Co, and Cd and significantly (P = 0.05) higher Cu and As contents than the dumpsite soils. The highest Cr, Ni, Co and Cd contents were recorded from dumpsite A while the highest Pb content was from dumpsite B.

Table.2: Concentration of heavy metals in Dumpsite Soil samples (mg/kg) compared with WHO/FAO certified standards.

Heavy Metal	Site A	Site B	Site C	Control	§WHO/FAO
Pb	*18.29b	21.18a	20.32a	5.56c	100.00
Cr	10.56a	9.89a	8.54b	5.88c	100.00
Ni	7.05a	6.64b	6.16c	6.06c	50.00
Cu	2.34b	2.21b	1.65c	5.11a	10.00

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Co	1.42a	1.38a	1.29b	0.20c	50.00
Cd	1.55a	1.51a	1.46a	1.20b	3.00
As	<0.01b	<0.01b	<0.02b	<0.05a	20.00

*Means of three replicates. Mean within each row followed by different letters are significantly different at P = 0.05 according to Duncan's multiple range test. §Maximum allowable limits of heavy metal in soils as defined by WHO and FAO. (Chiroma *et al*., 2014).

Concentration of heavy metals in fruits was significantly (P = 0.05) lower in samples from the control site than fruits from dumpsite soils (Table 3).

Table.3: Concentration of heavy metals in Dumpsite Fruits samples (mg/kg) compared with certified standards.

Metal	Site A	Site B	Site C	Control	§WHO/FAO
Pb	*8.67a	8.52a	7.63b	1.13c	0.30
Cr	7.44a	7.33a	6.59b	2.23	-
Ni	3.36a	2.85b	2.66b	1.14c	67.00
Cu	5.26a	3.66b	2.44c	0.02d	73.00
Co	1.36a	1.28a	1.25a	ND	50.00
Cd	0.32a	0.29a	0.22b	ND	0.10
As	< 0.02a	< 0.02a	< 0.02a	ND	-

•Means of three replicate. Mean within each row followed by different letters are significantly different at p=0.05 according to Duncan's Multiple range test. §Maximum allowable limits of heavy metal in plant as defined by WHO and FAO (Chiroma *et al*., 2014).

Accumulation factor calculations (Table 4) showed the highest values for Cu (2.25) in dumpsite A, followed by As (2.00) in dumpsites A and B. Accumulation factor was generally low in the control site fruits for all heavy metals than those from the dumpsites.

Table.4: Accumulation Factor of heavy metals in the experimental sites

Heavy Metal	Site A	Site B	Site C	Control
Pb	*0.47a	0.40bc	0.38c	0.20d
Cr	0.70c	0.74ab	0.77a	0.38d
Ni	0.48a	0.43b	0.43b	0.19c
Cu	2.25a	1.66b	1.48b	0.01c
Co	0.96a	0.93a	0.97a	0.00b
Cd	0.21a	0.19ab	0.15b	0.00c
As	2.00a	2.00a	1.00b	0.00c

*Means of three replicates. Mean within each row followed by different letters are significantly different at P= 0.05 according to Duncan's multiple range test.

IV. DISCUSSION

The proximate composition (% dry matter) of plantain fruits from both the waste dump and control soils differed from what has been reported by some earlier workers for crude protein (5.09-5.18%), fats (0.47-0.62%) and ash (2.03-2.33%) while carbohydrate content was however similar to some of the results (Odenigbo *et al.,* 2013). However, related work by Oko *et al.* (2015), gave lower carbohydrate contents (70.88 - 81.18%) than ours, also fibre and ash contents were higher in our control samples (3.04% and 3.04%) than what were obtained by them (0.19 – 0.16% and 0.55 – 2.53%) respectively. The variation in the nutrient composition of our results as compared to those of other workers could probably have been due to the differences in their growth environment, soil properties, varietal differences and even the prevailing climate. (Koyuncu *et al*., 2014; Zou *et al.*, 2015). Carbohydrate and fibre contents were significantly highest in the control soil fruit samples. Unripe plantain fruits are known to be high in carbohydrate contents (Makanjuola *et al*., 2013). Carbohydrates are energy yielding food nutrients. Thus plantain fruits harvested from uncontaminated soil will supply enough energy to the consumer. Dietary fibre such as contained in unripe plantain fruits has been implicated with such health

benefits as reducing blood cholesterol, slowing the rate of glucose absorption, improving the sensitivity of the body to insulin, relieving constipation and preventing the incidence of colon cancer (Okareh *et al., 2015;* Oko *et al.,* 2015).

With the exception of Cu and As, all the other heavy metals investigated showed significantly higher concentrations in the dumpsite soil samples than in the control. The heavy metals concentrations in the dumpsite soil samples were lower for Cd, Cu, and Pb than the mean obtained by Olufunmilayo *et al*. (2014) which ranged from 2.25 - 2.58; 2.58 - 3.30 and 60.00 - 91.67 mg/kg respectively. The concentrations of Co and Ni were however higher in our results than theirs which ranged between 0.42 - 0.72 mg/kg and 1.91 - 2.91 mg/kg respectively but our cobalt concentration was far lower than the values obtained by Awokunmi *et al.* (2010) which was 105 – 810 mg/kg. Heavy metals concentrations in any soil is known to be related to the biogeochemical cycles resulting from the anthropogenic activities such as agricultural, industrial and domestic wastes disposal (Olufunmilayo *et al*., 2014). The highest concentrations of Cr, Ni, Co and Cd were detected in soil samples from dumpsite A which is an age long dumpsite fed with various kinds of wastes from a popular Uyo urban market. The occurrence of these heavy metals in the soil makes them available for absorption by the plants roots (Okoronkwo *et al.,* 2015).

Heavy metals concentrations in plantain fruits varied markedly between those harvested from dumpsites and those from the control site. All the dumpsite fruits had significantly (P=0.05) higher heavy metals contents than those from the control site. Our results for Pb, Cr, Ni, Cd and As were however lower than what were obtained by Imasuen and Omorogieva (2013) 10.69; 37.71; 9.18; 21.90 and 13.20 mg/kg respectively for plantain fruits harvested from different types of polluted soils. Although with the exception of Pb in fruit samples from dumpsites B and C, the concentrations of all the other heavy metals in fruit samples were below the WHO/FAO maximum allowable limit. It is however worthy of note that their continuous consumption can lead to bioaccumulation resulting in lethal concentrations in the body. Lead is known to be very toxic even at very low concentrations (Okorononkwo *et al.*, 2005). It is a deadly carcinogen and is associated with renal tumour, cardiovascular, kidney, nervous, circulatory, skeletal and reproductive systems damages (Kibria, 2016). Cr is known to be both mutagenic and carcinogenic (Podsiki, 2008), causing asthma and shortness of breath as well as liver and kidneys damage with long term exposure. Ni is also a human carcinogen and constitutes a health hazard at high doses causing cancer of the nose, larynx, lungs and that of the prostrate, respiratory failure, birth defects and heart disorders (Kibria, 2016). Cu is considered as an essential trace element for humans, but at elevated concentrations such as result from bioaccumulation, it can cause cirrhosis of the liver and results in death in extreme cases (Nolan, 2003; Kibria, 2016). Co occurs as heavy metal in the plant or animal bodies without being bio-magnified up in the food chain due to the fact that a vast quantity of it ingested is passed out of the body unabsorbed (Lenntech, 2014). However, when a high concentration of Co is taken health effects such as nausea and vomiting, vision problems, heart and pulmonary problems and thyroid damage usually result (Lenntech, 2014). Cd is a toxic heavy metal even at low concentrations and is also considered a carcinogen causing bronchitis, emphysema and alveolitis (Kabata-Pendias, 2011). Arsenic is a deadly heavy metal which is considered a human carcinogen even at extremely low levels of exposure (ATSDR, 1999) with various other clinico-pathological conditions such as cardiovascular and peripheral vascular disease, developmental anomalies, neurologic and neurobehavioural disorders, diabetes, hearing loss, portal fibrosis, hematologic disorders (anemia, leukopenia and eosinophilia) carcinoma, nausea, vomiting, abdominal pain, muscle cramps and diarrhoea when taken in high concentrations (ATSDR,2000; Abdul *et al.,* 2015).

The accumulation factor (AF) calculations showed values greater than 1 for Cu in fruit samples from all the dumpsites while this was the case for As only for fruit samples from dumpsites A and B. Heavy metals are capable of moving from the soil to the edible parts of the food crop (Li *et al.,* 2012). Our results showed that the accumulation factor varied with the heavy metals thus agreeing with the findings of Adefemi *et al.,* (2012). Cu had the highest accumulation factor except in samples from the control site while Cd had the lowest. Those heavy metals with high AF point to the fact that much consumption of the plantain fruits from those dumpsites with time may result in some health problems.

V. CONCLUSIONS

The concentrations of Pb, Cr, Ni, Co and Cd were higher in all the dumpsite soils than in the control soil. Plantain fruits from dumpsite soils accumulated higher heavy metal concentrations than those from the control soil. In the three dumpsite soils investigated, Pb concentration was highest followed by Cr, while Cu and Ni followed in that order in site A and B. In site C, Ni was however, higher in concentration than Cu. The presence of these heavy metals in the dumpsite soils and their accumulation in the plantain

fruits harvested from there is sounding a cautionary note as to the health risk involved in continual cultivation and consumption of plantain fruits from these dumpsites. Proper and safer methods should be adopted by the Uyo municipal government to evacuate and dispose these wastes away from the immediate human settlement while at the same time creating awareness and discouraging the people from cultivating on waste dumpsite soils.

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Adaptation to Climate Change and Variability by Gender in Agro-pastoral Communities of Tanzania

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***Abstract*—** *Gendered division of responsibilities in agro-pastoral societies of semi-arid parts of Tanzania influence the exposure of women and men differently into various experiences, skills, knowledge, technology and resources in similar ways that they expose them to climate risks and opportunities. This paper examines gender based vulnerability and adaptation strategies to climate change in these communities. The study was undertaken in two villages of Chamwino District in Tanzania. Data collection involved focus group discussions, key informant interviews and household interviews (5%). Rainfall and temperature data for the past 30 years were also analysed. Indicators of climate change and variability were revealed from both climate and social studies. Annual mean rainfall decreased from 700mm in 1980 to 490mm in 2010 while average temperatures were increasing steadily. The findings indicate that recent climatic changes have favoured pest and diseases, which affects crops, livestock and people. Late onset and early end of rain season were also recorded which lengthened the hot season of the year and early drying of water sources. It was further established that, the change in gendered roles affected women and girls more than men and boys because activities related to chores that are women roles were most affected. Responses to climatic stresses also varied by sex because they had been exposed to different skills and experiences. Lack of resources in female headed households increased severity to impacts and hindered their capacity to overcome stresses.*

Keywords — Gender, Climate Change, Climate Variability, Adaptations, Tanzania.

I. INTRODUCTION

Scientific evidence have indicated that climate change and variability is really happening, affecting natural and human systems, especially in dryland and semi-arid areas (IPCC, 2007). In the drylands of the 'tropics and sub-tropics' where most crops are at their maximum climate tolerance, and particularly in non-irrigated farmlands, severe losses in production may result from even slight changes in climatic conditions (Olmos, 2001). However, the intensity of threats differs depending on geographical locations and at households and individual levels. Individuals of the same community or household may be affected differently depending on their roles (Heijmans, 2001; Olmos, 2001). Likewise, their responses may also vary due to their previous experiences, available resources, knowledge and skills (Rossi and Lambrou 2008; Nelson and Stathers, 2009). This has lead areas experiencing the inequalities especially those based on gender and where local norms regulate decisions, to devise adaptation strategies that differs between the households and individuals and making the entire adaptation process complex.

In the developing world, rural women comprise a larger portion (80%) of all smallholder farmers (Ngigi, 2009; FAO, 2010), and the majority of these are responsible for all domestic chores in their households. In Tanzania, agriculture, livestock, water and forestry are important sectors in provision of the majority rural households' livelihoods. These same sectors are highly sensitive to changes in climate (URT, 2003; URT, 2006b). Furthermore, it is estimated that over 80% of all Tanzanians live in the rural areas practicing these activities. Effects on such sectors therefore are felt by the majority Tanzanians.

The projection of the Intergovernmental Panel on Climate Change (IPCC) (2007) is that, Sub Saharan Africa will warm up to 4ºC come the end of this century. This projection points out higher warming in semi-arid areas than the rest. Likewise, semi-arid parts of central Tanzania are expected to get warmer with lower rainfall than the rest parts of the country (URT, 2003; URT, 2006b). Predicted impacts on farming and livestock systems due to this warming include a reduction in crop production of up to 80% - 84% and losses in livestock production (URT, 2003; URT, 2006b; Paavola, 2003). The estimated mean reduction of 0-20% in precipitation is predicted to extend dry seasons and affect pasture for livestock (Paavola, 2003; Yanda and Mubaya, 2011).

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Gender inequalities in resource ownership, in decisions and roles in households are also high in agro-pastoral communities of central Tanzania, and particularly in Dodoma Region. Rural communities in Dodoma have been traditionally agro-pastoralists; depending on the two major resources, that is, land and livestock for their livelihoods. These resources are mainly passed on to sons. Furthermore, most key decisions are made by husbands in those households. During a divorce the widow can be expected to leave the house without anything from the husband. The Tanzanian Law requires children younger than seven years to stay with their mothers before they go to live with their fathers. At this time, the widow who is land less and without livestock faces a difficult time to feed her family; and the situation is more serious in the context of climate change and variability. According to UNDP (2009), inequality in resource distribution amongst members of the society and the various social groups may enhance risks of climate change amongst those who are disadvantaged.

II. MATERIALS

2. 1 The Study Area.

2.1.1 Geographical location and administrative set up

This study was undertaken in two villages namely; Solowu and Mloda from Itiso and Makang'wa wards respectively, in Chamwino District (Figure 1). Chamwino District is one of six Districts of Dodoma Region. Other Districts within the region include Mpwapwa, Kondoa, Kongwa and Dodoma Municipal. Chamwino lies on the central plateau of Tanzania in the western bearing along Dar es Salaam road, latitudes 40˚ to 70˚ south and longitudes 35˚ to 37˚ north. Administratively, the District is divided into 5 divisions, 32 wards, 77 villages and 687 hamlets.

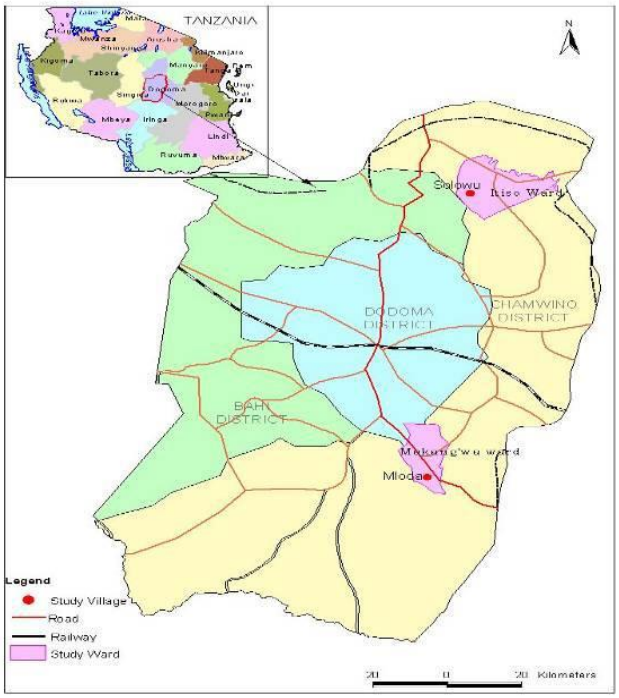

Fig.1: Location Map of the Study Area

Source: GIS Laboratory, Institute of Resource Assessment (IRA), University of Dar es Salaam

2.1.2 Demographic characteristics

Chamwino District has a total area of 8,056 square kilometers and is inhabited by around 289,959 people, of which 151,091 are female and 138,868 male (Chamwino District Profile, 2011). The Population density is 36 people per square kilometre and the average household size is 4.15 (ibid). District population growth rate is estimated at 2.3% per annum, which is relatively lower than the national annual population growth of 2.9. Solowu village has a population of 3,331 people, confined into 486 households whereas Mloda has 4,226 people and 1,128 households. The average household size in the two study villages was 5.3. The District is dominated by the Gogo ethnic group although other groups such as Mang'ati, Zigua and Nyakyusa were found during the survey as migrants to the area. Both customary as well as legal means were very powerful in regulating resource ownership and use and men were more powerful in accessing these than women - similar to a situation observed by Ngware (1997).

2.1.3 Biophysical features

The central zone of Tanzania, where Chamwino District is located is generally dry. The climate differs within the District, but it is generally warm. Rains fall between November and May forming two peaks in December and March. The District falls in two agro-ecological zones based on soils and climatic conditions, which includes a

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very dry flat undulating plain lowland area receiving an annual rainfall of around 400mm per annum. This part of the District is less populated and mainly used for grazing since rainfall is also very much unreliable. In this area crops such as sorghum, simsim, groundnuts and sunflower are grown (Chamwino District Profile, 2011). The other agro ecological zone has flat undulating hills. This area receives slightly higher rainfall - between 550-650mm per annum (Chamwino District Profile, 2011). In this zone crops such as sorghum millet, maize, castor, groundnuts, tomatoes, onion and vines are grown. Livestock keeping is common but the increasing population size limits the number of animals (*ibid*).

The physical climate of the area is influenced by Inter-Tropical Convergence winds, and due to its sentimentality, it has a long dry season and short intense wet season with erratic and unreliable rainfall patterns (Ngware *et al*. 1997, URT, 2006b). Soil fertility is variable, and always with localised salinity and hard pans (URT, 2006b). Vegetation cover of the area is characterized by thorn bushes that are either interspersed by sparse grass or bare ground in most parts of the area. Grass dominates some plains with miombo woodlands and inselbergs on the hilltops (Christianson, 1988, cited in Ngware 1997).

III. METHODS

This study employed several methods and techniques to collect qualitative and quantitative data from both primary and secondary sources. Relevant secondary data were obtained through reviews of published and unpublished literature from various sources including libraries and the internet. Data collected included climate, demographic, biophysical and policies related information. Results of the reviews were used to support arguments of the study findings.

3.1 Participatory Methods

Participatory methods including Focus Group Discussions (FGDs), Key Informant Interviews and Field Observations were used in this study to collect in-depth knowledge of local people's perception and experiences regarding climate change and variability; and also examining their inter-linkages with observed or perceived effects on the natural and human environments including livestock and agriculture. Such methods have the advantage of soliciting more information from local people; since they encourage participation and dialogue between local people and the researcher as well as amongst local people themselves (Lyimo and Kangalawe, 2010). Focus group discussions comprised of 12-15 people. Since it is a gender study, particular attention was paid to consideration of representation of various social groups of male, female, ethnic, marginalised and age groups. Key informants were government workers at the District and ward levels – those with field experience on agriculture, livestock, environment and climate, as well as elderly people who happen to have seen changes in the villages for longer. During focus group discussions and key informant interviews, a checklist was prepared to answer questions related to local perceptions and experiences on climate variability, climate change and their impacts on both humans and biophysical resources as well as adaptation strategies and available assets that are being employed by various groups of individuals to respond to those threats. Physical observations were done along with focus group discussions, key informant and household interview to capture and crosscheck issues identified in the field.

3.2 Sample Size and Household Interviews.

In this study, households were defined as basic units of production and consumption in the villages and were used as units of analysis as suggested by Lyimo and Kangalawe (2010); and hence were used as study units during household surveys. 5% of all households in each study village were randomly selected; and were considered adequate to measure reliably the characteristics of respondents as suggested by UN (2005). This was also considered so because other tools were used to complement household interviews.

3.3 Analysis of Climate Data

Climate data, that is, daily and monthly records of rainfall and temperature for the past 30 years were also studied. Temperature data were obtained from the International Research Institute for Climate Prediction (IRI) specific for the study area while rainfall data were obtained from the Chamwino District library, recorded daily from the local weather station. These daily and monthly weather data were analysed and then extrapolated to draw periodic trends such as annual, decadal and longer.

IV. RESULTS AND DISCUSSION

4.1 Socio Economic Activities and Gender

The main socio economic activities were sustenance farming and livestock keeping. Maize, millet, sorghum, sunflower and simsim were reported to be the main crops that were grown in the areas. Farming is mainly performed using hand hoe; although some families use oxen and a very few are changing to using tractors on occasional basis. Livestock keeping is by grazing where herds are taken on public lands normally far away from houses. More often, migration in search for grass to feed large herds of cattle during dry season is practiced. While both men and women undertook farm work; the larger portion of work is done by women who participate from farm preparation through planting, harvesting and marketing of crops. Men perform most of land preparation and

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marketing as well as cultivation when oxen is used. Women rarely involved in livestock keeping and management as this activity involved long walks, instead they were responsible to take care of children at home and performed almost all household chores. The findings further reveal that there were more female individuals (58.3%) than male (41.7%) in the study area. Females marry earlier at the age range between 16 and 19 while males marry at the age range 21 and 24 years. This has a connection with their exposure to education, whereby male are found to spend more time at school when compared to female who drop-off school to undertake other household activities. Similarly, boys spent more time in livestock keeping which kept them busy after completion of primary school education whilst girls were idle after completing primary education.

4.2 Perceptions and Established Facts on Climate Change and Variability

The findings of climate data analysed for the past 30 years as well as household interviews revealed indicators of climate change and especially climate variability. Temperature has been steadily increasing (Figure 1). Likewise, people perceive that this increase in temperature challenged the semi desert nature whereby it is too cold in nights and too hot during the day. Currently both day and nights were becoming hot. Rainfall trends show a sharp decline in intensity towards end of recording period in 2010 when compared to early 1980s (Figure 1). On the other hand, rainfall variability is higher in which fewer days receive heavy rainfall and there is a prolonged dry period in between. This was found to create unfavourable conditions to crops and even plants and grass. Therefore, the fall in crop yield that was also reported during interviews was most likely caused by this nature of rainfall behaviour. Furthermore, unlike in the 1980s where rainfall season started during the months of October or November and continued until April or May; towards 2010 the findings show that rains start in December and ends in March. This means that the rain season has been shortened. During interviews it was found that the time of the year that rivers run water has as well been reduced. This would most likely be linked to reduced rainfall and the shortened rain season. Moreover, it was found that, at times rains begin earlier in November, however, only few days or even one day has the rain, and is followed by a prolonged dry spell before the rains start again at the end of December or in January. This unpredictability tendency of the rains affected households' plans as to when they should grow their crops. The shortened rain season, forced communities to purchase and plant modern seeds with harvest circles. However, when they planted these seeds during early rains and the rains did not continue, the poor households could not afford to buy additional seeds as their stock ran out. Furthermore, it was revealed that the decline in rainfall was highly felt (100%) because all people participated in farming and this had a close link to low productivity in the farms, and similarly the drying of rivers and pastures for livestock. Interviews also revealed that the increase warmth attracted diseases and pests for animals, livestock and people. Variability in rainfall through the study period (1980 -2010) is well illustrated in Figures 4 and 5, which show that rainfall intensity, onset and end of the rain season has changed hugely between early 1980s and towards 2010. The overall trend of the rains over the years is shown in Figure 2, which depicts the variations in rains over the years, and further illustrates that the variations are more acute starting from 1990s. This similar understanding was captured during key informant interviews with the elderly people. The correlation between perceptions that the communities have, based on their experience on the local environment and the studied climate data indicates the validity of the information, and provides basis to model climate trends and predict the outcomes in future if economic activities and growth rates remains the same. Figure 3 indicates that although overall temperatures have risen, the cold months have become colder and hot months are becoming hotter as well.

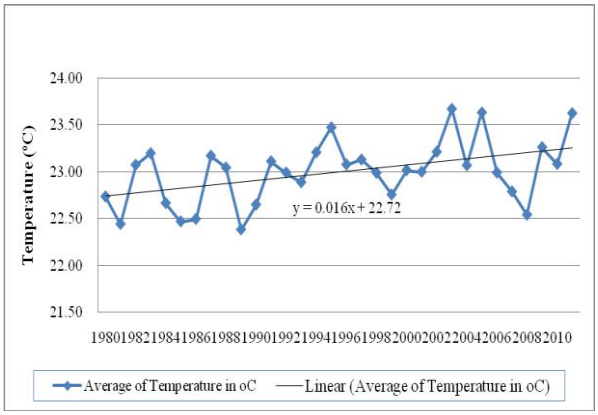

Fig.1: Average Temperature Trends in Chamwino from 1980 to 2010

***Source**: Fan Y., H. van den Dool (2004, 2008)*

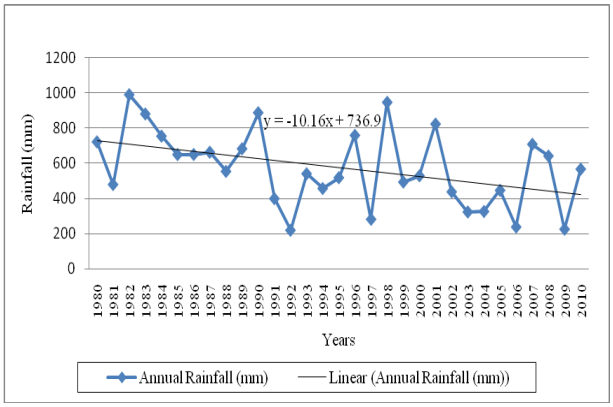

Fig.2: Total Annual Rainfall Trend from 1980 to 2010

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Source: Chamwino District Council, agriculture department

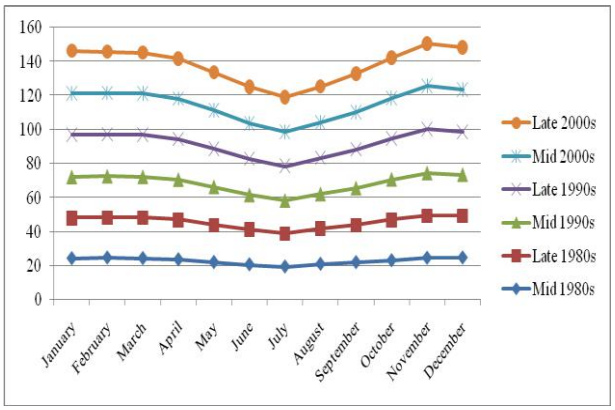

Fig.3: Five year temperature variations in Chamwino since 1980s

Source: Fan Y., H. van den Dool (2004; 2008)

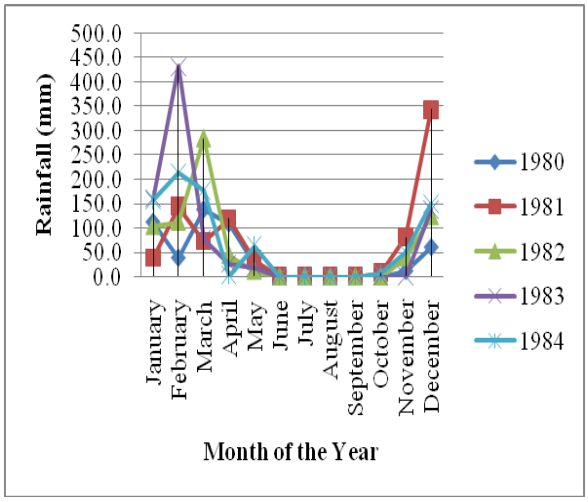

Fig.4: Total monthly rainfall (1980-1984)

Source: Chamwino District Agriculture Office.

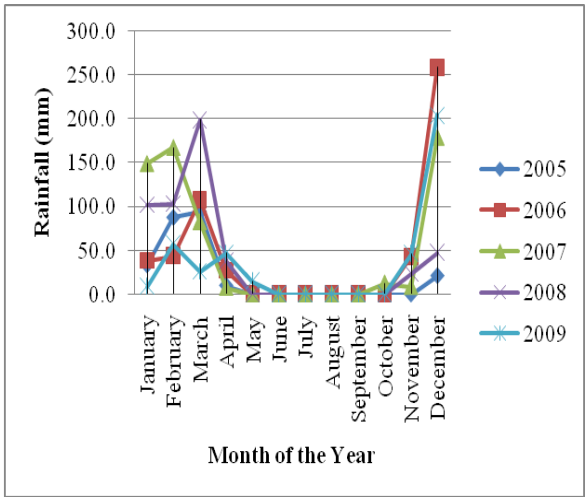

Fig.5: Total monthly rainfall (2005-2009).

Source: Chamwino District Agriculture Office.

This linkage between findings from socio-surveys and meteorological climate data analysis suggests a robust informal knowledge base possessed by the respondents. Therefore, local experience and knowledge deserves to be further exploited to create and improve scientific research on climate variability and climate change. Even with this note, male respondents seemed to be more knowledgeable about climate variability than females, probably because the male respondents had received higher education than females. This also indicated that the male respondents had combined their local experience with some formal education and exposure. It would thus appear that the community's level of understanding could shape the individual's perception on the cause or effects of climate risks. This finding somehow relates to the finding of Fabiyi *et al.* (2007), who concluded that education has the potential of making up some deficiencies in man as it enhances the understanding and communication in agriculture.

A. 4.3 Impacts of Climate Change

This study revealed several impacts due to climate change and variability on agriculture, livestock, water and forest resources as well as human beings. Respondents gave multiple responses when asked what they felt had been changing. In an order they indicated drought (54.6%), decreasing pastures and grazing lands (42.3%), animal and plant disease and pests and as well as human health problems. As a result of these, they reported that their produce in the farms were declining, and it was even worse in the years towards the end of the study period. Livestock were also decreasing due mostly to diseases. Others reported increased conflicts on resources and in households. People fought for water to feed their livestock during dry seasons because the same wells they dug for their livestock were also used for domestic water. Women and girls were most affected when compared to men because they had more roles (See also Table 1). This is because the roles are defined ethnically on gender basis. In this community all household chores are women responsibilities and men specialized more on livestock keeping and petty trade, including the sale of agricultural produce.

Due to early drying of water sources, water was obtained afar (normally 5 kilometres or more). This made it necessary for women to wake up very early to the well and spend 3 hours or more only to fetch one bucket of water because they carry it on their heads. Firewood, which was the source of fuel were now obtained afar as well because more areas have been cleared for faming due to increasing population and the need make big farms to offset crop failures. During dry season, men shifted to other villages in search for pasture or worked as labours in order to feed and find other needs for their families. A similar finding

was also observed by Liwenga (2003). During discussions, it was revealed that, the majority of men who left their villages did not come back earlier as they had promised. Some of them married or cohabited with other wives, where they went, and the whole household responsibilities were left to their wives back home. During rainy season, water is not scarce, however, women did the majority of the farm work especially when hand hoes were used. During this time, women did the majority of cultivation, planting, weeding and harvesting whereas men did more of land clearing. The participation of men in paying jobs than those that aren't, was also revealed. At the same time, men kept money when they sold out their produce, and made most of the decisions without involving their wives. Table 7 presents distributions of roles and responsibilities by gender within households in the study area.

The perceptions on impacts were based on the skills and line of experience that a person had been exposed to earlier on. This is why, when women and men were asked separately what of the selected set of indicators had worst effects, their responses varied. For instance, male respondents were more concerned about loss of their animals that had been occurring in recent years due to eruption of diseases such as East Africa Fever, which were not common in previous years. On the other hand, female respondents were more concerned about the reduced crop yields in which they identified the past three years were the worst years in record in crop production where a farmer could not have any harvest. This also explains how people are sensitive to loss of their properties when threats from external factors occur.

Table.7: Distribution of roles and responsibilities in households (in %)

Productive work	Male	Female	**Domestic work**	Male	Female
Farm preparation	68.9	48.5	Fetching water	21	100
Cultivation	95.6	87.9	Fuelwood collection	14	100
Planting	86.7	97	Cooking	16	94
Harvesting	88.9	97	Household cleanliness	4.7	94
Marketing	91.1	78.8	Baby sitting	33	94
Livestock feeding	37.8	6.1	Caring sick house members	58	79
Casual works	80	75.8	Breastfeeding	0	100
Bee Keeping	8.9	18.2	Grinding	2.3	94
	-	-	Looking for relish	67	94
Totals	558	509.1	Totals	216	849

Source: Own data from household Survey, May 2011

B. 4.4 Adaptation strategies to climate change

Several measures were taken to cope with effects of climate change and variability. These included the use of early maturing and drought-tolerant crop varieties; migrating with animals in search for new grazing land with good pastures; three-year interval deep ploughing with tractors, and ox-ploughs. All respondents (100%) indicated that farming activities were being carried out by hand hoes, and 65.9% male and 60.6% female farmers were changing from using hand hoe to ox-plough while 34.1% and 21.2% were changing to tractors respectively. However, ox-ploughs and tractors were not used regularly. For instance 60% of women who had used a tractor or an ox-plough could not afford hiring it each year. Therefore, the majority of households tended to apply tillage by tractor or oxen after a certain time interval. This was used as an adaptation strategy to improve land productivity. Normally heavy tillage involving deep soil turning with a tractor or ox plough was applied once every three years. This would break the hard pan, increase water infiltration and conserved soil moisture. It also aided nutrient mixing, increased water retention from run-offs, soil aeration and microbial activity which aided quick plant growth and increased yields. Farmers who had used these strategies, reported to have seen improvement in their harvest.

Additionally, during key informant interviews, famers reported that they had to opt for an ox-plough or a tractor to reduce the workload. The new technology also helped them to cope with short and unpredictable rains. Even though, due to lack of knowledge, all respondents were unaware that in so doing, they were tackling effects of short and unpredictable rains and thus they were responding to climate variability; because they failed to explain why they had to use ox ploughs at least after every three years. According to Smit and Pilifosova (2001) this types of adaptation is described as reactive and autonomous, in which people respond to climate risks without a clear knowledge of the reasons why. The present study revealed that low productivity in the area was also caused by hardpan formation resulting from livestock movements, which needed heavy tilling and that would not be regulated by hand hoes. As such, machinery was needed to break the hard soils and increase soil turning, which enhanced the capacity of the soil by improving water retention and storage as well as bringing up nutrients

to the subsoil essential for the early uptake of young seedlings.

Furthermore, farmers learnt that after the deep ploughing the farm does not take more than three years before it losses fertility and yields. On the other hand, this heavy tillage after three years was a strategy to reduce costs because the tractors and ox-ploughs were expensive to hire each year. On the other hand improved crop varieties, which are drought tolerant and resistant to diseases and pests, were being used.

Finally, it was further revealed that, the costs of adaptation were high. Nevertheless, some families (55.6%) diversified by practicing both pastoralism and farming together. These were more adapted to climate shocks than those that did only farming or livestock keeping alone. This is because, when crops failed, they could sell their animals and could use this money to buy food from nearby villages. They also used manure from their livestock to improve soil fertility or were able to till their land more frequently with oxen they owned.

However, only male-headed households were found to keep livestock. Even when they were asked during focus group discussions, there were only female-headed households that were reported to own livestock in the study villages. Likewise, families that had larger portions of land were found to be able to adjust during climate shocks. For instance, some used their farms that are closer to river courses, which were moist. But the majority of these were male-headed households. Most of female-headed households (63.6%) had smaller portions of land (less than 10 hectares), which means that they had to repeatedly grow crops on the same land, which finally lost its fertility. The reason was that, ethnically cattle and land are inherited to sons and not daughters. As a result of this, male-headed families had more options to adapt than female-headed families.

Additionally, the positive outcomes of climate variability that were found in the study area, such as business opportunities in commercial crops were taken up by wealthier households that had resources in place, such as a large amount of land or ox-ploughs, or those who could hire tractors. During divorce it was found that, wives did not inherit or receive a share of land or livestock from their ex-husbands. On the other hand the Tanzanian law requires that children younger than 7 years must stay with their mother before they live with their fathers. Therefore lack of resources increased threats to disadvantaged groups including women and the disabled. This supports argument by Olmos, (2001) that resource-rich households can be more resilient when risks or stresses occur.

4.5 Socio Economic Implications of Climate Change

There were direct and indirect social and economic consequences of impacts and adaptation strategies to climate change. The major ones included food insecurity, diseases, conflicts and increased workload in households. These issues hindered households' progress in different ways. Increased workload forced some children especially girls to drop off from studies in order to help their mothers with house chores. Likewise, when a married woman returns home late from fetching water or firewood that is caused by increased scarcity they would quarrel with their husbands. Young girls and boys were reported to migrate to small towns and Dodoma Municipal where they worked as waitresses in bars, or housemaids for girls and food and cloth vendors for boys. Because they did not have good education, they did not earn enough to feed themselves and their families back home. As a result it was reported that the majority of them ended up being prostitutes and were infected by sexually transmitted diseases such as HIV/AIDS. Furthermore, this copping strategy which involves migrating to other areas was highly practiced by men. Some men used this as an excuse to escape their family responsibilities, which were now left to their wives. Similar findings were obtained by Liwenga (2003) based on analysis of rainfall variability and mobility patterns in a semi-arid environment. This women to less active in social activities and had less time to rest when compared to men.

V. CONCLUSION

The study revealed clear evidences of climate change in the study area, as substantiated by people's perception and backed up by scientific climate data. Both of these suggest that, signs of climate variability started to be noticed in the early 1990s, particularly in 1992. Most common indicators in this area are a sharp decline in rainfall intensity, change in rainfall behaviour, its predictability, onset and end of the rain season. Also increase in warming whereby both day and nights were becoming hotter. These impacts have caused threats to livestock and farm produce that are a back borne to the economy of people in the study area. Women and men perceive and respond differently to the outcomes of climate variability, which follows their lines of experiences, and the roles they have been undertaking in their societies or households and which in return exposes them to different adaptation skills and experiences. This means that to effectively cope or adapt to threats of climate change adaptation strategies must take advantage of those skills in their totality and thus must involve both women and men at all stages. Responsible government institutions are advised to look into these varied dimensions. Furthermore, availability of resources enhances the ability to adapt, and thus women who had fewer resources were

most negatively affected. Moreover, the immense local knowledge that communities have, if well utilized, can aid researchers to model climate trends and adaptation strategies for a long time. Furthermore, analysis of gendered roles must be undertaken to be able to understand the roles that are most adversely affected and appraise these to identify most plausible solutions.

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Management of True Vaginal Prolapse in Bitch

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Abstract— *True vaginal prolapse is a rare condition in bitch. It occurs majorly following parturition or during estrogen risei.e during estrous phase of the cycle. A two year old Grey Hound female was presented with true vaginal prolapse. The prolapse mass was largeandhyperemic. By reducing the size and with bilateral pressure we reposed the mass in. Modified Buhner sutures were applied. Hormonal therapy using HCG were given for four days. The bitch recovered eventually.*

Keywords— Vaginal Prolapse, Proestrous, Hyperemia.

I. INTRODUCTION

True vaginal prolapse is very rare condition in bitch when compared to other vaginal pathologies like vaginal tumors or urethral tumors which protrude into vagina and obstruct the canal (Manothaiudom and Johnston, 1991). Vaginal prolapse usually occur in young bitch preferably less than 2 to 3 years age. It occurs majorly near parturition, as the serum progesterone concentration declines and the serum estrogen concentration increases (Konig*et al.*, 2004; Rani *et al.*, 2004). This condition is less common in diestrus, anestrus and normal pregnancy (Johnston *et al.*, 2001; Schaefers-Okkens, 2001). Under the influence of high serum estrogen levels edematous swelling of the vaginal mucosa may develop (Johnston *et al.*, 2001). This is accompanied by increased vaginal hyperemia and edema occurring during proestrus and estrus due to the estrogen stimulus (Schaefers-Okkens, 2001). An amplification of this high serum estrogenic response can lead to disproportionate mucosal folding of the vaginal floor just cranial to the opening of urethra, which ultimately results in protrusion of vaginal mucosa from the vulva. The protruded mucosa can eventually become necrosed, inflamed and can easily be ruptured (Suresh Kumar *et al.*, 2011).

II. CASE HISTORY AND OBSERVATIONS

A two year old female Grey Hound was presented in clinics with the history of mating. One day after mating due to vigorous straining the prolapse of vaginal fold and vagina has occurred (Fig 1). Physical parameters show a slight variation as compared to normal like sub normal rectal temperature (98.8 ^{o}F), increased heart rate (112 bpm) and congested mucus membrane. After carefully examining the mass it indicates the prolapse of vagina and its folds without the involvement of urinary bladder. The protruded mass has become edematous and hyperemic. The bitch has not urinated since last evening and straining was observed during defecation.

III. TREATMENT

Catheterization was done to empty the urinary bladder and we collected about 120 ml of urine. Prolapsed mass was washed extensively with mild Potassium Permanganate solution (0.1% $KMnO_4$ Solution). Cold water treatment was done to reduce the mass size. We use drapes dipped in ice and cold water. Lignocaine jelly and ointment Soframycin was applied to lubricate and desensitized the mass. Drapes were squeezed to reduce the edema and hence size of protruded mass. Bilateral pressure was applied with the fingers from the ventral floor and lateral sides to repose the mass into the body. After continuous squeezing and applying bilateral pressure we are able to reduce the size and repose the mass. Modified Buhner suture were applied on the external labia keeping an opening of one finger diameter for the urination. Bitch was kept on Inj. Intacef 500 mg o.d I/M, Inj. Neurobion 3ml o.d I/M and Syrup Immunol 10 ml P.O for five days. Apart from this hormonal therapy using Human chorionic gonadotropin (HCG) was administered intramuscularly daily for 4 days at a dose of 500 I.U to induce premature ovulation. Sutures were removed after two weeks and the bitch recovered eventually.

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IV. DISCUSSION

Vaginal prolapse mainly occur during proestrus or early estrous stages of the cycle (Johnston, 1989) andduring or shortly after parturition(Schaefers-Okkens, 2001). Increased abdominal pressure and excessive pelvic ligaments relaxation predispose the animal for pre partum prolapse (Markandeya*etal.*, 2004). Constipation, forced separation during mating and size incompatibility between breeding animals can also lead totrue vaginal prolapse (Purswell, 2005). Reports suggests that this condition may have some hereditary predisposition and is been seen in pure bred dogs (Johnston, 1989). Therefore it is advised to ovariectomizedthe affected bitches and they should not be used for breeding purpose(Troger, 1970). Vaginal prolapse usually occur during high serumestrogen concentration and is also connected with weakness of the perivulvar tissue of the bitch. Regression of the protruded mass begins in late estrus to early diestrus, as serum estrogenreturns to the normal basal level (Alan et al 2007;Feldman and Nelson, 2004; Johnston *et al.*, 2001;Sarrafzadeh-Rezaei et al 2008). In the present case, reoccurrence of prolapsed mass do not occur as the after the end of estrous phase.

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Figure

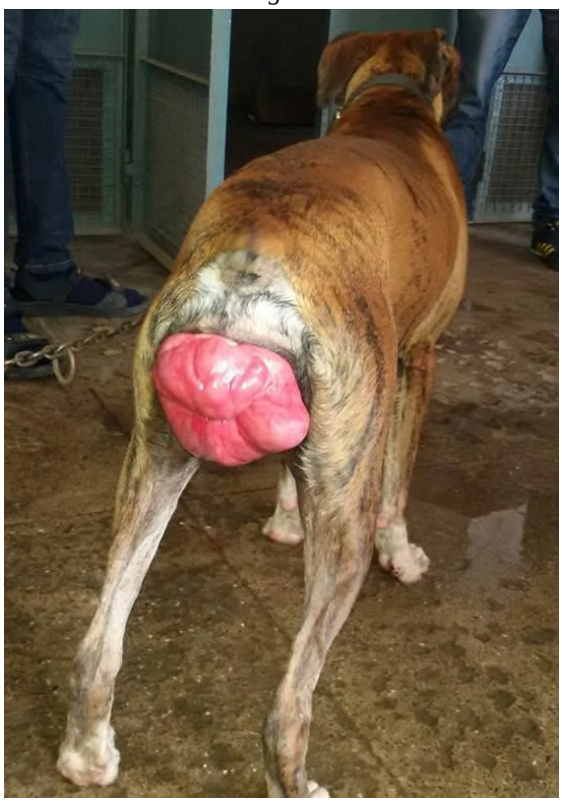

Fig.1: True vaginal prolapse

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Squalene Extraction: Biological Sources and Extraction Methods.

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Abstract*—Squalene is a terpenoid with great importance in cosmetic, food and pharmaceutical industry; it was originally isolated from shark liver oil but is easily found in animals, vegetables and microorganisms. Nowadaysis shark fishing is prohibited in some countries, that is the main reason to use renewable sources forsqualene extraction to protect marine life, since last decade, squalene is extracted from different sources and methods to achieve best yields at lower possible cost. Traditional extraction methods usually involve organic solvents as hexane which left residues on the extracted matrix, that can limit material use for human consumptionafter extraction. Separation and purification stages after extraction can elevate operations cost, one of the most interesting technology to obtain squalene from biological matrix is supercritical fluid extraction with CO_2as solvent because of economic, safe and easy removal characteristics.*

Keywords*—*Extraction, Renewable sources, Squalene, scale-up.

I. INTRODUCTION

Squalene is a very valuable compound common to found in vegetables and animal cells, because of its an intermediate on phytoesterolsand cholesterol biochemist-pathways and highly appreciated by its biological importance [1]. Squalene market is mainly divided un three industry sectors, cosmetics (69.2%), food (22.8%) and pharmaceutical (8%) (Fig. 1A) during 2014, squalene demand was about 267 000 ton that represents 102.4 billion dollars. Europe is the main squalene consumer followed by Asia Pacific and North America (Fig. 1B)[2]. Several investigations have been done to search new sources of squalene by different extraction methods to achieve greatest yield at lower possible cost. The aim of this work is to gather information about common and uncommon available animal, vegetal and microbial sources to extract squalene and methods or techniques to extract it as well and scaling-up experiments.

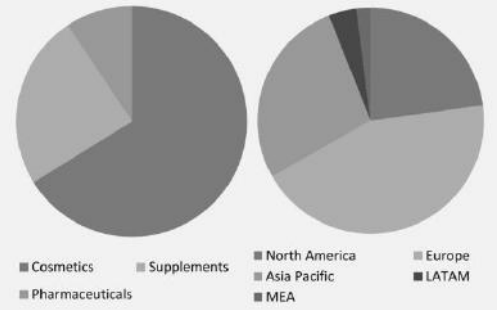

Fig.1: Market by industrial sector (A) and geographical area (B)[2].

II. SQUALENE

In 1916 by MatsumaruTsujimoto[3], identified a highly unsaturated hydrocarbon was identified from liver oils of the squaloid sharks, he proposes the name 'Squalene'. Squalene, is anhydrocarbonated chain ($C_{30}H_{50}$), a triterpene containing six unsaturated bonds with antioxidant nature[4]. Squalene has applications in various end-user industries such as cosmetic, food supplements, pharmaceuticals, and in other applications like high grade lubrication and fiber coating additives, however,the major data of commercial is referred to Shark Liver Oil (SLO). In USA SLO was used for vitamin A production but now is highly recommended in alternative medicine and ointment[5].In Europe, the cosmetic industry demanded SLO, as mentioned before, since product as lotions, eyeliner, eyeshadows, eye makeup remover and perfumes contains 0.1-10%squalene and foundation, lipsticks and other faces preparations contains up to 50% squalene[6],pharmaceutical, textile and leather industry also demands squalene. In Africa SLO is mainly use on fishing boats maintenance[7].

2.1 Squalene importance

Squalene is a molecule with a long carbon chain it tends to have hydrophobic properties that is of particular interest in industry because it can be used to transport liposoluble compounds in an effective and economic ways.[8] Squalene participates in the formation of steroid hormones, bile acids, steroids, and sterols synthesized though mevalonic acid pathway [9].

Human epidermal sebum is composed by triacylglycerides, free fatty acids (57%), wax esters (26%)

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and squalene (12%), the use of compounds present in human sebum as squalene in cosmetics reduces the possibility of allergies [10] and is highly appreciated in cosmetic industry due its emollient and antioxidant properties [1]. Squalene prevents H_2O_2 induced oxidative injury and protect against oxidative DNA damage [11]. Alcohol produces lipid peroxidation although, squalene reduces fetus retina during pregnancy [12]Squalene reduces serum cholesterol due this triterpenoid may act as a substrate for HMG-CoA reductase (3-hydroxy-3-methylglutaryl Co-A) [13]. Squalene has been studied along the years and has been reported with biological activity as antioxidant [11,14,15]chemopreventive[16]. The use of squalene in combination with antitumor drugs has been shown to decrease cancer cells growth [17,18].

2.2 *Squalene biosynthesis*

Squalene is found in both mammals and vegetable tissues because is an intermediary in cholesterol and sterols pathway, very important to any organism. Squalene biosynthesis initiates with enzyme thiolase that joins 2 units of Acetyl-CoA to form Acetoacetyl-CoA and by addition of another Acetyl-CoA by HMG-synthase, β-Hydroxy-β-Methylglutaryl-CoA (HMG-CoA) is formed, and by HMG-CoA reductase take place Mevalonate; then Mevalonate-5-phosphotranferse and phosphomevalonate kinase, 2 phosphates from Adenosine Triphosphate (ATP) are added and changes into Dimethylallyl pyrophosphate, Next phenyl-transferase made 2 head-to-tail unions and 3 isoprene units named as Farnesyl pyrophosphate that polymerizes by squalene synthase form squalene realizing inorganic Pyrophosphate (PPi) [19–21]. Those reactions can be observed in Fig. 2

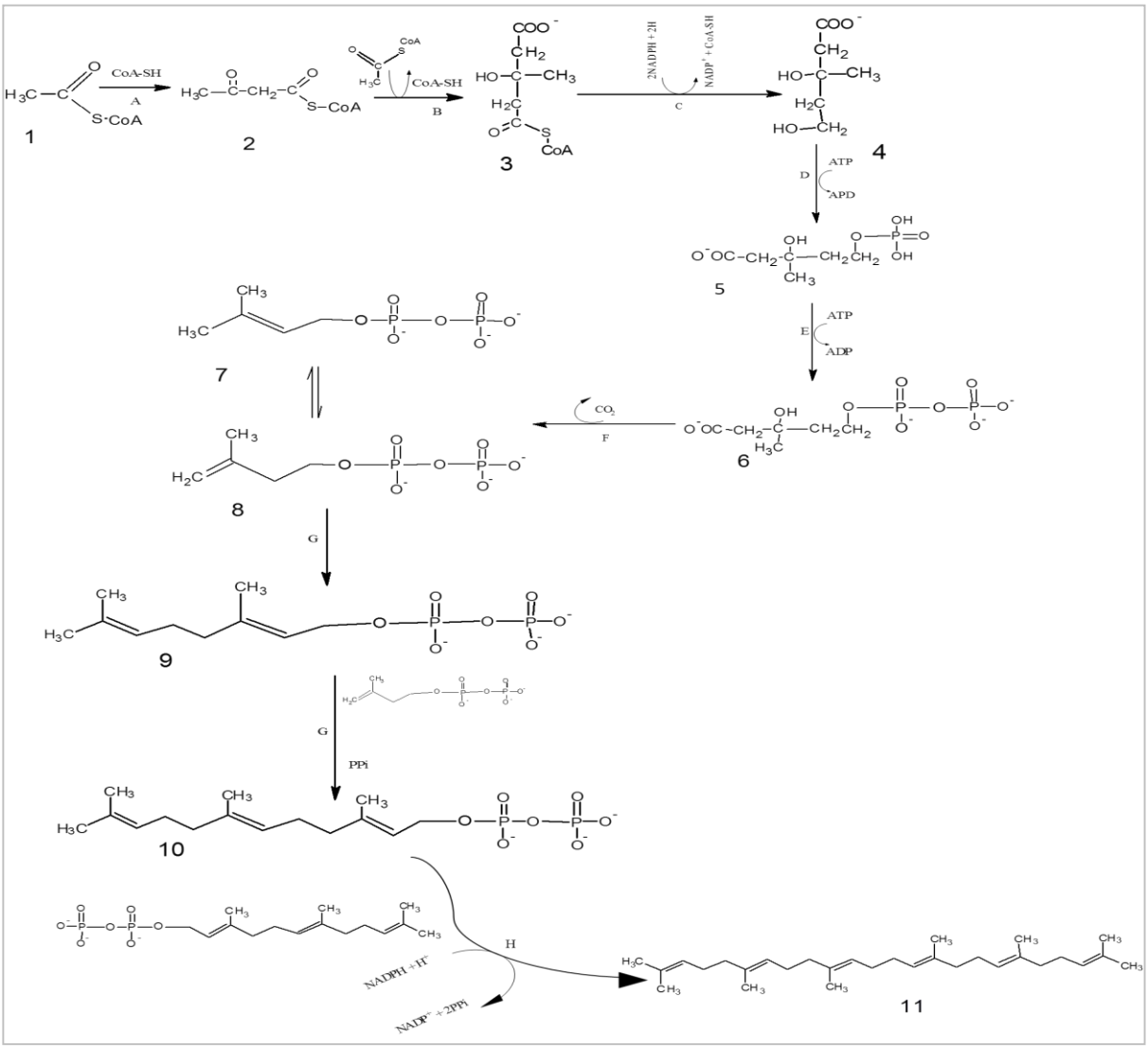

Fig. 2: Squalene synthesis pathway, adapted from reference [22]

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Intermediates and enzymes names, involved in squalene biosynthesis are listed in Table 1.

Table 1: intermediates and enzymes name involved in squalene biosynthesis

Intermediate		Enzymes	
1	Acetyl-CoA	A	Thiolase
2	Acetoacetyl-CoA	B	HMG-CoA synthase
3	β-hydroxi-β-Methylglutaryl-CoA	C	HMG-CoA reductase
4	Mevalonate	D	Mevalonate 5-phoosphotransferase
5	5-Phosphomevalonate	E	phosphomevalonate kinase
6	5-Pyrophosphomevalonate	F	Pyrophosphate mevalonate descarboxylase
7	Isopentenyl pyrophosphate	G	Prenyl transferase
8	Dimethyally pyrophosphate	H	Squalene sintase
9	Geranyl pyrophosphate		
10	Farnesyl pyrophosphate		
11	Squalene		

III. SQUALENE SOURCES

3.1 Shark Liver Oil

The richest source of squalene is abyssal shark livers even though shallow sharks' livers had lower squalene content than cod livers. New Zealander sharks livers contains about 50% by weight squalene[23]. Past decades studies were focused about shark livers and its squalene content. Some of these species are listed in Table 2.

Table 2. Squalene content in different shark liver oil

Shark specie	Squalene liver content (%)	Reference
Centroscymnuscrepidater	35.7-59.4	24
Centroscymnusowstoni	37.1-53.1	
Centroscymnuscoelolepis	31.1-47.1	
Deaniacalcea	43.4-66.1	
Etmopterusbaxteri	14.3-51.5	
Etmopterus sp. nov.	20.8	
Dalatiaslicha	43.4	
Centrophorussquamosus	<0.01	
Centroscymnusplunketi	0.9*	25
Etmopterusgranulosus	50.3-60.5*	
Deaniacalcea	69.6*	
Centroscymnuscrepidater	73*	
New Zelander shark	50-55*	26
Centrophorussquamosus	65.5	27
Cuban sharks	0.03	28

*Expressed as Hydrocarbon (predominantly squalene)

Cuban sharks, squalene determination was performed from a liver mixture of three species *Ginglymostomacirratum*, *Carcharhinuslongimanus*andCarcharhinus*falciformis*

Nowadays trade volumes of fishing sharks are close to exceed sustainable levels[29]. Onwards it become necessary to extract squalene fromrenewable sources[30].

3.2 Vegetable Sources

Squalene is present in all vegetable oils but in small amounts[31]. Olive is a well know squalene source and its content depends of it is associated with fruit maturation as autumn begins reach the higher concentration of squalene and the end of season there are no significant changes[32]. Nowadays olive oil become one of most vegetable squalene source commercially exploited, but its content is not enough to satisfy the demand[33]. Deodorized olive oil contains about 28% squalene[34]. Olive pomace which has been considered like a by-product in the olive oil production has residual (0.0023%) amount of squalene [35]. In other hand olive leaves that were found containing 0.0038-0.0152% squalene in hexane extracts[36]. Other products as palm oil contains only 1.8-2.3 % of squalene however, it is produced in huge mounts and so it can be use as squalene source [37]

Recently some other crops have been tested as possible new source of squalene. Cucurbit seeds squalene content reported is 10.97-40.27%, differences are due to variety of cucurbit, although is suggested that cucurbit seeds can have hypocholesterolemic effect on human diet [38].

Tobacco plant that, contains approximately 2% but; like it continues growing it accumulates up to 20 % in 8 years [39].

Residues from wineryindustry (lees)may be also valorized trough squalene recovery, yield achieved was 0.06± 0.008%, although seasonal production of raw material, labor requirement may limits its potential as a squalene source[40].

In Asia, ginseng is important because not need to grow in warm weather, and seeds oil content between 514-569 mg/100g squalene and represents about 60% of unsaponificable matter. [41] Nuts are an excellent source of vegetable oil; but some of them such as brazil, pecan, pine, pistachio and cashew nuts have a great squalene content due this nuts should be added to dairy diet. [42]

Essential oil obtained by hydrodistillation of the *Strychnosspinosa* leaves contains about 0.5% of squalene in oil fraction. [43] Using deodorized soy oil can be extracted 100% squalene content and up to 93% purity by solvent modified extraction[44]Rice bran is a co-product of milled rice and its contains approximately 20% and about 8.5% of squalene[45]. Bee pollen of lotus (*Nelumbonucifera*Gaertn) content 0.0084% of squalene extracted by supercritical fluid extraction[46]. Some

authors[30] have explored unconventional squalene sources as pumpkin, amaranth seeds, borageand walnut reported0.52, 5.22, 0.022, 2.83,% squalene in oil respectively.

In contrast there are crops that have been underestimated with industrial purposebut in some communities is common to cultivate as cultural heritage and it consumption is local, as amaranth, a pre-Columbian crop that contains relatively high amounts of squalene[47]. *Amaranthuscruentus*, oil extracts reports squalene content 6.95, 5.0 and 8%[30,48,49], respectively.

Five varieties of *A. cruentus* were cultivated at different altitudes and reported different squalene content ranged from 2.26 to 5.94% of the oil and statistical analysis showed significant difference for localities but not for varieties of plant so it is suggested that environmental conditions, such as temperature and water availability, may lead to a greater accumulation of squalene in the grain. [50]

Table 3: Squalene vegetable sources

Oil source	Squalenecontented in oil (%)	Reference
Olive oil deodorized	28	34
Cucurbit seeds	10.97-40.27	38
Olive pomace	0.0023	35
Olive leaves	0.0038-0.0152	36
Tobacco plant	2.00-20.00	39
Wine lees	0.06 ± 0.008	40
Gingseng seed	0.51-0.56	41
Brazilian nut	137.78	
Pecan nut	15.7	
Pistachio	9.14	42
Cashews	8.94	
Pine seed	3.95	
Strychnos spinose	0.5	43
Deodorized Soy oil	1.83	44
Rice bran	11.75	45
*Nelumbonucifera*Gaertn	0.0084	46
Palm oil	1.8-2.3	37
Olive oil	0.5-0.65	33
Camellia olifeira	7.62	52
Pumpkin seeds	0.52	
Amaranth seeds	5.22	30
Borage	0.022	
Walnut	2.83	
Amranthuscruentus	5-8	30,48,49
Amaranthushybridus	5.27-7.21*	51

*Expressed as unsaponifiable matter

Amaranthushybridus is reported to have between 5.27± 0.47–7.21±0.57% of unsaponifiable matter and can be assumed that it should content squalene[51]. *Table 3* summarizes squalene content in vegetables sources previously mentioned.Some of these renewable sources are not widely harvested or used industrially even when its squalene content is important, and others are wildly produced and made them better alternatives than shark livers.

3.3 Microorganism sources

Microorganism are an interesting squalene source since not need to be harvested in huge portions of land. Microalgae (*Schizochytriummangrovei*) represents a viable alternative source of squalene reach 33 mg/g of cell dry weight, even when biomass is a residue from biodiesel production[53].

A novel yeast strain classified in *Pseudozyma* genus,isolated from seawater is also an interesting squalene source produces 340.52 mg squalene/L with 40 g/Lof glucose and sodium nitrogen as nitrogen source[54].

The strain *Schizochytrium* sp. CCTCC M209059 reports similar squalene content as in fish oil. Due its fast growing and productivity is an alternative source to obtain squalene. High aeration is recommended to increase squalene synthesis, same authors determined squalene keeps oil stable[55].

Wild-type *Saccharomyces cerevisiae* can accumulate between 0.62 mg/L of squalene during the stationary growth phase and 3.4 mg/L of squalene until the exponential growth but an engineered strain (named FOH-2) can accumulate more than wild-type strain, since squalene biosynthesis mechanism is overexpressed[56].

3.4 Squalene Localization

Squalene(and other polyisoprenes)has the function of inhibit proton leakage through cell membrane, but its localization in cell membrane, was no clear until neutron diffraction experiments were performed[57]. Cell membrane is composed by hydrophobic/hydrophilic lipid bilayer, where squalane, a structural analogue of squalene with same number of carbon atoms ($C_{30}H_{62}$) as squalene,was found to be located between membrane monolayers (*Fig. 3*). Due squalane is a saturated compound may haveless stability between the bilayer than unsaturated molecule as squalene[57].According to this,during squalene extraction, saturated and unsaturated fatty acids could also be extracted.

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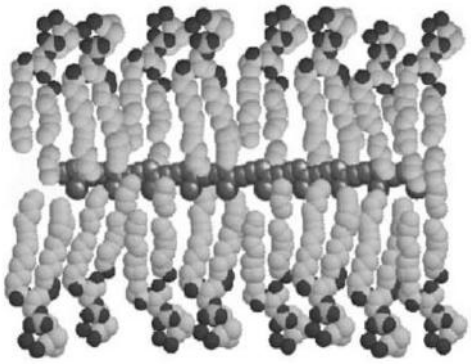

Fig. 3: Schematic representation of squalane in the middle the bilayer of the cell membrane[57]

Evidence of squalene enzymes obtained by immunofluorescence microscopy, suggested that squalene is synthesized in the smooth endoplasmic reticulum subsequently accumulated in small vesicles, some this material is incorporated to plasma membrane[58].Some squalene vegetables sources as amaranth seeds (*Amaranthushypochondriacus*), lipids fraction have been identified in embryonic cells (*Fig 4*), surrounding protein bodies (Pb) and cell nucleus (N). A considerable lipid fraction identified with selective colorants as Sudan Black B[59]. is possible to content squalene lipid bodies

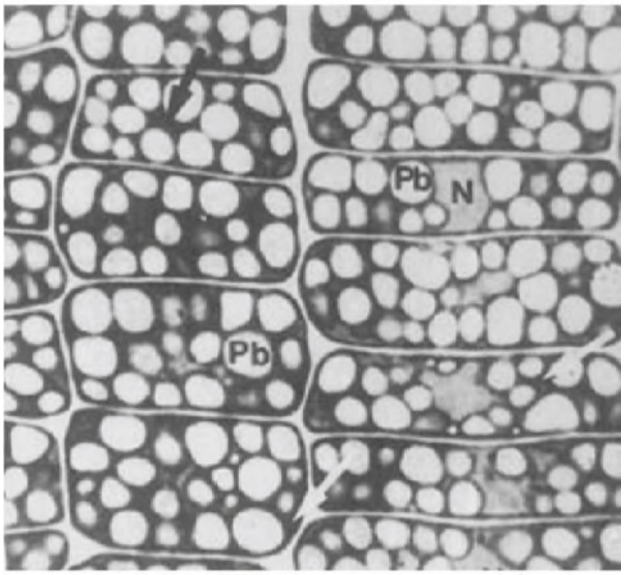

Fig. 4 Light micrograph of amaranth cells peripheric embryo of cytoplasm surrounding the protein bodies is full of lipids (arrows) which stains dark with Sudan Black B[59]

Identifying lipids reserves in vegetables matrix, which probably contains squalene are important to select suitable methods to extract squalene.

IV. EXTRACTION METHODS

Many techniques can be used to recover lipids from biological matrix, and obtain specific compounds[60].Soxhlet extraction (organic solvent extraction) is the most common method used as standard and extract is considered to be 100 % of extractable matter[61].Hexane is typically the solvent used for large scale extractions due to its relatively low cost and high extraction efficiency[62].

Lipid extraction usually involves organic solvents, at industrial scale is commonly used cold press to avoid thermolabile compounds degradation, since this methods are at low pressures, yield might be low, so development of new techniques at higher pressures may aid to increase yield and process time[63]. Ultrasonic extraction combined with organic extraction can achieve higher yield[64].

Cold press with new mechanisms that replaces hammer crusher achieved 90.1% extraction and oil reported till 65g/kg of squalene[33]. Cold press, organic solvent and Supercritical fluids extraction, were tested in order to compare its yields and the conclusion was supercritical fluids extraction reached the highest yield and purity[31].

Other separation method, is silver ion complexation based on the complexation reaction between Ag^+ and unsaturated carbon double bonds, it was tested on Camellia oil obtained from seeds of *C. oleifera*, optimal condition was 70% methanol (v/v),0.6 mol/L AgNO3, for12 h, at 0 °C. Purity of squalene extract reach 37.8%. advantages of this method are low cost, recycle of reagents and continuous operation[65],an disadvantage of this method, is saponification and esterified before extraction and chemical reagents removed from extract after extraction.

Supercritical fluid extraction (SCFE) can be used to extract polar compounds. Supercritical fluids have diffusivity as gas so can penetrate solid materials, high density and solvation power as liquids, these fluids are compressible and little pressure changes its properties. SCFE have been studied due its advantages against conventional extraction and extract have better quality, biostability and easy to remove from extracted matrix[66]. CO_2 is used to extract oil due its convenience characteristics as non-toxic, non-flammable, easy to remove and economic solvent and also reduces thermolabiledegradation in extracted compounds[48]. Squalene SCFE have been performed by several researchers even when is considered as an expensive technology and achieved extract with high purity. Amaranth seeds have been mainly tested by squalene SCE, some conditions are the next: 35MPa and yield was 0.305% and by adding a co-solvent is possible obtain more squalene[67]. In other work, CO_2 were used at 50°C and 300 bar reach 7.95% in oil[61] other optimal conditions reported to extract squalene were 30MPa and 40°C by 90-120 min in order to allow highest and faster oil and squalene extraction from *Amaranthuscruentus*. [48] At

higher temperature (100°C) best yield is reported to be at 55 MPa and 1.5 h extraction time from *Amaranthuspaniculatus*.

V. SCALED-UP EXPERIMENTS

At bench scale,embryonic tissue (as bran from amaranth) was separated from hole seed but amaranth bran was fine thin, therefore pellets were obtained by extrusion to be extracted. Large amounts of pellets (15 Kg) was immersed in hexane for 10 min, solvent was removed at vacuum at 65°C and then filtered[68] oil recovery reach up to 97.7%.

Scale up studies allows to establish methodology translate SCFE process from laboratory-scale to industrial scale, this behavior is not always approached or predicted, this is the mainly reason to observe differences at studies to avoid serious under or over estimates[69].Solubility of volatile solutes increases with temperature due an effect in their vapour pressure, this effect is pronounced multicomponent systems than binary systems. Pressure, temperature and solvent density had an effect on the extraction yield, due to the "enhanced solubility effect".SCFE Laboratory-scale units have a bed length/diameter of vessel ratio relatively high with those greater capacity units, these may affect overall lipid yield, reduced superficial velocity by increasing retention time consequently solvent is saturated [61]. Maintaining optimum condition extraction, solvent flow and biomass ratio not affect significantly the process efficiency even at 8 fold scaled-up[70].

Scaling-up SCFE process depends on extraction efficiency, a model capable to predict the extraction process and time operation which also depends of extracted matrix, batch size, retention time, time for load and unload extraction matrix and cycle of pressuring and depressing extractor to calculate extraction time cycle.[71]

VI. CONCLUSION

Squalene is a natural antioxidant very valuable in cosmetic, food and pharmaceutical industries, squaleneis also an important intermediate in animal and vegetables cells pathways, accordant to this there are several alternative sources for squalene extraction, many investigations have focused on obtaining best yield as possible, some of the most profitable squalene sources, would be by-products from industrial processes, since squalene is mostly used in human products for human consumption, is important to consider safe extraction methods as supercritical fluid extraction. Scaling-up experiments are important to estimate extraction yield and extract cost.The best source of squalene extraction will depend on the bioproduct and the available technology.

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Photosynthetic Pigments Content of Trapa Natans Specie in Skadar Lake

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***Abstract*— *Skadar lake is the largest lake in th Balkan Peninsula. The total area is 5500 m². Terretorialy the lake belong to Montenegro and to Albania. Both the sides of lake is a big development of acquatic vegetation, including a large variety of habitats and biological communities. The most important associations are Potameto-Najadetum, Trapetum natantis, Myriophyllo-Nupharetum, Nymphoideum peltata. These associations include lake shore macrophytes Najas, Vallisneria, Potamogeton, floating macrophytes Nuphar luteum, Trapa natans, Numphaea alba. Emersed water macrophytes are developed on both sides of lake predominantly in the eastern part of lake (Kamice Shegan) which go as far as 3 meter deep such Ceratophyllum, Najas marina, Najas minor, Vallisneria.Trapeum natantisi is largely widespread community in the Skadar lake. Waternuts develops wide population and communities in the inner and deeper part of the floating macrophyta zones. Plant samples were collected on the both sides of the lake. For Trapa natans specie is determined the content of photosynthetic pigments, chlorophyll a, chlorophyll b and carotenoids. Photosynthetic pigments were extracted with 80% acetone and their concentrations are expressed in mg/g dry absolutely leaf .Their measurement is made in the bands f 663, 645.470, of spectriphotometer. Trapa natans was found in the eastern shore and in western shore of Skadar lake. The highest values of chlorophyll a an b in the species Trapa natans was found in September 2014.*

Keywords*— *acquatic vegetation, habitats macrophytes, photosynthetic pigments, Skadar lake.

I. INTRODUCTION

Skadar lake is the largest lake on Balkan Peninsula. The drainage area of the lake is about 5500 km², 4.470 km² in Montenegro and 1030 km² in Albania. The lake area is 368 km². The lake volume varies between 1.8 km² in dry perods to 4.1 km³ during wet periods. The lake depth is about 7-10 m and the maximum lake depth reaches 44m.[1]

Skadar lake is known as a hight biodiversity ecosystem. The variety of the species per 100 m² is S/A=0.8752. We can mention important habitats like: the Eye of Shegan, the Eye of Viri, the underwater meadows in Shegan, the reeds-xunkth in Shkoder, Vrake and Buze Uje, the forests of the shores in the areas of Shegan - Kamice, Shkoder-Vrake, Zogaj-Shiroke.[5]

Aquatic and wetland flora is very rich. About 242 species of macrophytes are known from which 10 are algae(Characeae), 1 musk, 1 fir. 7 Species are members of Equisetaceae family, 115 are Monocotyledons and 107 Dicoyiledons. [2]

The most important associations of the vegetations types according to Pulevic et al(2001) are : Najadetum marinae ,Potameo-Najadetum, Potameo- Vallisnerietum, Potameo-natantis, Trapeum-natantis. Myriophyllo-Nupharetum lutei, Nymphoidetum peltata, Phragmitetum australis, Tyhpaetum latifolia, Ludwigetum palustris, Leucojo-Fraxinetum angustifolia.[5]

Summary of the most important asociations of the vegetation types

Najadetum marinae (Fukarek 1961) assemblage overgrow the Lake bottom in depths zones more than 3 m. This community is the most resistant on light deficiency. Dominant species is Najas marina but there are also species like Potamogeton perfoliatus, Myriophyllum spicatum, and Vallisneria spiralis. The mentioned species are typical hydrophyte.[3]

Potametum perfoliati association is common in the depth zones between 1 and 3 meters and is characterized by higher species diversity then others associations in the Lake. This association inhabits areas with colder water. Dominant species is Potamogeton perfoliatus and all other constituents are in hydrophyte type of plants. Constituents include: Myriophyllum spicatum, Myriophyllum verticillatum, Potamogeton crispus, Potamogeton pectinatus, Ceratophyllum demersum.[3]

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Potametum lucentis is a community that develops in inshore part of the lake .Dominant species of this community are Potamogeton lucens and Ceratophyllum demersum while others are far less abundant.[3]

Myriophyllo –Nupharetum lutei include species from Potamion association: Najas marina, Najas minor, Potamogeton perfoliatus, Potamogeton. crispus. It is characterized by presence of species Nuphar luteum,. This association inhabits colder water masses in littoral and are in contrast to association *Nymphoidetum peltatae* that inhabits wormer water masses of littoral

Floating vegetation is also represented by the communities Nymphaeto-Nupharetum lutei Lakušić 1965 and Trapetum natantis T h. Mull. Et Gors. 60.

Trapetum natantisi is largely widespread community in the /Skadar Lake. Water-nut develops wide population and communities in the inner and deeper part of the floating macrophyta zones as a continuous belt connecting this zone with that of the submerged vegetation .[3]

Between a large number of plant species it is necessary to make a selection of species that are considered «target species». Target species are defined the species which meet the one of the criteria of the Berne Convention. **In the area of Skadar lake are 3 globally threatened species Trapa natans, Marsilea quadrifolia dhe Caldensia parnassifolia** [2]

Trapa natans is an herbaceous, floating-leaf aquatic species that often grows in water around 60 cm deep. The floating leaves are arranged an a rosette,with leathery upper leaves up to 5 cm wide and broadly rhomboid,triangular. The species also produces submersed leaves that are morphologically different. The fruit is a horned nut- like structure that develops underwater and is approximately 3cm wide. The stems of plants is flexible and from 1to 5 m long,nodes of the stem have slender linear roots,while. The plant is anchored in the sediment by the lower roots that emerged from the propagating seed hull. Trapa natans is found world-wide in full sun and lowe energy, nutrient-rich fresh waters. The species is disturbance tolerant:it has been shown that sewage inputs create favorable conditions of increased alkalinity for the plant and that increased nitrogen is correlated with increased petiole and fruit biomass.[4]

Fig.1: Trapa natans found in Skadar lake

II. MATERIAL AND METHODS

It was collected plant samples in 6 stations from October 2013 to October 2014. In the laboratory it was made made the identification of collected samples using floras and keys of vascular plants. The samples collected are submited to the treatement for the determination of photosynthetic pigments.

For extraction we have taken the midsection of the leafs. Samples are placed in a PES filters to calculate the content of the pigments referred to the dry matter weight. As a solvent is used the acetone considered the most suitable for extraction in the case of tissue with high water content. Based on properties that have chlorophyll as volatile substances, all operations for the extractions are performed as soon as possible. During the work we have avoided the direct sun light. For each sample we determined the humidity to calculate the content of pigments referred in mg / ml and in mg / g dry matter. Determination of pigment is made on the basis of non-destructive spectrophotometric method. Absorption spectra of chlorophyll a, chlorophyll b and carotenoids allow to determine the content of the pigments in the extract without preliminary separation at 663, 645.470 wavelengths. To determine the content of pigments are used Rebelen equations.

III. RESULTS AND DISCUSSION

In the collected samples it was found; station Shkodra 1 Ceratophyllum demersum and Potamogeton perfoliatum, in Shiroke (Station 2) the specie Vallisneria spiralis. It was faund Najas marina species in western and eastern shore Zogaj(Station 3), Vrake, Koplik(Station 4). Trapa natans was found only in Shegan (Station 6) and Shiroke (Station 2).

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Table.1: Coordinates of Sampling Stations

Sampling points	Latitude	Longitude
S1	42◦ 3′ 14″N	19◦ 28′ 54″E
S2	42◦ 3′ 37″ N	19◦ 26′ 58″ E
S3	42◦ 4′ 19″ N	19◦ 23′ 53″ E
S4	42◦ 12′ 0.7″N	19◦ 47′ 65″ E
S5	42◦ 18′ 73″N	19◦ 41′ 50″E
S6	42◦ 27′ 23″N	19◦ 39′ 35″E

Fig.2: Coordinates of sampling points

Fig.3: View of the station six Shegan situated in eastern shore line (Photo by A. Temali)

Table.2: Photosynthetic pigments content of Trapa natans specie during 2013-2014

Photosynthetic pigments content of Trapa natans specie during 2013-2014							
	Tetor 2013	Qershor 2014	Korrik 2014	Shtator 2014	Tetor 2014	Mes	Devs
Chla (wet weight)	0.73	0.706	0.289	0.876	0.661	0.654	0.21
Chlb	0.31	0.192	0.140	0.347	0.150	0.229	0.09
Carotenoids	0.31	0.237	0.071	0.276	0.191	0.218	0.09
Chl(a+b)	1.05	0.898	0.42	1.224	0.812	0.883	0.29

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			9				
Chla (dry weight)	3.59	4.584	1.194	4.851	3.243	3.493	1.44
Chlb	1.54	1.248	0.580	1.921	0.736	1.206	0.55
x+c	1.52	1.540	0.295	1.529	0.938	1.166	0.55
Chl(a+b)	5.13	5.832	1.774	6.772	3.979	4.698	2.29
Chla/Chlb	2.36	3.818	2.092	2.598	4.502	3.074	1.03
(a+b)/(xc)	3.35	3.791	6.103	4.420	4.254	4.385	1.04

The maximum of chlorophyll a and b was observed in September 2014. The minimum of values of chlorophyll a and b was determined in july 2014 for wet and dry weight. Carotenoids have reached the maximum of values in October 2013 and the minimum values of carotenoids was obtained in july 2014 for wet dry. The carotenoids values for dry weight is higher in September 2014 while the lowest value for carotenoids(dry weight) is obtained in july 2014.

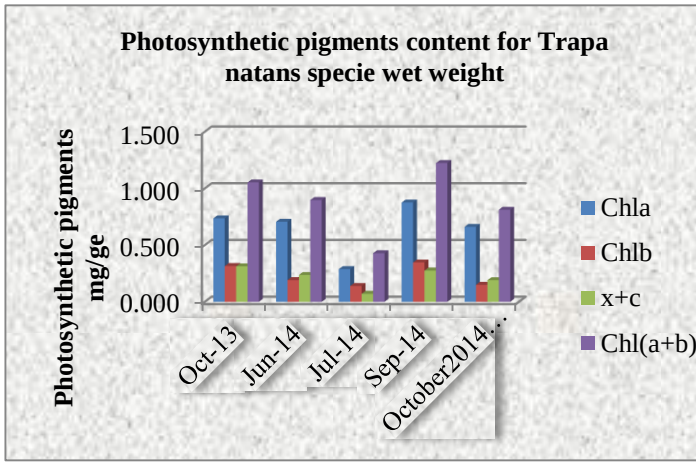

Fig.4: Photosynthetic pigment content in Trapa natans specie (wet weight)

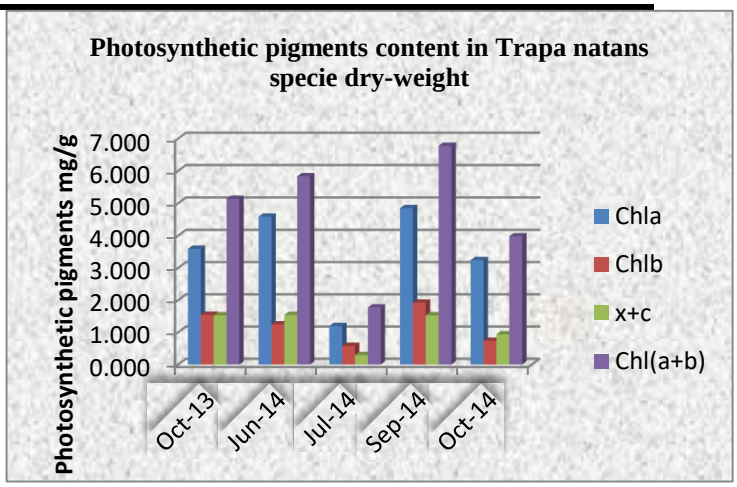

Fig.5: Photosynthetic pigment content in Trapa natans specie (dry weight)

IV. CONCLUSIONS

The species found in western and eastern shore lines are part of the primary associations of Skadar lake.

Trapa natans as endangered specie was found in both sides of Skadar lake.

The hightest value of chlorophyll a and b present in Trapa natans specie was determined in September 2014. Carotenoids have the maximum of values in October 2013.

The presence of considerable number of aquatic plants indicate that the quality of Skadar lake waters remains suitable to support the diversity of species.

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Bioremediating Effect of *Glomus Hoi* and *Pseudomonas Aeruginosa* on the Organic Content and Heavy Metals of Soil Polluted with Oil Refinery Effluent using *Amaranthus Cruentus* as a Test Plant

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Abstract— *This study analyzed the degrading effect of Glomus hoi and Pseudomonas aeruginosa on the organic content and heavy metals of oil refinery effluent polluted soil using Amaranthus cruentus as the test plant. This study was carried out to determine if agricultural activities can be improved using any or both of the micoorganisms.*

Eight different treatment layouts were used with three replicates for each level of pollution in the treatment layout. Ninety six (96) pots, each containing three kilograms of soil from both sterilized and unsterilized soil were used for the study. Fifty (50) grams of soil inoculum from propagated Arbuscular mycorrhiza was inoculated to a set of twenty four (24) experimental pots containing both sterilized and unsterilized soil before A. cruentus seedlings were transplanted to them. Another set of twenty four (24) pots containing both sterilized and unsterilized soil were injected with thirty (30) mL of P. aeruginosa inoculum solution before transplanting A. cruentus seedlings to them. The third set of twenty four (24) pots received dual inoculation of both fifty (50) grams of soil inoculum containing G. hoi and thirty (30) mL of P. aeruginosa inoculum solution before A. cruentus were transplanted to them. The residual twenty four (24) pots served as the control. Thereafter, pot preparation was arranged in the screenhouse in a randomized block design. The A. cruentus seedlings were raised in nursery for a period of two weeks before they were transplanted to the pots, seedlings were left for 3 days to overcome transplanting shock before contaminating the soil with refinery effluent at various concentrations of 0%, 2%, 4% and 6% v/w. The seedlings were allowed to grow for eight weeks before the termination of the experiment.

The pre planting analysis of soil showed that heavy metals analyses (zinc and iron) of sterilized soil had a lower concentration to the unsterilized. The soil pH ranged from 6.3 to 6.8.

It also revealed that organic matter and organic carbon content ranged from 0.8% to 1.3% and 0.4% to 1.7%. However, after the experiment, it was discovered in this study that treatments without any microorganism inoculation in sterilized and unsterilized soil had a higher level of % organic carbon and % organic matter content compared to the other treatments that were inoculated with one or two micro-organisms across all the levels of effluent concentration. Heavy metals of soil in all the soil samples were found to increase as the petrochemical effluent increased in concentration. The results obtained were analyzed using Duncan Multiple Range Test (DMRT) and other descriptive statistics.

This study opined that the combined use of G. hoi and P. aeruginosa was more effective in improving the organic contentand the reduce heavy metals of oil refinery effluent polluted soil than when either is used singly.

Keywords— Glomus hoi, Pseudomonas aeruginosa, Refinery effluent, Amaranthus cruentus, Bioremediation.

I. INTRODUCTION

Pollution of the environment with petroleum substances containing many highly toxic compounds is extremely dangerous to plant and animal lives. Petroleum substances, for example, include aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs which pollutes the soil (Turrio-Baldassarri *et al*., 2004). All over the world, scientists and environmentalists are faced with

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the challenge of overcoming the detrimentaleffects of the contamination of soil, air and water. Large-scalecrude oil spills on soil, leakages from pipelines, undergroundand surface fuel storage tanks, indiscriminate spills andcareless disposal and mismanagement of waste and otherpetroleum by-products of the society, constitute the majorsources of petroleum contamination in our environment. It hasbecome a topic of interest and attracted increasing attentionbecause of the carcinogenic, mutagenic and toxic effects. Various activities in crude oil exploration, exploitation, storage and transportation lead to spillage of oil to the environment (Nicolloti and Eglis, 1998). The spilled oil pollutes soils and the soils become less useful for agricultural activities with soil dependent organisms being adversely affected (Lundstedt, 2003). The effects of crude oil on the growth and performance of plants have been reported in many researches. These effects have been observed to occur due to the interference of the plant uptake of nutrients by crude oil and the unfavourable soil conditions due to pollution with crude oil (McGill and Rowell, 1977).

Bioremediation is a modern method in which the natural ability of microorganisms is employed for the reduction of the concentration and/or toxicity of various chemical substances, such as petroleum derivatives, aliphatic and aromatic hydrocarbons, industrial solvents, pesticides and metals (korda, 1997). The speed and efficiency of bioremediation of a soil contaminated with petroleum and petroleum products depends on the number of hydrocarbon-degrading microorganisms in the soil (Chen *et al.,* 2006). Bacteria, algae, fungi are some of the microorganisms that can be used to degrade oil polluted soil.

Glomus hoi which is an arbuscular mycorrhiza fungus is used for the treatment of polluted soils (Chen *et al.,* 2007). Mycorrhiza is the symbiotic association between fungi and the roots of vascular plants. The plant supplies the fungi with carbohydrate, while the fungi (mycorrhizal fungi) extends the surface area of the plant's roots and thus, increases their ability to absorb more nutrients (especially phosphorus) and water from the soil. Edwards *et al*. (2006) noted thatvarious bacteria produce surfactants such as *Pseudomonas aeruginosa* that aid in the biodegradation of fuels. The surfactant helps to decrease the surface tension and disperse the oil to allow maximum access to biodegrading microorganisms.

The interactions between the remediating organisms and the environment that leads to the process of bioremediation has not been well explored, hence this study, that sets out to investigate the degrading potentials of *Glomus hoi* and *Pseudomonas aeruginosa* on the physic chemical properties of soil polluted with oil refinery effluent.

II. MATERIALS AND METHODS

Experimental site

This study was conducted in the screenhouse of Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife

Collection of Materials

The petrochemical effluent was obtained from Warri Refinery and Petrochemical Company, Ekpan, Delta State. Soil inoculum of *Glomus hoi* and a culture of *Pseudomonas aeruginosa* were collected from the Mycology unit of Department of Crop Protection and Production, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. The test plant used for this study was *Amaranthus cruentus*, the seeds "cultivar variety NHAe-3" were obtained from National Horticulture Research Institute, Ibadan.

Propagation of Arbuscular Mycorrhiza

A sieved mixture of top soil and river sand in the ratio of 10 to 1 which was used for the propagation of arbuscular mycorrhiza was sterilized in the screenhouse using an autoclave; it was heated for 5 hours at 131^0c and left to cool for 4 days. Three hundred grams of soil inoculum containing *Glomus hoi* was obtained from the Mycology unit, Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. The inoculum was propagated using *Zea mays* cultivated variety IZEE-YPOP STRC5 for a period of four months. Chopped leaves of *Gliricidia sepium* were used every 2 weeks to mulch the soil throughout the four month period.

Determination and Estimation of Mycorrhizal Propagules in the Soil

The maize plants were removed after four months of propagation. The soil coupled with the root of the plant was air dried. The air dried soil was packed into sterile brown envelopes and taken to the laboratory for assessment. The population of arbuscular mycorrhizal spores in the soil inoculum collected was estimated using wet and sieving method. The soil (100 grams) was mixed with distilled water, stirred for two minutes and allowed to settle for 5mins, the soil suspension was then poured into the sieve of various mesh sizes (45 and 53 micrometer) arranged in descending order. A stream of wash bottle was used to wash down the spores into a centrifuge tube. It was then centrifuged at 2000 rpm for 3 minutes and the supernatant was decanted from the tube, the sediment was suspended in 40% sucrose solution and centrifuged again at 2000 rpm for

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1 minute. The supernatant which contained the spores was poured into a grid line plate.

Spores Counting

The counting of spores was done in 9cm diameter petri dishes with a grid line of 1cm per slide under a field dissecting microscope (mg. x 35).

Culture of *Pseudomonas aeruginosa*

A crude oil degrading strain of *P. aeruginosa* was isolated by preparing a bacterium culture of *P. aeruginosa* using nutrient agar in petri dishes and kept in the incubator for 48 hours at 37°C. This was followed by flooding it with sterile distilled water in order to harvest it. The inoculum was then added to a medium to which sterile crude oil acting as the sole source of carbon has been added and left at 37°C for 10 days. A pure colony of *P. aeruginosa* was isolated from this broth. The bacterium inoculum was prepared by streaking a single colony of *P. aeruginosa* on nutrient agar plate and then incubated at 37°C for 48 hours. Cells of *P. aeruginosa* was harvested from agar plates by flooding with sterile distilled water and standardized using a colorimeter to 10^8 CFU/ml.

Preparation of Pot for the Experiment

Sterilized and unsterilized soil was used for this experiment, there were ninety six (96) experimental pots comprising of a set of forty eight (48) pots with sterilized soil and another set of forty eight (48) pots with unsterilized soil. Each pot contained 3 kg of soil.

Planting and Inoculation of Soil with Microorganisms

Fifty (50) grams of soil inoculum from the propagated Arbuscular mycorrhiza containing 150 spores was inoculated to a set of twenty four (24) experimental pots containing both sterilized and unsterilized soil before *A. cruentus* seedlings are transplanted to them. Another set of twenty four (24) pots containing both sterilized and unsterilized soil was injected with thirty (30) ml of *P. aeruginosa* inoculum solution before transplanting *A. cruentus* seedlings to them. The third set of twenty four (24) pots received dual inoculation of both fifty (50) grams of soil inoculum containing *G. hoi* and thirty (30) ml of *P. aeruginosa* inoculum solution before *A. cruentus* seedlings are transplanted to them. The residual twenty four (24) pots served as the control. Thereafter, pot preparation was arranged in the screenhouse. Seedlings was left for a week to establish and overcome transplanting shock before contaminating the soil with petrochemical effluent at various concentrations of 0, 2, 4 and 6% v/w. Each treatment of the experiment was replicated three times and watered regularly to ensure adequate moisture.

Data Collection and Analyses

After the termination of experiment, %OC and %OM was analysed using soil test.Heavy metals (Zinc and Iron) of the soil were also analyzed both before and after the experimentusing Atomic Absorption Spectrophotometer (AAS). Data were analyzed using appropriate descriptive and inferential statistics.

TREATMENT LAYOUT

Sterilized and unsterilized soils were polluted with petrochemical effluent at a calculated percentage using the formula; Percentage soil contamination = (Volume of effluent/Volume of soil) x 100.

The layout of the experiment is as follows;

Treatment 1- sterilized soil + effluent + *A. cruentus*

Treatment 2- sterilized soil + *Glomus hoi* + effluent + *A. cruentus*

Treatment 3- sterilized soil + *Pseudomonas aeruginosa* + effluent + *A. cruentus*

Treatment 4- sterilized soil + *Glomus hoi* + *P. aeruginosa* + effluent + *A. cruentus*

Treatment 5- unsterilized soil + effluent + *A. cruentus*

Treatment 6- unsterilized soil + *Glomus hoi* + effluent + *A. cruentus*

Treatment 7-unsterilized soil + *P. aeruginosa* + effluent + *A. cruentus*

Treatment 8-unsterilized soil + *Glomus hoi* + *P. aeruginosa* + effluent + *A. cruentus*

Each of the layouts contaminated at 0, 2, 4 and 6% (v/w) petrochemical effluent concentration was replicated thrice. The experimental pots were irrigated regularly to ensure adequate moisture for proper growth of the test plant.

III. RESULTS

Table.1: Physicochemical Properties of Sterilized and Unsterilized Soil before Planting

Parameters	Sterilised	Unsterilised
OC (%)	0.4	0.7
OM (%)	0.8	1.3
Zn (ppm)	81.75	97.46
Fe (ppm)	5.89	9.34

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The physicochemical properties of sterilized and unsterilized soil before planting were found to show that heavy metals analyses (Zinc and Iron) of sterilized soil had a lower concentration compare to the unsterilized soil (Table 1). Organic matter and organic carbon percentages were also found to be lower in concentration in sterilized soil compared to the unsterilized soil. The textural class of the soil was loamy sand (Table 1).

Physico Chemical Properties of Soil after Planting

After the termination of experiment, the physicochemical properties of soil were analyzed to check for the change that occurred. % Organic carbon and organic matter content had the highest value at 6% effluent concentration in sample 1 and its lowest value was recorded at 0% effluent concentration in sample 7, the order of % organic carbon and organic matter content across the samples at 2% and 6% is 1 > 5 > 2 > 7 > 6 > 3 > 8 > 4 and 1 > 5 > 2 > 6 > 3 > 4 > 7 > 8 respectively (Fig. 1 and 2).

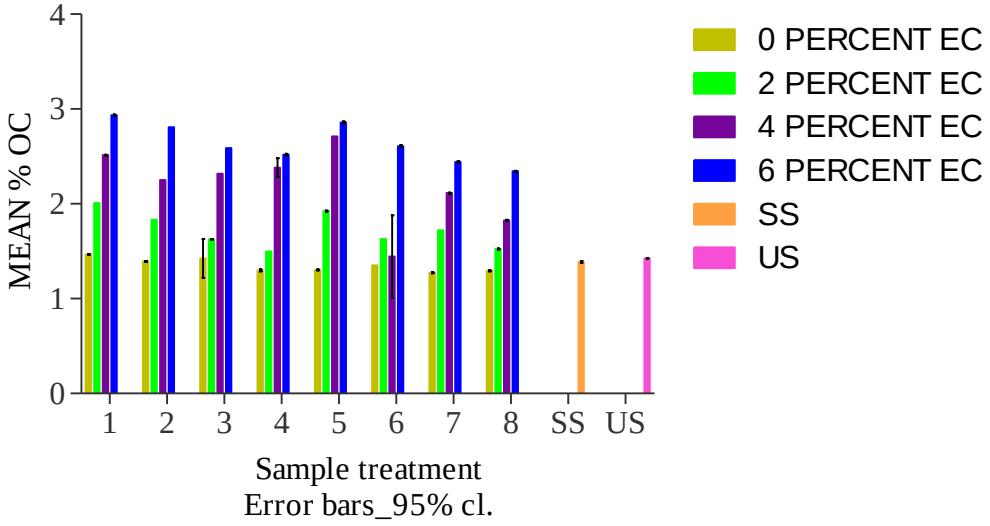

Fig.1: % Organic Carbon content of Pre and Post Planting Soil Samples

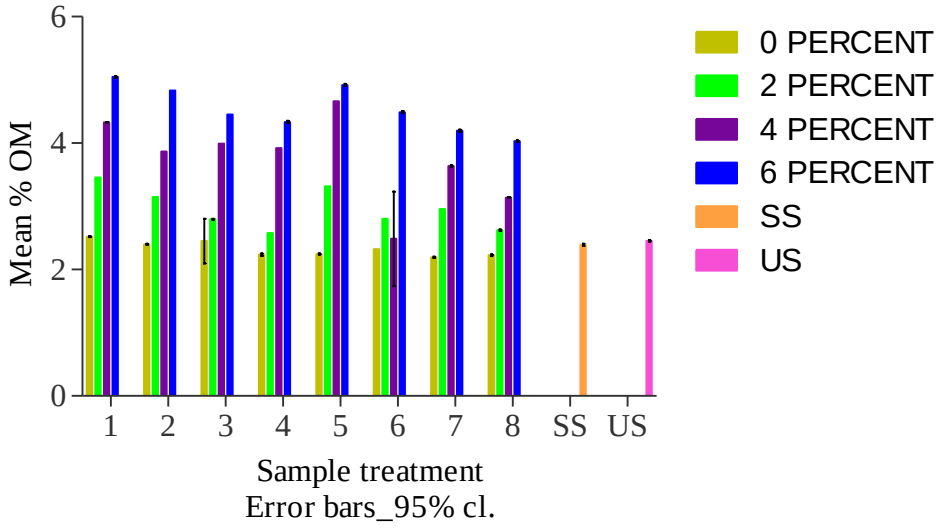

Fig.2: % Organic Matter content of Pre and Post Planting Soil Samples

Legend

1 - SS + TP
2 - SS + GH + TP
3 - SS + PA + TP
4 - SS + GH + PA + TP
5 - US + TP
6 - US + GH + TP
7 - US + PA + TP

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8 - US + GH + PA + TP

SS - Sterilized Soil before Planting
US - Unsterilized Soil before Planting
GH - *G. hoi*
PA - *P. aeruginosa*
TP - Test Plant
EC- Effluent Concentration

HEAVY METAL CONTENT OF SOIL AFTER PLANTING

Zinc analyses in the soil showed that the order of the concentration in 2% and 6% was 5 > 1 > 2 > 3 > 6 > 4 > 7 > 8 and 5 > 1 > 2 > 3 > 4 > 6 > 7 > 8, treatment 5 had the highest level of zinc concentration followed by treatment 1 both at 6% effluent concentration while treatment 8 had the lowest at 0% (Fig. 3). The heavy metal analyses of the soil were found to show that iron concentration had highest value in treatment 1 at 6% effluent concentration followed by treatment 5 at 6% while treatment 4 at 0% was the lowest. The order of iron concentration in 2% and 6% was treatment 1 > 5 > 2 > 3 > 6 > 7 > 4 > 8 and 1 > 5 > 2 > 6 > 7 > 3 > 4 > 8 respectively (Fig. 4).

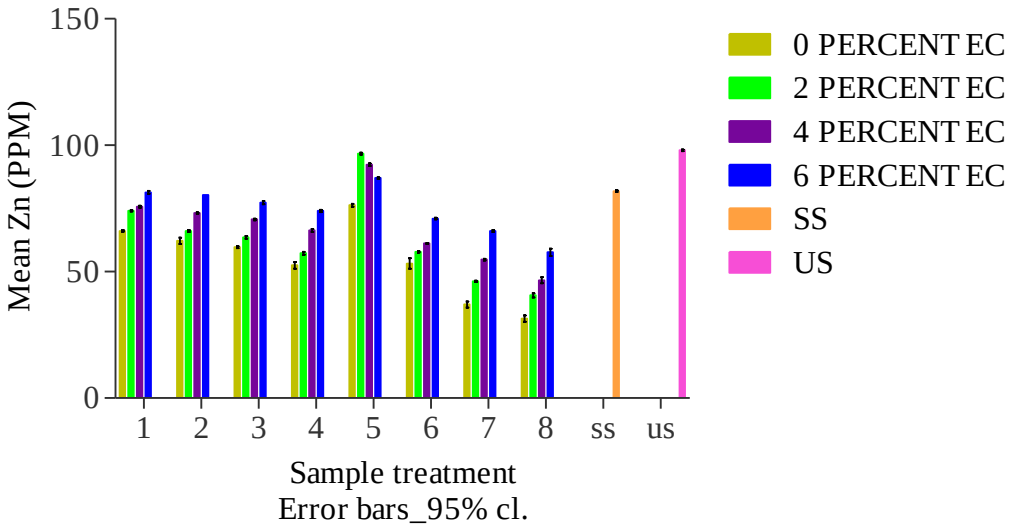

Fig.3: Zinc (PPM) Content of Pre and Post Planting Soil Samples

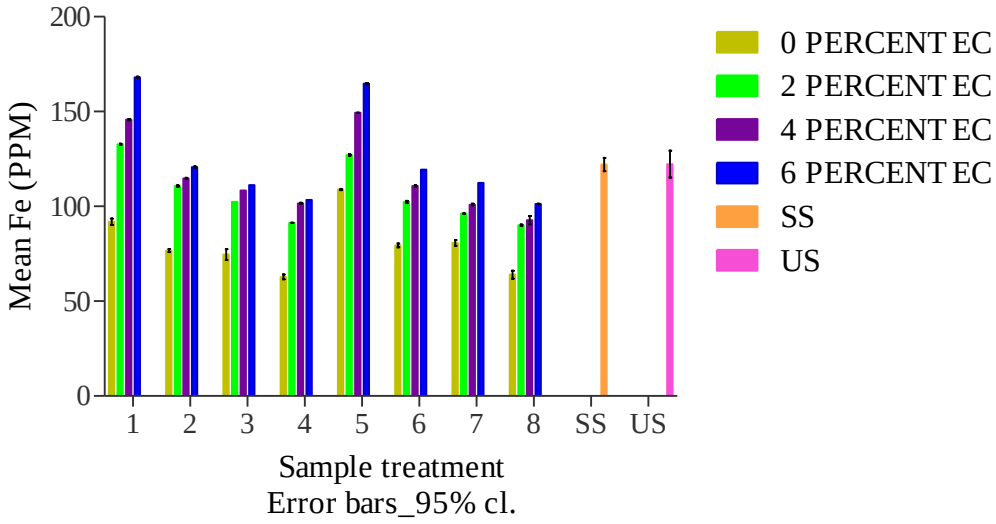

Fig.4: Iron (PPM) content of Pre and Post Planting Soil Samples

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Legend
1 - SS + TP
2 - SS + GH + TP
3 - SS + PA + TP
4 - SS + GH + PA + TP
5 - US + TP
6 - US + GH + TP
7 - US + PA + TP
8 - US + GH + PA + TP

SS - Sterilized Soil before Planting
US - Unsterilized Soil before Planting
GH - *G. hoi*
PA - *P. aeruginosa*
TP - Test Plant
EC- Effluent Concentration

IV. DISCUSSIONS

In this study, % organic carbon and organic matter content were found to increase as petrochemical effluent concentration increased across all the treatments, this may be due to the addition of effluent which increased petroleum hydrocarbon content of the soil thereby resulting into high carbon in the polluted soil. This is in line with the findings of Nwazue (2011) which reported that PHC polluted soil had lower pH value, low moisture content and more organic carbon than the unpolluted soil. It was discovered in this study that treatments without any microorganism inoculation in sterilized and unsterilized soil had a higher level of % organic carbon and % organic matter content compared to the other treatments that were inoculated with one or two microorganisms across all the levels of effluent concentration. This can be as a result of *Glomus hoi* and *Pseudomonas aeruginosa* in the soil which had utilize the carbon in the soil for their growth and metabolism which is one of the reasons for their ability to degrade the effluent.

Heavy metals are elements that exhibit metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity (Opaoluwa, 2010). They are characterized by relatively high density and high relative atomic weight with an atomic number greater than 20. Industrial effluents are usually considered as undesirable for arable soil, plants, animals and human health.

According to Gulfraz *et al.* (2003) some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V, and Zn is required in minute quantities by organisms. However, excessive amounts of these elements can become harmful to organisms. Other heavy metals such as Pb, Cd, Hg, and As (a metalloid but generally referred to as a heavy metal) do not have any beneficial effect on organisms and are thus regarded as the "main threats" since they are very harmful to both plants and animals. For other metals which are beneficial to plants, "small" concentrations of these metals in the soil could actually improve plant growth and development. However, it was discovered in this study that at higher concentrations of these metals, reductions in plant growth occurred. This may account for the decrease in growth parameters of *A. cruentus* as the effluent concentration increased in this study. However, heavy metals of soil in all the soil samples were found to increase as the petrochemical effluent increased in concentration, but treatments inoculated with *G. hoi* showed lower concentration in heavy metals compared to treatments without inoculation of micro-organism. This low concentration of heavy metals in this inoculated soil may be as a result of *G. hoi* ability to absorb and sequester some heavy metals in to their mycelia which is retained in the roots (Marques *et. al*., 2009). Due to a change in their oxidation state, heavy metals can be transformed to become either less toxic, easily volatilized, more water soluble (and thus can be removed through leaching), less water soluble (which allows them to precipitate and become easily removed from the environment) or less bioavailable (Marques *et. al*.,2009). He also noted that mycorrhizal fungi have been used in several remediation studies involving heavy metals pollution and the results obtained showed that mycorrhiza employs different mechanisms for the remediation of heavy metal polluted soils. Soils polluted with various heavy metals including As, Cu, Cd, Pb, U and Zn can be remediated via MAR. The MAR can also help with the transfer of elements such as carbon (Francis and Read, 1984), nitrogen (Haystead *et al*., 1988, Rogers *et al*., 2001), and phosphorus (Chiariello *et al*., 1982).

Treatments inoculated with *G. hoi* showed a lower concentration of zinc in the soil compared to the treatments without inoculation of microorganism, this may be due to the absorption of the zinc in the soil by the AM (*G. hoi*), this result is the same with the findings of Vogel-Mikus *et al.* (2005); Chen *et al.* (2006) which reported that AM fungi absorb N, P , K, Ca, S, Fe, Mn, Cu, and Zn from the soil and then translocate these nutrients to the plants with whose roots they are associated with. This report also confirmed the reason for the lower concentration of iron and copper in the soil samples inoculated with *G. hoi* compared to treatments without inoculation of *G. hoi*. Treatments with dual inoculation in this study however showed lower heavy metals concentrations compared to those treatments with single inoculation and treatments without inoculation of

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micro-organism. This revealed that there is positive and productive interaction between *G. hoi* and *P. aeruginosa* in bioremediation of heavy metals in petrochemical effluent polluted soil.

V. CONCLUSION

The arbuscular mycorrhiza Fungus, *Glomus hoi* was found to be able to give way for reduction in heavy metals in the soil which can make plants survive in petrochemical effluent polluted soil. The combination of the two microorganisms showed a better improvement in the overall reduction in heavy metal concentration. They also had a fruitful combination in increasing the organic matter of the soil compared to when they were inoculated singly. Hence, *G. hoi* and *P. aeruginosa* can enhance crop production in oil refinery effluent polluted soil. The microorganisms also worked better in sterilized soil than the unsterilized soil, this may be due to the no competition between native microorganisms in the sterilized soil with the inoculated microorganism.

This informs the reason of bioremediation being an acceptable approach for processing organic and inorganic wastes. The result of this study has shown that bioremediation is an environmental friendly and easy approach to degrade petrochemical effluent polluted soil.

VI. RECOMMENDATION

There should be adequate analysis of the site polluted with refinery effluent or petroleum hydrocarbon before and after bioremediation to enhance proper treatment of the soil.

However, government, oil refineries, farmers and other concerned individuals should embrace the use of bioremediation to treat oil refinery effluent pollution in the environment. This is because bioremediation requires no negative consequence or whatsoever. It is also easy to carry out. It is less skillfuland efficient.

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Relational Analysis of Profile of Beneficiaries of Farm Ponds and its Socio Economic Impact

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***Abstract*—** *The present investigation was conducted in Parbhani district of Marathwada region in Maharashtra State. The main objective of the study was relationship between profile of beneficiaries of farm ponds and its impact. The data were collected through personal interview with the help of interview schedule by contacting 80 beneficiaries. The result revealed that majority (75.00%) of the beneficiaries having middle farming experience, followed by 26.25 per cent of the beneficiaries were educated up to secondary school level, while 50.00 per cent of the beneficiaries were having semi-medium land holding .whereas 75.00 per cent of the beneficiaries having medium area under irrigation, While 80.00 per cent of the beneficiaries having medium family size. It was also found that 87.50 per cent of the beneficiaries having medium social participation, whereas 52.50 per cent of the beneficiaries having medium level of extension contact, and 52.50 per cent of the beneficiaries having medium level of economic motivation, followed by 63.75 per cent of the beneficiaries having medium risk preferences. Also the result showed that farming experience, education, land holding, area under irrigation, family size social participation, extension contact, economic motivation and risk preferences were found to be positively and significantly related with impact in technological change (i.e) crop production, cropping pattern and soil conservation structure of farm pond. Also the result showed that relationship of profile of beneficiaries with economic change in employment generation only economic motivation was positive and non-significant, followed by relationship of profile of beneficiaries with social change in material possession and implement possession i.e. economic motivation was non-significant also social participation was non-significant in change in education family member.*

***Keywords*— *Relationship of Beneficiaries, Farm Ponds, Economic Impact* .**

I. INTRODUCTION

The challenges before Indian agriculture is to transform rainfed farming into more sustainable and productive system by giving social, economical and technological backup to the people who depend upon it. Moreover, the economy is mainly dependent on stability of crop production in rainfed areas. Construction of farm ponds is one of the such beneficial programme for harvesting excess rain water during rainy season; which is implemented by the State Agricultural Development under National Agricultural Development Programme, Rashtriya Krishi Vikas Yojana (Aug 2007 In 11th five year plan) etc. The excess rain water harvested in farm ponds play a vital role in stabilizing crop production through recycling during dry spell in kharif season and for protective irrigation in rabi season. The major works of Rain Water Harvesting Structure adopted in the watershed are check dams, farm ponds, nala bunds, contour bunds, vegetative covers etc. which play major role in managing and conserving the soil and water resources. However, farm pond is perceived as best rain water harvesting structure by large majority of farmers. The present study was undertaken with the following specific objective

1. To study the profile of farm pond beneficiaries
2. To study the relationship between profile of beneficiaries of farm ponds and its Socio-economic impact

II. METHODOLOGY

The research study was selected by lottery method in Parbhani district of Marathwada region in Maharashtra State. The study was conducted in Parbhani district from selected district four talukas was selected and fro selected 4 talukas 5 villages from each talukas was selected on the basis of maximum number of farm ponds. From each selected village 4 beneficiary farmers was selected randomly those having 3 year before farm pond after receiving its beneficiaries list from the authority to make 80 samples of beneficiaries in total. All the respondents were personally interviewed at their home and farms and data was collected. The collected data was analyzed with the help of suitable statistical methods i.e. frequency, percentage, mean, standard deviation, coefficient of correlation and Z-test.

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III. RESULTS AND DISCUSSION

1. Profile of farm pond beneficiaries

Table 1 (n=80)

Sr. No.	Category	No.	%
1	**Farming experience**		
	1. Low	10	12.50
	1. Medium	60	75.00
	2. High	10	12.50
2	**Education**		
	1. Illiterate	14	17.50
	2. Primary school level	19	23.75
	3. Secondary school level	21	26.25
	4. Higher school level	19	23.75
	5. College level	07	08.75
3	**Land holding**		
	1. Marginal farmer	1	1.25
	2. Small farmers	23	28.75
	3. Semi-medium farmers	40	50.00
	4. Medium farmers	16	20.00
	5. Big farmers	00	00
4	**Area under irrigation**		
	1. Low	10	12.50
	2. Medium	60	75.00
	3. High	10	12.50
5	**Family size**		
	1. Low	4	5
	2. Medium	64	80
	3. High	12	15
6	**Social participation**		
	1. Low	70	87.50
	2. Medium	09	11.25
	3. High	01	01.25
7	**Extension contact**		
	1. Low	22	27.50
	2. Medium	42	52.50
	3. High	16	20.00
8	**Economic motivation**		
	1. Low	21	26.25
	2. Medium	42	52.50
	3. High	17	21.25
9	**Risk preferences**		
	1. Low	09	11.25
	2. Medium	51	63.75
	3. High	20	25.00

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Table.2: Distribution of relationship of profile of beneficiaries with Technological change i.e. (crop production, change in cropping pattern, and soil conservation structure).

Sr. No	Profile	Crop Production 'r' value	Cropping pattern 'r' value	Soil conservation 'r' value
1.	Farming experience	0.450**	0.504**	0.489**
2.	Education	0.687**	0.662**	0.701**
3.	Land holding	0.778**	0.821**	0.808**
4.	Area under irrigation	0.747**	0.705**	0.741**
5.	Family size	0.765**	0.841**	0.828**
6	Social participation	0.395**	0.480**	0.330**
7.	Extension contact	0.753**	0.698**	0.732**
8.	Economic motivation	0.281*	0.191*	0.197 *
9.	Risk preferences	0.672**	0.554**	0.621**

**Significant at 0.01 level of probability.

1.1 Profile of farm pond beneficiaries

It was found from Table 1 that majority (74.00 %) of the beneficiaries had medium farming experience and 12.50 per cent of the respondents had low and high farming experience each, followed by (26.25%) beneficiaries were educated up to secondary school level and 23.75 per cent of the respondents were educated up to primary school level and higher school level both, followed by (50.00%) of the beneficiaries were having semi medium land holding and 28.75 per cent of the respondents were small farmers, followed by (75.00%) majority of the beneficiaries had medium area under irrigation and 12.50 per cent having low area under irrigation, followed by (80.00%) of the beneficiaries had medium family size, and 15.00 per cent of the respondents had high family size, followed by (87.50%) of the beneficiaries had low social participation and 11.25 per cent of respondents had medium social participation, followed by (52.50%) of the farmers medium extension contact and 27.50 per cent farmers had low extension contact, followed by (52.50%) had medium economic motivation and 26.25 per cent had low, followed by (63.75 %) were having medium risk preferences and 25.00 per cent having high risk preferences.

2.1 Relationship of profile of beneficiaries with Technological change

It was noticed from Table 2 that farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, risk preferences was positively and highly significantly related with impact on crop production at 0.01 level of probability and economic motivation was also positively and significantly related with impact on crop production at 0.05 level of probability. Above relation indicated that after construction and using of farm pond most of the crop yield is increased due to the increased area under irrigation. Due to crop yield also increase annual income of farmers and they provide the more education to his children also increase social contact with extension workers to get more information about agriculture. Above findings are in line with, Ahire (2000), Erappa (2000), Nipanikar (2006) and Kulkarni (2009).

It was noticed from Table 2 that farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, risk preferences was positively and highly significantly related with impact on cropping pattern at 0.01 level of probability and economic motivation was also positively and significantly related with impact on cropping pattern at 0.05 level of probability. Before construction of farm pond respondents followed traditional cropping pattern i.e. they cultivated only one or two crops. After construction of farm pond cropping pattern changed to growing more than one crop due to increased area under irrigation, crop yield also increase due to crop yield annual income get increased by change in crop pattern.Above findings are in line with Ahire (2000), Erappa (2000), Nipanikar (2006) and Kulkarni (2009).

It was noticed from Table 2 that farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, risk preferences was positively and highly significantly related with impact on soil conservation structure at 0.01 level of probability and economic motivation was also positively and significantly related with impact on soil conservation structure. Soil conservation increased with increasing area under irrigation also increase in crop yield and cropping pattern. More land is used after construction of farm pond for crop cultivation due to this soil conservation practices also increased. Above

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findings are in line with Ahire (2000), Erappa (2000), Nipanikar (2006), Kulkarni (2009) and Deshmukh (2016). Farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, economic motivation and risk preferences this variables are positively and significantly associated with Technological change.

Table.3: Distribution of relationship of profile of beneficiaries with Economic change i.e. (employment generation).

Sr. No	Profile	Beneficiaries r value
1.	Farming experience	0.428**
2.	Education	0.707**
3.	Land holding	0.797**
4.	Area under irrigation	0.729**
5.	Family size	0.807**
6.	Social participation	0.344**
7.	Extension contact	0.716**
8.	Economic motivation	0.173 NS
9.	Risk preferences	0.555**

**Significant at 0.01 level of probability.

2.2 Relationship of profile of beneficiaries with Economical change

It was noticed from Table 3 that farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, risk preferences was positively and highly significantly related with impact on employment generation at 0.01 level of probability and economic motivation was also positively and non-significantly related with impact on employment generation at 0.05 level of probability. Due to change in cropping pattern work also increased for labour and also required more labour to done work in farm. Hence also increase the labour charges of labour. Before construction of farm pond respondents cultivated crop only in kharif season but after construction of farm pond they taken crop in rabi and summer season. Hence intensive crop cultivation increased the more number of labourer and additional employment is generated in the field of agriculture.Above findings are in line with Ahire (2000), Nakhate (2006), Ponnusamy and Gupta (2006), Kulkarni (2009) and Deshmukh (2016). Farming experience, education, land holding, area under irrigation, family size, social participation, extension contact and risk preferences this variables are positively and significantly associated with Economic change.

Table.4: Distribution of Relationship of profile of beneficiaries with Social change i.e. (material possession, change in to education of family member and implement possession).

Sr. No	Profile	Material possession r value	Change in to education of family member r value	Implement possession r value
1.	Farming experience	0.417**	0.371**	0.375**
2.	Education	0.653**	0.444**	0.621**
3.	Land holding	0.773**	0.354**	0.753**
4.	Area under irrigation	0.695**	0.545**	0.665**
5.	Family size	0.776**	0.375**	0.715**
6.	Social participation	0.439**	0.155 NS	0.378**
7.	Extension contact	0.694**	0.364**	0.728**
8.	Economic motivation	0.183 NS	0..474**	0.160 NS
9.	Risk preferences	0.560**	0.637**	0.558**

**Significant at 0.01 level of probability.

2.3 Relationship of profile of beneficiaries with Social change

It was noticed from Table 4 that farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, risk preferences was

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positively and highly significantly related with impact on material possession at 0.01 level of probability and economic motivation was also positively and non-significantly related with impact on material possession at 0.05 level of probability. The findings are supported by Ahire (2000), Shivanappan (2005), Nakhate (2006), Thakur (2014) and Deshmukh (2016).

It was noticed from Table 4 that farming experience, education, land holding, area under irrigation, family size, extension contact, economic motivation risk preferences was positively and highly significantly related with impact on change in education of family member at 0.01 level of probability and social participation, was also positively and non-significantly related with impact on change in education of family member at 0.05 level of probability. Due to this more yield are obtain from field and sold in the market. Income was available to educate the children with relation to construction of farm pond. Education is inversely propotional to the farm pond for improvement. The findings are supported by, Ahire (2000), Bhange (2005), Jugale (2006), Nakhate (2006) Chauhan et al. (2009) and Deshmukh (2016).

It was noticed from Table 4 that farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, risk preferences was positively and highly significantly related with impact on implement possession at 0.01 level of probability and economic motivation was also positively and non-significantly related with impact on implement possession at 0.05 level of probability. After construction of farm pond increased irrigated area result in increased area under cultivation of crops which result increased farm income also they require more implement for farm operation. Hence construction of farm pond result in increase in implement possession of respondents. The findings are supported by Ahire (2000), Erappa (2000), Nipanikar (2006) and kulkarni (2009).

Farming experience, education, land holding, area under irrigation, family size, extension contact and risk preferences this variables are positively and significantly associated with Social change.

IV. CONCLUSIONS

It is concluded that majority (75.00%) of the beneficiaries having middle farming experience, followed by 26.25 per cent of the beneficiaries were educated up to secondary school level, while 50.00 per cent of the beneficiaries were having semi-medium land holding, whereas 75.00 per cent of the beneficiaries having medium area under irrigation, While 80.00 per cent of the beneficiaries having medium family size. It was also found that 87.50 per cent of the beneficiaries having medium social participation, whereas 52.50 per cent of the beneficiaries having medium level of extension contact, and 52.50 per cent of the beneficiaries having medium level of economic motivation, followed by 63.75 per cent of the beneficiaries having medium risk preferences. Farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, and risk preferences were found to be positive and highly significant related with technological change and economic change. While only economic motivation was positive and significantly related with technological change and positive and non-significantly related with economic change. Also in social change Farming experience, education, land holding, area under irrigation, family size, social participation, extension contact, and risk preferences were found to be positive and highly significant related with material possession, whereas, economic motivation was positively and non-significantly related with material possession and implement possession. Also social participation was positive non-significantly related with change in education of family members. While only economic motivation was significantly related with technological change.

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Short Term Effect of Crop Residue and Different Nitrogen Levels on Grain yield of Wheat under Rice-Wheat System

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Abstract— *Crop residues are very important source of plant nutrients and recycling of crop residues with inorganic fertilizer increases the yield of rice and wheat in rice-wheat system. The objective of the study was to determine the production and productivity of wheat as affected by management of crop residues and different nitrogen levels. The field experiments were carried out in 2014 and 2015 at National Wheat Research Program, Bhairahawa, Nepal and the field was laid out in split plot design: two crop residue levels (with and without residues) as whole plot and seven nitrogen levels (0, 25, 50, 75,100, 125, 150 kg/ha) as sub-plot which were replicated three times. Significant difference was observed with crop residues incorporation in biological yield with the value of 5538 kg/ha as compared to without residue incorporation (4167 kg/ha) in 2014. Similar result was observed in 2015 as highest significant biological yield of 6629 kg/ha was recorded from residue incorporation plot. On the other hand, application of nitrogen @ 150 kg/ha resulted to significantly highest grain yield of 2593 and 3073 kg/ha in both years (2014 and 2015) respectively. The overall conclusion is that an improved crop residue management with appropriate dose of chemical fertilizer increases the grain yield of wheat in short term basis.*

Keywords— Crop residue, Nitrogen level and Yield.

I. INTRODUCTION

Crop residues are parts of crops left in the field after crops have been harvested and threshed. Crop residues are important natural resources and recycling of crop residues has the advantage of converting the surplus farm wastes into useful materials for meeting nutrient requirement of crops. it also maintains the soil physical and chemical condition (Powel et al 1997) and improves the overall ecological balance of the crop production system. As cereal crops, rice and wheat both are exhaustive feeders, and the double cropping system is heavily depleting the soil of its nutrient content. The combined use of rice or wheat straw and inorganic fertilizer can increase the yield of rice and wheat in rice-wheat systems (Mahapatra et. al. 1991). A rice-wheat sequence that yields 7 tons per ha of rice and 4 tons per ha of wheat removes more than 300 kg N, 30 kg P, and 300 kg K per ha from the soil (Singh and Singh, 2001). Another estimate shows that a 10 t ha^{-1} crop yield removes 730 kg NPK from the soil where the crop reisdue is often not returned to the soils (Gupta *et al.*, 2002). If this residue is not returned this may cause mining of soil for major nutrients leading to net negative balance and multi- nutrient deficiencies in crops. This is one of the reasons for the yield decline in the rice-wheat system. Thus, there are urgent needs to manage the residues of these crops for sustainability and stability of the system. Crop residues are very important source of plant nutrients. Literatures suggest that about 25% of nitrogen (N) and phosphorus (P), 50% of sulphur (S), and 75% of potassium (K) uptake by cereal crops are retained in crop residues, making them valuable nutrient sources (Singh and Singh, 2001). Unlike huge potential of crop residues in replenishing soil fertility, their importance has not been recognized by the farmers.

II. MATERIALS AND METHODS

The experiment was conducted in winter season of 2014 and 2015 at National Wheat Research Program, (NWRP), Bhairahawa and the field was laid out in split plot design: two crop residue levels (with and without residues) as whole plot and seven nitrogen levels (0, 25, 50, 75,100, 125, 150 kg/ha) as sub-plot which were replicated three times. The crop, Vijay variety was sown on December 3rd in 2014 and November 26 in 2015 at the spacing of 25 cm between rows with continuous seeding. The plot size was 5 × 3 m giving a net plot area of 15 m^2. Urea, single super phosphate, murate of potash and borax were the source of fertilizers used for supplying nitrogen, phosphorus, potash and boron respectively. Full dose of phosphorus, potassium and boron fertilizers was applied at the time of land preparation. Whole Crop residues were retained in the field

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as natural residues after rice harvesting. The recommended dose of 50 kg P_2O_5/ha, 50 kg K_2O/ha and 2 kg borax were applied as basal in all plots at the time of seed sowing. Half dose of N was used at the time of seed sowing as basal dose. The remaining half dose of N was side-dressed at 20 DAS and 40 DAS. The monthly mean maximum, minimum temperature and rainfall of both years were presented in table 1. Data were analyzed through GENSTAT statistical package and treatment means were compared using least significant difference (LSD) test at $P \leq 0.05$.

Table.1: Mean maximum, minimum temperature and rainfall values during the crop growing season, 2014/15 and 2015/16

Month	Maximum mean temperature (C˚)		Minimum mean temperature (C˚)		Rainfall (mm)	
	2014/15	2015/16	2014/15	2015/16	2014/15	2015/16
October	31.39	33.4	20.37	20.7	156	25.5
November	29.15	30.0	15.30	16.0	0	0.0
December	20.70	24.5	11.45	10.5	17.6	0.4
January	19.13	21.6	10.01	8.7	326	4.5
February	25.36	26.9	12.15	11.5	0	0.0
March	29.65	32.2	15.47	15.5	122.9	19.6

Source: National Wheat Research Program, Bhairahaw, Nepal.

III. RESULTS AND DISCUSSION

Effect of crop residues and nitrogen levels on growth and yield attributes of wheat, 2014

Significant difference was observed with crop residues incorporation in biological yield whereas no significant effects were observed in other yield attributing parameters maturity days, plant height, spike length, spikes per m^2, grains per spike, thousand grain weight and grain yield (Table 2). Result shows no incorporation of crop residues gave significantly higher biological yield (5538 kg/ha). Incorporation of crop residues gave higher maturity days (109.23), more grains per spike (24.57) and higher thousand grain weight (51.58gm) whereas taller plant height (93.93 cm), greater spike length (10.46 cm), more spikes per m^2 (221.4) and higher grain yield (2235 kg/ha) were observed in the plots with no crop residues incorporation.

Table.2: Effect of crop residues and nitrogen levels on growth and yield attributes of wheat at NWRP, Bhairahawa, 2014

Treatments	Maturity Days	Plant Height (cm)	Spike length (cm)	Spikes/m^2	Number of grains/spike	Thousand grain weight (gm)	Grain yield (kg/ha)	Biological yield (kg/ha)
Crop residues (A)								
Without crop residues	109.09	93.93	10.46	221.4	23.90	50.79	2235	5538
With crop residues	109.23	91.73	9.80	210.5	24.57	51.58	1946	4167
F-test of A	NS	NS	NS	NS	NS	NS	NS	*
LSD 0.05	0.35	6.7	1.63	27.37	3.111	6.10	336.6	826.9
Nitrogen levels (kg/ha) (B)								
0	108.16	88.40	9.53	175.7	20.45	50.60	1361	3050
25	108.0	92.40	10.33	176.8	22.60	51.57	1785	4150
50	108.33	92.20	9.27	214.8	23.55	51.50	1990	5017
75	109.33	95.0	10.57	221.0	25.15	49.97	2044	5033
100	109.66	95.13	10.37	218.7	25.92	53.40	2326	5317
125	110.0	95.17	10.50	230.2	25.53	51.93	2534	5567
150	110.66	91.53	10.33	274.7	26.47	49.33	2593	5833
F-test of B	***	NS	NS	***	**	NS	***	***

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LSD 0.05	0.52	4.77	1.22	37.09	3.16	4.63	496.6	1132.2
Interaction								
F-test of A × B	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	0.4	4.3	10.1	14.4	11	7.6	19.9	19.6

Results (Table 2) revealed significant (P<0.05) differences among different nitrogen levels on maturity days, spike per $m^{2,}$ grains per spike, grain yield and biological yield but was non-significant on plant height, spike length and thousand grain weight. Highest maturity days (110.66) was observed in application of nitrogen @150 kg/ha to the crop. Plots amended with nitrogen @125kg/ha gave greater plant height (95.17 cm) which was ad par with the application of 25 (92.40), 50 (92.20), 75 (95), 100 (95.13) and 150 (91.53) kg N/ha. Similarly, greater spike length (10.33 cm) was observed with N @125 kg/ha treated plots, whereas more spike per m^2 (274.7) was with 150 kg N/ha amended plots. Nitrogen application @150 kg/ha produced more number of grains per spike (26.47) which was at par with the application of N @ 50 (23.55), 75 (25.15), 100 (25.92) and 125 (25.53) kg N/ha. Higher thousand grain weight (53.40 gm) was produced with the application of 100 kg N/ha followed by 125 kg N/ha (51.93 gm). N application @ of 150 kg/ha yielded highest grain yield of 2593 kg/ha. Highest biological yield was observed in the plot with application of nitrogen @150 kg/ha (5833 kg/ha) which was ad par with N-application of 50 (5017 kg/ha), 75 (5033 kg/ha), 100 (5317 kg/ha) and 125 (5567 kg/ha) kg/ha. No significant differences were observed in the interaction between crop residues and nitrogen levels.

Significant difference was observed with crop residues incorporation in plant height, spike length, thousand grain weight and biological yield whereas no significant effects were observed in other yield attributing parameters maturity days, spikes per m^2, grains per spike and grain yield. Results showed that incorporation of crop residues gave significantly highest plant height (101 cm), spike length (8.9 cm), thousand grain weight (43.94 gm) and biological yield (6629 kg/ha).

Table.3: Effect of crop residues and nitrogen levels on growth and yield attributes of wheat at NWRP, Bhairahawa, 2015

Treatments	Maturity Days	Plant Height (cm)	Spike length (cm)	Spikes/m^2	Number of grains/spike	Thousand grain weight (gm)	Grain yield (kg/ha)	Biological yield (kg/ha)
Crop residues (A)								
Without crop residues	115	98	8.6	270.6	31.25	42.93	2376	4871
With crop residues	116	101	8.9	261.6	32.74	43.94	2410	6629
F-test of A	NS	*	*	NS	NS	*	NS	*
LSD 0.05	2.48	1.8	0.16	67.5	3.18	0.85	767.5	1172.7
Nitrogen levels (kg/ha) (B)								
0	115	92	7.9	196.3	29.30	43.63	1387	3433
25	115	98	8.1	213.8	31.00	44.07	1868	4567
50	115	100	8.9	239.3	34.33	45.27	2120	5200
75	115	100	8.9	272.7	32.62	44.60	2707	6150
100	115	102	9.2	294.2	32.22	44.37	2698	6700
125	115	103	9.1	302.8	31.78	41.83	2897	6850
150	114	104	9.5	343.5	32.72	40.30	3073	7350
F-test of B	NS	***	***	***	**	**	***	***
LSD 0.05	2.11	3.4	0.78	48.02	3.87	2.66	365.6	580.9
Interaction								
F-test of A × B	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	1.5	2.9	7.5	15.1	10.1	5.1	12.8	8.5

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Effect of crop residues and nitrogen levels on growth and yield attributes of wheat, 2015

Similarly, analysis of data revealed that significant differences among different nitrogen levels was recorded in the year 2015 on plant height, spike length, spike per $m^{2,}$ grains per spike, thousand grain weight, grain yield and biological yield but effect was found to be non significant on days to maturity (Table 3). Nitrogen application @150 kg/ha gave greater significant (P<0.05) plant height (104 cm) which was at par with the application of 50 (100 cm), 75 (100 cm), 100 (102 cm) and 125 (103 cm) kg N/ha. Similarly, greater spike length of 9.5 cm and maximum number of spikes per square meter (343.5) were recorded in the plots treated with N @150 kg/ha. N application @ of 150 kg/ha yielded highest significant (p<0.05) grain and biological mean yield of 3073 and 7350 kg/ha respectively. No significant differences were observed in the interaction between crop residues and nitrogen levels.

Similarly, figure 1 shows that the grain yield as affected by crop residues was found higher in second year as compared to first year. Lower yield in first year might be immobilization of soil N in presence of crop residues with wide C/N ratio, but in later year, straw incorporation did not affect wheat yields adversely. Incorporation of rice straw into the soil after its harvest leads to slow down of the decomposition and soil nitrate is immobilized (Bacon et.al 1987), reducing the N uptake and yield of subsequent wheat crops by about 40% (Bacon et. al. 1987; Sidhu et. al 1989).

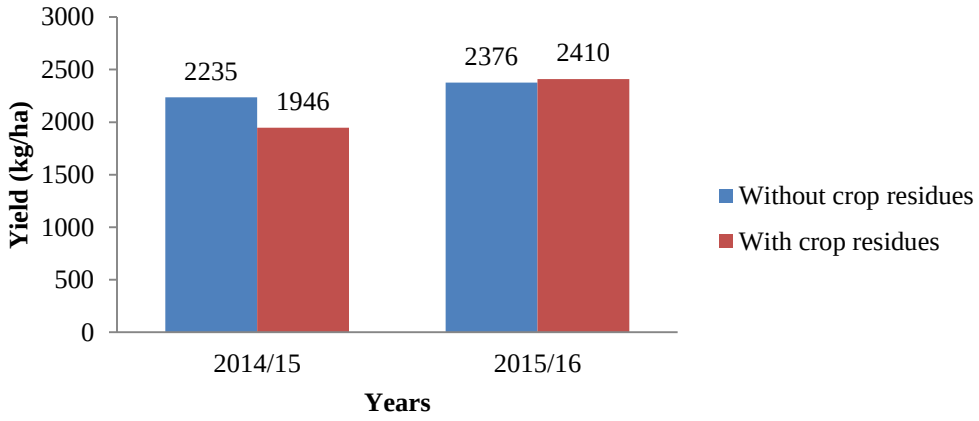

Fig.1: Comparison of mean grain yield of wheat as affected by crop residues, 2014/15 and 2015/16.

Grain yield was found highest with the application of Nitrogen @ 150 kg/ha in both years. While grain yield of wheat was obtained higher in second year as compared to first year in all levels of nitrogen (Figure 2).

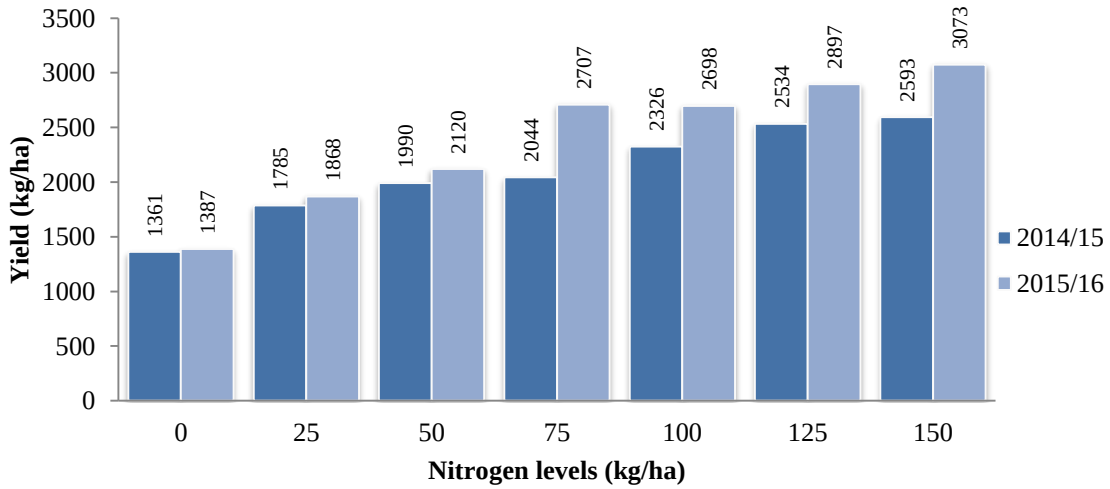

Fig.2: Comparison of mean grain yield of wheat as affected by nitrogen levels, 2014/15 and 2015/16.

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IV. CONCLUSION

Highest biological yield of both season wheat was observed in the plots with no incorporation of crop residues. Non significant differences on different plant growth and yield parameters were observed owing to very less decomposition of residues in the first year wheat production. Nitrogen application @150 kg/ha seemed beneficial in producing higher grain yield of wheat.

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Property Development and Land Use Planning Regulations in Nigeria

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***Abstract*—** *Several land use planning regulations have been enacted in Nigeria over the years to control property development so as to ensure sustainable human environment. Despite the existence of these regulations, property development is still being carried out in ways that constitute environmental challenges in cities. With samples drawn from Abia State, this study examined the level of compliance of property development with planning regulations in Nigeria. The study was based mainly on primary data which were collected through direct observation, questionnaires, and through measurement of geometric variables of the buildings and their immediate outdoor spaces. Cluster and simple random sampling techniques were used to proportionately select buildings and respondents that were surveyed. Data collected were analyzed with descriptive and inferential statistics. Specifically, the t - test for paired samples was used to test the hypotheses of the study. Findings show that the level of compliance of buildings to planning regulations is not significant, with mean compliance rate being less than 20%. It also reveals that there is significant difference in the level of compliance to planning regulations between buildings constructed in the urban areas (with mean compliance rate of 14.5%), and those constructed in suburban/ rural areas (with mean compliance rate of 42%). Certain factors were found to be responsible for the low level of compliance among which are low level of physical planning and inadequate funding for planning authorities. The researchers therefore recommend that government should embark on the preparation of up-to-date land use plans for various towns and villages; implement the autonomy of the town planning authorities; and create the enabling environment for effective development control across the country.*

***Keywords*— Abia State, planning regulations, property development.**

I. INTRODUCTION

Nigeria is one of the countries with high rate of urbanization in Sub-Saharan Africa, with many of her large towns growing at between 4 and 5% per – annum despite the economic downturn (Ogundele, et. al., 2011). As the cities are growing, buildings are springing up like mushrooms especially at the urban fringes, in agricultural land, and without formal planning or layout. Property development in Nigeria has evolved from crude indigenous structures which were fabricated with local building materials like mud, wood, and thatch during the pre-colonial/early colonial era, to sophisticated buildings designed to cover large expanses of land, with multiple floors, and advanced technologies/materials in present dispensation. The changing trend in property development has reflected the changing settlement structure occasioned by rapid urbanization globally. In some developed cities of the world like New York, London, Amsterdam, Beijing, Dubai, Tokyo, etc., property development has kept pace with urbanization trend hence the existence of high-tech buildings towering above fifty floors, and compensating adequately for the limitations posed by urban space inadequacy. But in most cities of the developing countries, Nigerian cities inclusive, technological development has not matched with rate of urbanization. Population explosion in cities has put urban housing under pressure, and property developers have had to maximize construction on their limited urban land without considerations to land use planning regulations and the implications of urban densification on environmental safety and convenience. Under such circumstances, property development in some cities of the developing countries has given rise to increased environmental challenges as exemplified by traffic congestion, flooding, overcrowding, and waste pollution. Underpinning the discipline of town planning and its instruments of land use regulation is the belief that allowing uncontrolled property development results in haphazard, and socially undesirable outcomes as mentioned above. State intervention is needed to curb and

shape market and human impulses, especially in land development, and this is the justification for land use regulations and development control by town planning authorities.

Land use regulations are rules which indicate how land in particular areas can be developed and applied (Goodfellow, 2014). Land use regulations serve the purpose of restricting development in order to give effect to urban plans. Land use planning regulations in Nigeria has its origins in British town planning activities that developed initially in response to the negative urban impacts of the industrial revolution. They were essentially aimed at improving health and safety by regulating overcrowding, pollution, inadequate services, facilities and amenities. The land use controls were intended to better organise urban space and produce ordered, safe, hygienic living environments (Ola, 2011). The British colonial administration used two major laws to achieve her planning objectives, and these were the 1917 Township Ordinance, and the 1946 Town and Country Planning Ordinance. Within the 1917 Township Ordinance the urban areas in Nigeria were divided into three classes of townships: the first class township of which Lagos was the only one at that time; the second class townships which were towns located on the rail lines; and the other towns which were regarded as third class townships. The 1946 Town and Country Planning Ordinance, which was fashioned from the 1932 Town and Country Planning Act in Britain, was meant to regulate the improvement and development of the different parts of Nigeria through planning schemes and planning authorities, (Arimah, & Adeagbo, 2000). The 1946 Town and Country Planning Ordinance became the mainstream legislation on land use planning in Nigeria for about 46 years, until it was replaced by the Nigerian Urban and Regional Planning law CAP 88 of 1992, which was later amended as Decree 18 of 1999. This legislation which is the extant law for physical planning in Nigeria conceptualized planning at the three tiers of government in Nigeria: Federal, State, and Local government, administered by three planning establishments: the Planning Commission, the Board, and the Planning Authority respectively. It equally assigned responsibilities of regulating property development to the Development Control departments of the various planning establishments. Other instruments used for land use regulation in Nigeria include: the national Building code 2006; land use zoning, minimum plot size and subdivision regulations promulgated by different state governments and the Federal capital territory Abuja. In Abia State, the local version of the urban and regional planning law of 1992 was enacted as the Abia State Planning Board and Planning Authority (ASPBPA) Law CAP 38 Volume II, 1999-2000. Based on this law, the public notice of March 7, 2006 that gave town planning form and impetus in Abia State was published (Umezuruike, 2015). This public notice established fifteen Town planning Authorities in the fifteen local government areas of Abia State, whereas the state capital territory continued to be administered by the Umuahia Capital Development Authority (UCDA). This marked the beginning of purposeful physical planning and development control in Abia State.

Despite the existence of these planning laws and regulations and the establishment of the planning authorities, there is a common perception in most states of Nigeria, particularly in Abia State, that property development is still being carried out in ways that constitute environmental challenges in cities (Aluko, 2011). It is believed that in new residential developments, internal and external space standards are being violated. It has been argued that property developers flagrantly contravene planning regulations in the course of development after they have duly secured planning approval, whereas some do not actually obtain approval before construction. A pilot study carried out by the authors in the year 2016 indicated general noncompliance to regulations relating to access and roads, building setbacks, building density, habitability of rooms, location and site plans, lot sizes, and parking. This has implications for both accessibility and sustainability, and for quality of life including health. Also, there has been growing concern that the internal space of new dwellings may be getting smaller, and that less family size housing is being provided; smaller sizes of windows, doors, internal storage spaces, and spaces for relaxation are being provided (compliance with internal space standards of buildings will be covered in subsequent studies). Unfortunately the level of compliance of property developments to land use planning regulations in Nigeria has not been empirically determined. Using geometric survey techniques and samples drawn from the seventeen local government areas of Abia State, this study therefore examined rate of compliance of property developments with town planning regulations in Nigeria, with the view to deriving recommendations that would guide government policy on development control.

II. THE STUDY AREA, ABIA STATE

Abia in south-east region became a State in the federal republic of Nigeria in 27th August 1991. Abia is located between latitudes 04°45' and 06° 07' north; and longitudes 07° 00' and 08° 10' east. It is bounded at the west by Imo State, at the south by Rivers State, at the north by

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Anambara and Ebonyi States, and at the east by Cross-River and Akwa-Ibom States. Abia State is made up of seventeen local government areas while the state capital is Umuahia. Abia State was among the first three states in Nigeria to domesticate the Nigerian urban and regional Planning Law CAP 88 of 1992 (Umezuruike, 2015), as the local version of the law (ASPBPA Law CAP 38 Volume II, 1999-2000) was passed in May 1999. This informed the choice of Abia State for this study. Figure1 is the map of Nigeria showing the thirty-six states and federal capital territory Abuja; and Abia State showing the seventeen local government areas. Upon the creation of Abia State in 1991 she inherited the Aba and Umuahia Area Planning offices from the old Imo State, and these two became the foremost planning agencies in the State. The Aba Area Town Planning Office superintended over the Aba town planning authority, the Obingwa town planning authority, Isialangwa town planning authority, and the Ukwa town planning authority. The Umuahia Area Town Planning Office supervised the Ikwuano/Umuahia town planning authority, the Isuikwuato town planning authority, the Bende town planning authority, and the Arochukwu/Ohafia town planning authority. The two area town planning authorities then at Aba and Umuahia coordinated physical planning activities at the eight planning authorities across the State, and reported to the director of planning, and then to the commissioner responsible for the ministry of lands, survey and urban planning. The passage of the Abia State Planning Board and Planning Authority law in 1999 abolished this old arrangement and made the whole of Abia State a planning area. It also established Town Planning Authorities in all the local government areas, with UCDA taking care of Umuahia north and south local government areas. However, the state planning board as envisioned by the law (CAP 38) is yet to be established till date, hence the department of planning under the act is currently operating under the auspices of the ministry of physical planning and urban renewal.

III. MATERIALS AND METHODS

The study was based mainly on primary data which were collected through direct observation, sampling of questionnaires, and through measurement of geometric variables of the buildings and their immediate outdoor spaces. The researchers adopted a triangulation of survey designs involving sampling of questionnaires, geometric survey, and oral interview. The geometric variables of buildings, their streets and outdoor spaces were measured using a handheld distance laser (SPECTRA QM55), and measuring wheels. Questionnaires were sampled on staff of the town planning authorities, while oral interviews were conducted on some developers.

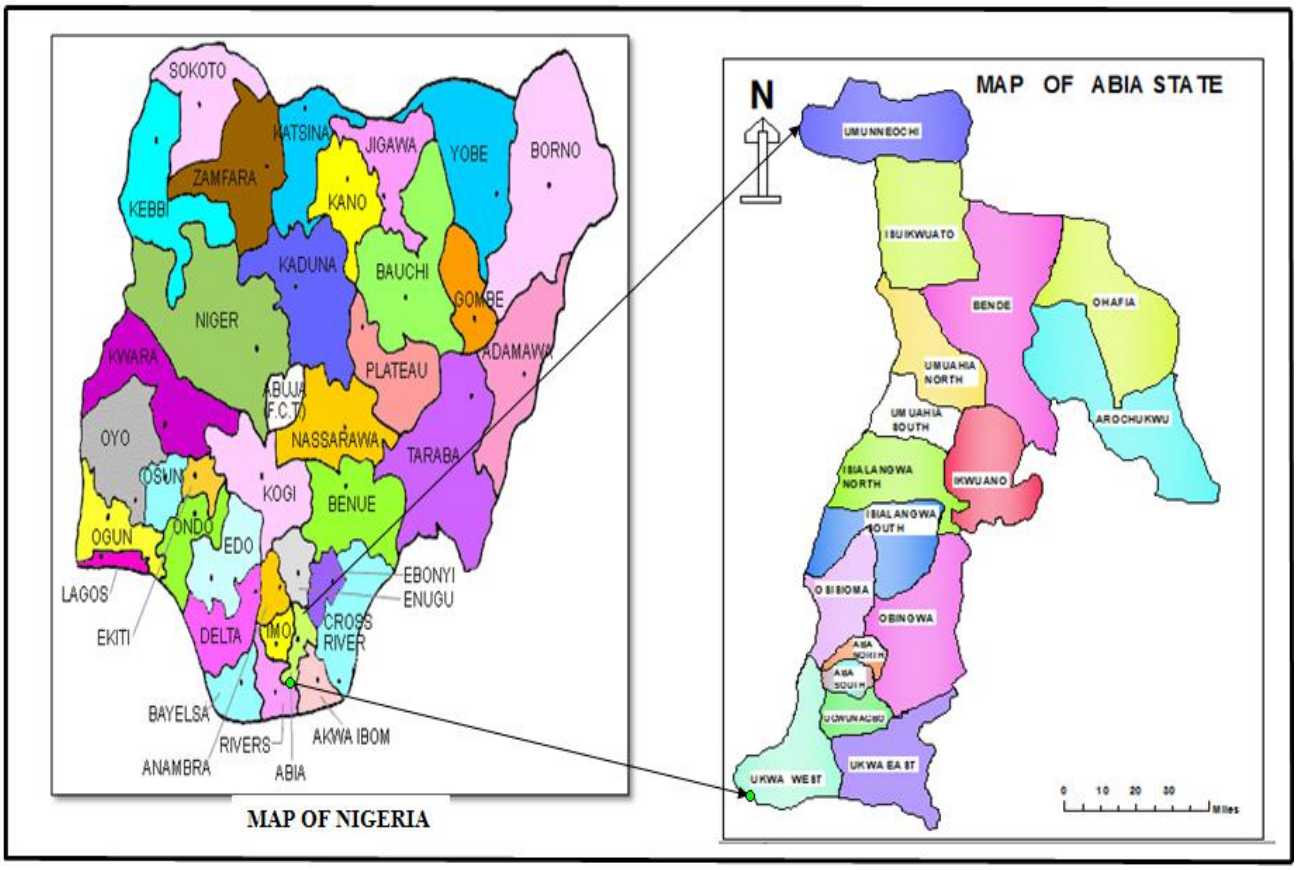

Fig.1: Map of Nigeria/ map of Abia State showing the seventeen local government areas

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The population of study is classified into two: the buildings constructed in Abia State in the past ten years; and the total number of planning staff in the town planning authorities in the state. The buildings constructed in Abia State within the past ten years (2006 – 2016) amounted to 31,099. The study adopted this time frame because it represents the period in which active town planning has taken place in the state following the public notice of March 7, 2006 that marked the implementation of the ASPBPA Law CAP 38 of 1999-2000. The population of professional planning staff in all the town planning authorities in the state is 64. These population data were collected from the town planning authorities in fifteen local government areas of Abia State and the UCDA. For the buildings, the sample size of approximately 156 was estimated from the population; and for the planning staff the sample size of 45 was also estimated using the model derived by Miller and Brewer (2003). Cluster sampling technique was used to divide the study area into sixteen regions following the local government territorial structure/ planning authorities, and a given number of buildings and planning staff were selected from each region proportionately, with regard to their respective populations (see table 1). Simple random sampling method was then used to select the buildings where measurements were carried out as well as the planning staff that were sampled questionnaires. Data collected were analyzed with appropriate parametric tests using SPSS for Windows, Version 17. Specifically, the *t* - test for paired samples was used to test the hypotheses, and P value of ≤ 0.05 was considered statistically significant.

IV. RESULTS AND DISCUSSION

4.1 Major Land Use Planning Regulations in Abia State

The extant land use planning regulations in Abia State are part of national planning regulations for physical planning, and building codes in Nigeria; as well as other regulations enacted at the state level through the ASPBPA Law CAP 38 1999-2000, the Umuahia Capital Development Authority law No 8 of 1992, and other regulatory standards in the relevant state ministries. Some of the major land use regulations are as follows.

i. Land use zoning: Regulations which segregate land into separate and often singular uses, such as residential, commercial, industrial, residential/commercial, and recreational. Zoning is usually articulated as part of layout schemes. Within each zone, particular activities are allowed or prohibited.

ii. Building set-backs and height requirements, including fencing requirements: The distance between any residential building and property boundary (beacons) at the frontline should not be less than 6metres with 3metres at the rear, right and left side airspaces respectively in all government reservation areas (GRA) and all private approved layouts. Building set-backs from road centreline for different categories of roads are: Highways (18m); Primary roads (14m); Secondary roads (10m); residential collector roads (8m); residential access roads (8m).

iii. Minimum plot size and subdivision regulation: Constraints relating to the minimum size which plots can be, and rules and laws pertaining to the subdivision of land into smaller plot sizes. These regulations aim to prevent excessive densities. High density plots are to be between $450m^2$ to $600m^2$; medium density plots ($600m^2$ – $750m^2$); and low density plots to be $750m^2$ up to $1,200m^2$

iv. Floor area ratios and limits (FAR): Floor Area Ratio is a measure of development intensity, which is expressed as a ratio of the gross floor area of a building to its total land area (net). The purpose of this ratio is to control the bulk of a building and intensity of activity to a level, which is consonant with the level of existing or proposed infrastructure facilities. The FAR is generated by dividing the building floor area by the plot area. The recommended floor area ratios are: Residential = 1:1 (high density); Commercial = 1:3; Industrial = 1:0.75 and Community facilities = 1:0.75

v. Plot Coverage: It measures the percentage of the total floor area of the plot covered by building. For high density area the maximum plot coverage is 50%; Medium density 40%, and low density 30%.

vi. Infrastructure standards (for soft and hard infrastructures): Minimum standards or guidelines for the provision of infrastructures (e.g. street width, public space, service levels). Any thoroughfare or public way shall not have right-of-way less than 10.0m in width (i.e. 6.4m for vehicles, 0.6m and 1.2m for drainage and pedestrian walkway on both sides respectively) which has been dedicated or deeded to the public for public use.

vii. Post - construction requirements: Certificate of fitness for habitation; As-Built Drawings; Changes in use and habitation. These are statutory documents to be submitted to the town planning authorities by the developer, in which the post construction state of the building and any possible change of use are

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assessed by the appropriate authorities, and duly certified.

4.2 Buildings Constructed in Abia State between 2006 and 2016

Data on total number of buildings constructed in Abia State in the past ten years were collected from the town planning authorities in the state and Umuahia Capital Development Authority (UCDA), and are presented on table 1. It shows that a total of 31,099 buildings have been built within the period, with only 8,431 (27.1%) of the building having obtained planning approval or undergoing the process of obtaining approval. This implies that about 72.9% of all properties developed in the state do not have development permit and are therefore in the contravention of land use planning regulations in the state. Table one also shows that the territory under UCDA recorded more growth in terms of number of buildings constructed within the period (28%), followed by Osisioma region (21%), Aba-north (6.8%), Obingwa (6.2%), and Aba-south region (6.0%). Regions with the least growth rate in property development are: Umunneochi (1.8%); Ukwa-west (2.0%); Ukwa-east (2.1%); and Bende (2.8%).

Table.1: Buildings developed in Abia State between 2006 and 2016

S/N	Local Government Area	Number of Buildings	Number of buildings with planning approval	% Number of buildings with approval	% of Total buildings	Sample size
1	Aba North	2100	786	37.4	6.8	11
2	Aba South	1852	801	43.3	6.0	9.4
3	Arochukwu	932	155	16.6	3.0	5
4	Bende	861	102	12.5	2.8	4
5	Ikwuano	1617	334	20.6	5.2	8
6	Isiala Ngwa North	1134	173	15.3	3.7	6
7	Isiala Ngwa South	1098	151	13.8	3.5	6
8	Isuikwuato	922	87	9.4	3.0	5
9	Obingwa	1914	448	23.4	6.2	10
10	Ohafia	772	113	14.6	2.5	4
11	Osisioma	6540	1720	26.3	21.0	33
12	Ugwunagbo	814	120	14.7	2.6	4
13	Ukwa East	655	73	11.1	2.1	3
14	Ukwa West	613	89	14.5	2.0	3
15	Umuahia Capital territory	8710	3214	36.9	28.0	44
16	Umunneochi	569	65	11.4	1.8	3
	Total	31,099	8,431	27.1	100	156

Source: Authors' Field Survey, 2017, Compiled from town planning authorities in Abia State

Figure 2 illustrates the percentage number of building with planning approval in each local government area of Abia state. The chart shows that Aba-south L.G.A has the highest percentage buildings with planning approval (43.3%) followed by Aba-north (37.4%) and Umuahia Capital Territory (36.9%). Incidentally these are the core urban areas of Abia State. The local government areas that recorded the least number of buildings with planning approval are Isuikwuato (9.4%); Ukwa-west (11.1%); and Umunneochi (11.4%). Generally the chart shows that the average percentage of buildings with planning approval in Abia State is less than 20%, and this indicates an abysmal failure of the town planning authorities in their development control responsibilities.

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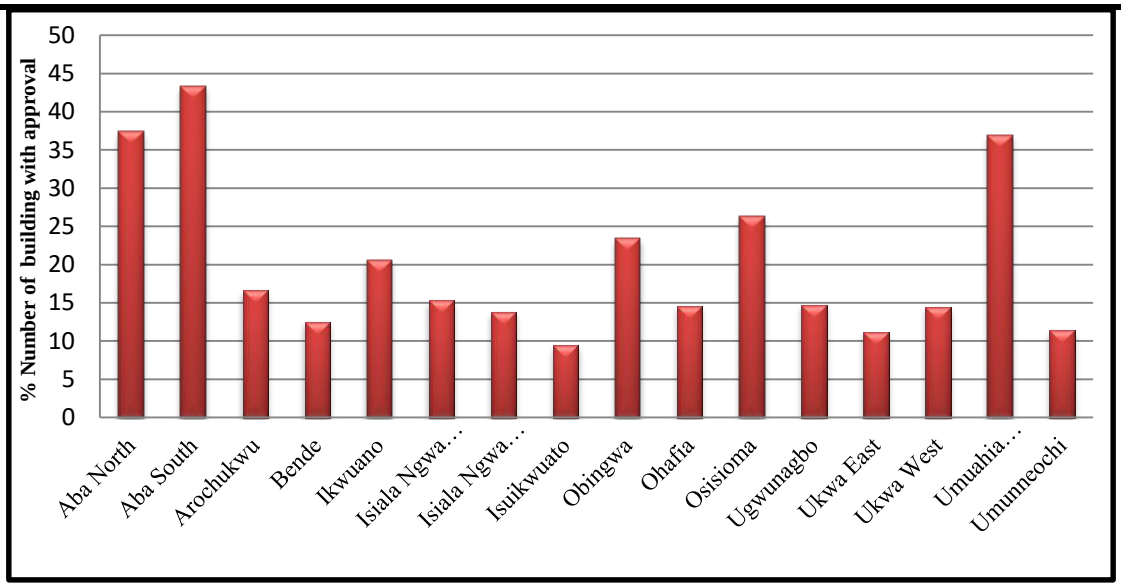

Fig.2: Percentage number of building with planning approval per local government area

4.3 Level of Compliance of Buildings to Planning Regulations in Abia State

The study examined the level of compliance of 156 randomly sampled buildings to a set of 9 land use planning standard in Abia State, and the data collected are shown on appendix –C, and have been summarized on table 2. The results could be reviewed as follows.

i. Set-back from road centreline: this regulation recorded 55.8% compliance. But a careful look at the data on table 3 reveals that 80% of the building that met this standard is in the rural areas, and this was because of the homestead settlement pattern practice in rural areas in Abia Sate. If the urban areas are taken in isolation, the rate of compliance to this standard falls below 20%.

ii. Set–back from property boundaries: the compliance rate to this standard is 39%. In considering properties that met this standard, every building in which its set-backs from property boundary are up to minimum standard in three out of the four directions of property line was considered to have complied with the regulation. Also, majority of the buildings that complied with the standard were in the rural areas.

iii. Floor Area Ratio: this showed a compliance rate of 91.6%. The significant compliance recorded on this standard was not as a result of enforcements, but rather a natural outcome since majority of the buildings fall in the category of bungalows, followed by one storey buildings. High rise buildings (those exceeding four floors) are not common in Abia State.

iv. Road/ Street Right-of-way: the compliance rate to this standard was very low (20%) across the state. Most of the roads are narrow. Some of the roads only have the carriage ways but lacking road shoulder, sidewalk, drainage channel, and utility lane, etc. The suburban and rural roads were worse off with less than 5% compliance. Some settlements were built along narrow roads that may simply be regarded as footpaths. Greater percentage of the roads is un-tarred and in very bad shapes.

v. Plot Coverage: this standard recorded 69.2%. The comparatively high compliance rate here was also as a result of buildings in suburban and rural areas, which have the pleasure of larger plots of land. But when the urban areas are taken in isolation, the compliance rate fell below 15% as can be seen from table 3.

vi. Zoning standard has 66.7% compliance, and again, it is also skewed in favour of rural areas which are purely residential and agricultural. Significant number of buildings in the urban areas violated zoning standard, especially with commercial land use playing prominent role in the city of Aba.

vii. Plot size standards showed 66.7% compliance. Buildings in the high density areas presented better compliance than those in the medium densities (Suburbs) and low density (rural) areas. This is because, government developed layouts are very few in number, whereas most developers buy land from private land holders whose concept of plot size is between 450 to 465 square meters irrespective of the density it falls.

viii. Certificate of fitness for habitation/ as Built Drawings (0.0%): the survey showed that no building complied with these standards. In fact, the town planning authorities were in ignorance of this

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regulation and therefore did not enforce it. Perhaps, this partly underscores the reason why developers freely modified their plans in the course of implementation after they had been given development approval.

ix. Change of use permit: significant percentage of the buildings has not changed usage since they were built. However for the few that changed, only 22.2% obtained change of usage permit, the rest did not.

Table.2: Compliance of buildings to planning regulations

S/N	Planning Standards	Minimum Standard	Number that met standard	Number that failed standard	Percentage compliance
1	Set-back from road centreline	*18m/ 14m/ 10m/ 8m	87	69	55.8
2	Set –back from property boundaries	**6m/ 3m/ 3m/ 3m	39	117	25
3	Floor Area Ratio	$^{+}$1:1 / 1:3 / 1:0.75	143	13	91.6
4	Road/ Street Right-of-way		31	125	20
5	Plot Coverage	$^{++}$ 50% / 40% / 30%	108	48	69.2
6	Plot size	$^{\#}$450 / 600 / 750m^2	75	81	48.1
7	Zoning		104	52	66.7
8	Certificate of fitness/ as Built Drawings	To be obtained by developer and submitted to town planning authority	0	156	0.0
9	Change of use	$^{@}$ N	6	21	22.2
Notes	* 18m for Highways; 14m for primary roads; 10m for secondary roads; and 8m for residential access roads				
	** 6m Front of property, 3m at rear of property; and at both sides of property				
	$^{+}$ 1:1 for residential high density; 1:3 for commercial; and 1:0.75 for industrial/ community buildings				
	$^{++}$ 50% for High density area; 40% for medium density; and 30% for low density				
	$^{\#}$ 450m^2 for high density; 600m^2 for medium density; and 760m^2 for low density areas				
	$^{@}$ N = not applicable, buildings which have not changed use = 129				

Source: authors' field survey 2017.

The study proceeded with the available data, to determine the significance of the rate of compliance to the planning regulations by property development in Abia State. Therefore a hypothesis was formulated thus: *Ho*, the level of compliance of property development to planning regulations in Abia State is not statistically significant. The *t* test for paired samples was performed to prove the hypothesis. The result is displayed in Appendix - A, and it showed ***t* = - 0.352**, and *P* value of **0.734,** which is not statistically significant ($P > 0.05$). Hence we did not reject *Ho*, which affirms that the level of compliance of property developers to land use regulations in Abia State is not significant. The mean compliance rate was 19.7%.

The study also considered the disparities in level of compliance to the planning regulations between urban areas and suburban/ rural areas in Abia State. The major urban areas in Abia state are Aba, and Umuahia, and parts of Osisioma and Obingwa. Total number of buildings sampled in urban areas is 77, while the building sampled in suburban/ rural areas amount to 79. Table 3 shows the result of this analysis. The findings reveal that properties in suburban/ rural areas have considerably higher level of compliance with set-back from road centreline regulations, plot coverage, set–back from property boundaries, floor area ratio, and zoning regulations than properties in urban areas. Properties in urban areas only showed better compliance rate on road/ street right-of-way standards, and plot–size regulations. The study further formulated a second hypothesis to test the significance of these variations as follows.

Ho: there is no significant difference in the level of compliance to planning standards between properties developed in the urban areas, and those in suburban/ rural areas.

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Table.3: Comparison of level of compliance to planning regulations between urban and suburban/ rural areas

S/N	Planning standard	Total number of buildings sampled	Number that complied with Standard		
			Urban Areas	Suburban / Rural areas	total
1	Set-back from road centreline	156	28	59	87
2	Set –back from property boundaries	156	13	26	39
3	Floor Area Ratio	156	62	81	143
4	Road/ Street Right-of-way	156	26	5	31
5	Plot Coverage	156	34	74	108
6	Plot size	156	53	22	75
7	Zoning	156	34	70	104
8	Certificate of fitness/ as Built Drawings	156	0	0	0
9	Change of use	156	4	2	6
	Total	*77 / 79	216	375	591
Notes	* 77 represents total number of buildings sampled in urban areas, while 79 is the number sampled in suburban/rural areas				

Source: authors' field survey 2017.

A *t* - test for paired samples was performed to prove the hypothesis (*Ho*): there is no significant difference in the level of compliance to planning standards between properties developed in the urban areas, and those developed in suburban/ rural areas. The result is shown in Appendix– B, and it presents ***t* = - 2.380**, and *P* value of **0.045** which is statistically significant ($P < 0.05$). Hence we reject *Ho*, signifying that there is significant difference in the rate of compliance to planning standards between buildings constructed in the urban areas, and those constructed in suburban/ rural areas. Buildings constructed in the urban areas showed mean compliance rate of 14.5%, and those constructed in suburban/ rural areas showed mean compliance rate of 42%. It is however observed that the higher level of compliance recorded in the rural areas is not as a result of development control but rather a natural adaptation of developers to more spacious land, which will eventually phase-out with increased urbanization. What this means is that, timely intervention in the suburban/rural areas to correct these planning aberrations through preparation and implementation of planning schemes would be of great benefit.

This is because, as these places get urbanized, the environmental challenges created by poor planning multiply, and may possibly reach catastrophic stages.

4.4 Factors Responsible For Low Level of Compliance to Planning Regulations

The study conducted a survey of 45 town planning officers in Abia state with structured questionnaires to determine the factors responsible for the low level of compliance to planning regulations, and the extent to which planning authorities carry out their statutory planning functions. The results are shown on table 4 and 5 respectively. Table 4 reveals the following factors responsible for low level of compliance, in order of importance.

i. Low level of physical planning. A 100% of the respondents identified low level of physical planning in Abia State as important reason for low compliance to planning regulations. They opined that planning in Nigeria and Abia State in particular, is presently synonymous with development control at its best. Other primary responsibilities of planning like preparation of planning schemes; land acquisition and creation of layouts; urban renewal and redevelopment are completely neglected. Planning in Nigeria is merely reactive rather than being proactive. Under this circumstance, there is no proper framework for planning regulation like the master plan or other planning schemes. Approved planning schemes are the fundamental basis for development control, and where they are lacking every other planning regulation lacks the basis for enforcement.

ii. Inadequate funding for planning authorities. 98.5% of respondents identified this factor as very important. The town planning authorities grapple with low funding from the ministry, resulting to their inability to pay staff salaries and to undertake planning activities.

iii. Enforcement risks. More than 90% of the respondents identified risks of mob attack during enforcement as a major hindrance to development control. The planning authorities are not attached with police unit thereby rendering the enforcement

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officers vulnerable to mob attack during field operations.

iv. Selective implementation of regulations (90.6%). The planning authorities simply focus on planning duties that generates fund without necessarily ensuring that developers adhere to standards. The planning authorities today are simply revenue collectors for government.

v. High cost of approval fees. About 80% of respondents indicated that the relevant state government fees charged for plan approval are very high relative to the economic conditions of an average developer in the state. It is common occurrence for developers to make only part payment as to initiate approval process, and thereafter commence development without having to come afterwards to complete their payment. This accounts to why greater numbers of buildings do not have planning approval.

vi. Court cases. More than 80% of respondents alluded to the fact that court litigations are often used by developers to frustrate enforcement of planning regulations.

vii. Political interference. About 70% of respondents indicated that interference by political actors in the ministries play a major role in frustrating planning regulation. Highly placed individuals often use their political connections to influence planning authorities over their properties which are in contravention of planning regulations.

Table.4: Factors responsible for low level of compliance to planning regulations

S/N	Factors	Number of / % Responses					
		Not important	Slightly important	Uncertain/ No answer	important	Very important	Total Repons
1	Poverty of residents	*31 / **69	13 / 29	1 / 2.2	0 / 0	0 / 0	45
2	Ignorance of residents	9 / 20	16 / 35.9	4 / 9.4	16 / 35.9	0 / 0	45
3	High cost of approval fees	0 / 0	0 / 0	3 / 6.3	36 / 80	6 / 12.5	45
4	Non flexibility of regulations	0 / 0	5 / 12.5	0 / 0	33 / 72	7 / 15.6	45
5	Selective implementation	0 / 0	1 / 2.1	0 / 0	41 / 90.6	3 / 6.3	45
6	Lack of up-to-date maps	0 / 0	0 / 0	0 / 0	11 / 24.5	34 / 75.5	45
7	Inadequate funding	16 / 35.8	11 / 25	8 / 17.2	10/ 22	0 / 0	45
8	Political interference	2 / 4.4	9 / 18.8	2 / 4.4	32 / 70.3	0 / 0	45
9	Shortage of professional staff	35 / 76.8	10 / 22	1 / 2.2	0 / 0	0 / 0	45
10	Low level of physical planning	0 / 0	0 / 0	0 / 0	20 / 44.4	25 / 55.6	45
11	Corruption of planning staff	1 / 2.1	15 / 32.8	10 / 22	19 / 42.2	0 / 0	45
12	Enforcement risks	0 / 0	0 / 0	0 / 0	4 / 9.4	41 / 90.6	45
13	Court cases	0 / 0	3 / 6.6	1 / 2.1	36 / 79.3	5 / 11	45
14	Delay in obtaining planning approval	1 / 2.2	4 / 8.9	1 / 2.2	31 / 68.8	8 / 17.8	64
	* number of responses; ** Percentage responses						

Source: authors' field survey 2017.

Among all the statutory duties of the town planning authorities, they only carry out development control and a little of staff improvement as can be seen on table 5. Their core duties which include plan preparation: creation of subdivision plans and other planning schemes to guide development; and urban renewal are not being carried out as responses on table 5 show. The primary reasons given by the authorities for this negligence are poor funding, lack of equipment, and lack of the enabling environment by government. This has far reaching implications as it makes it very difficult for planning authorities to enforce the planning regulations within a holistic statutory framework. Moreover, development control activities are simply reduced to revenue collection for government while illegal developments are allowed to go on.

The study further utilized oral interview survey to ascertain reasons why a good number of developers submit their plan to town planning authorities and yet do not follow it up to secure approval. Respondents identified five major reasons for this, and they are: high cost of fees charged for plan approval; bureaucratic bottleneck and unnecessary delays in obtaining approval; poverty and low income capacity of average developers in the country; corruption of planning staff, generally high level of ignorance of residents to planning requirements.

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Table.5: The extent to which planning authorities carry out their statutory planning functions

S/N	Planning responsibility	Number of / % Responses			
		Not a all	Very Slightly	Regularly	Total Reponses
1	Plan Preparation (Subdivision plans, Layout plans, etc)	*39 /**86.7	6 / 13.3	0 / 0	45
2	Development control	0 / 0	0 / 0	45/ 100	45
3	Urban renewal	31 / 60.9	14 / 39.1	0 / 0	45
4	Land Acquisition/ payment of compensation	45 / 100	0 / 0	0 / 0	45
5	Staff improvement	0 / 0	36 / 80	9 / 20	45
6	Research and Development	45 / 100	0 / 0	0 / 0	45
* number of responses; ** Percentage responses					

Source: authors' field survey 2017.

V. CONCLUSION AND RECOMMENDATIONS

The study examined the level of compliance of property development to land use planning regulations in Nigeria using samples drawn from Abia Sate. Findings show that the level of compliance of buildings to planning regulations is not significant. The mean compliance rate was less than 20%, and the planning regulations which recorded very low compliance are: set –back from property boundaries; road/ street right-of-way; plot coverage; plot size; certificate of fitness for habitation/ as built drawings; and change of use standards. Findings also show that there is significant difference in the rate of compliance to planning regulations between buildings constructed in the urban areas, and those constructed in suburban/ rural areas. Buildings constructed in the urban areas showed mean compliance rate of 14.5%, and those constructed in suburban/ rural areas showed mean compliance rate of 42%, and the better compliance shown by the latter is because of ample land spaces available in rural areas as well as the homestead settlement pattern that is practiced there. This result implies that development control activities of the planning authorities have failed to deliver a sustainable and functional built environment, and therefore needs to be re-examined. It also means that timely intervention in the suburban/rural areas to correct these planning aberrations through preparation and implementation of planning schemes would be of great benefit.

The study ascertained factors which are responsible for the low level of compliance to planning regulations as follows: low level of physical planning; inadequate funding for planning authorities; enforcement risks; high cost of approval fees; court cases; and interference by the political class. The study therefore recommends the following. Firstly, government should embark on the preparation of an up-to-date land use plan, and strategic plans for various towns and villages, including its utilities and facilities. This will effectively guide growth and development in a more sustainable manner, and provide the basic framework for a more realistic development control. Government as a matter of urgency should prepare and implement planning schemes for all fast growing suburbs and rural areas in Nigeria before urbanization fully catches up with them, while aggressive urban renewal should be used to correct the environmental challenges already created in the cities. Secondly, government should implement the autonomy of the town planning authorities as required by law, and ensure their funding through direct subvention as against the present situation where they are mere appendages to the ministries. Thirdly, the necessary logistics for the efficient functioning of the planning authorities (utility vehicles, tractors, and professional manpower) should be provided. There is also the need to create a police unit in the planning authorities to function with development control officers so as to minimize enforcement risks. Fourthly, government should cause there to be enforced the regulation requiring developers to carryout post construction assessment of their building, and prepare certificate of fitness for habitation and As-Built Drawings for submission to the planning authorities, as prerequisite for occupancy. This will greatly reduce the tendency of developers to deviate from their approved plans during implementation. Approval of development plan does not guarantee effective control of the built environment. It is just a part of the overall process of exercising control over the physical environment. Development control should end with the implementation of the approved plan, the use to which such structure is put into and the preservation of such structure in line with the planning scheme for such area. Fifthly, government should create the enabling environment for developers to be sensitized about the need to protect the environment by ensuring that their plans are approved prior to commencement of development. In this regard, government should place primacy on achieving

sustainable environment over revenue derived from plan approval. Fees charged in Nigeria to obtain development permit is very high and discourages an average developer. Part of the enabling environment would be to minimize political interference in planning duties, as well as reforming the judicial system to eliminate unnecessary technical grounds used by the courts to frustrate development control. Finally, the administrative machineries of the town planning authorities should be reformed to eliminate unnecessary bureaucracies in the process of plan approval, and to eradicate corruption.

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Appendix - A -

Paired Samples Statistics

			Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Building that met Standards		65.6667	9	49.76445	16.58815
	Buildings that did not meet Standards		76.0000	9	48.33994	16.11331

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Building that met Standards - Buildings that did not meet Standards	-10.333	88.19014	29.39671	-78.12227	57.45561	-.352	8	**.734**

Appendix - B –

Paired Samples Statistics

			Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Building that complied to standard in urban areas		24.7778	9	18.57268	6.19089
	Building that met standard in Suburban/ rural areas		41.1111	9	33.01683	11.00561

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		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Building that complied to standard in urban areas - Building that met standard in Suburban/ rural areas	-16.333	20.59126	6.86375	-32.16118	-.50549	-2.380	8	.045

Appendix C

S/N	Building Location	planning Approval	Setback Road	Setback Ppty Boundary	Floor Area Ratio	Road right-of way	Plot Coverage	Plot Size	Zoning	Certificate fitness/ as Built Drawing	Change of use
1	Nicholas street	1	0	0	1	1	0	1	1	0	N
2	Brass road	1	1	0	0	1	0	1	0	0	N
3	Diobu street	1	1	0	0	1	0	0	1	0	N
4	Eziama	1	0	0	1	1	0	0	1	0	N
5	Margaret Ave	1	0	0	1	0	0	0	0	0	0
6	Aba –Owerri Road	1	0	1	1	1	0	0	1	0	N
7	Railway	0	0	0	1	0	0	0	0	0	N
8	Behind PZ	1	1	0	1	0	0	0	0	0	N
9	Factory Road	1	1	1	0	1	1	0	0	0	N
10	Old GRA	1	1	0	1	1	1	1	0	0	0
11	Osusu	1	0	0	1	0	0	0	1	0	0
12	Ebenma	1	0	0	1	1	0	0	0	0	N
13	Industrial Layout	1	1	0	1	1	0	0	0	0	1
14	Umuola Road	1	0	0	1	1	0	0	0	0	N
15	Ukegbu Road	1	0	0	1	0	1	0	0	0	N
16	7 UP	0	0	0	1	0	0	0	1	0	N
17	Eziukwu	1	1	0	1	1	0	0	0	0	0
18	Milverton Road	1	1	0	0	1	0	0	1	0	0
19	Asa Road	1	1	1	0	1	0	0	0	0	N
20	Ngwa Road	0	0	0	1	0	0	0	0	0	0
21	East Road	0	0	0	1	0	1	0	0	0	N
22	People's Road	0	0	0	1	0	0	0	0	0	N
23	Nnentu	0	0	0	1	0	0	0	1	0	N
24	Umuagbai	1	0	0	1	0	0	0	1	0	N
25	Cemetery	1	0	0	1	0	1	1	0	0	0
26	Ihieorji	0	0	0	1	0	0	0	0	0	0
27	Azikiwe Road	1	1	1	0	1	0	1	0	0	0
28	Nkwo Ngwa	0	0	0	1	0	0	1	0	0	N
29	River Layout	1	1	0	1	1	0	1	0	0	N
30	Ndi Orji	0	0	0	1	0	0	1	1	0	N
31	Ndi Ama	0	1	0	1	0	1	1	1	0	N
32	Abam	1	1	0	1	0	1	0	1	0	N
33	Obinkita	0	1	1	1	0	1	0	1	0	N
34	Umuchi	1	0	0	1	0	0	1	1	0	N
35	Amuvi	1	0	1	1	0	1	0	1	0	N
36	Alayi	0	1	0	1	0	1	0	1	0	N
37	Egwueke	0	1	0	1	1	1	1	1	0	N
38	Onu Ibina	0	1	0	1	0	1	1	1	0	N
39	Eluokwu	0	1	0	1	0	1	0	1	0	N
40	Amaokwe Item	1	0	0	1	0	1	0	1	0	N
41	Okoko Item	0	1	0	1	0	0	1	1	0	N
42	Oloko road	1	1	0	1	1	1	0	1	0	N
43	Inyila	0	1	1	1	0	1	1	1	0	N
44	Ngwugwo	0	0	0	1	0	1	1	1	0	N
45	Amawom	0	0	0	1	0	1	1	1	0	N
46	Umudike	1	0	0	1	0	0	1	1	0	N

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47	Ogbuebule	1	0	0	1	0	1	1	1	0	N
48	Ariam	1	1	1	1	0	1	1	1	0	N
49	Okwe	1	1	0	1	0	0	1	1	0	N
50	Eziama Nsulu	0	0	0	1	0	1	1	1	0	N
51	Umuosu	1	1	0	1	0	1	1	1	0	N
52	Eziala	0	1	0	1	0	1	1	0	0	N
53	Osusu Isialangwa	0	1	1	1	0	1	1	1	0	N
54	Amapu Ntigha	1	1	0	1	0	1	1	1	0	N
55	Umuoha	0	1	1	1	0	1	1	1	0	N
56	Ihie	1	1	0	1	0	1	1	1	0	N
57	Umuekea	1	0	0	1	0	1	1	1	0	N
58	Egbelu Mbutu	0	1	0	1	0	1	1	1	0	N
59	Mbutu Ngwa	0	1	0	1	0	1	1	1	0	N
60	Nneise	0	1	1	1	0	1	1	1	0	N
	NOTE: * 0 = Building does not comply to minimum standard/ Not available ***N = , Not applicable ** 1 = Building complied to minimum standard / Available 0										

S/N	Building Location	planning Approval	Setback Road	Setback Ppty Boundary	Floor Area Ratio	Road right-of-way	Building Coverage	Plot Size	Zoning	Certificate of fitn/as Built Drawings	Change of use
61	Umuoba	1	0	0	1	0	0	0	1	0	N
62	Okpuhie	0	1	1	1	0	0	1	1	0	N
63	Isieketa	0	1	0	1	0	1	1	1	0	N
64	Ahaba	1	0	0	1	0	1	1	1	0	N
65	Ovim	1	0	0	1	0	1	1	1	0	N
66	Umuokogbue	0	0	0	1	0	1	1	1	0	N
67	Amaiyi Uhu	0	0	0	1	0	1	1	1	0	N
68	Umunnekwu Agbo	0	1	0	1	0	0	1	1	0	N
69	Osusu Amaukwa	1	1	1	1	0	0	1	1	0	0
70	Ukpakiri	0	1	0	1	0	0	1	1	0	N
71	Mgboko	1	0	0	1	0	0	1	1	0	N
72	Umuariama	1	0	0	1	0	0	0	1	0	N
73	Mgboko Itungwa	0	1	1	1	0	1	1	1	0	N
74	Ovom 1	1	0	0	1	0	0	0	1	0	N
75	Umuobiakwa	0	0	1	1	0	1	0	1	0	N
76	Osaa Ukwu	0	0	1	1	0	1	1	1	0	N
77	Umuagu	1	1	1	1	0	0	0	1	0	N
78	Ohanze Isiahia	0	1	0	1	0	1	1	1	0	N
79	Agburuike Isiugwu	0	1	1	1	0	1	1	1	0	N
80	Umuaro	0	1	0	1	0	1	1	1	0	N
81	Isiama Ohafia	1	1	0	1	0	1	0	1	0	N
82	Ebem Ohafia	1	1	0	1	0	1	0	1	0	N
83	Ugwujinba	0	1	0	1	0	1	0	1	0	N
84	Erinma Abiriba	0	0	0	1	0	1	1	1	0	N
85	Ndi Icho	0	1	0	1	0	1	0	1	0	N
86	Ndi Agbo Nkporo	0	1	1	1	0	1	1	1	0	N
87	Umuojima	1	1	0	1	0	0	0	1	0	N
88	Abayi Umungasi	1	0	1	1	1	0	0	1	0	0
89	Aro Ngwa	1	1	0	0	1	0	0	0	0	N
90	Osokwa	0	0	0	1	0	0	1	1	0	0
91	Amapu Ife	1	1	1	1	0	0	1	0	0	N
92	Okpu Umuobo	1	0	0	1	0	0	0	0	0	0
93	Ahiaba Umueze	1	0	0	1	0	0	0	0	0	N
94	Okpuala Umuogor	0	0	1	1	0	1	1	1	0	N
95	Umuokorocha	0	0	0	1	0	1	0	1	0	N
96	Urrata	1	0	1	0	0	0	0	0	0	0
97	Ekeakpara	0	1	0	1	0	0	1	1	0	N
98	Umule	1	0	0	1	0	1	0	0	0	N
99	Amasato	1	1	0	1	0	0	0	0	0	N
100	Tonimas	1	0	0	0	0	0	0	0	0	0
101	Umuode	1	0	0	1	0	0	0	0	0	N
102	Flyover	1	0	0	1	0	0	0	0	0	0
103	Ezenwagbara rd	1	0	1	1	0	0	0	0	0	N
104	Enyinba road	1	0	0	0	0	0	0	0	0	0
105	Ala Ojii	0	1	0	1	0	1	1	1	0	N
106	Alozie street	1	1	0	1	0	1	0	1	0	N
107	Samek Road	0	1	0	1	0	1	0	1	0	N

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108	Owerri Aba	1	0	0	1	0	0	0	1	0	N
109	Akanu Ngwa	0	1	1	1	0	1	1	1	0	N
110	Asa Umunka	0	1	0	1	0	0	0	1	0	N
111	Umugo	0	1	0	1	0	1	1	1	0	N
112	Ohambele	1	1	1	1	0	1	1	1	0	N
113	Obohia	0	0	0	1	0	1	1	1	0	N
114	Ohanku	0	0	1	1	0	1	0	1	0	N
115	Akwuete	0	1	0	1	0	1	1	1	0	N
116	Akanu - Ikwuriato	0	0	1	1	0	1	1	1	0	N
117	Owaza	0	1	0	1	0	1	1	1	0	N
118	Abayi Nchokoro	0	0	1	1	1	0	1	0	0	N
119	Okpuhie	0	1	0	1	0	1	1	0	0	N
120	Isieketa	0	1	0	1	0	1	1	1	0	N
	NOTE: * 0 = Building does not comply to minimum standard/ Not Available ***N = , Not applicable ** 1 = Building complied to minimum standard/ Available										

S/N	Building Location	planning Approval	Setback Road	Setback Ppty Boundary	Floor Area Ratio	Road right-of-way	Building Coverage	Plot Size	Zoning	Certificate of fitness/ as Built Drawing	Change of use
121	Clifford Road	1	0	0	1	0	1	1	1	0	N
122	Umuwaya Rd	0	0	1	1	0	0	1	1	0	1
123	War Meseum rd	1	0	1	1	1	0	1	0	0	1
124	Okigwe Road	1	1	0	1	0	0	1	0	0	N
125	Aba road	1	0	0	1	1	0	1	1	0	N
126	Ikot-Ekpene Rd	1	0	1	1	1	0	1	0	0	0
127	Okpara Avenue	1	1	0	0	0	0	1	0	0	N
128	BCA Road	1	0	1	1	0	0	1	0	0	N
129	Nkwere Street	0	0	0	1	0	1	1	1	0	N
130	Calabar Road	1	1	0	1	1	0	1	0	0	N
131	Mbaise road	1	0	0	1	0	0	1	0	0	N
132	Ohafia Road	0	1	0	1	0	1	0	0	0	N
133	Cameroun Street	1	1	0	1	1	0	0	0	0	N
134	Finberg's Road	1	0	1	0	1	0	0	0	0	0
135	Ndume-Otuka	1	1	0	1	1	0	1	0	0	0
136	Afara Road	1	1	0	0	1	1	1	0	0	N
137	Umuokehi Road	1	1	0	1	1	0	1	1	0	N
138	Umuire Road	1	0	0	1	1	0	0	1	0	N
139	Umuawa Road	1	1	0	1	0	0	0	0	0	N
140	Amachara Road	0	1	0	0	0	0	1	1	0	1
141	Mission Hill	1	0	0	1	1	0	0	1	0	0
142	Umuahia –Ndume	1	1	1	1	0	0	1	0	0	N
143	Afaraukwu Rd	1	0	0	1	0	0	0	0	0	N
144	Olokoro Road	0	0	1	1	0	0	0	1	0	N
145	Amakama	1	1	0	1	0	1	0	0	0	0
146	Apu,miri	1	1	0	1	0	0	0	0	0	N
147	Ohuhu	0	0	0	1	0	1	0	0	0	N
148	Amuzi	0	0	0	1	0	1	0	1	0	N
149	Nnono	1	1	0	1	0	1	0	1	0	N
150	Nsudimo	1	1	0	1	1	0	0	1	0	N
151	Ahia Ukwu	0	1	0	1	0	1	0	1	0	N
152	Amaba Ime	1	0	0	1	1	0	1	1	0	N
153	Ndoro	1	1	0	1	0	1	1	1	0	N
154	Amuda	0	1	1	1	0	1	1	1	0	N
155	Ngodo	0	1	1	1	0	1	1	1	0	N
156	Amubiri	0	1	0	1	0	1	1	1	0	N
	NOTE: * 0 = Building does not comply to minimum standard/ Not Available ***N = , Not applicable ** 1 = Building complied to minimum standard/ Available										

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Development of Indices for Effectiveness of Renewable Energy Technologies Impacting Change in Quality of Life of Rural Residents

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Abstract— *The history of economic development is on the cost of ecology rather than the sustaining environment and development. The countries that became centrally located in the stream of economic exchanges among people around the world impacted the environment in the long run. Newspapers and editorials include environmental horror stories almost on a daily basis and demand better management of natural resources (Jangu, 2014). But who is listening?*

The environment is not just lush green trees, threated plant and animal species. It is the entity on which humans primarily exist, and agricultural and industrial development depends. Development on the cost of the environment can never be sustainable rather it would take us to a point causing enormous ecological losses and human sufferings primarily because of the present rate of development in developing countries. In order to contribute to the overall development in India, access to modern energy and cleaner fuel for rural households is important. The Brundtland Commission in its 1987 report 'Our Common Future' coined the most quoted definition of the term sustainable development, i.e., development that meets the needs of the present without compromising the ability of the future generations to meet their own needs (Mathur and Goswami, 2016). Sustainable production and consumption of resources helps to satisfy necessities of life such as nutritious food, good health, clean water and sanitation, clean energy, education, employment creating sustainable communities while combating climate change.

Having a negative energy balance for decades, India is forced to purchase energy from other countries to fulfil the needs of the entire country. Hence, energy access is an important component of poverty alleviation and an indispensable element of sustainable human development. Government of India has initiated numerous development programmes focussing on providing sustainable energy solutions to rural communities often deprived of clean and uninterrupted energy supply for their daily energy requirements. The study entitled ***'Renewable Energy Options among Rural Households'*** *was conducted in Haryana and Himachal Pradesh states. The outcomes of the study provide a roadmap for future programmes promoting the use of clean, efficient and modern energy technologies, to be implemented more effectively. Findings would further benefit the primary and secondary key stakeholders involved in research and development, formulation of policies and regulations, promoting sale and purchase and provide financial assistance to future energy programmes meant to popularize the use of Renewable Energy Technologies.*

Keywords— Renewables, Sustainable energy, Rural households, Energy-use pattern, Solar energy.

I. INTRODUCTION

Energy is fundamental to survival of life in any part of the globe. The pervasive nature of energy related activities have vast impact on the environment world over. With the current pattern of energy production, distribution and consumption, the resources will be exhausted much faster that would cause accelerated environmental degradation and slow down the progress dramatically. The energy sector has to play a critical role, especially in developing countries due to the huge investments required to meet the growing energy needs.

For the present research the categorization of energy resources used is Non-Renewable and Renewable energy resources. **Non-renewable Energy Resources** refer to those sources of energy that are derived from finite and static stock of energy. They cannot be produced, grown, generated or used on a scale that can sustain its consumption rate. The fossil fuels such as coal, petroleum, natural gas, nuclear power are examples of non-renewable sources of energy. **Renewable Energy Resources** refer to those resources which are available in abundance, are infinite and environment friendly in nature. These resources include solar, wind, biomass, wave and tidal energy (Varun and Chauhan, 2014).

Energy Access to Rural Households in India

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India has transitioned from being the world's seventh-largest energy consumer in 2000 to fourth-largest within a decade and is the fifth largest power generators worldwide. India's energy basket has a mix of all the resources available including renewable energy resources (Pawar and Kaur, 2014).

Among the various sectors that use energy, household sector is the largest consumer of energy. Rural Households (HHs) in developing countries are often dependent on the use of traditional biomass resources such as fuel wood, crop residue and dung cakes for activities such as cooking, domestic lighting, water heating, cattle-feed preparation and indoor space heating. It provides for a minimum life-supporting energy service and also represents a high financial cost, negative effects on human health and stress on environmental resources.

There are many impediments to energy access for the rural masses despite the launch of several programmes and policies by the Government that aim to improve quality of life of people living in the remote and rural areas of the country. Some of these barriers are geographically-dispersed villages that are difficult to reach and hence, providing electricity (through conventional electric grid) becomes difficult. There is inadequate focus to explore local energy resources either due to lack of funds, technological know-how and appropriate organization. Adequate financial models to tap resources through Public-Private Partnership (PPP) are inadequate. Private sector investment is not sufficiently facilitated by the Government through an appropriate mix of subsidies and grants; incentives and tariff policies; and risk sharing. Due to low population density and fewer households in rural areas there is high transmission cost along with severe transmission and distribution losses. The lack of facility for domestic connection in initial stages, uncertainty of power, load has impacted the demand for power in rural area due to poor quality and unavailability. Long and cumbersome procedures for getting a connection, distant location of facilities for paying bills and repair affect acceptability of renewable energy resources (Kumar, 2012).

Need to Shift to Renewable Energy Technologies (RETs)

Traditional solutions often comprise relatively low efficiency and much of the energy output gets wasted due to use of age-old (inefficient) technologies. Therefore, sustainable energy services are seen as a necessity for improving the standard of living, facilitating development and reducing environmental impact. Use of decentralized and small-scale technologies that make use of new, locally available, renewable resources such as sun, biomass, wind, water etc. appear to be the ultimate solution. RETs can provide universal modern energy services which drive development and improve living conditions, particularly in rural communities (Mahapatra and Dasappa, 2012).

As mentioned by Kumar *et al* (2010), to meet the energy requirement for such a fast growing economy, India will require an assured supply of three to four times more energy than the total energy consumed today. RETs are being progressively adopted as an alternative to conventional energy resources to ensure a sustainable future. In India there has been vigorous pursuit of activities related to production, application, research and development, demonstration and awareness for a variety of RETs to be used in different sectors. The benefits of access to clean energy resources for rural areas are many, including reduced deforestation and carbon emissions; improved healthcare services due to reduced consumption of raw water and smoke from open fire cooking; clean energy generated from renewable resources; decreased use and dependency on kerosene, wood and coal; improved agricultural output and access to potable and clean water. Renewable energy sources create a momentum for increasing time available for productive, income generating tasks and wealth creation over time. This can help in poverty reduction in rural communities (Chaurey et al, 2004).

II. METHODOLOGY

The study was conducted in villages/hamlets from four districts of two states, viz-a-viz., Faridabad and Panchkula districts (Haryana); and Hamirpur and Bilaspur districts (Himachal Pradesh). The selection criterion for villages/hamlets for study was the presence of residents using RETs, i.e., either possessing or benefitting from RETs (since two or more than two years). The villages/hamlets from Haryana and HP were selected as the locale of the study because of **presence of HHs using similar types of RETs in both the states**, there was **availability of solar grid in Haryana for electrification of HHs** that provided an opportunity to the researcher to understand the effect of electricity on their quality of life. Few hamlets that were close to the border of HP, also benefitted from the solar electrification programme of Haryana. This gave an **opportunity to compare the ownership and usage of RETs in both the states**.

The **ex-post facto research design** included qualitative analysis and interview of the stakeholders, vis-à-vis., RET users, village representatives and RET programme implementation officers from *Akshay Urja (AU)* shops. **Purposive Sampling Technique** was used to select the key stakeholders for the study (i.e., RETs programme implementation officials, RET users and village representatives).To get an insight about the location of houses using RETs, community service and facilities, sources of biomass collection etc., **resource maps** were

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prepared by involving the village representatives, residents and programme implementation officials (field staff from *AU* shops and local repair technicians).

Significance of the Study

Achievement of goals at an individual, community and world level are possible only if access to affordable and reliable energy for rural areas is available. This would help to strengthen jobs, enhance security, provide hygienic food, increase income, help in betterment of health and education. United Nations have been working with Governments to ensure the sustainable development across the countries. **Millennium Development Goals (MDGs)** launched in 2000 primarily focussed on ensuring environmental sustainability by integrating the principles of sustainable development into country policies and programmes and reverse the loss of environmental resources (**Goal 7A**). Though there was no MDG specifically mentioning energy access and security. Building on the success and momentum of MDGs a smooth transition to the new global goals, i.e., **Sustainable Development Goals (SDGs)** launched in 2015 had proposed to confront the energy issues directly. These cover the three dimensions of sustainable development, namely, economic growth, social inclusion and environmental protection. In addition, these 17 SDGs are universal and apply to all countries, unlike MDGs that were intended for action in developing countries only. Each goal has specific targets that have to be achieved over the next 15 years. **Goals that focus specifically to energy access and mitigation of climate change are Goal 7** (Ensure access to affordable, reliable, sustainable and modern energy for all) **and Goal 13** (Take urgent action to combat climate change and its impacts).

In India almost 68.84% of the population resides in rural areas (Census Report, 2011). Also, India has highest percentage (35.4%) of population in the world that does not have access to the modern energy. Most of this population is from the rural areas of India. In addition, rural India is a power house of natural energy resources and provides great opportunity for production of renewable energy that can be utilized for the rural households, community at large and improving their built-environment such as schools and health centres. Also, improving the overall quality of life of residents w.r.t providing power to small businesses or cottage industries, income generation, financial security, health, education and reduction in drudgery of women. The research entitled **Renewable Energy Technologies among Rural Households** studied the energy use pattern in rural HHs of Haryana and HP; pointing towards two broad categories of resources used by rural households to fulfil their day-to-day energy requirements for various HH activities. These were **Non-Renewable Energy Technologies (NRETs) and Renewable Energy Technologies (RETs)**.In light of this, the study proposes a **Sustainable Development Model for Co-existence of NRETs and RETs** that can helpto integrate **efficient use of RETs** and **conservation of NRETs,** to achieve the Sustainable Development Goals (SDGs). At the same time provide solution to the existing barriers in adoption and sustenance of new and modern technologies in rural communities.

III. RESULTS AND DISCUSSION

This chapter briefly focuses on the development of the indices for **Effectiveness of RETs** and **Change in Quality of Life of Residents w.r.t RET Usage**.

3.1 Effectiveness of RETs Index

3.1.1 Rationale for Development of Index

The RETs effectiveness index was developed for the following reasons-

(a) RETs had been installed, distributed and purchased by selected users under various Government programmes and schemes. To find out the performance of RETs, it was necessary to develop a scale that could evaluate the effectiveness concerning programme implementation and sustenance, ease of use and operation, product affordability, product design, repair and maintenance and reliability (refer Table 3.1).

Table.3.1: RETs Effectiveness Index Parameters and Sub-Parameters Developed for the Present Research

S.No.	Effectiveness Parameters	Sub-Parameters
1	Product Affordability	• Presence of Subsidies • Loans and other incentives • Cost of RETs and components • Repair and Maintenance service costs
2	Ease of Operation	• Functioning and usage • Efforts to procure and install • Ease in transportation and storage • Availability of components
3	Repair and Maintenance	• User competence to troubleshoot faults • Repair service/*AU* shops

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		• Availability of trained technicians • Response time
4	Product Design	• Aesthetic appeal • Recurrence of faults • Simple and standardized design
5	Reliability	• Supplementing NRETs • Utility • Durability • Predictable
6	Initiation and Sustenance	• Awareness generation • Community participation • Effective management • Equitable access • RETs waste management • Role of women
Validity of Index		The validity of the index was attained through **Content Validity.**
Reliability of Index		**Cronbach's (alpha)** was calculated to measure the internal consistency and reliability. The index had high degree of internal consistency (Cronbach's alpha: 0.756).

(b) There were very few existing tools present to measure effectiveness of RETs as they focused largely on ownership, utilization and working status of RETs. All of these researches focused on integrated rural development with the use of energy efficient technologies including individual and community RETs. The scales that had already been developed had to be adapted for use in the present study because they were either too lengthy, specific for urban users, covered few RETs or did not cover all relevant dimensions in the present situation as revealed in the pilot study. The pilot study pointed out that various RETs were used by the rural residents.

3.1.2 Method of Development of Index

Development of Effectiveness of RETs index was executed in the following stages:

- **Concept clarification** involved review of literature and consultation of experts to finalize definition of effectiveness and the dimension that impacted the effectiveness of RETs.
- **Review of existing indices** on effectiveness of RETs helped in determining the relevant dimensions to develop the 'effectiveness of RETs index'.
- **Review of proposed 'Effectiveness of RETs index'** by the experts**.**

3.1.3 Selection of Parameters for RET Effectiveness Index

Various experts reiterated parameters that would contribute to the overall effectiveness of RETs in a rural set-up. An in-depth analysis of different parameters covered for selected RETs was done and relevant dimensions were included in the effectiveness index. Table 3.2 depicts the various indices that had been referred for formulating the RETs Effectiveness Index relevant for the study. Amongst the Effectiveness indices studied, following three indices seemed relevant in the context of the present study:

(a) **PV System Acceptance Test (2000):** RETs Effectiveness Score cards developed by New Mexico State University's Southwest Technology Institute and Winrock International. These score cards had been developed to study the effectiveness of electrification of households using solar grid. It was a comprehensive tool that focused on use, operation, repair and maintenance services, community participation etc. that helped in contributing towards sustainability of the PV electrification programme. It also helped to identify issues that acted as barriers towards overall success of the programme.

(b) **Village Energy Schedule (2002):** India Rural Energy Study by UNDP (United Nations Development Programme) and ESMAP (Energy Sector Management Assistance Programme) sponsored by World Bank named **Energy Strategies for Rural India: Evidence from Six States** surveyed six states to provide a wide range of climatic,topographic and socio-economic development. They were Andhra Pradesh, Himachal

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Pradesh, Maharashtra, Punjab, Rajasthan, and West Bengal. The schedule focussed on aspects related to energy-use practices, adoption of modern energy, user awareness and willingness to adopt newer technologies and attitude towards Government policies and programmes.

(c) **Village Level Schedule (2005):** This was formulated by Technology Projects and Market Research Group (TPMRG). It was based on the project under Integrated Rural Energy Programme and MNRE on cluster of villages that consisted of questions pertaining ownership, functioning and usage of RETs by rural residents in selected villages of Haryana. Based on the findings suggestions were given regarding potential technologies and strategies to be followed to save conventional energy resources used by rural residents.

Since, each of these indices were specific for the purpose for which they were formulated, therefore, they seemed limited for the present study.

Table.3.2: Parameters of RETs Effectiveness Index Covered in Other Researches and Developed for the Present Research

PV System Acceptance Test (2000) by New Mexico State University and Winrock International	**Village Energy Schedule (2002) by UNDP and ESMAP**	**Village Level Schedule (2005) by Technology Projects and Market Research Group**	**RETs Effectiveness parametersfor the present study**
1. Effectively Sustained Technology	**1. Users Awareness and Willingness towards RETs**	**1. Ownership of RETs**	**1. Product Affordability** • Presence of Subsidies • Loans and other incentives • Cost of RETs and components • Repair and Maintenance service costs
2. Effective Management	**2. User Attitude and Opinion about RET adoption** • Ownership and Functioning of RETs • Presence of Government Programmes to support RETs adoption and continuance • Reliability • Success Rating of Programmes	**2. Functioning and Usage of RETs**	**2. Ease of Operation** • User competence to troubleshoot faults • Repair service/*AU* shops • Availability of trained technicians • Response time
3. Effective Functioning			**3. Repair and Maintenance** • User competence to troubleshoot faults • Repair service/*AU* shops • Availability of trained technicians • Response time
4. Financial Viability			**4. Product Design** • Aesthetic appeal • Recurrence of faults • Simple and standardized design
5. Effective Use			**5. Reliability** • Recurrence of faults • Utility • Durability • Predictable
6. Equitable Access			**6. Initiation and Sustenance**

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			• Awareness generation • Community participation • Effective management • Equitable access • RETs waste management • Role of women
7. Participation and Decision Making			
8. Support for Poverty-Sensitive and Demand Responsive Participation			
9. Expertise of Agencies Involved			

RETs Effectiveness Index prepared for the study laid focus on various aspects that had an impact on adoption and sustainability of RETs in rural areas. These were divided into six parameters namely product affordability, ease of operation, repair and maintenance, product design, reliability, and initiation and sustenance. For each parameter and sub- parameters, set of questions were prepared by referring to the existing indices. After discussion with the experts, the questions were finally reduced to a set of twenty-five questions in RETs Effectiveness Index. It was observed during the pilot study and also mentioned by Krishna Kumar (2006) that questions should be kept short and succinct as a lengthy question can confuse respondents and cause them to miss its essential point. Hence, to maintain the reliability of the response the length of the questions was kept short as the questions addressed user opinions, judgments and attitudes towards RET effectiveness.

3.1.4 Validity of Index

Keeping in view the research objectives, the index was constructed. After formulating the preliminary index, it was examined for content or rational validity. The standardization was done by consulting the expert in the field of renewable energy, rural extension, home science, statistics and electrical engineering from different organization such as Ministry of New and Renewable Energy, Indian Agricultural and Research Institute (IARI) and Indian Institute of Technology (IIT Delhi), BSES Rajdhani Power Limited and BSES Yamuna Power Limited. The validity of the index for the study was attained through its content validity. Content validity is the extent to which the components within a measurement procedure are **relevant** and **representative** of the concept that they will be used to measure (Haynes et al., 1995). Establishing content validity was a necessary initial task in the construction of a new measurement procedure (or revision of an existing one).

The relevant changes were made in the index by reframing several questions after consultation with experts and hence, the final measure of RETs effectiveness was assembled with 25 questions.

3.1.5 Reliability of Index

Cronbach's (alpha) was calculated to measure the internal consistency and reliability. In statistics (Classical Test Theory), Cronbach's (alpha) is used as a (lower bound) estimate of the reliability of a psychometric test. It can be viewed as the expected correlation of two tests that measure the same construct. Cronbach's alpha was calculated using the following formula:

$$\alpha = \frac{K}{K-1}\left(1 - \frac{\sum_{i=1}^{K}\sigma_{Y_i}^2}{\sigma_X^2}\right)$$

Where,

K = number of items

σ_X^2 = variance of the observed total test scores

$\sigma_{Y_i}^2$ = variance of component *i* for the current sample of persons.

The index was found to have high degree of internal consistency (Cronbach's alpha: 0.76 for RETs Effectiveness Index, refer Table 3.3) ensuring the reliability of the index. Sattler (2001) has stressed that quotient above 0.7 but below 0.9 is considered relatively reliable while (Nunnaly, 1978) indicated 0.7 as an acceptable reliability coefficient.

Table.3.3: Reliability of RETs Effectiveness Index Developed for the Present Research

S.No.	Study Tools	Reliability Score	N of Items

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1	RETs Effectiveness Index	0.756	25

3.1.6 Scoring of the Index

Each of the (25) questions in the index were considered as specific indicators of a parameter. Four-point Likert scale was used for the responses ranging from total agreement to total disagreement to the statements. Scores were assigned from 1-4, 1 for the most negative response and 4 for the most positive response. The study was conducted using 4 point Likert scale as it was easier for the rural respondents to understand. In many marketing and inter-cultural researches, four-point Likert scale has been found to give more reliable results than five-point Likert scale instrument. Multi-dimensional four-point rating scales have been used in many researches to understand the attitude and perception of respondents on items in developed scales.

In a research by Azen and Walker (2011), a 27 item (multi-item) scale used a four-point Likert response mode to an array of items, a sequential use of multiple independent samples was found as an improvement.

Another citing of successful use of four-point Likert scale was found in Cai and Lester (2008), who tested the reliabilities of two versions of the instrument used in their study, with exactly the same items, but one with four-point response formats for the Likert scale (that is for each statement, there were four choices: 'strongly agree', 'agree', 'disagree' and 'strongly disagree'), and the other with five-point Likert scales used on a class of 48 (Year 7 students) in China, and it was found that four-point instrument was more reliable (Cronbach alpha 0.72) than five-point instrument (Cronbach alpha 0.59).

For the RETs Effectiveness Index, four items were included in **product affordability**, **ease of operation**, **repair and maintenance** and **reliability**parameter; with a possible score from 4-16. **Product design**parameter included three items, with a possible range of scores from 3-12 'and **initiation and sustenance**parameter comprised of six items, with a possible range of scores from 6-24. These items considered the ways in which rural residents assessed the overall effectiveness of RETs.

3.1.7 Calculation of Effectiveness of RETs Index

From the scores of six selected parameters of RETs effectiveness index, the overall scores were computed. Therefore, the total possible range of scores for the index was 25-100. The higher the scores for overall RETs effectiveness the more satisfied or optimistic the perceptions of the respondents. The index was translated in Hindi language for interview so that accurate responses could be obtained. The Hindi version had been verified by the experts in Hindi language. After development of index all the parameters were made into a single index for testing the effectiveness RETs on the basis of many parameters. The following formula was used for computation of index score.

Index = Summation of Actual Scores of each Sub-parameters/ Cumulative Maximum Score x 100

3.1.8 Description of Parameters and Sub-parameters of RETs Effectiveness Index

Effectiveness refers to the degree of correspondence between the actual and the desired outputs of a system. Peter Drucker (1954) pointed out that effectiveness is doing the right things and concentrates on results. To calculate the effectiveness of RETs six parameters comprising various sub-parameters were developed. These included Product Affordability; Ease of Operation; Repair and Maintenance; Product Design; Reliability; and Initiation and Sustenance.

Dimension 1: Product Affordability

The first parameter refers to the ability of the rural residents to purchase RETs and draw benefit from it in their day-to-day life. A durable product should be affordable to attract more customers to purchase it. Customers often compare the prices on the basis of the features provided such as safety, ease of use, serviceability, quality of parts and products, etc.

1(a) Presence of subsidies: This sub-parameter investigated the importance of subsidies for rural residents to purchase RETs. To make a product attractive to potential users especially in rural areas subsidies were important due to low purchasing power of majority of rural population. Since, RETs were novel in nature and had high initial cost, subsidies became all the more essential.

1(b) Loans and other incentives: MNRE had introduced schemes to provide financial support that was an important additional factor to attract rural consumers towards RET usage. Banks and microfinance organizations offered loans for purchasing RETs. The willingness to purchase product came with the availability of payback time.

1(c) Cost of RETs and components: It focused on the user perception of affordability of RETs on the basis of initial cost of RETs and cost incurred in repair/replacement of BoS components.

1(d) Service cost for repair and maintenance of RETs: Repair and Maintenance service cost of RETs could greatly impact the decision of potential RET customers towards purchasing the product(s).

Dimension 2: Ease of Operation

2(a) Simple functioning: Simple and easy to use products were welcomed by rural residents. Functioning of a device measured the ease with

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which it could be operated by a user without getting a formal training (M.M. Huq, 2003). It also highlighted the usability of the product by the rural users.

2(b) Procure and install: Ease in procurement of RETs was one of the major features to influence their adoption. If a device was easy to procure and install, the number of users to adopt the devices increase by a significant proportion (Wayne D. Hoyer, Deborah J. MacInnis 2008).

2(c) Ease of transportation and storage: Another factor which influenced the adoption of RETs was their ease of storage and transport. Users tend to get devices which were easily portable. This gave them flexibility in terms of usage of the product (Wayne D. Hoyer, Deborah J. MacInnis 2008).

2(d) Availability of components: Over the years of usage, the RETs had been subjected to wear and tear (especially the one placed outdoors). Availability of easy repair of products was important for the users. Availability of spare part was critical for higher acceptability of RETs.

Dimension 3: Repair and Maintenance

For good customer experience, getting an appointment (from technician) as soon as possible was important, i.e. transparency in approach, reasonable and efficient service window. With tighter margins and a more competitive landscape, efficient customer service could be used to attract new users and retain the existing ones. Not valuing customers' time by delay in scheduling additional visits (if needed to complete the work) was a sure way to let business go to competitors and in case of RETs, a switch back to NRETs or moving down the energy ladder.

3(a) User competence to troubleshoot faults: This sub-parameter explored user ability to troubleshoot problem on their own. As RETs were electronic products, it became easier for rural people to use and maintain them. If troubleshooting them was easy and minor faults could be mend by their own self without assistance of servicing staff, it would increase the adoption of RETs manifolds.

3(b) Presence of repair service /*AU* shops: Service refers to auxiliary or peripheral activities that were performed to enhance the primary product or primary service. Users were concerned not only about a product breaking down, but also about the elapsed time before service was restored, the timeliness with which service appointments were kept, the nature of their dealings with service personnel and the frequency with which service calls or repairs fail to resolve outstanding problems. Some of these variables could be analyzed quite objectively while others reflected differing personal standards of what constitutes to an acceptable service. For example, a recent study of consumer satisfaction with professional services found the major complaints to be that "the service was provided in a careless, unprofessional manner" and that "I feel I was treated as an object rather than as an individual". Users had different perceptions about the shops meant for repair and maintenance of RETs. Some users felt that Government maintained shops were more reliable as compared to the private shops.

3(c) Availability of trained technicians: In order to execute proper repair and service of the RETs it was imperative to have trained technicians who were skilled to resolve the problems that arise over a period of time. Trained technicians drove the growth of products and increased adoption of the products (Hawkins and Mothersbaugh, 2011).

3(d) Response time: Human factors research attempts to determine human capabilities in areas such as response time and flexibility (Hawkins and Mothersbaugh, 2011). The user expectation of response time was determined by the need of the equipment being used by them, as products vital for daily activities such as solar home lighting system, water heaters required quick response time for installation or repair. Users define response time to be one of the most important factors that impact the adoption of RETs.

Dimension 4: Product Design

4(a) Aesthetic appeal: Both aesthetics and perceived quality were closely related to the user-based approach. Aesthetics refers to how a product looks, feels, sounds, tastes or smells. It was clearly a matter of personal judgment and reflections of individual preferences.

4(b) Recurrence of faults: It was mostly dependent on the extent of usage and upkeep of the RETs which influenced the wear and tear of the product. These products required repair, hence perception of users about the recurrence of faults varied on the basis of individual usage and knowledge about maintenance. Products installed outdoors relatively had higher requirement of repair.

4(c) Simple and Standardized design: Users in rural areas preferred those RETs which were simple and easy to operate. Products that required skilled operation had lower adoption rate as compared to the ones that could be operated by anyone without assistance.

Dimension 5: Reliability

Reliability reflected the probability of a product failing or provide service for a specified period of time. To

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understand the reliability of RETs, it was required that a product had been used for some time. This was more relevant w.r.t durable goods than services that were consumed instantly.

Perceptions of quality could be as subjective as assessments of aesthetics since, users did not always possess complete information about the attributes of a product. Hence, as a solution they had to frequently rely on indirect measures such as comparing brands. Also both **reputation**, i.e., the historical strength of the department and **affiliation**, i.e., the quality of the university to which a department was attached were equally important in explaining the rankings (Knudsen and Vaughan, 1969). In case of RETs, products purchased from *AU* shops were reputed for their quality because the shops were affiliated to the Government.

5(a) **Supplementing NRETs:** One of the key aspects of reliability is resistance to failure. The lesser the probability of failing directly translates to higher reliability (Hawkins and Mothersbaugh, 2011). RETs should be more resistance to failure to increase their perception as reliable products by users.

5(b) **Utility:** Another important aspect the users look for in a product is utility. The products which help users in their core activities are considered to be of higher utilities than the products which have on and off usage. The lighting products are prime example of this as they help to perform core activities after sundown they are perceived to be of higher utility.

5(c) **Durable:** Durability is a measure of product life that has both economic and technical dimensions. It can be defined as the extent of usage one gets from a product before it physically deteriorates. A perfect example of this is the light bulb that requires replacement after specific hours of use as the filament burns up and the repair is impossible. Economists call such products 'one-hoss shays' and had used them extensively in modeling the production and consumption of capital goods (Bliss, 1975).

Durability becomes difficult to interpret when repair is possible as the concept takes on an added dimension, for the life of product will vary with the change in economic conditions. Durability becomes the amount of use one gets from a product before it breaks down and replacement was preferable to continued repair. The product-based approach focused on performance, features and durability of RETs along with the manufacturing-based approach focused on conformance to standards. MNRE had tried to take care of all these factors to ensure the durability of RETs by establishment of *AU* shops and introducing minimum specification for standardization of RETs and establishment of test centers to certify the same.

5(d) **Predictable:** This sub-parameter catered to find out the predictability of RETs as perceived by rural residents. Consumers purchased products for the ease and comfort they brought to the life. The predictability or the presence of service brought satisfaction and assured the use of RETs for longer duration.

Dimension 6: Initiation and Sustenance

6(a) **Awareness generation:** This sub-parameter focused on the association of awareness of RETs such as long-term benefits, cost-effectiveness, fuel-saving, presence of financial support, subsidies, etc. with increase in willingness of rural residents to purchase and use RETs.

6(b) **Community participation:** A community can derive considerable benefits from becoming involved in the developments and operation of energy projects in rural areas. This sub-parameter investigated the community involvement in renewable energy programmes. Despite their contribution to sustainability, the perception varied as few believed that the involvement of residents (potential users/beneficiaries) was sought by developers only when permission or space was required for installation. Community needs were not paid much attention and focus was just on achieving physical targets of the schemes. Hence, such schemes were considered as unwelcomed intrusion from the outsiders, exploiting natural resources and offering little in return to the community.

6(c) **Effective management:** In order to establish and make a renewable energy project successful, a well-planned strategy and management must be acquired. It was an important attribute for long-term sustenance of the RETs (Acharya and Aithal, 2015). The key parameters to ensure effective management were knowledge of product and user-friendly operation.

6(d) **Equitable access:** In order to increase the adoption of RETs in rural areas, it was imperative to ensure that all the residents had equitable access to products (to benefit from them) and also to repair and maintenance services (nearby their HH or community) to ensure that the products were used to their full potential.

6(e) **RETs waste management:** The main differentiator of RETs from NRETs was the pro-environment nature of the equipment. Residents

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believed in adopting RETs for they had positive impact on their lifestyle without causing harm to the environment. Though there were many bottlenecks in management of waste generated from RETs that required attention.

6(f) Role of women: RETs played direct role in improving the lifestyle of women in rural areas. Women were responsible for various HH activities such as cooking, cleaning and outdoor HH work which were vastly influenced by adoption of RETs (Remedios and Rao, 2013).

3.2 Change in Quality of Life (QoL) Index w.r.t RET Usage

3.2.1 Rationale for Development of Index

The QoL index was developed for the following reasons-

(a) During the review, various existing QoL indices were explored, most of which were relevant for elderly such as were disease specific, focused on psychological and spiritual well- being and so on. For the purpose of the study it was pertinent to device a scale that could measure impact of RETs on QoL of users. Therefore, the domains had to focus on the impact of RET usage on QoL of rural residents w.r.t education, health, income-generation, safety and security, comfort and convenience (refer Table 3.4).

(b) The present research focused on exploring the change in QoL of RET users in selected rural areas. Since, the residents had been using RETs from the past many years their perception was primarily based on experience on account of using RETs using recall method.

3.2.2 Method of Development of Index

Development of Change in QoL Index was executed in the following stages:

- **Concept clarification** involved review of literature and consultation of experts to finalize the various dimensions to study the change in quality of life of rural residents w.r.t RET usage.
- **Review of existing indices** on change in QoL and determine the relevant dimensions to develop an index for the present research.
- **Review of the proposed QoL index** and check its reliability and validity.

Table.3.4: Change in QoL Index Developed for the Present Research

S.No.	Quality of Life Parameters	Sub-Parameters
1	Education of Children	• Increased study time at home • Improved academic performance • Regularity to school/college • Participation in co-curriculum activities • Access to education services/utilities outside home
2	Healthcare	• Better access for elderly • Better access for women and children • Improved availability of medical facilities/service • Decreased indoor pollution • Better healthcare at home
3	Convenience and Social Life	• Increased leisure time • Ease in conducting HH activities • Improved social life • Better family relationships • Living an active life
4	Safety and Security	• Decrease in incidences of theft • Safety from wild/stray animals • Decrease in accidents • Safety inside home • Sense of security
5	Income Generation and Financial Security	• New-start ups • Ease in conducting existing work • Better time management • Regularity to work place • Increased productivity/profitability

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Validity of Index	The validity of the index was attained through **Content Validity.**
Reliability of Index	**Cronbach's (alpha)** was calculated to measure the internal consistency and reliability. The index had high degree of internal consistency (Cronbach's alpha: 0.84).

3.2.3 Selection of Dimensions for Change in QoL Index

Amongst the QoL indices studied, following four indices seemed relevant in the context of present study. An in-depth analysis of different dimensions covered in selected change in QoL index was done and relevant dimensions were selected for the study (refer Table 3.5).

(a) **PV System Acceptance Test(2000):** QoL Score cards were developed by New Mexico State University's Southwest Technology Institute and Winrock International. This test was comprehensive in nature as it comprised of questions pertaining to the assessment of effectiveness of PV electrification by the rural residents as well as the change felt by them in their lives with its usage. The test comprised of score cards and focused on grid-connected system only.

(b) **WHO QoL Scale, Extended Version (1991):** This version has been prepared by QoL Group, World Health Organization (WHO). The dimensions in this scale primarily focused on individual perception of inter-personal relationships as well as personal well-being in terms of level of independence, economic, health and psychological well-being.

(c) **QoL: A System's Model(2001)**: The University of Oklahama School of Social Work prepared this model. The approach to the measurement of the quality of life derived from the position that there were a number of domains of living. Each domain contributed to one's overall assessment of the quality of life. The domains include in this model were family and friends, work, neighborhood (shelter), community, health, education and spirituality. All of these domains were included in the developed index for the present research in the context of user perception of change in their quality of life as a result of RET usage (refer Figure 3.1).

(d) **QoL Index- Ferrans and Powers (1984):** Ferrans and Powers prepared this index. It comprised of thirty-three items on four domains, i.e., social and economic, psychological, health and functioning, spiritual and family. The subjects attributed scores on scales designed for satisfaction and importance with values ranging from 1 to 6. Hence, the responses were analyzed using qualitative methodology. These domains were included in the developed QoL index for the present research with a prime focus on satisfaction (significant change) or no satisfaction (no change) experienced by the rural residents w.r.t RET usage.

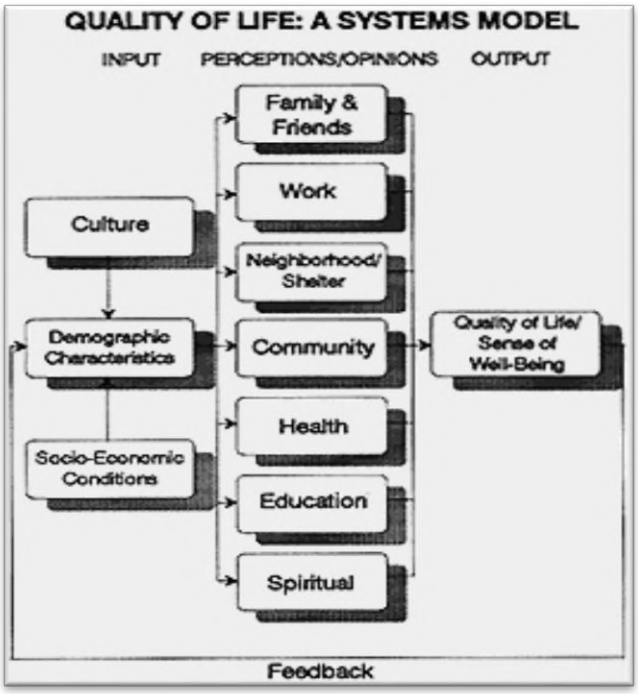

Fig.3.1: Quality of Life: A Systems Model by University of Oklahama School of Social Work (2001)

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For each dimension and sub-dimension, set of questions were prepared by referring to the existing indices. After discussion with the experts, the questions were finally reduced to a set of 25 questions in 'Change in QoL Index'. To maintain the reliability of the response the length of the questions was kept short as the questions addressed user opinions, judgments and attitudes of RET users towards their QoL. The index comprised of five dimensions, namely, **education of children; healthcare; convenience and social life; safety and security**; and **income generation and financial security** (refer Table 3.5).

Table.3.5: Dimensions of QoL Index Covered in Other Researches and Developed for the Present Research

PV System Acceptance Test (2000): Score cards developed by New Mexico State University and Winrock International	**WHO QoL Scale: Extended Version (1991) by QoL Group WHO**	**QoL: A System's Model (2001) by The University of Oklahama School of Social Work**	**QoL Index- Ferrans and Powers (1984) by Ferrans and Powers**	**Change in QoL dimensions for the present study**
1. Education	**1. Physical** • Energy and fatigue • Sleep and rest • Pain and discomfort	**1. Culture** • Work • Neighborhood • Family • Friends	**Health and Functioning**	**1. Education of Children** • Increased study time at home • Improved academic performance • Regularity to school/ college • Participation in co-curriculum activities • Access to education services/ utilities outside home
2. Income-Generation Activities	**2. Psychological** • Positive feeling • Thinking, memory and concentration • Self-esteem	**2. Demographic Characteristics** • Community • Health	**Social** • Family	**2. Healthcare** • Better access for elderly • Better access for women and children • Improved availability of medical facilities/service • Decreased indoor pollution • Better healthcare at home
3. Domestic Productivity	**3. Social Relationships** • Personal relationships • Social support • Sexual activity	**3. Socio-Economic Conditions** • Education	**Spiritual**	**3. Convenience and Social Life** • Increased leisure time • Ease in conducting HH activities • Improved social life • Better family relationships • Living an active life
4. Information and Communication	**4. Environment** • Physical safety and security • Home environment • Financial resources • Health and social care • Physical environment and transport	**4. Spiritual**		**4. Safety and Security** • Decrease in incidences of theft • Safety from wild/stray animals • Decrease in accidents • Safety inside home • Sense of security

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PV System Acceptance Test (2000): Score cards developed by New Mexico State University and Winrock International	WHO QoL Scale: Extended Version (1991) by QoL Group WHO	QoL: A System's Model (2001) by The University of Oklahama School of Social Work	QoL Index- Ferrans and Powers (1984) by Ferrans and Powers	Change in QoL dimensions for the present study
5. Convenience/ Comfort	**5. Spirituality/ Personal**			**5. Income Generation and Financial Security** • New-start ups • Ease in conducting existing work • Better time management • Regularity to work place • Increased productivity/ profitability
6. Health care and Safety	**6. Level of Independence** • Mobility • Activities of daily living • Dependence on medication or treatment • Work capacity			

3.2.4 Validity of Index

The validity of the QoL index was attained through the content validity. Keeping in view the research objectives the indices were constructed. After formulating the preliminary QoL index, it was examined for content or rational validity. The standardization was done by consulting the expert in the field of renewable energy, rural extension, home science, statistics and electrical engineering from different organization such as Ministry of New and Renewable Energy, Indian Agricultural and Research Institute (IARI) and Indian Institute of Technology (IIT Delhi), BSES Rajdhani Power Limited and BSES Yamuna Power Limited. The relevant changes were made in the index by reframing several questions after consultation with experts. Hence, the final measure of QoL was assembled with twenty-five questions.

3.2.5 Reliability of Index

Cronbach's (alpha) was calculated to measure the internal consistency and reliability. The indices were found to have high degree of internal consistency (Cronbach's alpha: 0.84 for QoL Index, refer table 3.6) ensuring the reliability of the index.

Table.3.6: Reliability of Change in QoL of Residents Index w.r.t RETs Usage Developed for the Present Research

S.No.	Study Tools	Reliability Score	N of Items
1	Quality of Life Score Cards	0.835	25

3.2.6 Scoring of Index

Each of the (25) questions in the index were considered as specific indicators of a dimension. Four-point Likert scale was used for the responses ranging from total agreement to total disagreement to the statements. Scores were assigned from 1-4, 1 for the most negative response and 4 for the most positive response. The study was conducted using 4 point Likert scale as it was easier for the rural respondents to understand.

In the QoL Index, five dimensions were included 'education of children'; 'healthcare'; 'convenience and social life'; 'safety and security'; and 'income generation and financial security'; with a possible score from 5-25. The QoL index included dimensions and sub-dimensions that considered the ways in which rural residents assessed the overall change in their QoL w.r.t RET usage.

3.2.7 Calculation of Change in QoL Index

From the scores of five dimensions of QoL index, the overall scores were computed. Therefore, the total

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possible range of scores for the index was 25-100. The higher the scores for overall change in QoL, the more satisfied or optimistic the perception of the respondents. The index was translated in Hindi language for interview so that accurate responses could be obtained. The Hindi version had been verified by the experts in Hindi language. The following formula was used for computation of index score.

Index = Summation of Actual Scores of Each Sub-dimensions/ cumulative maximum score x 100

3.2.8 Description of Dimensions and Sub-Dimensions of Change in QoL Index

Quality of life (QOL) has been defined by Ferrans (1990) as "a person's sense of well-being that stems from satisfaction and dissatisfaction with the areas of life that are important to him or her". The index was constructed to understand the user's perception of change in their quality of life with the use of RETs. There were 5 dimensions to determine change in the quality of life of users. These are discussed as follows:

Dimension 1: Education of Children

Change in education forms the basis of many renewable energy programs. This dimension investigated the changes witnessed by the residents of selected villages w.r.t change in home study time, academic performance, regularity to school, participation in co-curricular activities and access to educational facilities outside home.

1(a) Increased study time at home: Awareness for importance of education was increasing in rural areas hence, many families emphasized on education of their children. In many villages where electrification from conventional (electric) grid was either not present or the power supply was erratic, residents considered RETs as an alternative solution.

1(b) Improved academic performance: This sub-dimension was a result of various other developmental activities in the village. The perception of users about the influence of RETs usage on improving their child's academic scores was of importance for understanding the change in education with use of RETs.

1(c) Regularity to school/college: This sub-dimension was critical to understand the impact of RETs on regularity of children to school. For instance, hot water was required in the morning to take bath hence, solar water heaters provided convenience and were available even if power supply was not available, preventing any delay in reaching school on time.

1(d) Participation in co-curricular activities: Co-curricular activities formed a winning part of complete education. It provided opportunities for character development and valuable life lessons such as teamwork, sportsmanship, self-discipline and hard work. This helped students to become responsible adults, productive citizens and skilled professionals. This sub-dimension attempted to find out improvement in participation of children in co-curricular activities with the use of RETs.

1(e) Access to education services/utilities outside home: Many Government health centers were not able to operate after sundown. This sub-dimension measured the effect of RET usage on the functioning of utilities and education services. On adoption of RETs there was a possibility to keep utility services open even after sunset.

Dimension 2: Healthcare

Improvement in rural health service was important as the availability of electricity to support proper services such as provision of vaccines, medicines, healthcare professionals etc. was inadequate. An appropriate RET could help in expanding the opportunities for better healthcare available within or near the rural communities for residents.

2(a) Better access for elderly: Performing regular functions was difficult for elders in the rural areas where power supply was erratic and of poor quality. However, the adoption of RETs could provide them better access to various healthcare services.

2(b) Better access for women and children: Women and children had higher need of healthcare services in rural areas. RET adoption by the local healthcare institutions could provide better access to healthcare services to women and children.

2(c) Improved availability of medical facilities/service: This sub-dimension was directly related to the operational hours of local healthcare institutions. Adoption of RETs could provide flexibility to run them. This certainly resulted in the improved availability of medical facilities and availability of medical practitioners in rural areas.

2(d) Decreased indoor pollution: This sub-dimension focused on the importance of healthcare needs as it directly affected the health of women who cook using NRETs such as biomass/fuelwood. Adoption of RETs had a positive effect on the pollution levels in the house.

2(e) Better healthcare at home: Healthcare at home could be improved with the usage of RETs that played a significant role in ensuring the proper access to the medical facilities at home.

Dimension 3: Convenience and Social Life

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3(a) **Increased leisure time:** This sub-dimension was a key attribute to determine comfort and convenience. Usage of RETs provided additional time for leisure activities. The length of the workable day increased with the use of RETs such as solar home lights, solar street lights, solar water heaters, etc.

3(b) **Ease in conducting household activities:** RETs also provided convenience for conducting HH activities such as cooking, lighting, water heating, cleaning, etc. This dimensions focused upon the impact on RET usage on the ease and comfort in conducting HH activities.

3(c) **Living an active life:** Availability of more spare time resulting from RET usage could be used for socializing with friends, family, relatives and neighbors. Organizing late evening meetings under a tree was possible due to RET usage.

3(d) **Better family relationships:** Families could spend additional time together as many HH activities could be performed through a span of day and night therefore, the burden was not felt by women to finish all the chores within a short span of time (i.e, before sunset), that left no scope for rest, leisure, socialize or spend time with near and dear ones.

3(e) **Improved social life:** Since many public services such as schools and nursing homes were also adopting RETs, the access to facilities were enhanced.

Dimension 4: Safety and Security

This dimension focused on the perception of rural residents about change in safety and security with the use of RETs. Solar PV technology played a prime role in rural areas. Solar-powered lighting meant that children were able to go out and play after sunset, women felt safe venturing out at late evenings for HH shopping, temple or evening walks etc. Also, enhancing the feeling of safety among rural HHs from theft, wild animals, etc.

4(a) **Decrease in incidence of theft:** This sub-dimension was important to understand the user perception on decrease in theft as an impact of RET usage.

4(b) **Safety from wild/stray animals:** As revealed in the preliminary discussion with the villagers that the attack from wild animals was common since, the lanes and by-lanes were usually dark due to absence of street lighting. This sub-dimension focused on changes in safety from wild animals with presence of SPV based street lights.

4(c) **Decrease in accidents:** The incidence of accidents due to low visibility in rural areas was a problem. The introduction of RETs had been a boon to ensure safety from accidents especially in hilly terrain. The user perception of change in such incidences was found in this sub-dimension.

4(d) **Safety inside home:** The safety inside the home referred to the safety from mishaps (falling or hurting due to lack of visibility) at home especially for children, sick, elderly and pregnant women.

4(e) **Sense of security:** A positive feeling of safety and security with the use of RETs.

Dimension 5: Income Generation and Financial Security

For a renewable energy project to bring about considerable benefits to rural communities by way of enhancing the profitability and productivity of existing income-generation activities as well as assist in new start-ups. The resultant increase in income would help in better sustenance and adoption of RETs.

5(a) **New start-up:** This sub-dimension investigated the impact of RETs in the lives of rural people w.r.t taking up new work to enhance their existing HH income.

5(b) **Ability and ease in conducting existing work:** This sub-dimension referred to the user perception of change in their ability to conduct existing work with ease and comfort as a result of RET usage.

5(c) **Better time management:**Planning and exercising control over the amount of time spend on specific activities, especially to increase effectiveness, efficiency or productivity was important. The aim of this sub-dimension was whether rural residents were able to identify the impact of RETs in helping one exercise such control so that efficiency to conduct existing work and ability to take up new was increased.

5(d) **Regularity to work place:** To understand the contribution of RETs towards regularity of users to their respective work place was the aim of this sub-dimension.

5(e) **Increased productivity/ profitability:** A potential benefit of implementing sustainable energy options included strategies to improve rural conditions by linking RETs with productive uses. Most of the renewable energy projects implemented so far in the rural areas had concentrated on residential or HH applications. The use of renewable energy to increase productivity and profitability can help in sustenance and adoption of RETs in rural areas.

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Change detection analysis of Cropland using Geospatial technique -A case Study of Narsinghpur District

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Abstract**—** *Access to accurate and up-to-date information on the extent and distribution of individual crop types, associated with land use changes and practices, has significant value in intensively agricultural regions. Explicit information of croplands can be useful for sustainable water resources, land and agriculture planning and management. Remote sensing, has been proven to be a more cost-effective alternative to the traditional statistically-based ground surveys for crop coverage areas that are costly and provide insufficient information. Satellite images along with ground surveys can provide the necessary information of spatial coverage and spectral responses of croplands for sustainable agricultural management. This study strives to differentiate different crop types and agricultural practices to achieve a higher detailed crop map of the Narsinghpur district.*

Keywords**—** *Change detection, Satellite imagery, Crop cover, Supervised Classification technique, Remote Sensing and GIS.*

I. INTRODUCTION

Observation and assessment of crop status and development is a crucial topic for agronomic planning and management and for mitigating the effects induced by climate change and extreme events. In order to meet the need of increasing population demand, the timely and precise information on the area covered by different crops is quite necessary (Foerster et al., 2012; Conrad et al., 2014).

In particular, in-season crop type maps produced during early growth stages are the key information source for operational agricultural monitoring by both public authorities and private sectors. Early information on crop type and acreage is necessary to forecast agricultural water demand during the summer season (Mo et al., 2005; Reichstein et al., 2007). Despite the need for information delivered in near-real time during the crop season, official figure and statistics are usually provided after the end of the growing season, since data have to be collected, verified and compiled into a database.

Satellite remote sensing is a unique source of data for the identification of crop types over large areas, as described in the last two decades in scientific literature (e.g. Ortiz et al., 1997; Pohl and Van Genderen, 1998; De Wit and Clevers, 2004; Ok et al., 2012; Villa et al., 2015). A number of factors influence the accuracy of satellite-based crop maps: i.e. the spatial resolution of the imagery, the classification method, and the production time horizon, i.e. the temporal extent of the dataset and phenological stages covered (HubertMoy et al., 2001; Van Niel and McVicar, 2004; Duveiller and Defourny, 2010). Cultivated crops and site characteristics regulate the selection of the most suitable satellite dataset.

Medium resolution data (10-30 m, e.g. Landsat) gives better results at local/regional scales and over fragmented landscapes (e.g. Murty et al., 2003; Conrad et al., 2014). Duveiller and Defourny (2010) and Yang et al. (2011) demonstrated that spatial resolution in the range 10-140 m could be considered an optimal choice for a wide range of agricultural landscapes.

II. MATERIALS AND METHODS

Study area

The Study area lies between 23o16' to 24o36' N coordinates and 78o27' to 79o40' E coordinates with respect to the projection of zone no. 43N UTM on WGS 84 datum. It is covering approximately 5133 Sq. km. area. Its elevation range between 286.59 and 882.2 above MSL. The normal annual rainfall of Narsinghpur district is 1192.1mm. There are four tehsils fall under this district namely Narsinghpur, Gotegaon, Gadarwara & Kareli and the district further divided into six administrative blocks namely Saikhera, Babai Chichali, Chawarpatha , Kareli, Narsinghpur & Gotegaon. The study area is illustrated in Fig-1.

Data used

Satellite data of IRS P6 LISS III of 10th January, 2006 (Path 99 and Rows 55 and 56) and Landsat 8 of 1st & 8th February 2015 (Path 144 and 145 and Row 44) of the same season were used for the above case study. The study area falls in Sheet No. 55I and 55J with the Scale of

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1:250000 published by Survey of India. The other ancillary data, i.e. Land use/Land cover map of study area were used in the present study (Table-1).

Methodology

The methodology flowchart is illustrated in Fig-2. Different registrations for a specific sensor, after pre-processing, form a "multivariate image set". These data contain two types of information: spectral-temporal and spatial-contextual. The spectral-temporal information can be extracted with a supervised Maximum Likelihood algorithm (Duda et al.2000), resulting in a per-pixel classification.

The spatial information can be derived by means of a signature data set collection on the basin of histogram group. On the ideal situation, these group correspond with parcels on the grounds. In this study the segmentation was applied with supervised classification tool which is present in ERADAS Imagine software. Classification and the segmentation are combined using signature dataset collection of different crop type reflectance. This procedure determines for each parcel the pre-dominant class and assign all Pixels of the parcel to this modal class. By reducing speckle and errors in the vicinity of field boundaries, this application enhances the accuracy and legibility of the final map.

The ground truth data needed for the calibration of supervised classifications were collected in two field surveys in before classification and after classification. The garmin GPS handset and the associated software were used for the surveys. During the field surveys, large parcels or plots corresponding different crops were selected and identified and demarcated in FCC to form a ground truth vector GIS. Two mappings as per year 2006 and 2015 to estimate the areas changes of crops.

Classification Procedure

The digital image processing of satellite data has been carried out using ERDAS IMAGINE 9.2 software and crop cover maps of 2006 and 2015 were prepared following on screen visual interpretation method. In the level - I classification, six land use/land cover classes, i.e., built-up land, agriculture, forest, wastelands, open/fallow land and water bodies have been identified. In level - II classification, Extraction of agriculture area as area of interest (AOI) and overlay on False Colour Composite (FCC) image. In level - III classification, Agriculture class have been further classified. The wheat crop, gram crop, sugarcane and other crops have been identified under agriculture class. In level – IV, Change detection in the crop cover was carried out between 2006 and 2015.

III. RESULT AND DISCUSSIONS

Crop Cover Analysis for 2006 and 2015

The total agricultural area increased by about 6.31 per cent during the period from 2006 to 2015. In the year 2015, the agricultural area in the district was about 264284.8 ha which was about 32395.33 ha more than in 2006 (231889.47 ha).

The crop map of 2006 shows that the total wheat area in study area in 2006 was only 10.07% (51665.57 ha) of the total geographical area (Table-2). The dominant crop class in district was gram 136442.68 ha i.e. 26.58% of the total area under the district. This shows that the agricultural area of district was a gram dominant (Fig-3). Sugarcane and other crop classes cover an area of 25555.27 ha (4.98%) and 18225.95 ha (3.55) respectively. In year 2015, the dominant crop of the district was wheat 108112 ha i.e. 21.06% of the total study area. The total area under gram and sugarcane was 87428.6 and 54997.2 ha respectively (Fig-4). Only 2.68% of the total area was under other crops that means it has very thin vegetative cover.

The study shows a large scale change during 2006 to 2015 in Narsinghpur. The wheat area which was just 10.07% in 2006 increased to about 21.06% in 2015. Sugarcane area also increased from 4.98% to 10.71% during 2006 to 2015. This wheat and sugarcane area expanded on to the gram and other crops and hence the gram area reduced to 17.03 % in 2015 from 26.58% in 2006 and other crops area reduced to 2.68% in 2015 from 3.55% in 2006 (Table-2).

Crop cover changes

Fertile alluvial soils and the availability of water through canals as well as ground water sources support intensive agricultural activity in the region. The population pressure, economic potential of the region and the entrepreneurial attitude of a majority of the farmers in the region have significantly transformed the crop cover pattern of the area, especially during the recent years.

A study has been conducted by Rao et al. (1991) which was based on the interpretation of IRS-1A LISS-I image on 1:250,000 scale. They observed that due to changes in cropping pattern from seasonal crops to long term crops, there has been an increase in the cropping intensity by about 160% in the deltaic region during 1980s when compared to the earlier period. In the present study also the Landsat imagery of 2015 are found to be useful, especially for identifying broad categories of crop cover required for the purpose of this study.

Large-scale exploitation of groundwater resources during the recent years has increased the area of more water requirement crops possible instead of less water requirement crops earlier. Further, the area under

wheat and sugarcane has increased during the last ten years: This is evident from the observation of the satellite imagery. While 2006 image (Fig-3) shows maximum area under seasonal gram crop, the 2015 satellite image (Fig-4) shows wheat cover in many parts of the study area.

The progressive increase in the wheat crop as well as sugarcane crop extent in the district is revealed by the area statistics obtained from the G1S analysis of the two datasets (Table 2). During the period between 2006 and 2015, there has been an increase in the area under wheat by 56446.43 ha (109.25%) and sugarcane by 29441.93 ha (115.21%), respectively, while the extent of gram has decreased by 49014.08 ha (-35.92%) and other crops by 4478.95 ha (-24.57%), respectively. The increase in the sugarcane extent, is mainly due to the large-scale exploitation of groundwater resources and water available in the canal of the study area. In fact, an area of about 25555.27 ha, which was mainly occupied in 2006 by wheat, gram and other crops, has been converted into sugarcane area that contain water almost throughout the year. As such, the extent of sugarcane has increased considerably in the study area.

IV. CONCLUSION

Based on, a supervised classification method is proposed for identifying crops at the level in agricultural land by using multispectral data. It is concluded that the Agricultural practices in the study area have altered significantly in 10 years. The crop area overlapped in the fallow/barren area was evident by the development in of the canal in the area and augmentation of area covered by of wheat (109.25%) and sugarcane (115.21%) crop while decline in gram (-35.92%) and other cropped area (-24.57%).

The Change Detection analysis is an efficient way of describing the changes observed in each category. Study implies that in the year 2006 agricultural area were found 231889.47 ha, while in the year 2015 area were 264284.8 ha, which is easily identified through classification that open /fallow land converted in to Agriculture/other vegetation. The supervised method gives quite satisfactory results for vegetation varying in densities and also for scattered vegetation from a multispectral remote sensing image.

Our findings demonstrate that near real-time, in season crop mapping is feasible using satellite data with suitable spatial and temporal resolution in a simple, operational and inexpensive way. This early in-season crop map could be useful to support agricultural practices and management, especially for supporting the analysis of water demand for major crops during dry summer months.

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Fig.1: Location map of study area

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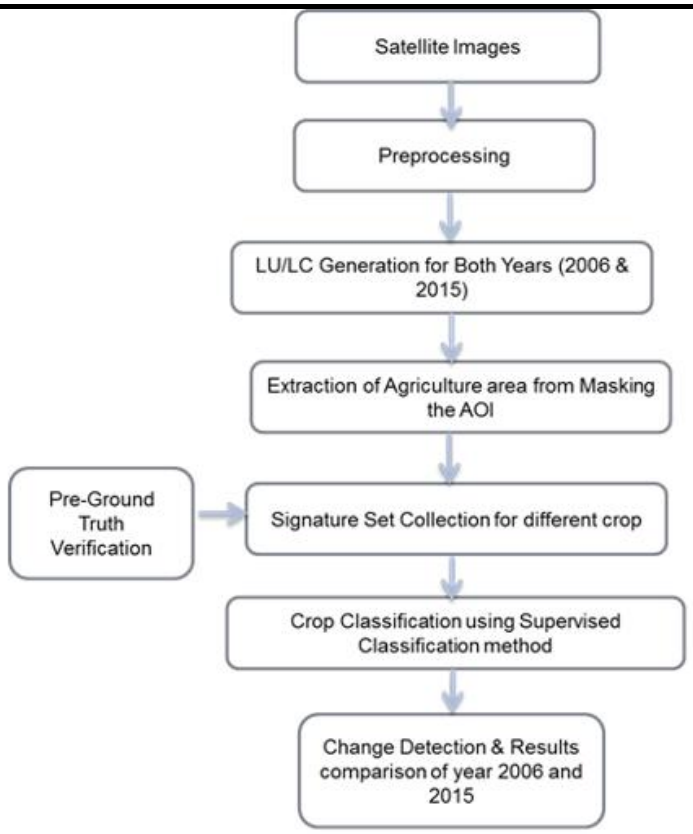

Fig.2: Methodology for extraction of crop data and change detection from imagery

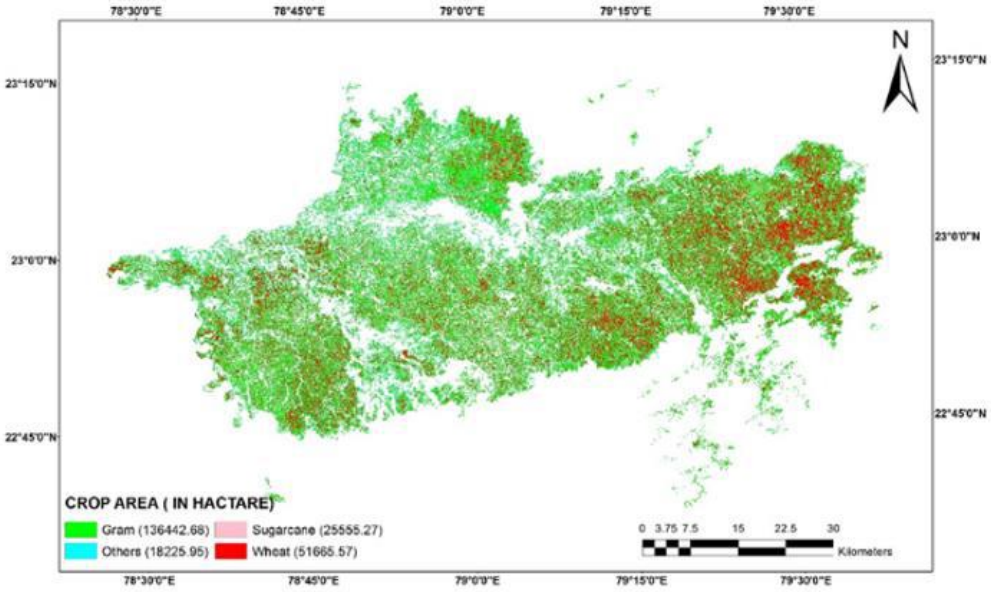

Fig.3: Crop Map of Narsinghpur District (Rabi Season-2006)

Table.1: Details of Satellite Image used for the study

S. No.	Year	Satellite	Spatial resolution	Source
1	February,2015	Landsat 8	30 meter	https://earthexplorer.usgs.gov/
2	January,2006	LISS III	23.5 meter	http://www.nrsc.gov.in/

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Table.2: Comparison of Crop Cover Changes

Crops Name	LISS 3 Data (2006)		Landsat Data (2015)		Difference	
	Area (in ha)	Area (%)	Area (in ha)	Area (%)	Area (in ha)	Percentage (%) change w.r.t. 2006
Wheat	51665.57	10.07	108112	21.06	56446.43	+109.25
Gram	136442.68	26.58	87428.6	17.03	-49014.08	-35.92
Sugarcane	25555.27	4.98	54997.2	10.71	29441.93	+115.21
Others	18225.95	3.55	13747	2.68	-4478.95	-24.57
Total Agricultural Area	**231889.47**	45.18	**264284.8**	51.49	32395.33	+13.97
Total Geographical Area	**513300**					

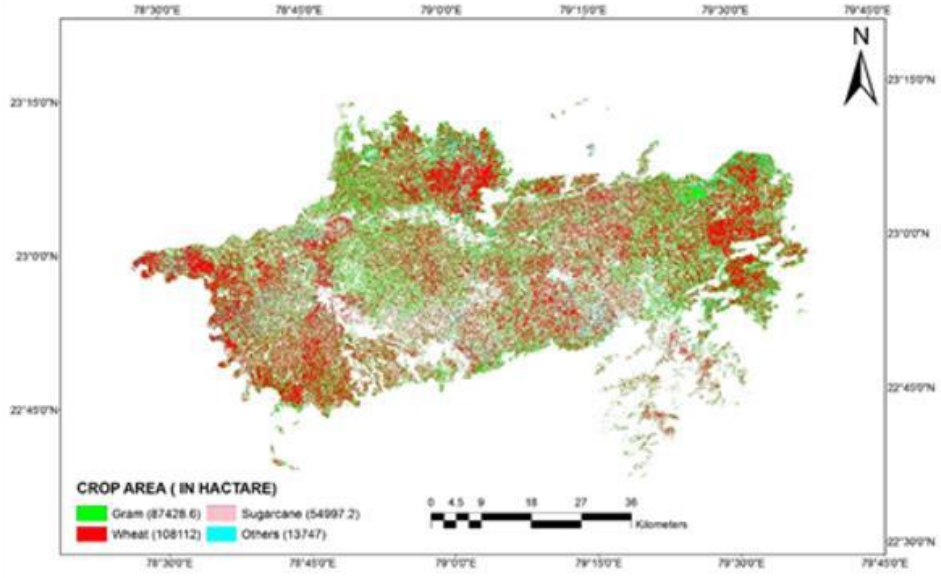

Fig.4: Crop Map of Narsinghpur District (Rabi Season-2015)

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Comparative Alterations in the Compositional Profile of Selected Root and Vegetable Peels Subjected to Three Pretreatments for Enhanced Saccharification

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Abstract— *Lignocellulosic feedstocks have gained worldwide interest as alternative biofuel source in the context of squeezing petroleum resources, enhanced environmental pollution from greenhouse gases and resulting climate change. The potential of agricultural processing residues such as root and vegetable peels (beet root, greater yam, pumpkin and vegetable banana) for bioethanol production was investigated through an understanding of their compositional profile and efficacy of three pretreatments in altering their composition and reducing biomass recalcitrance. Starch was the major polysaccharide in the residues (range: 25-37%), followed by cellulose (18-22%) and hemicellulose (15-20%). While dilute sulfuric acid (DSA; 121°C ; 0.102 MPa) hydrolyzed starch and hemicellulose to a high extent, steam pretreatment of moist residues (40 % and 50 % MC) at 100 °C also facilitated hemicellulose and starch solubilization. On the contrary, lime pretreatment retained most of the cellulose, hemicellulose and starch in the pretreated residues. Delignification was the highest (28- 37%) in steam pretreated residues, with minimal effect in DSA and lime pretreatments, necessitating lignin binding surfactants during saccharification in the latter. Reducing sugar content in pretreated liquors and Pretreatment Efficiency (%) were the highest (40-45 g* L^{-1} *and 57-64% respectively) in the DSA pretreatment. The study showed that as the pretreated liquor DSA and steam pretreatment was rich in fermentable sugars, whole slurry saccharification would be beneficial for maximizing the bioethanol yield.*

Keywords— Composition, peels, root and vegetables, pretreatment, steam, lime, DSA

I. INTRODUCTION

The increasing demand for transportation fuel especially from the oil-dependent nations of the world, due to enhanced population growth and rapid industrialization has necessitated the look out for alternative fuel sources which are relatively cheap and environment friendly. Excessive burning of coal and fuel has been associated with the global warming and climate change which are going to be the threatening issues of the near future [1]. Bioethanol is regarded as the best alternative to petroleum-based fuel, due to its high O_2 content (35%) and ability to reduce the emission of toxic gases which contribute to greenhouse effect [2,3]. Lignocellulosic biomass (LCBs) is identified as the best raw material for bioethanol production due to several factors such as abundant and cheap global availability, non-food resource and effective waste valorization potential [4,5]. Although a major part of LCBs is constituted by agricultural residues and woody biomass or dedicated crops such as switchgrass or coastal Bermuda grass [4, 6], there is also in increasing contribution from processing wastes resulting from enhanced industrial activities. Being waste byproducts, their effective valorization for bioethanol production could also help control pollution and health hazards from inadequate waste disposal.

Banana is an important fruit crop grown in the tropics and sub-tropics and currently India is the world's leading producer with a production of 27.58 million tomes accounting for 25% of the world [7]. Three common species of banana grown in the world are *Musa cavendishii*, *M .paradisiaca* and *M. sapientum.* Banana peel is a major agro-waste of most developed and developing nations which is currently utilized as animal feed source or for extraction of fiber, ethanol and pectins [8, 9]. Cooking (vegetable) banana falls under ABB group and very little research has been conducted on the utilization of its peel, although extensive studies have been conducted on the byproduct utilization of plantain and fruit (ripe) banana peels [10,11,12]. Hence, the potential of cooking banana peels as a source for bioethanol production was investigated. Besides, two other wastes generated from commonly consumed vegetables such as beet root (*Beta vulgaris*) and pumpkin (*Cucurbita moschata*) were also studied for their efficiency as a 2G ethanol sources. Beet root also known

as garden beet is extensively cultivated as an anti-oxidant rich vegetable in tropical countries and is also utilized for the extraction of betalains and natural colours [13,14].

The residue after extraction of pigments is a rich source of cellulose, hemicellulose and starch and hence could be a potential candidate for bioethanol production. *Cucurbita moschata* is cultivated extensively in the world with higher production yield and the pulp is used as vegetable, in soups, juices, puddings, breads etc. [15]. Thick peels comprising approximately 10-15% of fresh weight are thrown off and scanty literature is available in its utilization [15]. Although several studies have been conducted on the value addition of the pulp and seeds of *C. moschata* [16], very little is known about the potential of the peels which is a biowaste discarded during processing. Greater yam (water yam; *Dioscorea alata*) is another root crop species which is extensively cultivated in India and Africa for its starchy tubers. However, there is lot of processing waste due to the irregular morphology of the roots and more than 20% of the fresh weight is accounted towards peeling waste. Except for a few studies, the potential of yam peel as a bioethanol raw material remains largely untapped [17,18].The objective of the present study was to investigate the potential of peels from beet root, greater yam, pumpkin and vegetable banana for bioethanol production by designing appropriate pretreatment strategies which could help enhance the fermentable sugar yield from them.

Although bioethanol production from lignocellulosic biomass has long been recognized as a good option due to the cheap and abundant availability of the feedstock, its potential largely depends on the cost-effective processing by successfully overcoming the technological barriers. Biomass recalcitrance is the primary obstacle resulting from the highly crystalline nature of cellulose and its poor accessibility to cellulases due to shielding by lignin-hemicellulose matrix and has to be effectively tackled through appropriate pretreatment strategies[19]. Pretreatment cost has been identified as the second major contributor to ethanol production cost, first being raw material cost (including enzymes). Hence, research efforts have been intensified in the past few decades to develop cost-effective technologies that support the downstream processing operations with low enzyme dosages and shorter processing time. Variations in the physico-chemical characteristics of different lignocellulosic materials necessitate suitable pretreatment technologies to be developed for each of them [20]. Starch, being a major component of the selected biomasses, their pretreatment approaches and resulting compositional alterations may also be different from conventional LCBs.

Dilute sulfuric acid (DSA) has been widely used for the deconstruction of cellulose in agricultural residues, woody and herbaceous crops [3,20, 21,22] found that acid pretreatment causes disruption of covalent and hydrogen bonds as well as Vanderwaals forces which hold the biomass components, leading to solubilization of hemicellulose and reduction in cellulose crystallinity. Nevertheless, acid pretreatment has certain disadvantages such as the need for corrosion-resistant reactors, less efficiency of lignin removal and formation of inhibitors such as furfural, 5-hydroxymethyl furfural and acetic acid [23]. In order to overcome such problems, lime (calcium hydroxide) pretreatment has been attempted for several lignocellulosic feedstocks [24,25,26]. Being a cheap chemical that could be safely handled and recovered easily coupled with the low operational temperatures, lime pretreatment has currently regained interest. Removal of acetyl and uronic acid as well as ester linkages by alkali enhances cellulose digestibility and lignin solubilization [23, 27]. Besides, lignocelluloses are swollen in presence of alkali, which increases the accessibility of cellulose to cellulases [26].

We had reported earlier on the compositional variations in the peels from root crops such as sweet potato, elephant foot yam and tannia as well as from the vegetable, ash gourd and the changes they undergo during pretreatment techniques such as steam, dilute sulfuric acid and lime [28, 29]. The objective of the present study was to compare the effects of three pretreatment technologies for the starch-rich residues (lignocellulo-starch biomass) such as peels from beet root, greater yam, pumpkin and vegetable banana, on the compositional alterations brought about so that the most appropriate pretreatment and saccharification process for bioethanol production could be evolved.

II. MATERIALS AND METHODS

2.1. Raw materials and enzymes

Peels were collected by manual peeling from selected biomass such as beet root, pumpkin (yellow variety), vegetable (cooking) banana (ABB) and greater yam. These were immediately washed in running tap water to remove adhering sand and mud, drained and sun-dried for 36-48h to moisture content < 10%. The dry residues were powdered in a hammer mill (particles size: *ca.* 2-3mm) and used without screening for studies.

The enzymes used in the study for the precise quantification of starch included Spezyme ® Xtra and Stargen™ 002, supplied free of cost by M/S Genecor International Inc., USA (presently M/S Danisco US Inc., USA). As per the manufacturer's guide, Spezyme contained a thermostable α- amylase (E.C.3.2.1.1) with 14000 units of activity (1.0 AAU = amount of enzyme required to hydrolyze 10.0 mg starch/min under the assay conditions) and Stargen contained amylase and

glucoamylase (E.C.3.2.1.3) which synergistically hydrolyzed granular starch to glucose and had an activity of 570 Glucoamylase units (1 unit = amount of enzyme liberating 1.0g glucose/h from soluble starch under the conditions of assay [30].

2.2. Pretreatments

Three types of pretreatment strategies were adopted such as simple steam (100 °C), dilute sulfuric acid (121 °C) and lime (calcium hydroxide) at 121 °C, 50 °C and room temperature (30 ± 1 °C). In the case of simple steam treatment (herein after referred to as ST), the biomass powders were moistened to 40% and 50 % moisture content respectively and exposed to steam for 30 min, 45 min and 60 min in a vegetable steamer (M/S TTK Prestige India Ltd, India) [28] . Samples after pretreatment were suspended in distilled water (3:20 w/v) and the soluble fraction was separated from the water insoluble solids (WIS) by centrifugation at 8000 rpm for 30 min. Part of the WIS was lyophilized for ultrastructural studies while the remaining part was dried, powdered and used for the compositional studies.

In the second experiment, biomass samples were treated with dilute sulfuric acid (DSA; 0.5% v/v) in a pressure cooker (M/S TTK Prestige India Ltd.) for 30 min and 60 min at 121°C and 0.102 MPa pressure (time after pressure build up). Samples after pretreatment were adjusted to pH 6.0 using 10N sodium hydroxide and then separated to soluble and insoluble fractions, as described earlier (and subjected to studies).

In the third experiment, the biomass residues were subjected to three types of lime pretreatment (0.1g calcium hydroxide per gram dry biomass), such as high temperature (121 °C ; 0.102 MPa for 30 min and 60 min) low temperature (50 °C for 6 h and 24 h) and room temperature (30 ± 1°C for 24 h and 48 h). After each sampling period, the biomass slurry was adjusted to pH 6.0 using concentrated Hydrochloric acid and the soluble and insoluble fractions were separated and subjected to analysis as described earlier.

2.3. Compositional studies

2.3.1 Polysaccharides and lignin

The native and pretreated biomass samples were subjected to compositional analyses as per the methods described earlier [28]. Starch content was determined by enzymatic assay using Spezyme and Stargen by the standardized method [31]. Biomass (2.0% w/v) was digested sequentially with Spezyme (pH 5.5; 90 °C; 0.5 ml) for 30 min. and Stargen (pH 4.5; 40 °C; 0.5 ml) for 24 h. Sodium azide (0.25% w/v) was added to prevent microbial contamination and the released reducing sugars were quantified by the titrimetric method of [32] using potassium ferricyanide reagent. The interference from hemicellulose and cellulose during acid hydrolysis could be avoided in the enzyme method. Enzyme and substrate blanks were kept to eliminate the interference from reducing sugars already present in the enzyme and original biomass respectively. Starch content was calculated using the conversion factor, 0.9 and in the case of pretreated biomass, the content was worked back to the original dry biomass based on the dry solids recovery after pretreatment.

Neutral and acid detergent fiber were determined using the original method of Goering and Vansoest [33] with modifications incorporating amylolytic enzymes to avoid the interference from starch. The biomass slurry after treatment with neutral detergent solution in presence of sodium sulfite was digested with Spezyme and Stargen as described earlier. The contents after 24 h digestion with Stargen were filtered and residue washed with acetone and dried to quantify the NDF. The ADF content was determined in the NDF fraction by the method of Goering and Vansoest [33] and the values were worked back to the original dry biomass.

Hemicellulose content in the native and pretreated biomass was calculated as the difference between NDF and ADF. Cellulose was quantified as per the method of Updegroff [34] using acetic-nitric reagent with the difference that the ADF fraction was used, which helped to eliminate the interference from starch. Ash content in the native and pretreated biomass was estimated by the AOAC method [35]) by incinerating in a muffle furnace at 550 °C for 8 h. In order to avoid the overestimation of lignin due to the bound proteins, the protein content in ADF was determined by the Kjeldahl method [35] and subtracted from ADF to get the true ADF (TADF). Lignin content was then computed using the equation:

$$\text{Lignin (\%)} = \text{True ADF (\%)} - [(\text{cellulose} + \text{ash})\ \% \qquad (1)$$

2.3.2.Sugars and Pretreatment Efficiency

Total and reducing sugars in the original untreated and pretreated biomass were assayed in the 80% ethanol extract by the titrimetric method [32]. The reducing sugars in the pretreated liquors were also quantified by the same method. Pretreatment efficiency (%) was worked out from the total reducing sugars in the pretreated liquors and pretreated residue (value obtained from the substrate blank readings of starch assay) after nullifying the RS originally present in the untreated biomass using the following equation:

$$\text{PE (\%)} = \frac{[(\text{RSpt} + \text{RSr}) - \text{Rsob}]\times 100}{[\text{C+HC+S+TS in original biomass (\% dwb)}} \qquad (2)$$

where RSpt = RS released from the biomass due to pretreatment (expressed as % of the original biomass);

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RSr = RS held back in the pretreated residue (expressed as % of the original biomass); RSob = RS (%) originally present in the biomass; C: cellulose; HC: hemicellulose; S;- starch and TS: total sugars.

2.3.3 Statistical analysis

Three replicates were maintained for each experiment and duplicate analyses performed on each replicate. The data were subjected to Analysis of Variance (ANOVA) for statistical testing of the mean values and the least significant difference (LSD) for pair-wise comparison of mean values was worked out using the statistical package,[36].

III. RESULTS AND DISCUSSION

3.1. Compositional profile of native biomass

The compositional profile of peels from beet root (BP), greater yam (GYP), Pumpkin (PP) and vegetable banana (VBP) is presented in Table 1. Highest cellulose contents were observed in VBP and PP while the other two biomass residues had similar (18-19%) cellulose contents. Hemicellulose, on the contrary was higher in GYP and BP and the lowest in VBP. Very high starch content of 36.6% was obtained for VBP while the other residues had starch in the range of 24-29% (Table 1). Lignin content was the highest (10.6%) in PP and VBP, while BP had very low lignin content. Ash content ranged from 3.3 to 5.7%. Total and reducing sugar contents were the highest in the beet root peel, accounting for 17% and 7% of dry weight respectively. Despite the highest starch content, sugar content was the lowest in VBP.

Most of the studies reported on the bioethanol production potential of banana peel are related to the ripe fruit banana or plantain and hitherto no studies are available on cooking banana (ABB group) peel. Okareh *et al* [9] (2015) reported that *M. paradisiaca* peel contained 68% carbohydrate and 8.9% ash, besides 10.4% crude fiber. We found that the VBP had a potential sugar yielding carbohydrate (PSYC) content of 77% (comprising cellulose, hemicellulose, starch and total sugars), which is similar to that reported for plantain (AAB) group by Okareh *et al.* [9]. Besides there was very high starch content in VBP, while only 7.2% starch (dry basis) was reported in desert banana (AAB) group by Mohapatra *et al* [11] . Total carbohydrate and fiber contents of 59% and 8.2% were reported in *M. sapientum* peel by Xu *et al* [26]. They also reported that the peel contained 8.5% ash which was much higher than 3.40% observed in VBP in our study. Chantawongsa and Kongkiattikajorn [37] reported total carbohydrate content of 60.8 % in banana peel with a high starch content of 32.75% similar to our study. Lignin content in VBP (10.6 %) was in the range reported for plantain and fruit banana peels [11,37]. It was found that pumpkin (*C. moschata*) and greater yam peels were also rich sources of carbohydrate (72.13% and 71.2% respectively comprising cellulose, HC, starch and TS), while 74-75% carbohydrate has been reported for pumpkin peel by others [15]. Out of the total sugars, 74.5 % existed as reducing sugars (RS) in pumpkin peel which was much higher than 40.5 % in beer root peel, 50% in GYP and 61.7% in VBP (Table 1). There are no reports on the compositional profile of the peel of garden beet. Nevertheless, Zheng *et al*. [38] reported that the dry pulp from sugar beet after extraction of sucrose contained 86-87% carbohydrate and 1-2% lignin and its potential as a biofuel source has been reported [38]. The low lignin content in BP and GYP might be advantageous during the saccharification stage, as it could reduce the chances of inhibition of cellulase by lignin byproducts formed during pretreatment [39].

3.2. Polysaccharide changes during pretreatments

The changes in the structural (cellulose and hemicellulose) and non-structural polysaccharides in the selected biomass after the three pretreatment methods indicated that very high extent of starch hydrolysis occurred during DSA pretreatment in all the residues (Table 2). Proportionate increase in starch hydrolysis was observed when DSA pretreatment time was extended to 60 min. High starch hydrolysis was also observed in P2 (40% MC steam treated for 45 min.). It was found that approximately 94-95% starch was hydrolyzed in the various DSA pretreated (60 min) biomasses, while only 35-37% reduction occurred in P2 (Table 3). We had earlier reported 94% reduction in DSA pretreated biomass such as peels of sweet potato, elephant foot yam, tannia and ash gourd as well as in mixed vegetable wastes from households/ restaurants [28] while only up to 25% and 5% hydrolysis respectively were observed in steam (60 min.) and lime pretreatments. Maximum hydrolysis of hemicellulose occurred in ST (P3 and P6; Tables 2 and 3), which was similar to those reported earlier[28].

*Table 1: Compositional profile of the selected root and vegetable processing residues (expressed as g/100 g dry basis)**

Parameters	Beet root peel (BP)	Greater yam peel (GYP)	Pumpkin peel (PP)	Vegetable banana peel (VBP)
Ash	5.66 ± 0.10	3.29 ± 0.24	4.22 ± 0.06	3.40 ± 0.08
Lignin	3.87 ± 0.34	6.72 ± 0.17	10.66 ± 0.84	10.55 ± 0.33
Cellulose	18.94 ± 0.20	18.02 ± 0.58	21.05 ± 0.79	22.40 ± 0.64
Hemicellulose	19.17 ± 0.55	20.02 ± 0.57	17.74 ± 0.47	15.19 ± 0.56

Starch	27.13 ± 0.00	28.84 ± 0.44	24.61 ± 0.00	36.56 ± 0.00
Total sugars	17.07 ± 0.12	4.33 ± 0.00	8.73 ± 0.06	2.77 ±0.01
PSYC**	82.31	71.21	72.13	76.92
Reducing sugars	6.91 ± 0.04 (40.50%)***	2.17 ± 0.00 (50.00%)	6.50 ± 0.00 (74.46%)	1.71 ±0.00 (61.70%)
Others****	8.16	18.78	13.00	9.13

*Each value is Mean ± SD from three replicates; ** PSYC- potential sugar yielding carbohydrate comprises cellulose+ hemicellulose+ starch+ total sugars; *** figures in parentheses indicate percentage of TS existing as RS; **** Others include residual moisture, protein, extractives, bioactives such as phenols etc.

Cellulose was also hydrolysed to a higher extent (27-29 %) in these pretreatments, while only negligible hydrolysis was observed in the DSA and lime pretreated biomass residues (Table 3). There are reports that cellulose is only slowly attacked by DSA and is soluble in alkalis [39]. Approximately 42-43% hemicellulose was hydrolysed during DSA (60 min.) pretreatment (Table 3). Saha and Bothast [41]also found that pretreatment of corn fiber with 0.5% DSA (121 °C) had the highest effect in hydrolyzing hemicellulose and starch. Dilute sulfuric acid is reported to hydrolyze hemicellulose, leaving a residue that is rich in cellulose and lignin. Removal of hemicellulose is reported to weaken the carbohydrate-lignin matrix, thereby enhancing the accessibility of cellulose [42,43]. The findings from the present study are supportive of the earlier reports. Prolonging the exposure time of wet biomass to steam from 45 min. to 60 min. resulted in retention of more starch, possibly because of the transformation of starch to resistant form especially under the acidic pH due to the hydrolysis of hemiacetal groups from hemicellulose. Such a reversion was reported earlier in steam pretreated cassava starch factory waste and processing residues of cassava as well as in the peels from sweet potato, elephant foot yam, tannia and ash gourd [28,44,45].

Lime pretreatment resulted in the removal of only smaller quantities of cellulose, hemicellulose and starch, with much of the starch being retained in the water insoluble solid (WIS) fraction (Table 2) and the pattern was similar for the various residues irrespective of the variations in the original profile. While cellulose was removed to the extent of 1.2-10% by the three types of lime pretreatment, hemicellulose was solubilized to a higher extent (7.6-13%), with higher values for 24 h RT lime treatment and starch solubilization ranged from 0.20 to 4.8% only (Table 3). We had earlier reported similar pattern of removal of structural and non-structural polysaccharides during lime pretreatment of peels of sweet potato, elephant foot yam, tannia and ash gourd [29]. Kim and Holtzapple [46] reported solubilization of only 6.3% glucans and 21% xylan after 16 weeks lime pretreatment (0.5g/g biomass) of corn stover. Chang et al. [47] found that 0.1g/g dry biomass was the optimum for lime loading for sugarcane bagasse and the same was used in our study as well.

Among the three pretreatments, DSA resulted in the hydrolysis of very high amounts of starch and reasonably good quantities of hemicellulose, while hemicellulose was hydrolyzed to a greater extent in 60 min. steam pretreatment (40% and 50% MC). Since starch is a major component of the selected biomasses, DSA pretreatment (121 °C; 0.102 MPa) at moderate level (0.5% v/v) and time (30-60 min.) could be considered beneficial as it could reduce the amylase loading coupled with low xylanase requirement at the saccharification stage, leading to saving of enzyme costs.

3.3.Delignification in pretreated biomass

Maximum reduction in lignin was observed in P3 (40 % MC; 60 min.) and P6 (50% MC; 60 min.) for all the four biomasses (Table 4). Delignification percentage ranged from 28-37% in these pretreatments. Dilute sulfuric acid (60 min.) brought about only 8.7-14% delignification, while least effect was observed in the case of all the three lime pretreatments (Fig. 1 a-d). As the native untreated BP was found to contain only 3.87% lignin, the very low extent of delignification obtained in DSA and lime pretreatments might not pose a problem during saccharification.

Table 2: Structural and non-structural polysaccharide changes in steam, DSA and lime pretreated root and vegetable processing residues (expressed as g/100 g original material on dry basis)*

Pretreatments	**Beet root peel (BP)**			**Greater yam peel(GYP)**			**Pumpkin peel (PP)**			**Vegetable banana peel (VBP)**		
	C	**HC**	**ST**	**C**	**HC**	**ST**	**C**	**HC**	**ST**	**C**	**HC**	**ST**
Native	18.94[a]	19.17[a]	27.13[a]	18.02[a]	20.02[a]	28.84[a]	21.05[a]	17.74[a]	24.61[a]	22.40[a]	15.19[a]	36.56[a]
Steam pretreatment (40% MC)												

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P1 (30 min.)	17.71^{bc}	15.43^{d}	20.08^{d}	16.51^{b}	15.77^{d}	21.00^{d}	19.72^{b}	14.75^{c}	18.27^{d}	21.24^{bc}	12.63^{d}	27.15^{d}
P2 (45 min.)	16.96^{de}	14.97^{d}	17.27^{e}	15.87^{c}	15.37^{d}	18.09^{e}	18.94^{de}	14.47^{d}	15.63^{e}	20.09^{e}	12.37^{e}	23.57^{e}
P3 (60 min.)	13.70^{g}	9.25^{g}	20.67^{d}	12.83^{d}	9.45^{g}	21.76^{d}	15.43^{g}	8.24^{g}	18.77^{d}	16.40^{g}	7.14^{h}	27.56^{d}
Steam pretreatment (50% MC)												
P4 (30 min.)	17.55^{cd}	14.92^{b}	22.01^{c}	16.50	15.38^{d}	23.19^{c}	19.41^{cd}	14.39^{d}	20.00^{c}	20.60^{de}	12.43^{d}	29.30^{c}
P5 (45 min.)	16.02^{f}	14.42^{e}	20.08^{d}	15.04^{c}	14.86^{d}	21.13^{d}	17.86^{f}	13.29^{e}	18.21^{d}	19.30^{f}	11.48^{f}	27.12^{d}
P6 (60 min.)	13.65^{g}	8.74^{g}	22.16^{c}	12.78^{d}	8.92^{gh}	23.35^{c}	15.20^{g}	8.11^{g}	20.16^{c}	16.05^{g}	6.90^{h}	29.65^{c}
DSA (121 ºC and 0.102 MPa)												
30 min.	16.42^{ef}	14.61^{e}	4.48^{f}	15.76^{c}	15.39^{d}	4.89^{f}	18.03^{ef}	13.25^{e}	4.15^{f}	19.37^{f}	11.57^{f}	6.06^{f}
60 min.	16.04^{f}	11.10^{f}	1.61^{g}	15.40^{c}	11.72^{f}	1.84^{g}	17.38^{f}	10.22^{f}	1.25^{g}	18.79^{f}	8.66^{g}	2.15^{g}
Lime HT (121 ºC and 0.102 MPa)												
30 min.	17.51^{cd}	17.23^{b}	27.00^{a}	16.51^{b}	17.84^{b}	28.54^{a}	19.27^{cd}	15.75^{b}	24.47^{a}	20.52^{de}	13.46^{c}	36.37^{a}
60 min.	18.71^{a}	17.71^{b}	26.50^{a}	17.65^{b}	18.34^{b}	28.01^{a}	20.61^{ab}	16.19^{b}	24.00^{a}	21.94^{b}	13.84^{b}	35.70^{a}
Lime LT (50 ºC)												
6 h	17.82^{bc}	17.53^{b}	27.07^{a}	16.80^{b}	18.15^{b}	28.61^{a}	19.62^{bc}	16.03^{b}	24.52^{a}	20.89^{cd}	13.69^{b}	36.47^{a}
24 h	18.31^{ab}	17.34^{b}	26.00^{a}	17.27^{b}	17.95^{b}	27.48^{a}	20.16^{bc}	16.05^{b}	23.60^{a}	21.46^{bc}	13.54^{c}	35.10^{a}
Lime RT (30±1 ºC)												
24 h	17.21^{cd}	16.86^{c}	25.97^{a}	16.22^{b}	17.46^{b}	27.44^{a}	19.04^{d}	15.41^{b}	23.50^{a}	20.17^{e}	13.17^{c}	35.00^{a}
48 h	17.93^{bc}	17.45^{b}	26.90^{a}	16.91^{b}	18.07^{b}	28.44^{a}	19.74^{bc}	16.06^{b}	24.43^{a}	21.01^{cd}	13.63^{b}	36.33^{a}

*Each value is mean from three replicates; statistical comparison was made with the respective values in the native sample for each biomass; means with different superscripts in each column are significant at $p < 0.05$; MC- moisture content

Among the three types of lime pretreatments, highest delignification was in 24 h RT for the various biomasses (Table 4 and Fig.1 a-d). Lime is reported to enhance the removal of acetyl groups and breakdown the lignin-carbohydrate ester linkages, resulting in the reduction in cellulose crystallinity [4]. Besides, the divalent calcium ions are reported to form effective crosslinking with lignin, which therefore remains in the pretreated residue itself without getting solubilized [48]. Under the alkaline pH, carboxyl, methoxy and hydroxyl groups of lignin become ionized and assume negative charge, which facilitates its binding to calcium [49].

Table 3:Percentage reduction in the structural and non-structural polysaccharides due to steam, DSA and lime pretreatment in root and vegetable processing residues*

Pretreatments	**Beet root peel (BP)**			**Greater yam peel (GYP)**			**Pumpkin peel (PP)**			**Vegetable banana peel (VBP)**		
	C	**HC**	**ST**	**C**	**HC**	**ST**	**C**	**HC**	**ST**	**C**	**HC**	**ST**
Steam pretreatment (40% MC)												
P1 (30 min.)	6.50^{ef}	19.52^{d}	26.00^{d}	8.39^{g}	21.22^{f}	27.18^{d}	6.32^{cde}	16.83^{de}	25.79^{de}	5.20^{e}	16.87^{de}	25.73^{d}
P2 (45 min.)	10.45^{cd}	21.90^{cd}	36.33^{c}	11.95^{e}	23.25^{e}	37.26^{c}	10.03^{c}	18.44^{d}	36.51^{c}	10.34^{c}	18.59^{d}	35.54^{c}
P3 (60 min.)	27.65^{a}	51.73^{a}	23.82^{e}	28.82^{a}	52.78^{b}	24.55^{f}	26.73^{a}	53.54^{a}	23.73^{f}	26.81^{a}	53.02^{a}	24.62^{de}
Steam pretreatment (50% MC)												
P4 (30 min.)	7.30^{de}	22.15^{cd}	18.86^{f}	8.46^{g}	23.20^{e}	19.58^{g}	7.83^{cd}	18.86^{d}	18.73^{g}	8.06^{cd}	18.19^{d}	19.86^{f}
P5 (45 min.)	15.38^{b}	24.74^{c}	26.00^{d}	16.54^{b}	25.79^{d}	26.73^{de}	15.15^{b}	25.09^{c}	26.04^{d}	13.84^{b}	24.43^{c}	25.83^{d}
P6 (60 min.)	27.92^{a}	54.39^{a}	18.30^{f}	29.08^{a}	55.44^{a}	19.03^{g}	27.82^{a}	54.29^{a}	18.11^{g}	28.37^{a}	54.56^{a}	18.89^{fg}
DSA (121 ºC and 0.102 MPa)												
30 min.	13.28^{bc}	23.75^{c}	83.49^{b}	12.56^{d}	23.10^{e}	83.04^{b}	14.39^{b}	25.30^{c}	83.14^{b}	13.55^{b}	23.81^{c}	83.42^{b}
60 min.	15.28^{b}	42.09^{b}	94.06^{a}	14.56^{c}	41.44^{c}	93.61^{a}	17.44^{b}	42.41^{b}	94.94^{a}	16.15^{b}	43.00^{b}	94.11^{a}
Lime HT (121 ºC and 0.102 MPa)												

30 min.	7.51^{de}	10.10^{ef}	0.48^{j}	8.40^{g}	10.90^{h}	1.04^{j}	8.46^{cd}	11.23^{f}	0.60^{j}	8.41^{cd}	11.42^{f}	0.54^{j}
60 min.	1.17^{g}	7.59^{f}	2.32^{h}	2.06^{k}	8.38^{j}	2.88^{i}	2.12^{e}	8.71^{f}	2.50^{i}	2.07^{f}	8.90^{f}	2.36^{i}
Lime LT (50 ºC)												
6 h	5.88^{ef}	8.53^{f}	0.24^{k}	6.77^{h}	9.33^{i}	0.79^{k}	6.83^{cd}	9.66^{f}	0.40^{k}	6.77^{de}	9.85^{f}	0.26^{k}
24 h	3.31^{fg}	9.56^{ef}	4.17^{g}	4.19^{j}	10.35^{h}	4.72^{h}	4.26^{de}	9.56^{f}	4.12^{h}	4.20^{ef}	10.87^{f}	4.00^{h}
Lime RT (30±1 ºC)												
24 h	9.09^{de}	12.01^{e}	4.29^{g}	9.98^{f}	12.81^{g}	4.84^{h}	9.57^{c}	13.14^{ef}	4.53^{h}	9.98^{c}	13.33^{ef}	4.27^{h}
48 h	5.30^{ef}	8.93^{ef}	0.84^{i}	5.89^{i}	10.17^{h}	1.49^{j}	6.26^{cde}	9.50^{f}	0.74^{j}	6.20^{de}	10.25^{f}	0.63^{j}

* Means with different superscripts in each column are significant at $p < 0.05$

3.4.Reducing sugar (RS) changes and pretreatment efficiency

Highest reducing sugar levels (g L^{-1} pretreated liquor) were obtained in the DSA pretreated biomass slurries, due to the high starch and hemicellulose hydrolysis (Table 5). Prolonging the pretreatment time from 30 to 60 min. raised the RS level to 40-45 g L^{-1} in the various pretreated liquors. Among the ST pretreated liquors, the highest RS values were obtained for P3 (40% MC; 60 min). Least values were obtained for lime pretreated slurry, evidently due to the low starch and hemicellulose hydrolysis (Table 5). We had earlier reported similar trends for the RS content of ST, DSA and lime pretreated slurries of peels from sweet potato, elephant foot yam, tannia and ash gourd[28,29].

Table 4 : Lignin changes in steam, DSA and lime pretreated root and vegetable processing residues (expressed as g/100 g original material on dry basis).*

Pretreatments	**Beet root peel (BP)**	**Greater yam peel (GYP)**	**Pumpkin peel (PP)**	**Vegetable banana peel (VBP)**
Native	3.87^{a}	6.72^{a}	10.66^{a}	10.55^{a}
Steam pretreatment (40% MC)				
P1 (30 min.)	3.73^{abc}	6.14^{a}	10.42^{b}	9.73^{cd}
P2 (45 min.)	3.44^{cd}	5.70^{ab}	9.62^{cd}	9.03^{e}
P3 (60 min.)	2.71^{f}	4.49^{c}	7.61^{f}	7.57^{gh}
Steam pretreatment (50% MC)				
P4 (30 min.)	3.28^{de}	5.48^{ab}	9.02^{e}	8.43^{f}
P5 (45 min.)	3.11^{e}	5.19^{b}	8.69^{e}	7.89^{g}
P6 (60 min.)	2.56^{f}	4.23^{c}	7.11^{g}	7.19^{h}
DSA (121 ºC and 0.102 MPa)				
30 min.	3.63^{abc}	6.43^{a}	10.00^{bc}	10.00^{bc}
60 min.	3.46^{bcd}	6.14^{a}	9.15^{de}	9.37^{de}
Lime HT (121 ºC and 0.102 MPa)				
30 min.	3.84^{a}	6.52^{a}	10.40^{b}	10.29^{ab}
60 min.	3.75^{abc}	6.36^{a}	10.15^{bc}	10.04^{bc}
Lime LT (50 ºC)				
6 h	3.86^{a}	6.54^{a}	10.43^{b}	10.32^{ab}
24 h	3.71^{abc}	6.28^{a}	10.02^{bc}	9.91^{bc}
Lime RT (30±1 ºC)				
24 h	3.63^{abc}	6.14^{a}	9.92^{bc}	9.70^{cd}
48 h	3.83^{a}	6.50^{a}	10.37^{b}	10.26^{ab}

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* Statistical comparison was made with the native sample for each biomass; means with different superscripts in each column are significant at $p < 0.05$.

Accordingly, the pretreatment efficiency (%) was also the highest for DSA pretreatment.As high as 57-64% of the carbohydrates got converted to RS due to DSA pretreatment alone, which indicates that low enzyme loading might only be needed at the saccharification stage.Very low pretreatment efficiency (%) of 9-13% only was observed in the lime pretreatment, while ST for 60 min. gave PE (%) of 26-31% (Table 6). Solids recovery was also the highest (86-95%) from lime pretreatment, indicating the low biodegradation during pretreatment, while 40-68% and 40-60% residues respectively remained after ST and DSA pretreatments (data not shown) of the different biomasses.

Table 5: Reducing sugar content in the pretreated liquor from steam, DSA and lime pretreated processing residues (g L^{-1} pretreated liquor)*

Pretreatments	**Beet root peel (BP)**	**Greater yam peel (GYP)**	**Pumpkin peel (PP)**	**Vegetable banana peel (VBP)**
Steam pretreatment (40% MC)				
P1 (30 min.)	18.91^{g}	15.14^{g}	17.11^{e}	14.79^{g}
P2 (45 min.)	22.90^{e}	19.10^{e}	20.22^{d}	19.70^{e}
P3 (60 min.)	28.43^{c}	24.38^{c}	26.75^{c}	24.76^{c}
Steam pretreatment (50% MC)				
P4 (30 min.)	17.60^{gh}	13.36^{h}	16.02^{ef}	13.54^{gh}
P5 (45 min.)	21.55^{f}	17.39^{f}	20.00^{d}	17.78^{f}
P6 (60 min.)	27.51^{cd}	23.37^{cd}	26.34^{c}	23.11^{cd}
DSA (121 ºC and 0.102 MPa)				
30 min.	36.00^{b}	32.40^{b}	33.40^{b}	38.00^{b}
60 min.	43.00^{a}	39.48^{a}	40.56^{a}	45.00^{a}
Lime HT (121 ºC and 0.102 MPa)				
30 min.	10.32^{i}	6.07^{j}	10.30^{g}	5.50^{j}
60 min.	8.63^{k}	4.96^{k}	9.00^{gh}	4.20^{jk}
Lime LT (50 ºC)				
6 h	9.65^{ij}	5.40^{jk}	9.60^{gh}	4.50^{jk}
24 h	9.56^{ij}	6.27^{j}	10.00^{g}	5.60^{j}
Lime RT (30±1 ºC)				
24 h	10.52^{i}	7.84^{i}	11.58^{f}	7.30^{i}
48 h	9.65^{ij}	5.55^{jk}	9.58^{gh}	4.53^{jk}

* Statistical comparison was made between treatments for each biomass; means with different superscripts in each column are significant at $p < 0.05$.

These studies showed that the pretreated liquor from DSA and ST pretreatments being rich in reducing sugars and that from lime pretreatment being viscous due to the swelling of cellulose and starch, separation of the liquid fraction from the solid could lead to either loss of RS in the former two pretreatments or make filtration difficult in the latter case. Hence saccharification of the pH adjusted whole slurry would be advisable. Nevertheless,

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formation of fermentation inhibitors such as furfural, 5-hydroxyl methyl furfural and acetic acid has been reported in DSA pretreatments and an understanding of their levels in the selected biomasses, which is presently being studied and strategies to bring down the levels during downstream operations may be important.

Table 6: Pretreatment efficiency (%) in steam, DSA and lime pretreated root and vegetable processing residues*

Pretreatments	Beet root peel (BP)	Greater yam peel (GYP)	Pumpkin peel (PP)	Vegetable banana peel (VBP)
Steam pretreatment (40% MC)				
P1 (30 min.)	17.09^{g}	16.66^{g}	17.59^{g}	19.70^{g}
P2 (45 min.)	20.91^{e}	20.48^{e}	21.07^{e}	24.97^{e}
P3 (60 min.)	27.14^{c}	26.71^{c}	29.20^{c}	31.02^{c}
Steam pretreatment (50% MC)				
P4 (30 min.)	14.79^{h}	14.36^{h}	15.26^{h}	17.30^{h}
P5 (45 min.)	19.28^{f}	18.85^{f}	20.72^{f}	22.49^{f}
P6 (60 min.)	26.33^{d}	25.90^{cd}	28.99^{d}	29.21^{d}
DSA (121 ºC and 0.102 MPa)				
30 min.	58.60^{b}	57.13^{b}	57.40^{b}	60.00^{b}
60 min.	61.30^{a}	60.66^{a}	59.30^{a}	64.00^{a}
Lime HT (121 ºC and 0.102 MPa)				
30 min.	12.87^{i}	11.97^{i}	12.19^{k}	10.87^{k}
60 min.	11.38^{m}	10.48^{ij}	11.00^{n}	9.34^{m}
Lime LT (50 ºC)				
6 h	11.29^{n}	10.39^{ij}	12.19^{k}	9.16^{n}
24 h	11.86^{l}	10.96^{ij}	11.00^{n}	9.56^{l}
Lime RT (30±1 ºC)				
24 h	12.75^{j}	11.85^{i}	12.57^{i}	11.54^{i}
48 h	12.59^{k}	11.69^{i}	12.25^{j}	11.03^{j}

* Computed as given in Methods (Equation 2) based on the potential sugar yielding carbohydrates; means with different superscripts in each column are significant at $p < 0.05$.

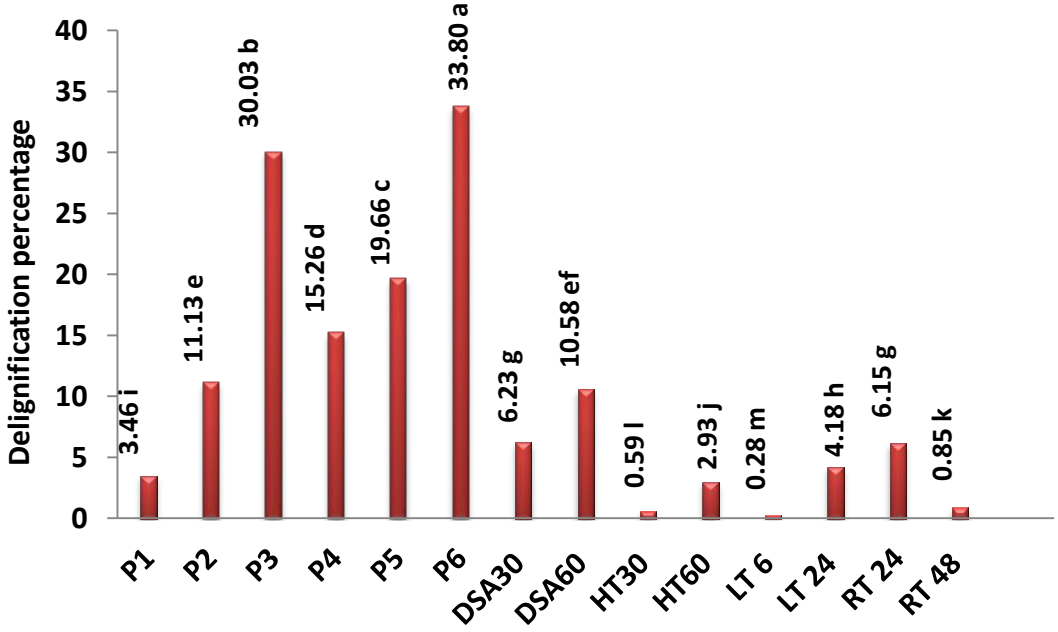

Fig.1a: Delignification in steam, DSA and lime pretreated beetroot peel Bars with different alphabets differ significantly at p <0.05

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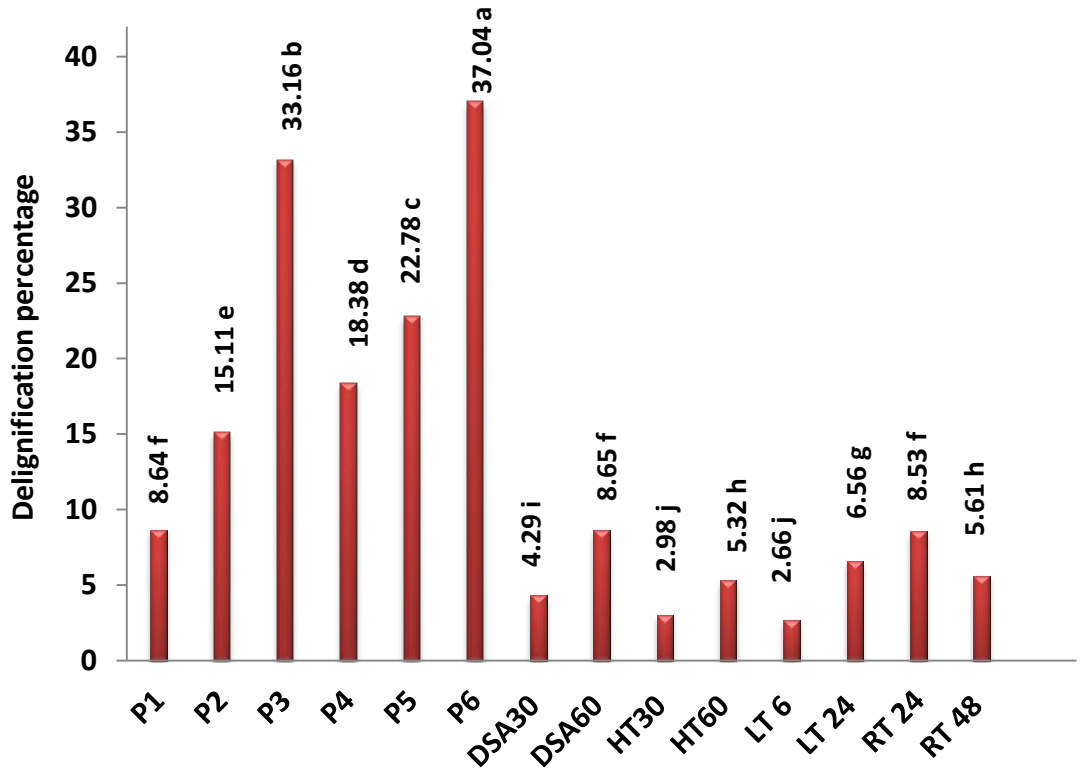

Fig.1 b: Delignification in steam, DSA and lime pretreated greater yam peel; other footnotes as in Fig. 1 a

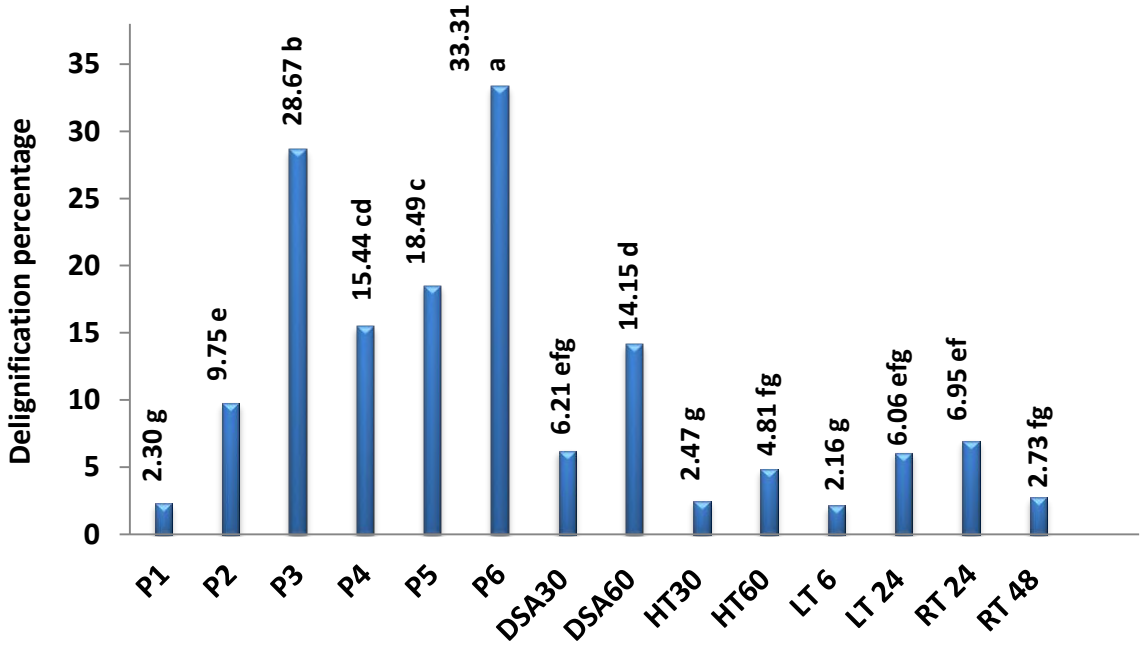

Fig.1 c: Delignification in steam, DSA and lime pretreated pumpkin peel; other footnotes as in Fig. 1 a

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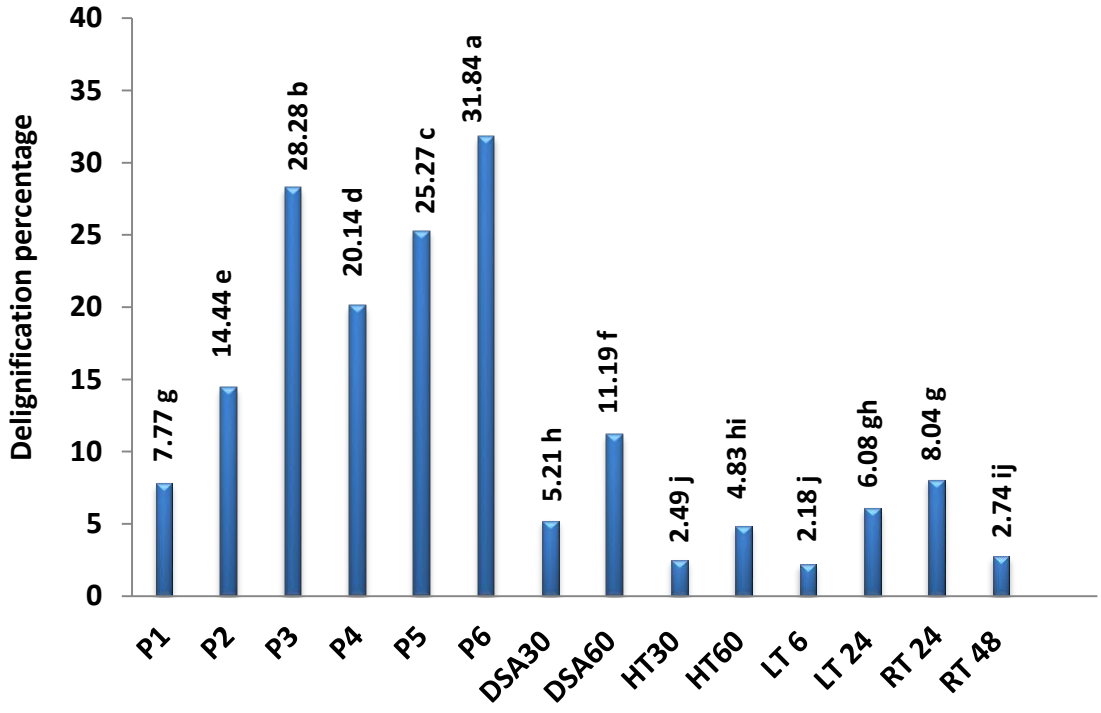

Fig.1 d: Delignification in steam, DSA and lime pretreated vegetable banana peel; other footnotes as in Fig. 1 a

IV. CONCLUSION

The effect of three pretreatments such as steam, DSA and lime on the compositional changes in root and vegetable processing residues was investigated. It was found that the peels from beetroot, greater yam, pumpkin and vegetable banana were rich in starch (25-37%) besides the structural polysaccharides, cellulose and hemicellulose. While DSA pretreatment for 60 min hydrolyzed starch to a very high extent (*ca.* 95%) followed by hemicellulose (*ca.* 43%), maximum hydrolysis of hemicellulose occurred in steam pretreatment (60 min). Lime pretreatment removed only small quantities of polysaccharides. Delignification was the highest in steam pretreated residues (28-37%) while only 8.7-14% lignins were removed from DSA pretreated biomass. Pretreated liquor from DSA treatment had the highest reducing sugar levels followed by steam treatment, indicating the need for whole slurry saccharification.

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Coping Strategies of Diabetic Yam Farming Households in Benue State, Nigeria

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Abstract— *This study engaged the Multinomial Logistic Model (MLN) to determine factors influencing te choice of coping strategies of diabetic yam farming households in Benue State, Nigeria. A multi-stage random sampling technique was used to select 340 yam farming households with emphasis on 2015 farming season. Primary data were obtained using a well structured and pretested questionnaire. The results of analysis shows that the most frequently used coping strategies were special diets such as millet, cocoyam, locust bean, groundnut, fruits and vegetables accounting for 39%, constant intake of drugs like metformin, biguarnide, sulphorylureas and insulin 25%, hired labour, 23.2%, routine exercise, 12.6%, while hawking was 0.3%. The choice of constant drug was -0.012, while the choice of hawking was significantly ($p < 0.05$) and negatively affected by education as a coping strategy. The marginal effect of education on constant drug was -0.012, while the choice of hawking was significantly ($p < 0.05$) and positively affected by the age as a coping strategy. The marginal effect of age on hawking was 0.04. It is recommended that government at Federal, State and Local levels with partners in progress should consider critical ways of managing diabetes by emphasizing healthy lifestyles such as ceasation of smoking, moderate alcohol intake, regular medical check-up and improvement of the socio-economic status of the diabetic farm households through good road network, steady supply of electricity which will better the quality of life of the farm households.*

Keywords— Yam Farming, FO, IDF.

I. INTRODUCTION

Available records from International Diabetic Federation (IDF), 2010) indicates that access to appropriate diabetes cure in Sub-Saharan Africa is extremely limited because of inadequate health care system, shortage of doctors, unaffordability of medication and other equipment. The poor, especially farmers, resort to traditional source or resign to fate, leading to improper management. Therefore, farmers who are victims or caregivers face farm labour supply shortage. This had impair food crop production. The study focused on the effects of diabetic scourge on yam crop farmers in Benue State. The choice of yam for the study was because yam is the predominant crop cultivated by farmers in the State.

According to Giwa and Tayo (2013), socio-economic characteristics such as family size, educational level, marital status, age, gender, and income level influence the ability of individual to cope with diabetes illness. This involves knowledge, self-care skills, household labour hiring, practice aimed at cushioning the effect of diabetes on income, increased efforts made by families members at the time of illness and death (Motala, 2013).

Diabetes represents a large financial cost to the society and individuals. The process of seeking treatment involves cost and expenditures on drugs and medication, transport, funeral rites of individual and families. The fear of contracting diabetes also urges people to protect themselves. The theory of averting behaviors predicts that a person will continue to take protective measure as long as the perceived benefits exceed the cost of doing so. The government also ensures that resources are provided to maintain and operate a good health system to enhance efficient and effective productivity.

Spencer (2002) showed that farmers must be assisted to increase production through more efficient use of resources. The full potential of land, capital and labour resources are yet to be fully tapped into due to poor health of farmers and related factors (Aboderin, 2010). Diabetes, therefore, is not only a public health problem but also a developmental problem. At the national level, apart from the negative effects of low productivity on the major sectors of the economy, diabetes had negative effects on saving, trade and investment potentials (Strauss & Thomas,1998).Some affected farm households and caregivers resort to soliciting claims from support network and untimely sale of farm produce, belongings, livestock, food reserves, capital assets, borrowing from money lenders and reduction in nutritional value of their food consumption. This could effects

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agricultural productivity, therefore the study examined the coping strategies of farm households.

II. METHODOLOGY

The study area was Benue State, Nigeria. "The Food Basket of the Nation", was created in 1976 with its name derived from River Benue, the second largest river in Nigeria. The administrative headquarters is Makurdi and it is composed of 23 Local Government Areas and 423 Council Wards. The State is located in the North Central region of Nigeria, which is the transition zone from the Northern and Southern ecologies. It lies between longitude $6^0 31'$ E and 10^0E and Latitudes $6^0 30'$ N and $8^0 10'$N (BNARDA, 2005). The State shares boundaries with five neighbouring states: Nassarawa to the North; Taraba to the East; Cross River and Enugu to the South-East; Enugu and Kogi to the West. The eastern part of the state is also bounded with the Republic of Cameroun.

Benue State has a total land mass of about 33, 955 km^2 (BNARDA, 2005). Agriculturally the State is divided into three zones:Zone A (Katsina-Ala, Ukum, Ushongo, Vandeikya, Logo, Kwande and Konsisha, LGAs); Zone B (Gboko, Tarka, Buruku, Gwer-east, Gwer-West, Guma and Makurdi, LGAs); and Zone C (Ado, Agatu, Apa, Otukpo, Ohimini, Okpokwu, Ogbadigbo, Obi and Oju, LGAs). The state has a total population of 4, 219, 244 people and 413, 159 households (National Population Commission (NPC), 2006; BNARDA, 2005).

The state has favourable agro-climatic ecologies for arable crops, tree crops and livestock production and enjoys two distinct seasons; rainy season, beginning from April to October, and dry season, from November, to March. Annual rainfall records vary from 1700mm in the southern part to 1250mm in the northern ecology of the state with annual temperature variations of 30^0C and 35^0C (Benue State Government (BNSG), 2011). The three major ethnic groups are the Tiv, Idoma and Igede. Other smaller ethnic group are Etulo, Abakpa, Akwaya and Jukun. Yam is the major crop produced in the State; and it is consumed in a variety of forms with sauce and soup, including pounded yam, roasted, fried and porridge (BNARDA, 2004). Yam is used as delicacy during marriage, birthday, funeral and other social and religious ceremonies in large quantities. Benue State, the Food Basket of the Nation with 70% of its population depending on agriculture as their main source of livelihood (BNARDA, 2004) ranked very high among the diabetic endemic States in Nigeria (DAN, 2014).

III. DATA AND ANALYTICAL TECHNIQUE

Data for this study were collected from primary sources. The primary data were collected from the diabetic yam farming households using a well structured and pretested questionnaire. The questionnaire was administered with the assistance of extension agents from Benue State Agricultural and Rural Development Agency and Diabetes Association of Nigeria, Benue State chapter with emphasis on 2015 farming season. The data collection instrument focused on prevalence and incidence of diabetes, socio-economic characteristics of households, direct and indirect cost in form of registration fees, consultation fees, laboratory test, transportation, productivity lost by diabetic patients, caregivers and substitute labour. The questionnaire also capture information on dietary habits, lifestyle, technical efficiency as well as factors influencing choice and coping strategies of the households. The instrument was administered to the household head.

The multinomial logistic regression model was used to determine household's socio-economic and demographic factors that influenced choice of coping strategies. The multinomial logistic regression was expressed as adopted by Sofoluwe,Tijani and Baruwa(2011) as:

$$P_{ij} = \frac{\exp(\beta_j X_i)}{1 + \sum_{j=1}^{5} \exp(\beta_j X_i)} for j = 1,2,3,4,5 \ldots\ldots\ldots\ldots (1)$$

P_{ij} is the probability of being in each of the groups 2,3,4 and 5.

$$P_{i0} = \frac{1}{1 + \sum_{j=1}^{5} \exp(\beta_j X_i)} for j = 1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

P_{i0} is the probability of being in the reference group 1.

In practice, when estimating the model the coefficients of the reference group are normalized to zero (Maddala, 1990; Greene, 1993; Kimhi, 1994). This is because the probabilities for all the choices must sum up to unity (Greene, 1993). Hence, for 5 choices only (5-1) distinct sets of parameters can be identified and estimated.

The natural logarithms of the odd ratio of equations (1) and (2) give the estimating equation (Greene, 1993) as:

$$ln \frac{[P_{ij}]}{[P_{i0}]} = \beta_j X_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

This denoted the relative probability of each of group 2,3,4 and 5 to the probability of the reference group (j =1). The estimated coefficients for each choice, therefore, reflected the effects of Xi`s on the likelihood of the farm household choosing that coping strategy relative to the reference group. However, following Hill (1983), the coefficients of the reference group might be recovered by using the formula:

$$\beta_1 = -(\beta_2 + \beta_3 + \beta_4 + \beta_5). \quad (4)$$

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For each explanatory variable, the negative of the sum of its parameters for groups 2,3,4 and 5 is the parameter for the reference group.

Xi are socio-economic and demographic variables which influence decision to choose a coping strategy. They were:

MS = Marital status of household head (married =1, otherwise=0);

SH = Sex of household head (male=1, female=0);

AH = Age of household head (years);

LE = Years of educational attainment of household head;

HS = Household size (number);

FS = Farming experience of the household head (years);

FI = Farm income of the household head (₦);

OF = Off-farm income of the household head (₦); and

RM = Remittance to the household head (₦).

IV. RESULTS AND DISCUSSION

Table 1 shows the percentage of households adopting particular strategy to cope with diabetic scourge in the study area. The most frequently used coping strategy by affected households was consumption of special diets such as millet, cocoyam, locust bean, groundnut fruits and vegetables making up to 38.8%. Weil (2004) found that special diets enhanced one's ability to cope with diabetes and stress. The high ranking of diet among other coping mechanisms might be related to the numerous advertisements on media on what to eat to reduce the incidence of diabetes mellitus by naturalistic, traditional healers and herbalist. About 25.0% embarked on constant intake of drugs such as biguarnides, insulin, sulphornylureas, statins and antihypentisives. Some authorities such as Enwere, Salako and Falade (2006), who investigated the prescription and cost consideration at a diabetic clinic in Ibadan, Nigeria, recommended constant drug intake as a prerequisite for coping with diabetes despite their high cost.

About 23.2% of respondents employed hired labour to cope with diabetes. This is necessary because diabetes scourge has significant adverse effects on household composition, labour supply, cropping pattern and food production. In assessing the effectiveness of households coping strategies with diabetes and heart-related ailments in Nigeria, DAN (2014) reported that hired labour played a central and crucial role while manual labour supply remained a major source of production.

The result further showed that 12.6% of respondents embarked on routine exercise to cope with diabetes illness while 0.3% survived by hawking. These findings are at variance with Jiang and Braun (2005) who reported households coping strategies with ill-health risk to include, selling of productive assets such as farm equipment, land, breeding animals, hired labour, borrowing from friends, withdrawing of children from schools, hawking, exercising, community based support. Variation in coping between households could be based on differences in asset base, age of household head, educational level as well as gender.

Table.1: Major Coping Strategies of Diabetic Farming Households (n=340)

Strategies	Frequency	Percentage (%)
Special diet	132	38.8
Regular exercise	43	12.6
Constant drugs	85	25.0
Hired labour	79	23.2
Hawking	1	0.3

Source: Field survey data, 2015.

The result of multinomial logit analysis showed the factors that influenced the choice of coping strategies by the respondents (table 2). The coefficients for each choice (special diets, constant drugs, hawking, hired labour) reflect the effects of socioeconomic and demographic variables such as age, sex, marital status, education, household size, farming experience, farm income, non-farm income and remittance on choice of a particular coping strategy. The significance of likelihood ratio chi-square (118.21) at 1% level implied that, the regression had a good fit. The result showed that the set of significant explanatory variables across the reference base group in terms of the level of significance and signs of the parameter estimate for factors influencing choice of coping strategies.

Age of the household head (0.09) positively and significantly influenced the choice of hawking as opposed to constant exercise as a coping strategy at 5%. As age of household head increased, the more likely they are going to send their children for hawking in order to raise money rather than embark on exercise. The marginal effect of age

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on hawking, as shown in table 3, was 0.004. This implied that a unit increase in the age of household head would increase hawking activities by 0.004%. Wu and Porell (2000) stated that most aged diabetes patients might even be too weak and with other complications to embark on constant exercise as a coping strategy.

The sex of the household head (-1.73) negatively and significantly influenced the choice of hawking as a coping strategy of diabetic farm households at 1% level.

Table.2: Parameter Estimates of Multinomial Logistic Regression Model for coefficient Factors Influencing Choice of Coping Strategies

Variables	**Special diet**	**Constant drugs**	**Hawking**	**Hired labour**
Age	0.04	0.04	0.09	-0.01
	(1.04)	(1.34)	(1.95)**	(-0.43)
Sex (male=1,female=0)	-0.60	-0.39	-1.73	-0.42
	(-1.09)	-0.78)	(-3.06)*	(-1.12)
Marital status	0.94	1.47	2.00	0.35
(married=1,	(1.09)	(1.99)**	(2.28)**	(0.74)
otherwise=0)				
Education	0.08	-0.08	-0.64	0.35
	(1.76)	(-2.12)**	(-0.36)	(0.74)
Household size	-0.11	-0.05	-0.06	-0.01
	(-1.53)	(-0.97)	(-0.88)	(-0.45)
Farming experience	0.001	-0.02	-0.08	0.35
	(0.03)	(-0.88)	(-2.46)**	(1.72)
Farm income	-4.29E-6	1.18E-06	-2.40E-06	-3.14E-06
	(-3.49)*	(-1.76)	(-1.78)	(-4.15)*
Non-farm income	-9.54E-6	-4.55E-06	-2.36E-06	-1.74E-08
	(-2.37)**	(-1.76)	(-1.17)	(-0.01)
Remittance	-1.1E-4	-1.20E-06	5.15E-06	-0.000*
	(-1.10)	(-0.23)	(1.14)	(-2.45)
Constant	-2.03	-2.95	-3.57	0.14
	(-1.000)	(-1.87)	(-1.53)	(0.12)
Number of observation	340			
LR chi-square	(36). 118.21*			
Prob > chi square	0.000			
Pseudo R^2	0.123			

Reference category = constant exercise
***, ** significant at 1% and 5% level**.
Numbers in parenthesis are z-values
Source: Field data analysis, 2015.

Table.3: Marginal Effects of Coefficients Influencing the Choice of Coping Strategies of Diabetic Farming Households.

Variables	$\frac{dy}{dx}$			
	Special diet	**Constant drugs**	**Hawking**	**Hired labour**
Age	0.002	0.0043	0.0042	-0.0062
	(0.90)	(1.26)	(1.92)	(-1.21)
Sex (male=1, female=0)	-0.017	-0.0045	-0.1080	-0.0205
	(-0.49)	(-0.09)	(-2.13)**	(-0.30)

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Marital status	0.032	0.094	0.049	0.0029
(married=1,otherwise=0)	(0.92)	(2.51)*	(2.90)*	(0.03)
Education	0.0051	-0.012	-0.0024	0.0143
	(1.76)	(-2.92)*	(-0.89)	(2.46)**
Household size	-0.0061	-0.0038	-0.0019	0.0015
	(-1.39)	(0.67)	(-0.61)	(0.23)
Farming experience	-0.0001	-0.0033	-0.0044	0.0092
	(-0106)	(-1.19)	(-2.92)*	(2.39)**
Farm income	-2.09E-07	2.92E-07	-6.31E-08	-5.68E-07
	(-2.81)*	(3.70)*	(-0.99)	(-4.00)*
Non-farm income	-5.47E-07	-4.02E-07	-5.30E-08	3.77E-07
	(-2.52)*	(-1.50)	(-0.55)	(1.43)
Remittance	-4.13E-07	4.55E-07	5.12E-07	-2.84E-06
	(-0.68)	(0.78)	(2.08)*	(-2.40)

(+)dy/dx is a discrete change of dummy variable from 0 to 1.
***,** significant at 1% and 5% level.**
Source: Field data analysis, 2015.

An increase in the number of male household heads would inrease the probability of choosing constant exercise as a coping strategy against hawking. The marginal effects of sex on hawking as shown in table 3 was -0.108. This implied that increase in the number of male headed households by 1% would reduce hawking as a coping strategy of diabetes by 0.108%. while increases in the number of female headed households by 1% will increase hawking as a coping strategy of diabetes by 0.108%. This is consistent with the findings of Oguntola (2011) who reported that men coped better with diabetes by embarking on routine exercise and other coping measures than women.

The coefficient of marital status was positively and significantly related with the choice of constant drugs (1.47) and hawking (2.00) as coping strategy of diabetic farm households at 5%. This is an indication that married household heads would rather embark on intake of constant drugs and hawking as coping strategies than constant exercise while the single household heads, (widow/widower) tended to favour constant exercise. The marginal effects of marital status on constant drugs and hawking as shown in table 3 was 0.094 and 0.049 respectively. This implied that a 1% increase in marriage among household heads would lead to an increase in constant intake of drugs and hawking by 0.094% and 0.049%, respectively.

This could be due to inflexible work schedule; unmet need for child care and lack play ground, park and public gym for constant exercise. Community may also be crime ridden and there may be no nearby indoor places for exercises. Basically, efforts to keep household members safe and indoor may encourage sedentary behaviour such as watching Nigerian movies and playing video games which are risk factors of diabetes. Single household heads may find it easier to hawk without cautions, support extracurricular activities and constant exercise as a coping strategy compared to married households head.

Similarly, the coefficient of education (-0.08) was negative and statistically significant on constant drugs at 5%. This implied that increase in the level of education of household head would increases the probability of choosing constant exercise against constant intake of drugs as coping strategy. The marginal effect of education on constant drugs was (-0.012) as shown in table 3. This means that a unit increase in education of household heads would reduce constant intake of drugs as a coping strategy of diabetes by 0.012%. This could be because educated people are more enlightened and informed on the danger of being constantly on drugs and would prefer to be involved in constant exercise as a coping strategy. The more people learn, the more they become experts in coping with ill- health by embarking on exercises and other physical activities. The role of education in coping with chronic illnesses, like diabetes, had been documented in literature. ADA (1999) reported that diabetic adults with high school education embark more on routine exercise as a coping strategy compared with college graduates. Chinenye *et al.* (2014) confirmed that the higher level of education increased the quantum of coping with diabetes scourge in Nigeria. The role of education and physical activity as a coping strategy

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in reducing the rates of disease and death from chronic illness has been well established (Ross, 2000).

The coefficient of farming experience (-0.08) was negative and statistically significant on hawking at 5%. This implied that increase in farming experience of household heads would increase the probability of choosing constant exercise as against hawking. The marginal effect of farming experience on hawking as shown in table 3 was, (-0.0044).This implied that increase in farming experience of household heads would reduce hawking as a coping strategy by 0.0044%. A well experienced diabetic farm households head would have a good knowledge of the impact of exercise over hawking. This would exert a positive influence on coping strategy of diabetic farm households. Using data on youth and diabetes care, Bundick (2011) found the same pattern among young individuals.

The coefficients of farm income (-4.29E-06) was negative and statistically significant on special diet and hired labour at 1%. This implied that an increase in farm income would increase the probability of choosing constant exercises as against special diet and hired labour. The marginal effects of farm income on special diet and hired labour, as shown in table 3 were -2.09E-7 and -5.68E-07. This showed that an increase in farm income of household head would reduce the consumption of special diet and hiring of labour as coping strategies by 2.09E-07% and 5.68-07%. This could be as a result of failed diet and failed hired labour. Also, as the income of diabetes household heads improved they could afford to purchase exercise and indoor equipment which were beyond their means previously. Many diabetes victims live in disadvantaged communities where healthy special diet like vegetables, fruits, groundnut, locust bean are available, but are considered unfashionable for consumption due to ignorance and lack of dietary education (IDF, 2012).

Likewise, the coefficient of non-farm income (-9.54E-6) was negative and statistically significant on special diet at 5%. This implied that increase in non-farm income of the household head would increase the probability of choosing constant exercise as against special diet such as millet, fruits and vegetables. The marginal effects of non-farm income on special diet as shown in table 3 was -5.47E-07. Thus increase in non-farm income of household head would reduce special diet as a coping strategy by 5.47E-07%. This could be because individual household heads who perceived themselves as capable of acquiring more income other than farming income are more likely to purchase exercise and indoor equipment and so may embark on exercise as a coping strategy, while looking fit and healthy without adding weight. This could also be because exercise as a coping strategy is relatively inexpensive, across gender and associated with reduced progression of diabetes and its complications. Umeadi and Chinenye (2014) concurred that exercise improved sensitivity of the body cells to insulin, improved lipid profile/cholesterol, cardiovascular respiratory health and increased metabolism.

The coefficient of remittance (-1.2E-4) was negative and statistically significant on labour at 1%. This implied that with an increase in remittance, would increase the probability of choosing constant exercise as against hired labour. The marginal effect of remittance on hired labour, as shown in table 3, was -2.84E-06. This suggested that increase in remittance of household head would reduce hiring of labour by 2.48E-06. This is likely the case where households receiving remittances do not see reasons to engage in farming anymore, but exercise as a form of leisure (Jiang *et al.,* 2002). A minimum of 150 minutes of moderate to vigorous intense aerobic exercise each week spread over at least three days in a week is ideal (Rashid *et al.,* 2006). Aerobic exercises such as walking and cycling had beneficial effects on diabetes and cardiovascular diseases.

V. CONCLUSION AND RECOMMENDATIONS

The study concludes that diabetic yam farming households adopt strategies such as embarking on different forms of exercise, consumption of special diets, hiring of labour, constant intake of drugs intake and hawking to cope with their illness. Variation in coping strategies between households could be based on difference in assets, age of households, educational level as well as gender, marital status, household size, farming experience, farm income, non-farm income and remittance. Its recommended that government at Federal, State and Local levels with partners in progress should consider critical ways of managing diabetes by emphasizing healthy lifestyle such as cessation of smoking, moderate alcohol intake, regular medical checkup and improvement of the socio-economic status of the diabetic farm households through good road network, steady supply of electricity which will better the quality of life of the households.

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First record of the Pacific bluefin tuna *Thunnus orientalis* (Temminck & Schlegel, 1844) from the coast off Sur, Sultanate of Oman

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***Abstract*—** *A single specimen of the Pacific bluefin tuna Thunnus orientalis was caught on 11 May 2017 in a long-line operated about 40 nautical miles off the coast of Sur atconfluent of Sea of Oman and Arabian Sea coast of Oman. This first record of its occurrence indicates the extension of distributional range of the species to the Arabian Sea coast of Oman.*

***Keywords*— coast off Sur, Pacific Bluefin, Arabian Sea.**

I. INTRODUCTION

Oman has a very long coastline of 3165 km with connections to three seas namely the Arabian Gulf, the Gulf of Oman and the Arabian Sea. The country has rich fish biodiversity (Al-Jufaily *et al*., 2010). The biodiversity of fish fauna of Omani coasts had been reported by several earlier workers (Boulenger, 1887; Steindachner, 1902; Regan, 1905; Norman, 1939; White & Barwani, 1971; Randall, 1995; Al-Abdessalaam, 1995; Fouda *et al*., 1997). In addition, there is scope for inclusion of several new species and new records to the list of known species of Omani fishes (McKoy *et al*., 2009; Jawad & Al-Mamry, 2009). As the result, a number of new records of fish species were reported from the Sea of Oman and Arabian Sea off Oman from recent studies (Jawad & Al-Mamry, 2009; Jayabalan *et al*., 2010; Al-Jufaily *et al*., 2010; Jawad *et al*. 2014).

Fishes of the genus *Thunnus*(Perciformes: Scombridae) are represented by eight species namely albacore, *T. alalunga* (Bonnaterre, 1788), southern bluefin tuna, *T. maccoyii* (Castelnau, 1872), bigeye tuna, *T. obesus* (Lowe, 1839), Pacific bluefin tuna, *T. orientalis* (Temminck & Schlegel, 1844), Atlantic bluefin tuna, *T. thynnus* (Linnaeus, 1758), yellowfin tuna, *T. albacares* (Bonnaterre, 1788), blackfin tuna, *T. atlanticus* (Lesson, 1831) and longtail tuna, *T. tonggol* (Bleeker, 1851) from world oceans (Froese & Pauly, 2009. Godsil, & Byers, 1945;Collette & Nauen 1983). Of these, Pacific bluefin tuna, *T. orientalis* is a commercially valuable species and is widely distributed in the Pacific Ocean(Froese & Pauly, 2009)and seasonally inhabiting subarctic, temperate, and tropical waters in the North Pacific Ocean as well as temperate waters in the Southern Hemisphere around Australia and New Zealand **(**Collette *et al*., 2014**)**.

Pacific bluefin tuna grows to a maximum weight of ~650 kg, a total length of ~300 cm and age of at least 20 years and it is the second largest species of tuna, after the Atlantic bluefin tuna (*Thunnus thynnus*) (Collette and Nauen, 1983; Kitagawa *et al*.,2007; Collette *et al*., 2014).Pacific blue fin tuna is highly prized in the Japanese sashimi markets and during 2014 about 17,076 t of fish were landed; however, *T. orientalis* is reported as 'Vulnerable' under IUCN red list of species (Collette *et al*., 2014).

(Collette and Nauen, 1983; Foreman and Ishizuk a, 1990; Bayliff, 1994)

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Fig.1: Map of Sultanate of Oman

From the Omani waters so far only three species such as *T. tonggol*, *T. albacares* and *T. obesus* are known (Godsil & Byers, 1945; Collette *et al.*, 2001; Randall, 1995; Froese & Pauly, 2011).In the present report, the occurrence of single specimen of Pacific Bluefin tuna *T. orientalis* off the coast of Suratconfluent of Sea of Omanand Arabian Sea coast of Oman(Fig.1) is recorded for the first time.

SYSTEMATICS
Order: PERCIFORMES
Family: SCOMBRIDAE
Genus: *Thunnus*
Species:*orientalis*

Fig.2: Specimen of T. orientalis collected from Oman

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II. MATERIAL EXAMINED

One specimen, Fig. 2, is 250 cm total length (TL), (204 cm standard length- SL; 224 cm fork length- FL); caught by long line; 11 May 2017. Atconfluent of Sea of Omanand Arabian Sea coast of Oman. It is difficult to say precisely because the fisherman did not have GPS (global positioning system).

The sample was brought to the Marine science laboratory at the Marine Science and Fisheries Centre, Ministry of Agriculture and Fisheries Wealth, Sultanate of Oman for analysis. After weighing,the morphometric and meristic characters of the fish were recorded following standard procedures (John& Schaefer, 1949). Then, the fish was cut open to identify the sex, maturity stage of the gonad and feeding intensity. The liver and air bladder structure were observed. Otoliths were extracted from head for subsequent age analysis. The muscle tissue was collected for genetic analysis. The fish sample was buried to preserve and display the bones in the museum.

Description

The fish weighed 237 kg and was a mature male. The feeding intensity was 1/2full stomach and the food was in fully digested state. Ventral surface of liver was striated with blood vessels. Air bladder was irregular. There were 36 gill rakers in the first left gill arch (Fig. 3)

Fig.3: First gill arch- Total gill rakers- 36.

The morphometric characters (Table- 1) and meristic counts (Table- 2) of the specimen agree with the descriptions of the species *T. orientalis* which was further confirmed by the genetic analysis of mitochondrial DNA with D-Loop (NCBI. Accession No. JN631213.1).

Body: Fusiform, almost round, very robust in front and tapering towards caudal peduncle. Head lengthless than body depth. Mouth large,teeth small and conical in a single series. The first dorsal height less (10.7% of FL) than the second dorsal fin height (16.1% of FL) in the present specimen; pectoral fin short and less than head length and about 17% of FL. In *T. orientalis*, the short pectoral fin and heights of 1st and 2nd dorsal fins are considered as the prominent morphometric characters to identify the species (Nelson, 2006).

Coloration: Lower side of belly was silvery white but no other colour pattern was visible. This might be due to the delay in bringing the specimen to the laboratory after three days. However, in fresh specimens lower sides and belly are with faint colourless transverse lines alternated with rows of faint colourless dots (Collette *et al*., 1983; Nelson, 2006; Tamura & Takagi, 2009).The first and second dorsal fins were dark bluish with dusky yellow tips. Dorsal and anal Finlets were yellowish, base reddish-brown towards middle and tips. Median caudal keel was black.

Table.1: Important morphometric measurements of T. orientalis

S.No.	Morphometric measurements	cm	In FL (%)
1	Head length	57	25.4
2	Head depth	51	22.8
3	Pre-opercle length	44	19.6

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4	Post orbital distance	33	14.7
5	Eye Diameter	6	2.7
6	Upper jaw length	20	8.9
7	Body depth	131	58.5
8	Girth	59	26.3
9	Distance snout to eye	19	8.5
10	Distance snout to nostril	17	7.6
11	Distance snout to 1st dorsalfin base	66.5	29.7
12	Distance snout to 2nd dorsalfin base	116	51.8
13	Distance between 1st and 2nd dorsalfin	22	9.8
14	1st Dorsalfin length	49	21.9
15	1st Dorsalfin height	24	10.7
16	2nd Dorsalfin length	20	8.9
17	2nd Dorsalfin height	36	16.1
18	Analfin length	15	6.7
19	Analfin height	36	16.1
20	Pectoralfin length	38	17.0
21	Caudal peduncle length	8	3.6
22	Caudalfin spread	71	31.7
23	Median keel height	5	2.2
24	Median keel length	25	11.2

Table.2: Meristic Counts of T. orientalis

S. No.	Meristic Counts	No.
1	1st Dorsal fin spines	XIII
2	2nd Dorsal fin rays	15
3	Dorsal Finlets	9
4	Pectoral fin rays	30
5	Anal fin rays?	12
6	Anal Finlets	8
7	Gill rakers	36
8	Keels	3

III. DISCUSSION

Thunnus orientalis is an epipelagic oceanic species that forms school by size and performs wide horizontal and vertical migrations and seasonally moves close to the shore (Magnuson, 1973; 1978; Kitagawa *et al*. 2007). This species is distributed in depth range of 1 to 550 m (Froese & Pauly, 2009; IUCN, 2014). The fish has great tolerance of sea-surface temperature ranging from 17ºC to 23ºC and be able to dive deep waters as cold as ~3°C(fish Base). It is a voracious predator feedingprimarily on a variety of small schooling fishes such as anchovies, sardines, herrings, menhaden and mackerels and also on squids, crabs and other less sessile organisms (Collette and Nauen, 1983; Allain, 2005; Swada *et al*., 2005).

The record of *T. orientalis*from the Arabian Sea coast of Oman is significant as the species has been found distributed in north and south Pacific (Ashida *et al.* 2015; Collette *et al*. 2014, Lewis, 2012; Itoh *et al*. 2003; Tanaka *et al*. 2006). Although, this species moves in schools, only single specimen was caught presently. This would lead to speculations such asincidental occurrence owing to disorientation or be linked to the ecological changes that occurred due to rise in water temperature in its distributional ranges causing a decline in prey organisms and hence, migrated to more abundant prey related regions or to unknown biotic and/or anthropogenic factors (Sharp & Dizon, 1978; Kimura *et al*., 2010).

The species *T. orientalis* is represented by a single Pacific-wide stock that is found primarily in the North Pacific

Ocean (Kitigawa *et al*., 2007). Hence, it may be presumed that the migration of the species from the north Pacific to the Arabian Sea coast of Oman might have occurred through the Bay of Bengal. Hence, it would be interesting to know whether the extension of the species distribution is very recent to the countries bordering the Bay of Bengal coast or already established populations are available in the region. Intensive research in this line is needed to better explain the occurrence of a solitary Pacific blue fin tuna from Oman.Tagging studies wouldreveal the possible routes of movement pattern of *T. orientalis* if any, from north Pacific to the western Indian Ocean.

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The Impact of Climate Change on Agriculture and Health Sectors in Tanzania: A review

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Abstract— *The impact of climate change in Tanzania is dynamic and differs among regions as they are impacted in different ways. While other regions experience normal rainfall and temperature patterns, others have continued to experience temperature extremes, severe droughts, decline in crops production coupled with food insecurity, extreme weather episodes of heavy rainfall associated with floods, loss of lives and infectious disease outbreaks. Despite the effects of climate change being recognized in the country, awareness is limited among local people, in particular the vulnerable communities. Thus, this review aims to raise awareness by giving a broader picture of impacts of climate change on agriculture and health sector. It reveals that in many parts of Tanzania, agriculture and health sectors may continue to suffer from the effects of climate change aggregated with limited awareness among communities. It is expected, that outbreaks of infectious diseases including malaria and cholera may increase as they correlate positively with high temperatures and rainfall. As a result, health problems and deaths of people, and reduced crops production will continue. Therefore, it is recommended that, the best way to overcome climate change is to invest effectively on the irrigation agriculture; and the health sector's budget should be enough to improve health care services and prepare for outbreaks of climate change sensitive diseases. Most importantly, provision of climate change awareness to the vulnerable communities must be seriously considered. About 50 peer-reviewed articles, government and international reports published between 2000 and 2017 were reviewed.*

Keywords— Agriculture, Climate change, Food insecurity, Health, Poverty.

I. INTRODUCTION

Wu et al. (2016) defines climate change as the long-term changes in weather conditions and patterns of unusual extreme weather events. On the other hand, IPCC (2001) describes a climate change as a change of climate which is attributed either directly or indirectly to anthropogenic activities that alter the global atmosphere composition and which is in addition to observed natural climate variability over comparable time periods. It is a global problem that defies and threatens global sustainable development, economy, food security, biodiversity, agriculture, human health and water availability as shown in Brown and Crawford (2008), Enfors and Gordon (2007), IPCC (2001; 2007), Majule et al. (2013), Mwakisunga et al. (2012), Ojoyi, (2017), URT (2003), Van der Werf et al. (2009) and Wu et al. (2016). Both natural and anthropogenic factors are implicated as causes for climate change (IPCC, 2007). Natural factors include volcanic eruptions, variations in solar output, natural aerosol emissions, and variations in the earth's orbital characteristics, whereas, anthropogenic factors include burning of fossil fuels, industrial activities, cement production, land use changes, deforestation and agriculture (IPCC, 2001, 2007; Van der Werf *et al.*, 2009; Wu et al. 2016). All these activities produce high emission levels of greenhouse gases (GHGs) which include carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Brown et al. 2007; IPCC, 2007; Mwakisunga et al. 2012). Thus mounting of GHGs concentration in the atmosphere is the leading cause of climate change (Hemp, 2009; IPCC, 2007). Several studies including IPCC (2001, 2007), FAO (2013) and Shikuku et al. (2017) clearly show that the GHGs are positively correlated with the burning of fossil fuels, oils, forest destruction and agriculture. It has been indicated that, climate change takes place in the context of developmental stresses, poverty, food shortage, drought, outbreaks of infectious diseases, environmental change and land degradation (Brown and Crawford, 2008; Brown et al., 2007; Enfors and Gordon, 2007; FAO, 2013; IPCC, 2001). Because of these factors and stresses, maintaining sustainable agriculture and better health sector becomes a challenge in the global climate change (Levira, 2009; Majule et al. 2013; Wu et al. 2016). Brown and Crawford

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(2008), Boon and Ahenkan (2012), FAO (2013), IPCC (2001), URT (2005) claimed that, climate change is the foremost global challenge to human prosperity that will be faced and experienced for several years. In Africa particularly sub-Saharan Africa, other studies indicate that climate change continues to distress agriculture, biodiversity, livestock production, environment, and health sectors (Burke et al. 2009; IPCC, 2007; Orindi and Murray, 2005; Shikuku et al. 2017; URT, 2005). Evidence from the European Environment Agency (EEA, 2008) revealed that global average surface temperature is increasing. For example, in the 20th century it has increased by 0.74 °C, while, since 1991 increase in global sea level is 1.8 mm per year. Additionally, researchers report that, future climate change will continue to be a significant driver of ecosystem stress and significantly impact agriculture and health sectors (Brown and Crawford, 2008; Brown et al. 2007; FAO, 2013; IPCC, 2007; Rowhani et al. 2011). IPCC (2001) also reports that, climate change causes a direct effect on the environment, economy, water resources, health, weather events, sea level rise and desertification.

Moreover, the climate change in Tanzania threatens sustainable development and other socio-economic activities (Majule et al. 2013; Rowhani et al. 2011). Unusual extreme temperatures and rainfall alterations have shown strong impacts on agriculture, health and other sectors in the country (Enfors and Gordon, 2007; Hemp, 2009; Majule et al. 2013). Climate change related scenarios such as severe droughts, floods, livestock deaths, crop failures and outbreak of disease such as cholera, malaria episodes and deaths are regularly observed (Levira, 2009; URT, 2007). Its impact also accelerates food shortage, poverty, deforestation and forest degradation, poor livelihoods and occurrence of infectious diseases (Hatibu, 2003; NAPA, 2005; Wolbring, 2009; Wu et al. 2016). The poor and rural communities are particularly chiefly vulnerable owing to their complete dependence on subsistence agriculture and forests resources coupled with limited capacity to adapt to climate change (Brown *et al.*, 2009). Researches have shown that more than 80% of Tanzanian population directly rely on agriculture for their livelihoods; thus, 10% decrease in rainfall would make most of areas unsuitable for cultivation (Hemp, 2009; URT, 2003, 2007). Moreover, Craparo et al. (2015) reported that, a minimum temperature change in future will be severe in the interior regions of Tanzania and will considerably affect crops production and health sector. This review paper provides an overview of the impacts of climate change on agriculture and health sectors in Tanzania. Recommendations to overcome them have also been discussed.

II. MATERIALS AND METHODS

2.1. Methods

Shemsanga et al. (2010) asserted that the best way to explain the effect of climate change is to focus on the context of crops production and outbreaks of infectious diseases such as cholera and malaria. Crop yields have been used in many studies to justify the impact of climatic change (Burke et al. 2009; Hatibu et al. 2003; Shemsanga et al. 2010). This is because climate change influences crop yields by decreasing soil moisture content, increase drought and floods, and support diseases affecting crops. A broad literature search was conducted using Google Scholar (http://scholar.google.com), Open Doar-directory of pen access repository (http://www.opendoar.org), Agora (http://www.fao.org/agora/en/), Springer Online Journals (http://link.springer.com/), Doaj-directory of open access journals (https://doaj.org) and Elsevier ScienceDirect (http://www.sciencedirect.com/). The attention was given on the peer-reviewed articles and government reports between 2000 and 2017. Other reports from different websites were also reviewed, these include Tanzania Climate Change Information Repository (TaCCIRe) (http://www.taccire.suanet.ac.tz), Tanzania Metrological Agency (TMA) (http://www.meteo.go.tz/), United Nations Environment Programme (UNEP), United Nations Development Programme (UNDP), World Health Organization (WHO), Group on Climate Change and Health (IWGCCH) and Intergovernmental Panel on Climate Change (IPCC).

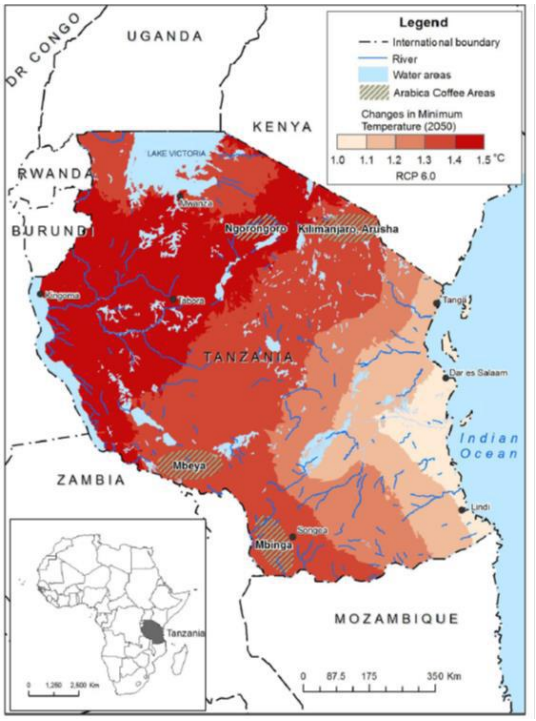

Fig.1: Map of Tanzania (Craparo et al. 2015)

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2.2. An overview of Tanzania

In the East Coast of Africa, Tanzania lies near to the equator between parallel 10S and 120S and meridians 300E and 400E (URT, 2007). It is the largest country in East Africa with a total area of 945,200 km2, of which 60,000 km^2 is inland water (URT, 2003). This area includes parts of Mafia, Zanzibar, and Pemba (Shemsanga et al. 2010). It borders Malawi and Zambia (South-West) Rwanda and Burundi (North-West), Kenya and Uganda in the North-East and North respectively, Mozambique (South) while the Indian Ocean in the East as shown in Fig 1.

III. DISCUSSION

3.1. **Impact of climate change on agriculture sector**

The agriculture sector in Tanzania is particularly vulnerable to climatic change because it is customarily dependent on rainfall (Ojoyi, 2017; URT, 2007; Yanda et al. 2008). This involves prolonged dry season, alteration in ecology of pests and diseases, unstable agro-ecological zones, poor or doubtful rainfall (URT, 2003), and uncertainty in cropping patterns (Shemsanga, 2010). Climate change catalyses competition between crops and weeds for light, moisture and nutrients (URT, 2007). Studies by Shemsanga et al. (2010) and Yanda et al. (2008) report that, increase or decrease in temperature and rainfall patterns in some cities accelerates shortage of food. This is because many crops die as a result of high temperature that increases evapotranspiration and moisture loss (Rowhani et al. 2011; URT, 2003, 2007). Furthermore, a decline in rainfall makes water inadequate for farming activities (Ojoyi, 2017), because many seasonal streams and rivers used for irrigation dry out (Craparo et al. 2015). For example, Rowhani et al. (2011) claim that, seasonal increase in temperature by 2^{o}C as projected by 2050 will reduce yields of rice, sorghum and maize by 7.6%, 8.8% and 13% respectively in Tanzania while a 20% increase in precipitation variability will decrease yields of rice, sorghum and maize by 7.6%, 7.2% and 4.2% respectively by 2050. In addition, increase in temperature between 2^{o}C and 4^{o}C will alter the ecosystem, causing ecosystem shifting (Rowhani et al. 2011). As a consequence, this will shift former areas suitable for cultivation of annual crops to perennial crops (Yanda et al. 2008). Severe droughts in Tanzania have been causing hunger as a result of dwindling in crops production. For instance, Shemsanga et al. (2010) reported that in Dodoma there was a decrease in harvest by 80% attributed to poor rainfall. In 2005, Kilimanjaro, Coastal and North-East regions experienced marginal rains, which led to food shortages and starvation. Besides, the food scarcity due to climate change, the phenomenon has also has resulted into an increase in malnutrition rate amongst children in the country (URT, 2005, 2007).

Furthermore, the rangelands which were suitable for use by pastoral communities for livestock keeping and settlements have declined because of climate change (Burke et al. 2009). Shortage of pastures and water due to shortage of rainfall and high temperature (DILAPS, 2007; URT, 2005) has caused considerable deaths of livestock which have been reported almost each year (Shemsanga et al. 2010; Yanda et al. 2008; URT, 2003). Escalation of tsetse flies has made the rangelands unsuitable for pastoralists and livestock settling (URT, 2007) and therefore decrease in their quality and size. As a result of this, pastoralists have been forced out of their former areas into farmers' areas to search for pastures and water for their livestock (URT, 2003). Subsequently, several conflicts between farmers and pastoralists that occur in the country have been reported in DILAPS (2007). These conflicts and associated deaths and injuries have been reported in Kilimanjaro district, Mara region, Morogoro region, Kilosa district, Mamba ward, and Arusha (DILAPS, 2007). Yet, livestock productivity, distribution and survival will continue to decline because of the present climate change variability, decrease in the quality of rangelands and prevalence of vector-born diseases.

3.2. **Impact of climate change on health sector**

It is not relatively simple to assess the effect of climate change on human health. This is because human health may be impacted due to extreme changes in cold and heat, droughts and floods. Other human health impacts may get up from ecological or social system alterations caused by climate change. The climate change affects the health sector in Tanzania by persistent burdens of diseases (Kibona, 2008; URT, 2007). This includes the basic elements of good health such as adequate food, clean air, safe water, adequate shelter, and health environment (Hulme et al. 2001). Climate change further impacts on health of people by multiplying the present health problems (Wu et al. 2016). Strong weather associated with heavy rainfall, landslides and floods destroy the infrastructures of health care services infrastructure and hence affect the health sector (URT, 2005; Yanda, 2005). In addition, it causes injuries and deaths, water supplies contamination, decrease in food production and disease outbreaks (Costello et al., 2009). The health sector is impacted in diverse ways, for example, the impact on food, system efficiency of local sewerage and the accessibility of fresh water supplies (Kibona, 2008). Furthermore, a decrease in food production also affects health of many people in form of malnutrition (FAO, 2013). A deficiency of water in most places of the country leads to consumption of unsafe water. This increases the chance of water borne disease outbreaks, a threat to

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health. There are many climatically sensitive illnesses in Tanzania; and they are very common during heavy rains, drought and flooding (Mboera et al. 2011; Shemsanga et al. 2010). Water related diseases linked to climate change are cholera, malaria, amoebiasis, cryptosporidiasis, giardiasis, leptospirosis, typhoid and schistosomiasis; and vector borne diseases such as dengue, encephalitis filariasis, leishmaniasis, Lyme disease, plague, rift valley fever, onchocerciasis, trypanosomiasis and yellow fever (Costello et al., 2009; Mboera et al. 2011; Paavola, 2003; Shemsanga et al. 2010; URT, 2007). Distribution of vectors causing water borne diseases can be limited by cold temperatures, however increasing global warming as a result of climate change accelerate the risk to human life (Mboera et al. 2011). This review focuses on two major climatic sensitive diseases (Cholera and malaria), however, other climatic infectious disease are also discussed in briefly.

3.2.1. Cholera

Cholera is a disease associated with climate change (Mboera et al. 2011; Trærup et al. 2011; Yanda, 2005). This means that climate change plays a positive role in spreading and escalating the disease (IPCC, 2001). It has been established in many parts of Tanzania that cholera outbreaks occur with increases in the amount of rainfall (URT, 2003; 2007; Yanda, 2005; Hulme et al. 2001). For example, Hulme et al. (2001) reported that cholera outbreaks in North East, South East, Lake Victoria basin and coastal areas of Tanzania were due to high rainfall. Trærup et al. (2011) also reported a significant relationship between cholera incidences and temperature in the country. He further showed that, initial risk of cholera increased by 15% to 19% for every 1^{o}C temperature increase. Additionally, he projected that in Tanzania by 2030, the total costs of cholera attributable to climate change variability will be in the range of 0.32% to 1.4% of national GDP. Similarly, Trærup et al. (2011) showed that the seasonal patterns that existed between June and October had the lower cholera cases which corresponded with lower minimum or maximum temperatures and total rainfall (Fig 2). Treatment and handling costs of people suffering from cholera as well as controlling the disease burden the country's economy. This is because more funds are injected in the health sector to combat it. In addition to the economic consequences, the disease also decrease labour force (URT, 2003; Yanda, 2005).

3.2.2. Malaria

Ahern et al. (2005) and Haines and Patz (2004) have shown that, incidences of malaria are highest during heavy rainfall and high temperature. The increase in precipitation and temperature is associated with global warming and therefore makes mosquitoes' habitats (such as ponds, pools, wells or bores, streams, rivers and canals) suitable breeding sites (Harrus and Baneth, 2005). Consequently, they grow and increase in population capable of spreading parasite causing malaria, *Plasmodium falciparum* (Lindsay et al. 2000; Kibona, 2008). Development rates of parasites and vectors are affected by temperature whilst mosquitoes' breeding sites are affected by the availability of rainfall (Craig et al., 2004; Zhou et al., 2004). Kibona (2008) in their study conducted in Lushoto district, Tanzania, reported that malaria cases were prominent during high rainfall seasons. For example, 249.1 mm of rainfall in April were associated with the increase of mean malaria cases in the same month (Fig 3). This is similar in other months in each rain season.

Kibona (2008) also reported an increase in malaria cases were linked with increased temperature (Fig 4). Largely, malaria cases tend to correspond with annual and monthly rainfall seasons and temperature (Lindsay et al. 2000). This correspondence is due to the temperature and rainfall patterns that favour the breeding and distributions of mosquitoes (Kibona, 2008; Ostfeld and Brunner, 2015; Reiter, 2001). This happens especially if the infrastructures such as pools, ponds, canals, streams and rivers are present to support their existence (Rodó et al., 2013). The burden of malaria on the health sector is very huge, it slows the health sector to provide better health care services. This is because the budget allocated in the sector is diverted to treatment and purchasing of malaria medicines every year. IPPC (2007) reported that a part from causing morbidity, malaria kills many children, elderly and pregnant women more than other diseases on the planet. Other studies indicated that the number of malaria cases are positively correlated with high extreme temperatures and rainfall (IPCC, 2001; Githeko and Ndegwa, 2001; Zhou et al. 2004). Moreover, Craig et al. (2004) reported that, incidences of malaria events are much more pronounced in rainy and warm days as a result of climate change.

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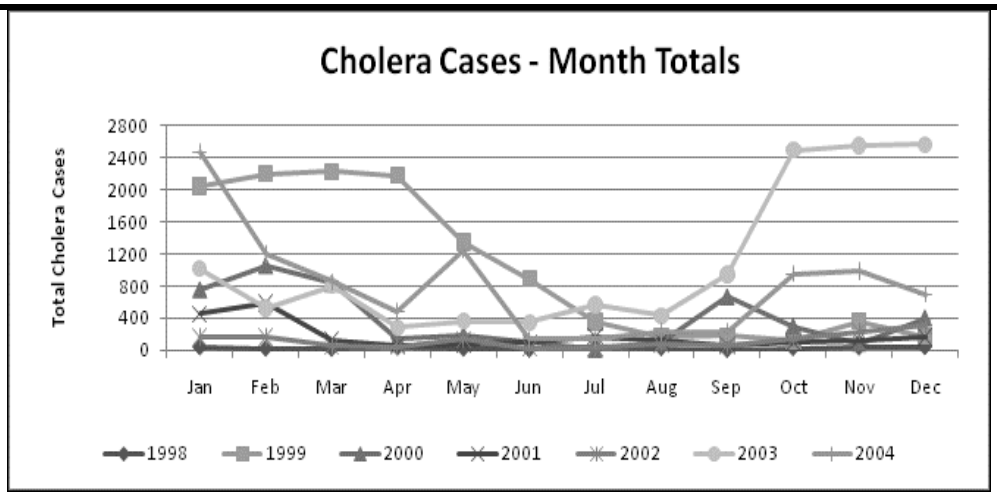
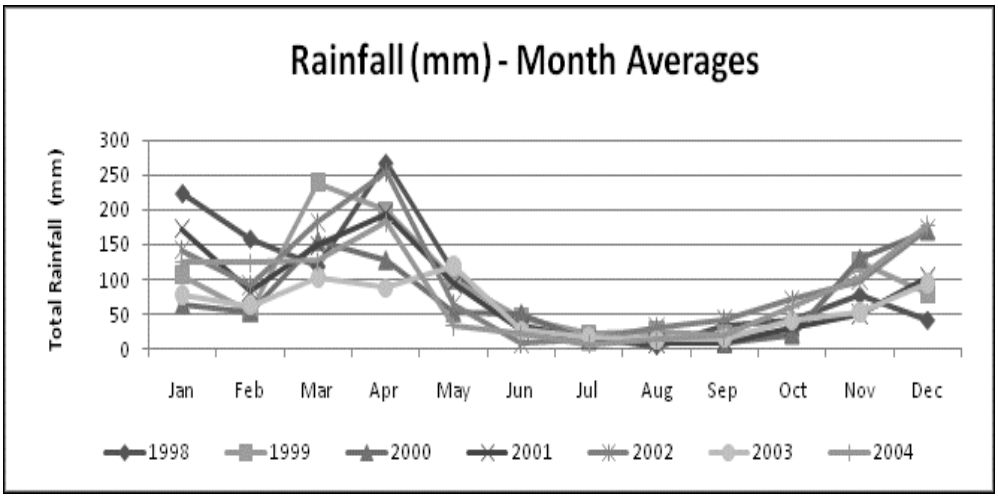


(a)



(b)

Fig.2: Seasonal distribution of cholera cases and rainfall in Tanzania (source: Trærup et al. 2011)

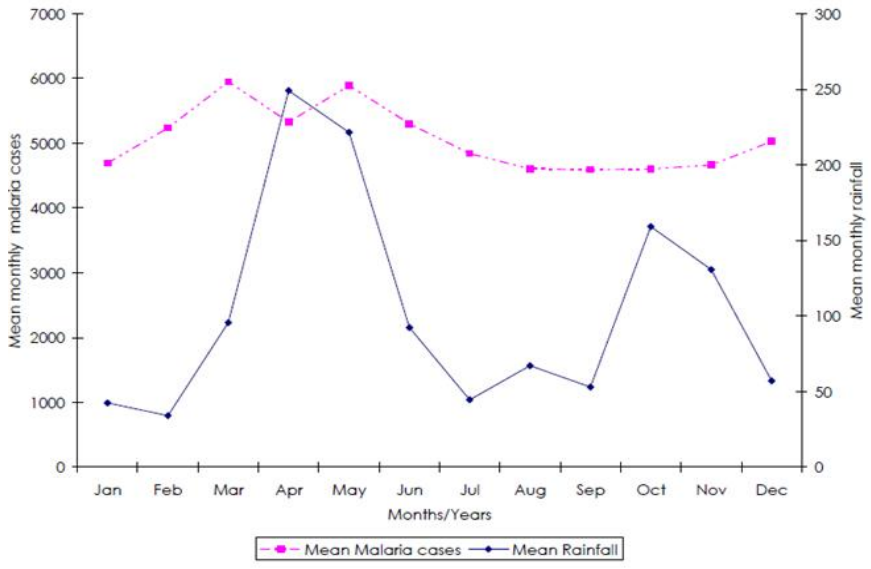


Fig.3: Mean monthly malaria cases and rainfall from Lushoto district, 1995-2004 (Source: Kibona, 2008)

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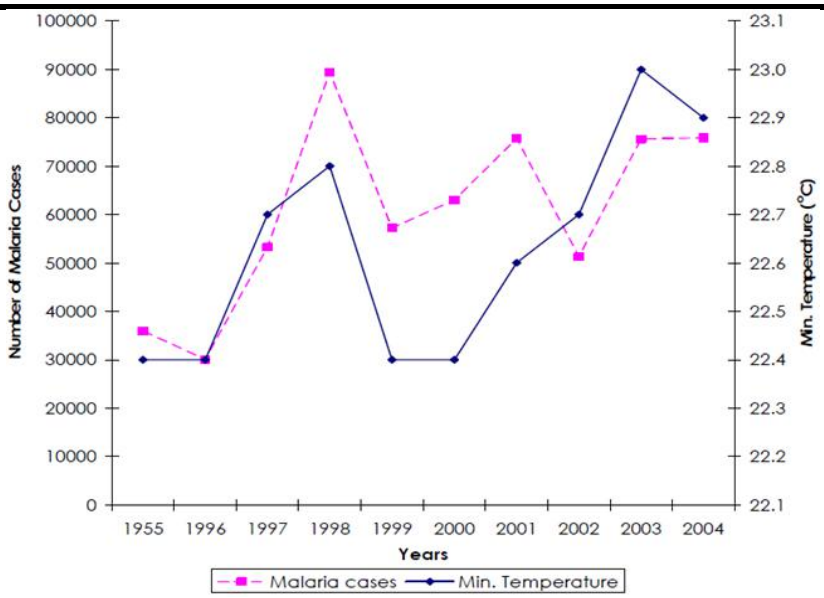

Fig. 4: Annual malaria cases and minimum annual temperature in Lushoto district, 1995-2004 (Source: Kibona, 2008)

3.3. Other climatic sensitive diseases which affect the health sector

There are other climatic associated diseases which have impact on health sector in Tanzania. These climatic diseases are summarised in Fig 5. Like cholera and malaria the rift valley fever is correlated to climate change variability (Mboera et al. 2011). It is documented that three quarters of the rift valley outbreaks occurred between 1950 and 1998 in East Africa during El Niño events (Patz et al., 2005). The outbreaks of the lift valley fever in Tanzania are usually correlated with high rainfall or El Niño and it is caused by Aedes and Culex mosquitoes (Zhou et al., 2004). Mboera et al. (2011) claims that, outbreak of rift valley fever was reported in January 2007 in Northern, Southwards and Westwards Tanzania districts while affecting about 511 people in 10 regions. On the other hand, African Trypanosomiasis caused by *Trypanosoma brucei rhodesiense* is transmitted by tsetse flies, a *Glosina* species (Moore et al. 2011). Its distribution is catalysed by climate change. When the ecosystem changes due to climate, vectors distribution also change. Factors such as high temperature and long term changes in rainfall affect the life cycle of tsetse flies and transmission of trypanosomiasis (Mboera et al. 2011). The tsetse flies distribution due to climate change and movement of people plus livestock accelerate the spread of sickness diseases caused by *Trypanosoma brucei* to different areas.

Dengue fever is caused by the arbovirus (Mboera et al. 2011; Mgonde et al. 2006). This disease is predominant in tropical and subtropical countries. Viruses causing dengue are transmitted to humans by mosquitoes, especially the two species, *Aedes aegypti* and *Aedes albopictus* (Mboera et al. 2011). Appropriate environment supported by warm conditions and rainfall is vital for these mosquitoes to breed and transmit dengue causing viruses. They breed in water contained in containers, flower pots, water tanks, tires filled with water, and in discarded cans and cups. Hence during rainfall, if these containers are filled with water they become suitable breeding sites for mosquitoes carrying dengue causing viruses. Urban communities with poor water and solid systems management are very vulnerable. Mboera et al. (2011) reports that, in Tanzania dengue occurred in February and May in 2010 in Dar es Salaam. In addition, Schistosomiasis disease is caused by *Schistosoma mansoni* and *Schistosoma haematobium.* The parasites are transmitted to humans via intermediate host, the snail (*Bulinus globosus*). Populations of the snails are dependent on water, temperature, water pH, water currents, and food availability. The density of the snails is influenced by rainfall patterns and therefore transmission of parasites. Human beings can have health problems if they use infected water or contaminated vegetable or fruits by *S. mansoni*.

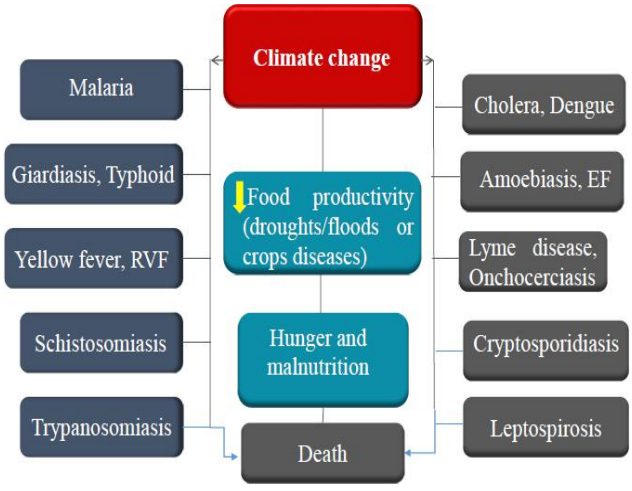

Fig.5: Impact of climate change on health sector and associated diseases (RVF and EF = Rift Valley Fever and Encephalitis Filariasis respectively). The yellow arrow pointing down mean a 'decrease'.

Another climate related disease is Leptospirosis. It is a zoonotic disease found mostly in tropical and subtropical countries favoured by extreme weather events (Biggs et al. 2011). It spreads via the urine of infected animals which gets into water or soil (Mboera et al. 2011). Areas experiencing repeated floods and typhoons particularly in urban slums and areas with poor sanitation are homes of leptospirosis infection. Biggs et al. (2011) reported cases of leptospirosis prevalence in two hospitals in Moshi region. This reveals that the disease affect health of people but also health sector in the country. Humans and animals are affected in a similar manner with this disease (Mgonde et al. 2006). They get infected through contact with contaminated soils or water, ingestion or inhalation of contaminated soils (Mgonde et al. 2006). Although the disease is climatic change related and affect people and animals, its epidemiological status to humans in the country is less considered probably due to its diagnostic complications and little awareness. Plague is also important climatic related disease caused by the bacteria known as *bacillus Yersinia pestis* (Stenseth et al. 2008). Distribution of the disease is regular with climate change (Drancourt et al. (2006; Stenseth et al. 2008; Nakazawa et al., 2008). For example, Pham et al. (2009) indicated that plague incidences usually tend to increase during hot and dry season and then followed by a period of seasonal rainfall. Drancourt et al. (2006) indicated that this bacteria lives in rodent hosts and transmitted to human and other animals through animal fleas. It can also be transmitted via predation, cannibalism or contaminated soils. In Tanzania, the disease is endemic in Lushoto district and causes human health problems, nevertheless, the plague cases are seasonal (Mboera et al. 2011).

IV. CONCLUSIONS

Human health is impacted by climate change directly or indirectly. Direct effects can cause mortality due to extreme heat and cold waves, flooding, droughts, and cyclones. Indirect effects are associated with increased health problems due to contaminated food and water, and malnutrition due to reduced food production. However, in Tanzania, mortality cases due to direct impacts are mainly caused by floods and droughts. In order to reduce the impacts of climate change on agriculture and health sectors, the government and other stakeholders should set up early warning system mechanisms and demonstrate optimal preparedness owing to the dynamic nature of climate change impacts. Preventive adaptation and mitigation measures against infectious diseases should be introduced, for example, good infrastructures for water and sanitation, and health service centres. These must be supplemented with Tanzania national goals of universal water and hygiene coverage. In agriculture sector, it is recommended that the best solution to overcome climate change is to effectively invest in the irrigated agriculture and also biotechnology whereby crops tolerant to various climate changes such as drought can be bred to ensure food security. It is also critical to provide education to farmers and livestock keepers about climate change adaptation and mitigation strategies. Most importantly, the government should massively sensitize and build the capacities of rural communities, who are also the chief victims of climate change, to practise sustainable and environmentally friendly agricultural technologies such as conservation agriculture, farrowing, agroforestry, afforestation, integrated plant nutrient management, integrated pest management etc. that have the capacity to increase resilience to, or mitigate effects of climate change. On a macro level, governments should consider investing in technologies with lower greenhouse gas emissions in key sectors of manufacturing, automobile, health and agriculture. Consequently, various global initiatives and agreements exist aimed at reducing climate change (effects), to which is Tanzania is a signatory, and must therefore remain committed to achieving such goals as Sustainable Development Goal(s) 13 (Climate Action). In accordance with this review, it is envisioned that the climate will continue to have impacts on both agriculture and health sectors. These impacts will extend to socio-economic aspects of rural communities which are most vulnerable to climate change. It must be noted that, climate change does not occur in void, as its effects and resulting environmental alterations interact with agriculture and health sectors.

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A case of Dystocia due to Fetal Ascites in Murrah Buffalo

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Abstract— *Dystocia in buffalo due to fetal causes is not common. However there are reports suggesting dystocia due to dropsical condition of fetus. Present case reports one of the fetal dropsical conditions in buffalo. In this case we report a successful management of dystocia due to fetal ascites in Murrah buffalo by incising the fetal abdomen to take out the fluid from peritoneum.*

Keywords— Fetal Ascites, dystocia, insufficient drainage.

I. INTRODUCTION

Chances of dystocia in buffaloes are less when compared to cattle due more voluminous pelvis. Fetomaternal disproportion and faulty disposition of fetus has been reported as commonest causes of dystocia in buffalo. Dystocia can also occur due to dropsical condition of fetus like hydrocephalus, ascites, hydrothorax and anasarca(Purohit et al., 2006; Purohit et al., 2012). However, the report of fetal ascites as a cause of dystocia in buffalo is rare (Luthra*et al*., 2001).Honparkheet al (2003) and Roberts(2004) reported the association of fetal asciteswith dropsical condition of the uterus, mesotheliomas of the fetal abdomen and brucellosis. Ascites can be caused by overproduction or insufficient drainage of peritoneal fluid and blockage of lymphatics(Sloss and Duffy, 1980). Ascites can alsooccurr due to reduced urinary excretion (Purohit*et al.,* 2012).

II. CASE HISTORY AND OBSERVATION

A six year old Murrah buffalo in second parity was presented in GADVASU clinic with the history of complete gestation period. Animal was straining from last night and progressed to second stage but after that no improvement has occurred. Buffalo was alert and active. Per vaginum examination revealed completely relaxed cervix with fetus in anterior longitudinal presentation and dorso sacral position and two forelimbs in birth canal. Thorough examination revealed fetus abdomen filled with fluid suggesting a case of fetal ascites.

III. TREATMENT

An epidural anesthesia with 2 % lignocaine is given to the animal to prevent excessive straining.A guided fetotome knife was inserted per vaginum to incise the fetal abdomen. After giving incision about 30 litre of brown colored fluid mixed with blood comes out (Fig 1). Partial fetal repulsion and adjustment of correct parturition posture was done to take out the fetus. Placenta was also taken out by rolling it on the hand. The fetus was comparatively smaller in size and kidneys and liver was showing some degenerative changes.However abnormal drainage or blockage of lymphatics can also aggreviate the problemBuffalo was given Inj. Gentamicin 20 ml I/M o.d. and Inj. Enrofloxacin 20 ml I/M o.d. on alternate days for five days, Inj. RL 4 Liter I/V, Inj. Metrogyl 1.5 Liter I/V and Intrauterine bolus Furea was kept.

IV. DISCUSSION

Arthur *et al.* (1986) stated that ascites may be due to hepatic lesions, general venous congestion or urinary obstruction with or without rupture of bladder. Placental dysfunction consequent to incompatibility of dam and fetus may predispose to fetal dropsy. Ascetic condition in this case may be due to cystic condition of kidney and rupture of urinary bladder or the overproduction or insufficient drainage of peritoneal fluid. The fetal ascites resulted into dystocia as a result of increase in abdominal diameter. Approaches similar to the present case for vaginal fetal delivery have been recorded in many previous studies (Roberts, 1971; Selvaraju*et al.,* 2009; Ravikumar*et al.,* 2013). It was concluded that ascetic fetus can be delivered by abdominal puncture. The etiology for polycystic kidney was not established yet, however, some etiologies responsible for renal cyst conditions are recognized to be

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related with autosomal recessive genes (Smith *et al*., 1996), chemicals like corticosteroids (Filmer*etal*., 1973) and diphenylamine (Thomas *et al*., 1957). Calves with polycystic kidney may be stillborn with other abnormalities or die shortly after birth without other abnormalities (Jubb and Kennedy, 1993).

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Figure

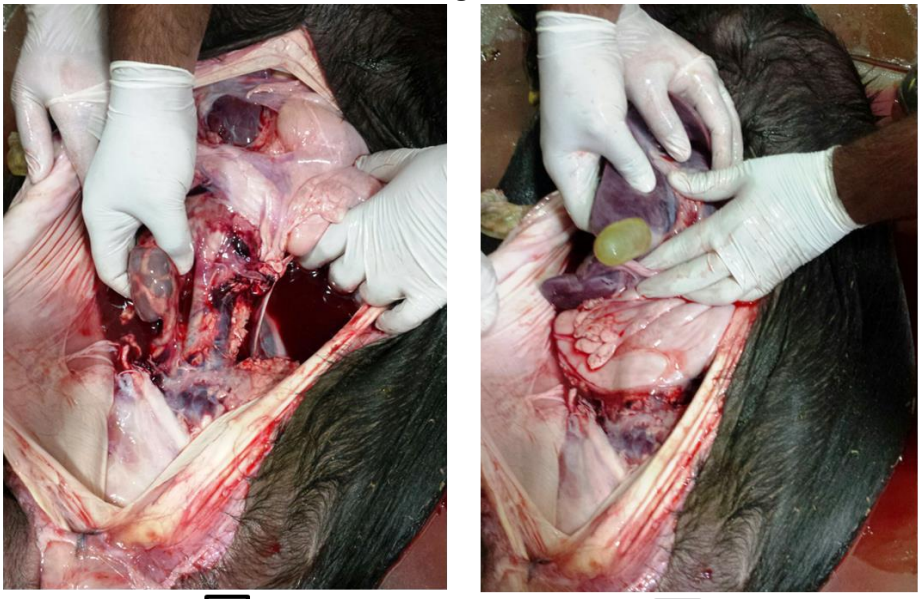

Fig 1 Abnormal pathological changes in kidney and liver of fetus. (a,b)

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Study of the quality of fruits of the *Hylocereusundatus* (Haw) Britton & Rose and *Hylocereusmegalanthus*(K. Schum ex Vaupel) Ralf Baue (Red and Yellow Pitahaya) during the maturationperiod.

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Abstarct— *In this work, the organoleptic and sensorial characteristics were determined, as well as the maturation pattern of the yellow and red pitahaya fruits grown in Ecuador. Several fruit quality indexes were evaluated for 15 days from the moment of harvest, such as: weight loss, dry matter, total soluble solids, titratable acidity, exocarp coloration and maturity relationship and related damages by attack of biotic agents. The results indicated that in storage conditions with a temperature of 25 ± 1 ° C, the red pitahaya presents a gradual loss of fruit quality up to nine days; after this period serious fungal damage to the fruit that affects its commercial quality begins. The yellow pitahaya has a time of storage that can reach up to 15 days, although the fruit looks externally dehydrated and aged; however internally the fruit maintains its quality for consumption. It presents organosensitive standards of quality and resistance to microbiological agents superior to those of the red pitahaya. Due to the behavior of the fruit in the various variables evaluated in this study, strong evidences are presented that suggest to consider it as a species of non-climacteric respiration.*

Keywords— Hylocereusmegalanthus, Hylocereusundatus, quality parameter's, maturation period.

I. INTRODUCTION

The name pitahaya refers to the fruits of the species known as red pitahaya *Hylocereusundatus* (Haw) Britton & Rose and yellow pitahaya *Hylocereusmegalanthus* (K. Schum ex Vaupel) Ralf Baue (*Selanthus* spp.), *Selenicereusmegalanthus* (K. Schumann ex Vaupel) Moran, belonging to the Cactaceae family (Rojas et al., 2008; Betancourt et al., 2010)

The cultivation of pitahaya for export is recent in Ecuador. *Hylocereusmegalanthus* (yellow pitahaya) and other native species have been cultivated for about 10 years in the Palora

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canton of the province of Morona Santiago, with an area of more than 70 hectares (MAGAP, 2013).

Fruits are affected in postharvest by environment conditions and the handling that has been given of them. Due to ignorance of these aspects, these fruits are exposed to inappropriate temperature and humidity, to inadequate handling and to cuts and compression, which accelerate fruit respiration and perspiration processes, reducing their quality and shelf life (Bolaños, 2002).

The quality of the fruit depends on the physical characteristics such as integrity, shape, taste, color, aroma, freshness, texture and health; Besides being free of biological and chemical contaminants; Must be clean (free of spines), free of visible foreign matter (mainly in the apical foramen), and pesticides must not exceed the maximum limits established by the Codex Alimentarius (FAO, 2014).

Although this species is gaining importance as a crop in Ecuador, there are still not enough basic post-harvest management studies, which has motivated the present investigation.

II. MATERIALS AND METHODS

Geographic location: The work was carried out in a post - harvest fruit laboratory, located at km 26, east of Guayaquil, on the Durán - Tambo road, Virgen de Fátima parish, Yaguachi canton, Guayas province.

Characteristics of the mother plant: The plant where the fruits of the yellow pitahaya were extracted for the study was three years and eight months, an average height of 2.70 m, with an average yield of 94 fruits at its highest peak in the First crop of the year. The mother plant of the red pitahaya, was two years and six months, with a height of 2.70 m, with a yield of 61 fruits in production time.

Vegetable material: 100 fruits of each species were extracted from a commercial plantation located in Cerecita, province of Guayas, which were representative of the generality of fruits of the plantation in terms of size and external quality.

Groups of 10 fruits were randomly collected, and the physical-chemical characteristics were analyzed. The weight loss during the post-harvest was carried out at the time of harvest and at 4, 8, 12 and 16 days after harvest. In order to establish the ripening pattern and the fruit changes in the post-harvest, the fruits were weighed in grams and the percentage of dry matter was determined through the relation between wet samples and dry samples; the concentration of total soluble solids, through a graduated refractometer on a scale of 0-32 degrees Brix, titratable acidity, by titration technique with sodium hydroxide and malic acid was used as the reference. The methods used were those referenced by the AOAC (2012).

The color evaluation was carried out using a digital colorimeter with Stellar Net fiber optics. The readings were described based on the three values of the equipment that express changes of coloration by means of the index LAB: L (luminosity), A (changes of Green to red) and B (changes from blue to yellow). The methodology consisted in taking the color reading of the skin and pulp on both sides of the fruit, to detect the changes produced during the maturation process in postharvest. A circular mark of approximately 3 cm in diameter was made on the fruits on each of their side faces and the color was read at the indicated times. With the data obtained, chromaticity was established based on the definition of Chroma and angle Hue described by Morris and Townsend (Alvarado, 2011).

III. RESULTS

Figure 1. shows the results of weight loss of fruits of the two species under study, during the 15 days of post-harvest maturation.

The weight loss of the yellow pitahaya was 11.34%, whereas for the red pitahaya was 44.85%.

Table 1. shows the dry matter concentration data during fruit ripening days.

The values obtained for the yellow pitahaya did not present significant differences for $p \geq 0.05$, whereas the red pitahaya from the sixth day, presented significant differences with the initial value.

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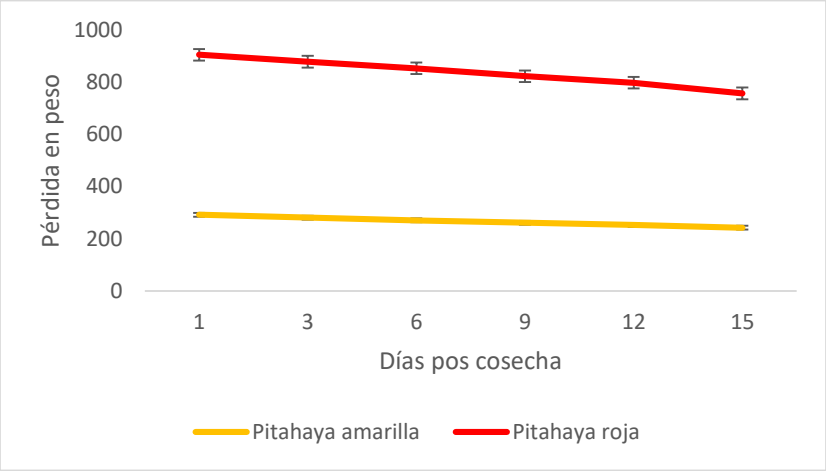

Fig.1: Weight loss of pitahaya fruits during 15 days of postharvest

Table.1: Changes in dry matter concentration of yellow and red pitahaya fruit during post-harvest maturation.

Treatment **Days after harvest**	**Yellow Pitahaya** **%**	**Red pitahaya** **%**
1	21,500a	27,200 b
3	21,500a	24,500 b
6	22,500a	23,060 bc
9	19,500a	22,000 bc
12	17,000a	19,500 bc
15	19,060a	15,000 c
CV	**27,65**	**26,66**

Legend: Different letters indicate significant differences for p≥ 0.95%

Figure 2 shows the results obtained in the determination of total soluble solids (sugars), of the fruits under study (red and yellow pitahaya) throughout the post-harvest period.

In the yellow pitahaya, the values of soluble solids were superior to the beginning of the experiment (day 1), compared to the red pitahaya, and during the maturation period an increase in both fruits was observed, but superior in the yellow variety with significant differences (p≥0.05) between the days of the study.

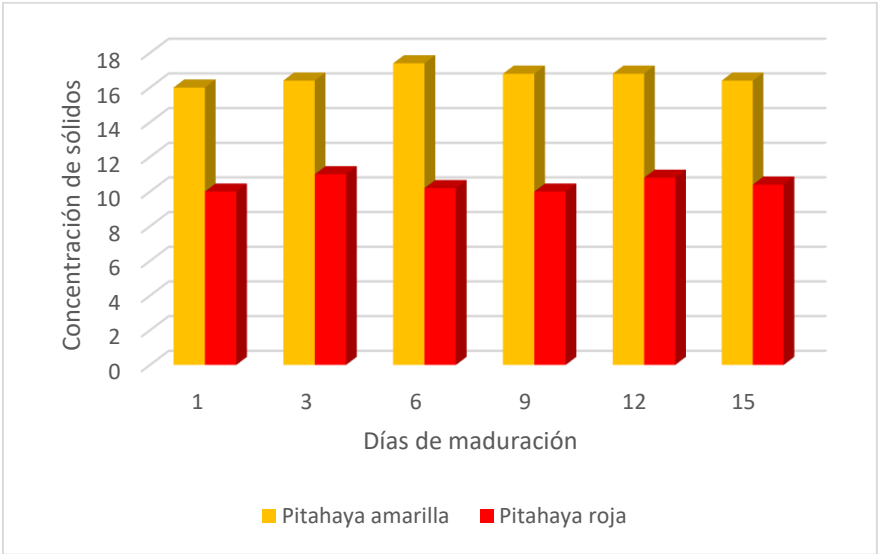

Fig.2: Changes in the concentration of soluble solids during maturation

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The values of titratable acidity can be observed in Table 2, in which there is no trend of change for any of the fruits under study during the post-harvest period analyzed. The acidity value of the fruits was similar in both cultivars and ranged from 1.20 to 1.28%.

Table.2: Percentage titratable acidity of yellow and red pitahaya fruits during post-harvest ripening.

Treatment Daysafterharvest	Yellow Pitahaya %	Red Pitahaya %
1	1,24	1,24
3	1,20	1,20
6	1,24	1,28
9	1,20	1,20
12	1,20	1,20
15	1,28	1,24
CV	11,90	11,90

The coloration of the epicarp calculated by the CROMA index is presented in figure 3, where it is observed that the fruits recently harvested from yellow pitahaya produce a slight increase in the color quality up to three days after the harvest (from 27.27 to 29.74); period after which the quality of the color begins to degenerate, a situation that was observed until the end of the experiment (from 29.74 to 6.65). In contrast, red pitahaya undergoes a slight improvement in color expression from the time of harvest to 15 days after harvest (from 27.5 to 37.2).

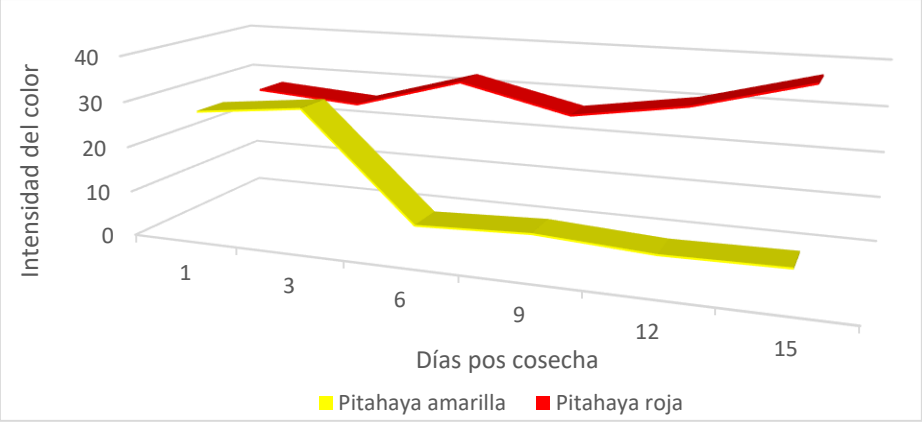

Fig.3: Changes in epicarp coloration of fruits during post harvest maturation

In the different periods of the evaluated post-harvest maturation, no statistical difference was observed between fruits in relation to maturity; however, the highest level of maturity ratio in yellow pitahaya was observed, where values between 13.04 and 14.69 were observed in comparison to red pitahaya, with values between 8.01 and 8.93.

During post-harvest maturation, the attack of cryptogamic agents was detected mainly in red pitahaya. On the sixth day of the experiment the presence of more or less regular spots of dark brown color was observed; these continued to grow until reaching a diameter of 5 cm and an extension of the spots as a whole that covered up to 90% of the fruit at the end of the experiment, which ended up completely collapsing the fruit, which had exposed deterioration of its tissues. The growth of fungal structures corresponding to a mixture of white, green and black mycelia that after being analyzed under the microscope were identified as *Collectotrichum*sp, *Alternaria*sp, *Rizophus*sp and *Fusarium*sp (Fig 4) . The fungi were also found in yellow pitahaya, but these were not as aggressive and did not determine the collapse of the fruit. The main fungi found after the corresponding phytopathological analysis were: *Colletotrichum*sp, *Cladosporium*sp, *Verticillium*sp and *Penicillium* sp. (Fig 5)

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Fig.4: Fungal damage on Red Pitahaya

Fig.5: Yellow Pitahaya fruit- 12 days post-harvest

IV. DISCUSSION.

The weight loss during the 15 days evaluation of the post-harvest maturation of the yellow and red pitahaya allowed to corroborate the universal pattern of weight loss that occurs in all the fruits during their maturation; which is basically due to the continuous removal of water and substrates in the form of CO2, through transpiration.

Cano et al. (2000) pointed out that the greater the difference between the relative humidity of the surrounding air and that of the product, the greater the dehydration. It has been suggested that the process of transpiration brings intrinsic weight loss, wilting, softening of fruit and progressive loss of nutritive value.

Robayo, (2002), confirmed this statement showing that even using modified atmospheres in the conservation of pitahaya, the loss of weight of the fruit during post-harvest could not be avoided.

Magaña-Benítez et al., (2010), a study on cooling processes of pitahaya fruits in controlled atmospheres, pointed out the inevitable loss of weight of the fruit during post-harvest.

Arias and Toledo (2007) and Pauli and Duarte (2011) indicated that if the fruit lost 5% of its fresh weight, it was no longer suitable for commercialization.

In this study, it was observed that in the conditions under which the experiment was carried out, the yellow pitahaya after three days maintained its initial characteristics intact, but at 9 days it had already suffered a weight loss of 9.3% and at the end of the experiment the loss reached 11.34%. In the case of red pitahaya, losses were higher, presenting at 3 days a 9.9% loss in weight and at the end of the experiment a 44.85%.

Fruit respiration follows a pattern that divides them into climacteric and non-climacteric. The perishability of fruits is directly related to the rate of respiration, the higher the rate of respiration, the shorter the shelf life (Arias and Toledo, 2007).

In the case of the pitahaya there is conflicting criteria. For yellow pitahaya Díaz (2005), it was observed that it was a non-climacteric fruit with a respiration rate of 95-144 mg CO2 / kgxh at 20 ° C, which contradicts Gallo's (1997) situation that placed it within the group of climaterics with a respiration rate of 20-80 mg CO2 / kgxh at the same temperature.

In this study and based on the observed results, it can be suggested that both yellow and red pitahaya have a behavior that inclines towards a non-climacteric respiration, with short lifespan.

The concentration of dry matter in post-harvest fruits gives a logical pattern of concentration this being high values immediately after harvest, where the fruit is composed of still hardened tissues. Since the membranes and cell walls are still rigid,while as the fruit matures, they become hydrolyzed and the cell walls collapse, leading to an increase in the moisture content of the fruit. After harvesting the fruits, the content of water and dry matter decreases. Adams-Phillips, et al. (2003) also point out that the levels of hydrolytic enzymes that cause the metabolism of plant cell components and the ripening of fruits after harvest are increased, generating a decrease in the values of dry matter as measured as the fruit advances in its maturation.

Similar concentrations of sugars in the fruit of the red dragon fruit throughout the period postharvest study marks a typical behavior of the fruits are not climacteric, while the yellow there is a tendency to increase their Brix until the sixth day after harvesting and after this period no increase is

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verified; In this variable the difference of degrees Brix present between the yellow and red pitahaya is remarkable, where the first of these it oscillates between 16 and 17,40 ° Brix and in the red pitahaya of 10 to 11 ° Brix. Studies carried out by Ramírez - Mora, et al. (2005), confirm that the lower soluble solids content was present in a fermented juice of red pitahaya, compared to the yellow one.

The concentration of organic acids of the fruit (titratable acidity) expressed in malic acid, showed an inalterability in terms of the concentrations during the whole maturation stage post-harvest of the fruit in the two cultivars studied. The values of titratable acidity of the fruit were similar in both and ranged from 1.20 to 1.28%, which is related to the study by Fernández et al. (2009), who stated that there was no variation of acidity Titrated in the pitahaya fruits of red, white and pink pulp.

Esquivel et al. (2007), indicated that in the pitahaya juice the main organic acid was malic acid, with concentrations of 8.20 and 6.08 g / l in the different genotypes of Costa Rica.

The titratable acidity values expressed by the fruits evaluated in this study are similar to the fruits studied by Camargo and Moya, (1995), harvested in state 5, those with titratable acidity percentages located in a range between 1.00 and 1 , 50%, at room temperature, similar to that applied in this investigation (25 ± 1 ° C).

V. CONCLUSIONS.

The results obtained allow us to arrive at the following conclusions:

In storage conditions with a temperature of 25 ± 1 ° C, the red pitahaya presents a gradual loss of fruit quality up to nine days. After this period, serious fungal damage to the fruit that affects its commercial quality begins.

The yellow pitahaya has a time of storage that can reach up to 15 days, although the fruit looks externally dehydrated and aged, internally the fruit maintains its quality of consumption.

The yellow pitahaya presents organ-sensorial standards of quality and of resistance to microbiological agents superior to those of red pitahaya.

Due to the behavior of the fruit in the various variables evaluated in this study and making a contribution to the lack of certainty about the type of respiration that this species possesses, strong evidences are presented that suggest to consider it as a species of non-climacteric respiration.

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Human Wildlife Conflicts to communities surrounding Mikumi National Parks in Tanzania: A case of selected villages

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***Abstract*—** *Human wildlife interaction is not a new phenomenon, it has existed since the beginning of humankind, it is evidenced by the fact that, many national parks are surrounded by human residents. The interaction between human and wildlife is of different nature depending on the culture of the surrounding human as well as wildlife community. For decade's human wildlife conflicts has been a great conservation challenge due to increased human population, international trade and change of policies. The challenge is more significant in a sense that it negatively affects both human and wildlife sustainability. Therefore a study was conducted to villages surrounding Mikumi national Park to assess reasons for conflicts between human and wildlife and account how communities prevent wild animals to destructs their agriculture products. Three villages were selected for study (Doma, Maharaka and Mkata, all villages surrounds Mikumi National Park Ecosystems. Different methodology includes: - Field observation, Household survey, Field interview, In-depth interview and Ethnography study were used. However descriptive analysis and non parametric test were performed by using SPSS 16 versions and Kruskal-wallis test respectively to compute mean, standard error, percentages and differences of wildlife consumption. Results suggests that, there is a gradual increase of human-wildlife conflicts which lead to loss of people's lives, as well as their livelihoods such as farms and farms product. Statistically results depicted that the average size of the farm affected at Doma, Maharaka and Mkata villages were 3.8 ± 0.1, 2.0 ± 0.1 and 2.2 ± 0.1 acres respectively, while at Mkata village 32 goats, 24 sheep and 76 cattle were reported to be killed by wild carnivores. In other way conflicts may result to poaching activities which may threaten the existence of huge herbivores such as Elephants and Rhinoceros. Apart from that, conflicts may lead to poor performances of tourism industry in the country. Research recommends that more efforts should be taken by the government and other stakeholders to prevent conflicts around all national parks so as to create good and conducive environment for human being life and wildlife in order to allow good performance of tourism industry for economic development of the country.*

***Keywords*— Human, Wildlife, Conflicts, National Park and village.**

I. INTRODUCTION

Globally, resource Conflicts have been a major threats for sustainable management and conservation of biological diversity sector since many years ago (Ruckstuhl, 2001). Currently it is recognized as one of critical and complex problem areas that have implications on the conservation of ecosystems in global environment and development discourse (Collier *et al*, 2003). Increasing resource competition at the global environment brings about social disparity and conflicts, these types of conflicts greatly impacted environmental quality, linked to human activities (Collier *et al.,* 2005). In many African countries such as Rwanda and DRC, Malawi and Tanzania which have many biodiversity species indicates that, resource conflicts are caused by competition of scarcity of resources and human made disturbance of ecosystems (Pearce, 1994; Winter 1997).

In East African the increasing of human-wildlife conflict are highly contributed by changing of land use in areas surrounding protected areas, which bring difficulties for community based conservation to succeed (Fowler, 2001). These areas experiencing expansion of small holder cultivation in wildlife dispersal areas, the situation has been reported to reduce animal home ranges, leading to increase human wildlife interaction, which may degenerate into

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human wildlife conflict (Little, 1994). In Tanzania, human problems constraining Wildlife Sector are responsible for increasing of resource conflicts (URT, 2012). Wildlife Conservation Authority is accused for marginalizing people, denying people access to traditional and legitimate rights, property damage and risk to human life through attack by wild animals and disease transmission (UNEP, 1995). In broad sense, the primary causes of resource conflict are demographic, economic, institutional and technological (UNEP, 1995), however (WRI, 1995) reported that the habitat loss in Tanzania was a serious problem for different ecosystems (WRI, 1995).

Conover (2002) explained Human-wildlife conflict as any action by human or wildlife that has adverse impact on each other whereas (Foreman, 1992 and Gittleman *et al*. 2001), defined Human-wildlife conflict as an issue of increasing conservation concern, particularly as burgeoning human populations move over further into wilderness areas. The negative impacts of wildlife on people may include crop damage, attacking and killing livestock and people, competing for game species or acting as disease reservoirs (Nyahongo, 2007). People may affect the wildlife through a wide range of lethal methods, such as shooting, poisoning, trapping or snaring, and habitat modification, encroachment or disease exchange between wildlife and livestock (Nyahongo, 2007). Although a remarkable variety of species cause conflicts with people, from rodents such as prairie dogs to mega-herbivores like African elephants (*Loxodonta africana*) (Hoare, 1999). Large carnivores are of particular interest in this conflict, where by their behavior put them in a direct competition with people for both livestock and wild game species or their ability to kill people (Baldus, 2004; Loe and Roskaft 2004; Packer et al, 2005; Silero-Zubiri and Laurenson, 2001). Carnivore's attack is a problem, and is reported in different parts; claim hundreds of lives each year globally, although no figures are available to prove it (Loe and Roskaft 2004).

To date, Human-Wildlife Conflict is a serious problem in different parts of the world (Bradshaw, 2007).This is simply because the human population increases but the resources available are fixed, also conflicts occur because every individual in those areas aims at fulfilling basic needs using the resources without caring for others and sometimes not caring even for the future generations (Damania, 2008). Close to the protected areas, the problem is very serious because the local communities' interaction with wildlife creates negative impacts to both sides often local communities kill wildlife to obtain bush meat for household consumption, and for income generation (Kombo, 2010). However, wildlife destroys crops and kills livestock and sometime injures or kills people, livestock keepers and crop producers elsewhere in the country are fighting for grazing land that actually are farms for crops (Geoffrey, 2005).

Areas around Mikumi National Park experience similar problems; crop damage by elephants and other herbivores, livestock depredation by wild carnivores, bush meat hunting by human, human injury caused by wildlife and conflict between crop producers and livestock keepers (Emanuel, 2004). Despite the fact that all events are vivid and known by conservationists and politicians, the level and extent of such conflict along the gradient from the park is not known, thus this study was conducted to the selected villages surrounding to Mikumi National Park to determine the level and extend of these natural resources conflict and its impacts to the communities surrounding Mikumi national park.

1.2 Global Impacts of Wildlife to human livelihood

The communities affected by carnivores must also bear the indirect costs of preventing attacks to livestock's and people live in constant fear of their lives (Roskaft *et al*. 2003; Loe and Roskaft 2004). Within the immediate buffer zones of the Selous Game Reserve or other protected areas, crop raiding by elephants, bush pigs (*Potamochoerus larvatus*) and other mammals is persistent problem that constitutes, like in most other regions, a major form of human-wildlife conflict (Ikanda, 2010). Each year, hundreds of acres are destroyed by crop-raiding elephants, hippopotami (*Hippopotamus amphibious*), bush pigs and vermin primates like baboons (*Papiocynocephalus anubis*) and monkeys (*Chlorocebus pygerythrus*) (Ikanda, 2010). In Alberta Canada, over a period of 14 years (1982-1996) wolves *(Canis lupus*) caused 2,086 deaths among domestic animals, mainly cattle and to a lesser extent dogs, horses (*Equus ferus caballus*), sheep (*Ovis canadensis*), chickens (*Gallus gallus domesticus*), bison (*Bos bison*), goats (*Capra hircus*), geese (*Branta canadensis*) and turkeys (*Meleagris gallopavo*) (Musiani *et al*. 2003). In Peru, in the Amazon Province of Tambopata, a population of 3200 people live inside the northern border of the 1.5 million ha protected area of the Tambopata –Candamo Reserve claim that the ocelot (*Leopardus pardalis*), hawks (*Accipiter spp., Leucopternis spp*.), jaguars (*Panthera onca*) and pumas (*Puma concolor*) were blamed for causing most of the depredation (Naughton-Treves *et al*. 2003).

In Zimbabwe, many areas of traditional agro-pastoralist bordering protected areas suffer from livestock depredation. In particular, in the Gokwe communal land, neighboring the

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Sengwa Wildlife Research Area, rural villagers experience a negative impact from the close proximity to the reserve, wild carnivores attack domestic livestock and the conflict is severe (Butler, 2000).It was reported that, between January 1993 and June 1996, 241 livestock were killed by baboons, lions (*Panthera leo*) and leopards (*Panthera pardus*), which contributed respectively to 52%, 34% and 12% of the kills (Butler, 2000).

In Kenya, Patterson *et al*. (2004) evaluate the level of impact of carnivore attacks on two private cattle ranches that lie adjacent to boundary of the Tsavo East National Park, the carnivores responsible were lion, spotted hyena and cheetah, they consumed cows, bulls and young cattle's. In a four-year study the ranches have lost an average of 2.4% of the total herd per annum, which represented 2.6% of their economic value and amounted to US$ 8,749.

In Zanzibar, the villagers in southern border of the Jozani Forest Reserve claimed that the red colobus (*Procolocus kirkii*) to consume their coconut (*Cocos nucifera*) (Siex *et al*. 1999). They consider red colobus as the third most serious vertebrate pest. However the red colobus is one of the most endangered primates in Africa and in Zanzibar (Siex *et al*. 1999). The explained manifestation of human wildlife conflict raises a concern to review human wildlife interaction and find a modality to suitably improve livelihoods and wildlife sustenance. Currently in Tanzania for example the population of elephants is going down rapidly more than ever, poaching triggered by increased international trade and demand for ivory is said to be the main reason. However, conflicts between wildlife and human are also adding fuel to the elephant's extinction fire.

A census report by the government of Tanzania (2013) conducted in (Selous-Mikumi and Ruaha Rungwa) shows the elephants population in the two ecosystems are 13,084 and 20,090 respectively, the figures indicates a notable decline in elephants population in these ecosysterms compared to previous census. For instances in 1976, the Selous-Mikumi had 109,419 elephants, the dropped dramatically to 22,208 in 1991. Although it rose again to 70,406 in 2006, the population has dropped again in the recent years; in 2009 the number stood to 38975 while right now the number dropped to 13,084. Similar situation appears in Ruaha-Rungwa ecosystem where the 1990 census recorded 11,712 elephants. This number rose to 35,416 in 2006 but as for now only 20090 was estimated. The figure shows a decline of 66% and 36.5% respectively from 2009 to present. This paper therefore is aiming at assessing the human interaction with wildlife with the assumption that the interaction (Conflicts) has to certain extent contributed to decline of elephant's population.

II. METHODS

2.1 Description of study area

This paper studied three villages surrounding to Mikumi national park, the villages are Doma, Mkata and Maharaka, each village studied separately, Mikumi National Park is described as a single ecosystem accommodating the three selected villages.

Mikumi National Park was gazetted as a national park in August 1964 and its boundaries extended in 1975. It is the fourth largest park in Tanzania covering 3,230 km^2 (1,250 square miles). The park is located in eastern Tanzania between 7°00' and 7°50'S, and between 37°00' and 37°30'E. The park is located in Morogoro Region, 283 km (175 miles) to the west of Dar es Salaam. It shares its boundary in the extreme south with the Selous Game Reserve – a world heritage site. Mikumi and Selous make one ecosystem where animals like elephant, buffalo and zebra normally migrate between each to the northern part of the Selous and Mikumi National Park (TANAPA 2004).

2.1.1 Biodiversity

Mikumi National Park has a unique combination of flora and fauna. It supports a wide range of large mammals, including elephants, lions, giraffe, zebra and buffalo and more than 300 species of birds (Mercer, 1983; Hawkins and Norton, 1998). The bird life is intermediary between north and south. . The park is located in an area where four vegetation zones intersect making it a diverse ecotone. The four vegetation types are miombo woodland in the south, arid bush land in the north, coastal zone in the east and mountain climate in the east and west (Hawkins and Norton, 1998) .The miombo woodland consists of mainly Brachystegia spp, while Combretum-Terminalia woodland dominates between hill areas and in floodplain (Mercer, 1983). The park is also dominated by other species like *Sclerocarya caffra*, *Cassia abbreviata*, *Borassus flabellifer* and *Hyphaene ventricosa* palms. *Balanites aegyptiaca* and Ficus spp. Mikumi National Park show seasonally local floods in Mkata floodplain. The floodplain and waterholes become a habitat for fish, freshwater crabs, and other aquatic wildlife in the wet season. There are also permanent waterholes with hippos in the center of the park.

2.2 Data Collection and Analysis

The data collection methods included field observation, household survey, field interviews and in-depth interviews.

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Field observation was important because it enabled the observation of a real situation of what is going on in three villages concerning the human elephant conflicts, human carnivore conflict and the agricultural (farmer) and pastoralist conflicts. Household survey was carried out in selected villages for the study basing on the research objectives. It was conducted through open ended questions and closed ended questions where a total of 156 households in three villages were involved. The data collected from household survey mainly focused on the socio-demographic characteristics of the respondents, implication of human-carnivore conflict, major carnivore causing conflict in the area, mitigation of human-carnivore conflict in the area, implication of Human-Elephant conflict on household income, effect(s) caused by elephant in the area, mitigation of human-elephant conflict in the area, possession of land, source of conflict between pastoralist and agriculturalist and what can be done to solve the conflict between pastoralist and agriculturalist. The household survey covered most of the field research time as it was one of the main data collection method. An In-depth interview was carried out; it was purposively directed to the village executive officers and also to pastoralist and some farmers, with the main issue to understand the behavior of elephants and how do they behave once they come in the villages, not only that but also, the behavior of different carnivores causing problems of killing livestock around the village. Furthermore the intention of doing in-depth interviews was to know what initiatives to solve those natural resources conflicts in their villages.

Descriptive analysis to compute mean and standard error and percentages were performed using SPSS 16 version for windows. Differences between the extents of elephant's consumption from 2008 to 2012 in each village were tested using non-parametric tests, Kruskal-Wallis tests and Mann-Whitney test were used to test non normality which highly existed. Summary statistics were quoted in tables to illustrate the distribution of data in respect to different parameters. Mean were reputed as Means ± Standard error. For all statistics, $p < 0.05$ were considered significant.

III. RESULTS AND DISCUSSION

3.1 Human carnivore conflict

Majority of the Respondents from the selected villages conduct agriculture as the main stay of their economies, agriculture activities are conducted around Mikumi National park where there is high interaction with wild animals like elephant, zebra etc, interaction creates conflicts between human being and wild animal, this always happen when wild animals destroys agriculture crops. In other way results depicts that wild animal specific carnivores kills and eat goats and cattle, for instance in Mkata village, results shows that, 5% (n=32) of the goats were killed by carnivores from January to June 2012, and 6% (n=24) of the sheep were also killed in that period of time and 4% (n=76) of the cattle were killed during the same period (January-June 2013). This situation raise conflicts between human and wild animals. It should be noted that, human being apart from agriculture also depends on animals like goats, cattle, etc (husbandry) they depends on them as commodities as they can sell whenever they face economic crises.

(Table 1) A total number of goat, sheep and cattle killed by wild animals in 2012-2013

Table.1: Livestock loss due to carnivores at Mkata village from January to June 2013

TYPE OF LIVESTOCK	NUMBER OF LIVESTOCK KILLED BY CARNIVORE		TOTAL NUMBER OF LIVESTOCK
	FREQUENCY	(%)	**N=1900**
GOATS	32	4.6	700
SHEEP	24	6	400
CATTLE	10	1.25	800

Among the three villages selected, which are Doma, Maharaka and Mkata, only Mkata village that is having livestock depredation by wild carnivores. This is simply because it is the only village keeping livestock. Other local communities in the other two villages do not keep livestock. Depredation cases found to occur in wet season involving spotted hyena, lion and wild dogs. During the dry season, herbivores concentrate within protected areas around permanent water sources whereas in wet season herbivores evenly spread around the area where situation makes hunting for carnivores more difficult enabling carnivores to hunt over larger areas. Similar observation was reported by Nyahongo (2004) and Bygott and Bygott (1975).

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Livestock grazing task in the field is usually attended by young individuals who might fall asleep or playing and not care for livestock. Thus makes easier for livestock to be killed by wild carnivores. In such cases, the animal may be attacked and killed without the knowledge of the herdsmen especially at night when most carnivores are active. Similar observation was reported elsewhere (Nyahongo 2004).

3.2 Human-elephant conflict at Doma village

Elephants are the animal species that had been claimed by majority to destroy crops in all villages, where at Doma an average of 3.8±0.1 acres of crops had been reported to be destroyed by elephants in 2012-2013. When comparing the mean values of each year, crop damage varied among years (Kruskal-Wallis test, $x^2 = 9.424$, df = 3, p = 0.0240). When data were splinted into two years period starting with 2008-2009 and 2009-2010 results suggest no statistical difference (Mann-Whitney test, U = 1.76, p = 0.230) again when comparing the next two years, 2010-2011 and 2011-2012 result suggests no statistical difference as well (Mann-Whitney test, U = 1.83, p = 0.425).

3.2.1 Human-elephant conflict

Furthermore, at Maharaka village the problem of elephant consuming crops had been claimed by majority of farmers where by an average of 2.0±0.1 acres of crops had been reported to be destroyed by elephants. The extent of crop damage varied among years (Kruskal-Wallis test, x^2 = 20.347, df =3, p = 0.000). When data were splitted into two years period and compared between two years period starting with 2008-2009 and 2009-2010 results suggest no statistical difference (Mann-Whitney test, U = 312.000, p = 0.153) again when comparing the next two years, 2010-2011 and 2011-2012 result suggests no statistical difference as well (Mann-Whitney test, U = 277.000, p = 0.027). Human-elephant conflict at Maharaka village was reported to be in extent where an average of 2.2±0.1 acres of crops had been reported to be destroyed by elephants. Meanwhile, the crop damage varied among years in the village (Kruskal-Wallis test, x2 = 60.974, df = 3, p = 0.000). Apart from that, when data were compared between two years period starting with 2008-2009 and 2009-2010 results suggest no statistical difference (Mann-Whitney test, U = 624.000, p = 0.00) again when comparing the next two years, 2010-2011 and 2011-2012 result suggests no statistical difference as well (Mann-Whitney test, U = 655.00, p = 0.001). Conflict exists in all the three villages in Doma ward which are Doma, Maharaka and Mkata because all people in these villages are practicing subsistence farming. Farmers cultivate different crops which are the same in all three villages including maize (*Zea mays*), tomatoes (*Solanum lycopersicum*), millet (*Panicum milieaceum*), paddy (*Oryza sativa*), water melon (*Citrullus lanatus*), oil seed and also different vegetables. They cultivate the crops throughout the year through irrigation scheme.

3.2.2 Effect caused by elephant in the area

The effect caused by elephant in all the three villages is very high each year. At Doma, Maharaka and Mkata the average size of the farm affected was 3.8 ± 0.1, 2.0 ± 0.1 and 2.2 ± 0.1 acres respectively. In various areas throughout Africa, elephants have destroyed more than 60% of crops in communal areas adjoining conservation areas (Anon, 2003). This situation might be due to global climatic change worldwide which leads to water shortage inside the park whereby elephants move outside the park to the nearby villages in search of food and water. .

Ways to Prevent Wild animals

Farmers in Maharaka were introduced to the new method of preventing themselves from elephant problems through the use of string, oil and paper, they were taught to surround their farms with string having oil and paper, so once elephant come across with that string they cough and then run away, this method was useful to them for a quite some time and it helped them a lot to prevent elephants from consuming crops, but from 2012 till now they claim that elephant no longer enter their farm using their front part, instead they enter the farm using their back part, and after cutting down the string having oil and paper, they start eating crops. So this technique used by elephant might be acquired by young elephant and it means after sometime this method of protecting the farms from being consumed by elephant will no longer be useful, so villagers claimed that their crops will continue being destructed by elephant as they used to destruct before the establishment of the technique.

Farmers in Mkata used pepper, oil and string to protect their crops from being destroyed by elephants. Soon after too much application of it the elephants adapted, they also enter the farm using their back and sometimes raise their head then after entering the farm they start eating the crops. This method is no longer suitable because it does not solving the problem. In Caprivi region in Namibia, fences lined with a mixture of grease and chili peppers are still being experimented (Brian and Barnes 2006).

However, farmers in Mkata are now using strong perfume to prevent elephants from entering to their farm and destroy

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the crops. They use perfume with strong smell like the perfume called "Kuluthum". They surround their farm with string and attach to the string pieces of cloth then they spray the perfume on the pieces of cloth, so once the elephant reach near the string having that piece of cloth with perfume, they go back because they dislike the sensation of strong smell. This method is used by most of farmers in Mkata and elephants are not entering to their farm once they come across with that smell. The problem a rise when farmers fail to buy that perfume because they cannot afford its price, one bottle is about 10US $ in the year 2012, so some farmers fail to prevent their farm since it is expensive.

Also one among the reasons put forward by farmers in Mkata is that the elephants move out of the park in search of fruits known as "ng'ongo" thus farmers suggested those tree to be planted inside the park so as to prevent the elephants from moving outside the park, this is not appropriate because planting the particular tree inside the park which is not there is like introducing invasive species inside the park. Exotic plants threaten the integrity of agricultural and natural systems throughout the world. Many invasive species are not dominant competitors in their natural systems, but competitively eradicate their new neighbors (Callaway and Aschehoug, 2000).

Most of the farmers in Mkata shift from agricultural activities to charcoal production activities; this is because of accumulation of farmers and pastoralist conflict and also the problem of elephant to consume crops. Most people now produce charcoal and Mkata area is now a famous place for producing charcoal. This is dangerous to the biodiversity found in the area and the survival of Mikumi National Park because too many trees are destroyed due to charcoal production hence disturbing the climatic condition of the area. Mkata was also among the villages which received food assistance from the government in the year 2013 because of being insecured. Although the area is having good and fertile soil, water is available in the area throughout the year due to presence of river Mkata, but still they asked food from the Government due to shortage of food security contributed by destruction of wild animals (Naughton-Treves and Treves, 2005).

IV. CONCLUSIONS

The problem of human-wildlife conflict increases each year and the loss that livestock keepers acquire due to depredation are very high if computed. Among the causes of the problem is poor construction of "bomas" for keeping the livestock in the area. Elephants destroy large area of crop field in all the three villages which are Doma, Mkata and Maharaka. Regardless of the local methods that have been used by the villagers the problem keeps on increasing year after year. Losses accounted by the villagers are very high from 2008 to 2012. Conflicts between livestock keepers and crop producers are only pronounced at Mkata village. It has been increasing year after year. The reason for the conflict is that the livestock keepers and crop producers coexist in the same area. The number of cases reported about the conflicts to the Village Executive Officer increases each year from 2008 to 2012.

In many situations, strategies or methods for addressing the human wildlife conflict issue are often constrained by local, national or international regulations, laws or treaties (Fall and Jackson, 2002). The ineffectiveness of some of the management practices is directly dependent on the establishment and application of policies and guidelines on a wide range of human activities. In various countries, existing wildlife policies are outdated, contradictory and require clarification, in particular those regarding land development planning and its impact on wildlife habitats. Policies on land tenure, controlled utilization of wildlife through hunting and trade of wildlife products, game farming, tourism development and compensation schemes should be strengthened and made to conform to the present national state of affairs and population requirements (Kenya wildlife Service, 1996).

V. GENERAL RECOMMENDATIONS

There is a need of trying to solve the conflict existing of natural resources in different places in our societies. This can be done by sometime using the bottom-top approach where by the solution for those problem should be initiated by the local people in the respective area. What can be done is to modify the idea brought by the local people. Apart from that the farmers should be introduced to other sources of income like bee keeping and also involving in entrepreneurship activities of which will raise their income. Also for the pastoralist, they should be provided with permanent areas where they will keep their livestock and also water sources for the livestock should be constructed in those areas. Nomadic pastoralism should be discouraged because it is environmental unfriendly. Additionally, the pastoralist should be provided with education about the minimization of the number of livestock they are having together with the ways of constructing strong "bomas" for keeping their livestock to prevent them from being consumed by the carnivores, example at Amboseli-Tsavo region in Kenya, where conflict between pastoralists and lions is a significant and growing conservation issue, a

scheme called 'Lion Guardians' was established, where young Maasai men were trained to track lions, provide advice to villagers in terms of where the tracked lions are, provide practical help in strengthening bomas, and talk to people about their problems and issues with large carnivores (Hazzah and Dolrenry 2007).

The bee keeping projects should be established on the buffer zone to minimize the extent of elephants from entering in the villages and consume the crops; this will also act as the source of income to local people. Placement of bee hives in strategic trees can be used to prevent the destruction caused by elephants as they are sensitive to the sound and sting of bees (Karidozo and Osborn 2005; Vollrath and Hamilton 2005). Elephants also have excellent hearing and the "buzz" from an active hive could also stimulate hive avoidance (O'Brien, 2002).

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Ecosystem Carbon Storage and Partitioning in Chato Afromontane Forest: Its Climate Change Mitigation and Economic Potential

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Abstract— *Forests trap carbon dioxide (CO_2) from the atmosphere, store in the form of carbon (C) and regulate climate change. In this study, C storage and climate change mitigation potential of Chato Afromontane forest was assessed from measurement of the major pools including the aboveground biomass, belowground biomass, dead tree biomass, plant litter and soil organic carbon (SOC). The result showed that biomass accumulation was comparatively larger for natural forest than plantations due to maturity, intactness and species diversity. The total C storage capacity of the forest ranged from 107.12 Mg ha^{-1} for acacia plantation to 453.21 Mg ha^{-1} for the intact natural forest. The mean C storage capacity by major pools ranged from 1.36 Mg ha^{-1} for the dead tree C to 157.95 Mg ha^{-1} for the aboveground C pool. The forest ecosystem accumulated a total of nearly 6371.30 Gg C in the vegetation plus soil to a depth of 60 cm. The large volume of annually trapped C by the vast channels of Chato forest makes it the most significant regulator of global climate change. Conservation of the sacred forest will have an imperative implication to the net positive C addition ensuring its viability for the international C market.*

Keywords— *Forest, Chato forest, Afromontane forest, carbon storage, carbon sequestration rate, climate change, carbon credit.*

I. INTRODUCTION

Forests are land use systems with high tree population and store large quantities of C (Lal 2005). Forest ecosystem store more than 80% of all terrestrial aboveground C and more than 70% of all soil C (Batjes 1996). According to Batjes (1996) the pedologic and biotic pools together are called the terrestrial C pools, and they are estimated at 2860 Pg or 2860 Gt (1Gt = 1Pg = 1 billion metric tons). Terrestrial C is the C stored in terrestrial ecosystems as living or dead plant biomass (aboveground and belowground) and in the soil along with usually negligible quantities as animal biomass. The main C pools in tropical forest ecosystem are the living biomass of tree and understory vegetation, dead mass of litter and woody debris, and soil organic matter. The vegetation of tropical forests is a large and globally significant storage of C because tropical forests contain more C per unit area than any other land cover (Hairiah et al. 2011). The forest resources of Ethiopia store 2.76 billion tons of C (about 10 billion Mg of CO_2) in the aboveground biomass (Yitebitu Moges et al. 2010). Forests can be both sources of atmospheric CO_2 when disturbed by natural or human causes, and sinks, when vegetation and soil C accumulate after disturbance, depending on land management thus potentially accelerating or mitigating climate change (Lal 2004).

The $REDD^+$ strategy, namely "reducing emissions from deforestation and forest degradation, and foster conservation, sustainable management of forests and enhancement of forest C stocks (through afforestation and regeneration) are keys to ensure net positive C addition that would then become a credit that could be sold in an international C market. However, the potential of C financing through REDD+ on forest C sequestration in tropical forests has not been systematically studied. The general allometric models developed by Pearson et al. (2005) and Chave et al. (2005) have been widely used, notably in the context of REDD+, and were recommended by the IPCC guidelines (IPCC 2006) for estimating C stocks in tropical forests. The general model developed by Chave et al. (2005) including tree height provided best biomass estimates specifically for moist tropical forests and reduce uncertainties as compared to other generic models (Ervan et al. 2013).

Afromontane forests are among the most species-rich ecosystems on earth (Schmitt et al. 2010). The study was conducted in Chato Afromontane forest ecosystem, one of the largest sacred forests in Ethiopia comprising of untouched natural forest and tree plantations. Although not studied so far, the wide range of tree plantations and high endemic plant species in Chato forest makes it the most

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powerful C sinks in the tropics. Therefore, the study was designed mainly to estimate C storage capacity and CO_2e sink of the forest ecosystem so as to unveil the climate change mitigation and economic prospective of the forest.

II. MATERIALS AND METHODS

2.1. The study site

Chato forest is situated between 9.62898256 to 9.810748292N and 36.90419252 to 37.06710714E (Fig. 1) in the western parts of Ethiopia with an elevation ranging from 1700 to 2350 m asl. It is found at about 30 km north-west of Shambu, the capital city of Horo Guduru Wollega Zone, Oromia Region. The forest was demarcated as National Forest Priority Areas (NFPA) and has been known by the name Chato-Sangi-Dangab forest in the country (EFAP 1994). The forest is classified under moist evergreen Afromontane forest consisting high diversity of endemic tree species and a variety of wildlife. Chato forest covers about 14,290.97 hectares (ha) of land comprising of species rich natural forest (13670.06 ha) and various tree plantations including 17 to 29 years old acacia spp. (6.05 ha), 18 to 31 years *Cupressus lusitanica* (434.21 ha), 25 to 31years old *Juniperus procera* (2.97 ha), 14 to 31 years old *Gravellia robusta* (3.43 ha) and 14 to 31 years old eucalyptus spp. (174.25 ha) such as *Eucalyptus citrodora*, *Eucalyptus saligna*, *Eucalyptus comandulus*. The area is characterized by having unimodal rainfall distribution with mean annual rainfall of 1566 mm and mean annual temperature of 16.7 ^{0}C.

2.2. Forest stratification and sampling techniques

Compartments or strata established during forest inventory by Horo Guduru Forest and Wildlife Enterprise, mainly based on forest stand type was used for biomass assessment. Besides, part of the forest that was not addressed during inventory by the Enterprise was stratified during the study. The area of each forest stand was tracked by using ground positioning system (GPS). In the stratum or forest stand, nested sample plots of 20 m x 20 m, 2 m x 2 m and 1 m x 1 m were randomly laid to measure the biomass of woody plants, herbaceous/saplings and litter biomasses, respectively. A total of 105 sample plots were taken for C stock inventory. Sample plots in the same stand, namely eucalyptus, acacia, *Cupressus lusitanica*, *Juniperus procera*, *Gravellia robusta* and natural forest were weighed to give average biomass and C stock for each stand type.

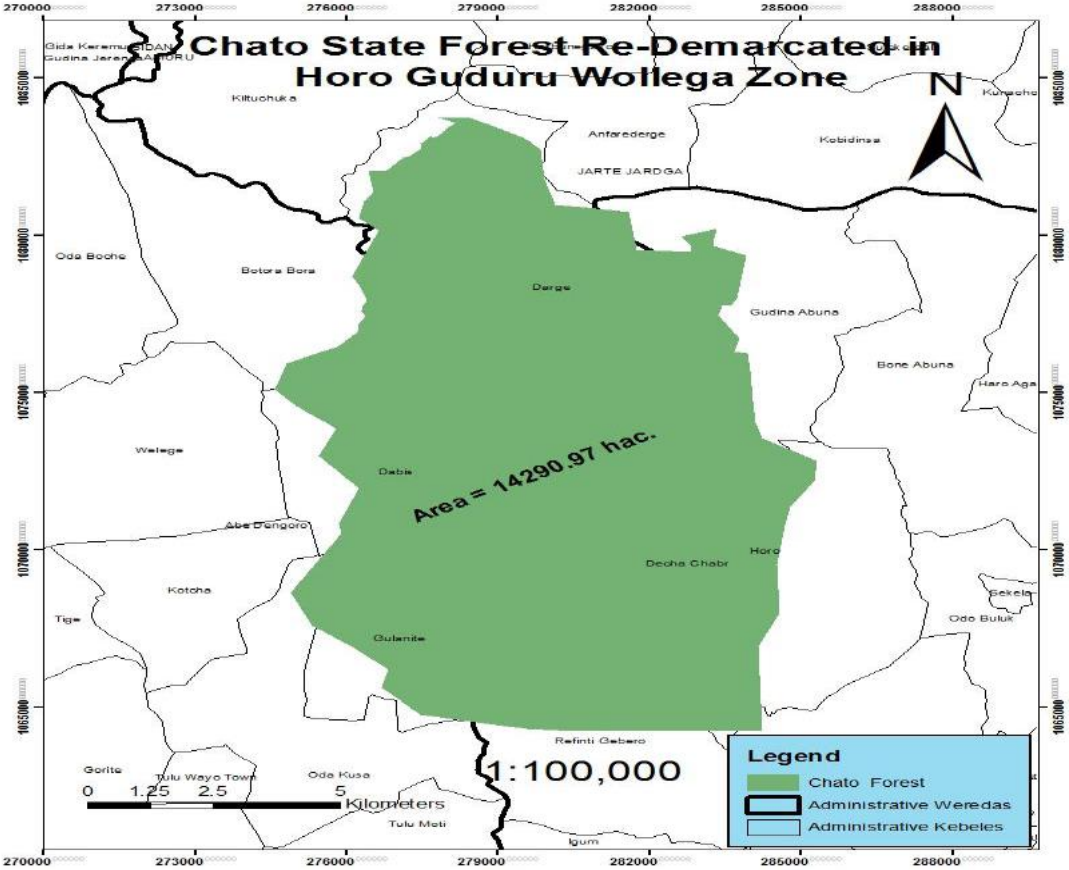

Fig. 1: Areal coverage and location map of Chato state forest

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2.3. Soil sampling and analysis

Carbon stock inventory for the soil was done for the upper 60 cm depth in the nested plot, by collecting samples from 0–30 and 30–60 cm layers at 20 locations. Following sample preparation, samples were analyzed based on the standard laboratory procedures. Bulk density was determined using core method (Blake and Hartge 1986) while SOC was determined using Walkley–Black oxidation method (Walkley and Black 1934).

2.4. Estimation of biomass in different pools

The major biomass components or pools assessed include aboveground live biomass, dead tree biomass, below ground biomass, and litter biomass.

2.4.1. Aboveground live biomass (AGLB)

In each 20 m x 20 m sampling plot, diameter at breast height (DBH) and tree height (H) were measured for every live tree using caliper and hypsometer, respectively. Then, the aboveground biomass of live trees with DBH ≥ 5 cm was estimated by using general allometric equation recommended by Chave et al. (2005) for moist tropical forest stands as indicated hereunder:

$$AGTB = 0.0509 * (\rho D^2 H) \quad (1)$$

where *AGTB* is aboveground tree biomass (kg), ρ is wood specific gravity (g cm^{-3}), *D* is tree DBH (cm), and *H* is tree height (m). Besides, live grasses, shrubs, herbs, saplings, and some tree seedlings from natural regeneration with a DBH < 5 cm (Pearson *et al*., 2005) were harvested in each 2 m x 2 m subplot located in every corner and center of the main plot (400 m^2) in the nest. In 4 m^2 subplot, total fresh weight of harvested plant material was measured, from which 500 g sample size was taken to the laboratory, oven-dried at 85 ^{0}C and reweighed to estimate the dry matter of aboveground grasses, shrubs, herbs and saplings biomass (AGHSB). Finally, aboveground live biomass was the sum of aboveground tree biomass and aboveground grasses, shrubs, herbs, and saplings biomass.

2.4.2. Belowground biomass (BGB)

Belowground biomass was estimated from aboveground biomass on the basis of root to shoot ratio of (0.24:1) recommended by Cairns et al. (1997) for moist tropical forests (woody and non-woody).

2.4.3. Dead tree biomass (DTB)

The dead tree biomass was estimated for standing and downed dead trees following (CSEMF 2011) equations. Standing dead tree biomass (SDTB) was estimated by classifying the dead tree into three classes. The first class works for standing dead tree with small and large braches and twigs but without leaves. In this case, general allometric equation was used to estimate biomass and 2% was deducted due to absence of leaves. The second class works for standing dead tree with no twigs but only some large branches. In this case:

$$VB = \left(\frac{\pi * H}{12}\right)\left(D_b^2 + (D_b + D_t) + D_t^2\right) \quad (2)$$

The third class works for standing dead tree with bole (trunk) only. In this case:

$$VB = \frac{(D_b^2 * D_t^2) + H}{8} \quad (3)$$

where VB is volume biomass, *H* is height of stem, D_b and D_t are diameter at base of the tree and top of the stump, respectively. Downed dead tree biomass (DDTB) was determined from volume estimate as:

$$VB = 0.25\pi\left(\frac{D_b + D_t}{2 * 100}\right)^2 * H \quad (4)$$

where VB is volume of dead wood (m^3), D_b is diameter of the base of the dead wood (cm), D_t is diameter of the tip of the dead wood (cm), H is length of the dead wood (m).

For standing dead trees of case 2 and 3 and downed dead trees, sample wood density was estimated by floating method (by cutting a disk of wood) and drying until a constant mass was obtained. Hence, wood density was estimated by dividing dry weight of the disk by volume of the disk. Subsequently, standing dead tree biomass of class 2 and 3 and downed dead tree biomass was estimated by multiplying volume biomass by their wood densities. Biomass of standing dead tree under each case was summed up to give total SDTB. Finally, SDTB and DDTB were summed to provide DTB.

2.4.4. Litter biomass (LB)

The dry matter of litter and finer plant debris was collected from 1 m x 1 m plot in every four corners and center of the main 400 m^2 plot in the nest. In the 1 m^2 plot, litter was collected and total fresh weight was recorded, from which 250 g sample size was taken to the laboratory, oven-dried at 85 ^{0}C and reweighed to estimate the dry matter.

2.5. Calculation of carbon stock from biomass

The amount of C stored in each pool (kg) was determined by multiplying the biomass of each pool (kg) to 0.50

(Payton and Waever 2011) as follows:

$$C_X = Biomass * 0.5 \quad (5)$$

2.6. Calculation of carbon storage capacity

Then, C storage capacity (Mg ha^{-1}) was calculated by dividing the C_x stored in each pool and each subplot (kg) by area of the subplot (m^2) and multiplying with 10 as follows:

$$C\ storagecapacity = \left(\frac{Cx}{A}\right) * 10 \quad (6)$$

where C storage capacity was estimated for each types of pools (i.e. AGB, BGB, DTB, and LB) expressed as Mg ha^{-1} and 10 is a conversion factor from kg m^{-2} to Mg ha^{-1}.

2.7. Estimation of soil organic carbon (SOC)

The SOC (Mg ha^{-1}) to specific soil depth was estimated as:

$$SOC = OC * \rho b * d * CFU \quad (7)$$

where *OC* is mg g^{-1} C concentration, *d* is soil thickness or depth i.e. 0–30 and 30–60 cm, ρ_b is bulk density of the soil (g cm^{-3}) and *CFU* is correction factor for units (= 10^{-1}).

2.8. Quantifying total carbon stock (TCS)

The total C stock in the nested plot expressed in (Mg ha^{-1}) was calculated by adding C stored in all pools in each subplot in the nest according to the equation:

$$C_{plot} = C_{AGLB} + C_{BGB} + C_{DTB} + C_{LB} + SOC \quad (8)$$

where C_{AGLB}, C_{BGB}, C_{DTB}, C_{LB}, and *SOC* were C stored in the aboveground live biomass, belowground biomass, dead tree biomass, litter biomass, and in the soil in the subplots expressed in (Mg ha^{-1}), respectively. The amount of C stored in each types of forest stand (Mg) was calculated as follows:

$$C_{st} = \left(\frac{\sum C_{plot}}{n_{plot}}\right) * A_{st} \quad (9)$$

where C_{plot} is the total C stored in each plots expressed in (Mg ha^{-1}), n_{plot} is the number of sample plots in the stand, A_{st} is area of each stand (ha). The total C stock in the whole forest was calculated as follows:

$$C_T = \sum C_{st} \quad (10)$$

where C_T is total C stock (Mg) and C_{st} is the total C stock of each forest stand (Mg).

2.9. Estimation of equivalent CO_2 sink

Finally, as 1 Mg of soil C = 3.67 Mg of CO_2 sequestered (Craig *et al*., 2010), the equivalent CO_2 sink (Mg) in Chato forest was estimated based on the total C stock as follows:

$$CO_2e = 3.67 * C_T \quad (11)$$

Values in Gg can be obtained by dividing Mg of OC or CO_2 by 1000.

2.10. Statistical data analysis

Descriptive statistics was used to summarize mean and coefficient of variation of measured parameters. Generalized biomass models developed for moist tropical forests were used to determine carbon stock of forests. Mean separation was carried out using least significant difference (LSD) at $p < 0.05$.

III. RESULTS AND DISCUSSION

3.1. Impact of stand type and biomass component on biomass accumulation

Biomass accumulation in the forest ecosystem is usually influenced by kind of forest, type of pool, tree size class and density, species composition, forest age, and level of protection, all of which determine the C storage level of the forest. The study result shows that Chato natural forest had accumulated large volume of biomass than plantation forest for similar pools (Table 1). Total biomass accumulation, the sum of biomass stored in all components, was highest for the natural forest followed by plantations including eucalyptus species, *Cupressus lusitanica*, *Juniperus procera*, *Gravellia robusta*, and lowest for acacia species. Larger biomass in natural forest might be attributed to maturity, species diversity and good understory cover.

The study result shows that the average biomass stored (Mg ha^{-1}) in different biomass pools decreased in order AGB > BGB > LB > DTB for all types of forest stands. The quantity of biomass accumulated in the aboveground biomass pool was significantly different from other pools at ($p < 0.05$) indicating more biomass was accumulated in the aboveground pool. The mean biomass accumulated in Chato forest by biomass components ranged from 2.73 Mg ha^{-1} in the dead tree to 315.90 Mg ha^{-1} in the aboveground biomass pools. Canopy cover, basal area, and height of trees might be attributed to the larger proportion of biomass in the aboveground biomass pool. The average value of the aboveground biomass for natural forest in the present study (603.72 Mg ha^{-1}) was higher than the findings of Brown and Lugo (1982) and Abel Girma et al. (2014) who reported a range of 225 to 446 Mg ha^{-1} for the tropical rain forests in Malaysia and a mean value of 475.51 Mg ha^{-1} for woody plants of Mount Zequalla Monastery in Ethiopia, respectively. However, the present result is almost similar

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with the aboveground biomass values of 607.7 Mg ha^{-1} reported for tropical wet evergreen forest of western India (Rai 1981) and less than 994.16 Mg ha^{-1} reported for forest in the lowland area of Simien mountains national park of Ethiopia (Tibebu Yelemfrhat et al. 2014). The average aboveground biomass for plantation forest in the present study (258.34 Mg ha^{-1}) was less than the aboveground biomass of plantation forest in the humid tropics in northeast India (406.4 Mg ha^{-1}) (Ratul et al. 2009) but greater than 223.6 Mg ha^{-1} reported by Wondrade et al. (2015). Nearly 78.99% of total biomass in the natural forest was allocated in the aboveground biomass (Table 2) while the remaining pools were accumulated only 21.01% biomass. In all forest stands, the smallest biomass was recorded in the dead tree/wood compared with other pools. Low tree mortality and decomposition of deed woods might be the causes for low dead tree biomass. The aboveground shrubs and saplings biomass was highly variable with stand type than other pools as depicted by larger coefficient of variation (76.73%) (Table 1). This could be due to differences in suitability of various forest stands for the understory growth and it was more vigorous in the natural forest than plantations.

Table.1: Average biomass accumulation in the different forest stands and biomass components

	Biomass storage (Mg ha^{-1}) in different components					
Forest category	AGTB	AGHSB	BGB	DTB	LB	Total
Eucalyptus spp.	396.34	13.80	98.43	3.18	6.50	518.25
Acacia spp.	90.35	9.13	23.87	0.67	2.80	126.82
Cupressus lusitanica	353.83	6.45	86.47	3.53	4.20	454.48
Juniperus procera	233.69	8.05	58.02	3.14	3.50	306.40
Gravellia robusta	175.73	4.33	43.21	0.31	2.30	225.88
Natural forest	574.54	29.18	144.89	5.52	10.20	764.33
Mean	304.08[b]	11.82[a]	75.82[a]	2.73[a]	4.92[a]	
CV (%)	57.20	76.73	57.42	71.50	60.61	

AGTB: aboveground tree biomass; AGHSB: aboveground grasses, herbaceous, shrubs, and saplings biomass; BGB: belowground biomass; DTB: dead tree biomass; LB: litter biomass; and CV: coefficient of variation. AGB = AGTB + AGHSB. Means within rows followed by different letters are significantly different at ($p < 0.05$).

Table.2: Percent biomass allocation in different pools for various forest stands

Type of forest	Biomass allocation (%)			
	AGB	BGB	DTB	LB
Eucalyptus spp.	79.14	18.99	0.61	1.25
Acacia spp.	78.44	18.82	0.53	2.21
Cupressus lustanica	79.27	19.03	0.78	0.92
Juniperus procera	78.90	18.94	1.02	1.14
Gravellia robusta	79.71	19.13	0.14	1.02
Natural forest	78.99	18.96	0.72	1.33

Previous research indicated that matured forests do not add up significant quantity of biomass because there is no net addition to the aboveground biomass density (Ratul et al. 2009). Instead, they are important for regeneration and sustaining a large volume of an already accumulated biomass and biodiversity. Newly established plantations are; however, add significant quantities of biomass to the ecosystem. The contribution of younger forests to the total biomass varied with the rate of growth suggesting fast growing trees have an increasing biomass storage rate than slow growing ones until the time of maturity.

3.2. Carbon storage capacity of different forest stands and pools

The total C storage capacity of different stands decreased in the following order: natural forest > eucalyptus species > *Cupressus lusitanica* > *Juniperus procera* > *Gravellia robusta* > acacia species (Table 3). The mean C storage

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capacity of the natural forest in the entire pools was 453.21 Mg ha^{-1} whereas that of plantations (viz. eucalyptus species, *Cupressus lusitanica*, *Juniperus procera*, *Gravellia robusta*, *and* acacia species) was 208.08 Mg ha^{-1}. Species richness, full ceiling canopy and several layers of understory might have contributed to the larger C storage potential of the natural forest. The average C storage capacity of Chato natural forest was greater than that of tropical rain forest of Malaysia (223 Mg ha^{-1}), Indonesian forests (161 Mg ha^{-1}) and Philippines forest (258 Mg ha^{-1}) but smaller than the intact natural forests in south-eastern Australia (640 Mg ha^{-1}) reported by Brown and Lugo (1982), Murdiyarso and Wasrin (1995), Lasco et al. (2006) and Brendan et al. (2008), respectively. Combining C stored in the natural forest and plantations, the mean C storage capacity of Chato forest in the entire pools was 248.93 Mg ha^{-1}.

Table.3: Average C storage potential in the different pools by major forest stands

Forest stand	C storage capacity (Mg ha^{-1}) in different pools					Total
	AGC	BGC	DTC	LC	SOC	
Eucalyptus spp.	205.07	49.22	1.59	3.25	41.70	300.83
Acacia spp.	49.74	11.94	0.33	1.40	43.72	107.12
Cupressus lustanica	180.14	43.23	1.77	2.10	53.16	280.40
Juniperus procera	120.87	29.01	1.57	1.75	62.01	215.21
Gravellia robusta	90.03	21.61	0.16	1.15	23.89	136.83
Natural forest	301.86	72.45	2.76	5.10	71.04	453.21
Mean	157.95^{c}	37.91^{ab}	1.36^{a}	2.46^{a}	49.25^{b}	
CV (%)	57.42	57.42	71.50	60.61	33.78	

Means within rows followed by different letters are significantly different at ($p< 0.05$). AGC: aboveground carbon; BGC: belowground carbon; DTC: dead tree carbon; LC: litter carbon; and SOC: soil organic carbon.

The C storage capacity varies with type of pool. The AGC pool and SOC were significantly different from other pools and from each other at ($p< 0.05$). The DTC and LC were also significantly different from AGC and SOC but not significantly different from each other at ($p< 0.05$). The study shows that the mean C stock of the major pools in each forest stand decreased as AGC > SOC > BGC > LC > DTC; implying more C allocation in the aboveground pool (Fig. 2). Nearly 63.45% of C was stored in the aboveground pool followed by 19.79, 15.23, 0.99 and 0.55% in the soil, belowground, litter, and dead tree, respectively. This was in line with Zerihun Getu et al. (2012) report that tropical forests in their natural condition contain more aboveground C per unit area than any other land cover type. The average C stored in the aboveground pool for the natural forest was 301.86 Mg ha^{-1} while that of plantation forest was 129.17 Mg ha^{-1}. Combining the C sequestered in the natural forest and plantations, the mean aboveground C storage capacity of the Chato forest was 157.95 Mg ha^{-1}. The average aboveground C for natural forest in the present study (301.86 Mg ha^{-1}) was larger than the average C in the aboveground biomass for tropical forests in Malaysia (149 Mg ha^{-1}) but smaller than estimates in the Phillipines (406 Mg ha^{-1}) reported by Tara (2012) and Lasco et al. (2006), respectively. The study result indicated that average aboveground C in the tree plantations was better in eucalyptus species (205.07 Mg ha^{-1}) and *Cupressus lusitanica* (180.14 Mg ha^{-1}) as they are relatively older than other tree plantations. The mean belowground C for the natural forest (72.45 Mg ha^{-1}) was much higher than that tropical forest in Malaysia (27 Mg ha^{-1}) (Tara 2012). The contributions of DTC and LC to the total C pool were minor which might be due to decomposition of dead wood over time leading to loss of C.

The mean SOC storage potential to a depth of 60 cm in Chato forest was 49.25 Mg ha^{-1}, where natural forest and plantations stored averagely 71.04 Mg ha^{-1} and 44.90 Mg ha^{-1}, respectively. The average SOC for the natural forest in the present study was a little higher than SOC stock range of 58.3 to 63.9 Mg ha^{-1} reported by Solomon et al. (2002) for humid tropical forest in southeastern Ethiopia and that of plantations is at par with 44.2 Mg ha^{-1} reported by Thomas et al. (2015). Mulugeta Lemenih et al. (2005) also found SOC storage of 23.4 Mg ha^{-1} for *Cupressus lusitanica* plantation which is lower than our findings (53.16 Mg ha^{-1}). The amount of C stored in the soil was greatly affected by species richness, age, size and density of forest and the understory cover. In forests with high plant diversity, it is likely that they would have litters with different degrees of chemical resistance; creating the possibility of longer residence of C through slower decomposition of litters and build up of soil C. As C is generally a more variable

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parameter, coefficient of variability (CV) was high for most C pools investigated within different forest stands (Table 3). Relatively, C stock variation within stand type was highest in DTC pool (71.50%) and lowest in SOC pool (33.38%). This implies C in the vegetation is more variable than C in the soil.

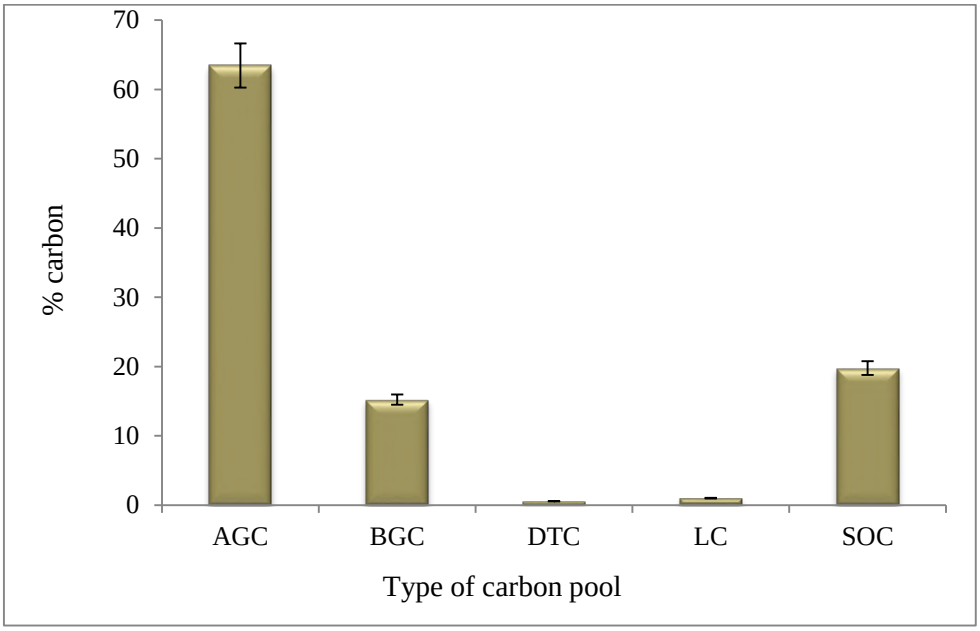

Fig. 2: Carbon partitioning/mean proportion of C stock in different pools in Chato forest

3.3. Net carbon sequestration rate

Plantation forest established in the study site some 31 years back had added nearly 175.93 Gg C to the forest ecosystem. Our result shows that the average C sequestration rate for the plantations of varying age was 8.65 Mg ha^{-1} yr^{-1}; the quantity higher than the average value of 3.98 Mg ha^{-1} yr^{-1} for mixed plantation forest in China reported by Yuanqi et al. (2015). Previous research indicated that plantation forests are a cost-effective means of sequestering C (Adams et al. 1999). Among plantations, eucalyptus species and *Cupressus lusitanica* were relatively matured and thus, stored more C than other plantations (Table 3). Young forest holds less C, but it is sequestering additional C over time. An old forest may not be capturing significant quantity of net new C but can continue to hold large volumes of C in the form of biomass over long periods of time. In line with this, Lewis et al. (2009) indicated that old natural forests may not be C neutral but continue to be C sinks and observed a slow increasing tree C storage rate of 0.49 Mg ha^{-1} yr^{-1} in African tropical old growth forests. Generally, a study by Popo-ola et al. (2012) indicated that planting new forests, rehabilitating degraded forests and enriching existing forests contribute to mitigating climate change as these actions increase the rate and quantity of C sequestration in biomass.

3.4. Climate change mitigation and economic potential of the forest

In the entire forest ecosystem, a total of 6371.30 Gg C was stored in the vegetation plus soil (Table 4).

Table.4: Total C stock and equivalent carbon-dioxide sink across different forest stands

Forest category	Total C stock (Gg)	Equivalent CO_2 sink (Gg)
Eucalyptus spp.	52.42	192.38
Acacia spp.	0.65	2.38
Cupressus lustanica	121.75	446.83
Juniperus procera	0.64	2.35
Gravellia robusta	0.47	1.72
Natural forest	6195.37	22737.00

*1 Gg = 1000 tons

Here, deforestation of each 1 hectare of natural forest and plantations would cause the loss of about 453.21 and 208.08 Mg C, respectively. Supposed deforestation of the whole Chato forest would emit 23382.65 Gg CO_2 to the atmosphere. Thus, sustainability of the forest has a clear implication to the global climate change. However, owing to protection of existing forests and expansion of plantations in the study area, there is rather a net addition of C to the forest ecosystem. Perez et al. (1997) suggested that the additional C sequestered from afforestation and reforestation could offset even the C release from deforestation.

As net gain is the main concern in climate change mitigation strategies, the jungle forests of Amazon cannot be qualified for REDD+ if there is no positive addition of C to the system. The C emitted to the atmosphere from industries and other anthropogenic activities need to be offset by removal of the C by vegetation and artificial means, if any. In our study, we recognized that the undisturbed Chato forest fulfills the key REDD+ strategic areas and would be eligible for the international C market. By continuing the current afforestation and forest management program, the forest will be a potential emission reduction center. Tree seedling plantations on bare lands around the forest need to be strengthened to add more C to the system. Community should be empowered to own the forest and protect from potential dangers. Local government authorities need to be transparent and strong enough to protect the forest from potential destruction by private firms and individuals who involve in timber and charcoal production, if any. Generally, organizations working on REDD+ projects need to be transparent enough to ensure sustainability of the Chato forest if they are really concerned with tackling global climate change through afforestation, reforestation and forest management.

IV. CONCLUSION

Forest type, density, maturity stage and status of protection affect level of biomass accumulation. We understood that the undisturbed and matured natural forest stored more biomass than plantations. Intactness and species diversity of Chato moist evergreen Afromontane forest makes it one of the largest C reservoirs in the tropics. Carbon allocation was by far larger for the aboveground pool than any other pools. Younger and fast growing plantations have better C sequestration rate than the old forest and ensures net positive C additions to the forest ecosystem. Large volume of annually trapped C by the vast channels of Chato forest makes it the most significant regulator of global climate change. Sustaining the afforestation and forest management programs will possibly ensure the viability of the forest for REDD+ projects. Lastly, more research is required to explore the untapped potential of the forest and give due attention to develop C models specific to the sacred Afromontane forests.

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Proximate analysis and *in-vitro* gas production of predominant forages in Afe Babalola University rangeland as feed resources for ruminant production

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Abstract*—In Nigeria, the major feed resource for ruminant production is the natural grazeland. However, most forage found on such lands cannot absolutely support ruminant production. Therefore, there is need to ascertain the nutritive values of predominant forages in Afe Babalola University before setting up a ruminant farm. Wet season forages: grasses, legumes, forbes and tresses were sampled and analysed for proximate composition and in-vitro gas production using standard techniques. It was found that crude protein ranged between 12.2 and 27.3% in Terminalia catappa and Leucaena leucocephala respectively. The ash content varied from 6.0-22.0% in Andropogon gayanus and Asclepias syriaca respectively. Crude fibre of the forages was between 12.5 and 28.0% in L. leucocephala and Centrosema pubescens respectively. Gas production was measured for 24hrs at 3hr intervals. At mid-fermentation, gas production ranged between 4.0 and 13.3ml/200mgDM (T. catappa and A. syriaca respectively). While at termination, it was from 9.0 - 22.67ml/200mgDM in T. catappa and A. syriaca respectively. Significant differences (P<0.05) existed among tested forages. Organic matter digestibility was from 37.7-58.54% in Tridax procumbens and A. syriaca respectively. Short chain fatty acid ranged from 0.27 - 0.6µmol in T. catappa and A. syriaca respectively. The methane gas ranged from 3.33-5.67mmol in Terminalia catappa and Calopogonium mucunoides respectively. In conclusion, most of the forages were found to be adequate for ruminant production in crude protein component. They were all noted to be very low in methane gas production which connotes energy loss in ruminant production. A good mixture of examined forages therefore, might serve as adequate feed resources for ruminant production in the area.*

Keywords*— *Forages, methane gas, crude protein, ruminants, native grassland.

I. INTRODUCTION

Low productivity of ruminant livestock is mainly hinged on poor quality forage and its unavailability in quantity. The major resources for ruminants are cereal crop residues and pastures from rangelands. Livestock graze on about 26% of the world's land area. Tchinda *et al.* (1993) reported that native pastures are the most widely available low cost feeds for ruminants in the tropics. Native rangelands offer the cheapest source of nutrients for the ruminants. It is however an accepted fact that for a greater part of the year, grasslands in the tropics do not supply sufficient nutrients to stocks for actualizing enhanced productivity. Forage digestibility is related to intake rate, and affects animal performance positively. In addition, leafy swards provide more suitable intake conditions in view of the characteristics of animal ingestive behavior (Benvenutti *et al*., 2008).

In Nigeria, ruminants slowly gain weight in the rainy season and rapidly loose it in the dry season, yet in the traditional animal husbandry, ruminants are mainly fed with grasses, so that improved livestock production is not likely attainable and sustainable by forage grasses alone (Babayemi and Bamikole, 2006). Babayemi *et al*., (2003) had earlier reported that the forages are unimproved and low in nutritive values during the wet season, while during the dry season proper, they are fibrous, lignified with low protein values and even in short supply. Lamidi *et al*. (2010) agreed with this by reporting that available forages for most part of the year are low in protein content which leads to marked decrease in voluntary intake and digestibility, and subsequently leads to substantial weight loss of the animals during this period. The success of the livestock industry anywhere in the world depends greatly on feed quantity and quality. However, the expensive nature of conventional feed as a result of competition between man

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and livestock (Ogunbosoye and Babayemi, 2010), makes this combination difficult. Afe Babalola University, Ado-Ekiti has a very large expanse of rangeland which might be suitable as feed resource for ruminant production. However, the nutritive value of predominant forages in the area is yet to be determined and documented.

II. MATERIALS AND METHOD

Description of the study area

Afe Babalola University is located in Ado Ekiti, Nigeria. Ado Ekiti is a city in southwest Nigeria, the state capital and headquarters of the Ekiti State. It is also known as Ado. The people of Ado Ekiti are mainly of the Ekiti sub-ethnic group of the Yoruba (Wikipedia, 2016). Ado-Ekiti is mainly an upland zone, rising over 250meters above sea level. Ekiti State lies between longitude 5°13'17 East of Greenwich meridian and latitude 70 37'16 North of the Equator. The weather condition of the study area is tropical climate with temperature 26ºC, humidity 74%, Rainfall 300-1100mm.

Sample collection for analysis

Predominant forages were collected at Afe Babalola University Ado Ekiti. Common forages (trees, legumes, Forbs and grasses) in the study area were collected in the wet season (June peak of rainy season). Two forages per type were harvested for the analysis (tree: *Terminalia catappa* and *Leucaena leucocephala*, legumes: *Centrosema pubescens* and *Calopogonium mucunoides*, forbs: *Tridax procumbens* and *Asclepias syriaca*, grasses: *Panicum maximum* and *Andropogon gayanus*). The fresh samples were weighed and air dried for 48 hours and oven dried to a constant weight at 105ºC. Oven dried samples were milled (2mm sieve) and kept for analysis.

Proximate analysis

Crude protein, crude fiber, ether extract and ash contents of forages were determined according to AOAC (2000). Kjeldahl procedure was used to determine the total nitrogen present in forage samples. It was effected through the breaking down of 2g sample in 25 ml concentrated H_2SO_4 acid plus selenium, using Gerhardt Kjeldahtherm until an opaque colour was obtained. The digested sample was rested for 12hours, diluted with distilled water and made up to the mark in a 250 volumetric flask. 5ml of digest was pipette and distilled with 40% NaOH solution and the ionized ammonium was trapped by boric acid. The distillate was immediately titrated (n = 3) with 0.01N hydrogen chloride. The crude protein was obtained by multiplying the nitrogen with factor: 6.25.

***In-vitro* fermentation procedures**

Preparation of the buffer and rumen liquor was carried out as described by Menke and Steingass (1988). The substrate was placed in calibrated gas tight plastic syringes fitted with a piston. The syringes were put in an incubator at 39±1^{O}C. Rumen liquor was collected from three female West African Dwarf (WAD) goats, sieved with a four layered cheese cloth and mixed with a sodium buffer (9.8g $NaHCO_3$ + 2.77g $(Na)2HPO_4$ + 0.57g KCl + 0.47gNaCl + 0.12g$MgSO_4$ $7H_2O$ + $CaCl_2$. H_2O per 1000ml) in a ratio1:2 v/v. 200mg DM of each sample with 30ml of rumen liquor and buffer were placed in each syringe and incubated in triplicate under continuous flushing with CO_2. A blank (rumen liquor + buffer) without substrate was incubated at the same time. The reading of the blank was subtracted from that of the other syringes. Gas production was recorded at 3, 6, 9, 12, 15, 18, 21 and 24h. After 24h of incubation, 4ml of NaOH (10M) was introduced into inoculums as reported by Fievez *et al*. (2005) to estimate the amount of methane produced. The value of gas produced at intervals was plotted against the using the equation Y= a + b (1-ect) (Ørskov and Mc Donald, 1979), where Y= volume of gas produced at time t, a= initial gas produced, b= gas produced from insoluble but degradable fraction, c = the rate constant for the degradation of 'b' and t= incubation time.

Statistical Analysis

Data collected were subjected to analysis of variance at p=0.05.

III. RESULTS AND DISCUSSION

Proximate composition of predominant forages in Afe Babalola University rangeland

Shown in Table 1 is the proximate composition of predominant forages in Afe Babalola University**.** The crude protein for the present study varied from 12.2-27.3% in *T. catappa* and *L. leucocephala* respectively. The ash content also varied from 6-22% in *A. gayanus* and *A. syriaca* respectively. Crude fibre of the forages was between 12.5 and 28.0% in *L. leucocephala* and *C. pubescens* respectively. The ether extract ranged from 2.0 - 9.5% (*A. gayanus* and *T. catappa* respectively*)*, while the Nitrogen free extract of the forages was observed to vary from 30.5 - 53.7% in *A. syriaca* and *A. gayanus* respectively. It was noted that there were significant differences (P< 0.05) in all the measured parameters among the forages.

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Table.1: Proximate composition (% DM) of predominant forages in Afe Babalola University

Forages	Ash	CP	CF	EE	NFE
C. pubescens	7.0^{g}	18.7^{d}	27.0^{c}	7.0^{b}	40.3^{c}
T. catappa	14.0^{d}	12.2^{f}	17.7^{e}	9.5^{a}	46.6^{b}
C. mucunoides	10.0^{e}	23.8^{b}	28.0^{b}	8.0^{b}	30.2^{e}
A. gayanus	6.0^{h}	13.7e	24.6^{d}	2.0^{f}	53.7^{a}
T. procumbens	20.0^{b}	18.9^{d}	18.5^{e}	7.5^{b}	35.1^{d}
L. leucocephala	16.0^{c}	27.3^{a}	12.5^{f}	8.0^{b}	36.2^{d}
P. maximum	8.0^{f}	13.6e	31.9^{a}	4.5^{e}	42.0^{c}
A. syriaca	22.0^{a}	20.4^{c}	18.1^{e}	9. 0^{a}	30.0^{e}
S.E.M	0.88	1.24	1.0	0.045	1.58

^{a,b,c,d,e,f}= Means on the same column with similar superscript letters are not significantly different($P< 0.05$).

Where, CP = Crude protein, CF = Crude fibre, EE = Ether extract, NFE = Nitrogen free extract. SEM = Standard error of means

Proximate composition is usually the basic and most common form of forages evaluation by animal nutritionist. There are many factors affecting proximate composition and mineral content of forages such as stage of growth maturity, species or variety (Agbagla-Dohnani *et al*., 2001; Promkot and Wanapat, 2004). The Crude protein (CP) content of *Asclepias syriaca*, *Calopogonium mucunoides* and *L. leucocephala* were higher than the other species. The CP of the forage species ranged from 12.2 to 27.3%, which is above the 7% CP requirement for ruminants which will provide ammonia required by rumen microorganisms to support optimum microbial activity. *Andropogom gayanus* had a higher CP of 13.7% compared to Odedire and Babayemi (2008) CP: 9.36% the ether extract and ash had lower values to that reported by the same authors. The ash content (8%) of *Panicum maximum* noted in this study was different and lower to the findings of Odedire and Babayemi (2008) however, the CP (13.6) was higher than that reported by the same authors.

Amata and Lebari, (2011) reported crude protein of 11.70% in *Terminalia catappa* as against 12.2% noted in the present study. The workers however reported lower level of crude fibre and ash than reported in the present study. The values obtained for proximate composition of *Calopogonium mucunoides* were lower in terms of crude protein: 23.8% and Nitrogen free extract: 30.2% but higher in ash: 9.80%, crude fibre: 28.0% and ether extract:8.0% compared to what Mecha and Adegbola (1980) reported; ash (9.80%), crude fibre (21.60%), ether extract(3.10%) crude protein (24.08) and Nitrogen free extract (41.42).

The nutrient composition of *Centrosema pubescens* as obtained in this present study differs from that of Nworgu and Egbunike, 2013 who obtained 9.14% (ash) which is considered higher than the present study ash: 7.0%, crude protein 23.4% was also found higher than crude protein 18.7% of the present study. However, lower values for crude fibre: 8.80 and ether extract: 3.32% compared to that of the present study: crude fibre: 17.7% and ether extract: 9.5% were observed.

Generally, the variation that existed between the present study and the past works on the forages considered may be traced to time and seasons of harvest, age of plant, leaf to petiole ratio, ecological location and edaphic (soil) (Makkar and Beacker, 1997; Babayemi and Bamikole, 2006).

***In-vitro* gas production of forages collected from the rangeland in Afe Babalola University.**

Presented in Figure 1 is the *in-vitro* gas production of forages from the rangeland in Afe Babalola University. At the onset of fermentation, gas production ranged from 0.00ml/200mg DM in C. *mucunoides, P. maximum, T. procumbens, C. pubescens, A. gayanus and T. catappa* to 2.33ml/200mg DM in L. *leucocephala*. However at mid fermentation, it ranged from 4.00ml/200mg DM (*T. catappa*) to 13.33ml/200mg DM (*A. syriaca*), while at termination, the gas production ranged from 9.00ml/200mg DM to 22.67ml/200mg DM in *T. catappa* and *A. syriaca* respectively. Gas production varied significantly ($P< 0.05$) in the forages from the 3rd hour to termination at 24th hour in the present study.

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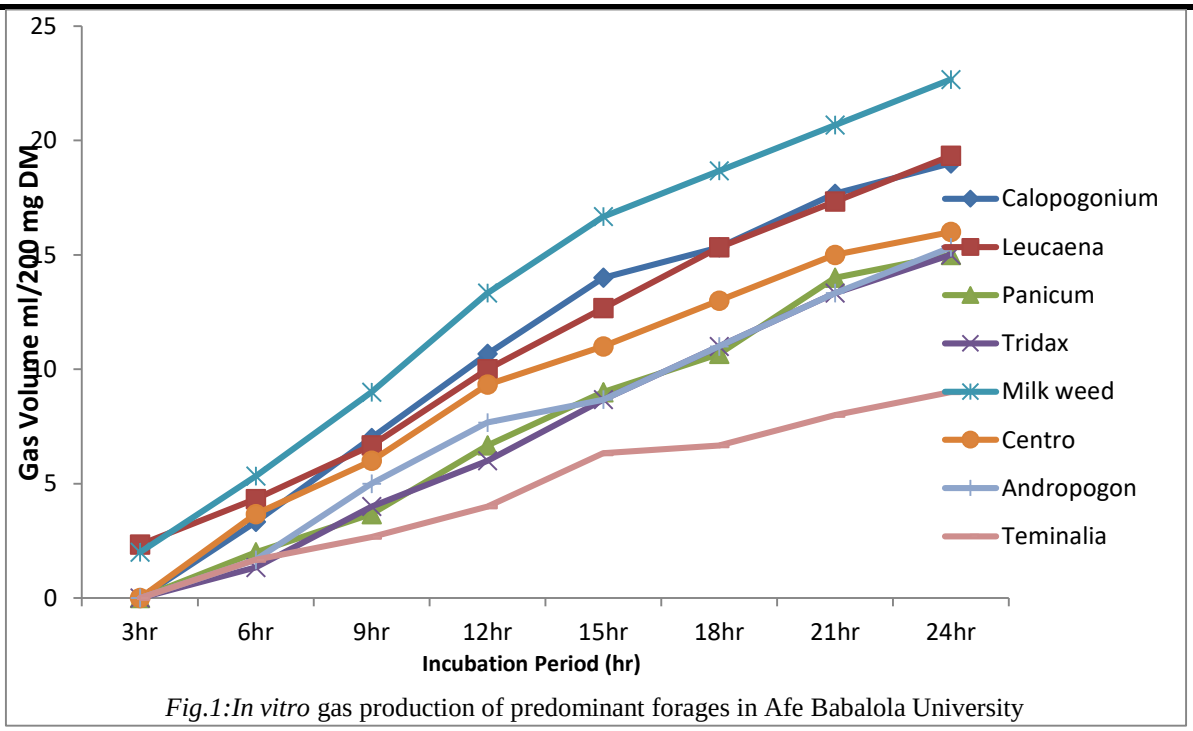

Fig.1:In vitro gas production of predominant forages in Afe Babalola University

Key

Terminalia catappa (Terminalia) *Leucaena* leucocephala (Leucaena), Centrosema *pubescens* (Centro), *Calopogonium mucunoides* (Calopogonium), *Tridax procumbens* (Tridax) *Asclepias syriaca* (milk weed), *Panicum maximum* (panicum) and *Andropogon gayanus* (Andropogon).

Presented in Table 2 is the Metabolisable energy MJ/Kg DM, organic matter digestibility (%) and short chain fatty acid (µmol) of predominant forages in Afe Babalola University. ME is an indication of energy and it ranged between 5.00 and 6.50 MJ/Kg DM (*Tridax procumbens* and *Asclepias syriaca* respectively). It was found to be significantly different among forages. Organic matter digestibility ranged from 37.7-58.54% in *Tridax procumbens* and *Asclepias syriaca* respectively with significant difference among forages. Short chain fatty acid which is an indication of energy made available to the host animal ranged from 0.27-0.60 µmol (*Terminalia catappa* and *Asclepias syriaca* respectively).

Table.2: Metabolisable energy (MJ/Kg DM), organic matter digestibility (%) and short chain fatty acid (µmol) of predominant forages in Afe Babalola University

Forages	ME	OMD	SCFA
C. pubescens	5.51^{bc}	50.63^{b}	0.44^{bc}
C. mucunoides	5.92^{b}	44.72^{c}	0.52^{b}
L. leucocephala	5.57^{bc}	46.67^{c}	0.52^{b}
A. syriaca	6.50^{a}	58.54^{a}	060^{a}
P. maximum	5.68^{b}	45.44^{c}	0.42^{c}
T. procumbens	5.00^{d}	37.70^{d}	0.40^{c}
A. gayanus	5.15^{cd}	39.85^{d}	0.43^{c}
T. catappa	5.02^{d}	45.48^{c}	0.27^{d}
SEM	0.07	2.90	0.0021

^{a,b,c}= Means on the same column with similar superscript letters are not significantly different($P< 0.05$).

Where ME – Methabolisable energy, OMD – Organic matter digestibility, SCFA – Short chain fatty acid, SEM = Standard error of means

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Norton (2003) justifies the use of forages in small quantities in order to supplement poor quality pastures and crop residues. It has been suggested that the gas production technique is more reliable than the nylon bag method for determining nutritive value of feeds containing anti-nutritive factors (Khazaal *et al.,* 1993). Nature and fibre levels, presence of anti-nutrition factor had been reported to influence the amount of gas produced during fermentation (Babayemi 2004). High level of crude fibre reduce digestibility which is synonymous to *in-vitro* gas production. In-vitro technique is a more reliable tool for evaluating ruminant forages. Though the two methods are independent of each other, they are interrelated (Babayemi *et al.,* 2004; Fievez *et al., 2*005).

Gas production is associated with volatile fatty acid production following fermentation of substrate (Blummel and Ørskov 1993). In addition, the application of models permits the fermentation kinetics of the soluble and readily degradable fraction of the feeds, and more slowly degradable fraction to be described (Gatechew *et al*., 1998). Moreover the gas production parameters of trees might demonstrate differences in their nutritional value that may be closely related to their chemical composition (Cerrillo and Juarez 2004). The inconsistency observed in the gas production is as a result of the different rate of different anti nutritional content as well as the forage degradability. *In-vitro* estimations of feed degradation are important tools for ruminant nutritionists. These methods measure either substrate disappearance or fermentation products (Blümmel *et al* 1997). In the present study, forages with high CP produced higher gas volume. Digestibility has been reported to be synonymous to *in vitro* gas production (Fievez *et al.,* 2005) that is, forages with high gas production will exhibit better digestibility.

In-vitro gas production characteristics of predominant forages in Afe Babalola University rangeland

Presented in Table 3 is the *in-vitro* gas production characteristic of predominant forages in Afe Babalola University. It was observed that 'a' which is the initial gas produced ranged between 1.67 and 3.67 ml in *T. procumbens, A. gayanus, T. catappa* and *C. pubescens* respectively. Significant differences existed among the considered forages (P< 0.05). The potential gas production from insoluble but degradable fraction 'b' varied from 7.33-20.65 ml *T. catappa* and *Asclepias syriaca* respectively, while the rate of potential gas production (a+b) ranged from 9.00-22.67 ml in *T. catappa* and *Asclepias syriaca* respectively. Rate at which gas is produced 'c' ranged from 0.032-0.059(ml/h) in *Andropogom gayanus* and *Calopogonium mucunoides* respectively with no significant differences among the forages.

Table.3: In-vitro gas production characteristic of predominant forages in Afe Babalola University rangeland

Forages	a	a+b	b	c	T	Y
C. pubescens	3.67^{a}	16.00^{b}	12.33^{d}	0.051	12.00^{ab}	9.33^{ab}
C. mucunoides	3.33^{ab}	19.00^{b}	15.67^{bc}	0.059	12.00^{ab}	11.00^{a}
L. leucocephala	2.33^{ab}	19.00^{b}	17.00^{b}	0.052	13.00^{ab}	11.33^{a}
A. syriaca	2.00^{ab}	22.67^{a}	20.67^{a}	0.043	9.00^{b}	9.67^{ab}
P. maximum	2.00^{ab}	15.00^{c}	13.00^{cd}	0.041	12.00^{ab}	7.33^{ab}
T. procumbens	1.67^{b}	14.33^{c}	12.33^{d}	0.037	11.00^{ab}	6.33^{b}
A. gayanus	1.67^{b}	15.33^{c}	13.67^{cd}	0.032	10.00^{b}	6.00^{b}
T. catappa	1.67^{b}	9.00^{d}	7.33^{e}	0.054	14.00a	5.67^{b}
SEM	0.96	3. 67	3. 00	0. 0022	4.88	4.88

^{a,b,c,d}= Means on the same column with similar subscript letters are not significantly different(P< 0.05).

a- initial gas produced/intercept, b- insoluble but degradable fraction, a + b- potential extent of gas production, c-rate at which gas is produced, Y- gas volume, SEM = Standard error of means.

The intake of a feed is mostly explained by the rate of gas production (*c*) which affects the passage rate of feed through the rumen, whereas the potential gas production (*a +b*), is associated with degradability of feed (Khazaal *et al*., 1995). Therefore the higher values obtained for the potential gas production in the *Calopogonium mucunoides, Leucaena leucocephala* and *Asclepias spp* might indicate a better nutrient availability for rumen microorganisms.

Methane gas (mmol) produced at 24 h of incubating predominant forages in Afe Babalola University

Presented in Figure 2 is the methane gas (mmol) produced from incubating predominant forages in Afe Babalola University. The methane gas ranged from 3.33-5.67mmol in *Terminalia catappa* and *Calopogonium mucunoides* respectively.

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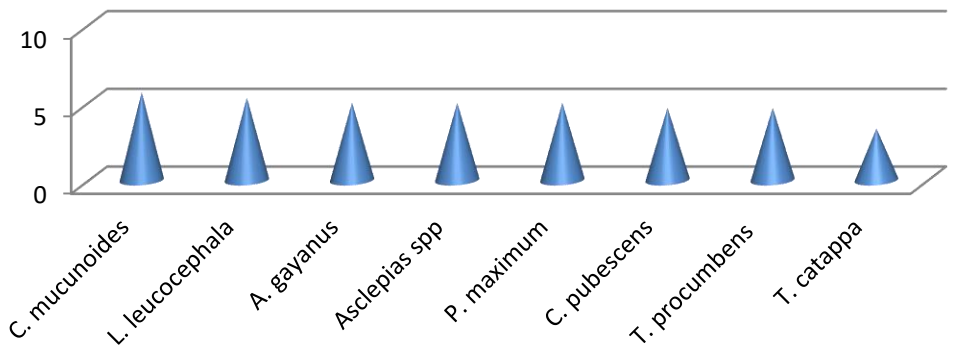

Fig.2: Methane gas (mmol) produced at 24 h of incubating forages

Methane production represent a significant energy loss to ruminants; it also contributes to global warming which is a worrisome phenomenon in the recent time and many tropical feedstuff have been indicated to increase methanogenesis (Babayemi *et al.*, 2004; Babayemi and Bamikole, 2006).

IV. CONCLUSION

The results revealed that *In-vitro* gas production techniques can be used to assess the nutritive value of forages. It unveiled the fact that most of the forages seem to be adequate in crude protein but deficient in ash content and fibre. Methane gas production was generally low and this is an indication that energy loss will be reduced and the forages will be environmentally friendly when fed to ruminants.

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Study of Intake, Growth and Nutrient Utilization of Growing Bulls Fed Forages as Sole Diets

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***Abstract*—** *The study was conducted to rank Napier, jumbo, maize and rice straw on the basis of their yield, production cost, nutritional value and productivity of native growing bulls. Thirty native bulls (Bos indicus) of 135 (±28) kg live weight (LW) were randomly allocated to five treatments in a completely randomized design and fed silage of maize (Zea mays; Hybrid, PG-1000), jumbo (Sorghum bicolor; Hybrid Sugar graze), Napier (Pennisetum perpureum; hybrid) and urea molasses straw of whole straw (UMS-WS) and UMS of stover (UMS-S) for a period of 90 days. The dry matter (DM) intake of Napier, jumbo, maize, UMS-WS and UMS-S was 2.08, 1.79, 2.01, 1.92 and 2.08 % LW, respectively which differed significantly (P<0.01). The DM digestibility of UMS-WS or UMS-S (45.49 and 44.37 %) was significantly (p<0.01) lower than that of Napier, jumbo and maize (50.22, 53.01 and 58.75 %, respectively). The LW gain was greater (p<0.01) in bulls fed maize silage (273.3 g/d) followed by Napier silage (81.4 g/d), UMS-S (75.3 g/d), jumbo silage (39.9 g/d) and UMS-WS (39.6 g/d). Considering the cost of beef production, maize may be ranked on the top followed by Napier, jumbo, UMS-S and UMS-WS, respectively which may be taken in profitable beef production system.*

Keywords*— *Feed efficiency, jumbo, maize, Napier, UMS.

I. INTRODUCTION

The efficiency of a fodder to animal production performance is important as about 55 to 75 % of the total costs of farming are associated with feed costs (1, 2 and 3). Feed evaluation systems are used to match the dietary nutrient supply with animal requirements for a specific level of production (4). These systems are important in order to optimize the efficiency of feed utilization, to improve animal performance and to reduce nutrient losses to the environment (4). Thus, the efforts aimed at improving the efficiency of feeding forage will have a large impact on reducing input costs associated with beef production.

Livestock is recognized as an integral component of rice based agricultural production system in Bangladesh and make multifaceted contributions to the growth and development in the agricultural sectors. Cattle fattening or beef enterprise is an important avenue for income generation for subsistence farmers as well as entrepreneurs. The shortage of feeds and fodder both in terms of biomass availability and nutritional quality are major concern to the producers and also considered a major constraint to animal productivity (5). An average 56.2% deficit of roughage DM and 80.0% of concentrate DM results in a very poor plane of nutrition for farm animals in the country (6). Any effort that i) explores quality feeds and fodders ii) generate production technologies for making their biomass available using agro-ecosystem sustainably and economically, and iii) value addition technologies for production and marketing of cost effective premixed feeds using available biomass may boost milk and meat production in the country. This requires qualitative evaluation of available roughages, and development of comparative nutritional weights of different roughages fed to ruminant animals. Moreover, scale of ranking available roughages (Napier, jumbo, maize and rice straw) based on their yield, production cost, nutritional value and productivity in the country is not developed yet. Such scale may help farmers feeding their animals cost effectively. Thus, the objectives of this study are to determine the effect of feeding different types of available straws and green fodders on the nutrition and growth performances of local bulls.

II. MATERIALS AND METHODS

2.1 Fodder cultivation

The seeds of jumbo grass (*Sorghum bicolor*; Hybrid Sugar graze) and maize (*Zea mays*; PG-1000; hybrid) were procured from BRAC Adventa Company, Dhaka,

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Bangladesh and, Progreen Seed Company, Hyderabad, India from their local authorized sources. Napier (*Pennisetum perpureum*; hybrid), jumbo and maize were grown under the recommended and identical agronomical management condition at Fodder Research Plot, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh.

2.2 Biomass production and cost of production

The annual fresh biomass yield per hectare land of Napier, Jumbo and Maize were determined under identical agronomic management condition. Napier was cultivated once and the number of harvest per year was considered 5 times. Similarly, there was a single cultivation of Jumbo and considered 3 harvest in a year. However, maize was cultivated separately 3 times in a year while calculating annual biomass yield.

The analysis of cost of cultivation of fodders included various components of costs. Here, only variable cost components such as cost of seed per cutting, land preparation, sowing cost, fertilizer, irrigation harvesting, silage preparation etc. were considered. The fixed cost such as rental value of land, depreciation of implements, interest on fixed capital, land revenue etc. are ignored.

2.3 Silage making

After harvesting, fodder was chopped into 6-8 cm using a chaf cutter machine and then ensiled in earthen pit. The silos were filled rapidly and compacted properly by hammering to remove air for maintaining a good anaerobic condition. Each pit was covered with 2 inches thick layer of rice straw, followed by covering with a plastic sheet. The plastic sheet was then plastered with mud to avoid any cracking. The silage was kept into the pit for 30 days.

2.4 Preparation of urea molasses straw (UMS)

Straws were procured from local sources and they were of two different types: one was the whole straw containing bottom and the top portion (WS) and the other was with only the bottom portion (stover). Both the straws were used for producing UMS (UMS-WS and UMS-S) according to the method described by Huque and Chowdhury (7).

2.5 Experimental design, animals and diets

Thirty local growing bulls (*Bos indicus;* Pabna & Red Chittagong Cattle) of 135 (±28) kg live weight were randomly allocated to five dietary treatments in a completely randomized design, having six animals in each treatment. The diets of the five treatment groups were maize, jumbo and Napier silage, and UMS-WS and UMS-S, respectively. At the onset of feeding trial, animals were dewormed according to the recommended doses of Endex ® (Levamesol BP 600 mg per bolus) at a rate of 20 mg per kg live weight. The animals were housed individually and fed the roughage diets *ad libitum* for a period of 90 days including a 7 days digestibility trial after 60 days of feeding. No supplementation was provided during the whole feeding trial. Fresh and clean water was made available in the sheds for the whole experimental period. The live weight gain (LWG) of bulls was calculated by measuring the live weight (LW) every ten days interval at 7 am in fasting condition during the whole experimental period.

2.6 Digestibility trial

The diets of bulls were supplied by morning (9 am) and evening (4 pm) meals by dividing the total amount into two equal amounts. The amount of daily feed supply and refusals found in each bull was recorded properly. Fresh samples of feed and refusals were analyzed in the laboratory to determine the daily dry matter (DM) intake of bulls. After 60 days of feeding, experimental bulls were transferred into metabolic stall, where faeces were collected separately for seven days. Records were kept on amount of feed offered, residue left and faeces excreted. During the collection period, composite samples of feed residue and faeces of individual bull were stored at -20 ^{0}C for further laboratory analysis.

2.7 Chemical analysis:

The samples of feeds, residue left and faeces were analyzed for DM, organic matter (OM) and crude protein (CP) following the method of AOAC (8). The acid detergent fibre (ADF) and neutral detergent fibre (NDF) was determined according to van Soest *et al* (9). Dietary metabolizable energy (ME) concentration was estimated from the digestible organic matter (DOM) intake as DOM kg x 15.58 = Mj ME (10)

2.8 Statistical analysis

The response to dietary treatments on intake, digestibility, nutritional quality and growth rate were compared statistically in an ANOVA of a Completely Randomized Design (CRD) using General Linier Model Procedures of SPSS, 11.1 for Windows (11) computer software packages.

III. RESULTS AND DISCUSSION

3.1 Chemical composition of experimental diets

Chemical composition of the roughages is shown in Table 1. Among the five different roughages the highest DM content was found in UMS-WS (67.65 %) followed by UMS-S, Napier, jumbo and maize silage (64.92, 22.95, 21.41 and 15.63, respectively) and the values differed significantly ($P<0.05$) except Napier and jumbo silage. In case of OM content, the highest values were found in maize and Napier silage (90.96 and 89.54 %, respectively) which varied significantly ($P<0.01$) with the values of jumbo silage, UMS-WS and UMS-S (86.48, 87.75 and 85.66 %, respectively). Maize silage (CP 9.65%) and UMS-WS (CP 8.75 %) had higher ($P<0.05$)

level of CP compared to others (varied from 8.08% to 8.57%). The ADF content of UMS-WS and UMS-S was similar (47.42 and 47.53 %, respectively) and differed significantly (P<0.01) with Napier, jumbo and maize silage (65.09, 69.05 and 56.31 %, respectively). Similarly, the NDF contents of UMS-WS and UMS-S did not differ, but significantly (P<0.01) less than the values of Napier, jumbo and maize silage (87.19, 75.56 and 75.39 %, respectively. The results with lower levels of CP in Napier (12) and Jumbo silage and higher levels of CP in maize silage is agreement with statements of Harris et al., (13) and Adewakun, et al. (14). Harris et al. (13) and Adewakun, et al. (14) also reported that Jumbo silage (Sorghum) had more structural polysaccharide than in Maize silage.

Table.1: Chemical composition of experimental diets (g/100 g DM)

Nutrients (% DM)	Experimental diets					SED	P-values
	Napier silage	Jumbo silage	Maize silage	UMS-WS	UMS-S		
DM (% fresh)	22.95^{d}	21.41^{d}	15.63^{a}	67.65^{b}	64.92^{c}	0.48	<0.01
OM	89.54^{a}	86.48^{bc}	90.96^{a}	87.75^{b}	85.66^{c}	0.30	<0.01
CP	8.08^{b}	8.53^{b}	9.65^{ac}	8.75^{bc}	8.57^{b}	0.18	<0.05
ADF	65.09^{a}	69.50^{b}	56.31^{c}	47.42^{d}	47.53^{d}	0.75	<0.01
NDF	87.19^{a}	75.56^{b}	75.39^{b}	65.81^{c}	67.29^{c}	0.66	<0.01

Means within the same row bearing different superscripts differ significantly; P>0.05, not significant

3.2 Nutrient intake

Nutritional responses of different roughages are presented in Table 2. The daily DM intake of Napier silage, maize silage and UMS-S was 2.68, 2.70 and 2.77 kg, respectively, or 2.08, 2.01 and 2.08 % LW, respectively. The daily DM intakes of jumbo and UMS-WS were 2.25 and 2.52 kg, or 1.79 and 1.92 % LW, respectively. The former three roughages had significantly (P<0.01) higher intake than that of the later two roughage. A similar trend in CP intake was also found among the roughages. The OM and CP intake were significantly (P<0.01) higher in bulls fed maize silage than bulls those fed other diets. Among the dietary groups jumbo silage fed group consumed significantly (P<0.01) lower OM and CP content. Bulls fed UMS-WS and UMS-S diets consumed significantly (P<0.01) lower ADF then bulls those fed other three diets. The intake of both ADF and NDF were significantly higher in bulls fed Napier silage diet. Keady and Gordon (15) reported that relative to grass silage as the sole forage, feeding maize silage as the sole forage increased (P<0.001) forage intake by 31 %. Similarly, Keady et al. (16) reported that relative to good quality grass silage as the sole forage, inclusion of average quality maize silage (28 % DM and 23 % starch) at 40% of the forage component of the diet (on a DM basis), increased (p<0.05) forage DM intake by 14%. Significantly higher DM intake in continental crossbred steers (424 kg LW) fed whole crop maize silage (9.54 kg DM/d) was also observed by Walsh et al. (17) compared to steers those offered grass silage only (7.41 kg DM/d).

Table.2: Nutritional responses of different roughages fed experimental animals

Parameters	Experimental diets					SED	P-values
	Napier silage	Jumbo silage	Maize silage	UMS-WS	UMS-S		
DM intake (kg/d)	2.68^{ac}	2.25^{b}	2.70^{ac}	2.52^{a}	2.77^{c}	0.05	<0.01
DM intake (% LW)	2.08^{ad}	1.79^{c}	2.01^{bd}	1.92^{bc}	2.08^{d}	0.03	<0.01
OM intake (kg/d)	2.35^{ac}	1.97^{b}	2.46^{a}	2.23^{c}	2.37^{ac}	0.04	<0.01
CP intake (kg/d)	0.25^{b}	0.22^{c}	0.28^{a}	0.23^{c}	0.25^{b}	0.004	<0.01
ADF intake (kg/d)	1.87^{b}	1.77^{b}	1.47^{a}	1.12^{c}	1.22^{c}	0.03	<0.01
NDF intake (kg/d)	2.37^{a}	1.71^{b}	2.06^{c}	1.63^{b}	1.93^{c}	0.03	<0.01

Means within the same row bearing different superscripts differ significantly; P>0.05, not significant

3.3 Nutrient digestibility

The apparent digestibility of different nutrients is presented in Table 3. The DM digestibility of UMS-WS or UMS-S was significantly (P<0.01) lower than that of the three fodders. Maize had the highest DM or CP digestibility (58.8 or 61.4 %), and they were significantly (P<0.01) higher than that of Napier or jumbo. The ADF digestibility of UMS-WS or UMS-S was significantly

(p<0.01) lower (55.83 and 39.41 %, respectively) than bulls those fed other three fodders. However, Jumbo had the highest ADF digestibility (81.43 %), and they were significantly (p<0.01) higher than that of Napier and Maize (76.85 and 66.56 %, respectively). Similar to ADF digestibility, UMS-WS or UMS-S had the lowest NDF digestibility (56.48 and 59.66 %, respectively) than that of three fodders. However, the NDF digestibility of Napier, Jumbo and Maize did not differ significantly (P>0.05).

The digestible DM, OM, CP and NDF intake (DMI, OMI, CPI, and NDFI) was higher (p<0.01) in bulls fed Maize silage than bulls those fed other roughages. Similarly, Maize had the highest intake of metabolizable energy (ME) or digestible CP (10.0 MJ/d and 168 g/d) and it differed significantly (P<0.01) with that of Napier (8.38 MJ/d and 142 g/d) and Jumbo ((8.42 MJ/d and 105 g/d) or with that of UMS-WS (7.48 MJ/d and 126 g/d) and UMS-S (7.65 MJ/d and 126.0 g/d). Balwani et al. (18) reported that DM, OM and CP digestibility of maize silage was significantly (P<0.05) higher than sorghum silages; the values for DM, OM and CP digestibility of maize and forage type sorghum were 68 vs 55; 69 vs 56; and 56 vs 55%, respectively. Garrett and Worker (19) found that sorghum silage were not conducive to higher quality feed. Similar conclusions were made by Owen et al. (20) and Meyer et al. (21).

Table.3: Apparent digestibility of nutrients by growing native bulls fed different roughages

Digestibility of nutrients	Experimental diets					SED	P-values
	Napier silage	Jumbo silage	Maize silage	UMS-WS	UMS-S		
DM	50.22^{ad}	53.01^{d}	58.75^{c}	45.49^{b}	44.37^{b}	0.86	<0.01
OM	52.56^{a}	63.87^{b}	61.72^{b}	50.17^{ac}	48.25^{c}	0.77	<0.01
CP	55.70^{c}	47.79^{a}	61.43^{b}	55.15^{c}	50.98^{d}	0.73	<0.01
ADF	76.85^{a}	81.43^{b}	66.56^{c}	55.83^{d}	39.41^{e}	0.93	<0.01
NDF	61.06^{a}	62.42^{a}	63.71^{a}	56.48^{b}	59.66^{ba}	0.82	<0.01
Digestible DMI (kg/d)	1.37^{a}	1.21^{cd}	1.58^{b}	1.15^{cd}	1.23^{ad}	0.03	<0.01
Digestible OMI (kg/d)	1.26^{c}	1.26^{c}	1.51^{a}	1.12^{b}	1.15^{bc}	0.03	<0.01
Digestible CPI (g/d)	142^{c}	105^{b}	168^{a}	126^{d}	126^{d}±	2.78	<0.01
Digestible NDFI (kg/d	1.46^{a}	1.08^{d}	1.30^{b}	0.92^{c}	1.15^{d}	0.03	<0.01
Digestible ADFI (kg/d	1.44^{b}	1.44^{b}	0.99^{a}	0.63^{c}	0.49^{d}±	0.02	<0.01
ME intake (MJ/kg DM)	8.38^{b}	8.42^{b}	10.05^{a}	7.48^{c}	7.65^{bc}	0.18	<0.01
MP intake (g/d)	45.60^{b}	45.82^{b}	54.68^{a}	40.71^{c}	41.62^{bc}	1.07	<0.01

Means within the same row bearing different superscripts differ significantly; P>0.05, not significant

3.4 Live weight gain and FCR

The LW gain of bulls fed different forage is presented in Table 4. Feeding maize silage had the highest daily gain of 273.3 g (P<0.01) compared to 81.4 g in Napier, 75.3 g in UMS-S, and 39.9 or 39.6 g in jumbo or UMS-WS diet. Except maize, the LW gains of other diets did not vary significantly (P>0.05). It had an average feed conversion efficiency of 9.87 followed by 32.9 of Napier, 36.8 of UMS-S, 56.4 of jumbo, and 63.6 of UMS-WS, and the differences among the diets varied significantly (P<0.01). Therefore, considering the beef production performances, maize may be ranked on the top of all, followed by Napier, UMS-S, jumbo and UMS-WS based on their coefficient of nutritional response to growth of 1.0, 0.30, 0.28, 0.15 and 0.14, respectively.

The higher DM, CP and ME intake and greater digestibility of DM, OM, and CP could be the reasons for exhibiting higher growth rate and better FCR of bulls fed maize silage than bulls those fed other roughages. Keady and Gordon, (15) in their study reported that feeding maize silage alone increased carcass gain by 31% than bulls those fed other grass silage. Keady et al. (16) also reported that relative to good quality grass silage as the sole forage inclusion of average quality maize silage (28% DM and 23% starch) at 40% of the forage component of the diet (on a DM basis), increased carcass gain by 17%. Keady et al. (16) and Walsh et al. (17)

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concluded that the FCR of the animals affected by the diet; animals those fed maize silage only had more efficient in utilizing energy than animals fed grass silage only. Walsh et al. (17) also reported that steers fed maize silage had a significantly better feed conversion efficiency compared to steers fed grass silage only (12.4kg DMI/kg carcass gain vs. 16 kg DMI/kg carcass gain) and maize silage had significantly higher LWG (1.200 compared to 0.802 kg/day), compared to steers fed grass silage only. Heifers fed maize silage alone had a significantly higher DMI than heifers fed grass silage only, 9.5 compared to 7.8 kg/day (22). Aston and Tayler (23) reported that at least an extra 2 kg of concentrates were required to enable cattle on grass silage to achieve comparable rates of LW gain to those on maize silage.

Table.4: Growth responses and FCR of growing native bulls fed different roughages

Parameters	Experimental diets					SED	P-values
	Napier silage	Jumbo silage	Maize silage	UMS-WS	UMS-S		
Initial LW (Kg)	133.9	135.1	134.8	134.7	135.8	8.05	>0.05
Final LW (Kg)	141.2	138.7	159.4	138.3	142.6	8.52	>0.05
Daily gain (g)	81.4^{b}	39.9^{b}	273.3^{a}	39.6^{b}	75.3^{b}	18.5	<0.01
FCR	32.92^{a}	56.35^{b}	9.87^{c}	63.62^{d}	36.78^{e}	0.76	<0.01

Means within the same row bearing different superscripts differ significantly (p<0.01); not significant, P>0.05

3.5 Biomass yield and the coat of production

The biomass yield and production cost of different fodders and silages are presented in Table. 5. The annual fresh biomass yield per hectare land of Napier, Jumbo and Maize were 150, 80 and 105 metric tons, respectively. It shows that the average cost of cultivation (total variable cost) per hectare per year required for Napier, jumbo and maize were 74905, 66545 and 122135 taka, respectively. The production cost per kg fresh and silages of Napier, jumbo and maize were 0.50, 0.83 and 1.16 Taka and 0.67, 1.09 and 1.36 Taka, respectively. The present findings agreed with Jabbari et al. (2011) who reported that the production cost of maize per unit land was higher than production cost of jumbo fodder. The higher cultivation cost of maize is due to use higher amount of seeds, fertilizer and increased cost for separate land preparation. The production cost of Kg.DM UMSs is shown in Table 6. The production cost including price of straw, molasses, urea and processing cost for UMS-WS and UMS-S were 9.98 and 8.98 taka, respectively. The production cost of UMS-WS was relatively higher than cost of UMS-S.

Table.5: Annual biomass yield and production cost of fodders and silages (Taka/ha)

Inputs	Napier	Jumbo	Maize
Seed/cutting	667	8,000	30,000
Land preparation	5,190	7,400	22,200
Sowing cost	4,167	500	2,000
Fertilizer	18,882	20,645	37,935
Irrigation	16,000	12,000	12,000
Harvesting	30,000	18,000	18,000
Silage preparation (pit, polyethylene, filling, chopping)	25,233	20,800	20,800
Total production cost (fresh, Taka/year/ha)	74,905	66,545	122,135
Total cost (silage, Taka/year)	1,00,138	87,345	1,42,935
Biomass production (Mt/year)	150	80	105
Production cost (fresh, Taka/kg)	0.50	0.83	1.16
Production cost (silage, Taka/kg)	0.67	1.09	1.36

Table.6: Production and preparation cost (Taka/Kg DM) of UMSs*

Inputs	*Production cost (Taka)	
	UMS-WS	UMS-S
Straw	6.00	5.00
Straw processing	1.00	1.00
Molasses	2.50	2.50

Urea	0.48	0.48
Total (Tk/kg)	9.98	8.98

**Market price, 2013*

3.6 Cost of feeding

The cost involvement of LW gain of bulls fed different roughage diets is presented in Table 7. It shows that the cost of per kg DM intake required for Napier, jumbo, aize, UMS-WS and UMS-S were 2.92, 5.10, 8.72, 9.98 and 8.98 taka, respectively. However, the total roughage cost of per kg LW gain required 103.6, 301.2, 87.8, 646.8 and 338.2 taka, respectively for Napier, Jumbo, Maize, UMS-WS and UMS-S diets. Considering diet, refusal, management cost and time or days required for LWG, the maize fed animals required less feed cost (Taka 114.2) for Kg LW gain followed by Napier (Taka134.7), Jumbo (Taka 391.5), UMS-S (Taka 439.6) and UMS-WS (Taka 840.9). Considering the cost of beef production, less cost is involved in maize feeding, followed by Napier, jumbo and UMSs, respectively. The present findings are in agreement with Keady and Gordon (15) who reported that feeding maize silage as the sole forage reduced feed costs by 37 penny/kg carcass gain ($P<0.001$) than bulls those fed other grass silage. Keady et al. (16) reported that relative to good quality grass silage as the sole forage, inclusion of average quality maize silage (28% DM and 23% starch) at 40% of the forage component of the diet (on a DM basis), reduced ($p<0.05$) feed costs by 25 penny/kg carcass gain.

Table.7: Costs (Taka) involvement in LW gain of bulls fed different roughage diets

Parameters	Silage/ UMS				
	Napier	Jumbo	Maize	UMS-WS	UMS-S
FCR	32.92	56.35	9.87	63.62	36.78
Cost (Taka/KgDM)	2.92	5.1	8.72	9.98	8.98
Refusal	0.23	0.24	0.15	0.19	0.21
Increase of cost considering refusal (Taka)	3.15	5.34	8.87	10.17	9.19
Cost of roughage diet (Taka)	103.6	301.2	87.8	646.8	338.2
Time (days for one Kg LWG)	12.3	25.0	3.7	25.0	13.3
Cost management	31.1	90.4	26.3	194.0	101.5
Cost per kg LW gain (Taka)	134.7	391.5	114.2	840.9	439.6

IV. CONCLUSIONS

It may be concluded that, considering beef production performances maize may be ranked on top, followed by Napier, UMS-S, jumbo and UMS-WS based on their coefficient of nutritional response to growth of 1.0, 0.30, 0.27, 0.18 and 0.16, respectively. On the other hand, considering the cost of beef production, the top fodder maize may be followed by Napier, jumbo, UMS-S and UMS-WS, respectively. Farmers may use this roughage scale in formulating cost effective diets for making more profit of cattle production.

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Immobilization of two endoglucanases from different sources

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***Abstract*—** *Cellulases are a important family of hydrolytic enzymes which catalyze the bond of cellulose and other related cello-oligosaccharide derivates. Industrial applications require enzymes highly stable and economically viable in terms of reusability. These costs can be reduced by immobilizing the cellulases, offering a potential solution through enzyme recycling and easy recovery. The covalent immobilization of enzymes is reported here: one is commercial cellulase from Aspergillus niger and other one is recombinant enzyme, named CelStrep it because was isolated from a new cellulolytic strain, Streptomyces sp. G12,. The optimal pH for binding is 4.6 for both cellulases and the optimal enzyme concentrations are 1 mg/mL and 5 mg/mL respectively. The support for immobilization is a poliacrylic matrix. Experiments carried out in this work show positive results of enzyme immobilization in terms of efficiency and stability and confirm the economic and biotechnical advantages of enzyme immobilization for a wide range of industrial applications*.

Keywords* — *Aspergillus. niger, endoglucanases, immobilization, Streptomyces.

I. INTRODUCTION

Cellulases (E.C. 3.2.1.-) are enzymes that hydrolyze β-1,4 glycosidic bonds in cellulose. Namely, they can be distinguished in endoglucanases (E.C. 3.2.1.4), exoglucanases (E.C.3.2.1.91) and β-glucosidases (E.C.3.2.1.21). They are mainly used in various industries including pulp and paper, textile, laundry, food and feed industry, brewing, and agriculture [1]. They are important also for the production of biofuel, for which there is an important demand to have a more sustainable life style worldwide. However, biomass refineries are not spread out in the world because the cost of cellulase highly affects the production of cellulosic-derived ethanol. To overcome this problem many researches are dedicated to both find new cellulases and also produce immobilized enzymes to make more inexpensive biofuel production. Cellulases are enzymes synthesized by a variety of microorganisms: bacteria and fungi (yeasts and molds) during their growth on cellulosic materials [2][3]. From the structure point of view, all of them contain a catalytic domain (Glycoside Hydrolase, GH) that can/may be linked through a simple sequence to a Carbohydrate Binding Domain (CBD) o Module (CBM). The catalytic domain is recognized as a Concanavalin-like domain that is common in both bacterial and fungal cellulases, while the CBD is present mostly in bacterial cellulases. Some bacteria possess "cellulosomes", cellulase complexes with both type of enzymes (with and without the CBD). In this work we have used two different endoglucanases. The first one is the commercial cellulase from the fungus *Aspergillus niger (A. niger)*, that possesses only the Concanavalin-like domain, while the second one is a recombinant protein isolated from a new cellulolytic bacterial strain, identified as belonging to Streptomyces sp. G12, isolated from mature compost obtained from agro-industrial wastes and named CelStrep by the authors [4]. Thus, the aim of this work was to immobilize both *A. niger* and Cel Strep cellulases and examine the activity of immobilized enzymes. In fact, enzyme immobilization provides the advantage of both re-use and increased enzyme stability and, consequently, reducing costs of specific industrial process (E. Cherian et al., 2015), i.d. biofuel production. The used covalent immobilization method recurs to Immobeads, namely COV-2 (150P), that have a polyacrylic matrix bringing an high amount of epoxide groups which bind the thiol, amine and hydroxyl groups of enzymes [5]. Furthermore we have studied the stability of the immobilized enzymes over the time.

II. MATERIALS AND METHODS

Materials

The supports used for immobilization process were Immobeads IB-COV-2-150P, (ChiralVision B.V., The Netherlands). Two different Cellulases (EC 3.2.1.4 - 1,4-β-endoglucanase) were used : the first from *Aspergillus niger* (*A. niger*, purchased by Sigma Aldrich – USA), and the second, a recombinant enzyme named CelStrep (Amore et al. 2012). *A. niger* cellulase activity was determined by using a kit purchased from Megazyme

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(Megazyme International Ireland) using AZO-CM-cellulose. CelStrep activity was determined according to Ghose (1987)[6] using 3.5-dinitrosalicylic acid (DNSA) purchased from Merck. Sodium acetate trihydrate, zinc acetate and other reagents were purchased from Sigma Aldrich (USA).

Methods

Immobilization procedure

The cellulases were covalently linked to epoxy groups on the beads (IB-COV-2-150P). These were equilibrated with sodium acetate buffer (0.1 M pH 6.2). 400 µL of 1 mg/mL enzyme solution from *A. niger* and 400 µL of 5 mg/mL enzyme solution from Celstrep were added to 100 mg of beads, in 2 mL vials and suspended in sodium acetate buffer (0.1 M, pH 4.6).

The beads-enzyme mixture was shacked for 3 h at 4 °C on the rotary mixer to allow the reaction. After that, the vials were let stand overnight at 4°C in order to promote the natural decantation of the beads.

Then the supernatants were removed from the sedimented beads and used to determine enzyme activity. Finally beads were washed on the rotary mixer four times with 1.5 mL sodium acetate buffer (0.1 M pH 4.6): first wash was performed for 1 min; second and third washes for 15 min; fourth wash for 5 min. The amount of immobilized and active enzyme (U_S) was determined by subtracting enzyme activity resolved in supernatants and washes (U_f) from the total enzymatic activity before immobilization (U_0).

Endoglucanase Assay using Megazyme kit

The enzymatic activity of free or immobilized endoglucanase from *A. niger* was measured using AZO-CM-Cellulose as a substrate according to the supplier instructions (Megazyme).

Endoglucanase Assay using DNSA

The enzymatic activity of free or immobilized endoglucanase Celstrep was measured using the 3.5-dinitrosalicylic acid based method (DNSA) [6].

Determination of protein concentration

Protein determination was carried out by the Bio-Rad Protein Assay (Bio-Rad), using bovine serum albumin as standard [7].

Calculation of the parameters of immobilization

The calculation of the parameters of immobilization and immobilized protein were calculated as follows according to R.A. Sheldon et al. [8].

Immobilized Protein Yield (IPY)(also defined as Protein Loading) - percentage of immobilized protein based on difference of proteins subjected to immobilization (P_0) and the proteins remaining in residual liquid after immobilization (P_f), divided by P_0 according to eq. 1.

$$IPY\ (\%) = [(P_0 - P_f)/P_0] \times 100 \quad (eq.\ 1)$$

Enzyme Immobilization Yield (IY) - percentage of immobilized enzyme based on the difference of enzyme subjected to immobilization (U_0) and that one remained in the liquid after the immobilization (U_f) divided by enzyme subjected to immobilization U_0 according to the eq. 2.

$$IY\ (\%) = [(U_0 - U_f)/U_0] \times 100 \quad (eq.\ 2)$$

Activity Recovery (AR) – percentage of immobilized enzyme in the support (Us) divided by U_0 according to the eq 3. AR represents the percentage of active immobilized enzyme over the total enzyme subjected to immobilization.

$$AR\ (\%) = [U_S / U_0] \times 100 \quad (eq.3)$$

Lost Activity (LA) - percentage of the lost enzymes in the total immobilization process, due to the difference of U_0 and the sum of U_S and U_f , according to the eq. 4.

$$LA\ (\%) = [(U_0 - (Usupport + U_f))/U_0] \times 100 \quad (eq.\ 4)$$

Efficiency (E) also named Recovered Activity (RA) - percentage of immobilized enzyme in the support (U_S) divided by the difference between U_0 and U_f, according to the eq. 5.

$$E\ (\%) = [(U_S)/(U_0 - U_f)] \times 100 \quad (eq.\ 5)$$

The efficiency can be considered more precise immobilization yield than IY since the first is based on the enzyme activity measured in the support (immobilized enzymes). There is a loss of activity in the immobilized enzyme, or not all enzymes remain active in the support.

Stability of immobilized enzyme

The stability of immobilized enzyme was monitored over the time, from time 0 (immobilization day) until 45 days after immobilization, using Efficiency parameter as equal to 100% of cellulase activity at time 0. The immobilized enzymes were stored at 4°C.

Analysis of protein sequences

Information on the sequences and domain composition of *A. niger* and of Streptomyces cellulase has been taken from UniProt database (codes A2R322_ASPNC and I7L8N7_9ATCN, respectively) [9].

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III. RESULTS AND DISCUSSION

In this study we have covalently immobilized two different cellulases , the first one from the mold A. niger commercialized from Sigma while, the second is a recombinant form previously cloned from a the cellulolytic strain *Streptomyces* sp. G12 isolated from compost [4]. The immobilization procedure has been realized using the same protocol reported in Fig. 1. The support for immobilizazion are beads made of a polyacrilic matrix with epoxide groups that promote covalent binding to amino, thiol or hydroxyl groups of the enzyme. In Table 1 are reported immobilization parameters obtained with the two different enzymes. Immobilization Yield , calculated as reported in M&M, is approximately the same for both enzimes, reaching 67% for A. niger and 55% for CellStrep. This parameter is calculated measuring U0 and Uf, without taking into account whether the enzyme molecules that have been bound to the support are all active. The last information is given from the Activity Recovery which is calculated as a ratio between the enzyme bound to the support (US) and the Enzyme subjected to immobilization (U_0). This parameter is equal to 53 % for *A.niger* and to 13 % for CellStrep. Conversely the Lost Activity is 14 % for the first cellulase and 42 % for the second one. Efficiency, which represents the percentage of the funtional immobilized enzyme over the total (funcional and not funcional) immobilized enzyme is 78 % for A.niger and 23% for CelStrep, while the Immobilization Protein Yield (also know as Protein loading) is equal to 42 % for A. niger and 70% for CelStrep. These results could be related to both molecular weight and sequence of the two different enzymes. In fact, fungal cellulase is smaller (26 kDa) than the bacterial one (which is 37 kDa). In fact, *A. niger* cellulase contains only the Concavalin-like Domain, while the Carbohydrate Binding Domain is missing. The Concanvalin-like Domain contains 113 residues out of 223 that possesses amino, thiol and hydroxyl groups, the ones classified as polar amino acids that are able to bing the epoxide groups of the Immo-beads 150P which represent 50.64% of the entire amino acid sequence (Table 2). On the contrary, the CelStrep contains both Cocanavali-like Domain and carbohydrate Binding Domain that are made of 228 and and 103 amino respectively, among which polar residues represent 45.13 and 39.78 % (Table 2). Thus, the latter enzyme exhibits an higher protein loading but a lower efficiency because most probably more residues are involved in the covalent binding to the support influencing enzyme functionality. In fact , it has been reported that high epoxide group content (tipycal of the Immo-beads 150P) provides quick binding but also multi-point attachment that is detrimental for some enzymes [5]. On the contrary for *A. niger* , even if the IPL is lower, the covalent binding occurs involving residues without effecting the terziary structure and thus the enzymatic activity. Similar results have been described in studies devoted to assess the immobilizazion efficiency in regard to the protein size [5]. In terms of stability, both immobilized endocellulases retained 100% activity after 45 days compared to the free enzymes (Fig. 2). In fact, A. niger lost 20% of its activity, while CelStrep exhibited only 60% of activity compared to the immobilized enzyme. The higher stability over the time of the free *A. niger* cellulase is not suprising since it is well know that fungal enzymes are usually more stable than bacterial ones [10].

IV. CONCLUSIONS

This study compares the activity of immobilized endocellulases of different origin. While *A. niger* endocellulase has been already immobilized by Huang et al 2014 [11], CelStrep was immobilized for the first time. The paper by Huang et al (2014)[11] refers of *A. niger* immobilized by recurring to magnetic nanoparticles that allow a re-use of the enzyme during idrolization of rice straw to produce ethanol. The process was judged efficient even if the immobilized enzyme only retained 80% of activity compared to the free one. CelStrep is a bacterial endocellulase isolated from a cellulolitic strain present in agro-industrial waste-derived compost by Amore et al 2012 [4] with the aim of selecting effective enzyme to treat cellulosic materials. Both endocellulases were immobilized on a polar polyacrilic matrix, previously used successfully in our laboratories to immobilize aspartic protease from *Carduus defloratus* [12], and retained the same activity compared to the free counterparts. Moreover, both enzymes exhibit a good stability over the time while, between the two different free enzymes, the *A.niger* cellulase retains 80% activity after 45 days, although CelStrep was 60% active in respect to the activity at time 0.

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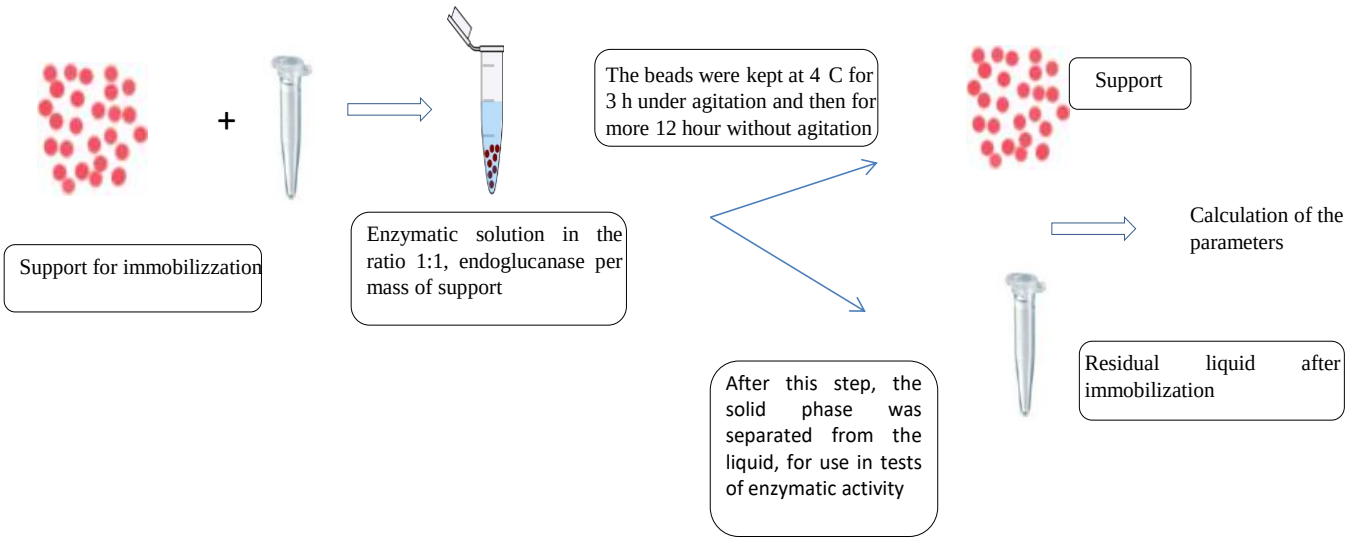

Fig.1: Flowchart of the enzyme immobilization process

Table.1: Parameters of immobilized cellulases

	IY (%)	AR (%)	LA (%)	E (%)	IPY (%)
Aspergillus niger	67.01 ± 3.78	53.57 ± 4.23	14.28 ± 1.89	78 ± 6.22	42.57 ± 5.07

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CelStrep	55.44 ± 3.63	13.04 ± 5.11	42.41 ± 1.45	23.23 ± 7.69	70.41 ± 5.76

Table.2: Percentage of polar amino acids (aa) in each domain of the studied endocellulases

	ConA-like Domain	**CB Domain**	**Total number of aa**
Aspergillus niger	223 aa	-	223 aa
% of polar aa	50.64	-	50.64
CelStrep	228 aa	103aa	331 aa
% of polar aa	45.13	39.78	43.5

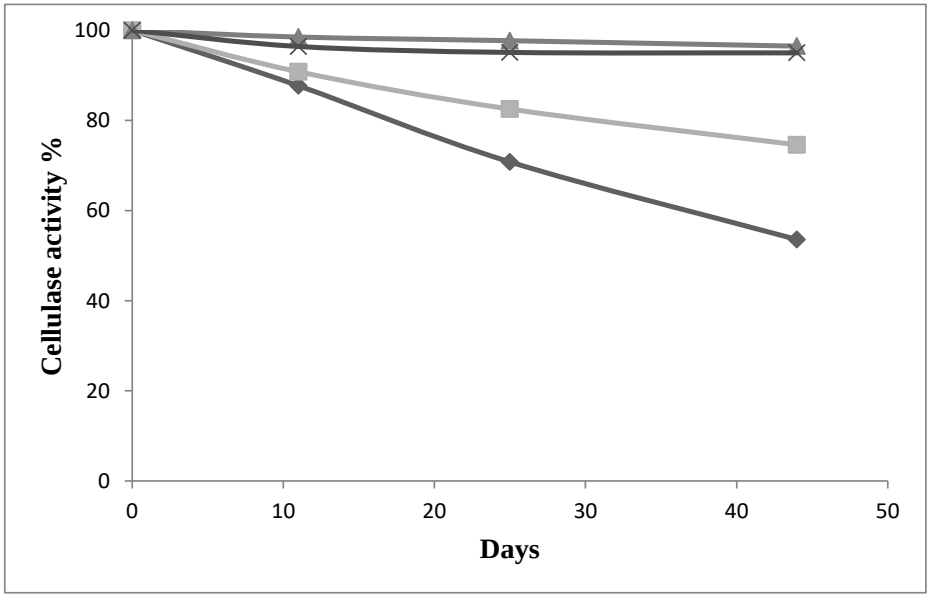

Fig.2: Stability of immobilized cellulases from Aspergillus niger (×) and Celstrep (▲) and of free enzymes, Celstrep (♦) and A.niger (■) during storage (at 4°C)

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Rapid and sensitive methods for detection of *Allorhizobium vitis*, causal agent of grapevine crown gall

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Abstract— *A rapid method and sensitive methods for extraction of bacterial DNA from pure culture and directly from plant materiel were compared in polymerase chain reaction with specific primers VCF3/VCR3 to see the reliable method that can used in the detection of tumorigenic strain of Allorhizobium vitis causal agent of grapevine crown gall. From the three tested methods of DNA extraction from pure culture, the alkaline method is the most effective technique for the extraction presenting a high sensitivity with a detection threshold equal to 5.10^4 CFU/ml. Five different protocols for extracting bacterial DNA from plant tissues of infected tomato, based on the use of an extraction buffer, were tested to see its usefulness in detecting pathogenic strain of A. vitisS4. Two protocols based on the use of Triton X-100 and Tween 20 were efficient for detecting A. vitis S4 directly from tomato tumors with a sensitivity of 10^3 CFU/ml for the both protocols. Consequently, these protocols were proposed as specific protocols for the detection of tumorigenic strain of A. vitis from symptomatic and asymptomatic plants.*

Keywords— Crown gall, grapevine, Allorhizobium vitis, DNA extraction, detection, detection threshold.

I. INTRODUCTION

Crown gall of grapevine caused by *Allorizobium vitis* (Moussavi et al., 2014, 2015), previously named *Agrobacterium vitis* (Ophel and Kerr 1990);is an economically important disease and one of the most serious bacterial disease affecting grape production in several countries (Burr and Otten, 1999). The tumorigenicity of *A. vitis* is an encogenes transfer into plant cells of the T-DNA (transferred DNA), part of a large tumor-inducing (Ti) plasmid (Thomashow et al. 1984).The T-DNA integrated into the plant cell genome, contains gens responsible for the biosynthesis of hormones (auxins and cytokines) leading to gall development and genes coded for biosynthesis of opines used as specific carbon and nitrogen sources for *A. vitis* development (Lacroix and Citovsky, 2013). The development of galls obstruct vascular tissue and restrict movement of water and nutrients into the vine above the gall, which affect grapevine growth and yield (Schroth et al., 1988).

A. vitis is adapted to survive in the plants tissues without causing tumors until conditions becomes favorable for gall development. Therefore, the disease spreads out by asymptomatic propagating materials (Kuzmanovic et al., 2014) which is necessary to develop a reliable detection methods of the pathogen in both symptomatic and asymptomatic plant materials and in the soil to efficiently prevent the disease (Bini et al., 2008). Generally, the detection methods of *A. vitis* are based on their isolation on the semiselective culture media and pathogenicity tests but these methods can take many weeks for results (Johnson et al., 2013, Shams et al., 2012). Polymerase chain reaction (PCR) it's the most reliable technique improve sensitivity, specificity and rapidity for the detection of *bacteria* which targets gens that is found only in *A. vitis* by the use of specific primers(Johnson et al., 2013; Shams et al., 2012, 2013).

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Several PCR protocols have been developed and successfully used for detection of tumorigenic strains of *A. vitis* directly from plant hostswith the use of primers, which target gens localized on Ti plasmid,*vir* or T-DNAregions (Bini et al., 2008). Generally,the methods of extraction of bacterial DNA from plant tissues are based to the use of a DNA purification Kit specific to *A. vitis* and characterized to inhibit the action of plant polyphenols that inhibit the PCR reaction (Bini et al., 2008). This method is very expansive and need several steps for purification of the DNA; for this reason, many study work was conducted to develop others techniques of DNA extraction directly from infected plants (Llop et al., 1999, Szegedi and Bottka, 2002).

The main purpose of this study was to compare the extraction procedures of DNA from pure culture for efficient routine detection of *A. vitis* and to evaluate and optimize protocols of detection of *A. vitis* used Bio-PCR and PCR reaction directly for infected plant with the study of threshold of detection of the pathogen.

II. MATERIAL AND METHODS

Bacterial strain and culture conditions

The bacterial strain used in this study is *A. vitis* strain S4 (sequenced strain) isolated from black raspberry in Hungary (Popoff et al., 1984). *A. vitis* S4 was cultivated on MG medium (Moore et al., 2001)(D-mannitol, 5g/L; L-glutamic acid, 2g/L; KH_2PO_4, 0.5g/L; NaCl, 0.2g/L; $MgSO_4 \times 7H_2O$, 0.2g/L; Yeast extract, 0.5g/L; Agar, 15g/L; pH=7) and incubated, for 24 hours, at 28°C.

Pathogenicity and hypersensibility tests

The pathogenicity of strains *A. vitis* S4 was studied by inoculating-plants of tomato (*Solanum lycopersicum* L.). The inoculation was made by 10 µl of suspension (10^7CFU/mL) of 24 hours bacterial culture in stem internodes of tomato 2-3 weeks after transplanting. Inoculated plants were maintained in greenhouse at 27°C during 3-4 weeks.

The hypersensibility reaction was determined on tobacco (*Nicotiana tabacum* cv. Xanthi). 200 µl of bacterial suspensions (10^7 CFU/ml) were infiltrated ontobacco leaf by a needleless syringe. Sterile water was used as a negative control. The tobacco plants were kept in a growth greenhouse. Development of necrosis was scored over a period of 4 to 5 days.

DNA extraction from pure culture

Three extraction procedures were used to obtain DNA for Bio-PCR analysis:

The first protocol was based to use alkaline method (Shams et al., 2013). From bacteria grown overnight at 28°C in MG medium, one colony of *A. vitis* S4 was mixed with 10µl of NaOH (20 mM) and incubated at 37°C for 5 minutes. The *A. vitis* lyses cells were stored at 4°C until they use.

The second protocol was based on the lysis of the bacterial cells by heating a bacterial suspension of 10^8CFU/ml at 100 °C for 15 min. the lyses cells were stored at 4°C until they use (Hannou et al., 2013).

The third protocol is based on the lysis of the cells by thermal shock by heating the bacterial suspension for 15 min at 100 °C then cooling in ice for 5 min and centrifuging at 10000 g for 1 min. The resulting supernatant was used for PCR (Pastrick and Rainay, 1999; Ameur et al., 2014).

Bio PCR-pTi

Specific primers VCF3 (GGCGGGCGYGCYGAAAGRAARACYT) and VCR3 (AAGAACGYGGNATGTTGCATCTYAC) were used to identify pathogenic strains of *A. vitis*by the detection of plasmids (pTi); they amplify a DNA fragment of 414bp of the *virC1* and *virC2* genes (Kawaguchi, 2009). Standard PCR was carried out in a 60µl reaction volume containing 38.6µL H_2O, 6µl (2mM) DNTPs, 1.2µl (2mM) $MgCl_2$, 3µl DMSO (Dimethyl sulfoxide), 1µl (10 µM) of each primer, 0.2µl Taq DNA polymerase (Invitrogen, France) and 3µl of lyses cells from each extraction protocols were tested. In order to test the possibility to detect *A. vitis* without carring extraction of the DNA, two others methods were tested; the first was to add a small colony directlyin master mix using toothpick; and the second method was to add 3µl of a bacterial suspension of (10^8 CFU/ml) into the mix. In all five protocols were tested to detect *A. vitis*.

The PCR was performed using the following program: initial denaturation at 95°C for 5min, followed by 35 cycles of denaturation at 95°C for 1min, annealing at 57°C for 1min and extension at 72°C for 1min, followed by an additional extension at 72°C for 3min. Electrophoresis was performed in 1.5 % agarose gel. The gel was soaked with ethidium bromide. Fragments were visualized with an ultraviolet (UV) transilluminator, and the gel was photographed.

Bacterial DNA extraction from tomato tumors

Five protocols were tested to obtain DNA from tomato tumors for PCR analysis. The samples used in this test was made from the tumors obtained during the production of symptoms on tomato plants. Firstly, the tumors were washed in running water and tumor fragment surface were disinfected with 70 % ethanol. The necrotic tissues were removed; and tumor was cut in small fragment. The pieces of tumor were ground in 2µl of sterile distilled water using a mortar and pestle to isolate the pathogen from plant tissues. After incubation during 30min, the macerates were filtered using sterile filter paper.

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The first protocol used in this study to extract the DNA from the macerates was been described by Bereswill et al. (1992). The macerates were shaken for two hours in a 0.9% NaCl solution. The supernatant was centrifuged during 10min at 10000g, the pellet was resuspended in 0.1ml of sterile water, and 10µl was used for PCR-pTi analysis.

The second protocol was described in the study work of Llop et al. (1999). 500µl of macerates were placed into an Eppendorf and centrifuged at 10000g for 10min. The pellet was resuspended in 500µl extraction buffer (200 mM Tris HCL pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS, 2% PVP), vortexed and left for 1 hour at room temperature with continuous shaking. Then it was centrifuged at 5000g for 5min, 450 µl of the supernatant were taken 450µl isopropanol added, mixed and left for 1 hour at room temperature. The mixture was centrifuged and the pellet dried under vacuum. Finally, it was responded in 100 µl water.

For the third protocol, we use the modified extraction technic by Taylor et al. (2001). 500µl of macerates were placed into an Eppendorf and centrifuged at 10000g for 10min. the pellet was suspended in 500 µl of plant extraction buffer (140 mM NCL, 50 mM KCl, 0.05% Tween 20, 2% PVP and 0.4% BSA). The mixture was left at room temperature during 15min and stored in 4°C until use.

The fourth technic tested in this study was described in the study work of Szegedi and Bottka (2002). 1ml of the macerate was placed in Eppendorf and centrifuged at 18000g in 4°C for 15 min. the pellet was suspended in 100µl of sterile water in mixture with Triton X-100 (1%) (V:V) and heated at 95°C for 10 min.

The last protocol tested in this study was the same protocol described previously but with some modifications. The Triton X-100 solution was replaced with Tween 20 (0.1%). This protocol is based on the work of Bini et al. (2008).

PCR analysis

For all extraction protocols from tumors, PCR analysis was made using specific primers of *VircC1* and *VirC2* gens (VCF3 and VCR3). The reactional mix was prepared separately for each protocol (Table 1). PCR program was performed with the same condition as before.

Determination of the detection threshold of *A. vitis*

In order to evaluate the sensitivity of detection protocols of *A. vitis* by Bio-PCR, two bacterial suspension of 10^9 CFU/ml were prepared, one in sterile water and the other in lysis solution of NaOH (20 mM). From the two bacterial suspensions, serial dilutions were made to a concentration of 10^2CFU/ml. The detection threshold of *A. vitis* was also made for the macerates obtained from the previous tests. The PCR was performed using specific primers VCF3 and VCF3 and according to the same condition as before.

III. RESULTS & DISCUSSION

Pathogenicity and hypersensibility tests

A. vitis strain S4 was tested for the ability to induce hypersensitive response (HR) on tobacco and tumors in tomato. The tested strain is capable to cause HR in leaf of tobacco after 2 days of inoculation (Figure 1A) and able to induce tumor on stems of inoculated tomato within 20 days of incubation in greenhouse (Figure 1B). The capacity of this strain to induce gall development in tomato is related to the presence of pTi plasmid, which is an important element for the pathogenicity and tumorigenicity of *A. vitis* strains (Shams et al., 2012). The necrosis in leaf of tobacco results in rapid cell death and it is a part of the plant defense response against pathogens (Rodriguez-Palenzuela et al., 1991, Heath, 1998). The HR induced in leaf of tobacco it resembles a disease reaction in grape tissues infected with *A. vitis* in natural conditions. It has been demonstrated that several plant pathogenic bacteria that cause necrosis on host plant are able to induce HR to another plants (Alfano and Collmer, 1996).The ability of *A. vitis* to induce HRis due to the capacity of this strain to produce specific enzyme, Plygalacturonase (PG) (Rodriguez-Palenzuela et al., 1991). Other research have demonstrated that when the concentration of *A. vitis*is greater than approximately 10^6 CFU/ml, the *A. vitis* was able to cause necrosis (Herlache, 1999).

Bio PCR-pTi

The effectiveness of three protocols of the DNA extraction and two others methods without the extraction of the DNA, used for detection of *A. vitis* S4 from pure culture, was evaluated by molecular test with the primers VCF3/VCR3(Figure 2). The results show that a 414 bp fragment of *virC1-virC2* gene (Sawada and Tsuchiya, 2003) was amplified with all the tested protocols of DNA extraction and without extraction using only a colony or a bacterial suspension; therefore, these protocols can be used for the different molecular tests to characterize and detect *A. vitis* strains. Moreover, the use of *virC* primers are able to detect pathogenic strains of *A. vitis* possessing pTi plasmid (Kawaguchi, 2009; Kumagai and Fabritius, 2008); and were used in several study work to identify tumorigenic strain of *A. vitis* in several countries(Kuzmanovic and al., 2012; Lamovšek et al., 2014).

Detection of *A. vitis* S4 from tomato tumors

The detection of *A. vitis* directly from infected plant represents an important technique for the diagnostic of the disease. In this work, five different protocols were used to extract the DNA from tumors produced in tomato plants.

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Moreover, to compare these protocols the macerate, the enriched macerate with the PBS and pure colonies were used as a DNA template. The obtained results show the presence an expected size amplicon (414 bp) which amplified using the protocols 3 (Taylor et al., 2001), 4 (Szegedi and Bottka, 2002) and 5 (Bini et al. 2008) of extraction of DNA from plant tissues and also from enriched macerate (Figure 3). However, we cannot detect *A. vitis* with use of the protocol1(Bereswill et al., 1992) and 2 (Llop et al., 1999) of DNA extraction from infected tomato and also the direct detection from pure macerate.

The three proctolos validated for the detection of *A. vitis* directly from plant tissues are based on the use of a buffer of extraction (Tween 20, the polyvinylpyrrolidone and bovine serum albumin) or only the detergent (Triton X-100 and Tween 20). These compounds can eliminate the inhibitors of PCR and also can cause lysis of bacterial cells. Plant compounds and in particularly the polyphenols and polysaccharides (De Boer et al., 1995) may limit the amplification of the bacterial DNA fragment extracted from plant material. Therefore, in the presence of these compounds, several authors suggest that the DNA should be purified using 2-mercaptoethanol or polyvinylpyrrolidone or by the use of commercial kits (Bereswill et al., 1992; Eastwell et al., 1995; Cubero et al., 1999; Taylor et al., 2001).

In the research work of Eastwell et al. (1995) they found that the bacterial lysis cells *in situ* in grapevine followed by DNA purification was more effective to detect *A. vitis* than then analysis of bacteria suspended in water. This observation may be due to the fixation of the bacteria to the cell walls of the grapevine cell. Moreover, in the study work of Kaufmann et al. (1996) they showed that the use of immunocapture culture of plant extract improve the sensibility and reliability of the method.

Determination of the detection threshold of *A. vitis*

For a large application of PCR for the detection of *A. vitis*, it is necessary to develop a rapid and simple protocol for the extraction of DNA for amplification and in the same time, the protocol must be sensitive for the detection of bacteria on low concentrations. For this reason, an evaluation test of the detection threshold of each protocol (from pure culture and macerate) was mad to know the minimum concentration detectable by various protocols applied to the *A. vitis* strain. Different concentrations of DNA ranging from 10^9 CFU/ml to 10^2 CFU/ml were test for the evolution of the detection threshold.

The determination of detection threshold by Bio-PCR of different protocols of DNA extraction indicate that the three protocols of lysis cell from pure culture show different results. For the alkaline method of DNA extraction, the detection threshold was determinate in a concentration equal to 5.10^4 CFU/ml (figure 4A). For the protocol of heating bacterial suspension, the detection threshold correspond to the concentration equal to 5.10^6 CFU/ml (Figure 4B). Moreover, for the third protocol of thermal shock the detection threshold correspond to the concentration 10^5 CFU/ml (Figure 4C). From the three tested protocols, the alkaline method was the more sensitive method for detection of *A. vitis* from pure culture. The alkalinity and high temperature cause the lysis of well cells and therefore the liberation of the DNA in the solution. The sensitivity of alkaline protocol can be explained by the additional NaOH property of the hydrogen bond perturbation between the DNA base pairs, which denatures genomic and plasmid DNA and allow their amplification. However the alkaline method is only used for the pure culture and isolated bacterial cells and cannot be used for the detection of *A. vitis* from plant material. In the study work of Burr et al. (1999) and Cubero et al. (1999) the detection threshold was determinate for 150 to 200 cells. Szegedi and Bottka (2002) were demonstrated that the detection threshold is equal to 10^5 CFU/ml using the protocol of heating bacterial suspension in sterile distillated water and in Triton X-100. The Triton X-100 solution was more effective than sterile distillated water.

For the determination of detection threshold, theprotocol 4 and 5 used for the extraction of DNA of *A. vitis* from plant material and based on the use of Triton X-100 and Tween 20 were selected due to the simplicity and rapidity of these techniques. Therefore, it is necessary to determinate their sensitivity in order to identify their detection threshold. The sensitivity evaluation test of each protocol was carried using a decimal dilution of the macerate solution. The obtained results of specific PCR-pTi show that tested protocols are able to detect bacteria with concentration equal to 10^3 CFU/ml (Figure 5). These techniques can identify *A. vitis* with a low concentration from macerate that contain different bacterial species.

IV. CONCLUSION

In conclusion, results obtained in the present work show that use of alkaline method for the extraction of DNA from pure culture are the reliable and sensitive method can be used in several study for molecular characterization and detection of *A. vitis* isolates. Moreover, the use of direct techniques to detect *A. vitis* from plant materials by specific PCR are important issues that can be used for the detection of the pathogens from symptomatic and asymptomatic grapevines.

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Table.1: Reactional mix preparation for the different detection protocols of A. vitis directly from plant tissues

Protocol	Volume	Reactional mix
1	50	- 24.5 ultrapure water containing: BSA, 160µg/ml, (NH4)2SO4, 16 mM and 2-mercaptoethanol, 10 mM. - 10 µl of DNA extract - 10 µl PCR buffer×10 (Bioline) - 2.5 µl dimethyl sulfoxide (DMSO) - 1µl of each primer - 0.5 µl Tween 20 (100%) - 0.5 µl Taq polymerase (5 U)
2	50 µl	- 29.76 µl ultrapure water - 10 µl PCR buffer×10 (Bioline) - 5 µl of DNA extract - 2 µl Formamid - 1.32 µl of each primer - 0.6 µl Taq polymetrase
3, 4 and 5	20 µl	- 9.8 µl ultrapure water - 4 µl PCR buffer×10 (Bioline) - 5 µl of macerate - 0.5 µl of each primer - 0.2 µl Taq polymerase

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From colony of *A. vitis* S4	20 µl	- 13.8 µl ultrapure water - 4 µl PCR buffer×10 (Bioline) - 1samll colony - 0.5 µl from each primer - 0.2 µl Taq polymerase

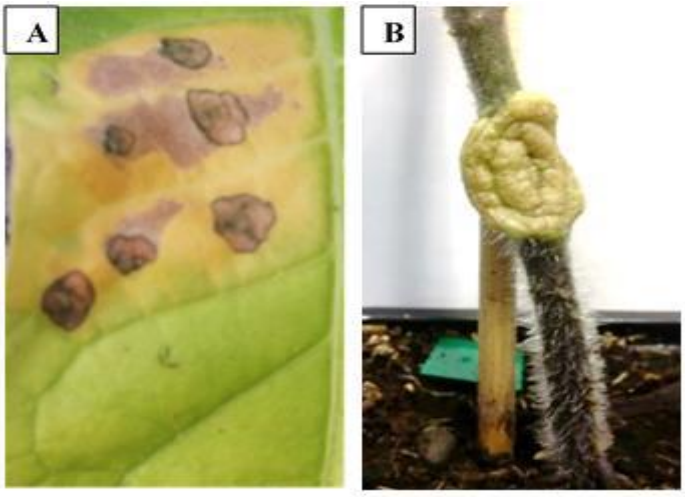

Fig.1*: Hypersensibilty and pathogenicity tests of A. vitis S4.* ***A****: hypersensitive response on tobacco leaf,* ***B****: development of tumor in the stem of tomato*

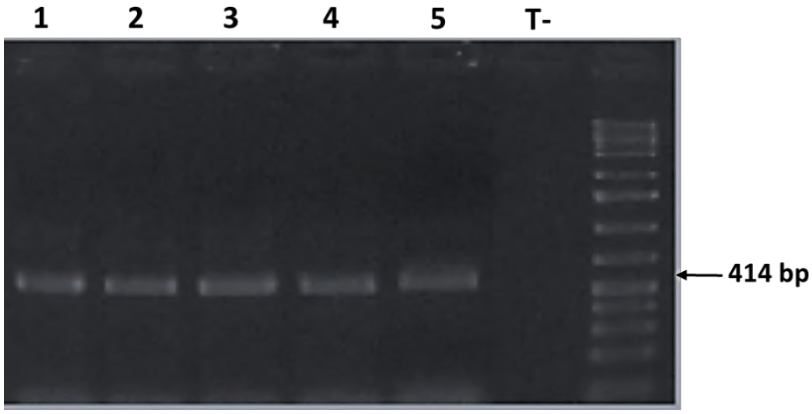

Fig.2: *Electrophoritic profile of A. vitis S4 amplified with VCF3/VCR3 primers and using different protocols of DNA extraction from pure cuclture.* ***1****: bacterial colony,* ***2****: bacterial suspension,* ***3, 4 and 5****: DNA extraction from pure culture using protocols 1, 2 and 3 respectively.*

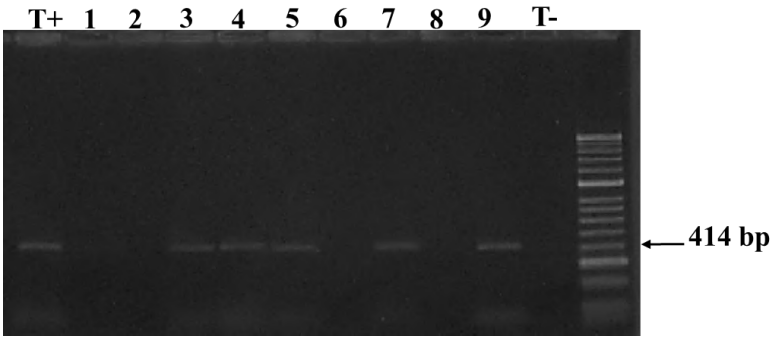

Fig.3*: Electrophoritic profile of A. vitis S4 amplified with VCF3/VCR3 primers and using different protocols of DNA extraction directly from plant material (tomato tumors).*

T+*: positive control representing by extracted DNA from pure culture;* ***1, 2, 3, 4 and 5****: bacterial DNA extract from plant material using protocols 1, 2, 3, 4 and 5 respectively;* ***6****: pure macerate;* ***7****: enriched macerate;* ***8 and 9****: isolated colony from culture medium;* ***T-****; negative control.*

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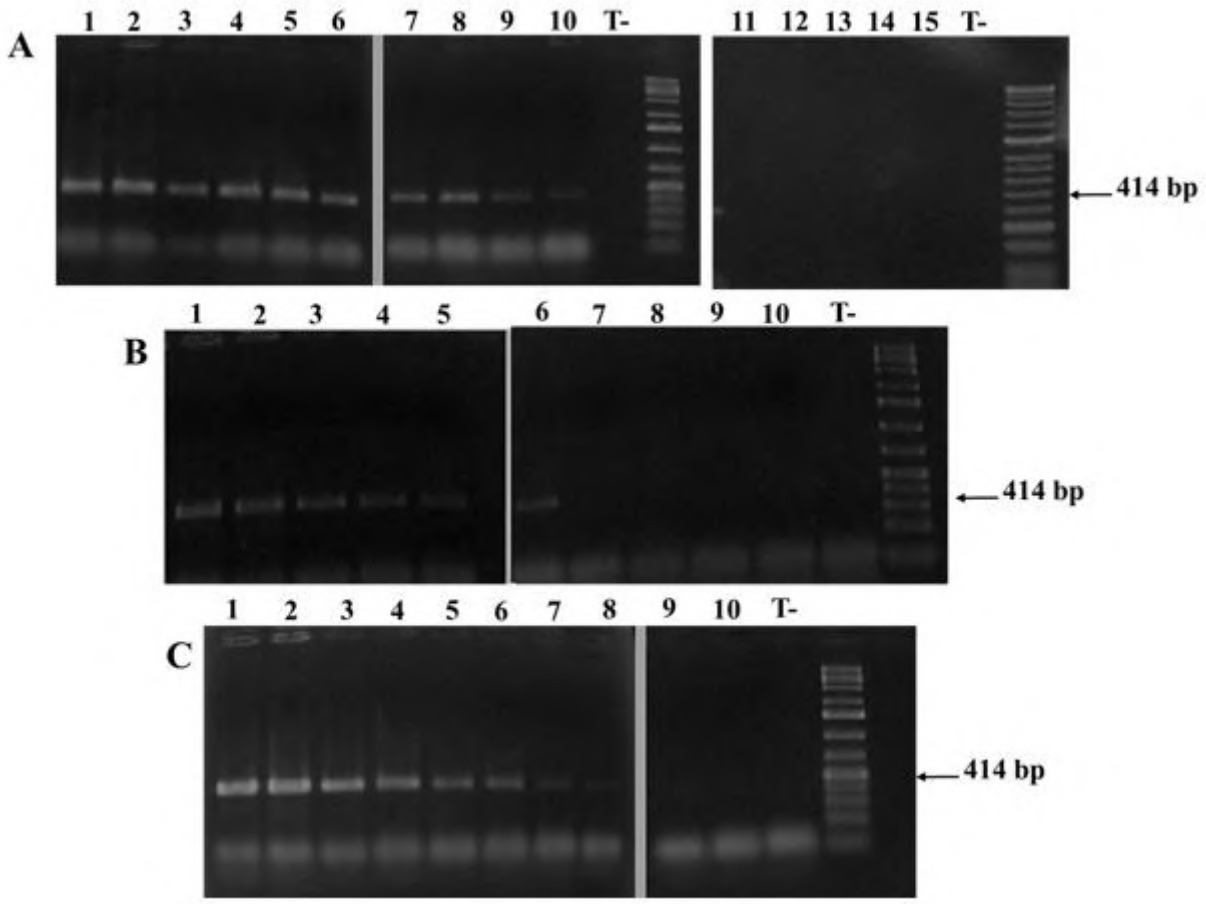

Fig.4*: Evaluation of the sensitivity of different protocols of DNA extraction from pure culture.* ***A****: DNA extraction with alkaline method;* ***B****: DNA extraction with heating bacterial suspension;* ***C****: DNA extraction using thermal shock. From 1 to 15: different concentration of bacteria (CFU/ml).* ***1****: 10^9;* ***2****: 5.10^8;* ***3****: 10^8;* ***4****: 5.10^7;* ***5****: 10^7;* ***6****: 5.10^6;* ***7****: 10^6;* ***8****: 5.10^5;* ***9****: 10^5;* ***10****: 5.10^4;* ***11****: 10^4;* ***12****: 5.10^3;* ***13****: 10^3;* ***14****: 5.10^2;* ***15****: 10^2;* ***T-:*** *negative control.*

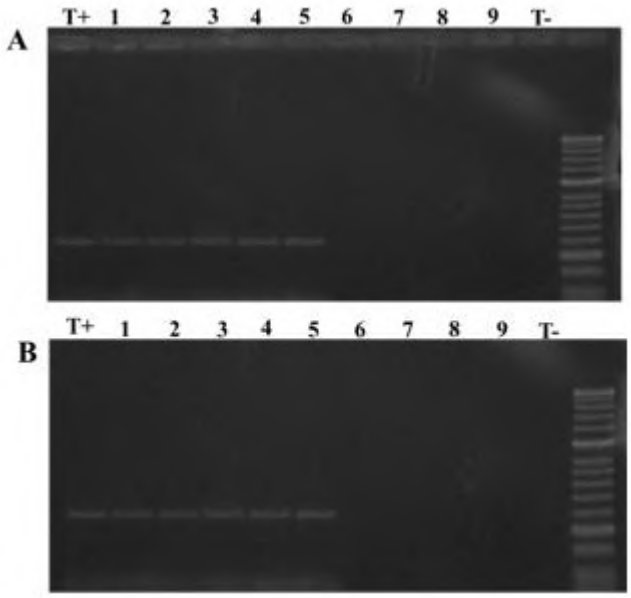

Fig.5*: Evaluation of the sensitivity of different protocols of DNA extraction from plant material using,* ***A****: protocol 4 of DNA extraction with Triton X-100 and* ***B****: protocol 5 of DNA extraction with Tween 20.*
T+ *: positive control ;* ***1*** *:10^7CFU/ml ;* ***2*** *:10^6CFU/ml ;* ***3*** *:10^5CFU/ml ;* ***4*** *: 10^4CFU/ml ;* ***5*** *: 10^3CFU/ml ;* ***6*** *: 10^2 CFU/ml ;* ***7*** *: 10 CFU/ml ;* ***T-*** *: negative control.*

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Economic profile of two species of Genus Euterpe, producers of açaí fruits, from the Pará and Amazonas States - Brazil

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***Abstract*—** *This study deals with an analysis of the production of the two assaih species (Euterpe precatoria Martius & Euterpe Oleracea) occurring in the states of Amazonas and Pará, respectively. In subject to delineate the economic behavior of two açaí pulp producing species, estimates were made based on temporal observations of the secondary data of produced amounts, prices practiced and respect analyses of growth market. The study made it possible to understand the market dynamics of the açaí economic activity from both species of the genus Euterpe. Analyzing data on the quantities produced and several studies on management, production and commercialization, it was possible to trace the apparent profile of the market structure of açaí fruit, where it was verified the strategic importance of investing in plantations of Euterpe precatoria species, because this species besides having higher anthocyanin contents, has its fruit production in the off-season months of E. oleracea. The harvest in alternate periods, adjust the annual supply and, certainly, will contribute to reduce the variation in prices. Over this perspective, was can perceive the social, political, technological, cultural, market and environmental transformations that need to be implemented and taken into account so that this activity may be able to follow an expansion of its demand and in an environment of perfect competition, find its point of balance, within the formats of the economic sustainability.*

***Keywords*—Assaih, Açaí Market, E. precatoria, E. oleraceae, production.**

I. INTRODUCTION

The Amazon has been a source of inspiration for several sectors of the world market, certainly due to the richness of its biodiversity and the exotic nature of its products. Therefore, it is considered a green reserve capable of providing innovative ingredients for the cosmetic, pharmaceutical or food & beverage industry, which are the main consumers of non-timber forest producers – NTFP [1]. Extraction of non-timber forest products (NTFPs) can provide important income for the inhabitants of tropical developing countries. The growth in the market for healthy products points to the need to obtain natural raw materials instead of synthetic or chemical components. In this consumption trend, "açaí", a native species of Amazonian biodiversity, has the potential to generate great business. Açaí is historically important for the survival of the native populations and capitalization of the northern region of Brazil. However, there is no standardization of data recorded in official bodies on production, marketing, among others, which are sometimes confusing and / or not reflecting reality. This negatively interferes with the creation of adequate marketing strategies, the concession of public incentives, necessary to subsidize the attraction of new ventures and job creation, being indirectly responsible for a repressed demand and consequent increase of prices of the final product.

Especially in the Amazon, the açaí is the fruit of the present and has a promising future. The market for this fruit began to conquer other regions of Brazil from the 1990s and its products are present on five continents [2]. In less than two decades, açaí pulp went through a process of expanding demand, conquering new markets, both geographically and in terms of target audience, reaching consumers with higher income. This change in demand occurred after the disclosure of its energy and nutritional properties, which resulted in the demand for the product by people interested in healthy foods [3].We have listed 22 different uses for all parts of the plant, from leaves to roots[4]. However, the main use is the preparation of a dark purple thick pulp called açaí wine obtained by maceration of the mature fruits of both species *E. oleracea* and *E. precatoria* [5]. From its pulp a great variety of products of market and of subsistence can be

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produced, being, therefore, a growing demand that according to experts, is far from being met.

In 2011, data from the Brazilian Institute of Geography and Statistics (IBGE) showed that the State of Pará is the largest national producer of açaí, with an annual production of about 850 thousand tons of the fruit, generating for the state economy an approximate value of R $ 677 million. According to the Secretary of State for Agriculture - SAGRI, more than 6 thousand tons of açaí pulp were exported from Pará in the previous year, which corresponds to an amount of more than U$D 17 million [6]. It should be noted that this market was established practically around the product extracted from a species, *Euterpe Oleraceae* Martius. However, the worldwide consumption trend and the growing demand for açaí indicate that there is room for the introduction of *E. precatoria* Martius, since it produces similar pulp and an increase in its supply, it may complement national production, Excellent option to supply the market's lack.Economic analysis studies for NTFPs generally present a partial approach, with emphasis on productive chains (processes and actors), rather than on the main input [7]. Contrary to this logic, we sought to trace the economic profile of açaí (fruit) production, analyzing the data of two species: *Euterpe precatoria* Martius. (Central Amazon) and *Euterpe oleracea* Martius. (Eastern Amazon).

II. METHODS

2.1 Description

2.1.1Gender

The genus Euterpe Martius. Is widely distributed in South and Central America [8]. The plants of this genus have great genetic variability, comprising seven species, five of which are native to Brazil [9]. The species *Euterpe edulis, Euterpe oleracea* and *Euterpe precatoria*, are considered as the most important of the genus due to their wide commercial usage [10].

2.1.2 Species

- *E. oleracea* Mart., Predominantly occurring in the eastern Amazon (Pará, Amapá, Tocantins and Maranhão), has as main characteristic, tillering, forming clumps and occurring in flooded floodplain areas.It is a palm tree with up to 25 strains per clump in differentdevelopment. The açaí flora and fruit practically all year round.However, the peaks of flowering and fruiting occur more frequently, duringJanuary to May and September to December [11].

- *Euterpe precatoria* Martius (açaí upland), is a palm widely distributed by Central America and north of South America, especially in Central and Western Amazonia [5, 12]. It is a common species in the forests of the Western Amazon, with a large occurrence in the states of Amazonas, Acre, Rondônia and Roraima [13]. Occurring naturally on the banks of rivers and lakes, in a non-flooded area of high várzea or upland / plateau. It is a solitary, monocaule palm, considered the most abundant palm tree in the Amazon [14].

2.2 Uses

From the fruits of the açaí palm is extracted wine, pulp or simply acai, as it is known in the Amazon region. Açaí is usually consumed with cassava flour associated with protein derived from fish, shrimp or meat, being the staple food for populations of riverside origin. Açaí berries are made with ice cream, liqueurs, jams, nectars and jellies, and can also be used to extract dyes and anthocyanins. The latest research shows the new organization chart of the use of the fruit of the açaí tree. The pulp represents 15% and the core corresponds to 85% of the total weight, from which the pulp is used in the production of cosmetics; The fibers in furniture, acoustic plates, xaxim, plywood, automobile industry, among others[15].

In addition, it is important to understand the role of organic matter in the generation of steam, charcoal and organic fertilizer. The “açaizeiro” has significantly impacted the commercial market. In addition to the uses described above, it may be a source of material for the manufacture of paper and cellulose [16]. It also presents ornamental potential [17, 18], in view of its morphological aspects. Highlights the therapeutic use[19]; The medicinal use[20]; Food use[21,22];Bioenergetic potential[23];High antioxidant activity [24,25,26].

2.4 Main differences

Taking as an example the State of Pará, where there are small orchards of Amazonian açai, implanted in areas of upland, without additional irrigation in the period of less precipitation of rains. It is possible to compare the behavior in the field of the two species *E. Oleracea* and *E.precatoria*, simultaneously submitted to the same edaphoclimatic conditions and it was observed that: The productivity is good, not being rare to obtain up to 50 kg of fruits / plant / year. The industrial yield of the fruits of *E. precatoria* is 30% to 40% higher than those of *E. oleracea* fruits and the production occurs in the first half of the year, ie in the off season of *E. oleracea*. As an alternative to complement the volume for export, due to its functional properties. The first studies have shown the superiority, in terms of the anthocyanin content of the fruits of *E. precatoria*, when compared to the fruits of *E. oleracea*[27].

2.5 Pulp production

Both species are producers of "açaí wine", name which is given to the pulp produced from its fruits. After processing the fruit consists basically of the addition of water and filtration, the pulp extracted from açaí receives the classification of type A, B or C, according to the amount of total solids (QST) present. Being considered respectively: Acai thick or special one that presents QST above 14% and a very dense appearance; Medium or regular acai berry is the one that presents QST above 11 to 14% and a dense appearance and, fine or popular acai berry that presents QST of 8 to 11% and a little dense appearance [28].

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2.6 Economic and biological importance

Euterpe oleraceae and *E. precatoria* produce products that have been presented at international fairs in Europe and North America, arousing the interest of the general public. Samples of pulp and by-products have been constantly shipped to Australia, Germany, USA, Italy and Japan [29].

Exports have been increasing significantly with annual rates of over 30% [30]. With the açaí export values coming from these two species of *Euterpe*, from 2004 this product reached the position of main fruit of the State of Pará in terms of income, employment and occupation of labor [31].

One of the great attractions for the drink commercialization is the presence of anthocyanins. Anthocyanins due to their anti-free radical, delay aging, extend cell life, increase immune defenses, promote better blood circulation and protect the body against lipid accumulation in Arteries. They also have the ability to delay vision loss and decrease the effects of Alzheimer's disease [32].

In a recent review, was presented comparative results of biological studies between the species *E. precatoria* and *E. oleraceae* that showed the antioxidant activities of the fruit pulp of *E. precatoria* are superior to those of the fruit pulp in *E. oleracea*. The results suggest that *E. precatoria* contains strongly water soluble antioxidants that can enter living cells and inhibit ROS (Reactive Oxygen Species) formation, with greater efficacy than those produced by *E. oleracea*[33].

Import data show that açaí, once totally destined for local consumption, has conquered new markets and become an important source of income and employment. The sale of frozen pulp to other Brazilian states has been increasing significantly. However, there are no consistent statistics on the amount of açaí exported to other regions of Brazil and abroad, but it is probable that something around 10% of production will be destined to these markets [34]

For the federal government, the extractive activity presents great potential in the generation and distribution of income, mainly in the rural and forest regions of the country, since it is still practiced by a large number of families. Therefore, the stimulus of this activity is seen as something important to [35].

III. DELIMITATION OF THE STUDY

In order to outline the economic profile of açaí productive activity, the study was based on secondary data from the following official bodies: Brazilian Institute of Geography and Statistics (IBGE); Sustainable Agriculture and Forestry Institute of the State of Amazonas (IDAM); Secretariat of Agriculture of the State of Pará (SAGRI); Brazilian Forest Service (SFB), National Supply Company (CONAB), Institute of Applied Economic Research (IPEA). Import and export data were collected from the Brazilian Export and Investment Promotion Agency, Aliceweb System and Secex / MDIC yearbooks.

3.1 Data processing

In this work, the IPA-DI-M, maintained by the Getúlio Vargas Foundation (FGV), was chosen. The estimates were made from the data [35,36, 37, 38]. The variables analyzed were: quantity produced by the extractivism, quantity produced by the plantation, total quantity exported, total quantity imported and respective prices, from 2005 to 2013, since, for one of the species object of this study, available data, prior to 2005, are inexpressive.

3.1 Data series

The Spearman coefficient was used to verify if the series of temporal data have constant mean and to evaluate if the trend movement is stationary or non-stationary. Spearman is a correlation of rankings and therefore is a non-parametric test, ie it does not require any assumption of normal distribution and can be used for ordinal variables. When the relationship between the variables is not linear but a monotonic function, Spearman may result in a higher coefficient than the Pearson correlation. This is good, but the downside is that Spearman's correlation coefficient is less sensitive to outliers.

IV. RESULTS AND DISCUSSION

The results obtained allowed comparisons between the economic activities of Acai in two states: Pará and Amazonas. Fig. 1 shows extractive production in all Brazilian Amazon states, where it is observed that Pará, the main producer, participates with 54.9% of this production and Amazonas with 35.5%. The largest variation in absolute values occurred in Acre. The production of açaí does not only come from extractivism and its cultivation is increasing.

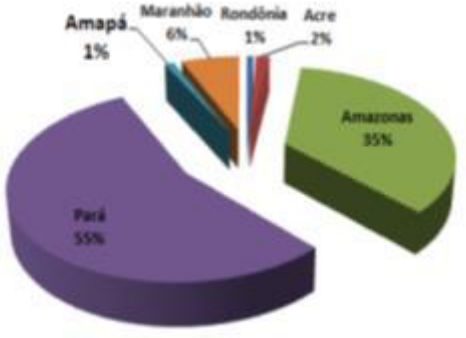

Fig.1: Production of Açaí (fruit) Extractivist in Brazil by States. Elaboration [39].

State of Pará is the largest producer of açaí (fruit) with a extrativist production of 111,073 tons, followed by the State of Amazonas with 71,783 tons, as shown in Fig. 2.

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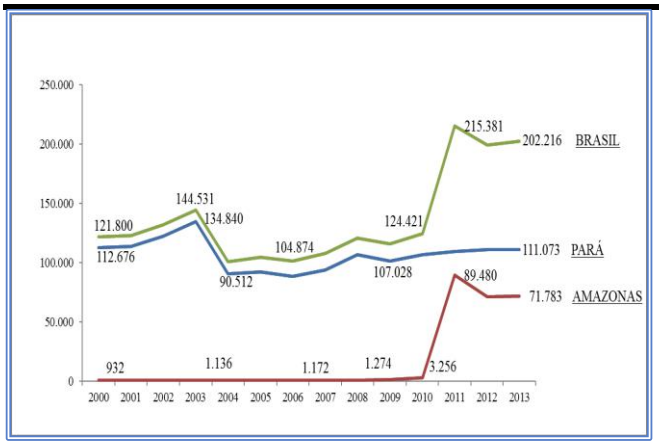

Fig.2: Production Evolution (ton) of Açaí (fruit) Extractivist in the States of Amazonas and Pará in 2013. Data Source: [36].

Analyzing the production data for the State of Pará (Figure 3), it is observed that in 2008 most of the production (in tons) was destined for domestic consumption, followed by consumption in the country and finally for export. This pattern of consumption continues to be followed, since the unofficial data of 2015, also show no signs of change.

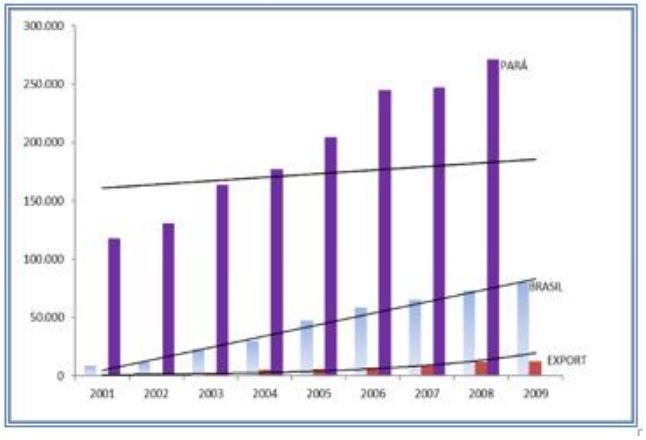

Fig.3 - Consumption (ton) of extractive açaí fruit in the State of Pará, Brazil and export. Data Source: [40]

Comparing the growth rate of the two states, Figure 4, it can be observed that for Pará it is below 20% pa, with negative peaks, indicating a severe fall in production in 1996 and 2004. In Amazonas, Variation is reversed, with the majority of the analyzed period remaining below 15%. However, it is observed that in 1996 and 2013, there was a significant ncrease in production.

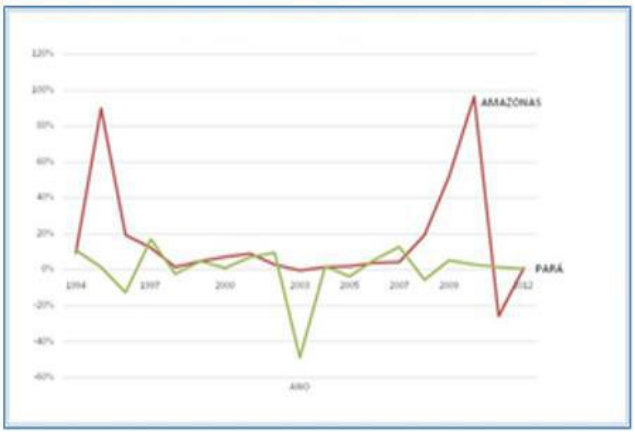

Fig.4 - Rate of production growth in tons of açaí between 1994 and 2012. - Production ofVegetable Extraction and Silviculture.Data Source: [41].

Calculating the Spearman coefficient for açaí production separately for the States of Pará and Amazonas (figure 5 e 6), the respective values were 0.96541 and 0.99098. The positive coefficients indicate that both sites have NON-STATIONARY series, that is, the production has very significant trend movements, not having a constant mean.

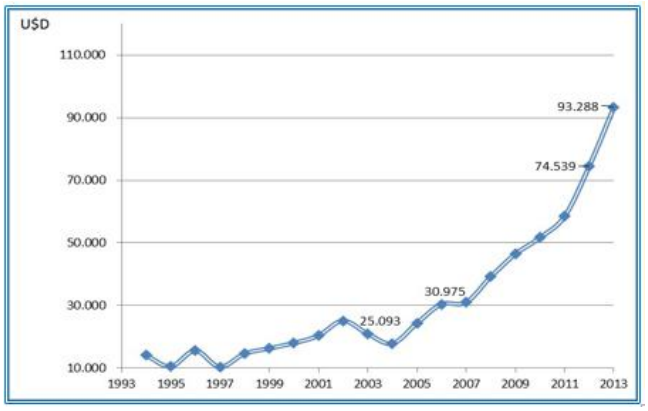

Fig. 5: Spearman coefficient of açaí production in (ton) x value (USD) in the State of Pará. Period analyzed 1994 - 2013. Data Source: [41].

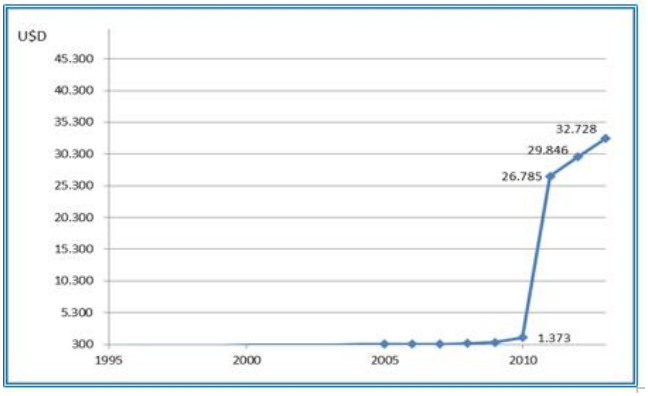

Fig. 6: Spearman coefficient of the production of açaí in (ton) x value (USD) in the State of Amazonas. Period analyzed 1994-2013. Data Source: [41].

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In terms of prices, the total production of açaí in Brazil, although it has undergone great variation during the studied period, presents visually a steady trend, with peaks and valleys around a central value. However, comparing the results presented for the two States, it is noticed that despite presenting series with non-stationary trends, the behavior of the means is quite different [42].

Evaluating the total production of açaí for the State of Pará (Table 1), we can see a change in production patterns. It is noted that the State produced 928 tons of fruit, being 817 tons from cultivation and 122 tons from extractivism. In the state of Amazonas, the State Department of Production SEPROR, empirically considers that 15% of the volume announced is from crop.

Table 1 *- Total production in the states of Pará and Amazonas (Cultivation / Extraction) * Estimated value.*

Production	***2012 (Ton)***		
	Total	***Cultivated***	***Extraction***
PARÁ	*928. 183*	*817.246*	*111.073*
AMAZONAS	*80.306*	*9.16**	*71.146*

Data Source: [40,42].

Pará remains the largest producer of the fruit, according to data from the Systematic Survey of Agricultural Production (LSPA), provided by the Brazilian Institute of Geography and Statistics (IBGE) and published by the State Department of Agriculture (SAGRI). Fig. 7 shows that in 2012, the municipalities of Paráina had reached a total of 817,246 tons of açaí, in an area of 91,426 hectares (planted and managed fruit), with an increase of area and production in relation to the interior year. SAGRI estimates that Pará is responsible for 80% to 90% of national production. Pará supremacy can be attributed to large-scale planted / managed açaí, an option that is not used in the same proportion in other states.

By associating total açaí production data for Amazonas and Pará in the same graph, it is possible to observe the real difference between the two places (Fig. 7).

Between 1994 and 2004, the data on extractive production in Amazonas are inexpressive, since they reach 1,000 tons only from 2005. It is also observed that in 2010 there was a peak of production around 90% (figure 7). However, it cannot be stated whether this situation was caused by increases in production or more likely by errors or omissions of public agencies in the collection of data from previous years.

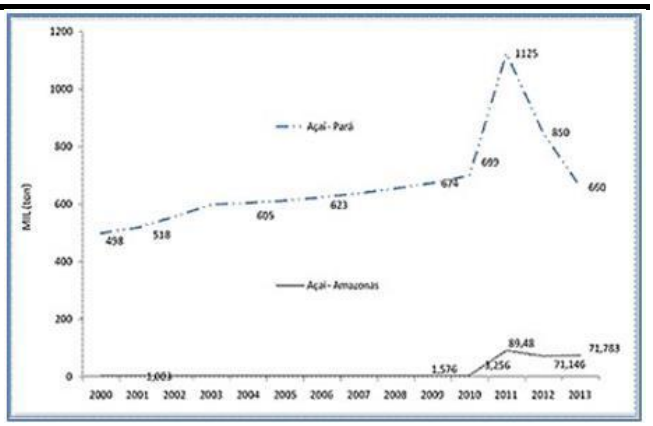

Fig. 7: Total production in tons the states of Pará and Amazonas (Cultivation / Extraction). Data Source: [40,42].

By making a parallel between the cultivated and extractivist quantities produced in Amazonas and Pará (figure 8), it can be verified that the percentage of participation of each State in the total production of açaí, differs from the situation shown in figure 8.

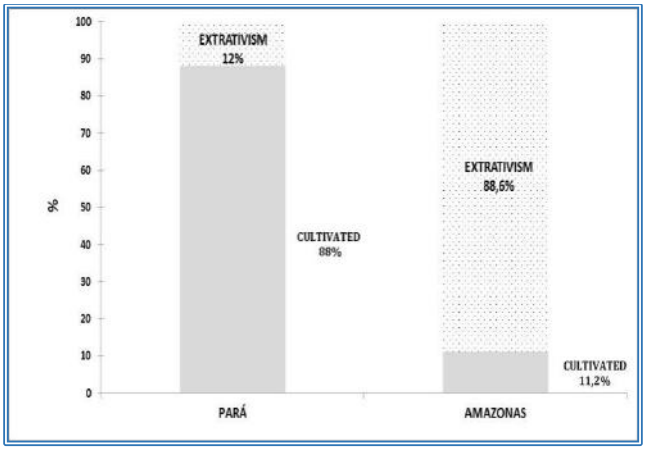

Fig. 8: Effective participation in the açaí market in 2012. Data Source: [36,40].

The results obtained by the State of Pará may be related to the investments in research for new cultivars, early and adapted to the upland, with high productivity. From the agreement signed with Embrapa Amazônia Oriental, the cultivar BRS Pará appeared in 2004. In 2013, it was the launch of the new cultivar that has as main characteristics the highest yield of pulp and production in the first semester, that is, in the off-season. Since 2008, according to the Secretariat's survey, about 19 tons of selected seeds of BRS Pará were distributed, which produced at least 9 million seedlings. "This development, coupled with the prospect of good business by private initiative, probably increased the area planted. Even with this initiative "Demand will still not be fully met, because it will take some years for the new plantations with technology (irrigation and new cultivars) to go into production."[44].

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V. CONCLUSIONS

Analyzing the quantities produced, the production methods and the marketing studies, it was possible to trace the apparent profile of the market structure of the açaí fruit where the following characteristics are perceived:The regional market revolves around the in natura consumption of pulp. The major consumer centers in Brazil, such as São Paulo and Rio de Janeiro, receive pulp processed and frozen, as well as ready-made beverages, with components such as guarana syrup and other fruits. The international market requires strict control, processes and analyzes, and it is necessary for the supplier to have agroindustry and technology.The product is homogeneous, but the seasonality of the extractive production causes great price variation. However, the commercialization of the fruit in natura (in the field) and pulp (in the cities) operates in perfect competition.The supply of açaí (*Euterpe oleracea*) has been shown in several studies as inelastic to the price, but it can not be said that it occurs for *Euterpe precatoria*, since the secondary data for this species are insufficient.It is strategic to invest in plantations of *Euterpe precatoria* species, because currently the volume produced in the state of Amazonas, represents about 1% of the national production and the fact that this species occurs in the off season of *E. oleracea*, will certainly contribute to maintain the supply of matter the whole year and consequently to reduce the variation in prices.

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Economic and Environmental Evaluation of Nitrogen Fertilizer Taxation: A Review

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***Abstract*—** *Nitrogen fertilizers is an essential input into modern agriculture, however the use of large amounts of this mineral fertilizers caused in the last three decades enormous environmental impacts such as eutrophication of waters and soils, loss of biodiversity, drinking water pollution and human health risks. The agri-environmental policy plays a crucial role to internalize pollution externalities from agriculture production and ensuring food production and food price remain affordable even to those with lowest income.*

To date, regulatory instruments, such as the Nitrate Directive in EU applied to reduce and manage nitrogen pollution run-off showed scarce results in terms of environmental protection and in many countries such as Ireland and Spain created evident loss of incomes and impose high costs on small to medium farmers to respect nitrogen fertilizers limits. Meanwhile in other countries economic instruments such as nitrogen taxation reach better results in terms of agriculture emissions reductions and environmental impacts due to their flexibility.

This review aims to document the current state of the knowledge of nitrogen taxation and gather experience from other countries for reducing nitrogen emissions assessing their effects on farmers' income productivity, food price stability and environmental outcomes.

***Keywords*—Nitrogen fertilizers, non-point source pollution, environmental regulation, nitrogen taxation.**

I. INTRODUCTION

The agri-environmental policy instruments that aims to achieve sustainable environmental outcomes such as protecting drinking water from fertilizers pollution, reducing soil acidification and loss of soil's fertility and eutrophication of waters plays a crucial role in the agriculture sector to manage and reduce nitrogen pollution.Regulatory instrument, showed scarce environmental results to mitigate contamination of groundwater. Discharges from agriculture (fertilizers and animal wastes) are the largest source of nitrate contamination of groundwater, but there is no current or historic regulatory program that was able to reduce fertilizers runoff. Overall, nitrate concentrations in groundwater have not decreased in the last three decades. In fact, concentrations have even increased in some areas, such as in US, where the Clean Water Act failed to reduce nonpoint pollution for the agriculture sector after more than 30 years action and evidence is clearly showed in Mississippi River, Lake Erie, Chesapeake Bay and other several American rivers. This was due mainly for the fact that the Clean Water Act in US gives to the federal government little power to regulate agriculture pollution. Some regulatory programs have recently introduced mandatory monitoring programs, but monitoring alone will not improve water quality. Many years are needed for regulatory actions to reduce nitrate in groundwater and improve drinking water quality.

The physical properties of nitrate in groundwater mean that regulatory actions on nitrate leaching today will not bring drinking water sources into water quality compliance for years to decades. In Europe, the Nitrate Directive introduced in 1991 showed some improvement form the environmental point of view, but still very far to solve this problem, considering that in some area, such as the Baltic Sea, eutrophication increased consistently in the last twenty years. Evidence shows that benefits, in terms of nitrogen reduction are strongly affected by the environmental conditions and by the farming systems in Europe. Many European countries struggled to respects the standard and limit for nitrogen application, and state that comply within the limits presented high farmer income losses. In particular, dairy sector shows serious problems to implement Nitrate Directive, respect the limits and large negative distributional effects to farmer income.

Ireland represents afailure of this directive in terms of farmers income impact and nitrogen reduction, where farmers were reluctant to introduce this regulatory mechanism due to the high costs and the lack of government policy that really compensate farmers that respect this regulation that aims to increase the quality of water. Few States adopt the right-based approach that

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typically involve the imposition of a limit or 'cap' on pollution or polluting activities, either by specifying a total nitrogen and phosphorous pollution limit.

One of the most important tradable emission permits created to reduce and manage nitrogen pollution from agriculture activities is the Water Quality Trading (WQT) adopted in United States and Canada. Benefit from WQT was highlighted only in some regional areas such as in the Ontario South Nation River in Canada and Michigan and Colorado in US, and include not only improving water quality but also strengthening community relationships. Overall, the biggest impediment to WQT was the lack of public knowledge of benefits and in many cases around United States, the transaction costs were extremely high and was one of causes that limited this mechanism. Successful trading required the development of institutions for organizing trade that are trusted by and effective for intended program participants. Positive results seems arising from New Zealand innovation program in Lake Taupo, North Island, where the Water Trust provide farmers the option to change agriculture activities or pursue alternative land uses. Anyway, this mechanism need long period of activities before producing environmental benefits and the Phosphate Quota System in Germany and Nitrogen Quota System in Denmark failed to produce positive results due to the short period activities. It comes clear that there is no ideal solutions or best environmental policy instrument to reduce nitrate in groundwater. Environmental taxes, and in particular nitrogen taxation shows some positive and interesting results in terms of environmental outcomes, farm costs and income redistribution. The next following section presents some empirical studies of nitrogen taxation.

II. EMPIRICAL ANALYSIS AND SIMULATIONS OF COMPARISON EFFECTS OF REGULATIONS AND HYPOTHETICAL INTRODUCTION OF NITROGEN TAXES

The application of a tax on nitrogen fertilizers seems more effective in terms of fertilizers demand reduction and consequently environmental benefits, even if some economists disagree about the price elasticity of demand fertilizers and expected very low reduction in nitrogen volume. This section present international case studies that emphasize economics and environmental outcomes of nitrogen taxation using both bio-economic models and econometric analysis to estimate the impacts of fertilizers taxation on fertilizers demand, production, farm income and environmental aspects.

Denmark

The use of mineral fertilizers in Denmark has increased consistently until the 1980s due to the intensive crop production and caused extensive environmental impacts such as water pollution and euthrophication. Denmark introduced a tax on nitrogen in fertilizers in 1998 but due of its exemptions, in practice only household users pay tax and these users were generally unaware of the tax. Since the 1980s a sets of regulatory instruments and Government action plans were introduced to limit and ban nutrient losses in agriculture in Denmark. From 1990 to 2011 the use of imported nitrogen fertilizers dropped from 390,000 tons to 200,00 tons reaching a reduction of 42%. The result of this environmental policy was considerably positive in terms of environmental benefits, but still very far from a sustainable solution.

In 2013,Skou Andersen *et al*. conducted an empirical analysis of extending nitrogen tax to farmers to reduce environmental impacts in Odense River Basin located in Denmark. The introduction of the nitrogen tax instead of the regulatory instrument would affect the price of imported mineral fertilizers, and therefore a reduction in their use (up to full elimination). This environmental tax would move farmers towards the use of organic fertilizers even if crop yields would decline relatively less however. So, nitrogen tax would not affect organic fertilizers such as waste product of animal husbandry, and their trade value could increase consistently. Skou Andersen *et al*. (2013) used an economic model to estimate the farmer income shock of introducing nitrogen taxation for the specific area and analyzed the impact on agriculture product prices.The results shown that the introduction of nitrogen taxation could increase the demand for organic fertilizers with a negative impact on imported mineral fertilizers and a significant environmental benefits in clean waters and drinking water.

Another important study conducted in Denmark on nitrogen taxation impact on crops and manure by Bernsten*et al.* in 2003. They used a whole farm model called FASSET (Farm Assessment Tool) developed by the Aarthus University in Denmark to evaluate consequence of changes in environmental regulations and the impacts on prices and subsides introducing nitrogen tax. In particular they analyzed the introduction of a tax on nitrogen in mineral fertilizer and a tax on the farm nitrogen surplus. In four different farm types such as arable on sandy soil, arable on loamy soil, pig production on sandy soil and pig

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production on loamy soil. From the empirical analysis none of the taxation measures was the most cost-effective for all farm types but they concluded that the environmental pollution reduction achieved with nitrogen taxation seems the best solution compared to other possible command and control mechanisms.

Spain

The Nitrate Directive and the more recent Water Framework Directive to limit nutrient losses to water bodies in agricultural land in Spain shown a very scarce results in terms of environmental benefits and imposed high costs on famers, especially those with small and medium size.

Martınez and Albiac (2006) developed and economic model to analyses the effects different environmental policy measures on agriculture production including the introduction of nitrogen tax. The economic model includes both corn production function and a nitrogen pollution function, in order to assess both the private benefits to farmers from corn production, and the damage cost to local communities from nitrogen pollution. Nitrogen taxation was considered the first best instrument of taxing N emissions, with a unit emission cost equal to 1.23€/kg (2005). The proposed nitrogen tax would strongly reduce nitrate losses, diminishing pollution levels by soil type between 10 and 60 percent. They also estimated that nitrogen tax could increase welfare in the district by 0.32 million euro. Their results indicated that a tax on mineral fertilizers and in particular the use of nitrogen results in more significant pollution reduction at much lower costs.

Gallego-Ayala and Gomez-Limon in 2009 compared the effects of the Common Agriculture Policy (CAP) in EU reform with alternative nitrogen taxation designed to mitigate nitrate pollution in agriculture sector in Spain. They estimated the economic, social and environmental impacts of the introduction of nitrogen fertilizers tax within the context of the new CAP. The first hypothetical scenario of an economic charge of €0.20 kg N-1 for nitrogen fertilizers would produce an irrelevant decrease in the use of mineral fertilizers and consequently irrelevant environmental benefits. However, an increasing value of taxation such as €0.40 kg N-1 would reach more than 50% reduction in the nitrate balance indicator, and reaching -64.4% for a charge of €1.00 kg N-1. The economic impact on farmer income seems consistent using nitrogen tax of €0.40 kg N-1 but could be compensated by the national Government incentives if the real aim is to reduce nitrate losses in to the waters.

Switzerland

Switzerland is another country that in the last two decades reported an increasing waters pollution caused by the losses of harmful nitrogen compounds from the agriculture. The agri-environmental policy in Switzerland to manage and reduce mineral fertilizers is based on regulation and restriction of nitrogen uses that vary on the type of farmer' activities and the regional areas. Due to the high farmers income compared to the other EU countries, the income variability is generally affected only by the extreme climatic events that rarely occurs. The idea of introducing nitrogen tax in Swiss agriculture is recently taken in consideration due to the scarce results from the currently regulations in terms of water quality improvement in rivers and lakes. Robert Finger (2012) in his study proposed the introduction of nitrogen tax in the Swiss Confederation, using a bio-economic model to investigate its economic and environmental impacts. The assumption was that if a nitrogen tax would be introduced, the nitrogen fertilizer demand decreases irrespectively of farmers' risk attitude. From his economic model simulation the three taxes option of 10%, 20% and 30% would reduce the nitrogen use respectively by about 5%, 9,65% and 13%. From this economic analysis is evidenced that in this particular case, a small amount of nitrogen taxation is required to reach reduction of nitrogen use without presenting evident farmer' income losses.

New Zealand

According to the data provided by the Minister for the Environment of New Zealand the 39% of groundwater monitored in New Zealand (2015) have level of nitrate that are above natural background levels caused by leaching of mineral fertilizers and stock effluent causing aquatic pant growth such as in Lake Taupo (Ministry for the Environment NZ, 2015). Various regulatory instruments were introduced in New Zealand since the 1991 such as the Resource Management Act (1991), the Agricultural Compounds and Veterinary Medicines Act (1997) (ACVM), Agricultural Compounds and Veterinary Medicines Regulations (2001), the Hazardous Substances and New Organisms Act 1996 (HSNO), that aims to reduce nitrate leaching and guarantee good level of waters quality. These regulations and the nitrogen-trading program created several economic impacts on small and medium maori pastoral agriculture activities and in some region shown scarce results of emissions reduction.

Ramilan*et al*., 2007 conducted an empirical study on the nitrogen taxation impact in New Zealand, focus on a

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Waikato River Sub catchment using dairy farm as case study, considering that this agriculture activity is the predominant land use in this country. In fact, dairy farming in North Island is the predominant agricultural land use and occupy almost the 70%. For the estimation of nitrogen taxation impact in dairy farming system, they used a whole farm model that takes into account agri-biological variable such as local climate, cow metabolism, pasture growth, paddock and economic variables. Nitrogen fertilizer application was limited at 200 kg/ha in the optimization process except the intensive farming systems. Cameron *et al* (2003) suggested that nitrogen applications to pasture are most efficient when applied at rates of between 20 and 40 kg N ha and should not exceed 150 to 200 kg N/ha. Even though the farming systems are not directly comparable due to the differences in soil and topographic characteristics, the results indicate differences among farms.

Ramilan*et al*. (2007) propose that the value of taxation should be differentiated by the different kind of farming systems and should be very high for extensive farms in New Zealand. An hypothetical tax of $5 kg N-1 will cause a considerable reduction in mineral fertilizers demand and environmental benefits for low to moderate concentration farms, while for high intensive farm systems the tax value should be $15 kg N-1 to reach the same results. They concluded that an efficient taxation scheme should be differentiated by farm types and level of nitrate emissions.

South Korea

South Korea agriculture sector is one of the most mineral fertilizers intensive users in the world. Water quality decreased consistently in the last three decades and agriculture emissions such as nitrogen and phosphorous are the principal pollutants causing eutrophication, losses of biodiversity in rivers and lakes and soil quality degradation. The use of phosphorous and nitrogen in the intensive agriculture excided the required for the optimal level and twice the amount used in EU countries.

To limit the environmental damages caused by the chemical fertilizers overuse, the Korean Government in 1993 introduced regulation and integrated nutrient management program to reduce the use of fertilizers. From 1995 to 2005 the average use of chemical fertilizers per hectare decreased from 424 kg to 376 kg (OECD, 2008). These regulation and eco-friendly management program show some interesting results in terms of chemical fertilizers demand reduction, but still very far from an efficient solution to protect the Korean waters from nitrate pollution.

Kim and Stoecker (2006) analyze the economic effect of the introduction of nitrogen tax on mineral fertilizers on rice production in South Korea, by using a partial equilibrium model. The model estimated the price elasticity of mineral fertilizers demand around 0.14 while the supply elasticity was 2.78. Introducing new nitrogen tax on mineral fertilizers farmers' welfare could decrease due to the increased fertilizers price and consequently the consumption should decrease.In relation to measuring the demand and supply elasticity for chemical fertilizers, they approached the demand side easily through survey data for the cost of rice production. The price elasticity of demand for chemical fertilizers was found to be 0.1456 and the supply elasticity was found to be 2.7875. The study presented three different values taxation of imposing 10%, 100% and 200% of tax increase and analyzed the impact on demand reduction and rice production.

The case of 10% tax increase, the fertilizers demand would decrease only by 1.5%. In case of a 100% tax, demand would drop by 14.6% and this level of decrease in fertilization had almost no influence on the quantity of yield. A 200% tax would decrease the demand at 29.1% reducing the yield of 22%.

They conclude that the case of 100% tax increase seems to be the more appropriate and efficient measure,because the decrease in farmer's income would be only 3% and the quantity of rice yield would be nearly unchanged.

Nitrogen taxation welfare impacts

Economic instruments such as nitrogen taxation to reduce and manage nutrient runoff from mineral fertilizer overuse have important advantages in environmental effectiveness and positive welfare effect to address water quality improvement, ability to raise public revenue and transparency. The aim of introducing nitrogen tax is to directly address the markets failure to take environmental impacts into account by incorporating these impacts into agriculture products prices. Considering that demand for mineral fertilizers and nitrogen in particular is very inelastic, to achieve reduction in application rate, high substantial taxation is required. Simulations and previous experience from countries that introduced nitrogen taxation, suggests that such tax should be applied at least at 100% rate. This taxation rate imposeslarge economic effects on farmers' income, especially farms with large livestock concentration. The most important aspect, before design and introduce nitrogen taxation, is to evaluate and differentiate the taxation rate related to the application rate

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and nutrient surplus per hectare. However, the negative effect of farmers' income loss caused by the tax since it increases their costs, could be compensated if government introduce a reimbursement to farmers. A large nitrogen taxation with reimbursement to farmers if well calibrated is a fair systems and able to strongly affect the demand of mineral fertilizers. En fact, fertilizers and feedstuff producers will be largely impacted, as farmers attitude will be to improve efficiency of nitrogen use to reduce their costs, and will substitute part of their fertilizers by manure and legumes and other organic feedstuff. This system will inevitably impact on mineral fertilizers sales and on mineral industry at large scale.

Increasing water quality and reducing the risks of chemical contaminations has a positive impact on society and in particular to rural residents. Estimating the monetary value of positive welfare effect, such as reducing algal blooms and eutrophication in a lake, can be estimated as the amount of society is willing to pay to gain water quality and save drinking water. In environmental economics, water quality improvement is defined as no-market value because there exist no markets and therefore no markets prices are available to estimate the economic value. The only way to assess the economic value of this environmental service is analyze the residents' willingness to pay to improve their environmental goods. Choice Modeling and Contingent Valuation represent the most important methodology approach to estimate in monetary value such environmental changes and nitrogen application reduction in agriculture areas. Poor *et al.* (2007) estimated the welfare benefit from increasing water quality of reducing nitrogen losses in Maryland waters in United States using hedonic price function where the price of residential property was regressed on the characteristics of environmental goods.This study show how water quality significantly influence residential property values and removing inorganic nitrogen from waters in Maryland increased the welfare household by $17,642 USD in (2007).Ahlroth (2009) estimated the welfare benefits of increasing water quality in Baltic Sea waters in Sweden using contingent valuation methodology to assess the willingness to pay for reducing nitrogen and phosphorous agriculture emissions. The willingness to pay for improved water quality was estimated at €270 per person per year and this amount aggregated to national level reach € 1.8 billion in 2009.Ik-Chang Choi*et al*. (2016) estimated the welfare benefits of improving water quality in tidal flat rural areas in South Korea using contingent valuation methodology, accounting $870 million USD in welfare economic benefit per year (2012).

Increasing water quality and reducing mineral fertilizers losses affects many aspects of human well-being and costs and benefits impact on different groups of beneficiaries at different level. Water quality is highly valued by the public and welfare benefit is consequently very consistent in terms of economic value. Therefore, agri-environmental measure to reduce the application of mineral fertilizers and the water degradation play a crucial role to increase the welfare benefits.

III. CONCLUSION AND POLICY RECOMMENDATIONS

Mineral fertilizers emissions from agriculture increased strongly within the last three decade and will continue to grow in the near future as shown in US and many other Nordic countries in EU. Hence, the agricultural sector will continue to be one of the main drivers of water pollution across the globe. To date, current agri-environmental policy that introduced regulatory instruments and water quality trade systems failed in manage and control fertilizers runoff. However, there are economic instrumentsthat could help to reach a sustainable level of water pollution, such as the nitrogen taxation. By putting the price on mineral fertilizers, this instrument aims to shift nitrogen inputs from polluting chemical fertilizers to less polluting substitutes such as organic fertilizer and gives incentives to improve nitrogen efficiency. Environmental benefits and the degree of control of environmental pollution achieved with nitrogen taxation vary with the market price of the agriculture products and are affected by the fertilizers demand elasticity that is correlated to the farmers income.Due to the fact that demand for mineral fertilizer proved to be very inelastic, -0,3 -0,5, therefore the tax rate needs to be high before the demand decreases. An appropriate level of charges is required to stimulate change in farmers' behaviour. This would make food more expensive because farmers would simply pass the increases cost of fertilizers onto the consumers and low income' people will be strongly affected. If we look for example the EU-28 economic situation in 2016, there are 122 million people at risk of poverty and increasing the food price to reduce fertilizers runoff seems completely inappropriate. So the crucial point is, considering that nitrogen taxation looks the more effective economic instrument to reduce mineral fertilizers demand, how we can compensate farmers losses and avoid that food price rise. Public financial support for eco-actions and trans-border cooperation programs are essential prerequisite to avoid

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impacts on farmer losses and food price. In fact nitrogen taxation can have adverse effects: unjustified income loss among certain groups of farmers, concentration of agricultural activity on a smaller area, and land abandonment in other regions, including regions where agriculture is vital for maintaining rural communities, cultural landscapes and biodiversity. Regressive impacts resulting from nitrogen pricing can be reduced through compensation, redistribute the revenues from the taxation to the farmers and by lowering taxes on employment and income. It comes clear, that the success and effectiveness of nitrogen taxation depends on how tax revenue will be distributed among the farmers (though tax rebate or incentives) who reduce mineral fertilizers demand and increase the demand of organic fertilizers. Same compensation mechanisms should be used among those farmers who invest in nitrogen recycle plants and removal technologies.

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Removal from wastewater and recycling of azo textile dyes by alginate-chitosan beads

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Abstract*—Alginate-chitosan beads were used as adsorbent to remove two azo anionic textile dyes, Direct Blue 78 and Direct Yellow 106, from aqueous solutions. Batch mode experiments of dyes adsorption were performed and the effects of various parameters such as contact time, adsorbent dosage, initial dye concentration, pH and temperature were examined.*

Successively, the dyes have been desorbed from the adsorbent and were recycled to dye a cotton fabric.

The maximum efficiencies in dye removal, performed at pH 6, 298 K and with 0.5 g of adsorbent, were found to be about 97% for Direct Blue 78 and about 86% for Direct Yellow 106, respectively. The adsorption isotherms fitted the Freundlich's model, the adsorption kinetics followed the pseudo-second order model and experimental data indicated an exothermic adsorption process. Moreover, the dyes desorption experiments from the alginate-chitosan beads demonstrated that about 50% of dyes were released in distilled water at high temperature (368 K) and the colored solutions obtained were so reused in dyeing tests.

The results demonstrated that the alginate-chitosan beads are very efficient systems able not only to remove dyes from wastewater, but also to recycle and reuse them in further dyeing processes.

***Keywords*—Adsorption, Alginate-chitosan beads, Desorption, Textile dye removal, Thermal analysis.**

I. INTRODUCTION

Several industrial sectors, such as paper, leather tanning, plastic, cosmetic, rubber, and textile productions, discharge great amounts of dyes into wastewater. These complex organic molecules cause an important source of pollution in hydrosphere, dyeing visibly the effluent waters [1].

Color is usually the first contaminant to be recognized in wastewater being highly visible to human eye even in presence of very small amount of synthetic dyes (less than 1 ppm) [2]. Colored water not only causes an objectionable aesthetic aspect, but also reduces sunlight penetration retarding the photosynthetic activity of aquatic species and inhibiting their growth. In addition, dyes are toxic, carcinogenic, mutagenic, or teratogenic both to aquatic species and to human beings due to the presence of metals, aromatic and azo groups in their molecular structures [3, 4].

Although dyes exhibit a considerable number of chemical structures, it is well-known that the azo dyes are one of the most widely used and represent approximately 65–70% of the total dye production [5, 6]. Azo dyes are toxic and potentially carcinogenic for the reduction of the azo groups with the consequent formation of aromatic amines in the wastewater [7]. Therefore, the dye removal from industrial effluents is a fundamental issue and appropriate wastewater treatments should be done to decrease the environmental impact, even though it is very difficult to realize because of the recalcitrant nature of azo dyes. Indeed, these molecules are resistant to aerobic digestion and are highly stable to light, heat and oxidizing agents [8]. In the last years, several physical, chemical and biological methods, such as adsorption, membrane-filtration, coagulation, flocculation, flotation, precipitation, oxidation, aerobic and anaerobic microbial degradation processes, have been developed for the removal of dyes from industrial effluents [9]. Some of these approaches are expensive, with a very low efficiency or are impracticable because of toxic by-products formation [10]. On the contrary, it has been proved that adsorption is one of the most effective and cheap methods which industries employ to reduce hazardous pollutants present in the effluent [11, 12]. Consequently, a lot of non-conventional and low-cost adsorbents, e.g. natural materials, biosorbents and by-products of industry and agriculture have been proposed by researchers [13-15]. Recently, it has been also demonstrated that the adsorption of dyes by means of natural and biodegradable polymers is one of the emerging methods for dye removal. Indeed, numerous studies based on the use of biopolymers, such as alginate [16, 17] and chitosan [18-20], have established that these biosorbents have a very high affinity for many classes of dyes. Sodium alginate (AL), the sodium salt of alginic acid, is a linear biopolymer extracted from brown algae containing β(1→4)-D-mannuronic acid (M) and α(1→4)-L-guluronic acid (G) residues. It has the properties to form stable three-dimensional hydrogel in presence of

bivalent cations in aqueous media, such as Ca^{2+} ions. The bivalent ions induce cross-linking of adjacent biopolymer chains, mainly at guluronic sequence (G-G) of polymer, following the so-called 'egg box model' [21, 22]. Several studies have showed that this polymer has been used for the removal of basic and disperse dyes [13, 16, 17]. The binding of these cationic dyes to anionic polymers can take place either by electrostatic or by other aggregation interactions, such as hydrogen bond and/or hydrophobic interactions [23]. Chitosan (CH) is a polysaccharide commercially produced by alkaline N-deacetylation of chitin, a N-acetyl-β-D-glucosamine polymer, the principal constituent of exoskeleton of crustaceans, insects, and arachnids. The chitosan polycationic structure allows to form a strong interaction with alginate negative charges based on electrostatic interaction between alginate carboxylic groups and chitosan amine groups [24].

Therefore, in this study, alginate-chitosan-based adsorbents were used to reduce the dye amounts present in industrial effluents. Despite the large number of papers dedicated to the removal of dyes by means of these materials, in the present work, not only the adsorption performance and its mechanism were evaluated, but also the dye recycling was demonstrated. Indeed, after dyes adsorption on the adsorbent material, the same dyes have been desorbed and reused for fabric dyeing tests.

In detail, beads with a solid core of alginate gel (AL beads) and alginate beads successively coated with a chitosan membrane (AL-CH beads) were prepared and used as adsorbent for the removal of two anionic azo dyes from aqueous solutions, Direct Blue 78 (DB78) and Direct Yellow 106 (DY106).

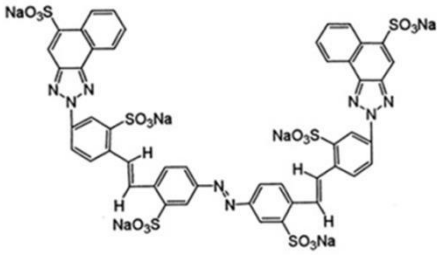
The chitosan coating not only reinforces the AL-beads, reducing their disintegration, but also endows beads with a positive surface charge, enhancing their ability to adsorb anionic dyes [25]. Indeed, several studies have demonstrated that the strong interactions between the amino groups present on chitosan chains and anionic dyes can be used to explain the adsorption mechanism [26, 27]. The DB78 and DY106 adsorption on AL-CH beads was performed and the effect of different variables, such as contact time, adsorbent dosage, initial dye concentration, initial solution pH and temperature were considered and discussed. The Langmuir and Freundlich equations were used to fit the equilibrium isotherms and the adsorption kinetics were determined by the pseudo first-order and second-order models.

FTIR-ATR measurements and thermal analysis, such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), were also utilized to understand the dye/AL-CH beads interactions.

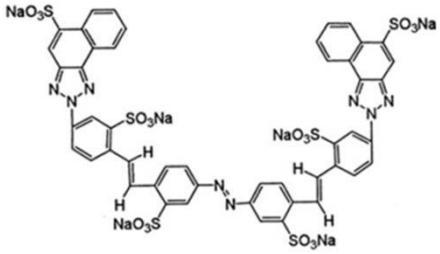
II. MATERIALS AND METHODS

2.1 Materials. Alginic acid sodium salt (AL) from brown algae (medium viscosity), calcium chloride ($CaCl_2$), Chitosan (CH) from crab shells (high viscosity, deacetylation degree ≥ 75%), acetic acid (99.9%) and sodium sulfate anhydrous (99.0%) were purchased from Sigma-Aldrich.

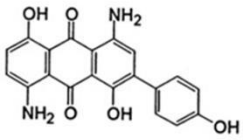
Different commercially available textile dyes, Direct Blue 78 (DB78), Direct Yellow 106 (DY106) and Disperse Blue 73 (DB73) were obtained by Colorprint Fashion S.L., a Spanish textile industry. Their chemical structures and characteristics are reported in Fig. 1 and Table 1, respectively.



Direct Yellow 106 (DY 106)



Direct Yellow 106 (DY 106)



Disperse Blue 73 (DB 73)

Fig. 1: Chemical structures of dyes.

Table 1: Chemical characteristics of dyes.

	Molecular Formula	Molecular Weight	Molecular structure	λ_{max} (nm)
DB78	$C_{42}H_{25}N_7Na_4O_{13}S_4$	1055.91	Tri-azo	601
DY106	$C_{48}H_{26}N_8Na_6O_{18}S_6$	1333.10	Mono-azo	418
DB73	$C_{20}H_{14}N_2O_5$	362.34	Anthraquinone	530

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DB78 and DY106 are direct dyes, a class of dyestuffs applied directly to the substrate in a neutral or alkaline bath and are defined as anionic dyes. DB78 and DY106 are tri-azo and mono-azo compounds and their chemical structures present four and six sulfonate groups respectively.

DB73, classified as a disperse dye, is a non-ionic molecule with an anthraquinone molecular structure which present a scarce solubility in water.

To prepare dyes stock solutions, calculate amount of dye were dissolved in double distilled water and successive dilutions were carried out to obtain solutions at desired concentrations. The pH of aqueous solutions was adjusted to the required value by adding either HCl or NaOH. AL (1% W/V) and $CaCl_2$ (2.5% W/V) solutions were prepared dissolving the required quantity of samples in double distilled water. Chitosan powder was added into an aqueous acetic acid solution (0.8% V/V) to obtain CH solution (0.1% W/V). All chemicals and solvents were used as received without further purification.

2.2 Instruments. UV-Vis absorption spectra were recorded using a Varian CARY 5000 UV-Vis-NIR spectrophotometer (Varian Inc. now Agilent Technologies Inc.).

A FEI Quanta FEG 250 scanning electron microscopy (SEM) was used to investigate the surface morphology of AL-CH beads placing the samples on an aluminum stub.

FTIR-ATR spectra were recorded by means of the Fourier Transform Infrared spectrometer 670-IR (Varian Inc. now Agilent Technologies Inc.) using the attenuated total reflection (ATR) method. Samples were scanned from 600 to 2000 cm^{-1} at a resolution of 4 cm^{-1} and 32 scans were summed for each acquisition.

The thermal analysis of AL and AL-CH beads along with DB78 and DY106 loaded AL-CH beads were performed with differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The experiments were carried out using Q200 TA Instruments and Pyris 1 TGA Perkin Elmer, respectively, under N_2 atmosphere with heating rate of 20°C/min.

2.3 Preparation of alginate and alginate-chitosan beads. Alginate beads were prepared using the method of external gelification. AL solution was extruded dropwise through a needle, with a diameter of 0.8 mm, into calcium chloride solution and the system was maintained under continuous magnetic stirring. The needle was placed at the output tube of a peristaltic pump and a constant flow rate (2 mL/min) was used. This procedure allows to obtain AL beads with approximately the same diameter, since the mean dimension of beads depend on several variable, such as the diameter of the needle used, the distance between the needle and the surface of calcium solution, the flow rate of dropping and the alginate and salt concentrations. AL beads were left in calcium solution for 30 minutes to complete the cross-linker process and to harden them. Then they were collected, repeatedly washed with double distilled water and dried in an oven at 60°C for about 5 hours.

To prepare AL-CH alginate beads, the wet alginate beads were further immersed into chitosan solution for 60 minutes and maintained under continuous stirring. Next, the produced AL-CH beads were collected, washed, and dried in an oven at 60°C.

2.4 Batch adsorption experiments. Dye adsorption processes were performed by batch mode experiments adding specific amounts of adsorbent to a fixed volume of dye solutions in controlling condition of agitation rate (150 rpm), pH and temperature. Every 10 minutes, the residual concentration of dye present in the aqueous solutions was determined by means of UV-Vis spectrophotometry at the maximum absorption wavelength (λ_{max}). Influence of different variables, including contact time, adsorbent dosage, initial dye concentration, pH and temperature were analyzed. These experiments were performed by varying the parameter under evaluation while all other parameters were maintained constant.

The values of dye removal (%) and amount of dye adsorbed onto beads, q_t (mg/g), at time t were respectively calculated using the following equations:

$$\% = \frac{(C_i - C_t)}{C_i} \cdot 100 \qquad \text{Equation (1)}$$

$$q_t = \frac{(C_i - C_t) \cdot V}{m} \qquad \text{Equation (2)}$$

where C_i and C_t (mg/L) are the liquid phase concentration of dye at initial and t adsorption time; V (L) is the initial volume of dye solution and m (g) is the mass of adsorbent.

All tests were performed in triplicate to insure the reproducibility of the results and the mean values were reported.

2.5 Adsorption equilibrium isotherms. The adsorption isotherms allow to understand how the adsorbate interact with the adsorbent putting in relation the concentration of dye in the bulk and that adsorbed on the adsorbent surface when the adsorption process reaches an equilibrium state [28]. So, accurate mathematical models of adsorption isotherms are indispensable to evaluate the adsorption behavior and to describe the equilibrium adsorption of substances from solutions. Although several isotherm models have been developed, in this study, the more common Langmuir and Freundlich models were used. Evaluation of the adsorption isotherms of dyes onto AL-CH beads were performed by adding various quantities of adsorbent to dye solutions. The systems were maintained

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at constant temperature of 298 K under continuous stirring until the equilibrium time. Measurements of dye concentration were conducted before and after the adsorption processes and the obtained experimental data were fitted with Langmuir and Freundlich models.

The values of the linear regression correlation coefficient R^2 give information about the best-fit model. In Table 2 are summarized the Langmuir and Freundlich values.

2.5.1 Langmuir adsorption isotherm. The Langmuir adsorption isotherm model assumes that adsorption takes place on homogeneous sites of adsorbent surface forming a saturated monolayer phase of adsorbate on the outer surface of adsorbent without interaction between adsorbed molecules [29, 30]. The Langmuir model is expressed by equation (3):

$$q_e = \frac{q_m \cdot K_L \cdot C_e}{(1+K_L \cdot C_e)} \quad \text{Equation (3)}$$

where q_e is the amount of adsorbed dye per unit mass of adsorbent at equilibrium (mg/g); C_e is the dye concentration in solution at equilibrium (mg/L); q_m is the maximum amount of the dye per unit mass of adsorbent (mg/g) to form a complete monolayer on surface and K_L is the Langmuir isotherm constant (L/mg) related to the affinity of the binding sites.

High value of K_L suggests much stronger affinity of dye adsorption. The equation (3) can be written in the following linearised form:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L \cdot q_m} \cdot \frac{1}{C_e} \quad \text{Equation (4)}$$

The intercept and slope of the plot between $1/q_e$ versus $1/C_e$ give the values q_m and K_L, respectively (Fig. 7).

2.5.2 Freundlich adsorption isotherm. The Freundlich adsorption isotherm is an empirical equation which describes heterogeneous systems that have unequal available sites on adsorbent surface with different adsorption energies. The Freundlich model can be represented by the equation (5) [31]:

$$q_e = K_F \cdot C_e^{\frac{1}{n}} \quad \text{Equation (5)}$$

The linearised form of Freundlich equation is:

$$\ln q_e = \ln K_F + \frac{1}{n} \cdot \ln C_e \quad \text{Equation (6)}$$

where q_e is the amount of dye adsorbed at equilibrium (mg/g); C_e is the concentration of dye in solution at equilibrium (mg/L); K_F is the Freundlich constant related to the maximum adsorption capacity of adsorbent (L/g) and n is the intensity of adsorption factor related to surface heterogeneity (dimensionless). The magnitude of n gives an indication of the adsorption favorability: values of n > 1 represent favorable adsorption condition [28]. A linear regression plot of ln q_e versus ln C_e (Fig. 7) allows to calculate the values of K_F and n respectively by the intercept and slope.

2.6 Thermodynamic analysis. Thermodynamic parameters, such as Gibb's free energy change (ΔG^o) (J mol^{-1}), enthalpy change (ΔH^o) (J mol^{-1}) and entropy change (ΔS^o) (J mol^{-1} K^{-1}), allow to understand the nature of adsorption. They can be calculated using the following relations [32]:

$$\Delta G° = -RT \ln K_c \quad \text{Equation (7)}$$

$$K_c = \frac{C_i}{C_e} \quad \text{Equation (8)}$$

$$\ln K_c = \frac{\Delta S°}{R} - \frac{\Delta H°}{RT} \quad \text{Equation (9)}$$

$$\Delta G° = \Delta H° - T\Delta S° \quad \text{Equation (10)}$$

where R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and T is the solution temperature (K).

The enthalpy change ($\Delta H°$) and the entropy change (ΔS^o) are obtained from the slope and intercept of the plot of ln K_c versus 1/T (Fig. 8).

2.7 Adsorption Kinetics. The mechanisms that control the adsorption process, such as chemical reaction, diffusion control and mass transfer, can be efficiently investigated by several models based on experimental data. Among these models, adsorption kinetic models are the most commonly used. The parameters obtained by these models allow to determine the uptake rate of solute which is a very useful value for design of full-scale batch adsorption process.

Thus, the adsorption kinetics of anionic dyes onto AL-CH beads were analyzed using the pseudo-first and second order kinetic models. The resultant values along with the corresponding linear regression correlation coefficients R^2 were reported in Table 3 and the best-fit model was selected based on the R^2 values.

2.7.1 Pseudo-first order model. The linearised integral form of the pseudo-first order model was described by Lagergren [33] and generally it can be written in the following form:

$$\log (q_e - q_t) = \log q_e - \frac{K_1}{2.303} t \quad \text{Equation (11)}$$

where q_e (mg/g) and q_t (mg/g) are the amounts of dye absorbed on beads respectively at equilibrium and at each time t and k_1 (min^{-1}) is the pseudo first order rate constant. The Lagergren's first order rate constant, k_1, and the theoretical q_e determined from the model, were calculated respectively by the slope and intercept values of plot log (q_e - q_t) versus t (Fig. 7a and 7c).

2.7.2 Pseudo-second order model. The simplified and linearised equation of pseudo-second order kinetic model is described by the following equation [34]:

$$\frac{t}{q_t} = \frac{1}{K_2 \cdot q_e^2} + \frac{t}{q_e} \quad \text{Equation (12)}$$

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where q_e (mg/g) and k_2 (g/mg min) are respectively the equilibrium adsorption capacity and the pseudo-second order rate constant.

The q_e and k_2 values were determined from the slope and intercept of plot t/q_t vs t (Fig. 7b and 7d). The applicability of the pseudo-second order model suggests that the chemisorption may be the rate-limiting step which controls the adsorption processes.

2.8 Desorption and dyeing experiments. The dyes desorption from AL-CH beads was also studied to determine the feasible reuse of dyes in other dyeing processes. Indeed, this study has the dual objective to remove the dyes from wastewater and to recycle them. After the adsorption step, the AL-CH beads loaded with DB 78 and DY106 were collected and then left in contact with distilled water for 120 minutes at 368 K, under continuous stirring. The final dyes concentration in the liquid phase was measured to determine the amount of dyes release. Then, this colored solution was used to carry out the dyeing experiments on cotton fabric without adjusting the pH of the baths.

The dyeing experiments were performed for 120 minutes at 368 K in presence of increasing amounts of sodium sulfate to promote the dye exhaustion, that is the process of dye transferring from the water to fibers.

III. RESULTS AND DISCUSSION

3.1 Comparison of dyes adsorption: AL vs. AL-CH beads. To identity the best material able to adsorb efficiently the anionic dyes, two different types of adsorbents, AL and AL-CH beads, were compared. 10 mL of DB78 (10.50 mg/L) and DY106 (13.30 mg/L) at pH 6 and 298 K were analysed using 0.5 g of AL and AL-CH beads as adsorbent.

Data reported in Fig. 2a shows that AL-CH beads resulted to be a better adsorbent than AL beads.

Indeed, for DB78, the dye removal efficiency was 32.53% with AL beads, in contrast to the 96.67% when AL-CH beads were used. In the case of DY106, the dye removal was 69.84% and 85.85% for AL beads and AL-CH beads, respectively. This behavior indicates that the possible mechanisms of the adsorption process is mainly based on ionic interactions between the positive amino groups of the chitosan surface and the negative charges of the dyes [35]. Furthermore, in this study, the AL-CH beads were chosen as preferential adsorbent to treat the anionic dyes.

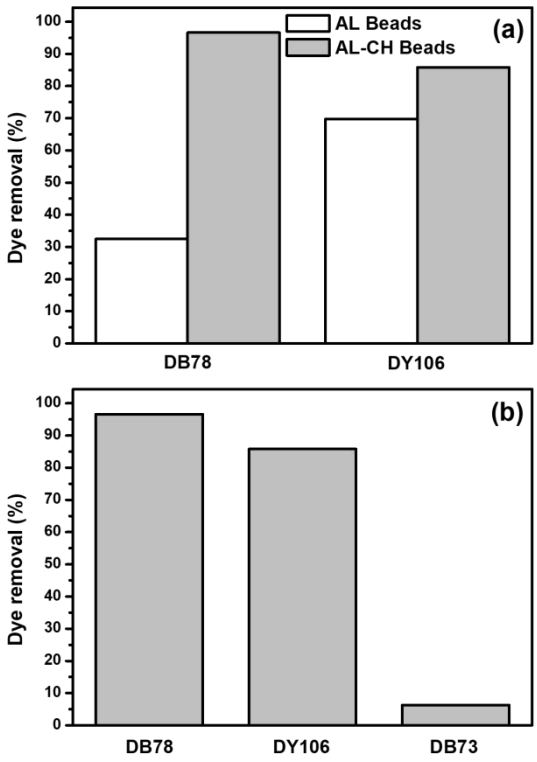

Fig. 2: (a) Adsorption comparison between different adsorbents, AL and AL-CH beads: 10 mL of DB78 (10.50 mg/L) and DY106 (13.30 mg/L) at pH 6 and 298 K using 0.5 g of AL and AL-CH beads, respectively.
(b) Adsorption comparison between different dyes: 10 mL of DB78 (10.50 mg/L), DY106 (13.30 mg/L) and DB73 (18.00 mg/L) at pH 6 and 298 K with 0.5 g of AL-CH beads.

3.2 Adsorption mechanism. To prove that the adsorption process depends on electrostatic attractions between the cationic groups of protonated chitosan and the anionic groups of dyes, the removal efficiency of the two ionic dyes (DB78 and DY106) was compared to those of DB73, characterized by the absence of ionizable groups.

The adsorption of DB78 (10.50 mg/L), DY106 (13.30 mg/L) and DB73 (18.00 mg/L) on 0.5 g of AL-CH beads were separately analyzed at pH 6 and 298 K.

As shown in Fig. 2b, the removal of non-ionic DB73 was a low 6.27% compared with the higher values relative to the anionic dye removal: 96.67% for DB78 and 85.85% for DY106. The great difference in the adsorption percentage indicates that the electrostatic interactions are the main responsible in adsorption, although other weak bonds between dyes and polysaccharide chains cannot be excluded [36].

Moreover, the better adsorption of DB78, in comparison to DY106, could be attributed to the dye chemical structure. Indeed, the presence of four sulfonate groups on

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DB78 structure (Fig. 1), allows to give a higher dye/chitosan molecular ratio than the six groups on DY106 [35].

3.3 Dye removal experiments.

3.3.1 Effect of contact time. 10 mL of DB78 (32.00 mg/L) and DY106 (40.00 mg/L) were stirred with 0.5 g of AL-CH beads at pH 6 and 298 K until 24 hours. To determine the best contact time of adsorption processes, the dye concentrations were measured at different times.

The data reported in Fig. 3a indicate that the removal of both direct dyes increased with time and, in the first minutes of adsorption process, the removal of DB78 was more fast than that of DY106. 84.05% of DB78 was removed from aqueous solution within the first 60 minutes and then the dye removal gradually increased to 95.65% in the next 60 minutes. Thereafter, no further appreciable adsorption occurred, so 120 minutes were deemed as the equilibrium time. Also, the percentage of DY106 removal increased from 67.80% to 83.21% when time was increased from 60 to 120 minutes.

Then, also for this dye, the time required to achieve the equilibrium was about 120 minutes. This could be attribute to the active site saturation of the adsorbent, which do not allow further adsorption [37].

3.3.2 Effect of adsorbent dosage. The adsorption of DB78 (32.00 mg/L) and DY106 (40.00 mg/L) solutions on AL-CH beads was also studied ranging only the adsorbent dosage, from 0.1 to 0.5 g, and maintaining constant the other parameters.

The systems, at pH 6 and 298 K, were stirred until the equilibrium was reached, and the remaining amount of the dye in solutions were measured. Fig. 3b indicates that the percentage of removal for the two dyes increased with the increase of adsorbent amount. Indeed, the dye removal from the initial solutions increased from 64.05% to 95.67% for DB78 and from 70.45% to 84.23% for DY106, as the adsorbent dosage increased from 0.1 to 0.5 g. This result is attributable to the increase in adsorbent surface area with the consequential increase in available adsorption sites [37].

A further increase in adsorbent dosage did not improve the removal percentage of both dyes, hence 0.5 g of AL-CH beads were selected as the optimum adsorbent dosage for the removal of the dyes.

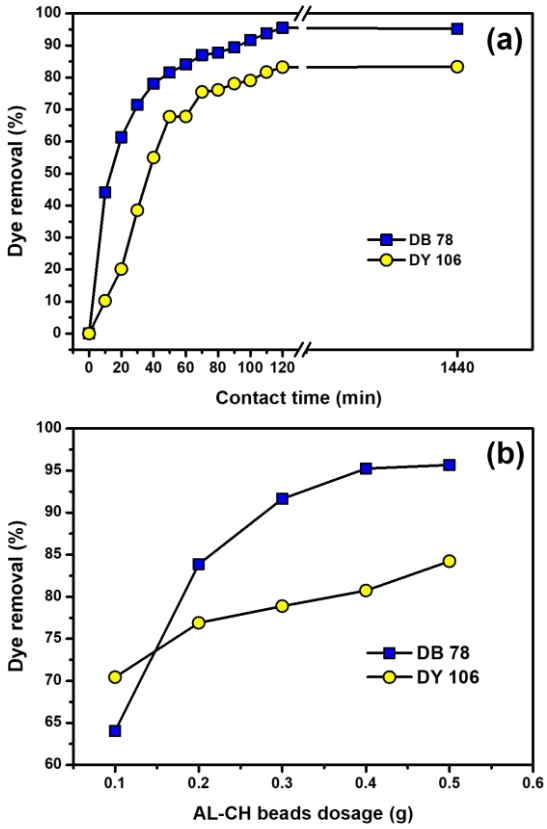

Fig. 3: (a) Effect of contact time on the removal of DB78 (32.00 mg/L) and DY106 (40.00 mg/L). 10 mL of dye solution with 0.5 g of AL-CH beads at pH 6 and 298 K were studied. (b) Effect of AL-CH beads dosage on the removal of DB78 (32.00 mg/L) and DY106 (40.00 mg/L) at pH 6 and 298 K. Increasing amount of adsorbent dosage, in the range from 0.1 to 0.5 g, were added into 10 mL of dye solutions.

3.3.3 Effect of initial dye concentration. Increasing concentrations of DB78 and DY106 solutions were used to study the effect of initial dye concentration on the adsorption mechanism. The experiments were performed at pH 6 and 298 K, using a constant volume of dye solution (10 mL) and a constant dosage of adsorbent (0.5 g). Fig. 4 shows that increasing the dye initial concentration, an increase in the dye adsorption capacity onto AL-CH beads was observed. As the initial concentration of DB78 increased from 10.50 to 52.80 mg/L, the amount of dye adsorbed onto beads at equilibrium, q_e, improved from 0.25 to 1.51 mg/L. In the case of DY106, q_e increased from 0.35 to 1.49 mg/L incrementing the dye initial concentration from 13.30 to 66.60 mg/g. These results suggest that higher initial concentrations of dye provide high driving force able to overcome the dye resistance to the mass transfer between the aqueous and the solid phase [38].

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Besides, observing the adsorption percentage (Fig. 4), it decreased as the initial dye concentration incremented. The dye removal decreased from 96.67% to 92.40% and from 85.85% to 72.20% for increasing concentration of DB78 and DY106, respectively, indicating a reduction in the availability of surface area due to the increment of dye amount. Indeed, for constant amount of adsorbent, increasing the initial dye concentration, the available adsorption sites become fewer, and hence the percentage of removed dye, which depends upon the initial concentration, decreases [39].

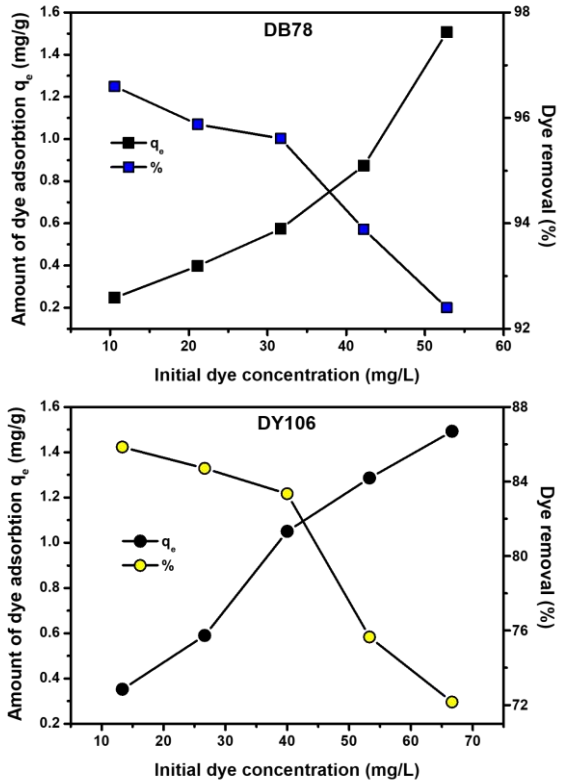

Fig. 4: Effect of initial dye concentration on the adsorption of DB78 and DY106 onto AL-CH beads (volume of dye solution 10 mL, adsorbent dosage 0.5 g, pH 6 and temperature 298 K). Increasing concentrations of dyes in the range from 10.50 to 52.80 mg/L, for DB78, and from 13.30 to 66.60 mg/L, for DY106, were respectively used.

3.3.4 Effect of initial pH. The dye solution pH affects the adsorption process acting not only on the adsorbent external surface charge, but varying also the ionization degree of the solubilized substances, the dissociation of functional groups on the adsorbent active sites, the chemistry of dye solution [37], and the interaction between the alginate and the chitosan surfaces.

Generally, as reported in literature, when chitosan supports are used as adsorbent materials, the percentage of anionic dye removal increases decreasing the pH [20, 26]. Indeed, at low pH, more protons are available to protonate the amino groups of chitosan molecules forming many positive charges ($-NH_3^+$), confirming the essential role of electrostatic attractions between protonated chitosan positive charges and the negative charges of anionic dyes. However, in the present study, a different behavior it was observed: the anionic dye adsorption onto AL-CH beads did not increase with the pH decrease. On the contrary, as shown in Fig. 5, the DB78 and DY106 removal, at different pH values, increased from 83.77% to 96.67% and 71.66% to 85.85%, respectively, when solution pH increased from 2 to 6 units. This result indicates that, probably, in the case of AL-CH beads, the solution pH affects also the ionic interaction between alginate and chitosan functional groups. At low values of pH, the carboxylic groups of alginate polymers are protonated and, consequently, are no longer able to interact with $-NH_3^+$ groups of chitosan. It causes a weakening of beads structure and a significant decrease in the adsorbent efficiency. Simsek-Ege *et al.* [39] have indeed demonstrated that the yield of the complex formation of chitosan coating on alginate was higher when the complex is prepared at pH 5 than at pH 2. Therefore, in our experiments, the solution pH 6 was used to perform all the adsorption processes, considering also that at pH > 6 the AL-CH bead structure becomes very instable.

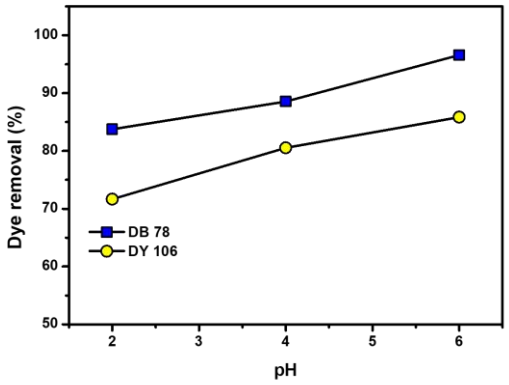

Fig. 5. Effect of initial pH on the removal of DB78 (10.50 mg/L) and DY106 (13.30 mg/L) at increasing pH values (from pH 2 to pH 6). The adsorption of 10 mL of dyes solutions was studied at 298 K using 0.5 g of AL-CH beads.

3.3.5 Effect of temperature. The effect of temperature on AL-CH bead adsorption capacity was investigated increasing temperature values, from 298 to 348 K, maintaining constant both adsorbent amount (0.5 g) and dye solution volume (10 mL) at pH 6.

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Fig. 6a shows that the amount of dye removal decreased from 96.65% to 71.67% for DB78 and from 85.80% to 61.24% for DY106 when the temperature increased from 298 to 328 K as the temperature incremented, indicating an exothermic process in the dye adsorption on AL-CH beads. Even if in the first minutes of process an increase in the temperature seemed to affect the adsorption rate, at the end of the process, when the equilibrium was reached, the increase in the temperature led to a decrease of dye removal (Fig. 6b and 6c).

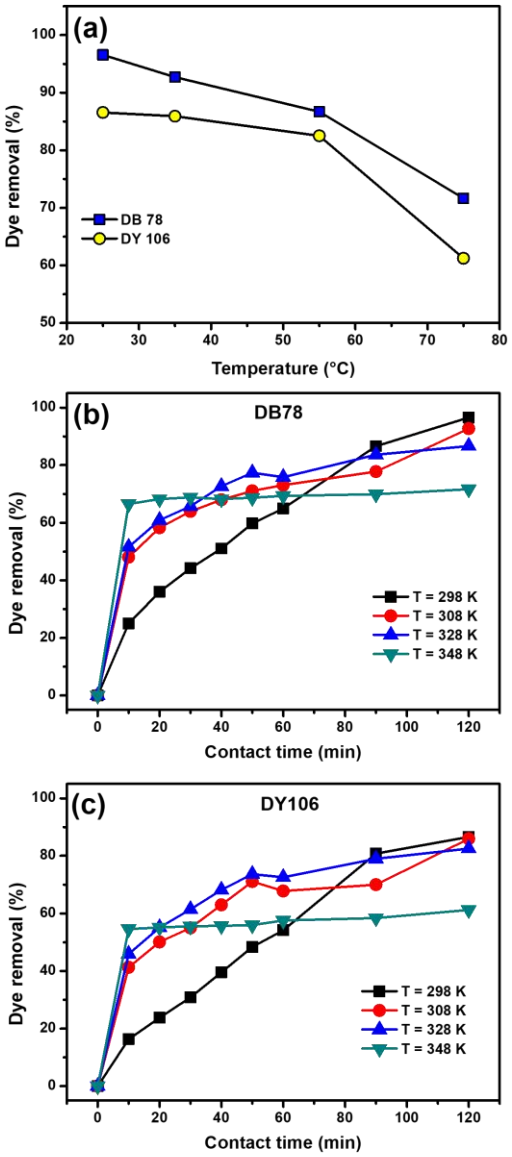

Fig. 6: (a, b, c) Effect of temperature on the removal of DB78 (10.50 mg/L) and DY106 (13.30 mg/L) at increasing temperature values (from 298 to 348 K). The adsorption of 10 mL of dye solutions was studied at pH 6 using 0.5 g of the adsorbent.

This result suggests that the temperature increase determines an increase in the dye diffusion which consequently induces an increase in the adsorption rate. Further, the increase in temperature provokes also an increase of dye solubility, making the interactions between solute and solvent stronger than those between solute and adsorbent. So, the dye adsorption on AL-CH beads becomes more difficult at high temperature. On the other hand, the temperature influences not only the adsorption, but also the desorption processes. Indeed, the release study confirmed the reversibility of the adsorption mechanism.

3.4 Adsorption equilibrium isotherms. The adsorption isotherms of DB78 and DY106 onto AL-CH beads were determined at pH 6 maintaining the system at 298 K. Various adsorbent quantities, in the range of 0.1-0.5 g, were added to 10 mL of DY78 (32.00 mg/L) and DY106 (40.00 mg/L) and the adsorption process was followed until the achievement of the equilibrium state.

The Langmuir and Freundlich adsorption isotherm parameter values for DB78 and DY106 and their plots are showed in Table 2 and in Fig. 7, respectively. The value of the linear regression correlation coefficient R^2 give information about the best-fit model.

Based on the Langmuir isotherm analysis, the maximum monolayer amount, q_m, of DB78 and DY106 adsorbed on beads was only 2.43 and 4.61 mg/g, respectively. These values were much lower than those calculated experimentally. These results suggest that the Langmuir model did not properly describe the anionic dye adsorption process on AL-CH beads. This is also confirmed by values of the linear correlation coefficients R^2 reported in Table 2. Applying the Freundlich isotherm model, the calculated R^2 coefficients resulted indeed higher than the previous ones for the adsorption of both dyes. This indicates that the DB78 and DY106 adsorption onto AL-CH adsorbent could be better described by the Freundlich model than the Langmuir model, suggesting that no monolayer adsorption of dye occurred, involving the heterogeneous surface of the adsorbent material. In addition, the Freundlich factors of heterogeneity, n, were determined as 2.4319, for the DB78, and 1.4688, for the DY106, indicating a favorable adsorption process (n > 1).

Table 2: Adsorption isotherm values for DB78 and DY106.

Dye model	Langmuir model			Freundlich		
	K_L (L/m	q_m (mg/	R^2	K_F (L/g)	n	R^2
DB78	0.3884	2.4325	0.9668	0.7438	2.4319	0.9942
DY106	0.0586	4.6147	0.9713	0.3586	1.4688	0.9842

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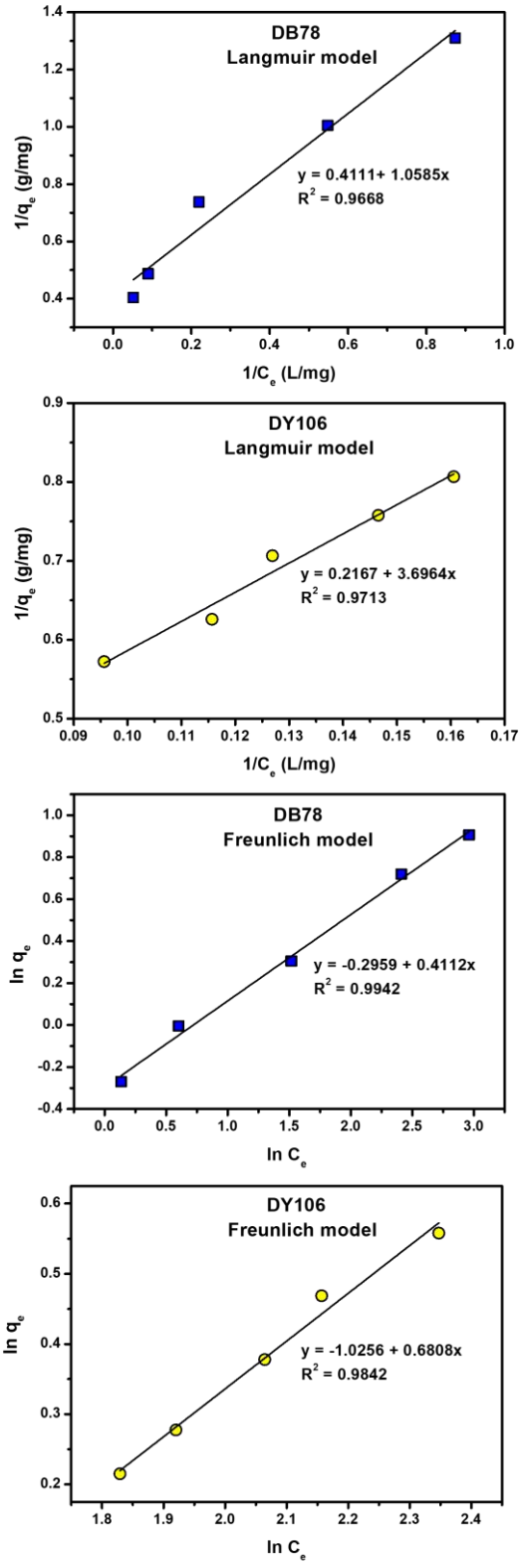

Fig. 7: Adsorption isotherm plots for the adsorption of DB78 (32.00 mg/L) and DY106 (40.00 mg/L) onto AL-CH beads at pH 6 and at constant temperature of 298 K.

3.5 Thermodynamic analysis. The plot of ln K_c versus 1/T, showed in Fig. 8, allowed to calculate the enthalpy change and the entropy change. The calculated ΔH° values of DB78 and DY106 adsorption by AL-CH beads were -34.65 kJ mol^{-1} and -17.77 kJ mol^{-1}, respectively. This indicates that the adsorption followed an exothermic process as already hypothesized. The corresponding values of ΔS° were -89.17 J mol-1 K^{-1} for DB78 and -42.87 J mol-1 K^{-1} for DY106. These negative values indicate that the disorder of the system decreased at the solid–solution interface during dyes adsorption on adsorbent. Moreover, the values of ΔG° at 298, 308, 328 and 348 K are -8.08, -7.18, -5.40 and 3.62 kJ mol^{-1} respectively for DB78 and -4.99, -4.57, -3.71 and -2.85 kJ mol^{-1} respectively for DY106. The negative values of ΔG° indicate the spontaneity and feasibility of the adsorption process. Since when the ΔG° values range between −20 and 0 kJ mol^{-1}, the adsorption is classified as physical adsorption, [32] in this study it is possible to affirm that the anionic dyes adsorption on AL-CH beads was mainly physical, involving electrostatic interactions.

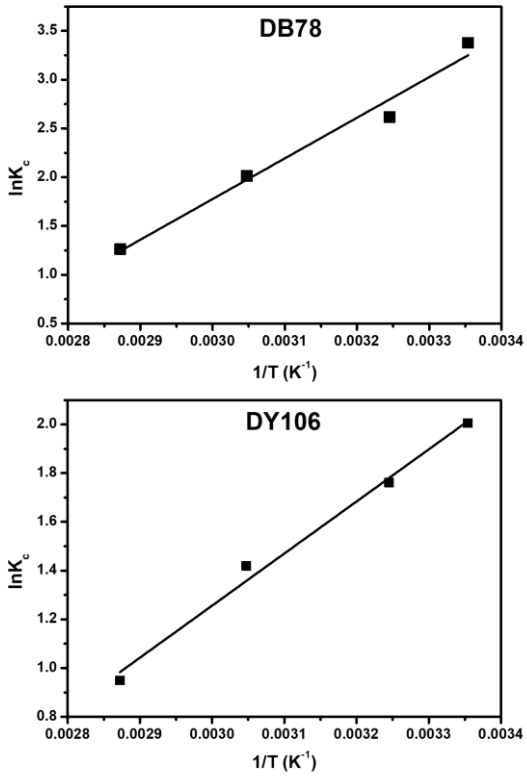

Fig. 8: Plot of ln Kc versus 1/T for DB78 (10.50 mg/L) and DY106 (13.30 mg/L) using 10 mL of dye solutions, pH 6 using 0.5 g of AL-CH beads.

3.6 Adsorption Kinetics. The applicability of the pseudo-first and pseudo-second order kinetic model was also tested for describing the adsorption process of anionic dyes on AL-CH beads.

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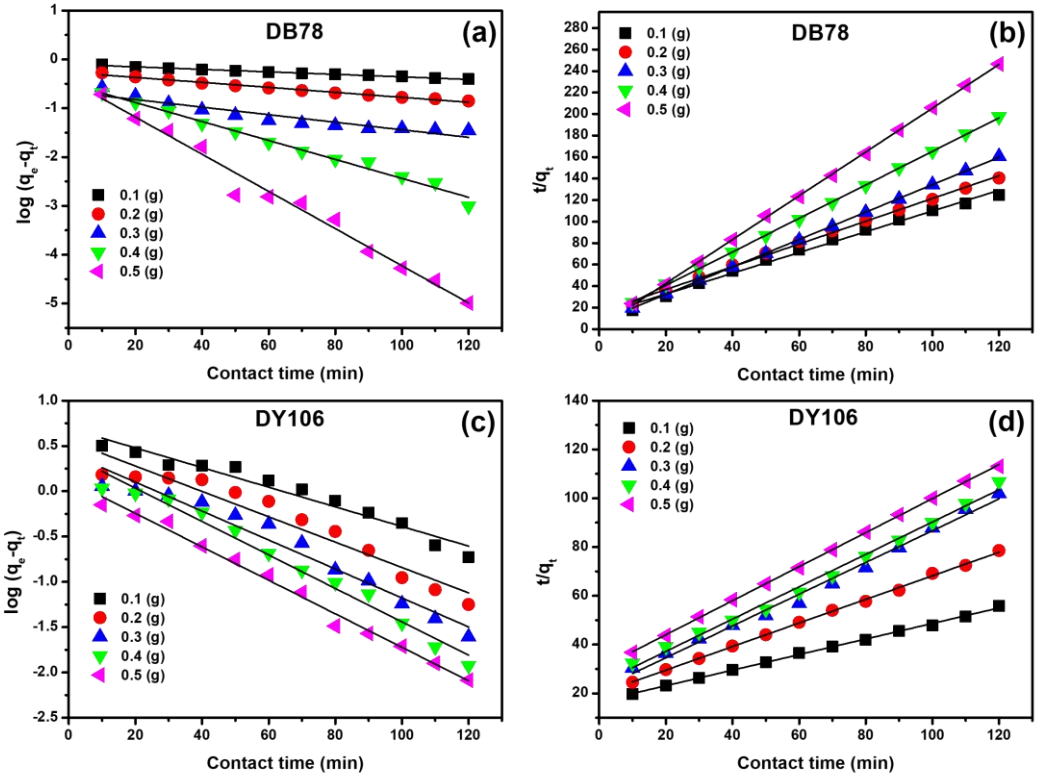

Fig. 9: Adsorption kinetic models of DB78 (32.00 mg/L) and DY106 (53.30 mg/L) onto AL-CH beads (from 0.1 to 0.5 g) at pH 6 and 298 K. Application of pseudo-first order kinetic model at (a) DB78, (c) DY106. Application of pseudo-second order kinetic model at (b) DB78, (d) DY106.

Table 3: Pseudo-first and pseudo-second order kinetic parameters for adsorption of DB78 and DY106 on AL-CH beads.

		Pseudo-first order model				Pseudo-second order model		
Dye	**AL-CH beads dosage (g)**	**q_e^{exp} (mg/g)**	**k_1 (min^{-1})**	**q_e^{the} (mg/g)**	**R^2**	**k_2 (g/mg min)**	**q_e^{the} (mg/g)**	**R^2**
DB78	0.1	1.3564	0.0059	0.1003	0.9884	0.0695	1.0366	0.9923
	0.2	0.9953	0.0117	0.5425	0.9868	0.0714	0.9448	0.9967
	0.3	0.7634	0.0178	0.2151	0.8815	0.2365	0.7844	0.9998
	0.4	0.6087	0.0447	0.3202	0.9858	0.2555	0.6416	0.9997
	0.5	0.4824	0.0877	0.3820	0.9824	2.3202	0.4909	0.9997
DY106	0.1	3.1992	0.0249	4.9709	0.9541	0.0061	3.1314	0.9984
	0.2	2.1961	0.0323	3.6261	0.9267	0.0118	2.0666	0.9987
	0.3	1.7473	0.0368	2.6402	0.9585	0.0197	1.5350	0.9909
	0.4	1.5067	0.0425	2.5417	0.9778	0.0184	1.5062	0.9933
	0.5	1.3684	0.0426	1.3379	0.9899	0.0162	1.4329	0.9997

These models were used for fitting (Fig. 9) experimental data recorded at pH 6 and 298 K using 10 mL of DB78 (32.00 mg/L) and DY106 (53.30 mg/L) onto different amount of adsorbent dosage (from 0.1 to 0.5 g). All kinetic parameters were presented in Tables 3. The linear regression coefficients (R^2) obtained by applying the pseudo-second order kinetics model were higher than those calculated by using the pseudo-first order kinetics model, suggesting that the DB78 and DY106 adsorption on AL-CH beads follows a pseudo-second order kinetics. In addition, as reported in Table 3, the corresponding calculated q_e^{the} values are very close to the experimental ones (q_e^{exp}). These data agree with other adsorption studies based on various chitosan-based adsorbent which reported similar kinetic trends [13].

3.7 Scanning electron microscopy (SEM) analyses. AL-CH beads and DB78 loaded AL-CH beads were analyzed by scanning electron microscopy to study their

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morphology. SEM images (Fig. 10) showed a highly porous and irregular structure, whose cavities are potentially able to adsorb the dyes molecules. This surface morphology agrees with the results obtained by Freundlich model of adsorption equilibrium isotherms, where a heterogeneous adsorption was demonstrated. Moreover, the presence of loaded dyes did not affect significantly the morphology of the samples.

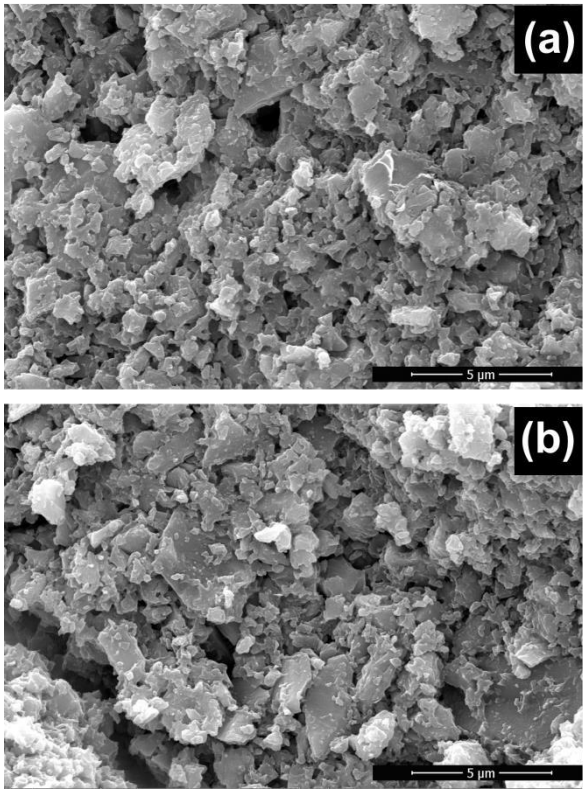

Fig. 10: SEM images. (a) AL-CH beads. (b) DB78 loaded AL-CH beads

3.8 FTIR-ATR spectroscopy measurements. Infrared (IR) spectra of studied samples (Fig. 11) were recorded to confirm the alginate-chitosan interactions and to better understand the dye-chitosan interactions.

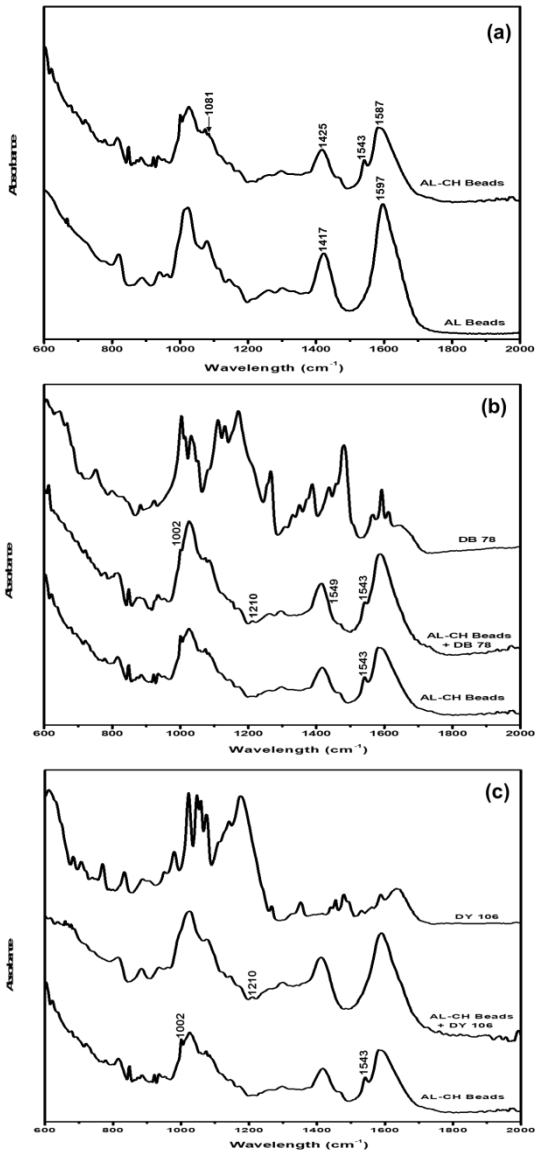

Fig. 11: ATR-FTIR spectra of adsorbent system. (a) AL beads and AL-CH beads spectra. (b) DB78, DB78 loaded AL-CH beads and AL-CH beads spectra. (c) DY106, DY106 loaded AL-CH beads and AL-CH beads spectra.

In Fig. 11a the spectra of AL beads and AL-CH beads were showed. AL beads displayed two intense absorption bands at 1597 cm^{-1} and at 1417 cm^{-1}, characteristic of alginate, assigned to the asymmetric and symmetric stretching of carboxylate groups [40, 41], that in the AL-CH bead spectrum, resulted shifted to 1587 cm^{-1} and to 1415 cm^{-1}, respectively [42]. In addition, in the AL-CH IR spectrum, a new less intense peak appeared at 1543 cm^{-1}, attributable to the N-H bending vibration characteristic of chitosan amide II band, although the signal was shifted respect to the corresponding one in pure chitosan (1599 cm^{-1}). These results confirm the chitosan coating of alginate beads by means of electrostatic interactions involving the carboxylic groups

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of AL-beads [41]. Moreover, new peaks were observed in AL-CH bead FTIR spectrum: a very weak signal at 1081 cm^{-1}, which may be attributed to the secondary hydroxyl group (C-O stretch mode relative to -CH-OH in cyclic alcohols) [41], and another one at around 1000 cm^{-1} corresponding to the variations of C-O stretching in the ring relative to the C-OH, C-O-C and CH_2OH moieties [43].

The FTIR spectra of AL-CH beads before and after the adsorption of DB78 and DY106 were also compared. In the spectrum of DB78 loaded AL-CH beads (Fig. 11b), a reduction in intensity of chitosan signal at 1543 cm^{-1} was observed compared to the corresponding signal in the AL-CH bead spectrum, suggesting the presence of dye-chitosan interaction through chitosan amide group. Additionally, a further intensity reduction of chitosan signal at 1002 cm^{-1} was also observed.

On the other hand, two new peaks were observed on AL-CH beads in presence of DB78: a very weak signal at 1459 cm^{-1} attributable to aromatic dye rings and another at 1210 cm^{-1}, corresponding to dye $-SO_3^-$ groups [44]. These signals confirm the addition of dye on the chitosan polymer shell.

A similar behavior was observed in the case of FTIR spectra of DY106 loaded AL-CH beads (Fig. 11c). The total absence of peaks at 1543 and 1002 cm^{-1} and the presence of a new weak peak at 1210 cm^{-1} were observed.

3.9 Thermal analysis. The thermal behavior of AL beads and AL-CH beads loaded with DB78 and DY106 was investigated by DSC and TGA. As shown in Fig. 12a, the DSC thermograms for sodium alginate powder, chitosan powder, AL beads and AL-CH beads exhibited a broad endothermic peak at about 90°C attributed to the loss of water associated to hydrophilic groups of AL and CH polymers [45]. The exothermic peak at about 245°C and the double exothermic endothermic peaks at about 180°C and 190°C for the AL powder, AL-CH beads and AL beads, respectively, indicated the beginning of a multistep decomposition process also confirmed by thermogravimetric curves (Fig. 13a), in agreement with literature [46].

The two dyes, DB78 and DY106, were thermally more stable than adsorbent beads as shown by DSC and TG curves in Fig. 12b and 13b. So, also AL-CH beads loaded with DB78 and DY106 were more stable than unloaded beads. Finally, these results confirmed the presence of interactions between the two dyes and Al-CH beads and showed that the loaded or unloaded beads have good thermal stability suitable for the practical application herein proposed.

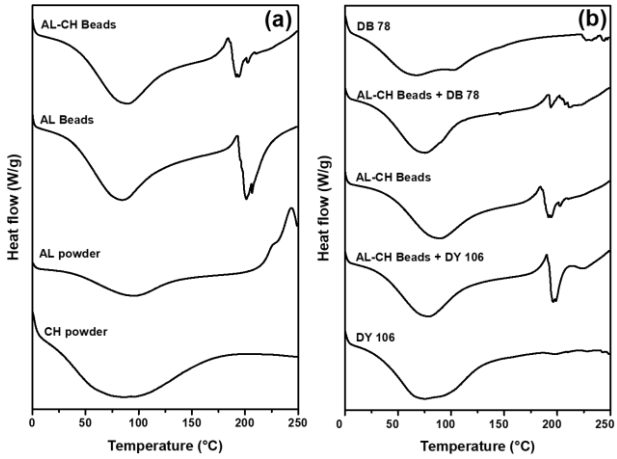

Fig. 12: Thermograms obtained by DSC for pure materials, beads and loaded beads. (a) Thermograms of AL powder, CH powder, AL beads, AL-CH beads. (b) Thermograms of DB78, DY106, DB78 loaded AL-CH beads, DY106 loaded AL-CH beads and AL-CH beads.

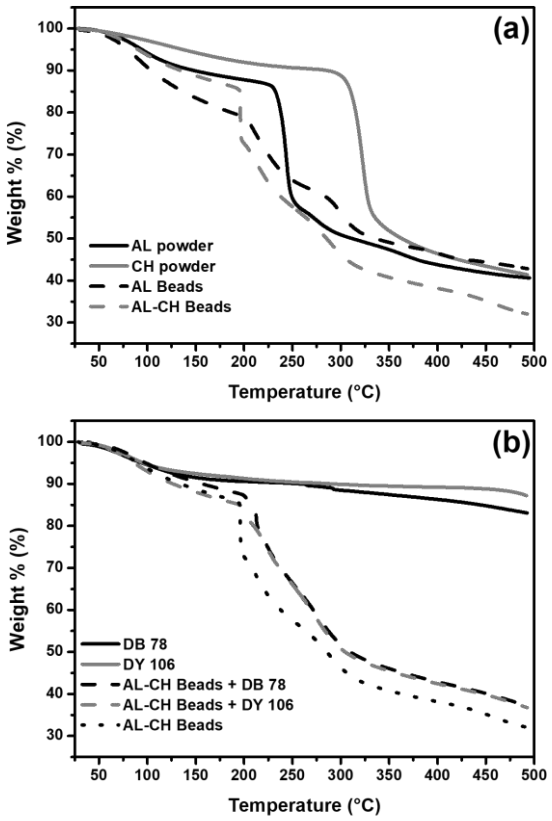

Fig. 13: Thermograms obtained by TGA for pure materials, beads and loaded beads. (a) Thermograms of AL powder, CH powder, AL beads, AL-CH beads. (b) Thermograms of DB78, DY106, DB78 loaded AL-CH beads, DY106 loaded AL-CH beads and AL-CH beads.

3.10 Desorption and dyeing experiments. The desorption of DB78 and DY106 from AL-CH beads was

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studied adding 0.6 g of dye loaded beads in 10 mL of distilled water for 120 minutes at 368 K, under continuous stirring. The final concentration of the adsorbate in the liquid phase was measured and the obtained amount of desorbed dye was 15.27 mg/L for DB78 and 20.16 mg/L for DY106. The amount of released dye was about 50% for both dyes, if compared to the initial quantity of adsorbed dyes.

These experiments confirm that the temperature affects both the adsorption and the desorption processes, indicating that the adsorption mechanism is a reversible process thanks to weak electrostatic interactions between adsorbate and adsorbent. Successively, the colored solutions obtained from desorption experiments, were directly used to dye some surfaces of cotton fabric. Small pieces (1.5×1.5 cm) of a white cotton fabric, with a superficial area equal to 2.25 cm^2, were immersed in 10 mL of colored solution for 120 minutes at high temperature (368 K) in presence of increasing amounts of sodium sulfate (10, 15 and 20 g/L) to promote the dye transferring from solution to fibers of fabric. Then, the amount of dye adsorbed on fabric (mg/cm^2) were measured and their values are reported in Fig. 14. The effect of the salt amount on the dye exhaustion is also clearly visible in the photos where the final outcome on fabrics was shown in Fig. 15. The results indicate that increasing the sodium sulfate concentration, the dyestuff coloring ability increments, suggesting a neutralization of cotton negative charge by sodium ions in the dye bath, favoring the fabric dyeing [47].

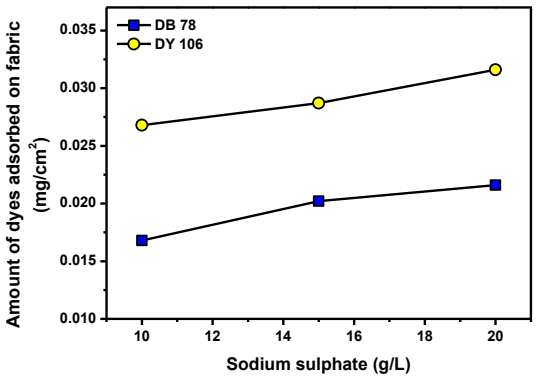

Fig. 14: Plot of amount of DB78 and DY106 dyes adsorbed on fabric vs. sodium sulfate concentration (10, 15 and 20 g/L). Dyeing experiments were performed at 368 K for 120 minutes.

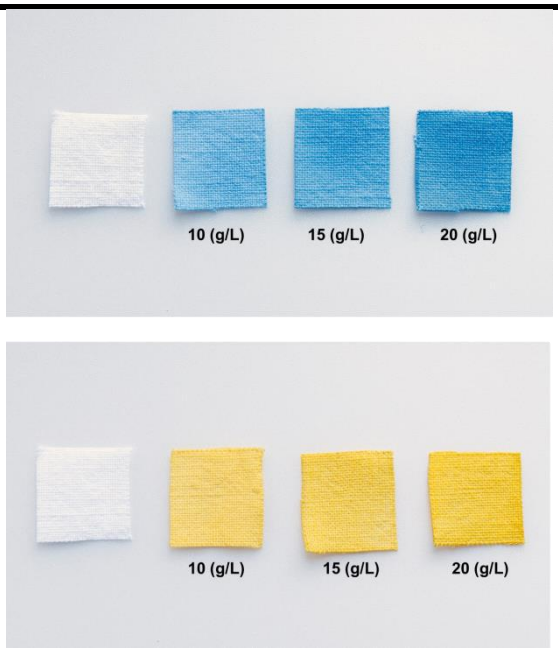

Fig. 15: Photographs of the outcome after the dyeing process on fabric in presence of increasing concentration of sodium sulfate (10, 15 and 20 g/L).

IV. CONCLUSION

Chitosan-alginate beads demonstrated to have a great potential as adsorbent material for the removal of anionic dyes from textile wastewater at room temperature and pH 6. The study conducted on alginate beads and on a nonionic dye established that the direct dyes interact with the chitosan shell by means of electrostatic interactions between the dye sulfonate groups and the cationic amino groups of protonated chitosan. The results showed a higher adsorption affinity of DB78 compared to DY106, owing to the differences in the molecular weight of the dye molecules and the number of sulfonate groups on each dye. About the isotherm analysis, the Freundlich isotherm model was found to provide the best prediction for the dye adsorption process, suggesting a heterogeneous adsorption on the adsorbent surface. Adsorption kinetics studies reported that a pseudo-second order kinetic provided the best correlation of the experimental data. Thermodynamic analysis demonstrated that the adsorption is an exothermic, spontaneous, and physical process.

Moreover, it was demonstrated that the studied system has not only the ability to remove dyes from wastewater, reducing the pollution, but also the ability to desorb them for further dyeing processes, considering the point of view of a sustainable recycling economy of textile dyeing process.

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Alleviation of Salinity Effects by Poultry Manure and Gibberellin Application on growth and Peroxidase activity in pepper

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***Abstract*—** *Capsicum is one of the most widely consumed vegetables and is also used as a spice for its pungency. Many species of Capsicum are being cultivated worldwide. Capsicum is considered as a commercial crop for their economic value. However, the yield of the crop suffers severely due to salt stress, Soil salinity reduces water availability of plant roots via negative (low) osmosis potential, as well as decrease of germination dynamics of plant seeds by ionic toxicity of Na and Cl , Significant differences in fruit-set, yield, photo synthetic rates, stomata conductance, total chlorophyll content, proline, In general, salinity affects almost every aspect of the physiology and biochemistry of plants.*

The aim of this study was to determine the salt tolerance of pepper (Capsicum annuum L) under salinity stress by saline irrigation water, Poultry and gibberellins applications were used to alleviated the negative effects on growth parameters and yield of Pepper under salinity stress.

The water salinity levels led to a significant elevation in the values of electrical conductivity of the soil with the peroxidase activity, and Sodium and proline contents in leaves, while resulting in decrease in growth parameters and leave contents of (NPK),The poultry and gibberellins applications increased the growth parameters (Dry weight of shoot and root &fruit weight) and (NPK) contents in leaves with slight dropping of peroxidase activity in leaves while a clear dropping of sodium and proline contents in leave.

That possible to mitigation the negative affect of salt stress by some application like exogenous hormones and Decomposed organic matter to solve the disruption of endohormons and lack of available nutrients under salt stress, and elevation of osmotic stress in soil solution in roots area.

The GA & poultry application improved the growth and it has increased the Pepper tolerance to the abiotic stress which was exerted by saline irrigation water.

Keywords*— *salinity, salt stress, pepper, Gibberellins, organic matter, poultry manure, nutrient availability.

I. INTRODUCTION

Growth and productivity of the plants are affected due to many abiotic stresses like salinity, heat, cold and drought etc.**(Sana et al., 2016)** Which are leading towards hundreds of billions of crop losses each year (Atkinson, N.J. and P.E. Urwin. 2012**).** Soil salinity is the most devastating among them **(Shahbaz, M. and M. Ashraf. 2013)** which not only limits plant growth and metabolism but also poses a foremost intimidation to sustainable agricultural production throughout the world particularly in arid and semi- arid areas **(Tayyab et al.,2016),**More than 400 million hectares of the total geographical area of the world are affected by high concentration of the soluble salts **(Sana et al., 2016)** ,Salt stress severely inhibits plant growth for two reasons: first by an osmotic or water- deficit effect of salinity and second by a salt-specific or ion-excess effect of NaCl. Moreover, plants subject to salinity stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, proteins, and nucleic acids (Abbaspour,2012), Salinization can also lead to excess intracellular production of reactive oxygen species (ROS) such as the superoxide radical ($O_2^{\cdot -}$), the hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) **6- (AL-Taey and Saadoon,2012)**,The tolerance of salinity is specific for each species or cultivar. Vegetables have a high sensitivity to the effects of NaCl **(Zhu, J.K. 2002)** which hinders growth because of its toxic and osmotic effects, respectively, causing accumulation of ions in the protoplasm and physiological drought**(Deuner et al.,2011)**,to defend against such oxidants, plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes that protect against the potentially-cytotoxic species of activated oxygen. Adaptation to salt stress requires alterations ingene.

Pepper (*Capsicum annuum*L.) is the second most widely consumed vegetable in the world and an excellent source of many essential nutrients for humans, especially vitamin C, phenolic compounds,

flavonoids, to copherols (vita-min E), carotenoids (pro vitamin A), capsaicinoids, and calcium. Additionally, some pepper cultivars contain significant quantities of capsaicinoids, a group of pungent phenolic derived compounds with strong physiological and pharmacological properties. Thus, the growing global demand of pepper fruits implies several strategies to increase crop production and fruit quality or promote the investigation to improve the plant resistance to environmental **stresses (Jimenez-Garcia et al.,2014),** Pepper is a moderately sensitive to salt stress **(Lee**,2006) and it is grown under protected glasshouse conditions in temperate regions and in the open field under warm Mediterranean climates, it is frequently exposed to saline conditions brought about by saline irrigation water containing amounts of salts including sodium chloride**(Kijne,2003)**

Salinization promotes an imbalance in the absorption of essential nutrients, causing metabolic disorders, which inhibit growth **(Maia, et al, 2012)**there are an extensive number of plant nutrition studies from all over the world, but the studies were mostly conducted to determine best management practices under non-saline conditions. Some studies have been conducted to determine if certain nutrients have alleviative effects on salinity tolerance **(El-Sidding and Ludders, 1994)**. Some studies indicated a positive effect of fertility on salt tolerance while some reported that there was no alleviative effect on salt tolerance, some Studies showed that application of fertilizers in saline soils might result in increased, decreased or unchanged plant salt tolerance. In other words, plant response to fertilizers depends on severity of salt stress in the root zone**(Faiza and Amin,2009)** However, in another similar study to **(Gomez, et al,1996)**, found a positive yield response for pepper at all three salinity levels by increasing nutrient N from 2 to 15 mM in a solution culture. However the effect of N on relative yield was not clear. The first salinity level above the control (25 mMNaCl) had a lower relative yield at lower N and with subsequent increases in salinity it had a higher relative yield.

phytohormons are considered the most important endogenous substances for modulating physiological and molecular responses, a critical requirement for plant survival as sessile organisms , Phytohormons act either at their site of synthesis or elsewhere in plants following their transport**(Shabir,et al,2016).**

The gibberellins (GAs) are a large group of tetracyclic diterpenoid carboxylic acids, The GAs show positive effects on seed germination, leaf expansion, stem elongation, flower and trichome initiation, and flower and fruit development , They are essential for plants throughout their life cycle for growth-stimulatory functions. They also promote developmental phase transitions. Interestingly, there is increasing evidence for their vital roles in abiotic stress response and adaptation **(Colebrook, et al., 2014).**Recently, experiments have been performed to investigate the role of GAs in osmotic stress response in Arabidopsis thaliana seedlings **(Skirycz, et al, 2012; Maggio, et al, 2010)**reported that GA3 treatment in tomato reduced stomata resistance and enhanced plant water use at low salinity. Likewise, GA3-priming increases grain yield due to the GA3-priming-induced modulation of ion uptake and partitioning (within the shoots and roots) as well as hormone homeostasis under saline conditions.

GAs are known to interact with all other phytohormons in numerous developmental and stimulus-response processes,the interactions between GA and ET include both negative and positive mutual regulation depending on the tissue and signaling case **(Munteanu, et al., 2014)**

Objectives:

The aim of this study was to determine the salt tolerance of pepper (Capsicum annuum L) under salinity stress by saline irrigation water, Poultry and gibberellins applications were used to alleviated the negative effects on growth parameters and yield of Pepper under salinity stress.

II. MARTIAL & METHODS

This experiment was conducted under glass house of horticulture department, collage of in AL- Qasim green university at Novemb1st 2015, the Sweet pepper (Capsicum annuum L.) of RIDA cultivar from Netherland was used. The seedlings were planted in plastic pots containing 10 kg of soil (six pots for each treatment).Each one supplied with 0.5 gm of NPK and granular fungicide. Seedlings were irrigated with river water (1.2 dS.m-1 /cm) for ten days twice a day before salinity treatment, followed by irrigation (half of seedlings) with salted water (6 dS.m-1 /cm) every day until seedlings were reaching 80 days old.

Plants were sprayed twice with of GA (0, 250 mg /L) the first spray was two weeks after germination, the second spray was 4 weeks after the first spray.

Experiment was conducted according to split-split plot design with threefactors, The main factor is the water quality (1.2 dS.m-1 represented river water (W1) & 6 dS.m-1 represented saline water (W2),the second factor (sub- plot) is the poultry fertilization levels with 10% (O1) &30% (O2),The third factor (sub-sub-plot) is gibberellin levels with (0, 250 mg/liter)The Gibberellin 0% (G1) & the 250 mg/liter (G2.) ,the data were analyzed statistically with Genstat discovery software. Means were statistically compared by L.S.D testat p<5%

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level.

The figure (1) below show the experiment planer, included 24 treatments

W1 O1 G1	W2 O2 G2	W1 O1 G2
W1 O2 G1	W2 O1 G2	W1 O2 G1
W1 O1 G2	W2 O1 G1	W1 O2 G2
W1 O2 G2	W2 O2 G1	W1 O1 G1

W2 O2 G2	W1 O1 G1	W2 O2 G1
W2 O1 G2	W1 O2 G1	W2 O1 G2
W2 O1 G1	W1 O1 G2	W2 O1 G1
W2 O2 G1	W1 O2 G2	W2 O2 G2

Measurement of growth attributes

Three plants were harvested randomly from four replicates at mature stage (90 days after sowing). Plant height, Root length, number of leaves, leaf area, number of fruits, fresh and dry biomass (g) were recorded in harvested plants Na,K Samples of leaf, stem and root were taken at grand period of growth for the analysis of different Cations (Na+, K+). Samples were dried and 0.5gm of each dry sample was taken for ash weight. Then solution of ash was made in 50ml of de-ionized water, and then dilutions were made in de-ionized water for mineral analysis. Concentration of Cations in samples was measured using PFP 1 Flame Photometer according to **(Wiessmann and Nehring, 1960)**,the nitrogen determination according to (**Jackson, 1958)** while the determination of phosphorus in leaves was measured according to (Page, et al, 1982)

Determination of Peroxidase Activity

This was determined by measuring the increase in absorbance at 510 nm resulting from the decomposition of hydrogen peroxide **(Trinder,1966)**the Lambda 25 UV/Vis spectrometer (Perkin Elmer) was adjusted to 510 nm. The blank was a mixture of 1.4 ml of phosphate buffer and 1.4 ml of H2O2 in the cuvette. The assay mixture contained 1.4 ml of phosphate buffer, 1.4 ml of H2O2 and 0.2 ml of the extract. The increase in absorbance at 510nm was recorded for 4 minutes. Then, ΔA240/min was calculated from the initial (45 second) linear portion of thecurve.

Determination of proline.

Proline colorimetric determination preceded according to **(Bates, et al., 1973; Marin, et al, 2010)**based on proline's reaction with ninhydrin ratio of 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. Thereaction was arrested in an iced bath and the chromophorewas extracted with 1 ml toluene and its absorbance at 520 nm was determined spectrophotometerically .0.1 gm of shoot and root tissues was suspended with 1 ml of 3% sulfosalicylic acid and after centrifugation (10min at12,000 rpm) was mixed in a 1:1:1 ratio with ninhydrin acid and glacial acetic acid. The reaction and determination of proline were carried out similarly tothat described above The concentration of proline in tissues were determined dependingon standard curve of pureproline.

III. RESULTS

1- Dry weight of shoot and root, fruit weight & chlorophyll content in leaves.

The figures (2,3,4&5) show a significant effect of poultry manure at 30% concentration on dry weight of shoot and root , fruit weight and chlorophyll content in leaves with boost rat was (130% ,93% , 99% & 13%) , sequentially according to 10% of poultry manure concentration , In a similar manner to gibberellin application of 250 mg /liter with boost rate was (31% ,42% , 84% & 14%) , sequentially according to 0 mg/liter of gibberellin concentration, but there is significant drop to dry weight of shoot and root &fruit weight with raising of water salinity ,the dropping rat was (45%, 34% , 58% & 7%) g , sequentially, the interaction treatment among (poultry 30% +gibberellin 250 mg + irrigation water 1.2 ds/m) achieved the highest means in dry weight of shoot and root, fruit weight & chlorophyll content in leaves while the lowest means at treatment (poultry10 %, gibberellin 0%,irrigation water 6ds/m) ,the application of poultry manure and gibberellin treatment alleviated the negative affect of saline water in dry weight of shoot and root , fruit weight &chlorophyll content with (3.76 , 2.11, 21.9& 49.9) , sequentially according to treatment which irrigated by saline water without poultry and gibberellin application which recorded (1.48, 0.65 , 9.8 & 44.9) sequentially.

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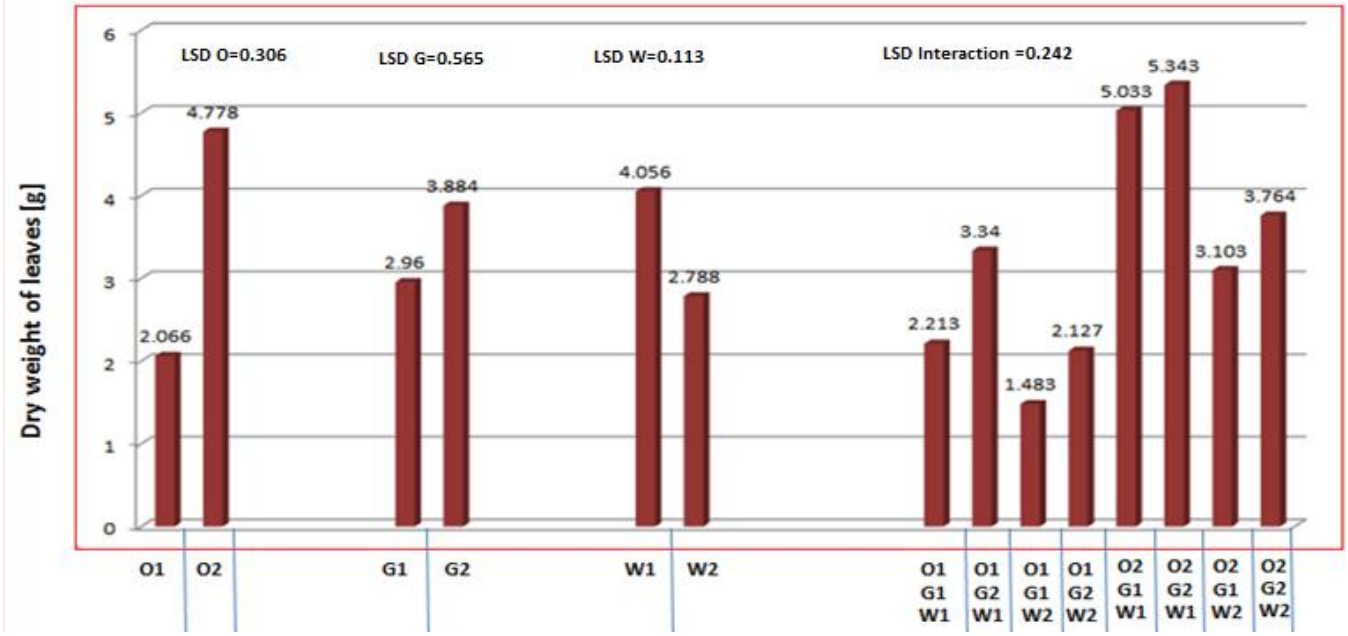

Fig.2: Shows the effect of water quality, poultry manure& gibberellin with interaction betweenthem on the dry weightofleaves Water quality (W) Poultry manure (O) Gibberellin(g)

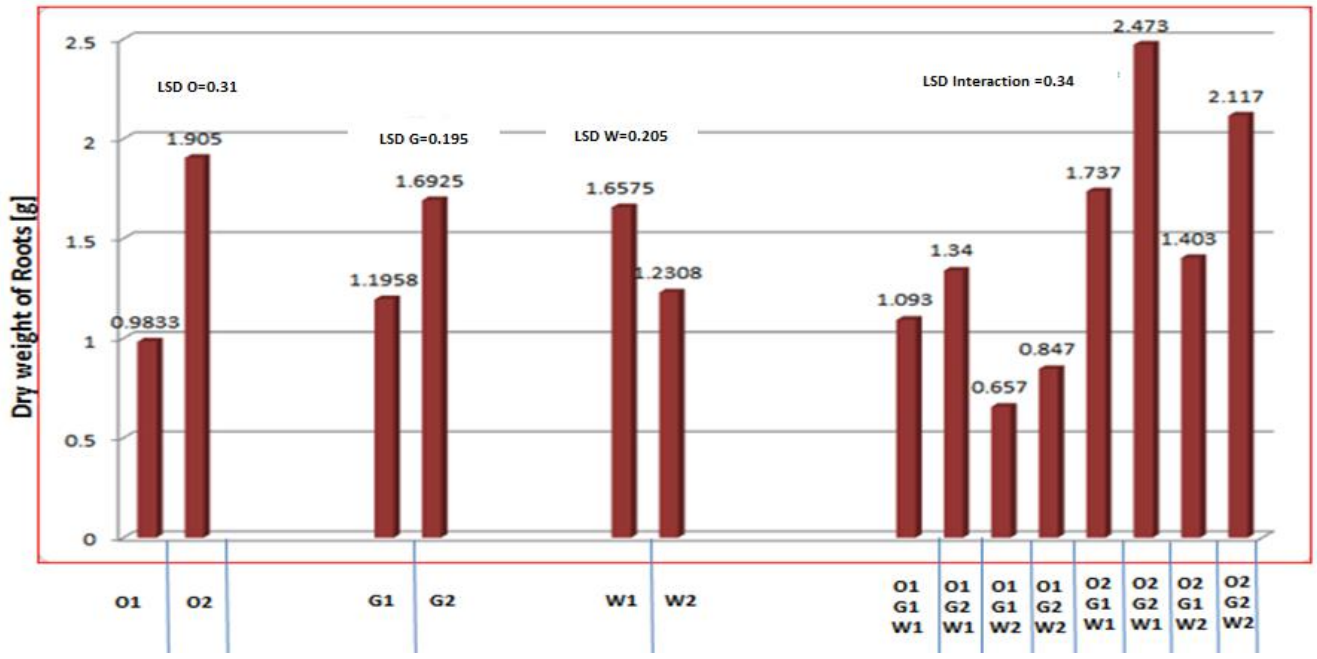

Fig.3: shows the effect of water quality, poultry manure& gibberellin with interaction betweenthem on the dry weightof root Water quality (W) Poultry manure (O) Gibberellin(g)

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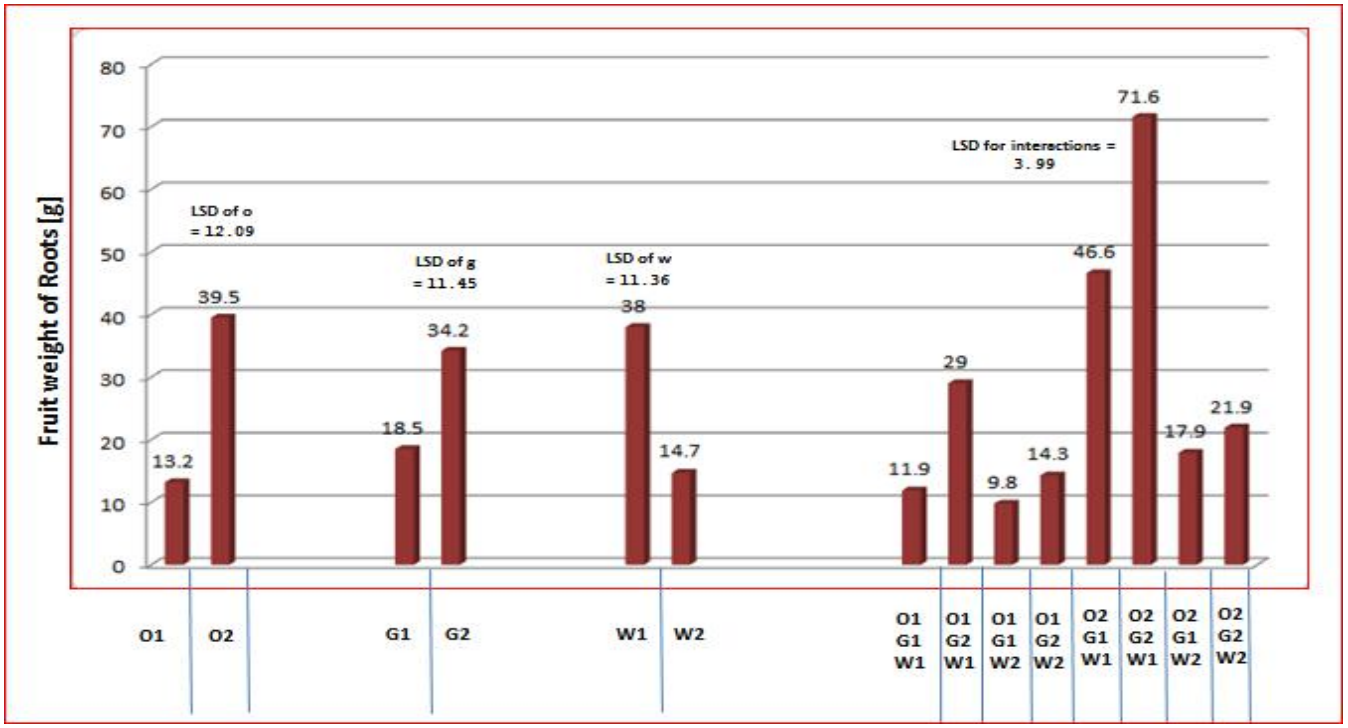

Fig.4: Shows the effect of water quality, poultry manure& gibberellin with interaction betweenthem on the fruit weight Water quality (W) Poultry manure (O) Gibberellin(g)

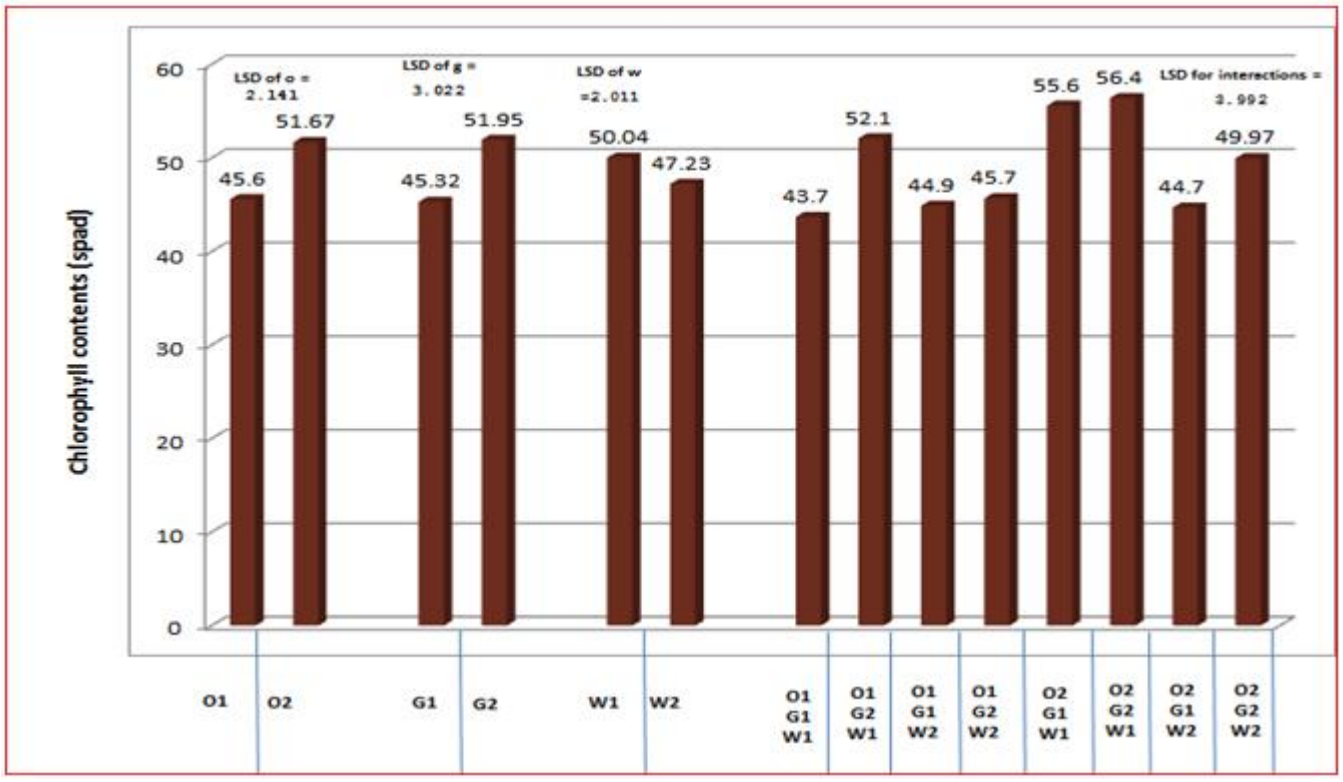

Fig.5: shows the effect of water quality, poultry manure& gibberellin with interaction between them on the chlorophyll contentinspad. Water quality (W) Poultry manure (O) Gibberellin(g)

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2-The Nitrogen, Phosphor, Potassium and Sodium in the leaves

The figures (6, 7, 8&10) show a significant effect of poultry manure at 30% concentration on Nitrogen, Phosphorus, Potassium content and K/Na ratio in the leaves with boost rate was (%70, 64%, 23%, &78 %) , sequentially compare with 10% of poultry manure concentration , while the poultry fertilization due to reduction of sodium uptake in root nearly (38%) figure(9), in comparison with treatments with 10% poultry fertilization. The gibberellin application of 250 mg /liter with increasing percentage of Nitrogen, Phosphorus, Potassium and K/Na (26%, 16%, 8% & 14%), sequentially according to 0% gibberellin figures (6,7,8 &10), same the way the gibberellin application led to reduction the Sodium content in leaves approximately 6%, figure(9).

The saline water led to reduction in nitrogen, phosphorus, potassium and K/Na ratio in the leaves compare to river water, there was anincrease differences in Sodium content in leaves when saline water was applied (figure9).

The interaction of water quality, poultry litter and gibberellin (W1O2G2) affected in nitrogen**,** phosphorus, potassium, content and the K/Na ratio compared with (W1O1G1), Treatment (W1O2G2) produced lowest Sodium content compared with treatment (W2O1G1) figure 9.

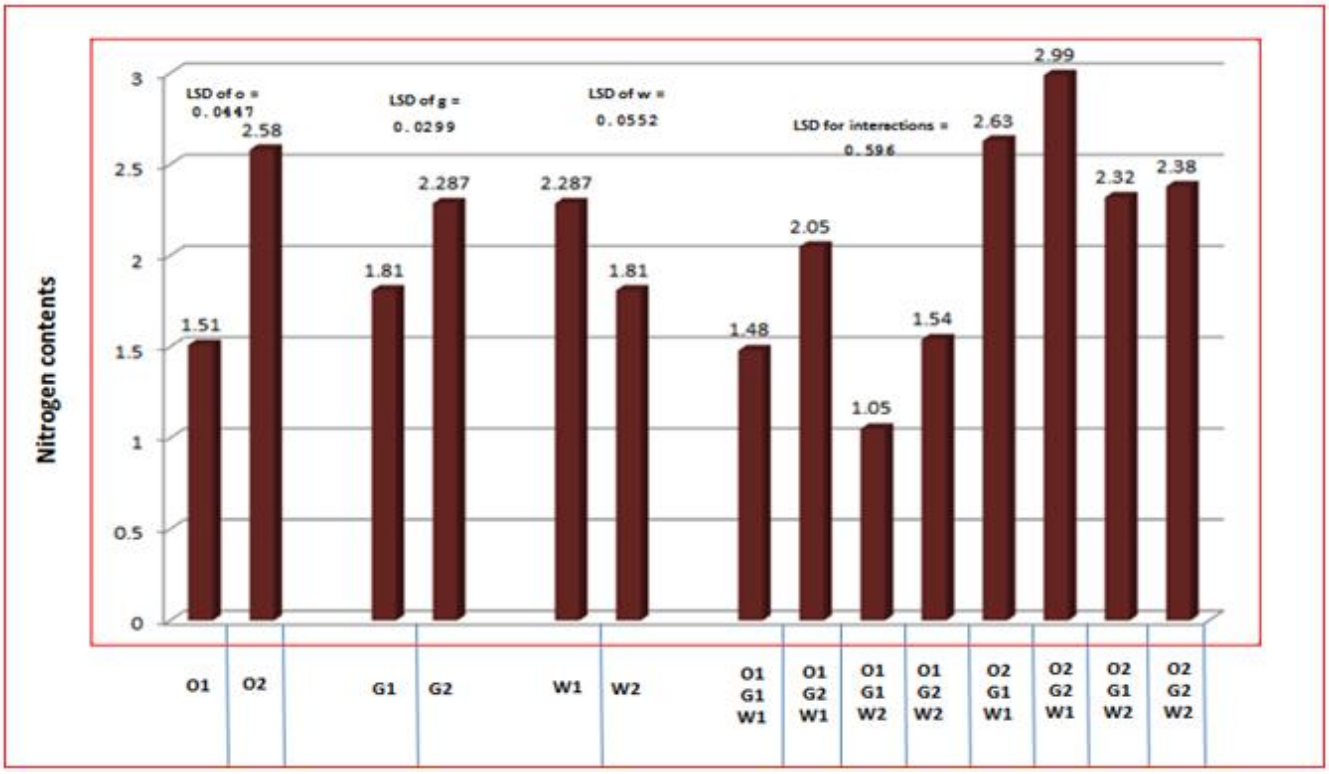

Fig.6: shows the effect of water quality, poultry manure& gibberellin with interaction betweenthem on the N contentinleaves. Water quality (W) Poultry manure (O) Gibberellin(g)

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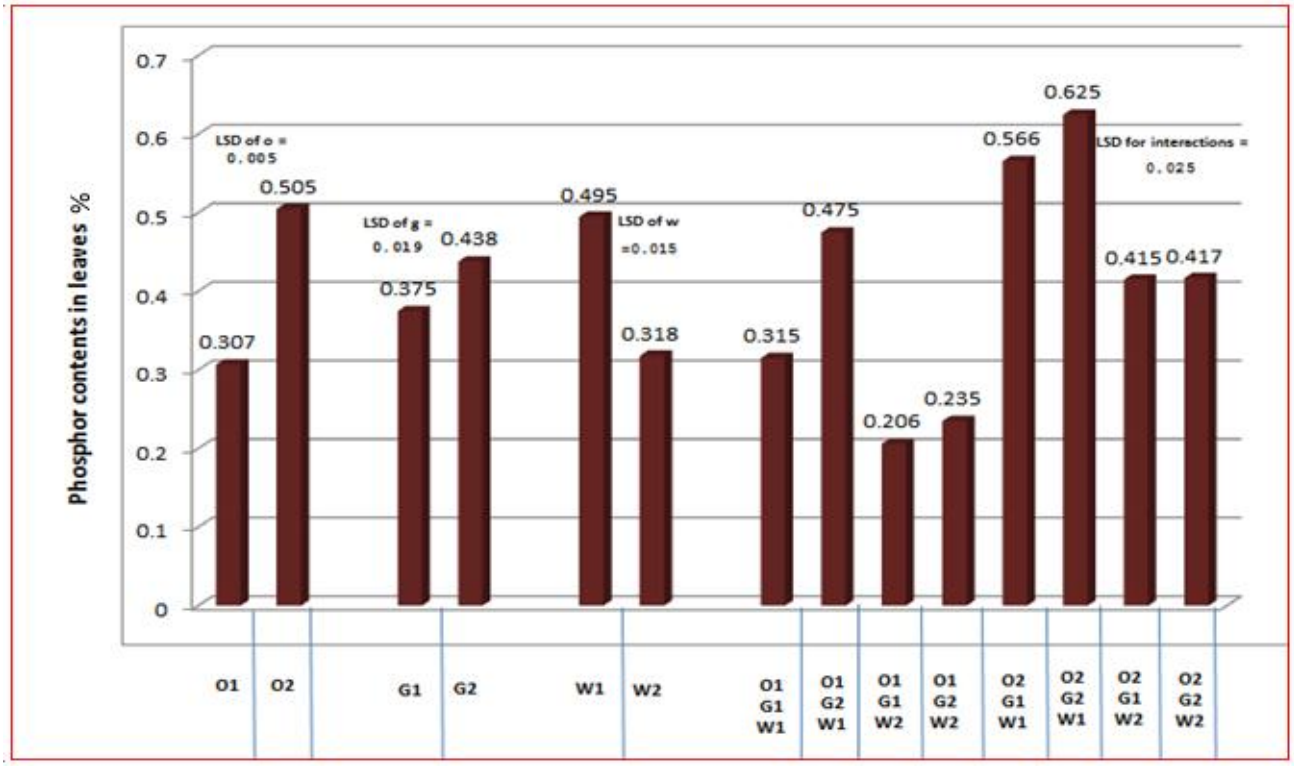

Fig.7: shows the effect of water quality, poultry manure& gibberellin with interaction betweenthem on the Phosphorus contentinleaves. Water quality (W) Poultry manure (O) Gibberellin(g)

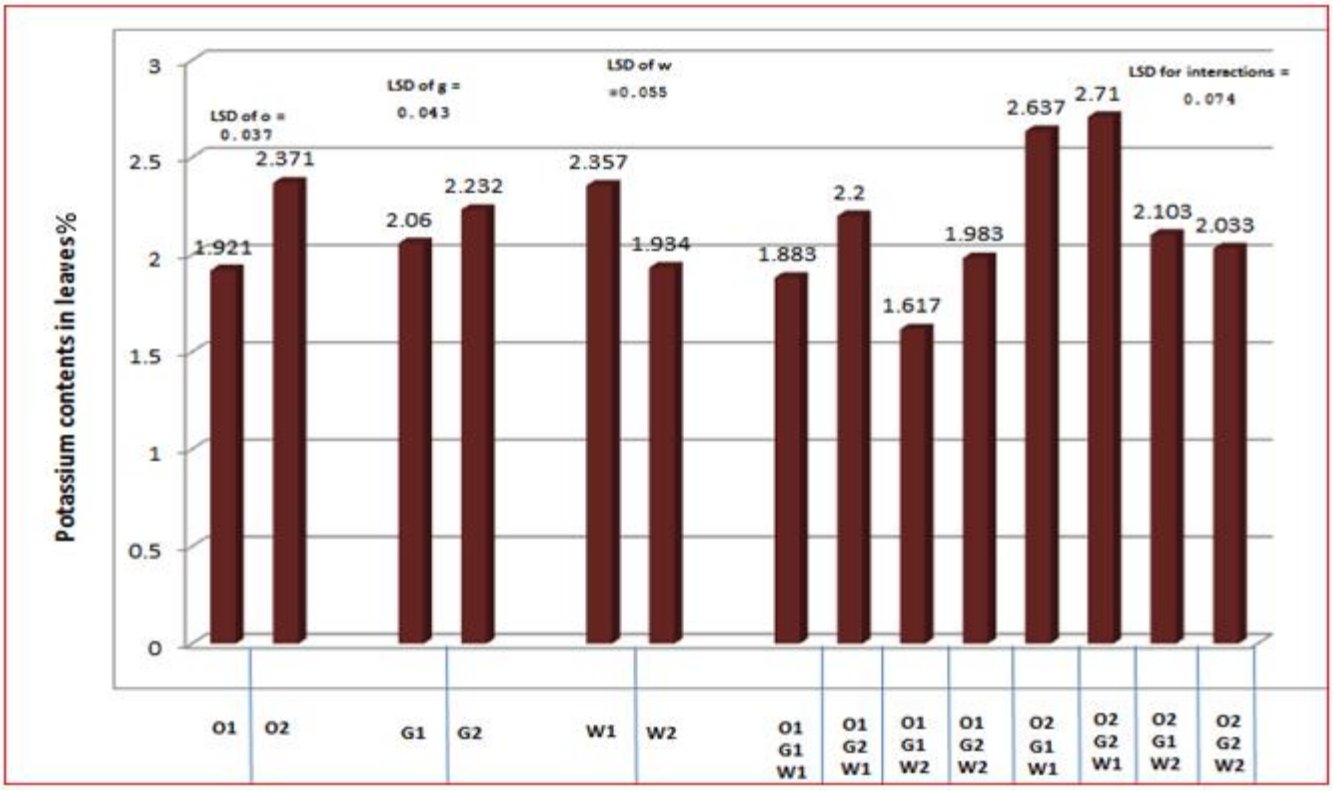

Fig.8: shows the effect of water quality, poultry manure& gibberellin with interaction betweenthem on the K contentinleaves.Water quality (W) Poultry manure (O) Gibberellin(g)

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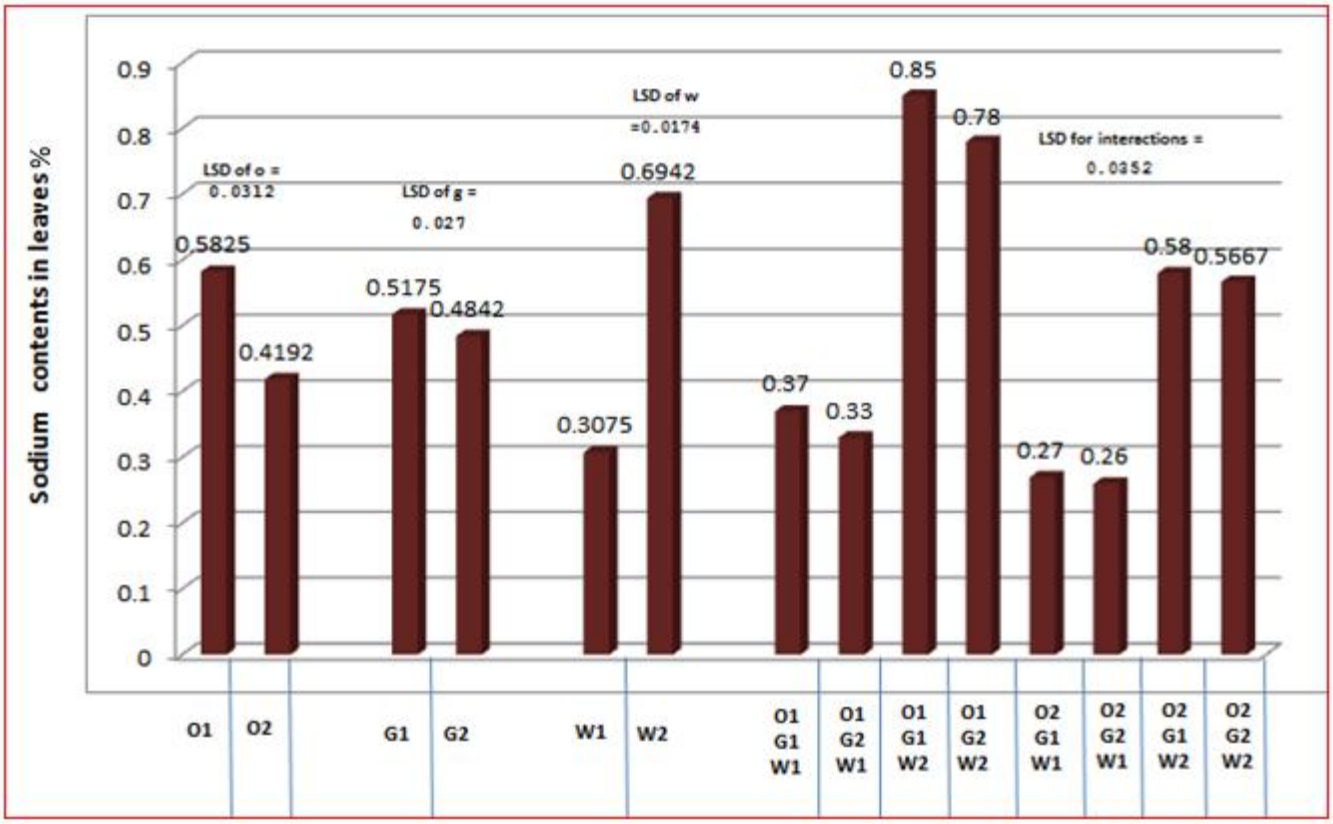

Fig.9: shows the effect of water quality, poultry manure& gibberellin with interaction betweenthem on the Na contentinleaves.Water quality (W) Poultry manure (O) Gibberellin(g)

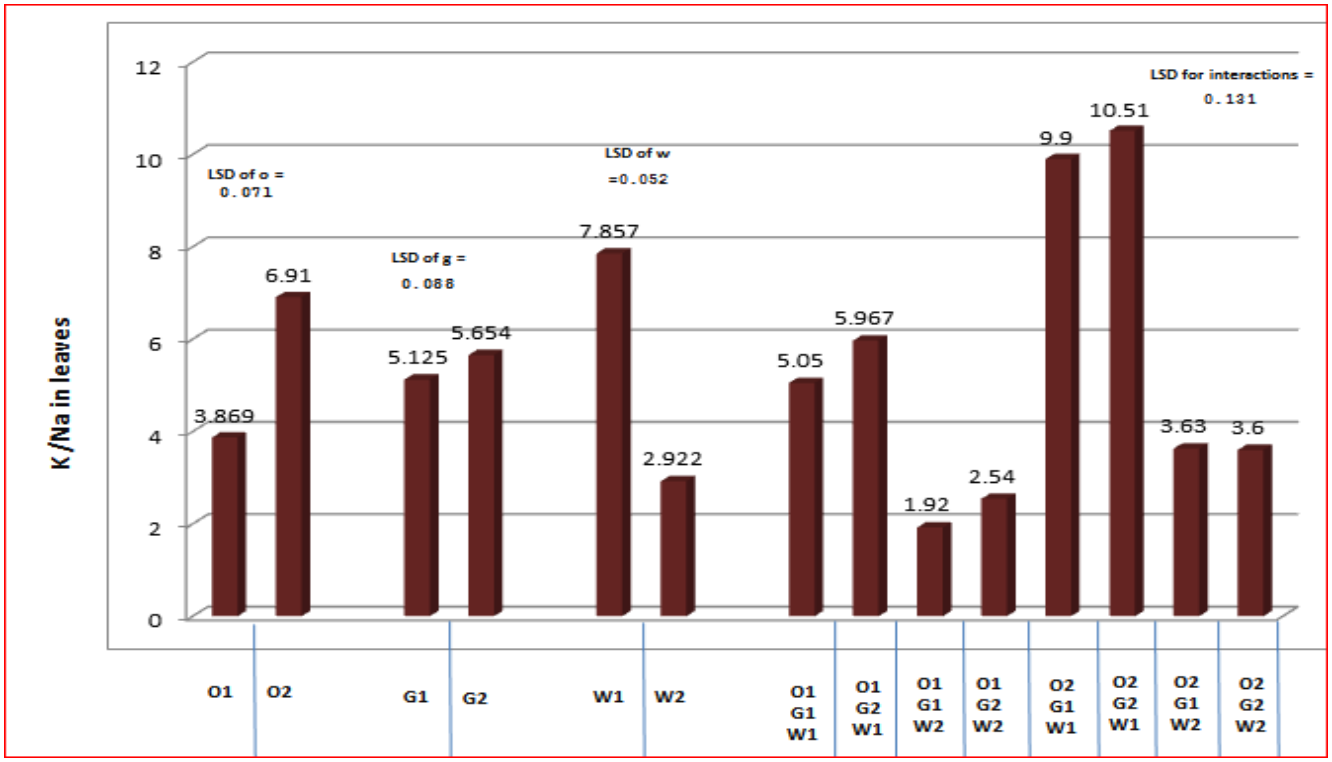

Fig.10: shows the effect of water quality, poultry manure& gibberellin with interaction between them on the K/Na contentinleaves.Water quality (W) Poultry manure (O) Gibberellin(g)

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3- Proline content in root & Peroxidase activity inleaves

The figures (11 & 12) show a significant difference from treatments with proline contents of root and peroxidase activity in leaves, the poultry manure 30% achieved significant values where boost rate was (9% & 13%) sequentially compared with treatment which 10% poultry manure fertilize, similarly the Gibberellin application by 250 mg /liter produced a significant increments with proline contents of root and peroxidase activity in leaves compared with treatment none Gibberellin treats , and peroxidase activities figures (11 & 12) shows reductive effect of saline water with dropping rate if (78% & 49%) sequentially compared with river water ,The tertiary interaction shows a significant affect amongst treatments of proline contents in leaves, the best result was (W2G2O1) & (W2G2O1) with (7.81, 7.58) mmole .g^{-1}, sequentially and the lowest result atthetreatment (W1G2O2) with (3.88) mmole .g^{-1}.and the best results of peroxidase activity was (W2G2O1) & (W2G2O2) with (102.67, 91.33) mg of protein $^{-1}$ sequentially, and the lowest results at the treatment (W1G2O2) with (54.33) mg of protein $^{-1}$ figures (11,12).

The application of poultry manure and gibberellins reduced the negative affect of salinity by saline water, the treatments (W2G1O1) recorded (7.58) mmole .g^{-1} of proline and (102.67) mg of protein $^{-1}$ of peroxidase activity compared with (W1G2O2) treatment which recorded (3.88) mmole .g^{-1} and (54.33) mg of protein $^{-1}$ sequentially.

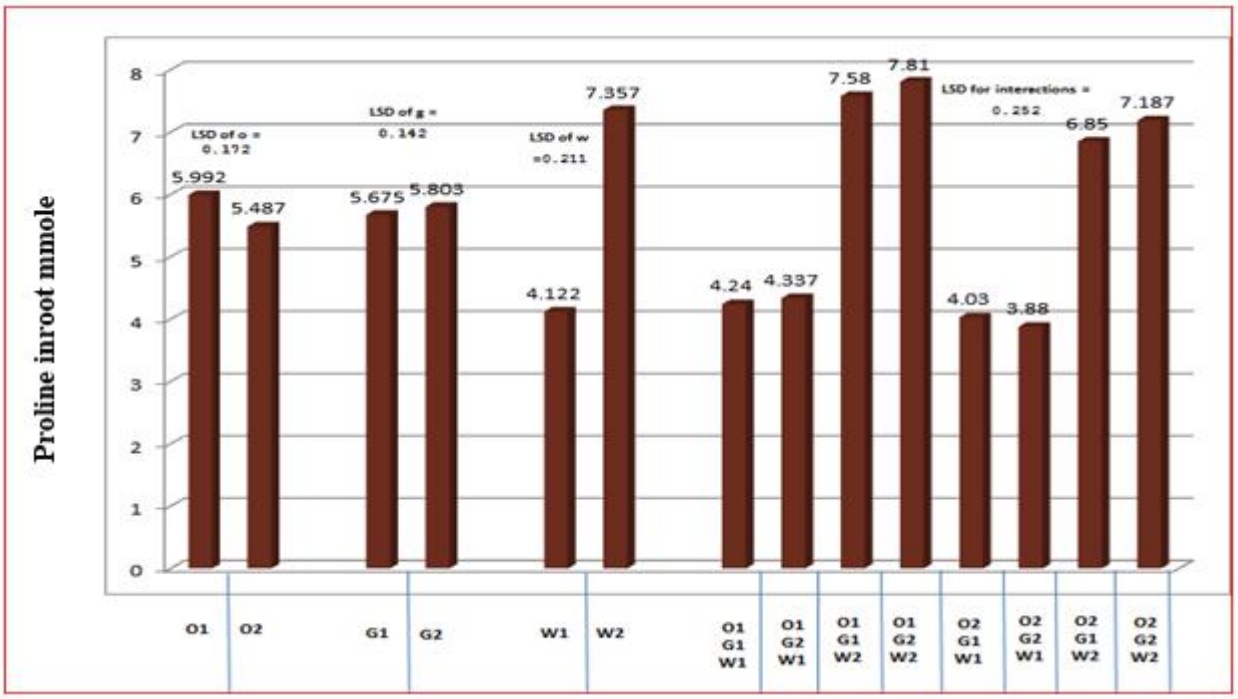

Fig.11: shows the effect of water quality, poultry manure& gibberellin with interaction between them on Proline content mmole .g^{-1} in root. Water quality (W) Poultry manure (O) Gibberellin (g)

IV. DISCUSSION

The figure (2,3,4,5,6,7,8,10) show a significant reduction in dry weight of shoot and root, fruit weight, chlorophyll contents of leaves, Nitrogen, phosphorus, Potassium & K/Na, when used saline water compared with river water, similarly ,**Jasim, et al (2012)**showed that salt stress was negatively affect wet weight, leaves number; leaves surface area and shoot length, The inhibitory effects of salinity on growth of pepper plant the effects of high soil salt availability and are probably due to decreased water absorption and disturbed metabolic processes leading to decreased meristematic activity or cell enlargement **(Kaydan and Okut ,2007).Hussein, et al(2007)**reported that there are two ways which salinity could retard growth, by damaging growth cells so that they cannot perform their functions or by limiting their supply of essential metabolites. Salinity stress is known to retard plant growth through its influence on several vital factors of plant metabolism, including osmotic adjustment **(Sakr, et al, 2009)**, nutrient uptake, protein and nucleic acid synthesis, photosynthesis **(Zaibunnisa,**et al., 2012) , organic solute accumulation, enzyme activity, hormonal

balance and reduced water availability at the cell level all of which result in reduced plant growth and ultimately reduced yield. Furthermore, increased salt content in the irrigation water may cause direct and indirect effects on leaf water relations and stomata closure which influence CO2 exchange and photosynthetic rate. Increased salt content in irrigation water may be directly toxic to plants, which in turn, lowered carbohydrate accumulation in the plants **(Morales**,et al.,2008) .

The proline contents in root and peroxidase activity were increased with saline water figure (11, 12) respectively, these are one of the role which plant followed to scavenge the reactive oxygen species, the effects of salt stress on plant growth to an increase in reactive oxygen species which play an important role in damaging all classes of biologically important macromolecules including DNA and the generation of H2O2 and lipid hydro-peroxides which cause membrane changes, To mitigate and repair damage initiated by reactive oxygen, plants have developed a complex antioxidant system. The primary components of this system include some enzymes such as peroxidase (POX), catalase (CAT) super oxidase dismutase (SOD) and proline**(Amira and Abdul**,**2015**)

The poultry and gibberellins applications alleviated from the negative affect of saline water figures (2, 3, 4, 5, 6, 7, 8, and 10). Organic fertilizer apart from releasing nutrient elements to the soil has also been shown to improve other soil chemical and physical properties which enhance crop growth and development **(Ikeh,et al,2012)**In addition, poultry manure has also been reported to increase soil pH, hence the acidic soil of the experimental site which could have caused the unavailability of nutrient element to the crops was checked by the limiting potential of organic manure **(Ogbonna, 2008)**Moreover, poultry manure contains essential nutrient elements associated with high photosyntheticactivities and thus promoted roots and vegetative growths**(John,et al,2004)**gibberellin play vital role in regulating developmental processes within plant bodies**(Gou ,et al,2010)**Gibberellin helps in cell growth of stem, leaves and other aerial parts by causing cell elongation, and increase in intermodal length. A higher concentration of gibberellins increases plant growth **(Bora and Sarma, 2007).**

Mckenzie and Deyholos ,(2011)reported that treatment of GA causes stem elongation, expansion and proliferation and cell wall thickening in bast fiber of linseed, GA3 counteracts with salinity by improving membrane permeability and nutrient levels in leaves which ultimately leads to better growth and also GA3 induced physiochemical changes responsible for induction of salt tolerance **(Amal,et al,2014)**

V. CONCLUSIONS

That possible to mitigation the negative affect of salt stress by some application like exogenous hormones and Decomposed organic matter to solve the disruption of endohormons and lack of available nutrients under salt stress, and elevation of osmotic stress in soil solution in roots area.

The GA & poultry application improved the growth and it has increased the Pepper tolerance to the abiotic stress which was exerted by saline irrigation water.

VI. KNOWLEDGE

That possible to mitigate the negative effects of salt stress by some application like exogenous hormones and Decomposed organic matter to solve the disruption of endohormons and lack of available nutrients under salt stress, and Osmotic stress elevation in soil solution in roots area..

We recommended that more researches about salt stress in arid and semi- arid zones to be conducted and the use of other applications from sources of organic matters with studying the phytohormones (Auxins gibberellins , cytokinis , ethylene ,ABA, etc.. in addition to studying the interactions between them on growth and yield to other plants for discovering and increasing the plant tolerance.

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Growth of Wheat Genotypes Influenced by Heat Stress

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Abstract— *Effect of heat stress on growth of eight wheat genotypes was evaluated. Total dry matter (TDM), leaf area index (LAI), crop growth rate (CGR) and net assimilation rate (NAR) were lower at the initial stage of growth and increased with plant age and all the genotypes showed higher values at normal growing condition compared to late and very late growing condition in both the years. Growth attributes such as LAR for all the genotypes declined throughout the advancement of growth stages in each growing conditions of both years. The HT genotypes showed higher values of TDM, LAI, CGR and LAD compared to MHS and HS genotypes in both the years.*

Keywords— Crop growth rate, Heat stress, Leaf area index, Total dry matter Wheat.

I. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a thermo sensitive cool season crop. But it is extensively cultivated in the world with latitudinal distribution from 30- 60º N and 27- 40 º S. The optimum temperature for wheat is 15- 18º C[1] while moderate high temperature (23- 32º C) for longer duration and very high temperature (33- 40 º C) for a shorter period are very common in the Mediterranean and subtropical environments particularly during grain filling [2]. Globally, about seven million hectares of wheat is affected by heat stress through the life cycle and about 40% crop faces terminal heat stress [3]. However, in some parts of Asian subcontinent where CIMMYT wheat germplasm has been successfully utilized, late planting is very common due to wide spread rice-wheat cropping pattern and crop damage due to high temperatures under late planting condition has become an important factor limiting wheat yield [4].

Bangladesh furnishes a good example of this process where wheat is grown in under hot and humid climate and in a short winter. Wheat is the second most important cereal crop next to rice in Bangladesh. Currently, wheat is grown about 0.706 million hectares with a production of 1.50 million tons [5].The national average of wheat yield is about 2 t/ha in Bangladesh which is about 50% lower than the potential yield of some released varieties. The yield gap between the potential and the national average is associated with many limiting factors of which high temperature stress is the vital factor [6]. About 80- 85% of wheat in Bangladesh is grown after transplanting aman rice following rice-wheat cropping system of which 60% of area is planted lately due to in harvesting of rice[7]. Thus the crop frequently encounters high temperature (mean air temperature >26 º C) stress during the reproductive stage of growth causing significant yield reduction. This problem will be further increased due to global warming. Such global warming would push the wheat cultivation further into heat stressed environment in future and may cause further yield reduction from the present yield level. Supra-optimal temperature during grain filling in the field associated with acceleration of phasic development [8], accelerated senescence [9], reduction of photosynthesis [10], increase in respiration and inhibition of starch synthesis in the growing kernel [11].The net effect of heat stress at the reproductive stage is to lower the kernel weight due to reduced grain filling period, grain filling rate or combined effect of both [12]. High temperature during grain filling stage has adverse effects on bread making quality [13].

In spite of low yield of wheat due to late planting post-anthesis heat stress, cultivation of wheat cannot be avoided. Because the increasing demands of wheat and irrigation dependent Boro rice cultivation may need to be replaced in future by partially or non-irrigated wheat cultivation to overcome arsenic problem. Therefore, effort to be made to minimize the late planting yield reduction by screening or developing heat tolerant wheat varieties. In this study, effect of heat stress on growth of wheat genotypes was analyzed.

II. MATERIALS AND METHODS

2.1 Location

The experiment was conducted in the research farm of Crop physiology and Ecology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during the period from November to April of 2011-12 and 2012-2013.

2.2 Materials

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For conducting the present investigation eight genotypes viz. Prodip, BARI Gom-25, BARI Gom-26, BAW-1143, BAW-1146, BAW-1147, BAW-1148 and Pavon-76, was used as experimental materials.

2.3 Experimental design

The experimental design was a split plot with three sowing dates. Three main plots were considered as treatment plots. Each main plot was subdivided into three replication plots each of 4×2.5 m^2 containing all the eight genotypes assorted randomly.

2.4 Cultivation procedure

Seeds were sown on 27 November (Normal), 17 December (Late) and 7 January (Very late). Three irrigation was applied at tillering, heading and grain filling stage.

2.5 Measurements of growth

In each year, there were 9, 8 and 8 harvests for normal, late and very late growing conditions, respectively with equal intervals of 10 days and the first harvest was taken at 20 days after sowing (DAS). At each harvest, the plants were cut off at the ground level and the tops were separated into leaves, stem and panicle (if present).The harvested plants parts were kept in an electrical oven (Model –E28# 03-54639, Binder, Germany) at about at 70º C for 72 hours and the dry weights of these plant parts were taken by an electrical balance (Model-AND EK-300i) and expressed in grams. Leaf area was determined as function of length maximum width×0.75 [14]. Dry weights of all the fractions were added to estimate total dry matter produced per unit land area.

2.6 Growth Attributes

Both the harvest interval method (classical technique) was followed to determine different growth attributes. From the dry weight of different plant parts and leaf area data, the following growth attributes were calculated between two successive harvests according to the classical technique of growth analysis [15].

1. Leaf area index (LAI) $= \dfrac{\text{Leaf area}}{\text{Ground area}}$
2. Crop growth rate $(CGR) = \dfrac{W_2 - W_1}{t_2 - t_1}$
3. Net assimilation rate $(NAR) = \dfrac{(W_2 - W_1)(\log_e LA_2 - \log_e LA_1)}{(LA_2 - LA_1)(t_2 - t_1)}$
4. Leaf area duration $(LAD) = \dfrac{(LA_2 + LA_1)(t_2 - t_1)}{2}$

Where, W_2 and W_1 are the total dry weights, LA_2 and LA_1 are the total leaf area per plant at t_2 and t_1, the later and the former harvest, respectively.

III. RESULTS

3.1 Total Dry Matter (TDM)

Influence of sowing time on total dry matter (TDM) of different wheat genotypes at different days after sowing (DAS) is shown in Figures 1.1 for 2011-12 and 1.2 for 2012-13. TDM increased slowly up to 50 DAS and then increased rapidly with the advancement of growing period of all the genotypes for both the years. At normal growing condition, all the genotypes produced higher TDM compared to late and very late growing heat stress condition. In second year (2012-13) all the genotypes produced higher TDM compared to the first year (2011-12) at each growing time due to lower temperature. Each growing conditions the heat tolerant genotype BAW-1143 produced the highest TDM whereas the heat sensitive genotype Pavon-76 obtained the lowest TDM at all the DAS. Genotypes BARI Gom -25, BARI Gom -26 and Prodip were intermediate producer of TDM. The moderate heat tolerant genotypes BAW-1146, BAW-1147 and BAW-1148 produced higher TDM than Pavon-76 but lower than those of the heat tolerant genotypes.

3.2 Leaf Area Index (LAI)

The effect of growing conditions on leaf area index (LAI) of eight wheat genotypes at different stages of growth is shown in Figures 2.1 for first year (2011-12) and 2.2 for second year (2012-13). LAI of all the genotypes for each the growing conditions started from a lower value and reached their peak at a certain stage of growth and declined thereafter in each years. At optimum sowing time (normal growing condition) all the genotypes showed higher LAI compared to late and very late sowing high temperature growing condition. At normal growing all the genotypes reached their highest value of LAI at 70 DAS, whereas for late and very late growing condition it was 60 DAS [23].They observed that leaf area of wheat genotypes decreased with late sowing. The decline in LAI following the flag leaf stage might be ascribed by aging of leaves, leaf senescence and thermal stress at later growth stages [24].

At normal growing condition heat tolerant genotype BAW-1143 showed the highest LAI at all the growth stages except at 20 DAS which was closely followed by HT genotypes BARI Gom -25, BARI Gom-26 and Prodip. At 70 DAS, BAW-1143 had the highest value of

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LAI. In this sowing time, heat sensitive genotype Pavon-76 had the lowest LAI which was closely followed by other MHT genotype BAW-1146, BAW-1147 and BAW-1148.

At late and very late growing condition the HT genotype BAW-1143 attained the highest LAI at all the growth stages.

3.3 Crop Growth Rate (CGR)

Crop Growth Rate (CGR) indicates the amount of dry matter accumulation rate per unit land area which the most significant term of analysis in field.

The effect of sowing time on crop growth rate of different wheat genotypes at successive days after sowing is presented in Figures 3.1 for 2011-12 and 3.2 for 2012-13. The CGR being controlled by canopy, photosynthesis and respiration, so, it is considered more meaningful function of crop growth. In each growing conditions, starting from a lower value, CGR of all the genotypes reached a certain peak and thereafter declined. At normal growing condition, all the genotypes in both the years attained their highest CGR at 70-80 DAS, whereas at late and very late growing condition it was at 60-70 DAS. In normal growing condition, all the genotypes showed higher CGR compared to late and very late growing condition. Heat tolerant genotypes BAW-1143 attained the highest CGR value at all the DAS in each sowings except at 90-100 DAS. At 90-100 DAS, HT genotype BAW-1143 had the highest CGR. Other MHT genotypes e.g. BARI Gom-25, BARI Gom -26 and Prodip closely followed BAW-1143. Whereas, heat sensitive genotype Pavon-76 showed the lowest CGR in all respects which was closely followed by the other HS genotypes BAW-1146, BAW-1147 and BAW-1148.

3.4 Net Assimilation Rate (NAR)

Net assimilation rate (NAR) of different wheat genotypes at different growth condition is presented in Figures 4.1 for 2011-12 and 4.2 for 2012-13. NAR in all the genotypes for both the growing conditions started from a lower value and reached their peak at certain stage of growth and thereafter declined slowly. At normal growing condition, all the genotypes attained their highest NAR at 70-80 DAS, whereas in late and very late sowing heat stress condition it happened at 60-70 DAS. At very late sowing heat stress condition, NAR was higher up to 60-70 DAS compared to normal sowing for all the genotypes. The figure showed that pattern of NAR was depended on the phenological stages at all sowing times. At normal growing condition, BAW-1143 attained the highest NAR at 70-80 DAS which was closely followed by, BARI Gom -25, BARI Gom -26 and Prodip . At this growth stage (70-80 DAS), BAW-1146, BAW-1147, BAW-1148 and Pavon-76 had the lowest NAR value. But in late and very late growing condition Pavon-76 attained the highest NAR at 60-70 DAS. In this conditions (late and very late sowing), Pavon-76 showed the lowest NAR at that growth stage (60-70 DAS).

3.5 Leaf Area Duration (LAD)

The effect of sowing time on Leaf Area Duration (LAD) of different genotypes of wheat at the different days after sowing (DAS) is shown in Figures 5.1 for the year 2011-12 and 5.2 for 2012-13. In all the genotypes for both the sowings and years, LAD started from a lower value and reached to their peak at a certain DAS and declined thereafter. At normal growing condition of both the years, all the genotypes reached their highest value of LAD at 70-80 DAS, whereas for late and very late growing condition it was 60-70 DAS. There existed similarities between two years growth pattern of LAD of all the genotypes. In both the years, at normal growing condition all the genotypes attained significantly higher LAD values compared to their respective late and very late growing conditions.

At normal growing condition of both the years, the HT genotype BAW-1143 showed the highest LAD at all the growth stages which were followed by Prodip, BARI Gom-25and BARI Gom-26. Whereas, the HS genotype Pavon-76 attained the lowest LAD values at all the DAS. Genotypes BAW-1146 and BAW-1147 was intermediate performer in LAD.

In both the years, at late and very late growing condition again HT genotype BAW-1143 attained the highest LAD at all the growth stages except at 20-30 DAS which was followed by Prodip, BARI gom-25 and BARI gom-26. At 20-30 DAS, the HT genotype BAW-1143 had the highest LAD, whereas HS genotype Pavon-76 attained the lowest LAD at all the DAS which was closely followed by other genotype BAW-1148.

IV. DISCUSSIONS

In this study, the maximum value of total dry matter was obtained for the maximum value of leaf area index in the first planting date or the least duration of comforting with terminal heat stress. Delayed sowing of wheat resulted in the reduction of total dry weight [16]. There were vitiations in TDM compared to late sowing (December) [17]. The rapid increased in total dry matter at the later stages of growth was due to the development of considerable number of late tillers [18]. After 90 days in case of normal and late sowing and 80 days in case of very late sowing until harvest time , it decreased due to hastened leaf senescence and decreased of leaf area index[19].

The appropriate sowing plants produced higher TDM due to longer duration of vegetative phase. The decreased in

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dry matter accumulation with the increase of terminal heat stress indicates the unfavourable response of wheat genotypes to terminal heat stress. It is perhaps related to the decreased of the photosynthesis activity that has led to decreased in dry matter accumulation. Dry matter accumulation decreased due to a decreased in leaf number, leaf area index and accumulation in leaf senescence in wheat [19].

It is perhaps related to a relationship between leaf area index and accumulation of dry matter, especially when wheat encounters to heat stress [12, 20]. Wheat yield and dry matter accumulation decreased with high temperature in during most reproductive stages when wheat enters the grain- filling period [22].

In the present study, LAI of all wheat genotypes for both the growing conditions started from a lower value and reached highest level at a certain stage of growth and declined thereafter. Leaf area index is a growth indicators used as a photosynthetic system measurement. LAI is related to the biological and economic yields and increase in LAI causes higher yield [25]. Under late sown condition, minimum LAI was recorded which might be due to sub-optimum temperature during the vegetative growth phase, as leaf development greatly depends on the prevailing temperature [26]. Thus in response to an increase in temperature, the leaf area remains too small to support the required growth of the seedling. In addition the low temperature slows down the rate of leaf initiation (Warrington & Kanemasu 1983), which may decrease the LAI. Leaf area index decreased due to increasing aging of leaves, hastens leaf senescence, shading and competition between plants for light and other resources, especially, when wheat encounters high temperatures. Increasing leaf area index is one of ways of increasing the capture of solar radiation within the canopy and production of dry matter [24]. Leaf area index was reduced differently in different wheat genotypes in response to high temperature stress [27, 28].

In the present study CGR was found higher for normal growing condition compared to late and very late growing condition. The comparatively higher CGR values at anthesis stage could be described by favourable temperature. Higher CGR for normal growing condition was due to higher production of dry matter owing to greater LAI [29]. Among the genotypes the heat tolerant group (BAW-1143, BARI Gom -25, BARI Gom -26 and Prodip) had higher CGR than MHT genotypes (BAW-1146, BAW-1147, BAW-1148) and heat sensitive genotype Pavon-76 in all the DAS for both the growing conditions. Highest CGR for optimum sowing compared to others genotypes and decreased with any delay in sowing [17]. However, CGR is regarded as the most meaningful growth function, since it represents the net result of photosynthesis, respiration and canopy temperature and canopy area interactions [29]. Maximum growth rate confirmed to the beginning of reproductive growth, which decreases with plant's higher maturity due to growth ending, leave's losses and withering. The great variation of CGR among genotypes indicates the problems of less efficient photosynthetic activity, their source to sink relation and managing of leaf area through plant population [30].

NAR increased slowly at the early stages of growth but at the later stages it increased sharply [17, 31, 32]. The rapid increase in NAR at the later stages may be due to increase ear photosynthesis in addition of increased photosynthesis of the developed leaf. In the present study, higher NAR values were observed in the late growing condition up to 60-70 DAS compared to the normal growing condition for all the genotypes.

It is due to plants of late growing condition passed through comparatively lower temperature at the early vegetative stages. Higher NAR was at the delayed sowing in wheat [17]. The maximum NAR figure at flag leaf can be attributed by higher leaf area[33]. In the present work, among the genotypes heat tolerant groups (BAW-1143, BARI Gom -25, BARI Gom -26 and Prodip) showed higher NAR than those of MHT genotypes (BAW-1146, BAW-1147, BAW-1148) and HS genotype Pavon-76 at all stages of growth in the normal growing condition. This is due to genetic characters of genotypes.NAR found variation among genotype and influenced by temperature [30].

In this study, it was observed that the flag leaves of some of the genotypes expanded lately but they senesced at the same time with others where flag leaves expanded earlier resulting a shorter leaf area duration in those genotypes**.** Leaf area duration (LAD) of all the genotypes showed essentially similar pattern to their LAI in both the years. Higher LAD was found in the normal growing condition compared to late growing condition in both the years. Heat tolerant genotypes (BAW-1143, BARI gom-26, BARI gom-25 and Prodip) attained higher LAD compared to heat sensitive genotype Pavon-76 in each growing conditions of every year. Optimum sowing (mid November) of wheat genotypes showed higher LAD compared to late sowing (mid December) and some wheat genotypes attained comparatively higher LAD than those of others[17]. Optimum temperature (20° C) at the vegetative stage enhanced the leaf initiation and leaf emergence for a longer period as revealed in the normal growing condition in wheat[34]. This duration decreased with delay in sowing prevailed by lower temperature at the early vegetative stage as found in the late growing condition.

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V. FIGURES

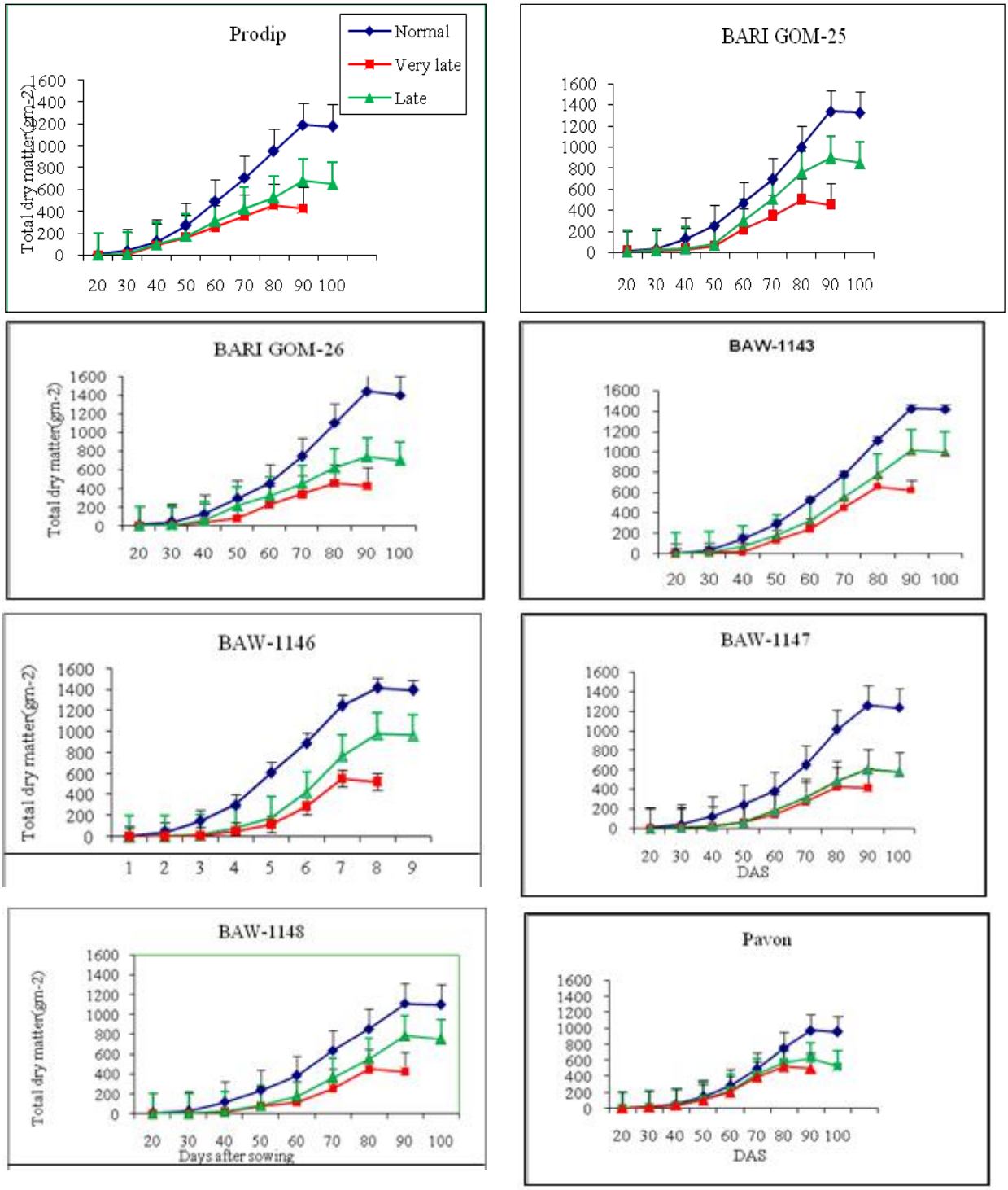

Fig.1.1: Effect of sowing time on total dry matter (TDM) of eight wheat genotypes at different days after sowing from original values (2011-12). Vertical bars indicate ± SE value.

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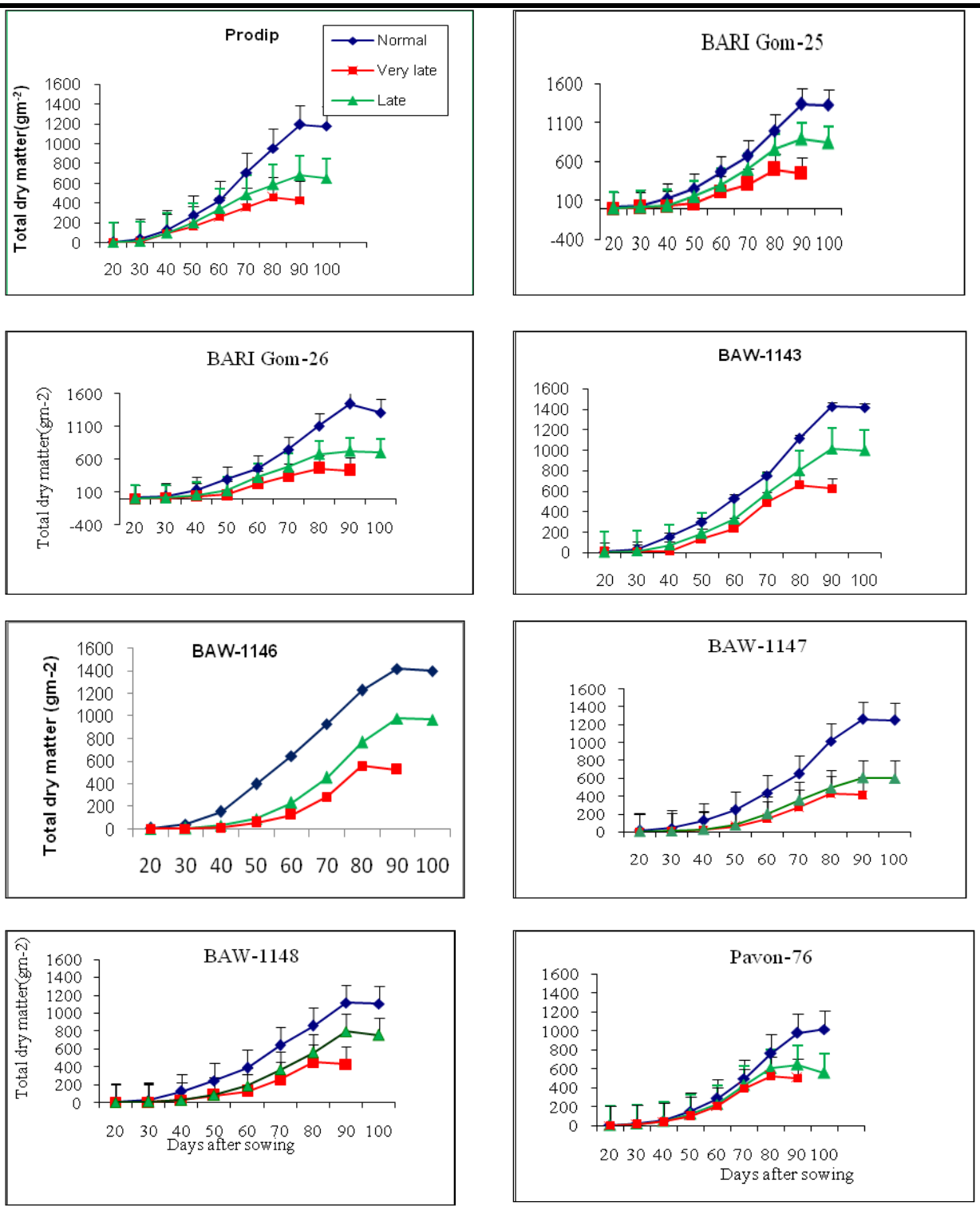

Fig.1.2: Effect of sowing time on total dry matter (TDM) of eight wheat genotypes at different days after sowing from original values (2012-13). Vertical bars indicate ± SE value.

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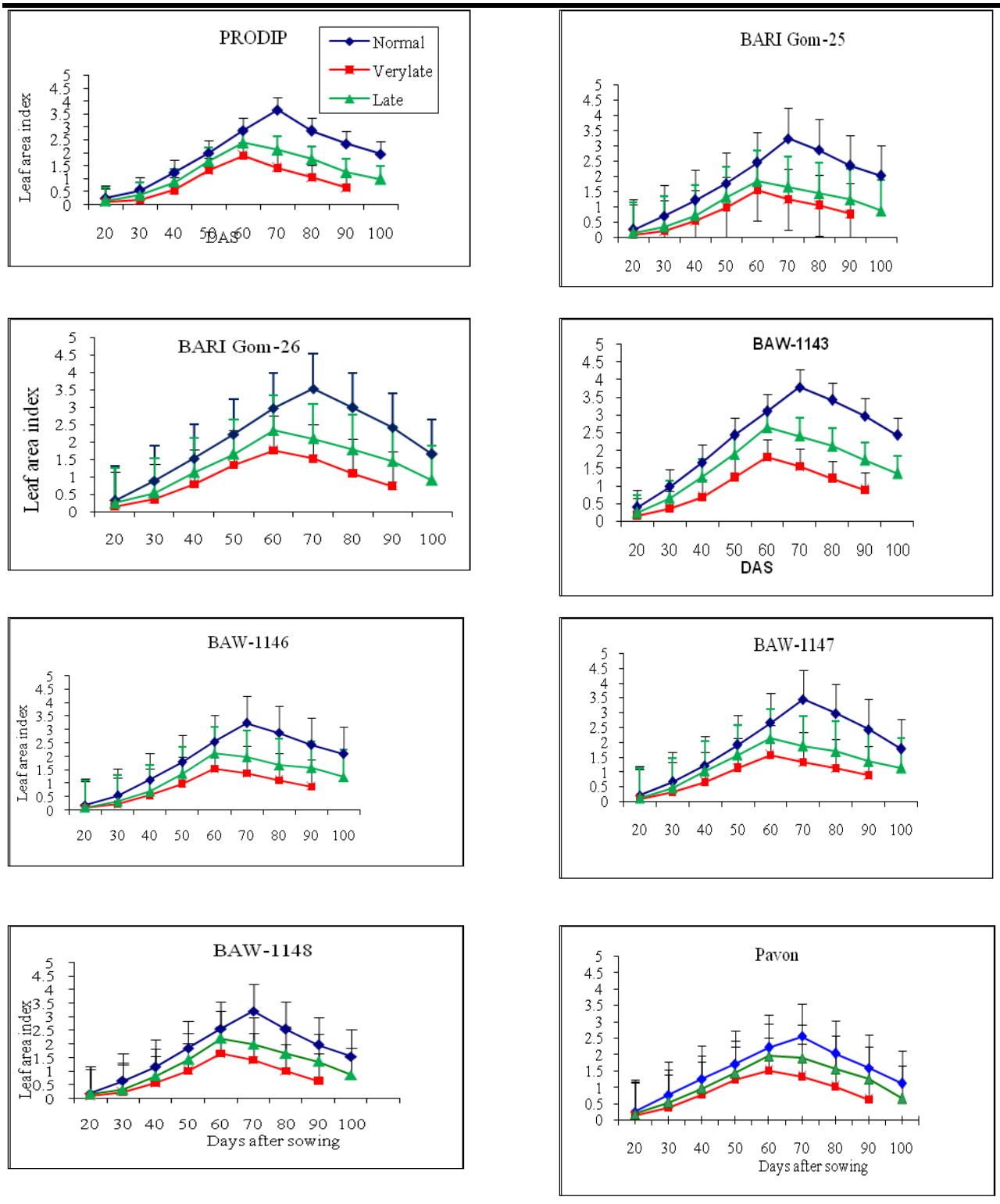

Fig.2.1: Effect of sowing time on Leaf area index (LAI) of eight wheat genotypes at different days after sowing from original values (2011-12). Vertical bars indicate ±SE value.

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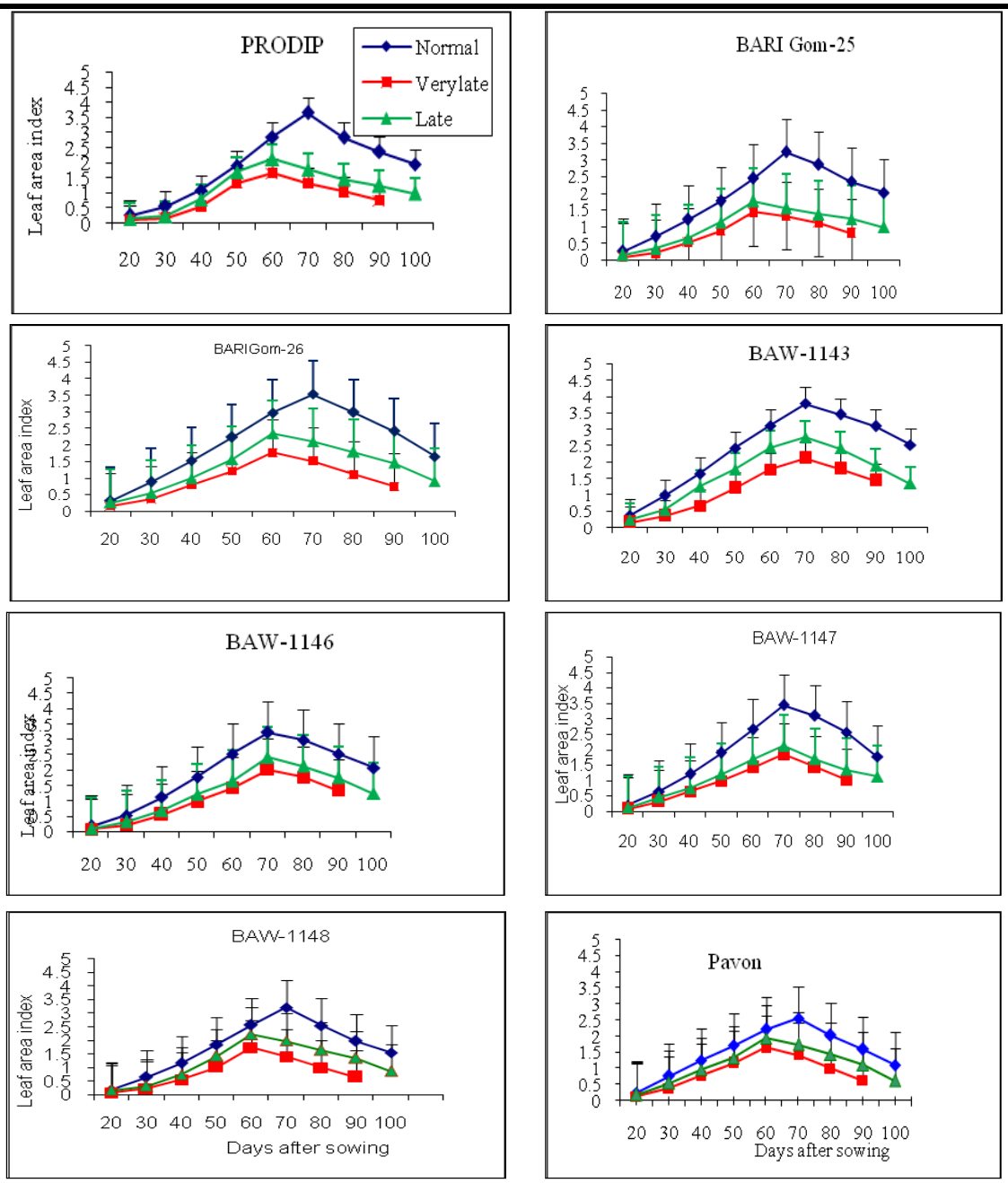

Fig.2.2: Effect of sowing time on leaf area index (LAI) of eight wheat genotypes at different days after sowing from original values (2012-13). Vertical bars indicate ±SE value.

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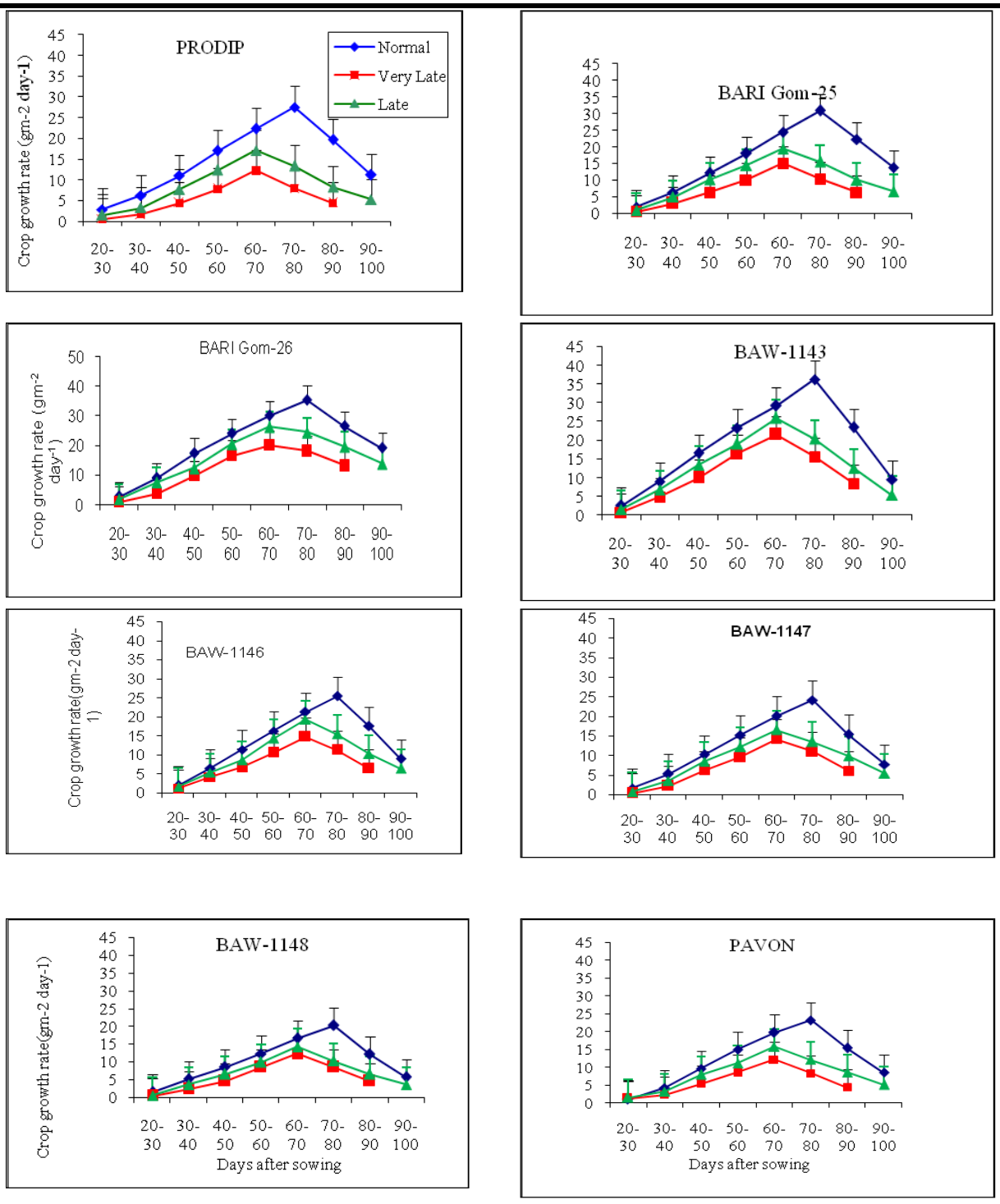

Fig.3.1: Effect of sowing time on Crop growth rate (CGR) of eight wheat genotypes at different days after sowing from original values (2011-12). Vertical bars indicate ± SE value.

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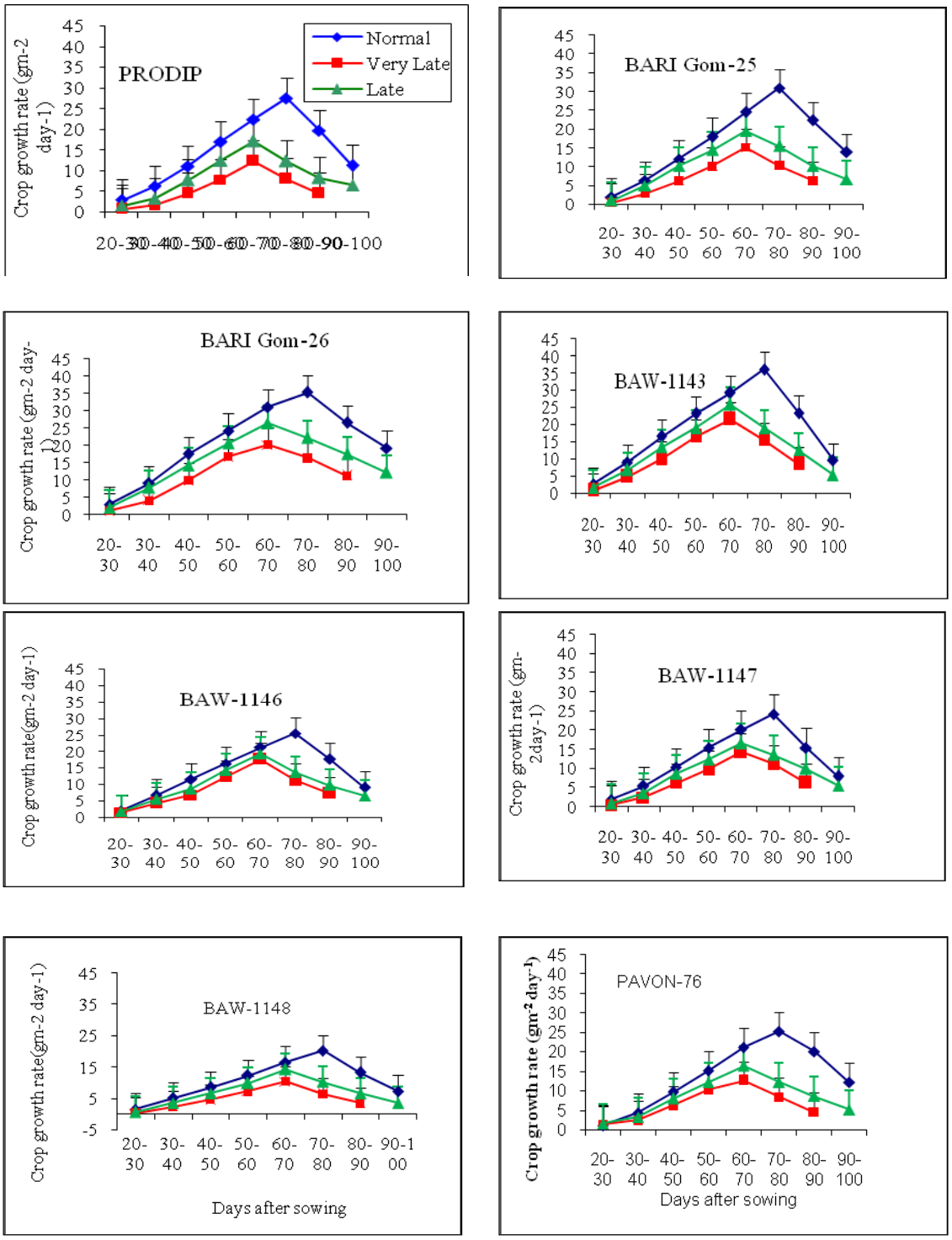

Fig.3.2: Effect of sowing time on Crop growth rate (CGR) of eight wheat genotypes at different days after sowing from original values (2012-13). Vertical bars indicate ± SE value.

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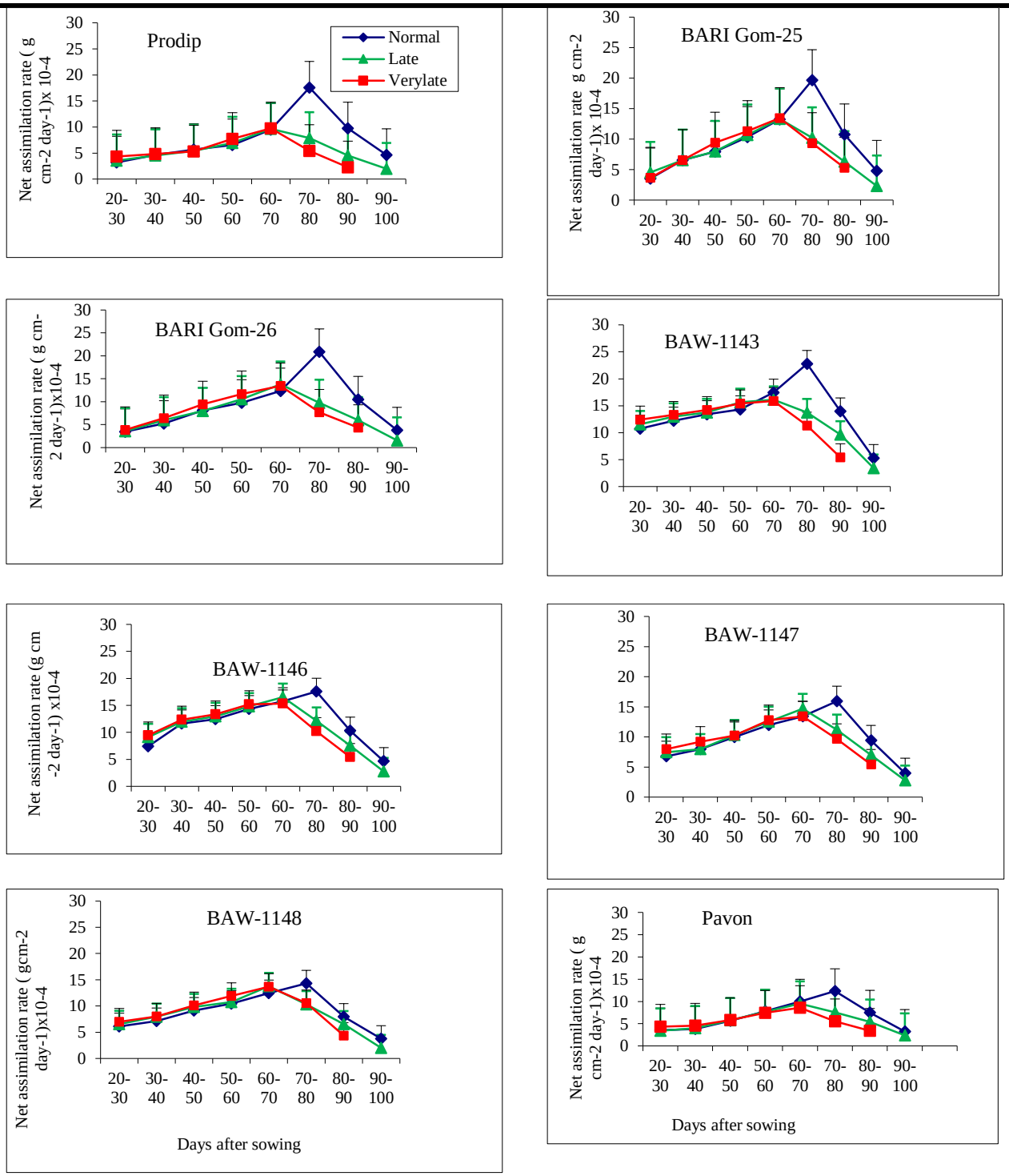

Fig.4.1: Effect of sowing time on Net assimilation rate (NAR) of eight wheat genotypes at different days after sowing from original values (2011-12). Vertical bars indicate ± SE value .

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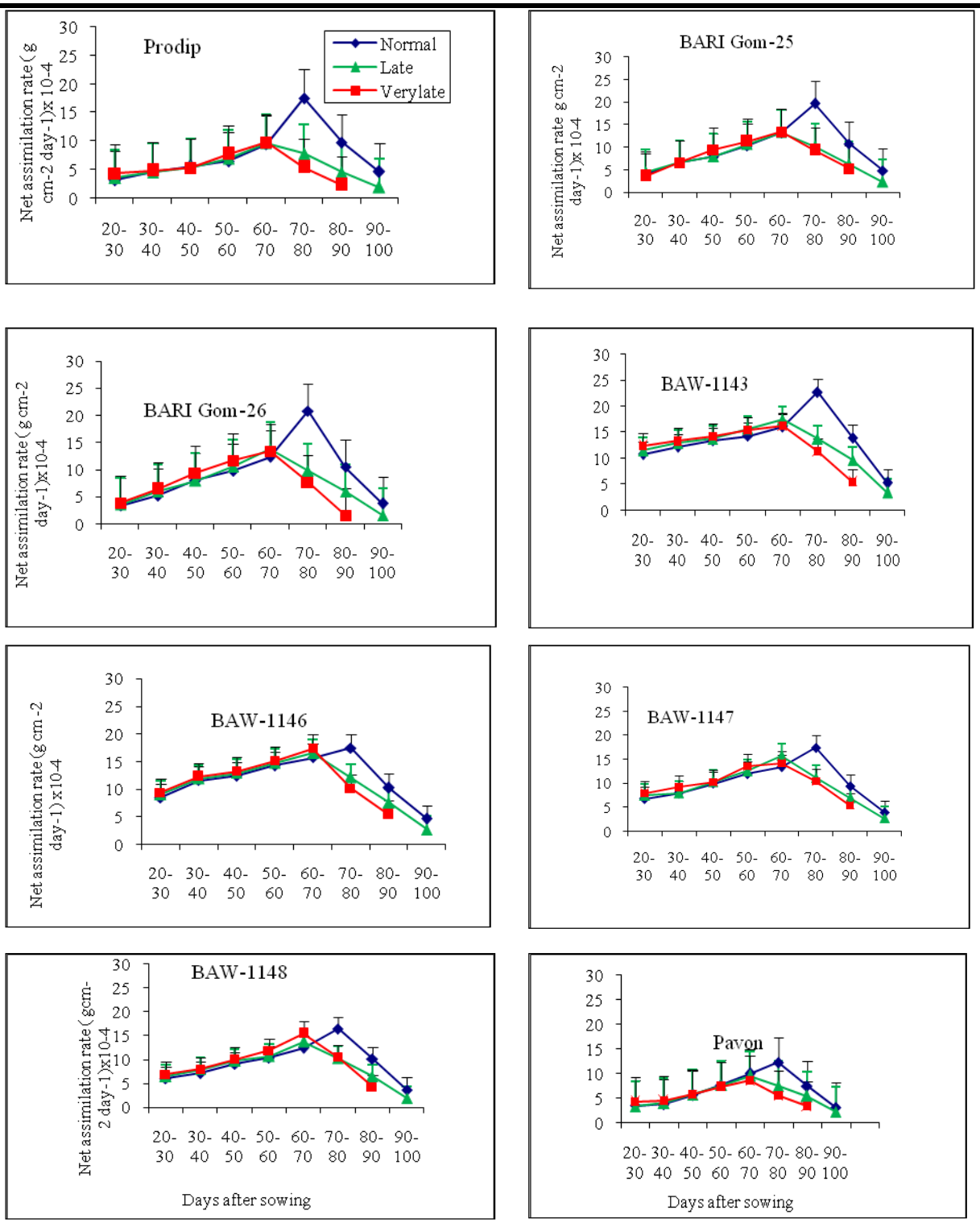

Fig.4.2: Effect of sowing time on Net assimilation rate (NAR) of eight wheat genotypes at different days after sowing from original values (2012-13). Vertical bars indicate ±SE value.

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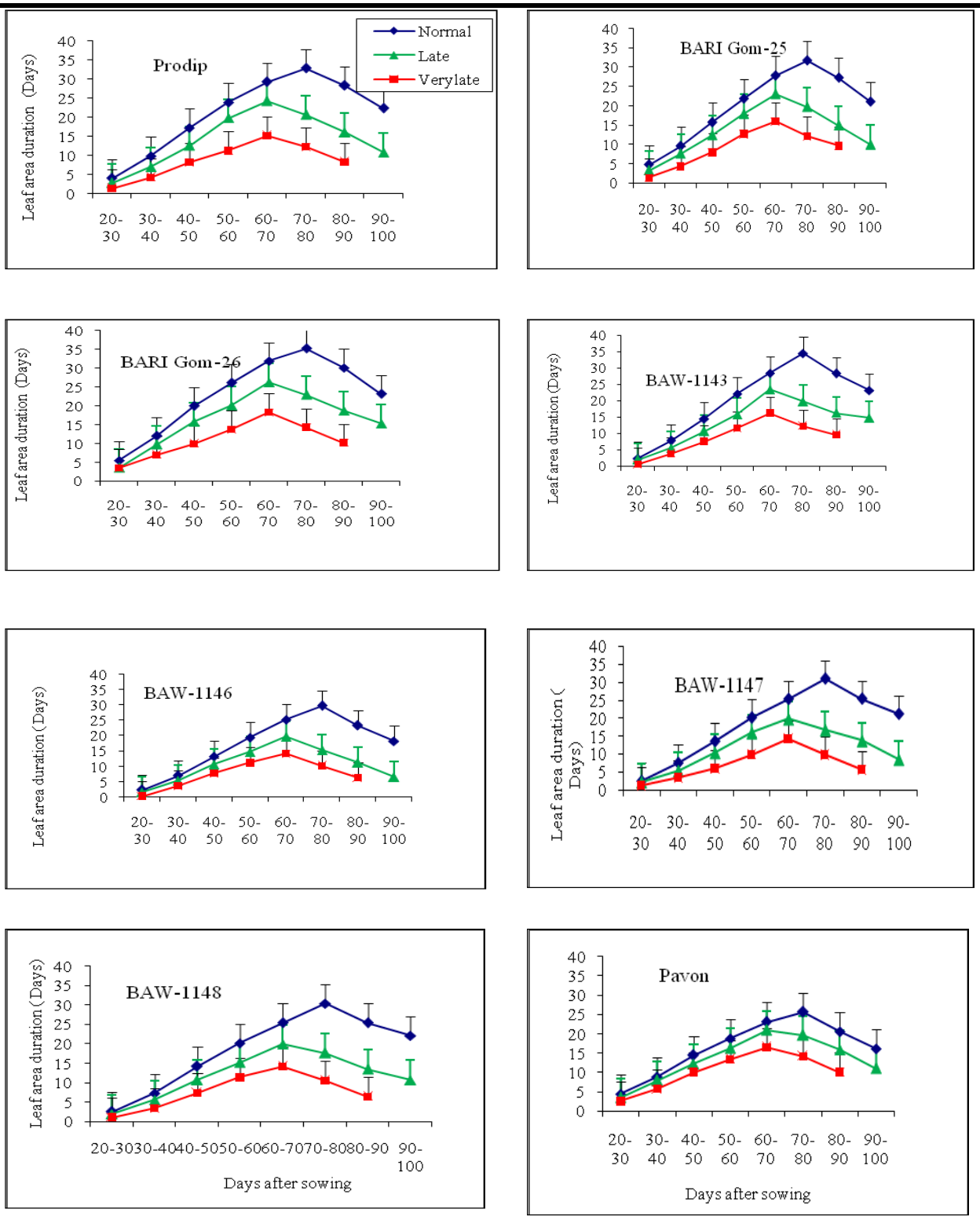

Fig.5.1: Effect of sowing time on Leaf area duration (LAD) of eight wheat genotypes at different days after sowing from original values (2011-12). Vertical bars indicate ±SE value.

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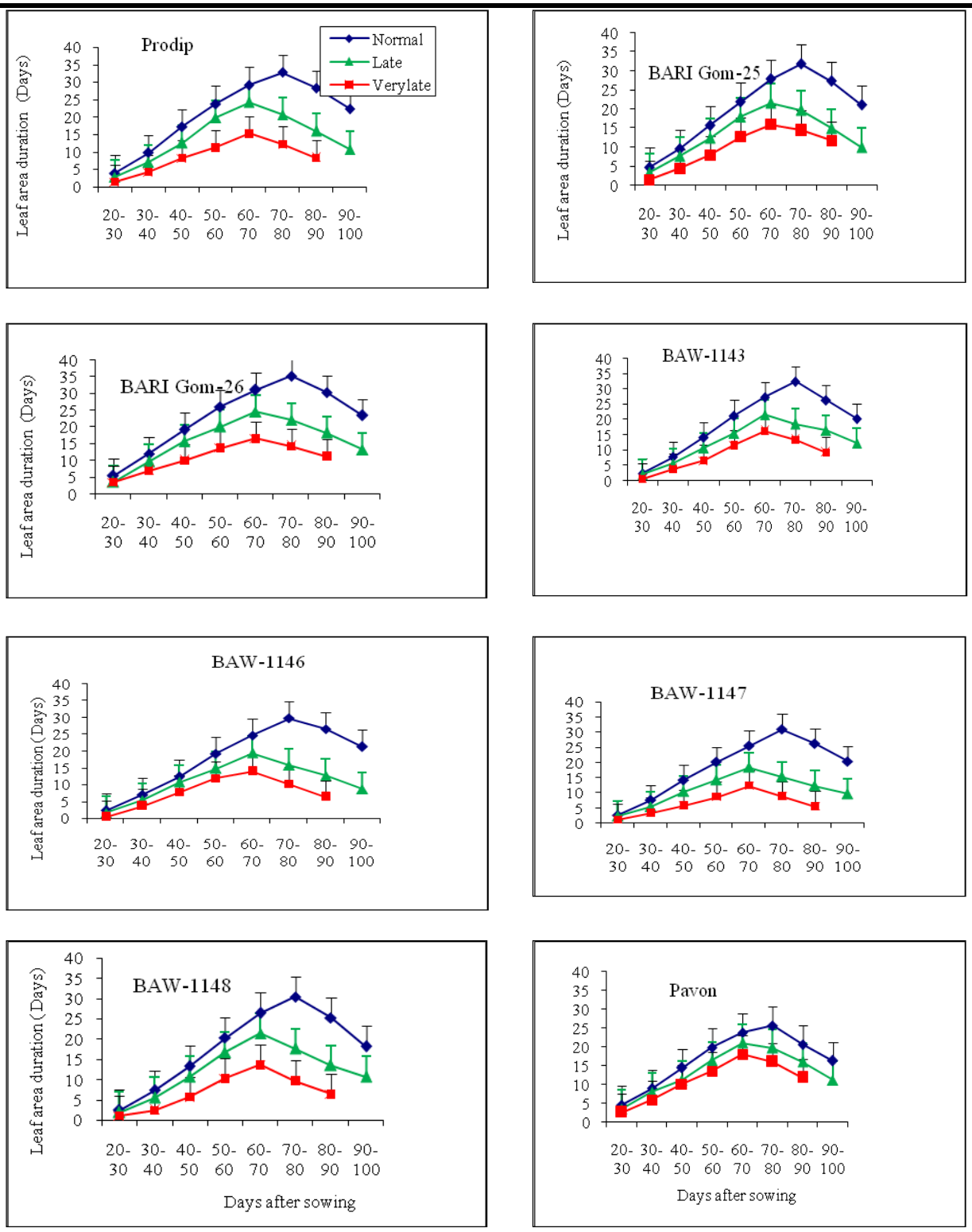

Fig.5.2: Effect of sowing time on Leaf area duration (LAD) of eight wheat genotypes at different days after sowing from original values (2012-13). Vertical bars indicate ±SE value.

VI. CONCLUSION

Total dry matter (TDM), Leaf area index (LAI), crop growth rate (CGR), net assimilation rate (NAR) increased slowly at initially stage and increased rapidly with the advancement of growing period of all the genotypes for both the years. At normal growing condition, all the genotypes produced higher value compared to late and very late growing heat stress condition. In second year (2012-13) all the genotypes produced higher value of these compared to the first year (2011-12). Each growing

conditions the heat tolerant genotype BAW-1143 produced the highest value. Whereas, the heat sensitive genotype Pavon-76 obtained the lowest value at all the DAS. Genotypes BARI Gom -25, BARI Gom -26 and Prodip were intermediate producer. The moderate heat tolerant genotypes BAW-1146, BAW-1147 and BAW-1148 produced higher value than Pavon-76 but lower than those of the heat tolerant genotypes**.** In all the genotypes for each sowings and both years, LAD started from a lower value and reached to their peak at a certain DAS and declined thereafter. At normal growing condition of both the years, all the genotypes reached their highest value of LAD at 70-80 DAS, whereas for late and very late growing condition it was 60-70 DAS. In both the years, at normal growing condition all the genotypes attained significantly higher LAD values compared to their respective late and very late growing conditions.At normal growing condition of both the years, the HT genotype BAW-1143 showed the highest LAD at all the growth stages which were followed by Prodip, BARI gom-25and BARI gom-26. Whereas, the HS genotype Pavon-76 attained the lowest LAD values at all the DAS. BAW-1146 and BAW-1147 was intermediate performer in LAD. In both the years, at late and very late growing condition again HT genotype BAW-1143 attained the highest LAD at all the growth stages which was followed by Prodip, BARI Gom-25 and BARI Gom-26. At 20-30 DAS, the HT genotype BAW-1143 had the highest LAD, whereas HS genotype Pavon-76 attained the lowest LAD at all the DAS which was closely followed by other genotype BAW-1148.

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The Content of Agar Seaweed *Gracilaria verrucosa* Fertilized with Vermicompost

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Abstract— *The economic value of seaweed G. verrucosa depends on the content of the agar it has. Cultivation Gracilaria verrucosa generally use inorganic fertilizers that are not environmentally friendly, inorganic fertilizer is not a wise step considering the recent increase in consumers who want a product that is free of pesticide residues. The purpose of this study was to analyze the optimal dose of vermicompost fertilizer to produce high quality of agar rendement, viscosity and gel strength seaweed Gracilaria verrucosa. From the result of the research, it was found that the quality of agar rendement, viscosity and gel strength were normal and homogeneous distribution (p>0,05). Then the ANOVA test showed that the fertilizer treatment gave a significant effect on the quality of agar rendement and viscosity (p <0,05), while the quality of agar gel strength did not give significant effect (p> 0,05). The highest level of viscosity and rendement of Gracilaria verrucosa seaweed was found in treatment A and the lowest in treatment F (control). The highest level quality of agar gel strength Gracilaria verrucosa was found in treatment F compared with other treatment.*

Keywords— G. verrucosa, vermicompost fertilizer, rendement, viscosity and gel strength.

I. INTRODUCTION

Gracilaria verrucosa is a plant widely distributed in tropical waters, can produce agar extracts (a commercial name for natural gelatin polymers containing carbohydrate and sulfate groups). The quantity and quality of agar derived from seaweed cultivation vary, not only by variety but also the age of the plant, rays, nutrients, temperature, and salinity [1], [2], [3].

Vermicompost is a 100% quality organic fertilizer and environmentally friendly derived from worm dung (vermics). Vermicompost contains various nutrients needed by seaweed and plays an important role in the process of photosynthesis. It also plays a role in preparing plasma cells and the formation of carbohydrates and proteins. During the vermicomposting process, essential plant nutrients such as nitrogen and phosphorus required by plants, which are present in the diet are converted through the activity of microorganisms into a form that is more easily absorbed by plants [4]. Improving the quality of crops on farms by extensive testing of vermicompost fertilizers has been done by Ohio State University, Cornell University in America, and SIRO in Australia. The tests show an increase in the size and quality of the plant, by 15-57% [5].

Vermicompost is a source of nutrients for nitrifying bacteria. With the existence of these nutrients microbes decomposing organic materials will continue to grow and decompose organic materials more quickly. Therefore, in addition to improving the quality of seaweed, vermicompost can also help the process of destruction of organic waste [6]. But there is no research data that provides the use of vermicompost to levels for seaweed, especially G. verrucosa. Thus, a study is needed to find out the optimal dose of vermicompost fertilizer to produce the high content of agar rendement, viscosity and gel strength G. verrucosa.

II. MATERIALS AND METHODS

2.1 Study site and sampling design

This research was conducted in open space in the pond area of Maliwowo Village, Angkona District, East Luwu Regency, South Sulawesi Province, April to July 2016 for 42 days. The experimental design used in this study was a complete randomized design (CRD) with 6 treatments and repetition 3 times so that there were 18 units of experiments, while the treatment performed was a dose of vermicompost fertilizer that was different from the treatment A dose of vermicompost fertilizer 300 g/m^2, Treatment B dose of vermicompost fertilizer 250 g/m^2, treatment C dose of vermicompost fertilizer 200 g/m^2, treatment D dose of vermicompost fertilizer 150 g/m^2, treatment E dose of vermicompost fertilizer 100 g/m^2 and F control treatment (without fertilizer).

2.2 The method of collecting data

Seaweed that was analyzed for quality of agar rendement by isopropanol (SNI, 01-26-1998), viscosity using a measuring instrument viscosimeter Brookfield [7] and gel strength using a measuring instrument Curd Meter [8]. Samples were analyzed kelp seaweed is wet, then dried and taken simultaneously for each treatment on the base or its branches. Water quality data collection for temperature, salinity and water pH is done every 7 days in

the morning (09.00 am) and afternoon (15.00 pm). Measurements of soil pH, nutrient content of water and soil in the form of nitrate, ammonium, and phosphate were carried out at the beginning and end of the study. The temperature is measured with a thermometer, salinity using a hand-refractometer and pH of the water using Fix pH 0-14, then to the pH soil laboratory test that was extracted using the H_2O ratio of 1:2.5. Analysis of nutrient content of water in the form of nitrate (NO_3) were analyzed with sulfuric acid phenol [9], while phosphate (PO_4) were analyzed by digested sulfuric acid-nitrate and ammonium (NH_4) was measured using a spectrophotometer [10], analysis of nutrient content of the soil in the form of nitrate (NO_3) were analyzed with sulfuric acid phenol with AAS method (Atomic Absorbance Spectrophotometer), the content of soil phosphate (PO_4) were analyzed with HCl solution AAS method, determination of ammonium (NH4) were analyzed using a standard solution of H_2SO_4 and distillation Semi-micro Kjeldahl [11].

2.3 Data analysis

Each parameter of rendement, viscosity, and gel strength was evaluated using two statistical analysis. First, test the normality of Kolmogorov-Smirnov (K-S) and Shapiro-Wilk (S-W), then test homogeneity using Levene test. Second, an Analysis of Variance (ANOVA) test if the data is normally distributed and homogeneous if the real effect is further tested by using the Tukey test. Data that is not normally distributed and homogeneous is transformed x = log 10(y). While the nutrient content of water and soil is analyzed descriptively based on the life eligibility for *G. verrucosa* seaweed.

III. RESULTS AND DISCUSSION

3.1 Quality of Agar Rendement

From the calculation of variance analysis that the application of vermicompost fertilizer gave a real effect ($p<0,05$) to the quality of agar rendement seaweed *Gracilaria verrucosa*. From Tukey's further test results that the different rendement between treatment F (control) and A (dose 300 g/m^2). But from the results obtained that the treatment of fertilizer provides quality of agar rendement is higher than the treatment without the provision of fertilizer (Table 1). [12] stated that factors affecting the quality of seaweed rendement are nutrients and water quality, both of which have a close relationship to the content of seaweed for 56.2%, while 43.8% is influenced by other factors. Other factors cause the high quality of agar rendement to be influenced by species, cultivation location and climate of their life [13].

3.2 Quality of Agar Viscosity

The variance analysis that vermicompost fertilizer gave a significant effect ($p<0.05$) on the quality of agar viscosity *Gracilaria verrucosa*, meaning that the application of vermicompost fertilizer had an effect on the increase of viscosity. From Tukey's test results, the viscosity of seaweed gave a difference ($p <0.05$) between treatment F (control) and treatment A (dose of 300 g/m^2), B (dose 250 g/ m^2), C (dose 200 g/m^2), D (treatment 150 g/m^2) (Table 2). [14] stated seaweed viscosity ranges from 5 to 800 cps. The highest viscosity content was obtained at treatment A (dose 300 g/m^2) and treatment B (dose 250 g/m^2) high viscosity level followed by the high level of agar rendement in treatment A that is 25,81% and B that is 23,51%. The lowest seaweed viscosity was obtained in F (control/ without fertilizer) treatment with 8.58% of the agar rendement content (Table 2). This is in accordance with the opinion of [15] the higher of rendement followed by increased viscosity. This is due to the high rendement causing the breaking of the agarose and agaropectin structures in *G. verrucosa* which causes molecular chains to tighten and envelop the water-immobilized molecules causing the solution to be viscous, which means the viscosity of the high solution. Other factors causing high viscosity are treatment, temperature, SO_4 content, concentration, dispersion level, the presence of electrolyte and nonelectrolyte [16].

3.3 Quality of Agar Gel strength

The result of verbal analysis with vermicompost fertilizer did not give statistically significant effect on gel strength *Gracilaria verrucosa* ($p>0,05$), so no further Tukey test was done. The highest gel strength was obtained at treatment F (control) of 79.0 g/cm^2 (Table 3). Gravity gel is the maximum load required to solve the polymer matrix in the burdened region [17]. High sulfate levels cause increased viscosity and decreased gel consistency. While on *G. verrucosa*, the higher viscosity will break down agarose and agaropectin structure of seaweed which is a factor to produce high gel strength. This shows that the viscosity value is inversely proportional to the gel strength value, if the viscosity is high then the gel strength tends to be low, and vice versa if the obtained viscosity value is low then the gel strength will be high [18].

3.4 Nutrient Water

Water quality is one of the important factors for seaweed quality. Temperature is an important physical factor, for the growth of seaweed. Temperatures directly affect seaweed in the process of photosynthesis, metabolic processes, and reproductive cycle [19] water temperature in the cultivation container ranges from 28-30^{o}C which is still in the range that is suitable for seaweed growth (Table 4).

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Low water quality range of salinity can cause seaweed growth to be abnormal. Water salinity during the study ranged from 14 to 16 ppt (Table 4). The results suggest that seaweed can still grow at a low salinity range, proving that Gracilaria verrucosa are a type of seaweed that can live on a wide salinity. According to [20] salinity range for seaweed cultivation ranges from 15-30 ppt and optimal for seaweed growth ranges from 20-25 ppt. Pondus Hydrogen (pH) is a measure of the hydrogen ion concentration and shows the acid or base properties of water [21]. Pondus Hydrogen (pH) of water during the study was 7 (Table 4). [22] stated that the optimum pH for seaweed cultivation ranged from 6.8 to 8.2. pH of water in this research was 7 hence container where the research belongs to waters with high productivity [23].

Phosphate (PO_4) water obtained during the study was 0.27 - 0.61 ppm (Table 4). From the concentration of phosphate obtained, belong to a high fertility rate. According to [24] states that the low fertility levels of phosphate levels range from 0 to 0.02 ppm, moderate fertility rates ranging from 0.021 to 0.05 ppm and high fertility above 0.05 ppm. According to [25], good phosphate values for seaweed growth range from 0.09 - 1.80 ppm. Seaweed also requires a nitrogen element, nitrogen is absorbed by seaweed in the form of nitrite, nitrate, and ammonium. Nitrogen serves to help the process of forming chlorophyll, photosynthesis, protein, fat and other organic compounds [26]. The range of ammonium (NH_4) water content was 0.15 – 0.90 ppm and nitrate (NO_3) water was 0.03 - 0.57 ppm (Table 4). The value is feasible for seaweed cultivation. According to [27], the concentration of nitrate and ammonium is good for seaweed ranged from 0.01-3.50 ppm.

3.5 Soil Nutrients

Soil pH range obtained during this study was 4.54 - 5.71 (Table 5), low pH in the soil showed that the soil is acidic. According to [28] states that the nitrification process can still occur at soil pH 3.8. With optimal growth obtained with a soil pH range of 5 - 8.5.

Phosphate (PO_4) of soil obtained during the study ranged from 10.98 to 20.25 ppm (Table 5). Based on soil fertility value of phosphate classified not appropriate with limiting factor of soil fertility. However, the phosphate content in the soil can be increased by providing basic fertilizer with fertilizer application at the time the water has not been filled in the cultivation container, so it will be marginal or suitable enough and even very suitable [29].

The soil NH_4 in this study ranged from 0.59 to 8.99 ppm whereas for NO_3 the soil ranged from 6.45 to 14.61 ppm (Table 5). An increase in soil NH_4 content during the study. NH_4 is an ion of NH_3 or ammonia that is toxic, this is related to low soil pH in this study which has an effect on the increase of NH_4 on the soil. While the soil NO_3 content decreased during the study. The decrease of NO_3 content is caused because soil pH becomes acid so that macro nutrient content decreases in the soil which has the function to stabilize soil pH into the base. If the soil pH acid, then SO_4 in the form of H_2S will increase in the waters so that nutrients such as nitrogen, phosphor and other macro nutrients will be bound and micro nutrients will increase [30].

IV. CONCLUSION

The research conducted the highest level of viscosity and rendement of *Gracilaria verrucosa* seaweed was obtained at treatment A (300 g/m^2) and the lowest in treatment F (Control). The highest level of *G. verrucosa* seaweed gel strength was found in the treatment F (Control). This proved the gel strength inversely proportional to viscosity and rendement. The nutrient content of water is within a reasonable range of seaweed cultivation activities of *G.verrucosa*. Water temperature in the cultivation container ranges from 28-30^{o}C, salinity ranged from 14 to 16 ppt, (pH) was 7, phosphate (PO_4) was 0.27 - 0.61 ppm, ammonium (NH_4) was 0.15 – 0.90 ppm and nitrate (NO_3) was 0.03 - 0.57 ppm. Then the nutrient content of the soil is categorized as less fertile for cultivation activities, but from research conducted, that seaweed *G. verrucosa* can still grow well. Soil pH range obtained during this study was 4.54 - 5.71, phosphate (PO_4) obtained ranged from 10.98 to 20.25 ppm, NH_4 in ranged from 0.59 to 8.99 ppm whereas for NO_3 from 6.45 to 14.61 ppm.

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Table.1: Average Quality of Agar rendement Gracilaria verrucosa was fertilized with different doses of vermicompost.

Dose of Vermicompost Fertilizer (g/m^2)	Rendement (%)	Production Agar (g)
(A) 300	25,81±5,54[a]	5,9
(B) 250	23,51±4,74[ab]	4,9
(C) 200	18,81±2,06[ab]	3,6
(D) 150	16,74±9,34[ab]	3,1
(E) 100	14,56±4,62[ab]	2,6
(F) Control	8,58±5,26[b]	1,2

Description: Different letters in the same column show significant differences between treatments at 5% level ($p<0.05$), ± (distance of minimum and maximum values). (Source: Rahmad, 2016)

Table.2: Average Quality of agar viscosity Gracilaria verrucosa was fertilized with different doses of vermicompost.

Dose of Vermicompost Fertilizer (g/m^2)	Viscosity (cps)
(A) 300	90,00±10,00[a]
(B) 250	90,00±10,00[a]
(C) 200	83,33±5,77[a]
(D) 150	83,33±15,27[a]
(E) 100	76,67±5,77[ab]
(F) Control	46,67±20,82[b]

Description: Different letters in the same column show significant differences between treatments at 5% level ($p<0.05$), ± (distance of minimum and maximum values). (Source: Rahmad, 2016)

Table.3: Average Quality of Agar Gel strength Gracilaria verrucosa was fertilized with different doses of vermicompost.

Dose of Vermicompost Fertilizer (g/m^2)	Gel strength (g/cm^2)
(A) 300	56,6±6,96[a]
(B) 250	46,1±5,60[a]
(C) 200	41,9±8,61[a]
(D) 150	41,7±2,80[a]
(E) 100	40,0±6,61[a]
(F) Control	79,0±34,33[a]

Description: Different letters in the same column show significant differences between treatments at 5% level ($p<0.05$), ± (the distance of minimum and maximum values). (Source: Rahmad, 2016)

Table.4: Water nutrient ranges during the study

Dose of Vermicompost Fertilizer (g/m^2)	Temperature range (^oC)	Salinity range (ppt)	pH range	PO_4 early (ppm)	PO_4 end (ppm)	NH_4 early (ppm)	NH_4 end (ppm)	NO_3 early (ppm)	NO_3 end (ppm)
A (300)	28 - 30	14 - 16	7	0,55	0,27	0,62	0,17	0,57	0,03
B (250)	28 - 30	14 - 16	7	0,46	0,28	0,67	0,15	0,56	0,03
C (200)	28 - 30	14 - 16	7	0,61	0,31	0,90	0,38	0,54	0,04
D (150)	28 - 30	14 - 16	7	0,45	0,28	0,43	0,16	0,06	0,03
E (100)	28 - 30	14 - 16	7	0,47	0,27	0,39	0,24	0,37	0,03
F (Control)	28 - 30	14 - 16	7	0,41	0,27	0,63	0,27	0,05	0,03

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(Source: Rahmad, 2016)

Table.5: The range of soil nutrients during the study

Dose of Vermicompost Fertilizer (g/m^2)	pH of Soil (H_2O) early	pH of Soil (H_2O) end	PO_4 early (ppm)	PO_4 end (ppm)	NH_4 early (ppm)	NH_4 end (ppm)	NO_3 early (ppm)	NO_3 end (ppm)
A (300)	5,71	5,02	12,35	11,98	0,92	8,3	10,28	6,59
B (250)	5,68	5,03	14,65	17,14	1,82	6,69	12,24	7,25
C (200)	5,63	4,92	16,02	19,32	1,96	8,99	13,05	9,95
D (150)	5,35	4,9	16,55	20,25	2,13	6,99	14,15	9
E (100)	5,44	4,54	18,08	17,99	1,94	7,28	14,61	8,58
F (Control)	5,65	4,94	12,22	19,49	0,59	7,3	9,76	6,45

(Source: Rahmad, 2016)

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A New Low Cost Biosorbent for a Cationic Dye Treatment

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Abstract— *The aim of our study consists to investigate the adsorption of Methylene Blue from aqueous solution by a new biosorbent prepared from Papaya seed. Adsorption behavior of the cationic dye was analyzed by variation of solution pH, contact time, adsorbent dose, and temperature. Adsorption isotherms were studied according to the Langmuir and Freundlich Model, and adsorption kinetics according to pseudo first and second order. Results show that the maximum adsorption is obtained at ambient temperature with the yield of 98.82% and was reached in first 20min (pH = 10, adsorbent dose of 100 mg in 50 mL). The Langmuir isotherm shows a correlation coefficient of 99.4% higher than 95.4%obtained for Freundlich model and the adsorption kinetic model follow a pseudo-second order with a maximum adsorption capacity of 52.28 mg/g.*

Keywords— Methylene Blue, adsorption, Papaya seed, adsorption isotherm and adsorption kinetic.

I. INTRODUCTION

Water pollution has been generated an enormous fund input and raised worldwide concern**[1]**. Textile wastewater, generally, contains a big amount of pollutants materials like colored materials or dyes, organic compounds and heavy metals ions.These materials can affect the physicochemical and the biological properties of sea, drinking water and globally the ecosystem. In addition to the undesirable colors of textile effluents, some dyes may degrade to produce carcinogens and toxic products **[2]**. Furthermore, the colored effluents reduce light penetration and potentially prevent photosynthesis. Dyes even in very low concentrations affect the aquatic life and food chain. In the recent years, researchers are indulging their interest in wastewater treatment by various processes such as precipitation, ion exchange, reverse osmosis and adsorption **[3]**. The adsorption of colored solution has been the main point of numerous researchesas the effective process because it's easy to do, produce sludge without chemical products like conventional wastewater treatment, selective and cost-effective**[3]**.In this work, we used a new biosorbent, to treat the Methylene Blue (MB) by the batch adsorption system; the work consisted on the study of the effect of different factors which influenced the adsorption process as: pH, adsorbent dose, contact time and temperature, to deduce the adsorption thermodynamic and kinetic behavior process.

II. MATERIALS AND METHODS

2.1. Material and reagents:

Different laboratories materials are used in this work like UV visible spectroscopy (HACH LANGE DR 6000) for determination of MB concentration at a wave number of 662 nm, multi-parameter Consort C 3040 to adjust the pH values by HCl (0.1N) orNaOH (0.1N) and multi agitator.Methylene Blue(MB) ($C_{16}H_{18}ClN_3S$ see **Fig.1**), obtained from Sigma Aldrich, with a molecular weight of 319.85 g/mol. Solutions were prepared by dilution with distillated water of the stock solution of MB with the initial concentration of 1000 ppm to reach the desired concentration.

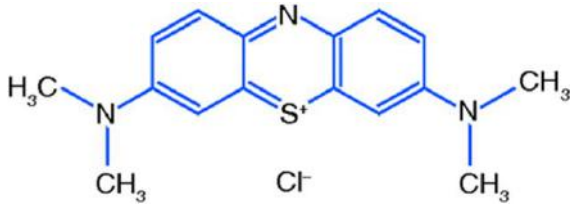

Fig.1: Methylene Blue structure

2.2. Characterization of the biosorbent:

Papaya seedare very abundant in Morocco and not valorized for any use were collected from a manufacturing process in the location of Settat- Casablanca, Morocco, washed several times to eliminate the impurities, and thencrushed to obtain a powder used as a new biosorbent. Our product was used in wastewater treatment without any chemical or physical activation treatment. The physicochemical properties of our biosorbent were determined by Langmuir and Freundlich model.

2.3. Adsorption experiments:

The adsorption experiments of MB on the biosorbent were reached after 2h of stirring at ambient temperature and

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agitation speed of 300 rpm. The suspensions were collected then centrifuged and the MB equilibrium concentrations were determined at 662 nm. The yield of adsorption and the amount of MB adsorbed at equilibrium noted R in (%) and q_e in $mg \cdot g^{-1}$, respectively, were calculated by the following equations (**Eq.1 and Eq.2**):

$$\mathbf{R}(\%) = \frac{\mathbf{C_0 - C_t}}{\mathbf{C_0}} . \mathbf{100} \quad \textbf{(1)} \quad \mathbf{q_e} = \frac{(\mathbf{C_0 - C_t})\mathbf{V}}{\mathbf{m}} \textbf{(2)}$$

Where C_0 is the initial dye concentration ($mg \cdot L^{-1}$), C_t is the equilibrium dye concentration ($mg \cdot L^{-1}$), V is the volume of the solution and m is the mass of the adsorbent (g).

2.3.1. Kinetic study:

Adsorption kinetic experiments were carried out using batch model. All of the dye solution was prepared with distilled water. Kinetic experiments were carried out by agitating 100 ml of solution of a constant dye concentration with 60 mg of MB at a constant agitation speed, ambient temperature and pH = 2. Agitation was made from 5 to 120 min.The experimental data will fitted by the pseudo-first-order and pseudo-second-order equation **[4]**

$$\text{Ln}\,(q_e - q_t) = \ln q_e - k_1 t \qquad \textbf{(3)}$$

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \qquad \textbf{(4)}$$

Where:

K_1: the rate constant of the pseudo-second order model (min^{-1}); q_t and q_e are the amounts of dye adsorbed on biosorbent in mg/g at time t and in the equilibrium respectively;

k_2 is the pseudo-second order kinetic model rate constant in g/mg. min

3.3.2. Isotherm study:

Different isotherm models provide us several information about the adsorption mechanism, the surface properties of the sorbent and the affinities between the sorbent and sorbate. In this work, two of the most used isotherms namely Langmuir and Freundlich, were used to fit the equilibrium experimental data of MBadsorption into our biosorbent. Langmuir theory assumes the existence of finite number of identical sites homogeneously distributed over the adsorbent surface, the linear form of this model is represented in the Eq.(**5**):

$$\frac{\mathbf{C_e}}{\mathbf{q_e}} = \frac{\mathbf{1}}{\mathbf{q_{max} K_L}} + \frac{\mathbf{C_e}}{\mathbf{q_{max}}} \textbf{(5)}$$

Where:

q_e: the equilibrium dye concentration on the adsorbent ($mg.g^{-1}$); C_e: the equilibrium dye concentration in the solution ($mg.L^{-1}$); q_{max}: the maximum adsorption capacity of the adsorbent ($mg.g^{-1}$); K_L: the Langmuir adsorption constant ($L.mg^{-1}$)

When the Freundlich isotherm model applies to adsorption on heterogeneous surfaces with interaction betweenthe adsorbed molecules, and is not restricted to the formation of a monolayer. This model assumes that asthe adsorbate concentration increases, the concentration of adsorbate on the adsorbent surface also increasesand, correspondingly, the sorption energy exponentially decreases. The well-known expression for the Freundlich model is given by the linear equation (**Eq.6**):

$$\mathbf{Log q_e} = \mathbf{\log K_f} + \frac{\mathbf{1}}{\mathbf{n_F}} . \mathbf{\log C_e} \qquad \textbf{(6)}$$

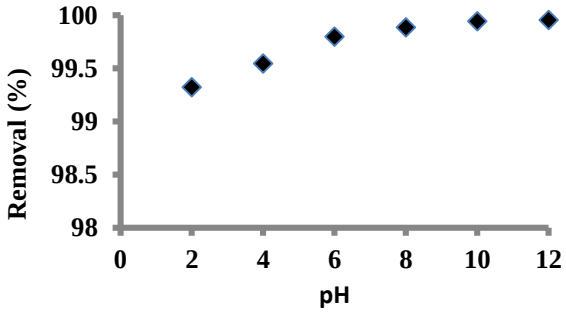
Where

q_e: the equilibrium dye concentration on the adsorbent ($mg.g^{-1}$); C_e: the equilibrium dye concentration in the solution ($mg.L^{-1}$); K_F: Proportionality constant for Freundlich equation [$(mg.g^{-1})(L.mg^{-1})^{1/n}$]; $1/n_F$: the adsorption intensity.

III. RESULTS AND DISCUSSIONS

3.1. Effect of pH on adsorption process:

In general, initial pH value may enhance or depress the uptake of solute. ThepH of adsorption medium influences not only the surface charge of adsorbent, but also, the degree of ionization of the material present in the solution and the dissociation of functional groups on the active sites of the adsorbent and the solution dye chemistry**[5]**.The effect of pH on MBremoval was analyzed over the pH range from 2-12. The pH was adjusted using 0.1 N (NaOH) or 0.1 N (HCl) solutions. In this work, 50 mL of dye solution was agitated with200mg of our biosorbentfor 120 min then the sample was centrifuged and analyzed using a spectrophotometer by measuring the absorbance changes at a wavelength of maximum absorbance 662nm. The **Fig.3**shows the effect of pH on Methylene Blue elimination, it's can be seen from the figure that the percentage removal of Methylene Blue by our biosorbent was optimum at basicpH =10, It could be as a result of the attraction between the positives charges of the cationic dye and the negatives of the sorbent. **Hassan et al., (2013)[6]**were obtained the similar basic adsorption (pH=8) of MB onto Haloxylonrecurvum plant stems



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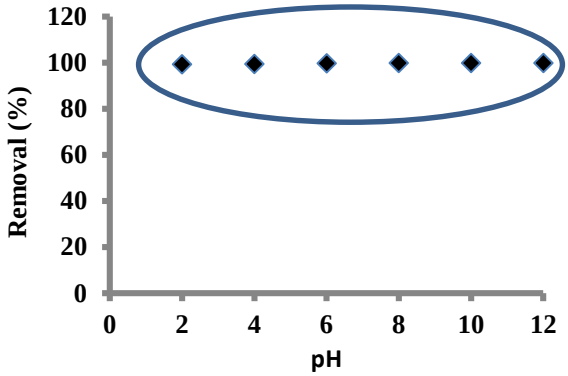


Fig.1: Effect of pH on Methylene Blue adsorption (V=50mL, t=2H, m = 200 mg)

3.2. Effect of adsorbent dose:

Adsorbent dose is a very important factor that influences the sorption process. Thebiosorbent product at various doses was added to 50 mL of MB solution with initial concentration of 100 ppm at optimal pH of 10 and atambient temperature.

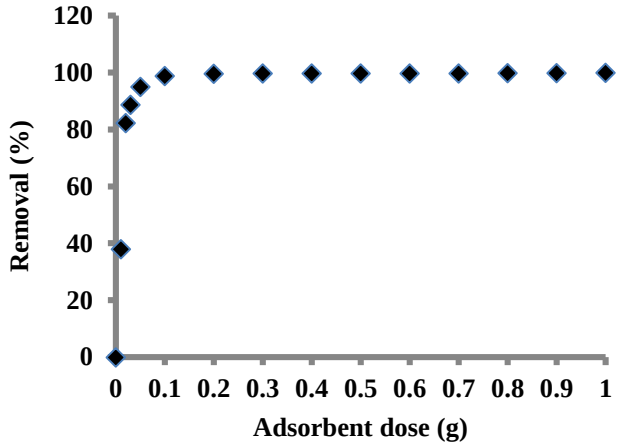


Fig.2: Effect of adsorbent dose on Methylene Blue adsorption (V=50mL, t=2H, pH=10)

The **Fig.2**revealed that the adsorption removal yield increase with the increasing of the adsorbent dose until the optimal value of adsorbent dose of 100 mg, after this value there was any significant change of the MB elimination.This can be explicated by the increases of the surface area and thus the number of available adsorption sites**. Mahammedi and Belkacem (2015)[7]**were obtained a maximum MB removal using4 g/L using natural clay.

3.3.Effect of contact time and kinetic study:

The plot of removal yield versus time is shown in **Fig. 3**.In this plot, it is apparent that MB removal by the adsorption increased rapidly in the initial stage (20 min) and became slower in the later stages until the attainment of equilibrium.

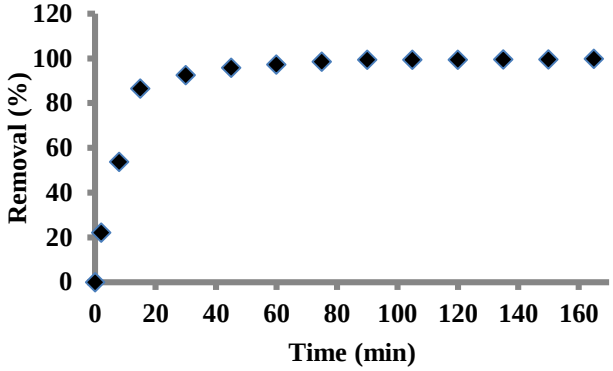
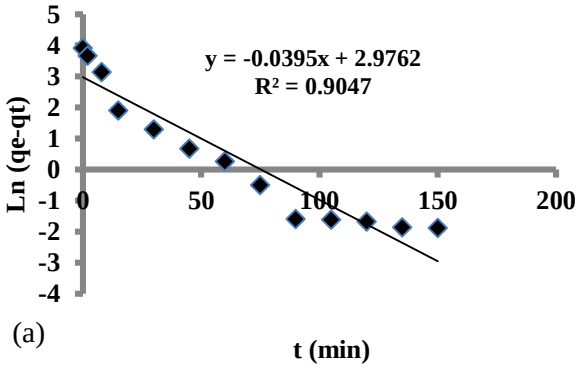


Fig.3: Effect of contact time on Methylene Blue adsorption (V=500mL, pH=10, m=1g)

As the surface adsorption sites become exhausted, the uptake rate is controlled by the rate at which the dye molecules are transported from the exterior to the interior sites of the adsorbent particles. Equilibrium time for the adsorption of MB found by **Amuda et al.,(2014)[8]**was found to be 60 min to reachonly an adsorption yield of 90% using Steam-Activated Carbon Produced from Lantana camara Stem.

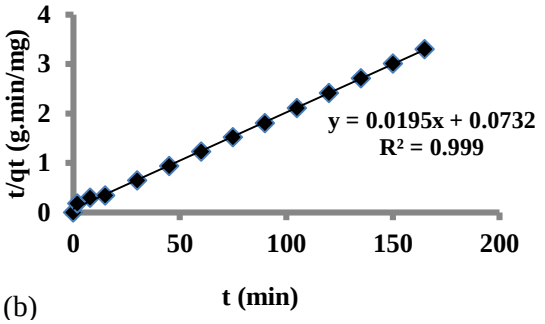
3.3.1. Kinetic study:

The plot of $t/q_t$and Ln (q_e-q_t) versus time t, were shown in the **Fig.4**



(a)

Fig.4: (a) Pseudo first order,



(b)

Fig.4: (b) Pseudo second order plot of papaya seed biosorbent

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From the results we observed that the adsorption of Methylene Blue was better fitted with the pseudo second order with a R^2=0.99 (**Table.1**).

Table.1: Adsorption kinetic parameters of Methylene Blue

Pseudo-second order			Pseudo first order	
Qe (mg/g)	K_2 (g /mg .min)	R^2	K_1 (min^{-1})	R^2
51.28	0.133	0.99	0.039	0.90

These results indicate that the kinetic model of the adsorption is based on the assumption that the rate-limiting step is a chemical adsorption involving valance force through sharing or exchange of electrons between adsorbent and adsorbate.Similar result was also obtained by **Han et al., (2017)** using Molybdenum Disulfide Nanostructure **[9].**

3.3.2. Adsorption isotherm study:

The adsorption capacity of the adsorbent, interaction between the solute-solution and the nature of adsorbed accumulation materials on the surface of the adsorbent can be explained using isotherm models. The **Fig. 5** shows the plot of Langmuir and Freundlich isotherm models.

Results shown in **Fig.5**revealed that the biosorption of MB on papaya seed adsorbent is the monolayer type since the correlation coefficient (R^2) calculated from Langmuir isotherm is below 0.99, which indicate lessapplicability for Langmuir isotherm.

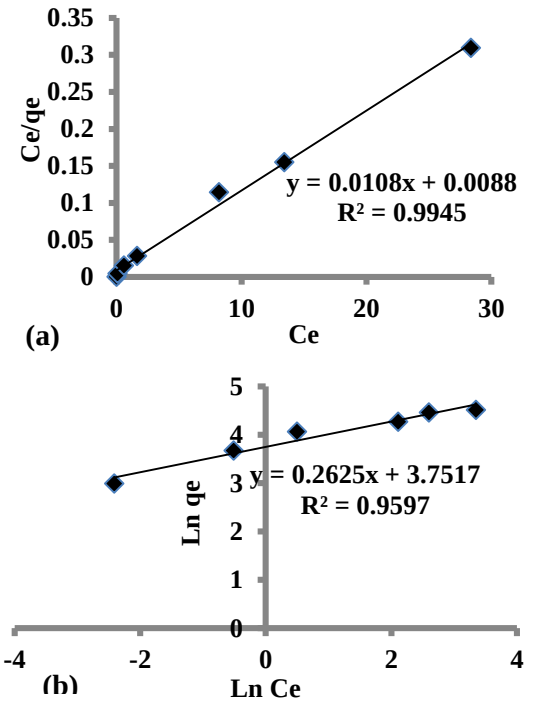

Fig.5 Adsorption isotherm of MB on papaya seed (a) Langmuir model, (b) Freundlich model

These results are in accordance with the result obtained in our Patent number PCT/MA2016/000003 for the removal of MB by combination of adsorption process into clay material and flocculation by polyelectrolyte extracted from cactus cladode.

IV. CONCLUSION

Papaya seed were selected as a suitable agriculture product thanks its abundance in Morroco and its disposal in the environment without any treatment. Several parameters were studied to deduce their effect on Methylene Blue adsorption. The adsorption of Methylene Blue increase with the increasing of time, temperature and adsorbent dose. The results show that the optimal condition of treatment of pH =10, adsorbent dose of 200mg in 100mL and very low contact time of 20 min are sufficient to eliminate 98.8% of Methylene Blue. The adsorption isotherm was well reproduced with Langmuir model and show that the adsorption is the monolayer type, the kinetic study shows that the adsorption follows the pseudo second order model.

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Proximate and Microbial Profile of Couscous Yoghurt Produced from Soya Milk

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***Abstract*—** *This study investigated the effect of milk type and mixture ratio on the proximate composition and microbial profile counts of couscous yoghurt. Yoghurts were first made from cow milk (CM), soya milk (SM) and equal mixture of both types of milk at ratio 50:50. Couscous was then mixed with yoghurts from cow milk (CMCY); soya milk (SMCY) and cow-soya milk (CSCY) at ratios of 90:10, 80:20 and 70:30 (yoghurt: couscous), w/w for the three respectively. The experiment was designed based on 2 factors (milk type and mixing ratio) at 3 levels, each resulting in a total of 9 treatments. Cow milk yoghurt without couscous was used as the control. Proximate compositions were determined using standard methods. Total viable microbial counts of samples were also determined. There were significant differences ($p<0.05$) in the proximate composition and CSCY at ratio 70:30 had the highest crude protein. In addition, CMCY at ratio 90:10 recorded the highest mean value for fat, while SMCY at ratio 80:20 and 70:30 recorded the least mean value for fat. All the couscous yoghurt samples had total viable cell counts of (<9 log CFU) that are within the acceptable range according to Codex Standards. In conclusion, the study has shown that CSCY at 70:30 had the highest nutrient content. Moreover, the products were also found to have low levels of microbial profile.*

***Keywords*— *Couscous, Microbial Profile, Proximate, Yoghurts*.**

I. INTRODUCTION

Yoghurt is one of the oldest fermented milk products that is consumed all over the world; and it is produced by fermenting milk with lactic acid bacteria which are responsible for the development of the typical yoghurt flavour[7]. Soya bean is economically the most important bean in the world providing vegetableprotein for millions of people and ingredients for hundreds of chemical products [2]. The key benefits are related to the excellent protein content (it contains all 8 essential amino acids) with high levels of essential fatty acids, numerous vitamins, minerals, isoflavones, and fibre[1]. The most nutritious and most easily digested food of the bean family, it is one of the richest sources of proteins in staple foods in the world today. Soya bean is one of the important crops taken into consideration as candidates for genetically modified (GM) foods due to its great demand worldwide [11].Studies carried out by [14] reviled that quality and shelf life of fermented dairy products greatly depends upon the quality of raw milk, low total bacterial counts, absence of antibiotics and bacteriophages. The product is said to be perishable in view of its unused lactose content [5].[13]reported that there is an apparent need for a valuable preservation method to control acid-tolerant spoilage yeasts and molds in yoghurt. Micotoxigenic fungi and pathogenic bacteria are able to grow at refrigeration temperature to numbers, which can result in an infection. Changes in the chemical, physical and microbiological composition of yoghurt determine the storage and shelf life of the product.

This study therefore was to determine the suitability of replacing cow milk with soya milk in couscous yoghurt production.

II. MATERIALS AND METHODS

2.1 Location of the Study

The study was conducted at the Crop Utilization Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, South-west Nigeria.

2.2 Materials

Soya bean seeds (variety TGX 1987, 62 F) were obtained from IITA headquarters Ibadan. Grains of millet (variety JARANI Brown) were obtained from IITA Kano, northern Nigeria. Fresh cow milk was obtained directly from the livestock farm of the Federal University of Agriculture Abeokuta, Ogun state, Nigeria. Commercially available yoghurt starter cultures (*Streptococcus thermophillus and Lactobacillus bulgaricus)* sugar and flavouringswere purchased from a reputable source in Abeokuta, Ogun State.

2.2.1 Soya Milk Preparation

Soya beans were cleaned manually to remove dust, damaged seeds, weeds, and metals. Pre-cleaned soya beans (1kg) were soaked in a 16 Litres clean tap water for 10-12 h. The soaked beans were de-hulled manually and milled into a smooth paste. The paste was mixed with 12 Litres of clean tap water to the thickness of milk and sieved through a muslin cloth into a clean fitted container, using method the described by [10].

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2.2.2 Preparation of Yoghurt

Soya milk and cow milk were pasteurized separately at 82 ºC for 30 min and allowed to cool to 42 ºC. Freeze-dried starter culture (*Streptococcus thermophillus* and *Lactobacillus bulgaricus)* was dissolved in a small quantity 75cl of lukewarm milk in a cup and poured into the two milk samples then stirred well. The milk was Incubated at 45 ºC according to manufacturer's instructions for the starter culture until it had reached the desired firmness. Sugar and flavourings were added to the coagulum and, stirred very well. Using method the described by [10].

2.2.3 Preparation of Couscous

Grains of millet (variety JARANI Brown) were cleaned, sorted and washed using tap water and were allowed to dry at 55^0C for 24h using box oven drier. Millet grains were then milled using fabricated milling machine into a smooth powder and sieved using 0.04mm sieve. Water was sprinkled on the milled millet powder and rolled by hand to form pellet, the pelletized millet was then dried for 5h at 55^0C using box oven to form couscous. The couscous was then steamed for 5min in a tight fitted container with boiled water [8].

2.3 Analyses of yoghurt couscous samples

2.3.1 Moisture content determination

Three grams of the sample was placed in a preheated and weighed metallic dish and dried in a Conventional Oven (Fisher Scientific Isotem oven model 655f) at 105 ^{0}C for 16h and then transferred to a dessicator at room temperature to cool. The loss in weight was then calculated, using method described by [3].

CALCULATION

$$\%\ \text{Moisture Content} = \frac{M_1 - M_2}{M_1 - M_0} \times 100$$

Where M_0 = Weight in g of dish and lid

M_1 = Weight in g of dish, lid and sample before drying

M_2 = Weight in g of dish, lid and sample after drying

2.3.2 Ash content determination

Three grams of the sample was weighed in a dried and pre -weighed crucible and ignited in a muffle furnace (Vulcan 3-1750) at 600 ^{0}C for 6 h to complete burning of all organic matter. The crucible was transferred directly to a dessicator, cooled and weighed immediately. Ash content was determined, using the method described by [3].

$$\% = \frac{(\text{weight of crucible} + \text{ash}) - (\text{weight of empty crucible})}{\text{Sample weight}} \times 100$$

2.3.3 Fat content

Fat from all the couscous yoghurt samples were extracted by adopting the [4]method using Soxtec extractor. Three grams of the sample was placed in the thimble and fitted into the extractor. The fat was extracted with 80 ml of hexane. The extracted fat in cups was weighed and calculated as percentage fat as indicated below

$$\%\ \text{Fat on oil} = \frac{(W_3 - W_2)}{W_1} \times 100$$

2.3.4 Crude fibre content determination

Crude fibre was determined according to [3] method No. 926.09. One gram of the sample was digested with 100 ml of 1.25 percent sulphuric acid with 2- 4 drops of n-Octanol added to prevent foaming and then filtered through a sintered glass crucible under vacuum. The residue was then washed with hot deionized water till neutralized; 150 ml of 1.25 percent sodium hydroxide was also used to further digest the samples. Digested material was again filtered and washed with hot water until neutralized. The washed material was dried at 100 ºC overnight, cooled in a desiccator and weighed. The dried residues were ignited for 3 h and the crucible was reweighed with burnt material. Crude fibre was calculated by using the following formula:

$$\%\ \text{crude fibre} = \frac{W_2 - (W_3 + C)}{W_1} \times 100$$

W_1 = Sample weight (g)

W_2 = Crucible + residue weight after drying (g)

W_3 = Crucible + residue weight after ashing (g)

C = Blank

2.3.5 Protein content determination

About 0.200 g of the dry sample was weighed into a digestion tube, 2.5ml of $H_2SO$and allowed to cool for 10 min, 1ml of 30% H_2O_2 was added to the sample and heated to 330^OC for 2 h and allowed to cool. About 0.200-0.800 ml of n: p solution was added to the five standards. The sample and standards were then diluted to the 50 ml mark into cups and N read on the auto- analyzer machine, using the method described by [6].

2.3.6 Microbial Determinations

The total viable count of yeast, mould and bacteria counts of the couscous yoghurt samples were determined using pour plate technique and the appropriate dilution was placed on nutrient agar plates. The plates were incubated for 3-5 days and colony forming units per ml sample (cfu/ml)using the method of [8].

2.4 Experimental Design and Statistical Analysis

The experiment was designed based on 2 factors (milk types and mixing ratios) at 3 levels each, i.e., a 3^2 factorial resulting in a total of 9 treatments. Cow milk yoghurt without couscous was used as the control

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The data obtained were subjected to One-way analysis of variance (ANOVA) using Statistical Package for Social Scientists (SPSS) version 21.0 while Duncan's multiple new range F test was used to compare the means and the least significant difference (LSD). Also the data were subjected to two-way ANOVA to investigate the interaction among the factors.

III. RESULTS

Table 1 shows the result for proximate composition of the different mixture ratios of the four different couscous yoghurt types. The values obtained for all the nutrients at different mixing levels of the products revealed significant ($p < 0.05$) differences. It was observed that only the moisture content of cow milk yoghurt (CMY) recorded the higher while cow-soya yoghurt: couscous (CSCY) at ratio 70:30 recorded the least value for moisture. CSCY at ratio 70:30 result showed the higher mean value for crude fibre. Also, soya milk yoghurt: couscous (SMCY) at ratio 70:30 and CSCY at ratio 80:20 recorded similar values for crude fibre. Crude fibre for CMY only recorded the least value. It was also observed in this study that ash at ratio 70:30 of CMCY yoghurt and CSCY recorded the highest, while SMCY at ratio 90:10 recorded the least mean value for ash. In addition, CMCY at ratio 90:10 recorded the highest mean value for fat, while SMCY at ratio 80:20 and 70:30 was seen to be lesser for fat. CSCY for carbohydrate at ratio 70:30 recorded the highest value, while the least value was recorded for CMY only. The result obtained for CSCY at ratio 70:30, recorded the highest mean value for crude protein. CMCY at ratio 90:10 and SMCY at ratio 80:20 recorded similar values for crude protein. The crude protein in cow milk yoghurt only was seen to be lower.Table 2 shows the results for microbial profile of the different mixture ratios of the four different yoghurt types. There were significant ($p<0.05$) differences in the mixture ratio of the products. CSCY at ratio 90:10, CMY that is 100% control and CMCY at ratio70:30 recorded the higher mean values for yeast. SMCY at ratio 80:20 recorded the least mean value for yeast. CMCY at ratio 70:30 had the highest mean value for mould, while CSCY at ratio 70:30 recorded the least mean value for mould. CSCY at higher inclusion of the couscous (70:30) elicited more bacteria counts. CMY at 100% (control), 80:20 and SMCY at 90:10 recorded similar values for bacteria counts. The least value was obtained for SMCY at ratio 80:20.

Table 1: Proximate Composition of different yoghurt mixes with millet couscous

Products	Moisture	Crude protein	Crude fibre	Ash	Fat	carbohydrate
CM yoghurt only	86.18±0.14^{a}	4.23±0.08^{h}	0.25±0.00^{h}	0.65±0.01^{d}	3.45±0.04^{b}	5.23±0.13^{j}
CM Yoghurt: couscous mix						
70:30	60.45±0^{i}	5.56±0.17^{b}	1.85±0.02^{b}	0.70±0.00^{a}	3.10±0.00^{d}	28.34±0.16^{b}
80:20	63.67±0^{h}	5.22±0.00^{cd}	1.51±0.03^{d}	0.67±0.01^{c}	3.22±0.00^{c}	25.71±0.06^{c}
90:10	67.52±0^{f}	4.80±0.03^{f}	1.24±0.02^{f}	0.68±0.01^{b}	3.56±0.00^{a}	22.19±0.01^{f}
SM Yoghurt: couscous mix						
70:30	69.36±0^{e}	4.95±0.07^{ef}	1.56±0.01^{c}	0.41±0.00^{g}	1.24±0.00^{i}	22.48±0.07^{e}
80:20	70.86±0^{c}	4.79±0.01^{f}	1.42±0.02^{e}	0.39±0.01^{h}	1.26±0.00^{i}	21.28±0.02^{h}
90:10	72.86±0^{b}	4.59±0.00^{g}	1.06±0.01^{g}	0.33±0.01^{i}	1.30±0.00^{h}	19.86±0.01^{i}
CM+SM Yoghurt: couscous mix						
70:30	56.79±0^{j}	6.28±0.26^{a}	2.56±0.03^{a}	0.69±0.01^{a}	1.76±0.04^{g}	31.91±0.27^{a}

80:20	65.48±0^{g}	5.29±0.13^{c}	1.57±0.04^{c}	0.56±0.00^{e}	1.86±0.00^{f}	25.24±0.10^{d}
90:10	69.86±0^{d}	5.05±0.03^{de}	1.09±0.00^{g}	0.50±0.00^{f}	2.00±0.00^{e}	21.50±0.03^{g}

$^{a-j}$ Means within the column with different superscripts differ significantly ($p < 0.05$).
CM: Cow milk only as control, SM: Soya milk, CM: Cow milk, Cow milk + Soya milk.

Table 2: Microbial profile of different yoghurt mixes with millet couscous

Products	Mould	Yeast	bacteria
CM Yoghurt only	8.60±0.02^{bcd}	8.63±0.02^{a}	8.41±0.03^{bc}
CM Yoghurt:Couscous mix			
70:30	8.71±0.05^{a}	8.64±0.02^{a}	8.30±0.09^{cd}
80:20	8.68±0.04^{ab}	8.62±0.04^{ab}	8.40±0.05^{bc}
90:10	8.61±0.03^{bc}	8.53±0.03^{bc}	8.46±0.05^{b}
SM Yoghurt:Couscous mix			
70:30	8.53±0.04^{cd}	8.44±0.02^{cd}	8.47±0.10^{b}
80:20	8.62±0.05^{bc}	8.35±0.10^{d}	8.21±0.12^{d}
90:10	8.59±0.07^{bcd}	8.51±0.07^{c}	8.43±0.07^{bc}
CM+SM Yoghurt: Couscous mix			
70:30	8.42±0.05^{e}	8.49±0.07^{c}	8.61±0.05^{a}
80:20	8.50±0.10^{de}	8.49±0.04^{c}	8.35±0.06^{bcd}
90:10	8.61±0.04^{bc}	8.66±0.03^{a}	8.31±0.07^{cd}

$^{a-e}$ means within the same column with different superscript differ significantly ($p<0.05$)
CM: Cow milk only as control, SM: Soya milk, CM: Cow milk, Cow milk + Soya milk.

IV. DISCUSSION

The lower moisture values of CMCY, SMCY and CSCY with different ratios when compared with CMY only could be due to the fact that the addition of couscous has increased the solid matter in the different blends of the yoghurt: couscous. This is in agreement with the work of [8] who reported that corn starch in the form of slurry thickened the soya yoghurt. The increased protein content with increasing levels of the couscous inclusion is in contrast to the work of [8] who reported a decreasing level of protein in the evaluation of soya-corn yoghurt. This could obviously be due to the significant quantity of protein in soya milk with couscous [9].The high protein content of the products in this study showed that consumption will contribute to the reduction of protein deficiencies in diets which have become a major challenge in poor nations and in children. It could be observed in this study that crude fibre, ash and carbohydrate assumed similar trends, as reported for protein. This corroborates with the findings of [8]. The increase in ash contents observed in all the products is due to the mineral contents caused by the addition of couscous as reported by [10]. The ash is an index of mineral content which is needed for bone development, teeth formation and body function [15] The low fat contents recorded for the ratios of SMCY and CSCY are an indication of the increased total energy available in the products and the longer shelf life which decreased the chances of rancidity.

The microbial profile count is an index of the level of sanitation and or water quality employed in the handling and processing of the products. All the couscous yoghurt samples had total viable cell counts of (<9 log Cfu/g) that are within the acceptable range according to Codex alimentarius standards which stated that a maximum count of 10.0 Cfu/g microbes is allowedin yoghurt. The products were also entirely found to have low levels of microbial count. Observations in this study indicated that the handling and processing of the various yoghurts mixes with couscous was done under proper hygienic conditions. The levels of mould and yeast obtained in this study were also within the recommended level of 10.0 log cfu/g for yeast and mould reported by [12] who stated that levels above 10.0 log cfu/g are capable of producing toxic metabolites (mycotoxin e.g., aflatoxin) leading to food poisoning and can cause cancer of the liver in humans.

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V. CONCLUSIONS

At the end of this study, the following conclusions were made;

1. According to the results of proximate composition it can be concluded that products prepared from CMCY and CSCY yoghurts at ratios 70:30 had highest nutrient contents than those of SMCY and CMY. The results obtained in this study indicated that the nutrient composition of both yogurt types changed similarly.
2. The products were also found to have low levels of microbial profile which is good for human consumption.

VI. RECOMMENDATION

It can be recommended that Cow-soya couscous yoghurt should be added in the ratio 70:30 because of its high nutrient contents which will help in overcoming the issue of malnutrition in children.

VII. ACKNOWLEDGMENTS

I am grateful to the Almighty God for his divine opportunity and inspiration given to me throughout the conduct of this research. My sincere gratitude also goes to my Supervisors, their guidance, encouragement and constructive criticism which have immensely contributed to the success of this research work.

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Microbial Effect of Refuse Dump on the Composition of Leafy Vegetables Grown in the Vicinity of Dump Site Along River Benue, Mubi Road, Yola

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Abstract— *Microbial quality of vegetables grown in the vicinity of dumpsite along river Benue basin Yola Adamawa state was investigated to determine the effect of the wastes. A total of twenty samples were studied, from each of vegetable, soil and water at different distances 50, 100 and 200m from the dumpsite. Microbial analysis showed that total bacterial, mold and yeast, and coliform bacteria counts exceeded the 1,000 CFU/100ml guideline for water used in fresh produce. The result shows that total bacterial count was found to be significantly higher in the soil ranging from 4.3 x 10^5 – 4.78 x 10^6 followed by irrigation water ranging from 1.0 x 10^4 – 3.66 x 10^6 and the least was the vegetable ranging from 1.0 x10^4 – 9.0 x 10^4. Coliform bacteria count was found to be higher in the irrigation water ranging from 2.0 x 10^4 – 1.2 x 10^5 followed by the vegetables ranging from 1.0 x 10^4 – 2.0 x 10^4 and no growth of coliform was found in the soil. Mold and yeast was found to be significantly higher in the soil ranging from1.0 x 10^4 – TNC and was absent in the vegetables and water respectively. The higher level of microorganism observed in the dump site vegetables compared with the control vegetables show that refuse dump contribute to the microbial load in the study site. This implies that the microbial quality of vegetables may pose a health risk to the people who consume them if not properly prepared.*

Keywords— Microbial, Refuse dump, Vegetables, Yola.

I. INTRODUCTION

The nutritional significance of vegetables in healthy diet cannot be over emphasized. Vegetables are known to be good source of vitamins, minerals and dietary fiber. They also add flavor (color and taste) as well as aesthetic appeal to diet. Eating vegetables provides health benefits, people who eat more vegetables as part of an overall healthy diet are likely to have a reduced risk of some heart diseases like stroke, cancers, obesity, type 2diabetes etc. That is why the consumption of fresh fruits and vegetables is encouraged by government health agencies (James J.B and T. Ngarmsak, 2011)

However, leafy green vegetables were identified as the commodity group of highest concern from a microbiological safety perspective. This commodity grouping was considered to include all vegetables of a leafy nature and of which the leaf is the intended for consumption such as lettuce (all varieties), spinach, cabbages, chicory, leafy fresh herbs (e.g. cilantro, basil, parsley) and watercress. (WHO/FAO, 2008)

The land around river Benue basin stretching to Lake Gerio, has long been used for both wet and dry season farming. This area also served as a dumpsite for refuse or domestic waste. Waste water from the neibouring wards passes down the area through connecting drainages. During the rainy season, when there is so much water, contamination of vegetables may take place at all stages during pre and post-harvest stages (De Roever, 1999). Row fruits and vegetables are known potential for a wide range of microorganisms, including human pathogen (Tambekar and Mundhada, 2006). Food-borne bacterial pathogens commonly detected in fresh vegetables are coliform bacteria, E. coli, staphylococcus aureus and Salmonella sp. (Tambekar and Mundhada, 2006).

Microbiological risk assessment is an emerging tool for the evaluation of the safety of food and water supplies. Different organizations have suggested that microbiological risk assessment should be carried out so that appropriate remedial measures can be adopted to curtail the incidences of food-borne illness as a result of consumption of contaminated foods. Microbes, mainly the coliforms group have been used extensively as indicator of microbiological quality of water and food. Their presence indicates improper treatment or post-disinfection contamination (Ciira, 2003).

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The main objectives of these study was to investigate the potential hazard of microorganism associated with vegetables grown in the vicinity of refuse dump along river Benue basin Yola, Adamawa state and highlight the importance of proper preparation before consumption and also investigate the source of contamination and prevalence of pathogenic microorganisms their consumption is encour-aged in many countries by government health agencies toprotect against a range of illnesses such as cancers and cardiovascular diseases. However, fruits and vegetables, and in particular leafy greens that are consumed raw, are increasingly being recognized as important vehicles for transmission of human pathogens that were traditio- nally associated with foods of animal origin. Their consumption is encour-aged in many countries by government health agencies to protect against a range of illnesses such as cancers and cardiovascular diseases. However, fruits and vegetables, and in particular leafy greens that are consumed raw, are increasingly being recognized as important vehicles for transmission of human pathogens that were traditio- nally associated with foods of animal origin.

Source of materials

Representative samples of vegetables, water and soil were obtained from the study site along the River Benue Basin, Shinko site. Instruments, tools and general laboratory glass wares were obtained from Food science and Technology laboratory Modibbo Adama University of Technology Yola.

II. STUDY AREA

Yola is the capital city and the administrative center of Adamawa State Nigeria. Located on the Benue river, it has a population of 336,648 (2010). With coordinates 9º13˙48”N 12º27˙36”E/ 9,23000ºN 12,46000ºE and its elevation is 1,965ft. above sea level (599m) The specific site is in river Benue bank in Yola bye pass where variety of vegetables are cultivated and it is chosen because the area is currently undergoing severe degradation resulting from municipal waste disposal.

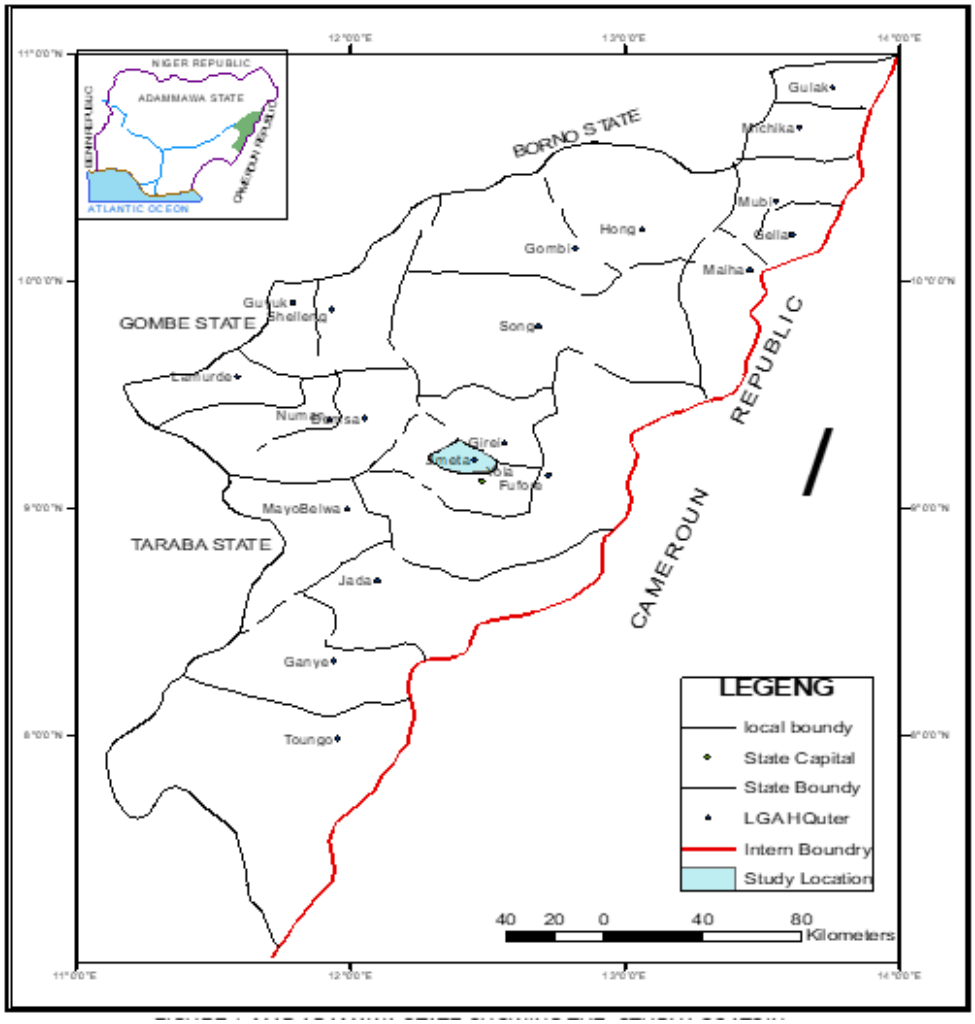

Fig.1: Map Adamawa state showing the study Location

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Study Design

The research was carried out in two phases; the first phase was preliminary investigations involving the history of the dumping site, the variety of materials dumped at that site and the estimate of the size or volume of the dump.

The second phase was the analytical process which involves the environmental media, sample collection, preparation and laboratory work.

Preliminary investigation of the refuse dump

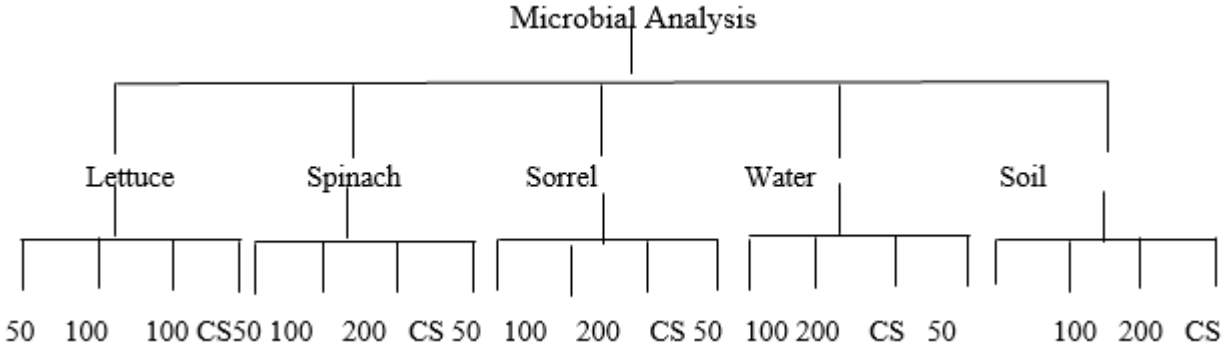
The information was collected through interviews, field survey measurements, and direct observation of the type and nature of waste, as well assess the volume of the dump site and then evaluate the role of government and community in managing the site.

III. ANALYTICAL METHODS

The analytical phase involved microbiological analysis viz: total bacterial plate count (TBPC) was determined by the procedure described by V.A Jideani and I.A Jideani (2006). Yeast and Mould count was determined by the procedure described by Harrigan and McCance(1976). Coliforms bacteria was determined by the procedure described by Harrigan and McCance(1976).

Experimental Design

Figure 3 is a 5x4 factorial design for the study of microbial contamination of vegetables, water and soil.



CS=Control sample, m = meters

Sampling Procedure

Three (3) popular vegetables namely Lettuce, Sorrel and spinach with their associated soil, water was taken at a distance of 50 meters, 100 meters, 200 meters from the edge of the selected area. Reference vegetable sample was taken from a farm in Loko Song L.G.A where refuge is not close by. All the Soil samples were collected from the upper soil layer of 0-5cm to the laboratory for analysis.

IV. RESULTS

Table.1: Results of the microbial analysis of vegetables (lettuce, spinach and sorrel) water and soil samples (cfu/ml)

Enviro. Media	Distances	coliform count	total bacterial count	mold & yeast count
Lettuce	50m	0.0 x10^4	9.0 x 10^4	0.0 x 10^4
	100m	1.0 x 10^4	2.0 x 10^4	0.0 x 10^4
	200m	2.0 x 10^4	7.0 x 10^4	0.0 x 10^4
	Control	0.0 x 10^4	5.0 x 10^4	0.0 x 10^4
Spinach	50m	1.0 x 10^4	3.0 x 10^4	0.0 x 10^4
	100m	0.0 x 10^4	8.0 x 10^4	0.0 x 10^4
	200m	1.0 x 10^4	4.0 x 10^4	0.0 x 10^4
	Control	0.0 x 10^4	2.0 x 10^4	0.0 x 10^4
Sorrel	50m	0.0 x 10^4	2.0 x 10^4	0.0 x 10^4
	100m	0.0 x 10^4	3.0 x 10^4	0.0 x 10^4
	200m	2.0 x 10^4	1.0 x 10^4	0.0 x 10^4

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	Control	0.0×10^4	1.0×10^4	0.0×10^4
Water	50m	1.2×10^5	3.66×10^6	0.0×10^4
	100m	2.0×10^4	2.52×10^6	0.0×10^4
	200m	3.0×10^4	6.0×10^4	0.0×10^4
	Control	0.0×10^4	1.0×10^4	0.0×10^4
Soil	50m	0.0×10^4	7.3×10^5	TNC
	100m	0.0×10^4	7.7×10^5	4.0×10^4
	200m	0.0×10^4	4.78×10^6	3.0×10^4
	Control	0.0×10^4	4.3×10^5	1.0×10^4

Analysis was performed in triplicates
TNC: too numerous to count

V. DISCUSSION OF RESULT

Vegetables

The average total bacterial count, coliform count and the mold and yeast count from four distances were represented in table4.

Total bacterial count of the vegetables range from 1.0×10^4 –9.0×10^4 cfu/ml and the coliform count range from 1.0×10^4 – 2.0×10^4 cfu/ml. it is observed that the overall microbial load of the vegetables was found to be independent of the distance from the dumpsite. This implies that the microbial source is not due to the dumpsite soil but, it is coming from the irrigation water because, irrigation water was observed to have the highest microbial growth and which serves as a potential source of contamination of vegetables. The result is in agreement with that reported by Chaturvedi *et al*. (2013) And Halablab *et al.,* (2011). They reported presence of microbial contamination in vegetables

There were significant difference in the total bacterial count growth found in each distance but, in the coliform count there were no difference. There were also significant difference between the samples in the study site and the control. This indicates that there is indeed effect of the dumpsite (effluent water) on the quality of the vegetables. These findings correspond with the report recorded by Abakpa *et al.,* (2013) on the microbial quality of irrigation water and irrigated vegetables in Kano state Nigeria. Majority of farmers use water from drains with low water quality.

Irrigation water

Total bacterial count and coliforms count are higher in the irrigation water compare with the vegetables and soil samples. The microbial load was found to decrease with distances increasing from the dumpsite. Total bacterial count range from 1.0×10^4 – 3.66×10^6 cfu/ml and the coliform count range from 2.0×10^4 - 1.2×10^5 cfu/ml. Mold and yeast were not found in the irrigation water.

The higher growth showed by water samples is an indication that the irrigation water serves as the potential source of contamination these result therefore, reflect the exposure of the vegetables to contamination during irrigation and in particular, the existence of favorable conditions for the multiplication of microorganisms. Previous studies by Abakpa *et al.,* (2013). Reveals that irrigation water source received pollutant from various effluents discharged from industries, domestic sewages, abattoir and other non-point source of pollution.

Soil

Total bacterial count range from 4.3×10^5 -4.78×10^6 with the highest count coming from 200m distance and lowest count from the control sample. Soil inhabit microorganism under favorable condition therefore, the soil serves as a potential source of contamination Many vegetables grow low to the ground where they are likely to come in contact with the soil. If the soil has been treated with improperly treated animal manure as fertilizer or irrigated with contaminated water vegetables are also likely to be contaminated.

Coliform bacteria count showed no growth this indicates that most of the coliforms obtained in vegetables are coming from the source of irrigation water. The result showed that bacterial count was higher in the soil than water. Previous studies indicated that Mold and yeast count range from 1.0×10^4 – TNC with the highest coming from 50m distance and lowest from the control sample. Mold and yeast growth was only found in the soil samples. The high mold and yeast count in the soil sample might be due to dumpsite waste rich organic matter as stated by Oyedele *et al.,* (2009)

Summary

A total of twenty samples were collected for the study, three samples from each of vegetable, soil and water were taken

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from three different distances and each with their control sample.

The result shows that total bacterial count was found to be significantly higher in the soil ranging from 4.3 x 10^5 – 4.78 x 10^6 followed by irrigation water ranging from 1.0 x 10^4 – 3.66 x 10^6 and the least was the vegetable ranging from 1.0 x10^4 – 9.0 x 10^4. Coliform bacteria count was found to be higher in the irrigation water ranging from 2.0 x 10^4 – 1.2 x 10^5 followed by the vegetables ranging from 1.0 x 10^4 – 2.0 x 10^4 and no growth of coliform was found in the soil. Mold and yeast was found to be significantly higher in the soil ranging from1.0 x 10^4 – TNC and was absent in the vegetables and water respectively.

Conclusion

Regular monitoring of contamination is very important in food industry to prevent food poisoning and other health hazards. In the present work, the microbial loads of soil, water and three different vegetables grown around dumpsite were examined. The result was found to be higher than the acceptable limits. This implies that the microbial quality of vegetables may pose a health risk to the people who consume them if not properly prepared.

Recommendation

The high bacterial load in the vegetable samples could serve as an indicator for the need to promote awareness about the possible health hazards that could be due to proximity of these vegetables to the dumpsite. There is therefore, the need for regulatory bodies to ensure that microbiological standards are established and practiced by farmers of vegetables.

Since the dumpsite was found to directly contribute to the pollution of the soils, vegetables and irrigation water, and the fact that it is an illegal entity, as such dumping should be stopped and the site properly closed.

Again, public enlightenment on proper handling of wastes in the society should be intensified in order to reduce wastes related problems along the food chain.

Lastly people should thoroughly wash the vegetables with tap water or salt water in other to reduce the microbial load especially lettuce that is consume fresh.

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Increased Potential of Protein Content of Waxy Corn

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***Abstract*—** *The purpose of this research is to gain a potentially waxy corn strains of high protein content. Specific targets to be achieved in this study are promising lines of F1 that have potentially sticky and high protein content. The method used is cross-pollinated plant breeding methods, the hybridization between maize Variety of Srikandi Putih (♀) and the Local Waxy Corn (♂). Characters F1 compared to corn Variety of Srikandi Putih and the Local Waxy Corn. The results showed that character of plant height, number of leaves, and leaf area are higher in Srikandi Putih Variety compared to Local Waxy Corn but age flowering male and female Local Waxy Corn faster than Srikandi Putih Variety. Character length of ear, diameter of ear, weight of 100 seeds, seed weight plant^{-1} and protein content higher in Srikandi Putih compared to Local Waxy Corn. F1 values on all observation characteristic of plant height, leaf number, leaf area, male and female flowering age, ear length, ear diameter, weight of 100 seeds, seed weight plant^{-1} and protein content were generally among the values of Srikandi Putih Variety and Local Waxy Corn.*

***Keywords*— corn, sticky, protein, hybridization.**

I. INTRODUCTION

Generally corn crops are used for animal feed, only a small portion is used for food and food industries. Along with the increasing number of population then the basic food needs must also be met. Therefore it is necessary to diversify the staple food, one of them by utilizing corn as an alternative staple food. To make corn as an alternative staple food needs to be improved quality and flavor to be tasty and nutritious. The merging of two good properties of two different corn varieties can be done if their genetic diversity is available. High quality protein maize (QPM) is available and already on the market, but the flavor is not delicious, making it less delicious for human consumption. The beginning of genetic improvement on protein quality was triggered by the discovery of opaque and floury genes which were reported to alter the lysine and tryptophan content of the seed endosperm [15].. Of the genes that have been identified, only opaque-2 (o2) and floury2 (fl2) genes are often used in improving the properties of corn endosperms [6], [8]. Similarly, local waxy corn is also available and can be obtained in the South Sulawesi Region. This local waxy corn is one of South Sulawesi's typical corn with a taste that is very tasty and popular people, both young corn and processed old seeds because it contains high amylopectin, but the lack of production is low and protein content is less. Disadvantages of both types of corn need to be removed with certain plant breeding techniques to obtain high protein corn and feel sticky.

Corn with special properties can be established through repetitive plant breeding programs [2]. Amylopectin content in waxy corn nearly 100%. Ordinary corn endosperms consist of a mixture of 72% amylopectin and 28% amylose [5]. According to Bates et al. (1943) *in* [1], the endosperm content of waxy corn is almost entirely amylopectin. In waxy corn there is a wx recessive gene in homozygous (wxwx) that affects the chemical composition of starch, causing tasty and savory taste. Backcross breeding methods can be applied to integrate the donor genes from specific corn of amylopectin-rich to high quality protein corn and high productivity. Thus, corn will be obtained which has the desired special properties.

Research Purposes

The purpose of this research is to obtain high potential protein content of waxy corn strain as a forerunner of high quality corn and delicious and nutritious flavor to be consumed as an alternative staple food that the price is affordable

II. METHODS

The research was conducted in the Village Antang, Sub-District Manggala, Makassar, which took place in September 2016 until March 2017. Materials used in this study are: Seed corn of Variety of Srikandi Putih, seed corn of waxy corn, Urea, SP-36, KCl, compost, pesticide, paper bags, labels, paper HVS, rope, plastic bags. The tools used are: hoes, shovels, scissors, tape measure, digital scales, electric oven, shove, hand sprayer and hype.

This study was designed using Cross-breeding methods in cross-pollinated plants, that is hybridization between Local waxy corn with Variety of Srikandi Putih. Model planting as follows: Variety of Srikandy Putih as female parents (P1) planted 3 rows while Locally waxy corn as

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the male parent (P2) planted one row. Each row consists of 15 plants. Repeated 5 times. The crosses between the Srikandi Putih variety and the local waxy corn will produce F1 generation.

Implementation of Research

Land to be planted conducted soil analysis to determine the level of soil fertility and pH. Further prepared seeds, compost, Urea, SP-36 and KCL and all the necessary equipment. Then the land is plowed and uniformly distributed and then made a plot of 2.5 m x 3 m size of 5 plots, then given a compost of 20 kg per plot.. Srikandi Putih of corn seed and waxy corn previously given Ridomil fungicide to prevent disease. Because the age of flowering maize Variety of Srikandy Putih longer than local waxy corn, seed of Srikandi Putih is planted early 10 days and then waxy corn by way of as much as 2 seeds/hole with a spacing of 70 cm x 20 cm. After that the seed of waxy corn is planted with the same way. Fertilizing Urea, SP-36 and KCl done after the corn crop was 7 days after planting (DAP) at a dose of 200 kg urea. ha^{-1}, 150 kg of SP-36.ha^{-1} and 100 kg KCl.ha^{-1}. Urea special given 2 times, 50% at 7 DAP and 50% at the age of 50 DAP. After the plant was 7 DAP thinning to leave one plant. Weeding every two weeks.

Plant Hybridization

After both types of flowering corn crops, pollination is performed. Pollen mature waxy corn collected in paper bags then powdered into mature pistil head (silk) Srikandi Putih Variety. After that the pollinated female flowers (silk) are wrapped in paper bags that have been inscribed on date and the name of the genotype.

Data Collection

Data were observed: 1) Plant height; 2) leaf number; 3) Leaf area; 4). Age of male flowering ; 5) Age of female flowering; 6) Length of the ear; 7) Diameter of the ear; 8) The weight of 100 seeds. 9) The weight of seeds per plant; 10) The protein content

Statistic analysis

The data were analyzed descriptively of all the characters phenotype observed.

III. RESULTS AND DISCUSSION

1. Plant Growth

The average of observed growth of waxy corn and Srikandi Putih on various observation parameters is presented in Table 1.

Table 1. Average Waxy corn Growth and Srikandi Putih Growth Parameters

No.	Plant height (cm)		Number of leaves (sheet)		Leaf area (cm)		Age of flowering ♂ (day)		Age of flowering ♀ (day)	
	WC	SP	WC	SP	WC	SP	WC	SP	WC	SP
1	144.5	159.8	8.0	10.0	395.5	421.3	47.0	56.0	49.0	58.0
2	155.8	160.2	9.0	7.0	392.6	418.4	47.0	57.0	48.0	58.0
3	158.2	162.5	7.0	8.0	378.9	412.8	46.0	55.0	48.0	56.0
4	156.8	170.3	8.0	8.0	400.8	420.5	47.0	55.0	49.0	57.0
5	156.4	168.2	9.0	9.0	388.8	416.7	46.0	57.0	47.0	59.0
Rataan	154.3	164.2	8.2	8.4	391.3	417.9	46,6	56.0	48.2	57.6

Note: Wc=Waxy corn, SP= Srikandi Putih

Table 1 shows Srikandi Putih Variety tend to provide higher growth ranging from plant height, leaf area, male flowering age and female flowering age are longer compared to Local Waxy Corn. Only the relatively equal number of leaves between the Srikandi Putih Variety and Local Waxy Corn. This is appropriate with the description of Srikandi Putih Variety. On the other side of Local Waxy Corn, the height of corn depends varieties, generally ranges from 100 - 300 cm. The corn leaves extend and come out of the stem nodes. The number of leaves consists of 8-48 sheets depending on the variety [11]. Other studies have shown that the type of Srikandi Putih seed is generally pearl (flint corn), seeds like pearl, bloated and hard. White Waxy Corn is a variant of white corn. Status of white Waxy Corn by some farmers in South Sulawesi, NTB, NTT and Maluku is considered the same as white corn. Farmers grow white Waxy Corn to harvest young, eaten in the form of stew [10]. White Waxy Corn is white transparent, 85 days old and contains low amylose (<10%) in seed endosperm.

2. Production and Quality of Maize

The average observed parameters of production and quality of corn are presented in Table 2. Table 2 shows Srikandi Putih Variety generally production tends to be higher compared to Waxy Corn and F1 generation. This can be seen in the production parameters ranging from length of ear, ear diameter, weight of 100 seeds and weight of seeds per plant. The results reported that Waxy Corn is a local maize that has low yield potential, ie less than 2 t / ha, small cobs with a diameter of 10-11 mm and highly susceptible to disease [4]. Furthermore, reported that yield of Waxy Corn is about 35 percent lower than normal maize seed production and if planted it must be isolated from surrounding plants at least 200 meters [14]. Waxy corn is special type of corn is more and more needed, consumers and industries. Corn special marked its own advantages, such as amylopectin content high above 90 percent [9]. Similarly, in the parameter of protein content, where protein content in F1 generation is higher than Local Waxy Corn but still under Srikandi

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Putih. Interesting things can be observed between F1 with both parents (Lokal Waxy Corn and Srikandi Putih) which shows the value on each parameter of production and quality that tends to be between the value of both parents. This gives an indication that the properties of both parents have been inherited to F1, but the properties are still partial and not yet stable so still need further research to obtain new varieties. In line with the results of research that one of the efforts to increase the level of corn kernel protein is to utilize the xenia effect. The xenia effect itself can be interpreted as the pollen effect of the male elder from the male crosses with the females growing on the seed [3]. Furthermore, it is stated that the varieties of maize that exist in Indonesia have a hard seed properties as developed in the framework of protection against pest attack. This kind of variety has low protein content characteristics because it does not have an opaque-2 gene that controls protein levels. According to [12] the presence of opaque-2 gene, can increase protein content, but on the other hand causes corn kernels are soft, and fragile. Breeding experts start developing corn plants that have high levels of protein by inducing opaque-2 genes into a variety, but they lead to undesirable traits such as low production and the fragility of seeds.

The result of the research shows that crossbreeding Local Waxy Corn with Srikandi Putih Variety produces seeds of F1 generation different from both parent, changes in shape and seed size (show Figure 1). Xenia can be seen from the character of its kernel color visually. On qualitative traits, the xenia symptoms affect the color of the seed, the shape of the seed, the shape of the fruit, and the cooking time [13]. The crossover results with the JTK-3, JU, A-1 and Bonansa parent with the female elder genotype, xenia appear only in the kernel color and kernel characters. These results differ from those reported by [7] in thorny palm, where the xenia affects almost all the quantitative characters of the observed fruit.

Table.2: Average Parameter of Production and Quality of Waxy Corn and Srikandi Putih and F1 genotype

No.	Length of the ear (cm)			Diameter ear (cm)			Weight of 100 seeds (g)			Weight of seeds per plant (g)			Protein Content (%)		
	WC	SP	F1	WC	SP	F1	WC	SP	F1	WC	SP	F1	WC	SP	F1
1	12.4	15.4	14.2	3.4	4.7	3.7	30.7	35.2	31.2	18.2	80.2	26.5	8.4	10.4	9.2
2	13.2	16.8	13.6	3.2	4.6	3.5	32.1	35.4	32.3	17.4	82.1	28.4	8.8	10.2	9.4
3	12.8	17.9	14.4	3.4	4.5	3.7	30.5	36.2	32.8	17.6	81.5	25.8	9.1	10.5	9.2
4	13.5	16.6	13.8	3.5	4.4	3.6	31.5	35.8	33.2	16.8	78.8	24.6	8.2	10.8	9.4
5	12.6	17.2	15.0	3.5	4.7	3.6	30.3	36.5	31.6	18.5	79.6	26.7	8.3	10.1	9.1
Average	12.9	16.8	14.2	3.4	4.6	3.6	31.0	35.8	32.2	17.7	80.4	26.8	8.6	10.4	9.3

Note: Wc=Waxy corn, SP=Srikandi Putih, F1=Genotype F1 (hybridization Wc x SP)

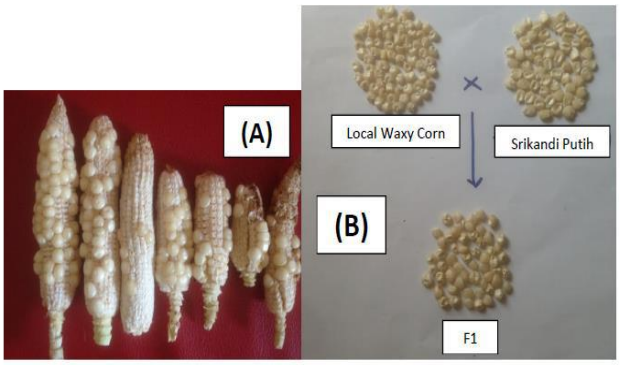

Figure 1. F1 Seed from the Local Waxy Corn Crossing with Srikandi Putih Variety (A): Cob F1; (B): Local Waxy Corn Seeds, Srikandi Putih Variety seeds, and F1 seeds

IV. CONCLUSIONS

Based on the results of observations that have been done then it can be concluded as follows:

1. Character of plant height, number of leaves, and leaf area are higher in Srikandi Putih Variety compared to Local Waxy Corn
2. Age Flowering male and female Local Waxy Corn faster than Srikandi Putih Variety
3. Character length of ear, diameter of ear, weight of 100 seeds, plant seed weight and protein content higher in Srikandi Putih compared to Local Waxy Corn
4. In general, the value of F1 generation on each character of production and quality is between the value of both parents (Srikandi Putih Variety x Local Waxy Corn)
5. F1 protein content has increased 8.1% compared to Local Waxy Corn

NOMENCLATURE

F1 Filial 1 (Generation 1)
♂ Male
♀ Female

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Study on Toxic Impact of Sugar Factory Effluent on the Gill of the Fresh Water Fish Rasbora Daniconius

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Abstract— *The fresh water fish Rasbora daniconius were exposed to two sublethal concentration of sugar factory effluent for 4 weeks studied. The concentration were record 1/5(2.2%) and 1/10(1.1%) of the 96 hrs* LC_{50} *values of sugar factory effluent. The gills of R. daniconius showed the curling and degeneration and breaking of epithelium cells of the secondary gill lamellae, destruction of blood cells, blood capillaries and nuclei were the prominent features of the gill.*

Keywords— R. daniconius, Sugar factory effluent, Histopathological changes and tissues of gill.

I. INTRODUCTION

Fishes have most widely been used as a test organism to evaluate the toxicity of west and other pollutants may be due their adaptability to laboratory condition, availability and varying degree of sensitivity to toxic substances.

In the present study an attempt has been to determine pathological changes induced by sugar factory effluent.

Histologically, gills have a large superficial area through which gaseous exchanges between the blood and the external medium take place (Newstead, 1987). The direct contact between gills and water promotes the interaction with toxic substances present in the water as they have sites of ionic link of performing normal functions. Adsorption of metal and other pollutant with charges may eventually bring about toxic effect on the organism (Hollis and Playle, 1997). The thin lamellae that cover the secondary lamellae represent the largest site for gaseous exchanges. The chloride cells, responsible for ionic exchanges, are usually distributed among the secondary lamellae under condition of low ionic concentrations, besides transporting Na^+, Cl^+ and other substances.

Many workers have studied histopathology of tissues after exposing fishes to different pesticides or heavy metals or industrial effluent. Mitrovic and Brown (1968) reported congestion in blood vessels and changes in gills due to heavy metal poising.

Srivastava (1984) studied the histopathology of gills of *Channa gachua* after exposure to sublethal concentrations of Malathion and Chloradene. Kulshrestha *et al.,* (1984) studied histopathological changes due to exposure to pesticides in *Channa striatus.* Singh and Sahai (1984) recorded gill damage in *Rasbora daniconius* exposed to BHC. Histopathological changes in gill, kidney, liver and intestine of *Garra mullya* due to mercury exposure have been studied by Gokhale (1984).

The review of the literature shows the effects of effluent bring alterations in the structure of important body tissues of gill. The present investigation was undertaken to explore, observe, and record the changes in different tissues of *Rasbora daniconius***.**

II. MATERIALS AND METHODS

The fresh water fish, *Rasbora daniconius* were brought from river Bhima near Kangaon, Tal. Daund, Dist. Pune and acclimatized to laboratory conditions for about two weeks. The healthy fishes of uniform length and weight were selected and were exposed to two sub lethal concentrations of sugar factory effluent. The sublethal concentrations were selected on basis of the results of acute toxicity vaules. The LC_{50} value for 96 hours was estimated at 11%. The Sublethal concentrations for chronic test were selected at 2.2% (1/5) and 1.1% (1/10).

The food was supplied every alternate day and weekly observations were made, where as water was renewed every week during the exposure period. The aquaria were, kept away from mechanical disturbances.

At the end of exposure, survived fishes were taken out from aquaria and decapitated; tissues of Gill were fixed in Bouin's fluid, processed and embedded in paraffin wax. Section at 4-6 µ thickness were cut and stained in Harris

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haematoxylin and eosin as suggested by Bancroft and Stevens (1977) and Bancroft and Cook (1984). After the staining the slides were mounted in DPX and observed under the light microscope for histological details and subsequently photomicrographs were taken.

III. RESULTS

NORMAL HISTOLOGY

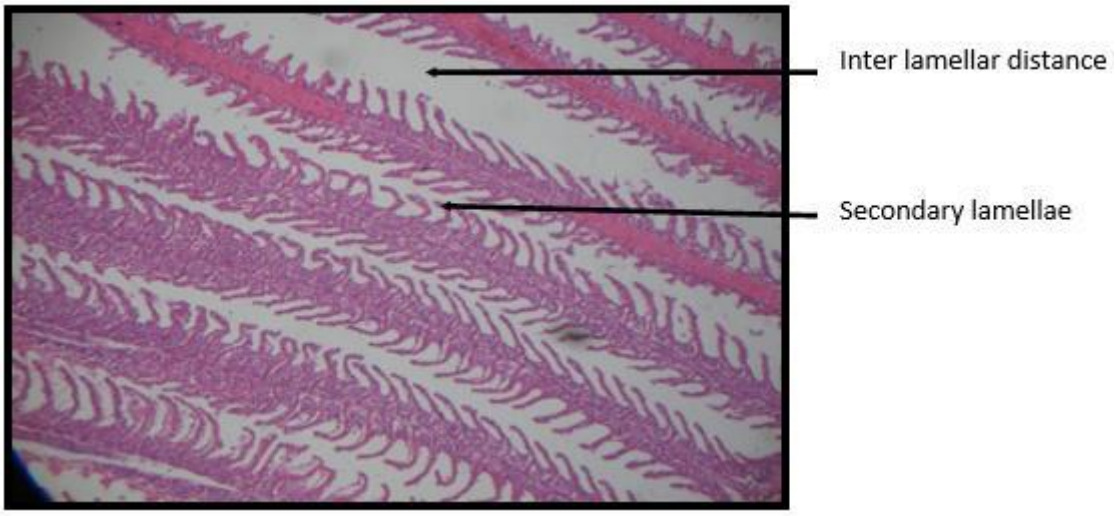
1. Gill (Photographs No. 1)

Rasbora daniconius possesses four pairs of gills situated at branchial chamber, as in other teleosts. Each gill arch, has a double row of elongated, laterally projecting structures, the primary gill filaments. On the upper and lower surface, leaf like projection at right angle to its axes is the secondary gill lamellae. A delicate flattened structure comprising of two epithelial sheets are continuous to its free end secreted along its length as large number of widely separated pillar cells there is the continuous blood space between two pillar cells. The pillar cells are arranged in rows occupying the whole area of secondary gill lamellae.

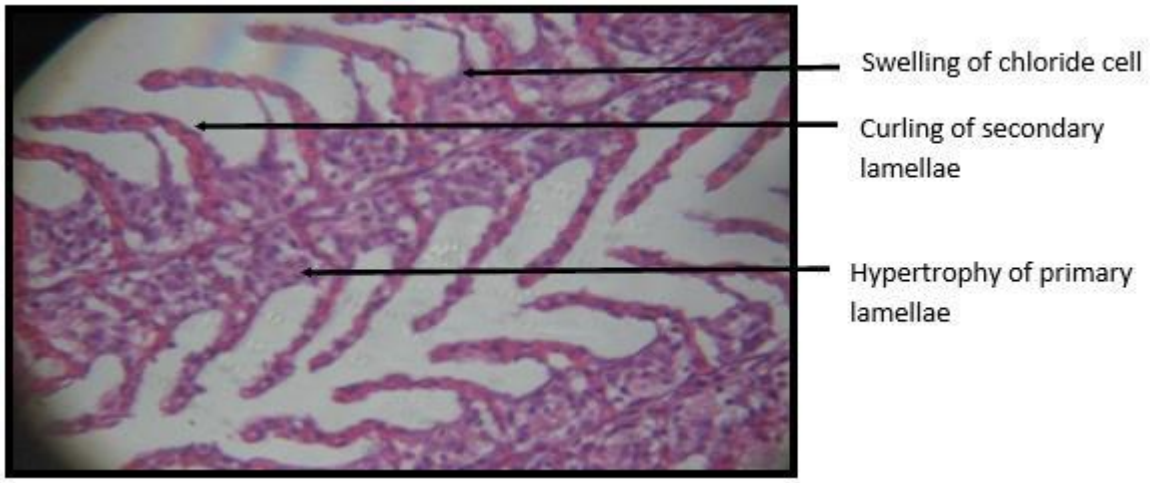
HISTOPATHOLOGICAL CHANGES

2. Gill: - (Photograph No.2&3)

The gill of *R. daniconius* exposed to sub lethal concentrations of sugar factory effluent showed curling, the degeneration and breaking of epithelium cells of the secondary gill lamellae, hypertrophy, destruction of blood cells, blood capillaries and nuclei were the prominent features of the gill.



Photograph No.1: L.S. of gill of R. daniconius (Control). Hematoxyline /Eosin



Photograph No.2: L.S. of gill of R. daniconius after 1.1 % conc. (Chronic) exposed to sugar factory effluent Hematoxyline/ Eosin

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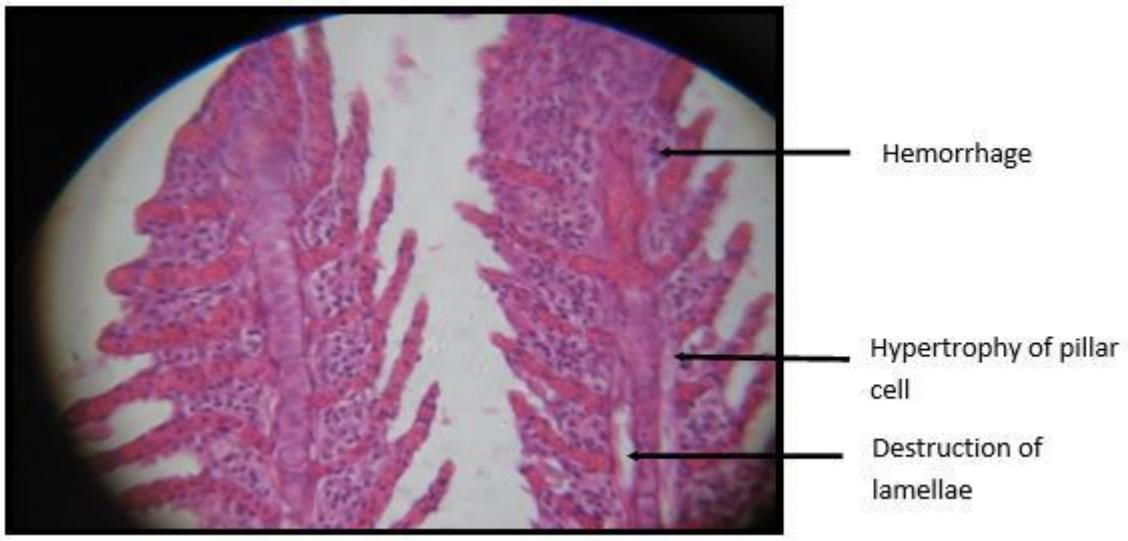

Photograph No.3: L.S. of gill of R. daniconius after 2.2% conc. (Chronic) exposed to sugar industry effluent. Hematoxyline/Eosin

IV. DISCUSSION

Study of the literature on fish histopatholgy reveals that many workers contributed on different aspects of fish tissues. Carpenter *et al.,* (1927) found fish dead due to coagulation of mucous over gills which impaired respiration. The trouts exposed to sulphonate detergent were found with damaged gill (Schimid and Mann, 1961) and sulphonate detergent effect was recordable on the gills of *Lepomis gibbosus* exposed to acute and chronic bioassays (Cairns and Scheiner, 1964) sulphonate effect was recorded on the blue gill, *L. macrotochirus* by Lamke and Mount, 1963) Brown *et al.,* 1968 studied the damage of gills by detergent done and by natural detergent and Zinc.

The histopathological changes in heart, liver and gills exposed to sub lethal concentration of sodium arsenate were studied by Giderenus (1966). Khangarot and Somani (1980) studied the mercury toxicity on *Puntius sophore* and reported that the gill epithelium is separated from the basement membrane and pillar cells. Haniffa and Sundaravadhanam (1984) observed partial destruction of gill epithelium, pillar cells, acidophil, mast cells, blood cells, blood capillaries, cartilage cells, separation of epithelial layer of secondary lamellae from basement membrance, mucous cells destroyed and gill filaments were seen completely covered by thick mucous layer on distillery effluent treated *Barbus stigma.*

Paulose (1989) reported fusion of adjacent lamellae in the gill of *Labeo rohita* within 15 days of exposure either to Methyl mercury or mercuric chloride 65.

Nath and Kumar (1989) reported changes in gills of *Colisa fasciatus* exposed to sublethal concentration (64 ppm) of Nickel Sulphate, like hypertrophy of respiratory and mucous cell, separation of epithelial layer from pillar cell system, extensive necrosis and hyperplasia layer from pillar cell system, extensive necrosis and hyperplasia leading to clubbing at the tip of lamellae.

Moza *et al.,* (1993) reported pathological changes in gills of *Carassius auratus*, induced by Cadmium and found epithelial lifting hyperplasia, lamellar fusion, vacuole formation in pillar cells was observed on 10^{th} day of exposure; more prominent hyperplasia, congestion of blood stasis, necrotic epithelia cells, depletion of cells in between inter-lamellar zone i.e. complete cell necrosis and vacuole formation in pillar cells was observed on 20^{th} day of exposure. Saksena and Pandey (1993) reported the hyperplasia, hypertrophy, fusion of secondary gill lamellae, desquamation of epithelium, increased number of mucous cells in the gill of Copper Sulphate exposed *Labeo rohita.*

Ashok and Vinod (1995) studied changes in gill surface of *R. daniconius* exposed to sub lethal concentration of 0.05 mg/l of Mercury ($Hgcl_2$) 96h and found damage, fusion and dumping in the swollen, deterioration and modification of ring ridges into more expanded surface area in the secondary lamellae.

Adsorption of metal and other pollutant with charges may eventually occure bring about toxic effect on the organism (Hollis and Playle, 1997). The thin lamellae that cover the secondary lamellae represent the largest site for gaseous exchanges. The chloride cells, responsible for ionic exchanges, are usually distributed among the secondary lamellae under condition of low ionic concentrations, besides transporting Na^+, Cl^+ and other substances.

Erkman *et al.,* (2000) studied histopathological changes induced by cyphenothrin in gill of *Lebistes reticulutus* and found lifting of the eqpithelial layer from gill lamellae, degeneration of secondary lamellae due to edema, shortening of secondary lamellae and club shaped lamellae.

Prasad (2002) studied the effect of Copper and Zinc on the gill of *Channa marulius* and found necrosis, exudation of erythrocytes from the secondary lamellae, vacuolization and separation of basement membrane from the epithelial cells curling and fusion of some secondary lamellae after 2160 hours exposure. Thophon *et al.,* (2003) noticed aneurism with rupture of respiratory epithelium of secondary lamellae and breakdown of pillar cell system in the gills in Cadmium exposed *Lates calcarifer.*

Vutukuru *et al.,* (2005) observed architecture changes in the gill morphology like loss, fusion, clubbing of secondary lamellae and detachment of gill racker following softening of gill shaft in Copper treated fish, *Esomus danicus.*

Olojo, (2005) was observed fingerlings of the fish *Clarias gariepinus* were exposed to continuous exposure to sub lethal concentrations (0.006mg/l and 0.008mg/l) of lead for three weeks, showed distortion of gills, the swimming became slower and there was reduction in their rate of feeding primary and secondary lamella overlapping occlusion of inter lamella spaces, epithelium is completely disrupted owing to the lyses of the cells. The increase intracellular vaculation signals onset of edematous changes.

Soni and Gupta (2006) studied histopathological changes due to mercuric chloride and influence of EDTA on the gill of *Heteropneustes fossilis* and recorded histopathological changes in the gill due to long term exposure of the fish to mercuric chloride, are severe. Oedematous condition and vacuolatization in the cells of gill rays, hypertrophy in gill septum, breakage in the epithelium covering enlarged micropillaries and small separated pillar cells have been clearly observed. Altinok and Capkin (2007) observed lesions in the gill of rainbow trout exposed to 0.6 or 1.3 mg/l endosulfan concentration consisted oedema, separation of epithelium from lamellae, lamellar fusion and swelling of the epithelial cells. Suchithra *et al.,* (2007) noticed bulging of the hyperemic secondary lamellae in to the lumen of the accessory respiratory organ, necrosis and swelling of the respiratory epithelium leading to hemorrhages and fusion of secondary lamellae of cadmium chloride exposed fish, *Heterapneustes fossilis.*

Aniladevi *et al.,* (2008) reported that, after 21 days of pesticides exposure gill become edematous with prominent clubbing. Separation of primary gill lamellae and hemorrhage in the vessels outside the secondary gill lamellae were observed. Hadi and Alwan (2012) studied the histopathological changes in gill of fresh water fish, Tilapia zilli, exposed to aluminum shows that the cellular hypertrophy in the epithelial layer of primary filaments and fusion of secondary lamellae, epithelial lifting, interstitial edema and blood congestion in axis of the primary filament, necrosis of gill epithelium tissue.

The present study revel extensive damage to the inter gill architecture on fish have been noticed compared to control fish. Changes like bulging at tip of primary lamellae, epithelia hypertrophy. Fusion, curling and reduction of secondary gill lamellae, disorganization and rupture in secondary lamellae, swelling in pillar, mucous, and chloride cells and their nuclei appear swollen and pyknotic. Hemorrhage at primary and secondary lamellae in the sugar factory effluent treated fish in contract to control fish. The pathological changes in the gills might have resulted due to shifting from aerobic to anaerobic pathway in tissues respiration of fish. Histological evidences in the present study are correlated to some extent with the work of Sonawane and Khillare (1992), Singh and Karpagaganapathy (1988), Usha Rani (1999), Suchithra *et al.,* (2007) Aniladevi *et al.,* (2008).

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Egg quality characteristics of pullet chickens fed Neem (AzdirachtaIndica) leaf meal (NLM) managed under two housing systems

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Abstract— *A study was carried out with 180 dominant black strain pullet birds to evaluate the effect of neem and housing types on egg quality characteristics of pullets at point of lay. The birds were randomly assigned to two housing types (deep litter with run and deep litter housing) of 6 treatment groups comprising of 30 birds and 3 replicates of 10 birds each. The experiment was arranged in a 2 x 3 factorial layout in a completely randomized design. Diets containing neem leaf meal (NLM) at 0, 0.5 and 1.0% was administered to birds. Data were collected egg external and internal characteristic at point of lay. Highest egg weight (45.53 g) was obtained in birds fed 0.5% NLM. Bright yellow yolks were obtained from birds managed on deep litter housing with run. It was concluded that up to 1.0% NLM could be included in the diets of laying pullets to trigger early egg production and improve egg yolk colour.*

Keywords—Egg, Housing, Neem, Pullets, Quality.

I. INTRODUCTION

The utilization of several medicinal plant as feed ingredient to reduce production cost in poultry diet is not new but the inclusion levels at various ages and physiological conditions varies [10, 5]. Among these leaf is neem. Neem leaves is believed to have relieved so many different pains, fevers, infections and other complaints that it has been called the "village pharmacy[9] reported that eggs are good source of low cost high quality protein, providing 6.3grams of protein (13% of the daily value of protein) in one egg for a caloric cost of only 68calories. The structure of humans and animal is built on protein. We rely on animal and vegetable protein for our supply of amino acids and then our bodies rearrange the nitrogen to create the pattern of amino acids we require. According to [8] lutein a carotenoid thought to help prevent age related muscular degeneration and cataracts may be found in even higher amounts in eggs than green vegetables such as spinach, which have been considered its major dietary sources as well as supplements. However, the use of Neem leaf meal is limited due to bioactive compounds (Azadirachtin, limonoids and tannin) that have deleterious effects on nutrient utilization of monogastric animals [6, 2].

The present study was therefore undertaken to investigate the effects of varying levels of dried neem (*Azadirachtaindica*) leaf meal (NLM) in layers diets on the egg quality characteristics of
pullets at point of lay.

II. MATERIALS AND METHOD

2.1 Location of the Experiment

The experiment was carried out at the Poultry unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State. It fell within the rain forest vegetation zone of South-Western Nigeria at an altitude of 127m, latitude 7° 13'N and longitude 3° 26' E. The climate is humid with a mean annual rain fall of 1037mm. The annual mean temperature is 34.7° C and relative humidity is 82%. [4].

2.2 Experimental diets

The fresh green neem leaves were harvested from mature Neem trees within the environs of the university farms. The leaves were cleaned, made free of stems and sun-dried on a polyethylene sheet until they became crispy. They were milled and stored in sealed polyethylene bags until they were ready for diet formulation. Three experimental diets were formulated with neem leaf meal inclusion at 0, 0.5, and 1 .0% to partially replace wheat offal and was offered to the birds from start to the end of the experiment. The ingredient compositions of the experimental diets are shown in Tables1

Table.1: Gross composition (%) of grower's diets

	% Inclusion levels of neem leaf meal (NLM)		
Ingredients	0	0.5	1.0
Maize	50.00	50.00	50.00
Soybean meal	12.00	12.00	12.00
Wheat offal	33.00	32.5	32.0
Azadirachtaindica	0	0.5	1.0

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Bone meal	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00
Lysine	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Grower's vit./trace mineral premix	0.25	0.25	0.25
Common Salt	0.25	0.25	0.25
Total	100.00	100.00	100.00
Determined analysis (%)			
Crude protein	16.49	15.03	14.88
Ether Extract	3.11	2.96	2.92
Crude fibre	4.10	3.50	3.02
Ash	2.55	2.06	2.03
Calcium	0.06	0.06	0.06
Phosphorus	0.28	0.28	0.28
Lysine	0.77	0.77	0.77
Methionine	0.25	0.25	0.25
Energy (MJ/Kg)	5.52	5.52	5.52

Vit./Min. Premix contained: Premix (Embavit No 90) contained Vit. A, 10 000 000iu; D_3 , 2 000 000iu; E, 12 500iu; K, 1.30g; B_1, 1.30; B_2, 4.00g; D Calcium-Pantothenate, 1.30g; B_6, 1.30g; B_{12}, 0.01g; nicotinic acid, 15.00g; folic acid, 0.05g; biotin, 0.02g; Co, 0.20g; Cu, 5.00g; Fe, 25.00g; I, 0.06g; Mn, 48.00g; Se, 0.10g; Zn, 45.00g; choline chloride, 200.00g; BHT, 50.00g.

2.3 Experimental birds and management

Two hundred and thirty four (234) four weeks dominant black strain pullet chickens were brooded for four weeks and allotted to two housing systems of six treatment groups, each comprising of thirty nine (39) randomly selected birds in three (3) replicates of thirteen (13) birds each. Birds in treatments 1, 2 and 3 were managed on deep litter with run while those in treatments 4, 5 and 6 were managed in exclusive deep litter housing. Birds in treatments 1 and 4 fed control diet were given antibiotics from start to the end of the experiment, while those on treatments 2 and 5 and 3 and 6 were offered diets with NLM inclusion at 0.5 and 1.0%, respectively. Newcastle, Infectious Bursal diseases and coccidiosis vaccinations were carried out routinely via drinking water. Vitamin was given prior to vaccination and at the end of each vaccination programme. The experiment lasted for a period of seventeen weeks.

2.4 Egg External Qualities

Weight of each first egg average over the number of first egg per group was measured with a balance sensitivity of 0.01g. The length and width of each egg were measured using vernier calipers. The width was measured as the distance between two ends of the egg at the widest cross sectional region using vernier calipers. The length was measured as the distance between the broad and narrow ends of the egg. The Egg Shape Index (ESI) was calculated as the percentage of the egg breadth/broad end (width) to the egg length. The thicknesses of individual air-dried shell were measured to the nearest 0.01mm using micrometer screw gauge.Egg shells were air dried in crates for three (3) days and weight of the dried shell was measured using a sensitivity balance of 0.01g.

2.4.1 Egg Internal Qualities

The eggs were gently broken and the maximum albumen heights were measured with tripod micrometer.The Albumen weight was determined the difference between the egg weight and the sum of weight of yolk and dry egg shell.% Albumen weight was calculated as the percentage of the albumen weight to the egg weight.Yolk weightwas measured in grams using Mettler top-loading weighing balance. % Yolk weightwas calculated as the percentage of the yolk weight to the egg weight.Yolk colourwas determined using yolk colour fan after the egg was broken and the yolk placed in Petri dish. Haugh unit (HU) was calculated using the values obtained for the egg weight and albumen height.

III. RESULTS

Table 2 shows results of the main effects of housing systems and varying inclusion levels of NLM on egg quality characteristics of grower pullets. The results showed that housing systems had no significant ($P>0.05$) influence on all the egg parameters measured except yolk colour. Likewise, inclusion of the NLM had no significant ($P>0.05$) effect on shell thickness, shell weight, % shell weight, albumen height, % albumen weight, % yolk weight and haugh unit. Birds on diet containing 0.5% NLM laid eggs with the highest ($P<0.05$) values of egg weight, length, width, albumen weight, % albumen weight, yolk weight and % yolk weight, however, the values are similar to egg laid by the birds fed the control diet. The birds fed 1.0% NLM laid eggs that had highest ($P<0.05$) value of egg shape index. The values of Haugh unit ranged ($P>0.05$) from 92.87 (control) to 96.21 (0.5% NLM).

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Table.2: Main effects of housing systems and NLM inclusion on egg quality characteristics of grower pullets

Parameters	Housing systems		% NLM inclusion level		
	Deep litter with run	Deep litter	0	0.5	1.0
Age at first Egg(days)	144.33±2.80	142.00±10.5	147.5±4.51^{a}	146.5±1.29^{a}	135.5±8.85^{b}
Weight at 1st lay (kg)	1.18±0.10	1.19±0.08	1.27±0.10^{b}	1.56±0.03^{a}	1.13±0.05^{b}
Egg weight (g)	39.32±7.90	39.30±7.05	40.88±5.20^{a}	45.53±4.12^{a}	31.53±2.71^{b}
Egg length (mm)	50.18±4.32	49.82±3.60	50.62±2.77^{a}	53.61±1.38^{a}	45.78±1.26^{b}
Egg width (mm)	37.26±2.52	37.49±2.17	38.02±1.83^{a}	39.09±1.36^{a}	35.01±1.16^{b}
Egg shape index (%)	74.38±2.82	75.34±1.75	75.14±0.89^{ab}	72.95±2.93^{b}	76.48±1.18^{a}
Shell thickness (mm)	0.47±0.61	0.47±0.73	0.49±0.05	0.46±0.07	0.46±0.08
Shell weight (g)	4.32±1.29	4.02±0.80	4.23±0.54	4.40±0.73	3.88±1.70
% shell weight	11.36±4.67	10.20±0.57	10.34±0.61	9.63±1.00	12.36±5.62
Albumen height (mm)	8.63±1.01	7.43±1.00	7.73±0.86	8.68±1.00	7.69±1.51
Albumen weight (g)	26.18±5.60	26.28±5.45	7.73±0.86^{a}	8.68±1.00^{a}	7.69±1.51^{b}
% Albumen weight	66.33±4.38	66.69±2.42	26.70±3.19	31.38±4.02	20.63±2.38
Yolk weight (g)	8.82±2.25	9.00±1.28	9.95±1.53^{a}	9.75±0.83^{a}	7.03±1.00^{b}
% yolk weight	22.31±3.37	23.11±2.38	24.28±0.71	21.62±3.36	22.23±1.55
Yolk colour	5.17±1.17^{a}	3.17±1.17^{b}	5.00±1.63^{a}	4.25±1.71^{ab}	3.25±0.96^{b}
Haugh unit	97.74±5.09	91.92±4.15	92.87±4.71	96.21±4.49	95.40±7.45

^{abc}Mean in the same row with uncommon superscript differed significantly (P<0.05) ; NLM= Neem Leaf Meal

Table 3 shows the results for the interactive effect of housing systems and NLM inclusion levels on egg quality characteristics of grower pullets. The results revealed significant (P<0.05) differences for almost all egg parameters. The interaction for means weight of bird at lay, shell thickness, shell weight, 5 shell weight, % albumen weight and % yolk weight were not significant (P>0.05). Birds on the deep litter fed diet that contained 1.0% NLM inclusion came on lay earlier (128 days) than other groups. Parameters such as egg weight, length and yolk weight were more pronounced (P<0.05) in birds fed with diet contained 0.5% NLM inclusion for the two housing systems. Results for yolk and % yolk weights are comparatively similar for birds fed with the control diet and 0.5% NLM inclusion in deep litter housing with run.

Table.3: Interactive effects of housing systems and NLM inclusion on the egg quality characteristics of grower pullets

Housing systems	Deep litter with run			Deep litter		
NLM inclusion (%)	0	0.5	1.0	0	0.5	1.0
Parameters						
Age at first egg (g)	144.5±4.95^{ab}	145.5±0.71^{ab}	143.0±2.83^{b}	150.5±0.71^{a}	147.5±0.71^{ab}	128.0±1.41^{c}
Weight at 1st lay((kg)	1.29±0.23	1.15±0.04	1.11±0.03	1.25±0.11	1.17±0.02	1.16±0.06
Egg weight (g)	41.95±8.27^{ab}	45.40±1.98^{a}	30.60±0.28^{b}	39.80±2.83^{ab}	45.65±6.86^{a}	32.45±4.31^{b}
Egg length (mm)	50.86±4.57^{ab}	53.98±1.63^{a}	45.71±0.35^{b}	50.38±1.36^{ab}	53.24±1.60^{a}	45.86±2.16^{b}
Egg width (mm)	38.40±2.82^{ab}	38.83±1.25^{ab}	34.56±0.19^{b}	37.65±1.24^{ab}	39.36±1.92^{a}	35.47±1.78
Egg shape index (%)	75.55±1.24^{ab}	71.99±4.49^{b}	75.60±1.00^{ab}	74.73±0.45^{ab}	73.91±1.39^{ab}	77.36±0.25^{a}
Shell thickness (mm)	0.50±0.02	0.42±0.80	0.51±0.04	0.49±0.08	0.51±0.04	0.40±0.07
Shell weight (g)	4.15±0.92	4.20±0.85	4.60±2.55	4.30±0.14	4.60±0.85	3.15±0.35
% shell weight	9.86±0.25	9.22±1.47	14.99±8.18	10.82±0.41	10.05±0.35	9.72±0.20

Albumen height (mm)	7.52±0.33^{bc}	9.48±0.10^{a}	8.90±0.10^{ab}	7.93±1.39^{abc}	7.88±0.62^{abc}	6.48±0.06^{c}
Albumen weight (g)	27.60±4.95^{ab}	31.35±2.47^{a}	19.60±2.26^{b}	25.80±1.70^{ab}	31.41±6.51^{a}	21.65±2.76^{ab}
% Albumen weight	65.91±1.20	69.00±2.44	64.09±7.99	64.84±0.34	68.49±3.96	66.74±0.37
Yolk weight (g)	10.20±2.40^{a}	9.85±1.34^{a}	6.40±0.00^{b}	9.70±0.10^{ab}	9.65±0.49^{ab}	7.65±1.20^{ab}
% yolk weight	24.22±0.95	21.78±3.91	20.92±019	24.35±0.76	21.46±4.31	23.54±0.58
Yolk color	6.0±1.41^{a}	5.5±0.71^{abc}	4.0±0.00^{abc}	4.0±1.41^{abc}	3.0±1.41^{bc}	2.5±0.71^{c}
Haugh unit	91.70±0.90^{bc}	100.02±0.92^{ab}	101.49±4.06^{a}	94.05±7.75^{abc}	92.39±1.17^{abc}	89.32±1.33^{c}

^{abc}Mean in the same row with uncommon superscript differed significantly (P<0.05) ; NLM= Neem Leaf Meal

IV. DISCUSSION

The weight difference of the birds at first lay observed in this study could be attributed to the inclusion levels of the test ingredient and age at which the birds came to lay. The age of the birds could be a major factor that influenced the size of eggs produced. The values for shell weight and thickness were in close range with those reported by [3] who obtained non-significance effect of varying inclusion levels of NLM across treatments. The possibility of yolk score becoming a factor to the different sizes of egg has been proven false. The housing systems and inclusion levels of the NLM were relevant to yolk colour, yolk weight and % yolk weight. The size of the egg affects the proportion of the yolk and other contents inside the egg. The colour score of egg from the control diets indicates that there was improved yolk coloration due to its rich xanthophyll content. This contrasts the works reported by [7]. The range of values obtained for haugh unit in this study was higher than those reported by [1] who reported 83.63-87.02. The average value of Haugh unit obtained in this study conforms to the values reported for standard commercial egg production guides. [9]also reported that haugh unit score of 72 and above has been graded as the best quality. This implies that the Haugh unit obtained in this study is of good standard.

V. CONCLUSIONS

Inclusion of NLM at 1% induced early egg production and quality egg shape index. Improved albumen and yolk colour were obtained in birds fed 0.5% NLM. And Bright yellow yolk was obtained from birds managed in deep litter housing with run.

VI. RECOMMENDATION

From the results it can be concluded that neem leaf meal be included up to 1% in the diets of pullet chickens to induce early egg production.

VII. ACKNOWLEDGEMENT

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A report on Tuberculosis in Monkeys (*Macaca mulatta*): A case study at Chittagong Zoo

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***Abstract*—** *Simian tuberculosis is one of the most important bacterial diseases of captive monkey in Bangladesh. A prevalence study to characterize Mycobacterium infecting tuberculous monkeys in captive managemental systems in Chittagong Zoo was carried out. In the present study, 14 rhesus monkeys which were newly arrived in the zoo and kept in the quarantine were used for the tuberculin skin testing (TST) to determine the prevalence of tuberculosis. An overall of 28.57% (4/14) was recorded by the TST. There were also marked differences in the prevalence of the disease within different age groups. In the tested positive animals, one was died within two days and showed tubercle in the lung and other organs in the post-mortem examination. The lung sample was collected for Zeihl-Neelsen revealed red colored tubercule bacilli.The above examination confirmed that, the macaques were suffering from tuberculosis.*

***Keywords*—Mycobacterium tuberculosis, Rhesus monkey, Tuberculosis, Tuberculin, Prevalence.**

I. INTRODUCTION

Simian (Primate) tuberculosis (TB) is a major health problem in most of the developing countries. As in many other hosts, simian tuberculosis is also caused by bacterium *Mycobacterium tuberculosis*(Avicenna, 2011). Outbreaks of tuberculosis have been reported in many captive monkey colonies around the world. In Japan, tuberculosis in monkeys has been reported in zoos (Yumi Une, 2007). Many species of *Mycobacteria* can cause disease in primates and other species (Yumi Une, 2007). Recently, two outbreaks of tuberculosis occurred in four different kinds of monkeys and humans were also infected with the disease in Japan (Yumi Une, 2007). In zoos, tuberculosis was reported not only in monkeys but also in several different kinds of animals (Yumi Une, 2007).TB bacteria can live in the body for years long in non-active form (Moreland, 1970). Primates acquire classic tuberculosis (TB) by contact with other infected nonhuman primates (NHP) or humans through inhalation or the digestive route (Moreland, 1970). It has shown that about 60% of infected NHPs develop Latent Tuberculosis Infection (LTBI); latency is confirmed by a positive tuberculin skin test (TST) (Patel, 2011). This disease and the causative agent *Mycobacterium tuberculosis* have been intensively studied, yet the basis for protection, as well as many of the microbial and immunologic factors that contribute to disease, is not well understood. In Bangladesh outbreak of tuberculosis has been reported in humans and domestic animal but, not yet been reported in captive monkeys. In our study area (Chittagong zoo) outbreak was also not specifically recorded. But it is known that geographically this area is at a risk of tuberculosis, especially in the Chittagong zoo due to the possibility of direct contact of zoo animals with visitors. In our study, we only focused on the prevalence of tuberculosis in Chittagong zoo. The tuberculous monkey is a health hazard especially to other monkeys in the group, but (re)transmission of the infection to humans has been reported as well (Yumi Une, 2007). Due to lack of proper diagnostic and treatment facility TB is increasing and zoonoses transmission occur. In spite of reasonable precautions, outbreaks continue to occur and tuberculosis remains a serious threat to the health of captive monkey and their care takers. To the best of knowledge, there is no published comparative report on the prevalence of tuberculosis in captive monkeys (rhesus monkey) in Bangladesh. Therefore, the following study was carried out with an aim of estimating the prevalence of tuberculosis in captive monkeys (rhesus monkey) with these following objectives:

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1) To study the prevalence of tuberculosis in captive primates (Rhesus monkey).
2) To identify the risk factor associated with the disease.

II. MATERIALS AND METHODS

(1)The survey area: The study was done at Chittagong zoo, Bangladesh.

(2) Study population and study period: In the present study, 14 rhesus monkeys which were newly arrived in the zoo and kept in the quarantine were used to determine the prevalence of tuberculosis. The study time was October 2014 to November 2014.

(3) Study design:A cross-sectional study design was followed in the present study with a view of estimating from the record book.

(4) The capture of Monkey: In the quarantine shed the monkeys were live captured by the caretaker with the use of the net. During the capture, no monkey was injured and all safety measures had taken to check any critical condition. All the monkeys are captured in a humane way.

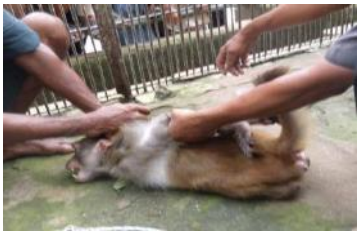

Fig.1: Capture of the monkey

The original plan was to inject the tuberculin immediately after capture, but the restrictions on entry to two-thirds of the area made the monkey afraid and showed angriness. Following the identification of infected animals in the group of the monkey caught, was injected tuberculin and observed the swelling.

(5) Ante-mortem examination and tuberculin test: All the monkeys were included in the sample size were examined physically before they are slaughtered. Age, sex, and weight of the animals were recorded. Additionally, body temperature, pulse rate, respiratory rate, type of nasal discharge (if present), the condition of regional lymph nodes, and visible mucous membranes were examined. Besides purified protein derivative (PPD) tuberculin was injected intradermally to record the swelling above 10 mm for confirmed tuberculosis.

(6) Post-mortem examination: Only one infected monkey has died after two days of injecting tuberculin and through post-mortem of the monkey was done. After post-mortem visual examination of intact organs like kidneys, lung, the liver was done. The tuberculous nodule was found in the visceral organ and grossly diagnosed as tuberculosis.

(7) Laboratory diagnosis: The impression from the tentatively diagnosed TB nodules found in different visceral organs was taken and on glass slide stained following acid fast staining procedures described in Literature review section. Examination of stained smear was carried out in microbiology department of Chittagong veterinary and animal science university.

(8)Data collection: The individual animal identification marking, breed, sex, and age of the animal were recorded. After injecting tuberculin the swelling site was measured at 0 hours and 72 hours in mm.

(9)Data analysis: The recorded raw data were entered into Microsoft excel data base system to be analyzed using Statistical Program for Social Science (SPSS) version 20. Descriptive statistics were computed. Prevalence of tuberculosis was calculated as the number of monkeys found infected with tuberculosis, expressed as the percentage of the total number of monkey examined. Fisher's exact test was used to evaluate the association between the tuberculin positivity and different risk factors. The p-value less than 0.05 (at 5% level of significance) were considered significant in all analyses.

III. RESULTS AND DISCUSSION

Description of the study population:

There were 29 monkeys in the primates quarantine section of the Chittagong zoo. For the study, we captured 14 monkeys which were marked by shaving (Fig.2) different area of the body for identification. Among 14 monkeys 6 were male and 8 female. The average age of male is 1.8 years and female is 2.1 years. Average body weight of the male was 3.02 kg and female 3.45 kg.

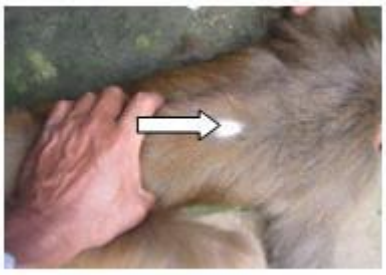

Fig.2: Marking for the identification of monkey

Antemortem examination: The diagnosis of simian tuberculosis by clinical examination is of very limited value given that most animals infected with the bacterium do not show clinical signs of the disease and that there are no pathognomic signs of simian tuberculosis in the monkey. Monkey with latent TB is not infectious and may appear healthy for years, but eventual reactivation of latent TB can result in secondary transmission and outbreaks of disease in

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established colonies. Reactivation of latent infections that were not detected using traditional screening methods during primary quarantine is emerging as an important factor in the epidemiology of TB in the monkey. Only one monkey was observed emaciated with consistent coughing and the monkey died 2 days after the injection of tuberculin. Besides body temperature, pulse rate, respiratory rate, type of nasal discharge, the condition of regional lymph nodes, and visible mucous membranes were examined.

Intradermal Skin Test: A tuberculin Sensitivity test (TST) was carried out for the diagnosis of M. Tuberculosis in the captive monkey. The sample was provided by the respective authority. For this study, 14 monkeys were live-trapped by using the net. After recording their age, sex the monkeys were then weighed and marking was done. The hair was clipped without traumatizing the skin and the injection site noted. Then an intradermal tuberculin test was performed and injecting intradermally 0.1 ml of tuberculins. Then the swelling of the side was measured (Fig.3). The site was inspected at 24 and 48 and 72-hour interval. In the observation we found 3 monkey's skin swelling above 10 mm. During diagnosis process, a 27 gauge needle was used for intradermal injection of tuberculin. The injecting site was the skin of fore arm region. At first, the animal was captured by the animal caretaker using the net. Then the injecting site was prepared for tuberculin injection. All the monkeyswere given 0.1 ml PPD intradermally and then swelling was measured immediately in mm and a wide verity of swelling length was recorded. During the observation, a diseased animal died on the spot. The carcass was subjected to a detailed post-mortem examination and some portions of lesions found were fixed in buffered formalin for histological examination and some are frizzed for microbiological examination. For the microbiological test, the sample was taken in the dept. of microbiology of Chittagong veterinary and animal science university (CVASU) and acid fast was done.

Fig.3: Swelling measurement after 72 hours

Table.1: Prevalence of tuberculosis in captive monkeys (Rhesus monkey):

No. of tested animal	No. of positive case	Prevalence (%) with 95% Confidence interval
14	3	21.42 (−0.07% - 42.91%)

A similar type of skin test was performed in the United States and the considerable animal was found infected (Narasimhan, et.al; 2013). In the study of zoonosis of Non-Human Primates reported 0.5% (9/1621) prevalence of tuberculosis in Rhesus monkey in the USA which is much lower than present study (Narasimhan, et.al;2013). The variation of these two studies was probably due to differences in study design and the number of sample size.

Post Mortem examination:

For determination of the cause of death of the monkey, the postmortem was done on the Post-Mortem room of Chittagong zoo. Before the PM all organs and tissues, including external body, examined in a systematic way (position color, size, weight, shape, consistency, content, smell, an extension of lesions, and aspect of the section). Protective clothing was worn according to bio hazard level of tuberculosis.

The instrument pack also include post-mortem knives, forceps, two scalpel handles (one for cutting, one for burning organ surfaces before taking a microbiology sample), stout scissors (for cutting bones), and fine scissors for dissection.

After finishing the PM disposal of the carcass, appropriate disinfection of self and equipment, to avoid further spread of the disease was ensured.

Post-mortem finding:

The animal was opened aseptically and gross examination of the visceral organ was done.The tubercles had a yellowish appearance and an abscess with necrotic focus and caseation was found. Other findings at post-mortem examination were numerous small focuses usually in the lung, liver, lymph node etc (Fig.4). Tubercles were found in bronchial, mediastinal lymph nodes and after that sample were collected (Fig.5). Tubercle nodule was also found in the lungs, liver, spleen, body cavities and female genitalia as it was chronic tuberculosis.In a study by Francis, (1958) reported the same kind of nodular elevation in lymph node found in the cow. Thus, a tentative diagnosis of TB was made based on the similarities of post-mortem sign found in the dead monkey and the referred study.

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Fig.4: Tubercle nodule in the lung, Liver and Lymph node

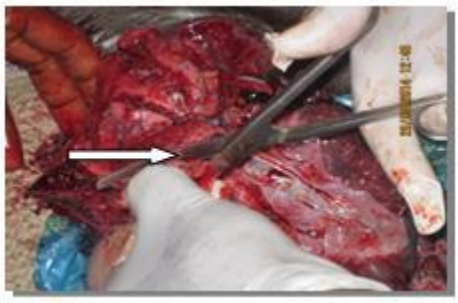

Fig.5: Sample collection after post-mortem

Acid fast finding:

For the study, were made total 10 slides where smear was taken from the lung. Tubercle lesion site was smashed and grinded, then taken a smear from them. After staining, red colored tubercle bacilli were observed in the lung specimen indicating that the monkeys were exposed to tuberculosis. The cell wall of tubercle bacilli contains (Fig.6) waxy material so it did not take dye at room temperature. But the bacteria took up stain with dye by prolonged application or by heating. When once the bacteria were stained, they resisted decolorization with acid alcohol, but the tissue could be decolorized and took the color of methylene blue. For this reason, bacilli looked red and the tissue looked blue in the acid fast staining result. In a study of Forero, M. et al., (2004), similar identification of red color TB organism was found by Ziehl-Neelsen method for acid-fast staining.

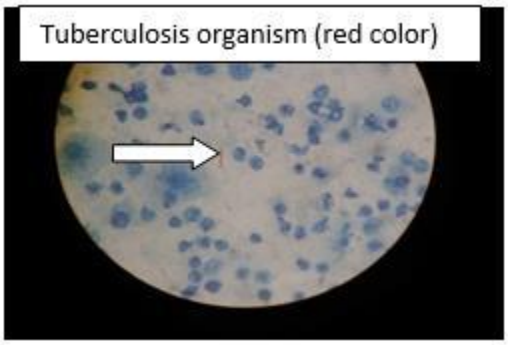

Fig. 6: Tuberculosis organism after acid fast staining

Table.2: Risk factors of tuberculosis:

Variable	Category	No. of animal tested	No. of positive animal	Odds Ratio (95% Confidence interval)	P value
Gender	Male	6	2	3.5(.24-51.89)	0.54
	Female	8	1	1	
Body weight	High(≥ 4 kg)	6	3	9.00*(.75-108.3)	0.06
	Low(< 4 kg)	8	0	1	
Age	Adults (≥ 3 years)	5	3	13.33*(1.05-169.56)	0.03**
	Young (< 3 years)	9	0	1	

*Odds ratio was calculated by adding 1 in each cell.

** Significant at 5% level

Variables identified to be significantly associated with status (tuberculosis) in Fisher's exact test included age, sex, body weight (Table 2). Plasma cells can occasionally be seen in smears of peripheral blood. Hypoalbuminemia and hyperglobulinemia are common findings. Serum protein has the nodulous portentous effects and protein electrophoresis. The organ affected by the lymphosarcoma and upper respiratory distress. Tuberculosis was found to be more prevalent in males as compared to females. Similar gender relationship of tuberculosis was found where the ratio of female to male tuberculosis cases notified was 1:1.5–2.1 and 70% more smear-positive male than female tuberculosis patients are diagnosed every year and notified to the WHO (Diwan, and Thorson, A., 1999). Adult individuals showed higher prevalence than the young. A strong association ($P<0.05$) was observed between age and risk of tuberculosis (Table 2). Similar age relationship with tuberculosis was found in human where more than 65 percent of the residents of New York City who died of the disease were over 45 years of age (Robins, 1953). The exposure time probably

increased with increasing age. Thus the older the monkey the higher chance of being TB positive. However, the gender of the animals was not significantly associated (P>0.05) with tuberculosis and there was no significant difference between tuberculin positivity and body weight of the monkey.

IV. CONCLUSION

M. tuberculosis is well known to have the widest species range, infecting an extensive range of animals, from cattle to humans, domestic animals to feral or wild ones. In addition, captive animals infected with tuberculosis create problems in the management of zoological collections, increasing the risk of infection to other valuable animals as well as to their keepers. Although tuberculosis in such animals is an important problem, there is a dearth of well-validated data for the diagnosis of the disease in the monkey. Although the epidemiology of simian TB is well understood and effective control and elimination strategies have been known for a long time, the disease is still widely distributed and often neglected in most developing countries. Its public health consequences, although well documented from the past experiences of industrialized countries, have scarcely been investigated and are still largely ignored in these regions. Prevalence (21.42%) of tuberculosis in captive monkeys indicates that the area is at risk of transmission of TB of zoonotic concern. Research is needed to determine when M. tuberculosis is of zoonotic importance and what the underlying mechanisms of transmission are. TB was not possible to find out the possible source of infection and mode of transmission due to the short duration of the study. Further long-term study needed to find out the possible source of infection and specific organism. Outbreaks have severe economic consequences due to animal losses, disruption of research and costs related to disease control. Therefore persons working with monkey should be familiar with the disease and preventive measurements. The increase of TB in such areas calls for stronger intersectoral collaboration between the medical and veterinary professions to assess and evaluate the scale of the problem. Any vaccination research and development program should therefore also take into account the possible application of vaccines to the monkey, particularly in developing countries.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR'S CONTRIBUTION

All authors contributed equally and approved the final manuscript.

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Effect of dose and timing of application of different plant growth regulators on lodging and grain yield of a Scottish landrace of barley (Bere) in Orkney, Scotland

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Abstract— *The effects of dose and the timing of the application of three different types of plant growth regulators on lodging and grain yield of a landrace of barley (Bere) were investigated. Results indicated that the application of full dose of plant growth regulators at Zadoks growth stage 31 had improved lodging resistance by reducing the stem length. Amongst plant growth regulators Upgrade caused the highest reduction in stem length and lodging index compared with other plant growth regulators while Adjust was the least effective plant growth regulator. The results indicated that Upgrade was less effective in lodging control at the higher nitrogen level (90 kg ha^{-1}). Although this plant growth regulator improved lodging resistance, grain yield was not enhanced in any of the trials. This outcome was due to a delayed lodging and/or absence of severe lodging in the control plots. Further investigations on the effect of timing of lodging incidence on grain yield would be useful extension of the present study. A separate trial investigating the effectiveness of Upgrade in lodging control under a range of nitrogen levels is recommended.*

Keywords—landrace, Bere, plant growth regulator, dose, timing of application.

I. INTRODUCTION

Bere is a landrace of barley (Jarman, 1996) and has been an important part of Orkney's Agriculture for hundreds, possibly thousands of years (Theobald *et al.,* 2006). In Scotland particularly during 18th and 19th centuries it was a versatile crop that provided flour for baking, malt for brewing and distilling and straw for animal bedding and thatching (Newman, 2006). Once widely grown, Bere is now only grown on a very small scale in Orkney, Caithness, Shetland and a few areas on the Western Isles (Scholten *et al.,* 2007). This decline was partly due to changes in market demand from grain production to grass for the beef industry and the introduction of high yielding modern varieties of barley (Thompson, 2001). The Agronomy Institute, Orkney College, Scotland, which opened in 2002 has put considerable efforts in the development of high value niche products in order to revive the demand for Bere's grain. Until today, two new products, *Bere* whisky and *Island beer*, have been developed in collaboration with *Bruichladdich distillery-Inverness* and *Valhalla distillery-Shetland* respectively (Martin, Wishart and Scott, 2013; Martin and Wishart, 2015). As a result of this development, Bere is now an economically viable crop and a few farmers are interested in growing Bere because they can get a higher price for their produce than they could before. However, farmers are concerned about Bere's susceptibility to lodging due to its long and weak straw (Martin *et al.,* 2010). Severe lodging can interfere with the speed and efficiency of harvesting operations (Tripathi *et al.,* 2003) and, most importantly, it can cause significant economic losses by reducing grain yield (GY) (Pinthus, 1973) and grain quality of barley (Stanca *et al.,* 1979; Birggs, 1990). In order to avoid lodging related negative effects on harvesting and grain yield, Bere is presently grown with no or low nitrogen inputs (30 kg N ha^{-1}) on marginal land in Orkney (Dr. Peter Martin, personal comm.). Plant growth regulator (PGR) can reduce stem length and improve the standing ability of the barley (Kust, 1985; Sanvicente *et al.,* 1999) and wheat (Jung, 1964; Tripathi *et al.,* 2003). Amongst PGRs *Ethephon* (2-chloroethyl phosphonic acid) (ET) and *Chlormequat chloride* (CCC) have been effective in decreasing plant height and reducing lodging incidence in wheat (Crook and Ennos 1995). However, the effectiveness of PGRs in controlling lodging depends on many factors including variety, type of growth regulator, its application rate (Bahry, 1988), crop growth stages at the time of application (Caldwell *et al*., 1988) and its dose (Simmons *et al*., 1988). There was no published information on the effects of timing, type

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and dose of PGRs on lodging and yield of Bere. This paper is first of its kind which reports the results obtained from three different trials carried-out in 2008 and 2009. In *Trial 1*, the effectiveness of different doses of PGRs in lodging control and yield enhancement was investigated. *Trial 2* examined how the timing of application of PGRs affected lodging related trait, yield and yield components of Bere. These two trials were carried out under low N-level (30 kg N ha^{-1}). *Trial 3* was essentially a repetition of *Trial 2* except that it was carried-out at a higher N-level (90 kg ha^{-1}).

II. METHODS AND MATERIALS

Bere was sown using standard seed rate (160 kg ha^{-1}) recommended by Martin *et al.* (2010) at an experimental site near Orkney College, Kirkwall, Orkney (Grid reference: HY 456 114) in two successive growing seasons (2008 and 2009). The soil of the experimental plot was classified as clay loam, with organic matter (3.9 %), NO_3-N (17.25 mg kg^{-1}) and NH_4^+ (0.96 mg kg^{-1}), P (28.2 mg kg^{-1}), K (70mg kg^{-1}) and acidic in nature (pH=5.5). Plots were planted using a Pneumatic Accord Combine Seed Drill. Weed control was achieved in all trials by applying a mixture of Mecoprop (1.5 l ha^{-1}) and 4-chloro-2-methylphenoxy acetic acid (MCPA) (1.0 l ha^{-1}) in 200 l of water. The plant growth regulators (PGRs) used were *Adjust* {[chrolmequat chloride], Mandops, a.i 620 gl^{-1}}, *Cerone* {[2-chloroethylephosphonic acid], Bayer CropScience, a.i 480 gl^{-1}} and *Upgrade* {[chrolmequat chloride + 2-chloroethylephosphonic acid], Bayer CropScience, a.i 360:180 g l^{-1}}. All PGRs were sprayed using Knapsack sprayer in sufficient water (160 l ha^{-1}) and with a wetting agent "Banca" at the manufacturer's recommended rate (10 ml 20 l^{-1}) to give good foliage coverage. The agronomic details of all the trials are illustrated in Table 1.

Table.1: Agronomic detail for 2008 and 2009 trials

	Trial 1	***Trial 2***	***Trial 3***
Date Sown	6th May 08	6th of May 08	27th April 09
Seed Rate	160 kg ha^{-1}	160 kg ha^{-1}	160 kg ha^{-1}
Row Spacing	9.5 cm	9.5 cm	9.5 cm
Previous crop	Bere	Bere	Bere
Fertilizer (N-P-K kg ha^{-1})	30-30-40	30-30-40 kg	90-30-40
Date Harvested	24th Sep 08	9th Sep 08	11th Sep 09

Since the trials were not complete factorial experiments, it was not possible to statistically compare means across PGRs, growth stages or doses. However, means were manually calculated to aid in the interpretation of the effect of treatments. In all the trials, ears m^{-2} (EPSM) was recorded in a representative 50 cm x 50 cm quadrat. A representative sample of 20 stems was manually harvested from each treatment plot. A sub-sample of 10 stems was used to record stem length from the bottom of the stem to the base of the ear as described by Schittenhelm and Hartmann (2006). The ears of the remaining stems (10 stems) were then manually threshed to record grains ear^{-1} (GPE). All plots were visually monitored after every rainfall event to record the onset of lodging. Final lodging assessments were made just before final harvest in the un-sampled half of each plot area. A frame marked with different angles was used to visualize the angle of deviation of stems from vertical. These observations were then converted into lodging index (LI) with slight modification to the formula developed by Berry *et al.* (2003) so that intermediate angles of 0-15, 15-30, 30-45, 45-60, 60-75, and 75-90 could be included.

Lodging Index = {1/6 (% at 0^0-15^0) +2/6(% at 15^0-30^0)+3/6(% at 30^0-45^0)+ 4/6(% at 45^0-60^0)+5/6(% at 60^0-75^0)+(% at 75^0-90^0)}.

Grain yield (GY) was estimated by harvesting the plots either manually or by combine harvester. A sub-sample (100 g) of grain was drawn to measure grain moisture content (GMC). A Contador counter (Hoffman Manufacturing Inc, Germany) was used to count the grains required for 1000-grain weight (TGW). The GY and TGW were adjusted to 15 % GMC. Statistical analysis of the data was performed separately for each of the trials using Genstat 9.1. Means of treatments were compared using Fischer's protected least significant differences (LSD) at 5% level of probability. The relationships between yield, yield components, lodging and lodging related traits were investigated by regression analysis.

2.1 *Trial 1*

The seven treatment combinations for this trial are provided in Table 2. These treatments were applied when 75% of the plants were at ZGS 33 while 25% at ZGS 37 on individual subplot plot 6 m x12 m (72 m^2). In all plots,

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data were collected for stem length (StL), lodging index (LI), ears m^{-2} (EPSM), grains ear^{-1} (GPE), grain yield (GY) and 1000-grain weight (TGW). Plots were assessed for lodging on 11th Sep 2008. GY was estimated by combine harvesting two strips, 2.3 m wide and 12 m long on 24th September 2008.

Table.2: List of plant growth regulators and their abbreviations

Treatment	***Abbreviation***
Adjust (half dose)	A ½
Cerone (half dose)	C½
Upgrade (half dose)	U½
Adjust (full dose)	A1
Cerone (full dose)	C1
Upgrade (full dose)	U1
Control	No-PGR

2.2 *Trial 2*

This trial was sown along with *Trial 1* on similar date. A list of treatments is shown in Table 3. The treatments were applied at two different growth stages i.e ZGS 31 (1st node detectable) and ZGS 37 (flag leaf just visible) on 19th and 30th June 2008 respectively. All the treatments were replicated 5 times and randomly assigned to individual plots of size (2 m by 3 m) in a randomized block design. Soon after the onset of stem elongation, 5 main stems of the plants in each plot were tagged with cable ties to ensure that main stems were used for recording stem diameter (SD) and stem length (StL) at maturity. The tagged main stems were harvested on 6th September 2008. The leaves and ears were removed from the stems and StL was recorded. SD was measured using calipers at 1 cm above the stem base. Plots were assessed for lodging before being manually harvested on 9th Sep 2008 to record yield and other parameters.

Table.3: List of plant growth regulators and their abbreviations applied at different growth stages in 2008

Treatment	***Abbreviation***
Adjust at ZGS 31	A31
Cerone at ZGS 31	C31
Upgrade at ZGS 31	U31
Adjust at ZGS 37	A37
Cerone at ZGS 37	C37
Upgrade at ZGS 37	U37
Control	No-PGR

2.3 *Trial 3*

This trial was a randomized block design with 4 replications. Seven treatments (Table 4) were applied to individual sub-plots (3 m x 6 m). All the PGRs were sprayed at ZGS 31 and ZGS 37 on 15th June 15th and 21st June 2009 respectively. Plots were mechanically harvested using combine on 11th Sep 2009. Data recorded for this trial were StL, LI, TGW and GY.

Table.4: List of plant growth regulators and their abbreviations applied at different growth stages in 2009

Treatment	***Abbreviation***
Adjust at ZGS 31	A31
Cerone at ZGS 31	C31
Upgrade at ZGS 31	U31
Adjust at ZGS 37	A37
Cerone at ZGS 37	C37
Upgrade at ZGS 37	U37
Control	No-PGR

III. RESULTS

3.1 *Trial 1*

The data recorded for this trial are presented in Table 5. StL was significantly (*P= 0.016*) affected by the PGR treatments. *Upgrade* was the most effective PGR which caused the greatest reduction in StL compared with *Cerone* and *Adjust* (averaged over both doses). The control treatment resulted in the highest StL. Interestingly, the half dose and full dose of the PGRs produced almost identical StL. Visual assessments of the crop made on 16th and 30th August 2008 showed no apparent sign of lodging-flat (angle of deviation of stem from vertical > 76^0). However crop leaning (angle of deviation between 16^0-45^0) was seen in all the plots and was comparatively higher in the control than in the PGR treatments. PGR treatments had a significant (P< 0.001) effect on LI. *Upgrade* was the most effective PGR in reducing the LI followed by the *Cerone* and *Adjust* treatments. When the effects of individual doses of the PGRs were examined, it was observed that the full dose gave a better lodging control than the half dose. PGR treatments had significant (*P= 0.027*) effect on EPSM and full dose of the PGRs resulted in higher EPSM than half dose and the control. The highest EPSM was produced by the plots treated with *Cerone* followed by *Upgrade* (averaged over both doses). *Adjust* was the least effective PGR. GPE was also significantly (*P= 0.009*) affected by the PGRs. The highest GPE was recorded from the *Upgrade* treatment followed by the *Adjust* while the lowest was from the *Cerone*. When the effects of individual doses of the PGRs were examined, it was noted that the full dose of *Cerone and Adjust* reduced the GPE by 16% and 13% respectively compared with the half doses. The correlation analysis revealed that there was a significant (*P= 0.007*) negative association between

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EPSM and GPE (Fig 1). TGW differed (*P= 0.002*) between the treatments. The plots treated with *Adjust* produced the highest TGW, followed by the control and *Upgrade* treated plots while the lowest from *Cerone*. It was also noted that the full dose of *Cerone* reduced the TGW by 6% compared with its half dose. The correlation analysis indicated that there was a negative association between EPSM and TGW (Fig 2). GY was not significantly affected by the PGR treatments.

Table.5: Effect of half and full dose of plant growth regulators on selected parameters of Bere in 2008

Trial 1			**2008**			
Treatments	StL (cm)	LI	EPMS	GPE	TGW (g)	GY (kg/ha)
A1	91.4	43.5	404.3	30.9	35.6	3596
C1	89.6	28.5	443.7	28.8	33.8	3254
U1	76.6	27.8	416.0	36.5	35.1	3418
A ½	89.8	45.3	336.0	35.1	36.5	3080
C ½	86.3	44.0	412.8	33.6	35.9	3368
U ½	78.2	40.2	382.9	34.9	34.9	3548
Control	94.1	67.5	362.7	34.4	36.1	3192
Probability	0.016	<0.001	0.027	0.009	0.002	0.384
LSD(0.05)	10.70	11.4	61.8	4.0	1.2	519.4
S.E	3.7	3.9	21.2	1.4	0.41	177.9

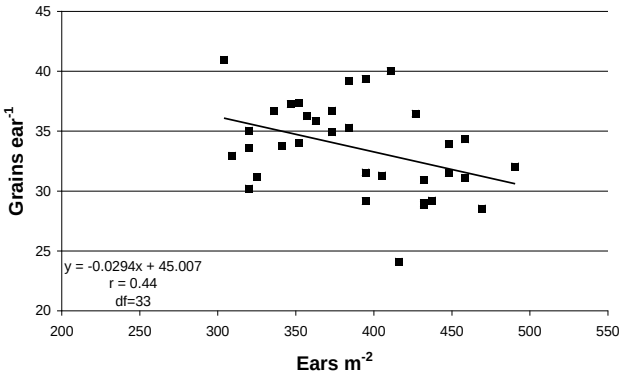

Fig.1: Correlation between grains ear^{-1} and ears m^{-2}

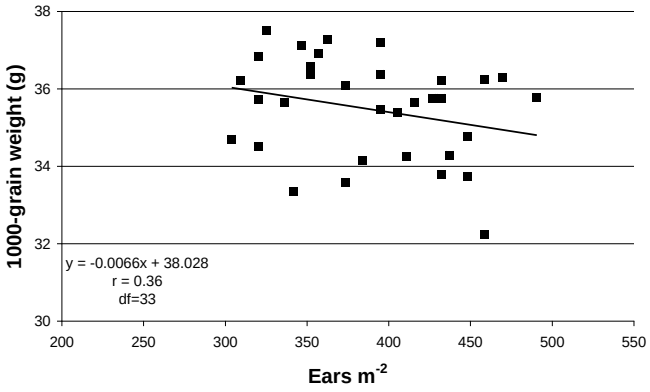

Fig.2: Correlation between 1000-grain weight and ears m^{-2}

3.2 *Trial 2*

SD was not significantly affected by the timing of application and the type of PGRs (Table 6). There were significant (*P= 0.007*) differences in the StL between the treatments. Amongst PGRs, *Adjust* was the least effective treatment in reducing StL (averaged over all growth stages). When the effects of the timing of application were examined it was noted that the earlier application of

Adjust and *Upgrade* at ZGS 31 caused more reduction in StL than their later applications (ZGS 37). SWCM was significantly (*P= 0.033*) affected by the PGR treatments. Early application at ZGS31 tended to reduce SWCM than at ZGS 37 (average of all three PGRs). *Upgrade* applied at ZGS 31 was the only treatment that showed significant decrease in the SWCM compared with the control. This treatment reduced the stem dry weight proportionally more than the length. In contrast, the remaining PGR treatments had a higher SWCM than the control. The LI assessment made on 9th September 2008 showed significant (*P< 0.001*) differences between the treatments. All the three PGRs reduced the LI compared with the control, and were more effective at ZGS 31 than ZGS 37. *Adjust* was the least effective PGR at ZGS 37. The highest LI was observed in the control (70) while the lowest value was recorded for the U31 treatment. The PGRs had a significant effect (*P< 0.001*) on EPSM. Earlier application of PGR at ZGS 31 increased the number of EPSM compared with those plots treated at ZGS 37 (average of all three PGRs) and the control. The highest EPSM was from the U31 treatment followed by the C31 treatment while the lowest was in the U37 treatment. EW was significantly affected by the treatments. The earlier application of the PGRs reduced the EW more than the later application. The highest was from the control treatment while the lowest from the U31 treatment. GPE was also significantly (*P< 0.001*) altered by the treatments. Earlier application of PGRs at ZGS 31 produced lower GPE than later application. The highest GPE was from the control treatment followed by the A37 and A31 treatments while the lowest GPE was from the U31 treatment. TGW was significantly (*P= 0.002*) affected by the treatments. Earlier application at ZGS 31 resulted in a lower TGW than at ZGS 37 (averaged over PGRs). Among the PGRs treatments, the *Adjust* treatment produced the highest TGW followed by *Cerone* treatment and the lowest was from the *Upgrade* treatment (averaged over growth stages). The control treatment had the heaviest TGW followed by the A37 treatment and the lowest was from the U31 treatment. It was noted that none of the plant growth regulators caused any significant effect on GY irrespective of the timing of application when compared with control. Simple linear regression analysis revealed that the interrelationships between yield and its components were not significant (Table 7). This was due to the negative correlations between EPSM and both GPE (Fig 3) and TGW (Fig 4). There was no significant association between GPE and TGW (Table 4.3). The multiple regression analysis, considering all the yield components as yield predictive variables, showed that EPSM together with EW explained 56% of the variations in grain yield (Table 7). There were no significant correlations between SWCM or SD and LI (Table 8). A step wise inclusion of additional variables in a multiple regression model improved the correlation and a regression model comprised of StL, EW and SD as predictive variables correlated most closely with the LI (Table 8).

Table.6: Effect of timing of application of plant growth regulators on selected parameters in 2008

Trial 2				2008				
Treatments	StL (cm)	SD (mm)	SWCM (mg cm^{-1})	LI	EPSM	GPE	TGW (g)	GY (kg ha^{-1})
A31	85.3	3.98	11.6	52.5	400.8	32.3	36.1	4407
C31	83.1	3.88	10.7	25.5	471.2	25.5	33.9	4200
U31	68.3	3.49	9.5	17.5	544.0	23.1	33.5	4221
A37	87.8	3.78	12.0	65.5	367.2	32.9	36.6	4400
C37	75.5	3.5	10.3	33.7	413.6	29.6	36.1	4270
U37	77.6	3.83	11.2	37.2	357.6	30.8	34.9	4172
Control	94.1	3.52	10.2	69.7	376.8	33.8	37.4	4616
Probability	0.007	0.411	0.033	<0.001	<0.001	<0.001	0.002	0.150
LSD(0.05)	13.5	0.57	1.52	10.5	68.8	2.7	1.9	575.2

Table.7: Values of the co-efficient of determination (R^2) and probability (P) for linear regressions of yield and its different components of yield.

Yield components	No. of independent variables	R^2	Probability (P)
Ears m^{-2} (EPSM)	1	0.0698	NS, df=33
Grains ear^{-1} (GPE)	1	0.0201	NS, df=33
1000-grain weight (TGW)	1	0.0828	NS, df=33

EPSM, GPE	2	0.4945	<0.001, df=32
EPSM, TGW	2	0.2857	=0.004, df=32
EPSM, EW	2	0.5692	<0.001, df=32
GPE, TGW	2	0.0341	NS, df=32
EPSM, GPE,TGW	3	0.5546	<0.001, df=31

NS: Not significant (*P> 0.05*)

Table.8: Values of co-efficient of determination (R^2) and probability (P) for linear regression of lodging index and lodging related traits

Lodging related trait	No. of independent variables	R^2	Probability (P)
Ear weight (EW)	1	0.6601	<0.001, df=33
Stem length (StL)	1	0.2863	<0.001, df=33
Stem diameter (SD)	1	0.0011	NS, df=33
Stem weight per cm (SWCM)	1	0.0920	NS, df=33
EW, SD, StL	3	0.7635	<0.001, df=31

NS: Not significant (*P> 0.05*)

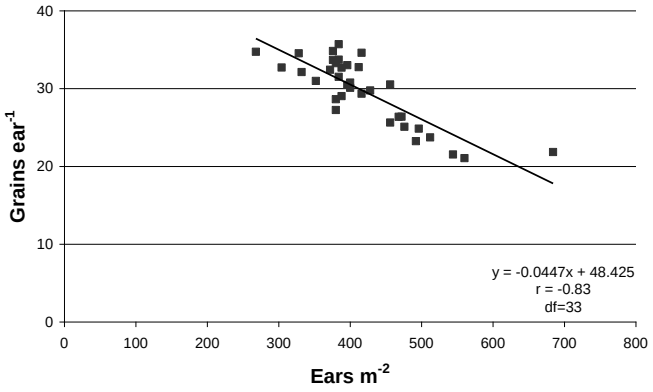

Fig.3: Correlation between grains ear^{-1} and ears m^{-2}

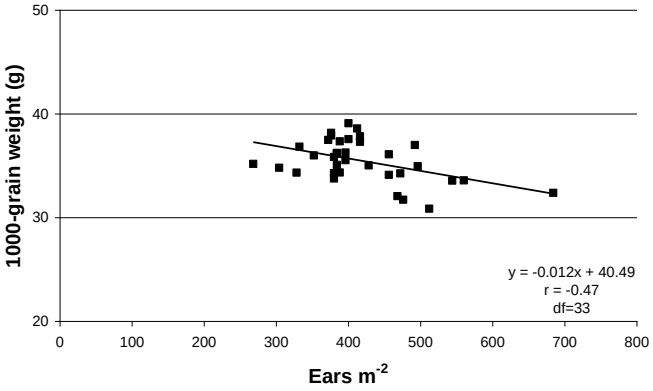

Fig.4: Correlation between 1000-grain weight and ears m^{-2}

3.3 *Trial 3*

Results for the C31 treatment are not reported because this treatment was contaminated with a chemical herbicide which resulted in severe damage to the plants. *Upgrade* and *Cerone* reduced the length of stem and LI more than the control plots (average over both application times) while *Adjust* was the least effective PGR (Table 9). GY was not affected by any of the PGR treatments. A simple regression analysis revealed that LI had no significant associations with GY or TGW and it was also not related to StL (Table 10).

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Table.9: Effect of application of plant growth regulators at different growth stages on lodging and yield of Bere in 2009.

Trial 3	2009			
Treatment	StL	LI	TGW (g)	GY (kg ha^{-1})
A31	118.0	95.4	30.6	4585
U31	112.6	76.0	29.7	4189
A37	123.	97.7	29.4	4186
C37	111.0	90.0	29.8	4166
U37	110.3	72.3	31.1	4870
Control	124.7	95.4	29.8	4854
Probability	<0.001	0.032	0.012	0.250
LSD(0.05)	6.8	18.1	0.93	833.9

Table.10: Values of co-efficient of determination and probability for selected parameters

Dependent variable	Independent variable	R^2	Probability (P)
GY	LI	0.005	NS, df=22
TGW	LI	0.093	NS, df=22
LI	StL	0.092	NS, df=22

NS: Not significant (*P> 0.05*)

IV. DISCUSSION

Reduction in StL was influenced by the type of PGRs and application rate. In agreement with the work of White (1991), *Adjust* (CCC) was found to be the least effective in shortening the length of stem. This response was thought to be due to poor absorption of CCC by the barley plant (Skopik and Cervinka 1967). *Upgrade* caused the greatest reduction in the length of stem and the half of the recommended rate was as effective as the full dose. Although a half dose and full dose of PGRs produced similar StL, the LI did not follow this pattern and the lowest LI was achieved from the full dose of *Upgrade* and *Cerone*. This outcome suggested that the mechanism by which PGR increased resistance to lodging may not be related to StL alone. Other lodging related stem traits such as SD (Easson *et al*., 1993), SWCM (Zuber *et al.,* 1999) as well as EW (Tripathi *et al.,* 2003) were investigated in *Trial 2*.

It has been reported that a higher SD is an indication of lodging resistance (Mukherjee *et al.,* 1967) but the *Trial 2* results showed no evidence that PGRs affected SD. This outcome was consistent with the findings of Stanca *et al.* (1979) on different barley varieties. Dunn and Biggs (1989) suggested that lodging resistance in barley was associated with thicker stem walls rather than a larger SD. White (1991) and Zuber *et al.* (1999) considered SWCM as a measure of stem strength. These results suggest that PGRs, such as *Cerone* and *Upgrade*, might increase the stem strength by concentrating dry matter into shorter stems which would result in a lower LI. In contrast, the lowest LI was recorded in those plots which had the lowest SWCM. This outcome may suggest that lodging resistance may not be solely related to stem strength or that SWCM was not a good indicator for the stem strength. Pinthus (1967) found that EW and StL were strongly related to lodging. This was because when stems were displaced from vertical position due to the wind, a second base bending moment resulted from the centre of gravity which increased with increase in EW and StL (Pinthus, 1973). In our study, the simple regression analysis indicated that EW and StL were strongly correlated with LI and 76% of variation in LI was explained jointly by EW, StL and SD.

Higher levels of N result in higher lodging incidence in susceptible varieties (Jordan and Stinchcombe, 1986; Newton *et al.,* 1998). In *Trial 3* we used 90 kg ha^{-1} N was applied with the objective to increasing the lodging risk and to investigate the effectiveness of PGRs in controlling lodging. The results indicated that *Upgrade*, which had reduced StL by 34% and lodging by 75% than the control in *Trial 2*, caused only a 10% reduction in StL and 20% in lodging in *Trial 3*. This suggested that the stem shortening efficiency of the PGR was lower at the higher N-level which may have been reason why the PGR was less effective in reducing LI. However, differences in weather conditions during the two growing seasons and sowing date can affect StL and LI (Leitch and Hayes, 1989; Amir and Sinclair, 1994). A set of trials investigating the effect of sowing dates and seasons on lodging related traits and lodging incidence would be useful extension of the present study.

It is often reported that PGRs enhance GY by increasing EPSM (Ramos *et al*., 1989). In this research, whilst full dose and earlier application of PGRs at ZGS 31 increased EPSM, GY was not significantly enhanced. This was due

to a negative association between EPSM and GPE. The increase in EPSM decreased TGW resulting in non-significant effects of PGRs on GY. Although higher N-level increases GY (Pietola *et al.,* 1999), severe lodging can significantly reduce yield in susceptible varieties (Tripathi *et al.,* 2004). The results obtained from *Trial 3* revealed that GY between the PGR treated plots and the control was not different. This outcome suggested GY was not affected by the detrimental effect of lodging at the higher N-level (90 kg ha^{-1}). This may have been due to late lodging which occurred after crop had lost its green colour. It has been reported that lodging at the early milk stage can cause the greatest yield losses while lodging at the soft dough to hard dough stages has a negative effect on grain weight but a less severe effect on yield reduction (Jedel and Helm, 1991). However the duration between the lodging event and harvesting must not be overlooked. A long duration between pre-harvest lodging and harvesting operation due to wet conditions may result ear sprouting. In Orkney controlling pre-harvest lodging is very important because rain can delay the harvesting operation for several days which may result in severe yield and quality losses. The present study indicated that PGRs (*Cerone* and *Upgrade*) application always resulted in low LI. Although, in the absence of severe lodging or significant yield enhancement, the PGR may not justify its expenditure, it may facilitate easier harvesting operations.

One of the objectives of this paper was to identify suitable PGR and the optimum growth stage for its application on Bere. It was not possible to definitively identify and recommend a PGR suitable for all conditions from the results of the limited number of trials undertaken in this study. But taking into account the effects of ET (*Cerone*) and CCC (*Adjust)* on StL, LI and yield components, the most suitable choice seems to be the *Upgrade* which is a mixture of ET and CCC. The presence of CCC in a commercial formulation of *Upgrade* can antagonize the negative effect of ET on TGW and GY (Caldwell *et al.,* 1988). Also a combination of CCC and ET has been recommended for the varieties that are sensitive to brackling (buckling of middle internodes) (Sanvicente *et al.,* 1999) which could be beneficial to Bere. The results revealed that *Upgrade* consistently caused the highest reduction in StL and LI under the lower N-level (30 kg ha^{-1}) but its application at the higher N-level (90 kg ha^{-1}) was not so effective in lodging control. A set of trials investigating the effectiveness of this PGR under different fertility levels ranging from medium (60 kg ha^{-1}) to high (90 kg ha^{-1}) would help to determine its potential use.

V. CONCLUSION

The findings reported in this paper have implications for the use of PGR. Whilst PGR may be required to control lodging in Bere, its use may reduce the economic benefit and profitability unless the PGR increases yield. Since lodging was not severe in trials 1 and 2, it can be commented that Bere does not require PGR application under low N-level (30 kg ha^{-1}). Whilst at a higher N-level (90 kg ha^{-1}), the PGR,*Upgrade* improved the standing ability of Bere, had no effect on GY. This suggests that in the absence of severe lodging, the economic benefit of PGR is likely to be low. However, considering the susceptibility of Bere to lodging, PGR may be considered for yield protector rather than yield enhancer. It application may be recommended to avoid lodging-flat and to facilitate the harvesting operation. Further investigation on the effectiveness of *Upgrade* on lodging incidence and grain yield under a range of N-levels would assist in estimating the cost-benefit of integrating PGR in the production guidelines for growing Bere in Orkney.

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Effect of nitrogen, phosphorous, potassium, plant growth regulator and artificial lodging on grain yield and grain quality of a landrace of barley

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***Abstract*—** *Landraces of different crops are still preferred due to their stable yields under low inputs and adverse climatic conditions to which most modern varieties are not adapted. In the UK, a landrace of barley called Bere is currently grown in extreme climatic conditions of Orkney to which most of the modern varieties are not adapted. Although this landrace is probably the oldest barley under cultivation in the UK, very little research has been conducted. In this paper the effects of nitrogen, phosphorous, potassium, plant growth regulator and artificial lodging on grain yield and quality of Bere were investigated in the Orkney's short growing season. Higher nitrogen application resulted in a higher lodging incidence but grain yield was not reduced by the severity of lodging. The artificial lodging applied at Zadoks growth stage 77 resulted in the greatest yield losses which indicated that control measures may be required to avoid lodging at this critical growth stage. Phosphorous and potassium had no significant effect on lodging resistance. Whilst plant growth regulator improved lodging resistance it was less effective in controlling lodging at the highest nitrogen level (90 kg ha^{-1}). The trials indicated that higher level of N caused marginal increase in grain yield when nitrogen level was raised from 45 kg to 90 kg ha^{-1}. This tended to suggest the use of medium N-level (45 kg N ha^{-1}) for producing Bere. Plant growth regulator increased lodging resistance but had an inconsistent effect on grain yield. This study recommended the use of plant growth regulator as a means of easing harvesting rather than for enhancing yield and quality. The study concluded that phosphorous and potassium could be used to improve disease resistance and grain yield but not for lodging control.*

***Keywords*— Landrace, Bere, nitrogen level, plant growth regulator, artificial lodging.**

I. INTRODUCTION

Until the arrival of modern plant breeding in the 19th Century, landraces and old varieties significantly contributed to meeting the world food needs (Harlan, 1975). However, the development of high yielding modern varieties led to the virtual disappearance of landraces in the 20th Century in developed countries (Newton *et al.*, 2009). In several developing countries and a few peripheral areas in developed countries, landraces of different crops are still preferred due to their stable yields under low inputs and adverse climatic conditions to which most modern varieties are not adapted (Pswarayi *et al.*, 2008). In Orkney, Scotland, a landrace of barley, Bere, is cultivated on a very small scale for milling (Theobald *et al.*, 2006) and to supply a niche market for distilling (Martin and Chang, 2008; Martin, Wishart and Scott, 2013; Martin and Wishart, 2015). It has been reported that growing season in Orkney is short (4 to 5 months) (Bond and Hunter, 1987). The wetness of the climate coupled with low temperature can complicate early field preparation and sowing. Harvesting can become increasingly difficult through September as a result of increasing rainfall. Most of the modern varieties are not suitable to produce grain crop in the short growing season of Orkney. The best strategy would be planting of a short duration variety which can make rapid growth. Bere grows rapidly in spring and matures early (Martin and Chang, 2008) and this is probably one of the reasons why a few farmers still grow Bere in Orkney. However, farmers are generally concerned about its susceptibility to lodging due to its long and weak straw (Peachy, 1951; Martin *et al.*, 2008). Lodging is undesirable because of its detrimental effects on grain yield and quality (Pinthus, 1973; Stanca *et al.*, 1979; Birggs, 1990). Plant growth regulator (PGR) can reduce stem length and improve the standing ability of the barley (Kust, 1985; Sanvicente *et al.*, 1999) and wheat (Jung, 1964; Tripathi *et al.*, 2003). A few researches have

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used phosphorous (P) and potassium (K) for controlling lodging (Casserly, 1957; Arnold *et al.*, 1974). However, the effect of these treatments under a range of N-levels has not been investigated on Bere. There are also no published estimates of lodging related yield losses that could help to predict cost and benefits of integrating lodging control measures. It is difficult to accurately estimate yield and quality losses caused by lodging under natural conditions due to the unpredictable nature of lodging events (Kelbert *et al.*, 2004). Several researchers have used artificial lodging inducement as a mean to estimate yield and quality losses (Bauer, 1964; Stanca *et al.*1979). This paper reports the results obtained from two different trials. The first trial investigated the effects of a range of treatments on lodging related traits, yield components, grain yield and grain quality. In the second trial, yield and quality losses associated with lodging were estimated by inducing artificial lodging at two critical growth stages.

II. MATERIALS AND METHODS

Two separate trials were carried-out in 2009 and 2010 using standard seed rate (160 kg ha^{-1}) recommended by Martin *et al.* (2008) at an experimental site near Orkney College, Kirkwall, Orkney (Grid reference: HY 456 114). The soil of the experimental plot was classified as clay loam, with organic matter (3.9 %), NO_3-N (17.25 mg kg^{-1}) and NH_4^+ (0.96 mg kg^{-1}), P (28.2 mg kg^{-1}), K (70mg kg^{-1}) and acidic in nature (pH=5.5). Plots were planted using a Pneumatic Accord Combine Seed Drill. Weed control was achieved in all trials by applying a mixture of Mecoprop (1.5 l ha^{-1}) and 4-chloro-2-methylphenoxy acetic acid (MCPA) (1.0 l ha^{-1}) in 200 l of water by a tractor-mounted hydraulic nozzle sprayer (RAU-SPRiMAT L, Netherlands) having a 12 m boom length. A knapsack sprayer was used to apply the PGR (*Upgrade*) [{chlormequat chloride + chloroethylphosphonic acid}, Bayer CropScience, a.i 360:180 g l^{-1}] at Zadoks growth stage (ZGS) 31 in *Trial 1* and ZGS 37 in *Trial 2*. Ears m^{-2} (EPSM) was recorded by counting the number of fertile ears (ears with at least one fully filled grain) in a representative 50 cm x 50 cm quadrat in each plot. A representative sample of 20 stems was manually harvested from each treatment plot. A sub-sample (10 stems) was used to record stem length (StL) from the bottom of the stem to the base of ear as described by Schittenhelm and Hartmann (2006). The remaining stems were dissected into ear and stem and then dried separately at 80^0C for 72 hrs. Stem weight (SW) was used to calculate stem weight per cm (SWCM) by dividing SW by StL. Ears were weighed to record ear weight (EW) and then manually threshed to record grains ear^{-1} (GPE). A random sample of 10 stems was selected from each plot at ZGS 71 to assess the severity of disease. A scoring system developed by Large and Dolling (1962) was used to describe the severity of disease from 1 (disease not present) to 9 (dead leaves with no green area left) of the top four leaves. To record the onset of lodging all plots were visually monitored after every rainfall event. Final lodging assessments were made just before harvest. A frame marked with different angles was used to visualize the angle of deviation of stems from the vertical. The percentage area of the crop that was leaning at various angles was made. These observations were then converted into lodging index (LI) with slight modification to the formula developed by Berry *et al.* (2003) so that intermediate angles of 0-15, 15-30, 30-45, 45-60, 60-75, and 75-90 could be included.

$$\textit{Lodging Index} = \{1/6\ (\%\ \text{at}\ 0^0\text{-}15^0) + 2/6(\%\ \text{at}\ 15^0\text{-}30^0) + 3/6(\%\ \text{at}\ 30^0\text{-}45^0) + 4/6(\%\ \text{at}\ 45^0\text{-}60^0) + 5/6(\%\ \text{at}\ 60^0\text{-}75^0) + (\%\ \text{at}\ 75^0\text{-}90^0)\}.$$

The grain nitrogen content (GNC) was estimated on a grain sample of 300 g using Infratec Grain Analyzer (FOSS, Denmark). Grain yield (GY) was estimated by harvesting the plots either manually or by combine harvester. A sub-sample (100 g) of grain was drawn to measure grain moisture content (GMC). A Contador counter (Hoffman Manufacturing Inc, Germany) was used to count the grains required for 1000-grain weight (TGW). The GY and TGW were adjusted to 15 % GMC. Statistical analysis of the data was performed separately for each of the trials using Genstat 9.1. Means of treatments were compared using Fischer's protected least significant differences (LSD) at 5% level of probability. The relationships between yield, yield components, lodging and lodging related traits were investigated by regression analysis.

2.1 Trial 1

In this trial Bere was planted on 28th April 2009 in a strip-split plot design with 5 replications. There were 24 treatments resulting from the factorial combination of three levels of nitrogen (N), two levels of phosphorous (P) and potassium (K) and two levels of plant growth regulator (PGR) (Table 1). The N treatments were randomly allocated to 3 columns and the combination of P and K (P0K0, P0K45, P45K0, and P45K45) were assigned to four double rows. The fertilizer treatments were drilled along with the seed. The PGR treatments (GR0 and GR1) were randomly assigned to sub-plots. Sub-plot size was 3m x 6 m with a 6 m wide guard between replicates. Data were collected on stem length (StL), stem weight (SW), stem weight per cm (SWCM), lodging index (LI), disease score (DS), ears m^{-2} (EPSM), ear weight (EW), grains ear^{-1} (GPE), 1000-grain weight (TGW), grain yield (GY) and grain nitrogen content (GNC). GY and EPSM were determined by harvesting a 1m x 1m quadrat from each

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plot on 8th September 2009. Ears were counted and threshed manually to record GY.

2.2 *Trial 2*

This trial was sown on 27th April 2010 in a randomized block design with 4 replications. A basal fertilizer treatment i.e 45 kg ha^{-1} N and 30 kg ha^{-1} each of P and K was drilled along with the seed. There were six treatments resulting from the factorial combination of plant growth regulator (PGR) at two levels and lodging treatment (LT) at three levels (Table 2) which were randomly assigned to individual plot (3m x 12m). The artificial lodging (AL) treatments were applied by walking through the crop while rolling a barrel on the plot flatten the crop. This method was advantageous in that it required few resources in terms of cost, equipment, time and skills compared with other methods such as wind tunnel, airplane propeller or weighted plywood board used by other researchers Bauer (1964); Harrington and Waywell (1950); Stanca *et al.* (1979). LI was assessed immediately following application of artificial lodging treatment and again at harvest. Other data collected from all the plots were StL, LI, EPSM, GPE, GY, TGW and GNC. GY was estimated by combine harvesting of one strip 2.3 m wide and 12 m long on 11th September 2010.

III. RESULTS

3.1 Trial 1

StL was significantly affected by N ($P< 0.001$) and PGR ($P< 0.001$) while the effects of P and K were not significant. N increased the length of stem (28 % for N45 and 33 % for N90) compared with the N0 treatment. A decrease of 13% in StL (averaged over all treatments) resulted from the GR1 treatment when compared with GR0 (Table 3). There were no significant interactions amongst treatments (Tables 4 and 5). SW was significantly affected by N ($P< 0.001$) and PGR ($P< 0.001$) but not by P and K. The heaviest SW was obtained from the N45 treatment and the lowest from N0. The GR1 treatment resulted in 12 % reduction in the SW compared with GR0 (averaged over all other treatments) (Table 3).There were no significant interactions amongst the treatments for SW (Tables 4 and 5). SWCM was significantly ($P= 0.021$) affected by N but not by PGR, P and K (Table 3). The no-nitrogen plots (N0) had the highest SWCM, which declined as N-level increased (averaged over the other treatments). There was a significant ($P= 0.002$) PGR x N interaction because, without PGR, the SWCM was constant at all N-levels, where as when PGR was added, it decreased as N-level increased (Fig 1). There were no significant interactions amongst the remaining treatments (Tables 4 and 5). The LI assessments made on 1st September 2009 indicated significant effects of N ($P< 0.001$) and PGR ($P< 0.001$). However, there were no significant effects of P and K (Table 3). LI rose as the level of N increased. A significant ($P< 0.001$) PGR x N interaction was caused by the proportionately larger reductions in LI when GR1 was applied at N0 than at the other N-levels (Fig 2). There were no significant interactions amongst the remaining treatments for LI (Tables 4 and 5). There were significant effects of N ($P< 0.001$), P ($P= 0.041$) and K ($P= 0.008$) on DS while PGR had no significant effect (Table 3). The DS increased with the application of N and was higher in the N90 (6.6) and N45 (6.0) treatments than with N0 (5.7). Small but significant reductions in DS were obtained from the P45 and K45 treatments compared with P0 and K0 respectively. There were no significant interactions amongst the treatments for DS (Tables 4 and 5). N ($P< 0.001$), PGR ($P= 0.008$) and K ($P= 0.041$) had significant effects on EPSM while P had no significant effect (Table 3). EPSM increased from 296 (N0) to 412 (N90) with increasing N-level. This increase corresponded to 16% for N45 and 40% for N90 compared with N0. The K45 treatment increased EPSM by 4% compared with K0 while the GR1 treatment resulted in 5% higher EPSM (averaged over all treatments) compared with the GR0 treatment. There were no significant interactions amongst the treatments (Tables 4 and 5). EW was significantly affected by N ($P= 0.016$) and K ($P= 0.035$) but not by P and PGR. The highest EW was from the N45 treatment (1.25 g) compared with 1.13 g for the N0 and N90 treatments. The K45 treatment increased the EW by 5 % compared with the K0 treatment (Table 3). There were no significant interactions amongst the treatments (Tables 4 and 5). GPE was significantly ($P< 0.001$) affected by N but not by P, K and PGR. Averaged over all treatments, the N45 treatment increased the GPE by 17 % compared with the N0 treatment. However, doubling the N-level to 90 kg ha^{-1} caused a non-significant 3 % reduction in GPE compared with N45 (Table 3). There were no significant interactions amongst the treatments (Tables 4 and 5). TGW (g) was significantly ($P< 0.001$) affected by N but not by P, K and PGR. Averaged over all other treatments, values of TGW were 35.3, 33.4 and 30.9 g for N0, N45 and N90 respectively, indicating a decrease in TGW with increasing level of N (Table 3). There was a significant ($P= 0.020$) N x PGR interaction which resulted because application of GR1 at N90 decreased TGW while at N0 and N45, the GR1 treatment increased TGW (Fig 3). There was a significant (P= 0.039) interaction effect of N x P x K x PGR on TGW due to the different effect of PGR at different N-levels (Fig 4). The GR1 treatment had a significant negative effect on TGW at N90 with high levels of P and K but not at N0 or N45. There were no significant interactions amongst the remaining treatments

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(Table 4 and 5). GY (kg ha^{-1}) was significantly affected by N (*P< 0.001*), K (*P= 0.011*) and PGR (*P= 0.013*) while there was no significant effect of P. Averaged over all treatments, there was a 26 % increase in yield from the N45 and a 38% from the N90 treatment compared with N0. The application of K45 and GR1 treatment increased GY by 8% and 6% compared with K0 and GR0 treated plots respectively (Table 3). There were no significant interactions amongst the treatments (Table 4 and 5). GNC was significantly affected by N (*P< 0.001*) but not by P (*P= 0.834*), K or PGR. The mean values for GNC (averaged over P, K and PGR) were 1.62, 1.71 and 1.99 from the N0, N45 and N90 treatments respectively. An increase in GNC when GR1 was applied with K45 resulted in a significant (*P< 0.001*) interaction. There were no significant interactions amongst the remaining treatments (Tables 4 and 5). Simple linear correlation analysis between yield and its components for individual plot data from all the treatments revealed that EPSM accounted for the largest amount of variation in GY, while EW, GPE, and TGW had lower values for coefficient of determination (R^2) (Table 6). When these components were fitted against GY by stepwise multiple linear regression, the combination of EPSM and GPE or EW accounted for 73 % of the variations in GY. The inclusion of TGW in the regression model had very little impact on the coefficient of determination which was due to the negative association between TGW and EPSM (Fig 8). GNC was also negatively associated with TGW (Fig 9). Variation in StL accounted for 61 % of the variation in LI (Table 7). LI was also significantly (*P< 0.001*) associated with EPSM (R^2= 0.41), SW (R^2= 0.28) and SWCM (R^2= 0.08). However, there was no significant association between EW and LI (Table 7). A step wise inclusion of additional variables into the regression model improved the correlation and a maximum of 70 % of the variation in LI was explained by the combinations of (1) StL, SWCM and EPSM and (2) StW, SW and EPSM.

3.2 Trial 2

StL was significantly affected by PGR (*P< 0.001*) and LT (*P< 0.001*) (Table 8). The GR1 treatment resulted in 14 % shorter stems compared with GR0 (averaged over lodging treatments). Plots subjected to AL treatments produced shorter stems than from the AL0 treatment. The overall reductions in StL (averaged over PGR treatment) due to the AL59 and AL77 treatments were 18 % and 5 % respectively compared with AL0. There was a significant (*P< 0.001*) LT x PGR interaction which was due to StL not being affected by GR1 when AL59 had been applied (Table 9). There was a significant (*P= 0.023*) effect of LT on SWCM (mg cm^{-1}). The highest SWCM was recorded from the AL59 treatment (10.71 mg cm^{-1}) and the lowest from the AL77 (9.64 mg cm^{-1}) which was not significantly different from AL0 (9.79 mg cm^{-1}) (Table 8). The PGR treatment had no significant effect and the interaction of PGR x LT was also not significant (Table 9). LI was significantly affected by PGR (*P< 0.001*) and LT (*P< 0.001*). Averaged over LT, the GR1 treatment improved the standing ability of the crop compared with the GR0 treatment (Table 8). There was a considerable recovery following the AL59 treatment and LI of AL0 and AL59 were almost similar. However, AL77 was the most severe lodging treatment. There was a significant (*P< 0.001*) LT x PGR interaction because with AL77, the LI of the GR1 treatment was very similar to that of the GR0 treatment while with AL59 and AL0, the GR1 treatment always resulted in a significantly lower LI than GR0 (Table 9). Ear sprouting was noticed at harvest in the plots subjected to the AL77 treatment. There were no significant effects of PGR and LT on EPSM but GPE was significantly (*P= 0.011*) reduced by LT and the AL77 treatment caused the largest reductions (11 %) compared with AL0 (Table 8). There were no significant interactions amongst the treatments (Table 9). PGR and LT had no significant effect on TGW, GMC and GNC but GY was significantly affected by LT and the AL59 and AL77 treatment caused 9 % and 17 % reductions in GY respectively, compared with AL0 (averaged over PGR treatment) (Table 9).

IV. DISCUSSION

In cereals, several morphological characteristics such as StL (Pinthus, 1973), EW (Tripathi *et al.,* 2003) and SWCM (White, 1991) are related to lodging. The results in *Trial 1* showed that StL was the main characteristic strongly associated with lodging. Higher N-levels (N45 and N90) increased StL and the propensity of Bere to lodge compared with the untreated plots. Together with StL, EW is associated with lodging (Pinthus, 1967). When plant is displaced from its vertical position due to wind a second bending moment results from the force of gravity which rises with increase in StL and EW (Pinthus, 1973). This consequently increases lodging incidence. In the present research EW and StL increased when N-level was raised from N0 to N45 which consequently increased lodging incidence. Increasing N-level also increases tiller density (Lauer and Partridge, 1990) which results in the production of taller and weak stems which are susceptible to lodging (Crook and Ennos, 1995). This study obtained similar results and the reduction in strength, measured as SWCM, due to the higher levels of N may have been a consequence of higher EPSM. This resulted in higher LI in the N90 treated plots compared with N45 and the untreated control plots. PGR reduces lodging risk by decreasing StL (Tripathi *et al.,* 2003). In this research the PGR also reduced StL and resulted in a lower LI compared with the

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untreated control plots. But the PGR was less effective in controlling lodging at the highest N-level (N90). This outcome was partly due to changes in crop density in response to PGR and N. Higher application of N-level and PGR increases stems per plant and per unit area (Christensen and Killorn, 1981; Foster *et al.,* 1991; Ma and Smith, 1992). As stems per plant increases, the risk of root lodging increases (Baker *et al.,* 1998). This is attributed to the increased leverage on the anchorage system of the aerial parts of all the stems belonging to one plant (Berry *et al.,* 2007). In this study the plots which received combined treatment of N90 and GR1 had higher EPSM but weaker stems which consequently increased lodging risk and incidence. This partly explained why the PGR was less effective in lodging control at N90 than at N45 or N0. P and K have been reported to improve lodging resistance (Casserly, 1957). However in this trial the two elements had no effect on LI. This outcome was partly because the two elements increased EPSM which consequently reduced stem strength resulting in a higher LI than the plots which did not receive P and K. This tended to suggest that the two elements may not be ideal for lodging control which is in agreement with the findings of Mulder (1954) and Gasper *et al.* (1994) on cereals.

N application can increase disease incidence due to changes in crop density (Jordan and Stinchombe, 1988). One mechanism for this could be increased tillering which can result in favourable conditions for disease spread (Howard *et al.,* 1994). Results reported in *Trial 1* showed that DS rose with increased N-levels which was probably associated with increased EPSM. Increased DS may also be attributed to higher lodging incidence (Kono, 1995). PGR can reduce disease incidence by protecting the crop from lodging (Jordan and Stinchcombe, 1986). However, in this study, the PGR tended to increase DS (although statistically not significant). This outcome may have been due to increased tillering and short stems which favoured disease spread and development. Johnston and Macleod (1987) have proposed a similar explanation to this outcome for spring barley. P and K have the potential in lowering the DS (Mitchell and Walters, 2004; Amtmann *et al.,* 2008). In this study P addition tended to reduce DS which may have been the result of changes in plant metabolism reducing food supply to pathogens (Walters and Bingham, 2007). K application reduced the incidence of powdery mildew partly because the soil was deficient in K and its application may have allowed the crop to grow and defend itself against biotic and abiotic stresses better than the crop which did not receive K. The study concluded that the addition of P and K would be useful in improving resistance in Bere against powdery mildew.

It has been reported that when available N-levels in soil are low the relationship between GY and added N is linear (Kramer, 1979). In this study, there was a 26 % increase in GY when N-level was raised from N0 to N45. This increase was attributable to increased EPSM and high TGW. Doubling the N-level from N45 to N90 caused only a 12 % increase in GY. This limited increase was due to reduced TGW and decreased GPE probably caused by the negative association of EPSM with GPE and TGW (Foster *et al.,* 1991). Moreover, the higher LI from the highest N-level (90 kg ha^{-1}) may have reduced GPE and TGW (Jedel and Helm, 1991). P and K can have significant positive effect on GY (Gately, 1968). The results reported in this paper indicated that K had a significant effect on GY while the effects of P were not detected. This outcome was due to a difference in the availability of the two elements as the soil of the experimental site was deficient in K but contained sufficient in P. Perrenoud (1990) and Gately (1968) gave similar explanations for the non-significant effects of P and K respectively on GY. The results indicated that the combination of high levels of N, P and K produced a higher yield than when either was applied individually alone. This was partly due to the positive effect of N on EPSM and a higher TGW due to the application of P and K. PGR had a significant positive effect on GY in *Trial 1* but not in *Trial 2*. This may have resulted from differences in the timing of application of PGR. The earlier (ZGS 31) application of PGR in *Trial 1* significantly increased EPSM which resulted in high yields. This is in agreement with the findings of Tripathi *et al.* (2003) on several wheat varieties. The later application (ZGS 37) in *Trial 2* had no significant effect on EPSM and therefore the yield enhancing effects of PGR were not consistently observed. Higher levels of N result in higher lodging incidence in susceptible varieties (Jordan and Stinchcombe, 1986; Newton *et al.,* 1998) which negatively affect GY (Fischer and Stapper, 1987). In this study LI increased with N-level but GY was not lowered by its detrimental effects probably due to late occurrence of lodging. It has been reported that when lodging occurs at heading, grain number ear^{-1} and grain yield may be reduced (Day, 1957; Weibel and Pendleton, 1964; Briggs, 1990). Lodging at the early milk stage can cause the highest yield losses while lodging at the soft dough to hard dough stages has a negative effect on grain weight, but a less severe effect on yield reduction (Jedel and Helm, 1991). The low level yield losses from lodging at heading may be associated with the ability of crop to regain its vertical position (Stanca *et al.,* 1979). The results reported in *Trial 2* obtained similar results in which Bere was able to recover its erect position following the AL59 treatment. This recovery may have allowed the crop to photosynthesize normally which resulted in a lower yield depression compared with plots which were unable to recover from the AL77 treatment. The highest yield

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reduction (*ca* 20%) caused by AL77 treatment was attributed to grain germinating in the ear and the inability of the combine harvester to pick up lodged stems (personal observation). However, yield losses figure reported in this paper may not represent what would occur naturally because the barrel-method for inducing lodging may have caused stem breakage and stoppage of assimilates translocation towards ear. But the level of yield losses in this paper were either lower or comparable with the losses reported by Pinthus (1973) (34%), Stanca *et al.* (1979) (38%) and Briggs (1990) (21%) who also used artificial lodging techniques to estimate yield losses. Therefore, the predicted yield losses in this study were used for estimating cost and benefit of using a PGR for controlling lodging. Data of the present trial indicated that AL77 could have incurred a loss of £160 ha^{-1}. This estimate was based on the market price of Bere's grain in 2010 (£200 t^{-1}) and average GY (4.0 t ha^{-1}). The calculation indicated that an investment of £75 ha^{-1} to cover the cost of a PGR and its application may save up to £85 ha^{-1} farm income by protecting the crop from the lodging at ZGS 77. This figure may be over exaggerated because under natural conditions the type of damage caused by AL77 is unlikely to occur, and unless PGR results in significant yield enhancement it may not justify its cost of application.

Higher inputs of N to increase yield can reduce malting quality by affecting TGW and GNC (Widdowson *et al.*, 1982). Higher N-levels reduce TGW (Christensen and Killorn, 1981) and may raise GNC due to low carbohydrates accumulation (Lauer and Partridge, 1990), which is undesirable for malting (Grashoff and d'Antuono, 1997; Conry, 1997). GNC followed the increasing level of N in *Trial 1* which was also associated with decreased TGW. Moreover, high N-levels can result in increased translocation of N from vegetative parts to grains which raises GNC (Papakosta and Gagianas, 1991). P and K had no significant effect on GNC, an outcome consistent with the results of Gately (1968) for malting barley. The non-significant effect of the PGR treatment on GNC suggested that it could not be used to manipulate grain quality. Lodging can decrease TGW which may raise GNC (Caierao, 2006) due to low carbohydrates content in the grain (Grashoff and d'Antuono, 1997). However in this *Trial 2* there were no significant alterations in TGW and GNC in response to artificial lodging treatments. It is possible that the grain sample used for TGW and GNC determination may not have been representative of a lodged flat crop. Since combine harvester was unable to pick-up all lodged stems and sprouted ears, it is possible that the sample from the lodged flat plot did not contain poor quality grain. This consequently resulted in a non-significant effect of artificial lodging treatment on TGW and GNC.

V. CONCLUSION

Based on the results reported in this paper it can be concluded that Bere responds positively to N and K. However, the marginal increase in crop yield from N45 to N90 suggested the adoption of N45 to achieve reasonable yield (*ca* 4.0 t ha^{-1}) and acceptable grain quality (GNC< 1.80 %). The results indicated that an adequate supply of P and K may be required to improve disease resistance and GY; however, these nutrients did not contribute to control lodging. PGR resulted in low LI but did not result in higher yield. Although, in the absence of severe lodging or significant yield enhancement, the PGR application may not justify its expenditure, it may facilitate easier harvesting operations. In Orkney controlling pre-harvest lodging is very important because rain can delay the harvesting operation for several days which may result in severe yield and quality losses. Considering the susceptibility of Bere to lodging, PGR may not be applied for enhancing yield and quality but to avoid late-season lodging. Its inclusion in the production guidelines is recommended to avoid pre-harvest ear sprouting and to ease harvesting operation.

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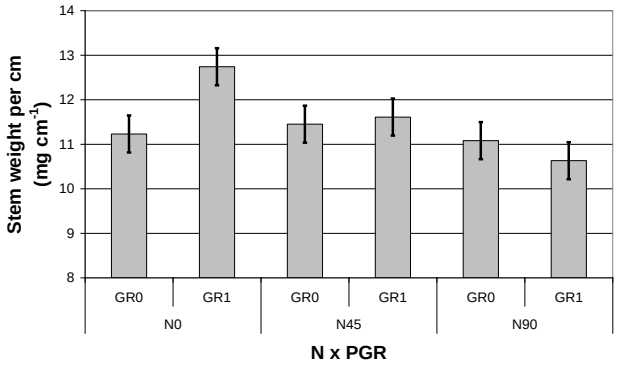

Fig.1: Effect of N x PGR interaction on SWCM
Bars on columns represent standard error of differences of means

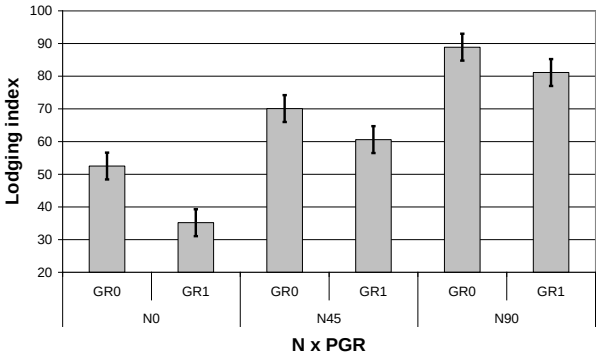

Fig.2: Effect of N x PGR interaction on lodging index
Bars on columns represent standard error of differences of means

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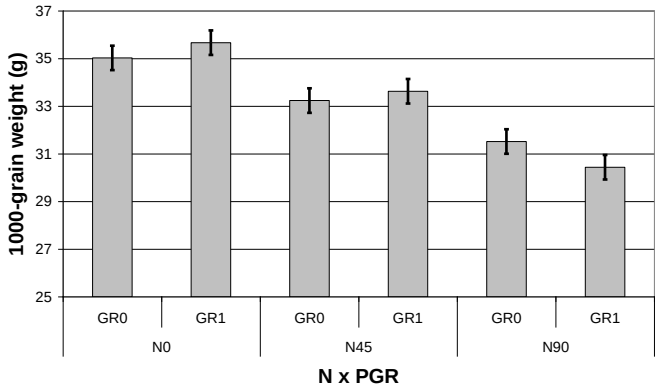

Fig.3: Effect of N x PGR on 1000-grain weight
Bars on columns represent standard error of differences of means

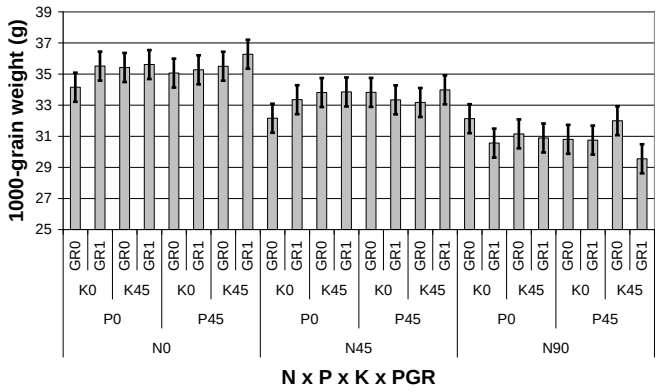

Fig.4: 1000-grain weight as affected by the interaction of N x P x K x PGR
Bars on columns represent standard error of differences of means

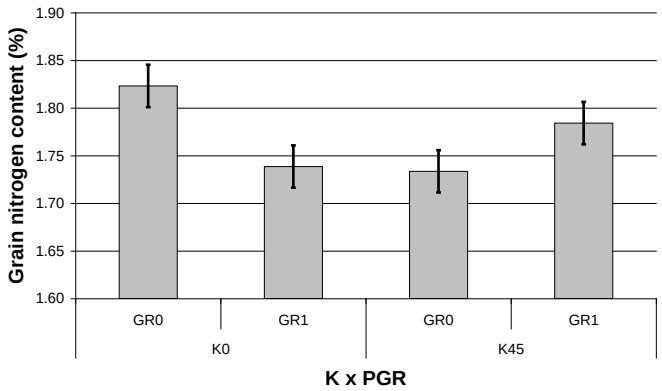

Fig.5: Grain nitrogen content as affected by the interaction of PGR x K
Bars on columns represent standard error of differences of means

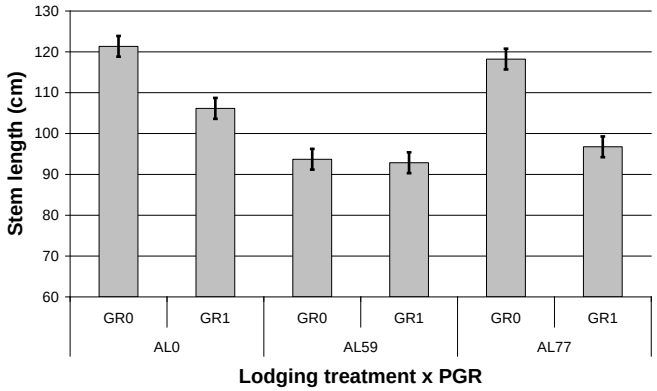

Fig.6: Stem length as affected by interaction of lodging treatment x PGR
Bars on columns represent standard error of differences of means

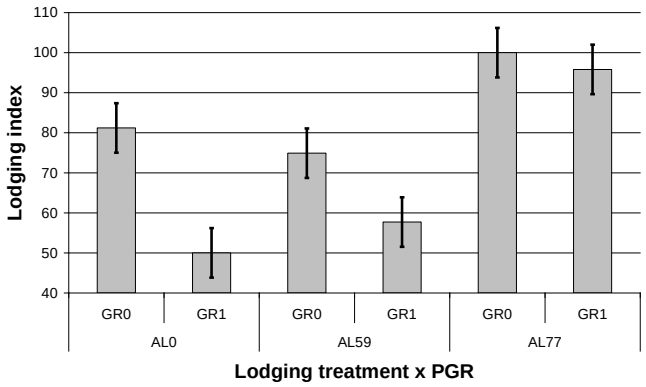

Fig.7: Lodging treatment x PGR effect on lodging index
Bars on columns represent standard error of differences of means

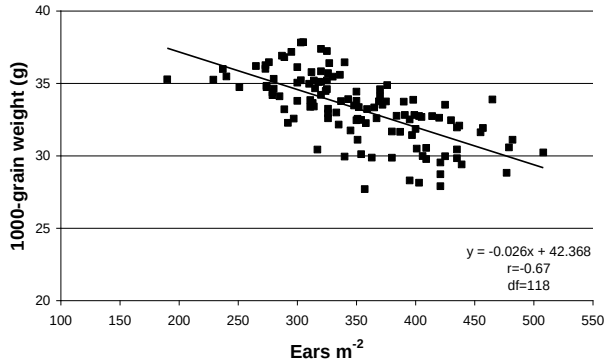

Fig.8: Correlation between 1000-grain weight and ears m-2

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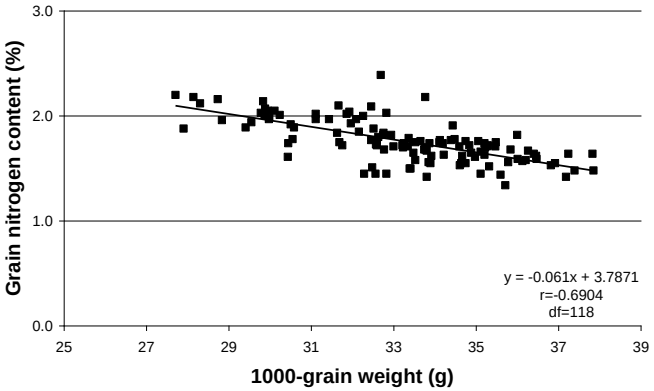

Fig.9: Correlation between grain nitrogen content and 1000-grain weight

Table.1: List of treatments and their abbreviations

Treatment	Level	Abbreviation
Nitrogen (N)	0 kg ha^{-1}	N0
	45 kg ha^{-1}	N45
	90 kg ha^{-1}	N90
Phosphorous (P)	0 kg ha^{-1}	P0
	45 kg ha^{-1}	P45
Potassium (K)	0 kg ha^{-1}	K0
	45 kg ha^{-1}	K45
Plant growth regulator (PGR)	No-PGR	GR0
	PGR at ZGS 31	GR1

Table.2: List of treatments, their abbreviations and application dates

Treatment	**Abbreviation**	**Date of application**
PGR treatment		
No-PGR	GR0	-
PGR (Upgrade) (2 l ha^{-1}) at ZGS 37	GR1	21 June 2010
Lodging treatment		
No AL (Natural lodging)	AL0	-
AL at fully emerged ear stage	AL59	4th July 2010
AL at milk stage	AL77	21st July 2010

Table.3: Main effect of N, P, K and PGR on lodging, yield and quality related traits of Bere in 2009

Treatment	StL (cm)	SW (g)	SWCM (mg)	LI	DS	EPSM	EW (g)	GPE	TGW (g)	GY (Kg ha^{-1})	GNC (%)
N0	86.9	1.04	11.98	43.8	5.7	296.4	1.13	32.77	35.35	3060	1.6218
N45	111.2	1.3	11.53	65.3	6.0	344.0	1.25	38.23	33.44	3860	1.7007
N90	115.3	1.26	10.85	85.0	6.6	412.1	1.13	37.14	30.98	4297	1.9867
F-Probability	<0.001	<0.001	0.021	<0.001	<0.001	<0.001	0.016	<0.001	<0.001	<0.001	<0.001
LSD (5%)	4.6	0.10	0.73	8.19	0.36	31.6	0.08	2.05	0.93	284.4	0.14
P0	104.6	1.21	11.6	66.7	6.2	349.4	1.18	36.3	33.22	3715	1.7682
P45	104.6	1.17	11.31	63.8	6.1	352.4	1.17	36.86	33.29	3763	1.7713
F-Probability	NS	NS	NS	NS	0.041	NS	NS	NS	NS	NS	NS
LSD (5%)	3.3	0.08	0.65	5.41	0.12	12.4	0.06	1.71	0.59	200.1	0.05
K0	103.5	1.17	11.35	64.7	6.2	344.4	1.14	35.24	33.08	3599	1.778

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K45	105.7	1.22	11.56	64.7	6.0	357.4	1.20	36.86	33.43	3879	1.7615
F-Probability	NS	NS	NS	NS	0.008	0.041	0.035	NS	0.011	0.207	NS
LSD (5%)	3.3	0.08	0.65	5.41	0.12	12.4	0.06	1.71	0.59	200.1	0.05
GR0	112.3	1.79	11.25	70.9	6.1	342.8	1.18	36.3	33.26	3633	1.7805
GR1	96.8	1.72	11.66	58.9	6.1	357.4	1.16	35.79	33.25	3845	1.759
F-Probability	<0.001	<0.001	NS	<0.001	NS	0.008	NS	NS	0.014	NS	NS
LSD (5%)	2.9	0.06	0.45	3.39	0.14	11.7	0.05	1.32	0.52	168.1	0.04

NS: Not significant (*P> 0.05*)

Table.4: Interaction effects of N, P and K on lodging, yield and quality related traits of Bere in 2009

Treatment			StL (cm)	SW (g)	SWCM (mg)	LI	DS	EPSM	EW (g)	GPE	TGW (g)	GY (Kg ha^{-1})	GNC (%)
N0	P0	K0	87.81	0.99	11.41	42.42	5.55	291.0	1.14	33.47	34.83	2842	1.62
		K45	87.20	1.07	12.24	45.83	5.77	311.3	1.10	31.66	35.52	3158	1.63
	P45	K0	92.96	0.96	11.67	39.17	5.82	284.1	1.10	31.94	35.16	2966	1.62
		K45	89.71	1.13	12.62	47.92	5.66	299.3	1.21	34.03	35.89	3272	1.62
N45	P0	K0	108.54	1.34	12.34	66.92	6.23	337.9	1.21	37.91	32.75	3714	1.73
		K45	114.11	1.26	11.06	67.75	5.94	347.5	1.32	39.90	33.83	4049	1.66
	P45	K0	111.17	1.23	11.08	67.33	6.04	348.6	1.21	36.68	33.58	3851	1.71
		K45	112.20	1.31	11.62	59.33	5.87	342.1	1.26	38.43	33.57	3826	1.71
N90	P0	K0	116.29	1.31	11.24	90.75	6.95	389.4	1.06	34.38	31.35	4057	2.0
		K45	113.73	1.29	11.29	80.25	6.64	419.0	1.23	40.51	31.02	4468	1.97
	P45	K0	113.99	1.19	10.37	81.83	6.63	415.2	1.13	37.06	30.78	4162	1.99
		K45	117.36	1.23	10.51	87.17	6.34	424.9	1.10	36.61	30.77	4503	1.98
F-Probability			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LSD (5%)			7.887	0.173	1.335	12.521	0.382	39.16	0.146	2.427	1.377	469.8	0.149

NS: Not significant (*P> 0.05*)

Table.5: Interaction effects of N, P, K and PGR treatment on lodging, yield and yield related characteristics of Bere in 2009

Treatment				StL (cm)	SW (g)	SWCM (mg)	LI	DS	EPSM	EW (g)	GPE	TGW (g)	GY (Kg ha^{-1})	GNC (%)
N0	P0	K0	GR0	98.20	1.05	10.67	53.50	5.705	279.2	1.13	33.99	34.15	2648	1.66
			GR1	77.42	0.94	12.15	31.33	5.395	302.8	1.14	32.94	35.51	3036	1.59
		K45	GR0	93.52	1.12	11.95	51.17	5.884	326.0	1.08	31.28	35.42	3202	1.62
			GR1	80.89	1.01	12.53	40.50	5.655	296.6	1.12	32.04	35.61	3115	1.63
	P45	K0	GR0	93.97	0.98	10.30	48.33	5.735	279.2	1.09	31.66	35.06	2937	1.68
			GR1	71.95	0.941	13.04	30.00	5.902	289.0	1.12	32.22	35.27	2996	1.56
		K45	GR0	101.86	1.22	11.98	57.00	5.435	285.4	1.17	33.60	35.50	3040	1.60
			GR1	77.55	1.03	13.25	38.83	5.890	313.2	1.23	34.46	36.27	3504	1.63
N45	P0	K0	GR0	112.65	1.35	12.03	71.00	6.300	334.8	1.25	39.70	32.16	3611	1.83
			GR1	104.42	1.32	12.66	62.83	6.255	341.0	1.18	36.12	33.35	3818	1.63
		K45	GR0	121.09	1.33	10.99	70.67	5.930	340.6	1.32	39.62	33.81	4151	1.64
			GR1	107.12	1.19	11.13	64.83	5.955	354.4	1.33	40.18	33.85	3948	1.68

			GR0	116.92	1.30	11.13	67.50	6.000	327.8	1.16	35.12	33.82	3577	1.77
		K0	GR1	105.42	1.16	11.04	67.17	6.085	369.4	1.25	38.24	33.34	4124	1.64
	P45		GR0	121.04	1.41	11.64	71.17	5.850	333.6	1.23	38.16	33.17	3550	1.66
		K45	GR1	103.35	1.20	11.60	47.50	5.895	350.6	1.29	38.70	33.98	4101	1.75
			GR0	121.68	1.45	11.92	94.83	7.135	374.6	1.15	36.38	32.13	3976	2.02
		K0	GR1	110.90	1.17	10.56	86.67	6.770	404.2	0.97	32.38	30.56	4137	1.98
	P0		GR0	121.02	1.37	11.30	81.00	6.560	409.4	1.23	40.54	31.15	4292	2.00
		K45	GR1	106.44	1.21	11.29	79.50	6.720	428.6	1.22	40.48	30.89	4600	1.95
N90			GR0	122.29	1.30	10.68	89.17	6.682	416.8	1.13	36.76	30.80	4203	1.98
		K0	GR1	105.68	1.07	10.07	74.50	6.585	413.6	1.13	37.36	30.75	4121	2.00
	P45		GR0	124.36	1.29	10.42	90.50	6.160	406.6	1.21	38.80	32.00	4405	1.90
		K45	GR1	110.36	1.17	10.60	83.83	6.530	443.2	1.01	34.42	29.55	4644	2.06
F-Probability				NS	NS	NS	NS	NS	NS	NS	NS	0.039	NS	NS
LSD (5%)				10.44	0.239	1.700	14.830	0.5608	616.7	0.195	5.169	1.85	616.7	0.166

NS: Not significant (*P> 0.05*)

Table.6: Values of co-efficient of determination (R^2) and probability (P) and degrees of freedom (df) for linear regression of yield and its components

Traits	No. of independent variables	R^2	Probability (P) Df
Ears m^{-2} (EPSM)	1	0.6565	<0.001,df=118
Grains ear^{-1} (GPE)	1	0.2351	<0.001,df=118
Ear weight (EW)	1	0.2431	<0.001,df=118
1000-grain weight (TGW)	1	0.1950	<0.001,df=118
EPSM,GPE	2	0.7291	<0.001,df=117
EPSM,TGW	2	0.6760	<0.001,df=117
EPSM, EW	2	0.7311	<0.001,df=117
EPSM,TGW,GPE	3	0.7422	<0.001,df=116

NS: Not significant (*P> 0.05*)

Table.7: Values of co-efficient of determination (R^2), probability (P) and degrees of freedom (df) for linear regression of LI and lodging related characteristics

Traits	No. of independent variables	R^2	Probability (P)
Ear weight (EW)	1	0.001	NS, df=118
Stem length (StL)	1	0.6059	<0.001,df=118
Stem weight (SW)	1	0.2794	<0.001,df=118
Stem weight per cm (SWCM)	1	0.0816	<0.001,df=118
Ears m^{-2} (EPSM)	1	0.4101	<0.001,df=118
EW, StL	2	0.6242	<0.001,df=117
EW,SW	2	0.3084	<0.001,df=117
EW,SWCM	2	0.0851	<0.005,df=117
EW,EPSM	2	0.4111	<0.001,df=117

StL,SW	2	0.6206	<0.001,df=117
StL,SWCM	2	0.6203	<0.001,df=117
StL,EPSM	2	0.6894	<0.001,df=117
SWCM,EPSM	2	0.4433	<0.001,df=117
StL,SW,EPSM	3	0.6985	<0.001,df=116
StL,SWCM,EPSM	3	0.6996	<0.001,df=116

NS: Not significant (*P> 0.05*)

Table.8: Main effect of PGR and LT on lodging, grain yield and quality related traits of Bere in 2010.

Treatment	StL (cm)	SWCM (mg cm^{-1})	LI (%)	EPSM	GPE	GY (kg ha^{-1})	TGW (g)	GMC (%)	GNC (%)
GR0	111.1	10.3	85.4	390.0	43.7	3834	32.2	19.2	1.83
GR1	98.6	9.8	67.8	407.3	43.2	3862	31.8	19.0	1.81
F-Probability	<0.001	NS	<0.001	NS	NS	NS	NS	NS	NS
LSD (5%)	3.1	0.64	7.6	41.1	2.3	274.5	1.5	0.53	0.04
AL0	113.7	9.8	65.6	419.0	46.1	4220	31.6	19.3	1.82
AL59	93.3	10.7	66.3	396.0	42.6	3831	32.9	18.9	1.80
LT77	107.5	9.6	97.9	381.0	41.6	3493	31.5	19.1	1.85
F-Probability	<0.001	0.023	<0.001	NS	0.011	336.2	NS	NS	NS
LSD (5%)	3.8	0.79	9.3	50.3	2.8	475.4	1.8	0.65	0.06

NS: Not significant (*P> 0.05*)

Table.9: Interaction effect of PGR x LT on lodging, grain yield and quality related traits of Bere in 2010.

Treatment		StL (cm)	SWCM (mg cm^{-1})	LI (%)	EPSM	GPE	GY (kg ha^{-1})	TGW (g)	GMC (%)	GNC (%)
GR0	AL0	121.3	9.71	81.2	391.0	46.23	4259	31.96	19.58	1.85
	AL59	93.7	11.26	74.9	399.0	42.13	3794	33.03	18.93	1.81
	LT77	118.2	9.95	100	380.0	42.82	3450	31.74	18.98	1.85
GR1	AL0	106.2	9.87	50	447.0	45.98	4182	31.31	18.95	1.79
	AL59	92.8	10.17	57.7	393.0	43.10	3868	32.94	18.97	1.80
	AL77	96.7	9.33	95.8	382.0	40.48	3536	31.25	19.21	1.85
F-Probability		<0.001	NS	0.025	NS	NS	NS	NS	NS	NS
LSD (5%)		5.4	1.1	13.2	71.1	4.1	475.4	2.7	0.92	0.08

NS: Not significant (*P> 0.05*)

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Characteristics of Nutraceutical Yoghurt Mousse Fortified with Chia Seeds

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Abstract**—** *Fortification yoghurt mousse with Chia seeds as a novel neutraceutical dairy product was studied. Chia seeds were added with the ratios (1,2and 3% to yoghurt mousse and compared with yoghurt mousse with 1.25% gelatin as control. The physicochemical and functional properties for Yoghurt mousse were evaluated. Fortification of 3% chia seed can be recommended for production novel dairy products with high neutraceutical properties and high acceptable sensory properties. Evaluation the preventive role of chia on lipids in normal and isoproterenol (ISO)-induced myocardial infarction was studied in rats. Chia at two doses (3% as one serving and 6% as double serving concentrated ratio in yoghurt mousse) was orally administered to rats for a period of 28 days. Isoproterenol (5 mg/kg) was injected intrapreitoneal to male wistar rats at last 7 days. ISO-treated rats also showed a significant increase ($p < 0.001$) in the levels of triglycerides, and very low-density lipoprotein cholesterol (VLDL-C) level in plasma with subsequent decrease ($P<0.01$) in the level of HDL as compared to yogurt-administered rats. Pretreatment with (43mg/kg BW) chia to ISO-treated rats showed a significant decrease ($p < 0.05$) in the levels of triglycerides, and very low-density lipoprotein cholesterol (VLDL-C) level in plasma as compared to ISO-induced rats. While Pretreatment with (86 mg/kgB.W) chia showed a significant increase ($p < 0.01$) in High -density lipoprotein cholesterol (HDL-C) level. The results of the present study indicated that the overall cardioprotective effect of chia seeds is probably related to its ability to inhibit lipid accumulation by its hypolipidaemic property.*

Keywords— Chia- Nutraceutical – lipid profile- Yoghurt Mousse - Physicochemical and Functional properties.- Cardio protective effect.

I. INTRODUCTION

The concept of "nutraceutical" was introduced by Stephen DeFelice in 1989, by combining the terms "nutrition" and "pharmaceutical". The term refers to raw foods, fortified foods or dietary supplements containing biologically active molecules, also known as bioactive molecules Palthur et al., (2010). That provide health benefits beyond basic nutrition Liu (2003). These bioactive compounds include certain polysaccharides, peptides, phytochemicals, vitamins, and fatty acids that are naturally present in foods. Since fermented milk products are among highly-consumed food in the world, they have been used to deliver nutritional components into human diet. Furthermore, fortification of these products such as yogurt, is a good way to improve nutrient intake in daily food products Preedy et al., (2013). Seeds from Salvia hispanica L. or more commonly known as chia is a traditional food in central and southern America.

Currently, it is widely consumed for various health benefits especially in maintaining healthy serum lipid level. This effect is contributed by the presence of phenolic acid and omega 3/6 oil in the chia seed. Chia seeds, contain the richest botanical oil source of ALA (Alfa Linolinic Acid) and high amounts of fiber and minerals. Poudyal *et al.,* (2013) have recently shown that the administration of chia oil improved heart left ventricular dimensions, contractility, volume and stiffness as well as hypertension, glucose tolerance and insulin sensitivity in rats fed a high fat-high fructose diet. Therefore, Chia is considered an important nutraceutical product and one of the most efficient polyunsaturated fatty acid (PUFA) sources for enriching foods and producing functional foods and gluten-free products, such as bread and baked products. Ayerza and Coates (2001), Martnez and Paredes (2014).

Hydrocolloids or food gums are widely used in different applications in the food industry due to their ability to retain water. Chia seeds are strongly hydrophilc, capable of absorbing several times their weight in liquids such as water Vazquez *et al.,* (2009), this hydration capability is due to the structure of the outer seed coat. Chia seed can be utilized as a functional coating with improved functional properties Munoz *et al., (2012).* The approval of chia seed as a Novel Food by the European Parliament has led to high degree of usage of chia seed in a wide range of foods. It is already well established that chia does not have anti-allergic, anti-nutritional and toxic effect on human health. Biscuits, pasta, cereal bars, snacks and yoghurt and cake are usually supplemented with chia seed. Borneo et al. 2010). Chia is consider as one of the few medicinal plants that produce essential oil

in a great concentration, which is used for the preparation of omega-3 capsules.

Probiotic dairy products such as dahi, yoghurt, ice cream, cheese, and kefir are appropriate vehicles to deliver beneficial bacteria to human host in addition to the available medical health supplements either in the form of pills or capsules Prajapati and Nair (2003); Shah and Prajapati (2013). The probiotics market has been one of the prime beneficiaries in the recent fad for functional foods. The global probiotic products market was estimated at $26 125.9 million in 2012. According to one survey probiotic market have risen up to worth $1732.8 million by 2019, incorporating probiotics in different kinds of food products (functional foods, dietary supplements, specialty nutrients, animal feed); in medicinal relevance regular, therapeutic, preventive health care; or by any other convenient mode of application. Anonymous (2014).

It is revealed from studies that chia seeds beside their neutracutical effect, These seeds had possess many important functional properties(water-holding capacity, oil holding capacity, solubility, viscosity, emulsion stability and foaming stability) which prove its potential to be used as a thickening agent, gel forming agent, chelator, foam enhancer, emulsifying agent, clarifying agent, rehydrating agent and as suspension formers in the formulation of food products at both home and commercial level. All these properties make chia a promising functional food for the future. Chia seeds offers a great future perspective for feed, food, medical, pharmaceutical and neutraceutical sectors. From this point of view the objectives of this study were to develop Yogurt mousse as a novel, high added-value dairy product fortified with chia seed *(Salvia hispanica L)* and study the changes in the physiochemical composition, microbiological, and functional properties as cardioprotective effect and its ability to inhibit lipid accumulation by its hypolipidaemic property.

II. MATERIALS AND METHODS

1. Materials:

Chia seeds *(Salvia hispanica L.*) in the form of packed whole seed was obtained from Bob's Red Mill Natural Foods Inc. Milwaukie, OR97222U.S.A. Commercial lyophilized mixed yoghurt starter culture (YCX11) was obtained from Chr. Hansen's laboratories, Copenhagen, Denmark. Buffaloes' milk was used for manufacture of yoghurt Mousse. The milk was obtained from Animal Production Research Institute, Ministry of Agriculture. Cream 30% fat were prepared. Gelatin Dr.Oetker (UK) Ltd., Sugar and vanilla were obtained from local market. All common chemicals used for experimental animals were purchased from one of the following suppliers Sigma Co. (St. Loius, MO, USA).All other reagents were of the highest grade commercially available. (All chemicals used in the study were of analytical grade).

2. Preparation of Yoghurt Mousse:

Yoghurt Mousse was prepared according to Menendez et al., (2006). 75 gram buffaloe' milk 3% fat and 15 gram Cream milk (30% fat content) were pasteurized and inoculated with 3% w/w of yogurt culture at 42ºC then incubated to 4 h . The mixture was cooled to 6o C and left to stand for 12h. It was then whipped with 10 gm of sugar in the presence of 1.25g (1.1% w/w) of powdered gelatin previously prepared in 15ml of water at 80º C. The product was finally packed in yoghurt cartons and stored at 6ºC.

The same assay was repeated adding 1, 2 and 3% g from chia seeds instead of gelatin. Four treatments of yoghurt mousse were as follows:

1- Yoghurt mousse with 1.25 % gelatin. (control)
2- Yoghurt mousse with 1% Chia seeds
3- Yoghurt mousse with 2% % Chia seeds
4- Yoghurt mousse with 3% % Chia seeds

3. Analytical Methods for Yoghurt Mousse

3.1. Methods of chemical analysis

Chia seeds were determined by AOAC (2005) contents, total standard methods for total solid, fiber, fat, protein, and ash. Carbohydrate contents were calculated by difference. The iron, zinc, Phosphorus and calcium contents of Chia seeds by the atomic absorption method by the HPLC method (AOAC 2012). The Yoghurt Mousse Chia Seeds fortified formulations were analyzed for total solids (TS), fat, crude protein and carbohydrates were calculated by difference (AOAC 2012). The pH values of the fermented products from the different treatments were measured using a digital pH meter (Model HI9321) (Hanna, Germany). Fatty acids composition were determined according to AOAC (2012).

3.2. Rheological and syneresis analysis

Water-holding capacity of yoghurt was determined using a procedure by Guzmane *et al.*, (1999). 20g of yoghurt (Y) was centrifugated for 30 min at 1250×g at 20°C. (h = 4.8 cm). The whey expelled (WE) was removed and weighed. The water-holding capacity (WHC) was determined as:

$$WHC = \frac{100\times(Y\ WE)}{Y}$$

- The viscosity was measured using a Brookfield DV digital viscometer (Brookfield Engineering, Middleborough, MA, USA) with spindle No 92 at 50 rpm.
- Assessment syneresis, the whole yoghurt samples (50g) were transferred to a stainless steel sieve, 120

mesh and left in a 4° C to drain in graduated cylinder. The total whey drained after 2 hrs, was measured Williams, *et al.*, (2004).

The following formula was used to calculate syneresis.

$$\text{Synersis} = \frac{\%\ V1 \times 100}{V2}$$

Where: V1 = volume of whey after draining
V2= volume of yoghurt sample

3.3. Microbiological analysis

Total bacterial count, Lactic acid bacteria ,Total coliform count, yeast and molds, Spore form, *Staphylococcus aureus* and *Bacillus cerues* were determine according Marshall (1993).

3.4. Sensory analysis

Yoghurt Mousse sample were evaluated by experienced members of the staff of the dairy department, APRI Egypt after 1,4and 8 days of cold storage at 6°C. The samples were scored as 60, 30 and 10 for flavor, body& Texture and Colors & appearance.

Experimental Animals

Adult male Wistar rats, weighing (150±20) g. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in plastic cages with good aerated covers at 25°C ± 0.5°C as well as 12 h light/dark cycles. Animals were allowed free access to water and were supplied daily with a standard diet.

Experimental design

Forty two male Wistar rats weighing (140–160) g were randomly allocated into seven groups having **sex in each as follows:**

- Group I (Control): Rats received only distilled water for 28 days.
- Group II (ISO + low dose chia): Rats received yogurt by oral gavage for 28 days.
- Group III (low dose chia): Rats received chia seeds (3%) by oral gavage for 28 days.
- Group IV (high dose chia): Rats received chia seeds (6%) by oral gavage for 28 days.
- Group VI (ISO): Rats received daily ISO (5 mg/kg, ip) for the last 7 days.
- Group VII (ISO + low dose chia): Rats received chia (3%) by oral gavage for 28 days and ISO (5 mg/kg BW, ip) for the last 7 days.
- Group VIII (ISO + high dose chia): Rats received chia (6%) by oral gavage for 28 days and ISO (5 mg/kg BW, ip) for the last 7 days. At the end of the experiment, animals were sacrificed and blood samples were collected and centrifuged to separate plasma. Plasma were then kept at -80°C for subsequent biochemical assays.

Blood Samples preparation

Blood was collected from sacrificed rat in vacuontainer and centrifuged 3000g for 10 min. Plasma samples were collected and stored at -80 until.

Determination of lipid profile and cardiovascular risk indices

Plasma total cholesterol, triglycerides, and high-density lipoprotein (HDL)-cholesterol were assayed using commercial diagnostic kits (spectrum diagnostics Egyptian company of biotechnology, Cairo, Egypt). Very low-density lipoprotein (VLDL)-cholesterol concentration was calculated according to the following formula:

VLDL-cholesterol = triglycerides/5.

Cardiovascular risk indices were calculated according to Ross (1992) as follows:

Cardiovascular risk index= total cholesterol/HDL-cholesterol.

Statistical analysis

Statistical evaluation was conducted with Instat Program GraphPad. Software, Inc, San Digeo, USA, version3. 6, Copyright©1992-2003 Results were expressed as mean ± S.E. The results were analyzed for statistical significance by one way ANOVA followed by Tukey-Kramer & Duncan's multiple comparison post-test. Values of $p < 0.05$ were regarded as significant.

III. RESULTS AND DISCUSSION

The Chia seeds used in this study had the following nutrient composition as shown in Table (1) Dietary fiber recorded 24.60 g /100 g, which higher than previously reported by Tosco (2004). The fat content, protein and ash were 33.16 , 21.34 and ash 4.6 g/100 g respectively. These results are in accordance with that reported by Michele and Myriam (2014)

Chemical analysis of yoghurt mousse fortified with Chia seeds

Table (2) shows Chemical analysis of yoghurt mousse fortified with chia seeds at the ratio of (1, 2 and 3%). The total solids (TS) content increased with the increase in the percentage (w/w) of Chia seeds. Significantly increase could be observed in fat, protein and fiber due to the high content of these nutrient components in Chia seeds. These results are in accordance with those reported by Safaa (2017), who found the total solids were increased in soft cheese and its formula by the increasing of chia flour ratios. Protein content of chia seeds is greater than protein content of all the cereals. The absence of gluten in chia is another unique feature of chia that it can be digested by the patients suffering from celiac disease. Rahman *et al.*,(2015).

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Chia seed contains between 34 and 40 g of dietary fiber per 100 g, equivalent to 100 % of the daily recommendations for the adult population Mohd *et al.*, (2012). Fortifying yogurt mousse with Chia seeds increase the fiber content as chia seeds ratio increased. Ratio of 3% recorded 1.26% fiber content in the end product which provide nearly 8.5 % from the fiber daily recommended for one serve . Dietary fiber is one of the important components of healthy diet. Intake of adequate amount of dietary fiber is associated with the prevention of cardiovascular diseases like stroke, myocardial infarction, vascular diseases, obesity, hypertension, hyperglycemia, and hyperlipidemia. Dietary fibers also cannot be digested and absorbed by the small intestine but get fermented in the large intestine. The total dietary fiber content of chia seeds ranges is much higher than that present in several grains, vegetables and fruits such as corns, carrot, spinach, banana, pear, apple, kiwi . Reyes *et al.,* (2008), Ovando *et al.,* (2009). The insoluble dietary fiber of chia is capable of retaining water several times of its weight during hydration and thus provides bulk and prolongs the gastro-intestinal transit time. Increased gastro-intestinal time is directly related to gradual increase in post-prandial blood glucose levels and decrease in insulin resistance over a period of time. Munoz *et al.,* (2012). Edwards and Garcia (2009) explained the health effects of food hydrocolloids which dependent on how they are incorporated into foods and in the diet. There are many hydrocolloid carbohydrates naturally present in plant foods as part of the cell wall, such as hemicelluloses and pectin, with all these features, chia seeds can be used as emulsifiers and stabilizers due to their high fiber content, and as an ingredient for products gluten-free, and with low carbohydrate.

Obtained results in Table (2) also indicate that there were significantly decrease in pH values for Yogurt mousse fortified with 1, 2and 3% chia seeds respectively than control treatment .This is agreement with Tamime and Robisons (2007) who mentioned that The concentration of lactic acid in milk during fermentation increases .

The minerals content of yoghurt mousse

The incorporation of chia seed in yoghurt mousse improved the nutritional value of the product. Chia is an excellent source of minerals, it contains 13 to 354 times more calcium, 2 to 12 times more phosphorus, and 1.6 to 9 times more potassium than 100 g of wheat, rice, oats and corn. The iron content of chia is also quite high compared to most other seeds: it has six times more iron than spinach, 1.8 times more than lentils, and 2.4 times more than liver. Bushway *et al.*, (1981); Beltrán-Orozco and Romero, (2003). As illustrated in Table (2) ,it could be observed that the three yoghurt mousses which fortified with chia seeds are richer in minerals with an increase by the ratio increase compared with the control yoghurt mousse.

All dairy products, contains very little iron. Therefore, dairy products are logical vehicles for iron fortification because they have high nutritive values, reach target population and are widely consumed. The quality of iron-fortification dairy products depends on the iron sources used, levels of iron and properties of dairy products utilized for iron fortification. Fortification with iron is technically more difficult than with other nutrients because iron reacts chemically with several food ingredients. For these reasons, the ideal iron compound for food fortification should be one that supplies highly bioavailability iron and in the meantime does not affect the nutritional value or sensory properties of the food and should be stable during food processing and of low cost. For all this consideration fortification yoghurt mousse with chia seeds reach to 3% can be a safety source of iron and it can provide 1.14mg/100g witch nearly 14% from the daily recommendation in one serve. Same trend with zinc content in chia seeds, ranging from 4.58 to 4.58 mg per 100g Sukhneet *et al*., (2016) which can consider as an excellent source for zinc .Added chia to yoghurt mousse Significantly increased zinc content reach to 0 .68 mg/100g which about (6% DRI) for one serve, this highest score recorded with the ratio of 3%. Zinc is important in order for the body to function effectively. Zinc controls the enzymes that operate and renew the cells in our bodies. The formation of DNA. Zinc deficiency is the fifth leading risk factor for disease in the developing world. In a recent survey by WHO .Bimola *et al*.,(2014).

Physicochemical analysis of yoghurt mousse fortified with Chia seeds during storage.

-Viscosity

Viscosity is defined as the resistance of the fluid to flow caused by internal friction Malcolm (2002). Fortification yoghurt mousse with (3%) chia seeds resulted a significant ($P<0.05$) increase in viscosity, followed by yoghurt mousse with (2%) chia seeds and yoghurt mousse with gelatin as control respectively. The lowest record was obtained by yoghurt mousse with 1% chia seed .Table (3) .These results could be due to the low concentrations of chia and the week jelly nature formed by chia in low concentration. Viscosity of yoghurt mousse gradually increase during storage periods in all treatments. markedly increase could be observed with added of chia seeds especially in the ratios 2% and 3% , it could be attributed to the chia mucilage properties which can change the size and shape of the chia, and give yoghurt mousse smooth and stable texture. Campos (2015) suggested that chia mucilage can be used as an

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emulsifier and stabilizer in an ice cream. He also mentioned that extracted mucilage of chia has been used to improve and maintain the quality of ice cream during storage.

-Syneresis

Synersis is the shrinkage of the gel, which lead to whey separation. Table (3) shows that the syneresis of yoghurt mousse decreased in parallel with viscosity the. Also it could be observed that less syneresis has also been reported for yoghurt mousse with gelatin as control than (yoghurt mousse fortified with 1% chia seeds. Significant syneresis decreased ($P<0.05$) recorded by advancing storage .These effects of Chia seeds on syneresis could be attributed to the presence of fiber retaining more aqueous phase. Chia seeds contain 5-6% mucilage, which can be used as dietary fiber. Reyes *et al.,* (2008). Muñoz et al. (2012) studied the hydration of chia mucilage, finding that a 100 mg sample of mucilage absorbs 2.7 g of water, which is 27 times its own weight.

-Water holding capacity (WHC)

Water holding capacity WHC is the amount of water absorbed and held by the hydrated sample after an external force is applied. A principal feature of chia seeds is that when placed in an aqueous medium, it secretes a mucilaginous polysaccharide that surrounds the seed. It has been reported that consumption of this mucilage aids digestion and that, together with the seed, constitutes a nutritious food source .Salgado-Cruz *et al.,* (2005). As shown in Table (3) there were significant differences between the water holding capacity (WHC) of yoghurt mousse fortified with chia seeds and control. The highest WHC when fresh was obtained for yogurt mousse samples made using (3% chia) it was recorded 65.25 % which was 61.4 % higher than that of control yogurt mousse (40.41 %). Same trend can be observed during storage period. These results is in agreement with Ranil *et al.,* (2014) who found that chia seed gel had the highest WHC when compared against commercial guar gum and gelatin, which are 2 commercial food ingredients used to improve WHC. According to Galla and Dubasi (2010), WHC depends on a number of factors including ingredient and water interactions, number of hydration positions, protein configuration and fat. High WHC of Chia seeds can be explained by its higher protein and fiber contents as these components could bind water as stated by Ragab et al., (2004). Fortified yoghurt mousse with different concentration of Chia seed enhanced water holding capacity when fresh and during storage period the high levels of dietary fiber in chia mucilage help maintain the moisture in the product, and avoid the loss of moisture during storage because of the numerous free hydroxyl groups in fiber which can form hydrogen bonds with water.Oakenfull (2001), Wang and Cui, (2005)

Microbiological Characteristics

Data in Table (4) showed microbiological analysis of yoghurt mousse fortified with chia seeds. It could be observed that yoghurt mousse fortified with chia seeds (3%) recorded the lowest total bacterial count .From the same table it could also be noticed that total bacterial counts were decreased with increasing of chia percentage. These results could be due to phytochemical caused inhibition of microbial growth Safaa (2017). On the other hand lactic acid bacteria in yoghurt mousse with 3% chia seeds showed increasing than the control sample when supplement with chia seed. This increasing were also in parallel with the increasing of chia concentration .Same trend were found by Carmen *et al.*,(2015) who reported that yogurt supplementation with 1,4 % chia seeds and 7, 6 % cranberries significantly improves the stability of lactic acid bacteria . High fiber contents in chia seeds lead to increase the population of LAB and the fecal water content and reduce microbial harmful enzyme (β-glucosidase, β-glucuronidase and tryptophanase) activities. Lee *et al.,(*2011). From the same table it could be also observed that spore form count recorded highest number in yoghurt mousse with 1% chia seeds, followed by the control treatment, while yoghurt mousse with 2% and 3% recorded the lowest spore counts. Same trend were noted during storage period. This is could be due to the reducing on the pH by fermenting the lactose to lactic acid; adding acids or other approved preservatives; adding sugar or salt to reduce the water activity (aw); removing water Loralyn *et al.,* (2009). Mould and yeast are not detected in yoghurt mousse fortified with Chia seeds when fresh and during storage (8 days/6^{o} C).

Sensory properties of yoghurt mousse fortified with Chia seeds

Sensory analysis indicated in Table (4) that yoghurt mousse fortified with 3% chia seeds has got highest scores for all sensory parameters during whole storage period , followed by yoghurt mousse with 2%chia seeds and the control treatments .yoghurt mousse with 1% chia recorded the lowest total scores this is due to the low concentration of of chia seeds 1%. Treatment yoghurt mousse with gelatin 1.25% concentration recorded higher scores than yoghurt mousse with 1% chia. Panelists preferred yoghurt mousse with chia seeds regarding to its nutty flavor , crunchy texture and gelling properties which fits the mousse properties . Chia possesses many important physiochemical and functional properties which makes it more suitable in the food industry. Chia acts as a good thickener, gel former, chelator, foam enhancer, emulsifier, suspension formers, clarifying agent and as a

rehydrating agent .These results is in agreement with Sukhneet Suri *et al.,* (2016).

Effect of incorporation chia seeds on the Microstructure of Yoghurt mousse

Based on the microscopic investigation Yoghurt mousse fortified with 3% chia seeds effect on microstructure and in appearance than Yoghurt mousse control. Bubbles play a key role in foamed food products, including confectionery mousses because they create fine texture with a light, smooth and creamy mouth feel. The quality of such products is directly impacted by the formulation and processing conditions. As can be seen in the micrographs, the microstructure of yoghurt mousse using 3 % chia seeds which gained the highest scores greatly differed than that using 1.25 % gelatin. No strands could be seen in the gel structure with 3% chia seeds , while for yoghurt mousse with 1.25% gelatin, the structure was much looser and the strands could be seen clearly (Fig 1. A) Some particles were observed with 1.25 % gelatin gels. This results could be due to the low concentration of gelatin and the low pH in yoghurt mousse .this is in agreement with Zhihua Pang *et al.,(*2014) ,Who mentioned that the hardness of gelatin gels increased at high gelatin concentration (5.0%),and the fracturability of the gels was greatly influenced by pH, which is minimum at its isoionic point (IP) because of the maximum molecular folding at that pH. Petrie and Becker (1970). It was reported that aggregation of gelatin increased and the gelatin gel turned from transparent to opaque as the pH was increased from 5.4 to 7.5 (Walkenstrom and Hermansson, 1997) .On the other hand chia seed gel had higher emulsion activity and stability than gelatin gel. The chia seed gel and chia flour gel could have higher OHC due to the concentrated gels sponge-like nature which could absorb. The gel would have greater space in it spongy structure to absorb and trap the oil. Such sponge-like structures are not formed by gelatin and guar gum which could explain the much lower oil holding capacity. Gelatin is a protein which allows its gel to set at lower temperature and it works best at low temperature. Chia seed is polysaccharide based and their gels generally do not set at low temperature similar to guar gum, which is a different gel behavior compared to gelatin. However, interestingly chia seed gel had a lower spread value (3.38 mm) compared to gelatin (7.17 mm) at 3 °C, indicating that chia seed has the potential to replace gelatin in chilled food formulation. Ranil *et al*., (2014).

Fatty Acids Composition

The major fatty acids in buffalo milk are the palmitic acid, the oleic acid, the stearic acid and the miristic acid. The content of essential C18:2 (omega 6), known for its atherogenic properties decreased slightly in the coagulation of milk, while C18:3 (omega 3) increased. These fatty acids are essential for growth and development and are beneficial in the maintenance of human health and prevention of chronic diseases and neurological disorders. Yashodhara *et al.,* (2009). Fatty acids are essential nutrients for many lactic acid bacteria, and supplementation of growth medium with fatty acids can influence the membrane composition and growth rate Johnsson *et al*., (1995) ; Muller *et al.,* (2011). In our studies it could be observed in Table (6) that Oleic acid (C18:1) was in the highest percentage (27.53, 28.35) among the unsaturated fatty acids in each control and fortified yoghurt mousse with 3% chia seeds. This is in agreement with Sheisa *et a*l.(2013) who reported that chia seeds showed the most significant amounts of oleic and linolenic acids, 197.76 and 174.73 mg/ g-1, respectively . Lopez (2010) mentioned that the incorporation of milks enriched with oleic acid into the diet has resulted in reductions in total cholesterol, LDL-cholesterol and triglyceride levels, the effects of which were observed among healthy individuals, those with increased risk for cardiovascular disease and individuals with CVD. From the same table it can also illustrated that the content of essential C18:2 (omega 6) and C18:3 (omega 3) in yoghurt mousse sample fortification with chia seeds at the ratio (3%) showed a higher records (7.54 and 4.80) compared to control samples which were (5.26 and 1.45) for C18:2 (omega 6) and C18:3 (omega 3) respectively.

The presence of higher concentration of polyunsaturated fatty acids in chia oil has increased its popularity and cultivationmany folds. Omega-3 fatty acids are comprised of three essential fatty acids; alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid whereas omega-6 is comprised of linoleic acid and arachidonic acid . Pawlosky *et al*. (2003). Chia seed with appreciabl amounts ofω-3 alpha-linolenic acid (ALA) andω-6 linoleic acid. Of all the known food sources chia contains the highest concentration of these fatty acids. On an average it contains about 64 % ω-3 and 19 % ω-6 fatty acids. Ali *et al*.,(2012). The seed is appropriately known as power house of omega fatty acids. Eicosapentaenoic acid, an docosahexaenoic acid have cardio-protective effects Manzella and Paolisso (2005). Diets must be balanced in the omega-6 and omega-3 fatty acids to be consistent with the evolutionary understanding of the human diet. This balance can best be accomplished by decreasing the intake of oils rich in omega-6 fatty acids (corn oil, sunflower, safflower, cottonseed, and soybean) and increasing the intake of oils rich in omega-3s (canola, flaxseed, perilla, and chia). Artemis (2010). It can be take in consideration that the total ω-3 PUFAs available from milk is only 0.33 mg/100 mL, so in order to achieve 0.5

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g/d ω-3 PUFA which consider as recommended daily allowance of omega-3 the intake of at least 150 L of milk or 15 kg cheese or 5 kg butter is required, suggesting that unfortified milk and dairy products supply inadequate amounts of dietary PUFAs. Commercial dairy products such as liquid milk and yoghurt are currently fortified with ω-3 PUFAs obtained from flaxseed, fish oil, or marine microalgae. Balasubramanian Ganesan *et al*. (2014). But it can be taken in consideration that atypical organoleptic characteristics such as flavor and smell from marine sources were not found in chia .Ayerza (2002) This showed the superiority of chia seed against other nutritional sources rich with PUFAs.

The ω-6/ ω-3 ratio was at level 3.63 and 1.57 for yoghurt mousse with gelatin as control and yoghurt mousse fortified with chia seeds with the ratio of 3% this ratio is commonly used to assess the nutritional value and healthiness of food lipid material for human consumption. Simopoulos(2008) recommended that ratio ω-6/ ω-3 from 4:1 reach to 1:1 as an ideal ratio in human diets to prevent the development of cardiovascular diseases and some chronic diseases including cancer. The incorporation of ingredients with high PUFA content into fermented dairy products like chia seeds provides numerous health benefits .

Effect of Chia on lipid profile and cardiovascular risk indices

Data represented in Figure (2) show the effect of chia on lipid profile and cardiovascular risk indices of normal and ISO-induced hypertrophic rats. Compared to the control group, rats supplemented with chia at both doses and ISO-induced rats exhibited insignificant change in all studied parameters. While rats supplemented with yogurt mousse alone showed a significant decrease ($P<0.001$) in levels of TG and VLDL-C [fig.2 a&b] associated with a significant increasein levels of TC and HDL-C [fig.2 c&d] ($P<0.05$, $P<0.001$, respectively).

In addition, Compared to yogurt mousse group, ISO-administered rats showed significant (P, 0.001) increase in TAG and VLDL-C compared with a significant decrease ($P< 0.001$) in HDL-cholesterol (Fig2a,b&c). On the other side, Pretreatment with chia to the ISO-induced rats showed a significant decrease ($P<0.001$)in the level of TAG and VLDL (Fig2 a&b) in low dose chia group with subsequent increase ($P<0.01$) in the level of HDL at high dose chia group in comparison to the ISO-treated group. (Fig 2d).The cardiovascular risk indices TC/HDL-cholesterol showed non significant ($P<0.05$) variation between all studied groups (Fig.2 e).

The variation in omega-3 fatty acid chemistry might cause a difference in important factors mediating effects on blood lipids like distribution to tissues, distribution among the various lipoproteins in blood and effects on regulators of lipid metabolism in the liver. The main finding in the study presented here was that the chia seeds rich altered the blood lipid profiles in dyslipidemic, ISO-treteated rat markedly and in a health-beneficial manner by reducing circulating triglycerides and VLDL-cholesterol total, while it increased HDL-cholesterol.

IV. CONCLUSION

According the results from this study it could be recommended to incorporate chia seed with the ratio 3% in the production of yoghurt mousse as a novel neutraceutical and desirable dairy product.. In addition, based on the current research findings, chia seed is a good choice of healthy to maintain a balanced serum lipid profile. Furthermore, details on the mechanisms of chia seed's hypolipidemic effects need to be studied with reference to its health content of the omega 3 and omega 6 fatty acids ratio.

Table.1: Composition of chia seeds

Nutrient	**Amount/ 100g**
Total solid	**96.60**
Dietary fiber	**24.50**
Fat	**33.16**
Protein	**21.34**
Ash	**4.60**
Other carbohydrate	**16.0**
Caloric value (Kcal/100 g)	**(545.8)**

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Table.2: Chemical composition of yoghurt mousse fortified with Chia seeds

Components	Yoghurt Mousse (1.25 gelatin)	Yoghurt Mousse (1% chia seeds)	Yoghurt Mousse (2% chia seeds)	Yoghurt Mousse (3% chia seeds)
Total solids	26.83^{b}	27.35^{ab}	27.86^{ab}	28.53^{a}
Fat	7.53^{b}	7.80^{b}	8.23^{a}	8.56^{a}
Protein	6.42^{b}	6.63^{ab}	6.95^{ab}	7.21^{a}
Fiber	-------	0.34^{c}	0.79^{b}	1.26^{a}
Ash	1.16^{a}	1.28^{a}	1.45^{a}	1.58^{a}
pH	4.80b	4.76ab	4.73a	4.69a
Calcium (mg/100g)	185.3^{ab}	193.6^{ab}	202.4^{a}	210.0^{a}
Phosphorus(mg/100g)	93.5^{b}	115.4^{ab}	126.0^{ab}	135.0^{a}
Irion (mg/100g)	0.53^{b}	0.65^{ab}	0.78^{a}	1.14^{a}
Zinc (mg/100g)	0.47^{b}	0.52^{ab}	0.61^{a}	0.68^{a}

Table.3: Physicochemical analysis of yoghurt mousse fortified with Chia seeds during storage.

Treatments	Storage Days	Yoghurt Mousse (1.25 gelatin)	Yoghurt Mousse (1% chia seeds)	Yoghurt Mousse (2% chia seeds)	Yoghurt Mousse (3% chia seeds)
Viscosity (Cp)	**1**	**3552^{ab}**	**1040 ^{b}**	**3080^{ab}**	**5784^{a}**
	4	**3326^{ab}**	**1489^{b}**	**4882^{ab}**	**6164^{a}**
	8	**3210^{ab}**	**1730^{b}**	**6480^{a}**	**6568^{a}**
Synersis%	**1**	**0.88^{ab}**	**1.12^{a}**	**0.54^{b}**	**0.40^{b}**
	4	**1.21^{ab}**	**1.60^{ab}**	**0.72^{a}**	**0.53^{a}**
	8	**2.15^{ab}**	**2. 20^{ab}**	**0.80^{a}**	**0 .60 ^{a}**
WHC%	**1**	**40.41^{b}**	**46.32^{ab}**	**49.63^{ab}**	**65.25^{a}**
	4	**44.63^{ab}**	**50.14^{ab}**	**58.37^{ab}**	**67.47^{a}**
	8	**47.52 ^{b}**	**52.56 ^{b}**	**67.47^{ab}**	**70.32 ^{a}**

Table.4: Sensory properties of yoghurt mousse fortified with Chia seeds during storage period

Treatments	Storage Days	Yoghurt mousse (1.25%gelatin)	Yoghurt mousse (1% chia seeds)	Yoghurt mousse (2% chia seeds)	Yoghurt mousse (3% chia seeds)
Flavor (60)		**54^{a}**	**52^{b}**	**54^{a}**	**55^{a}**
Body&texture (30)	**1**	**23^{a}**	**21^{b}**	**23^{a}**	**25^{a}**
Colors&appearance (10)		**8^{a}**	**7^{ab}**	**8^{a}**	**8^{a}**
Total (100)		**85**	**80**	**85**	**88**
Flavor (60)		**52^{ab}**	**51^{ab}**	**55^{a}**	**56^{a}**
Body&texture (30)	**4**	**23^{a}**	**20^{b}**	**24^{a}**	**25^{a}**
Colors&appearance (10)		**8^{a}**	**7a^{b}**	**8^{a}**	**8^{a}**
Total (100)		**83**	**78**	**87**	**89**
Flavor (60)		**52^{ab}**	**50^{b}**	**56^{a}**	**57^{a}**
Body&texture (30)	**8**	**23^{ab}**	**20^{b}**	**24^{a}**	**26^{a}**
Colors&appearance (10)		**7^{a}**	**6^{b}**	**8^{a}**	**8^{a}**
Total (100)		**82**	**76**	**88**	**91**

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Table.5:Microbiological analysis of yoghurt mousse during storage period

Treatments	Storage Days	T.B.C Log CFU/10^{-3}	L.A.B log CFU/10^{-6}	Spore form log CFU/10^{-1}	T.C.C	Yeast and Mould
Yoghurt Mousse (1.25 % gelatin)	1	80	49	1.3	ND	ND
	4	52	76	1.7	ND	ND
	8	36	123	2.6	ND	ND
Yoghurt Mousse (1% chia seeds)	1	77	65	1.4	ND	ND
	4	63	93	1.9	ND	ND
	8	32	156	3.2	ND	ND
Yoghurt Mousse (2% chia seeds)	1	62	75	1.2	ND	ND
	4	44	125	1.5	ND	ND
	8	28	175	1.8	ND	ND
Yoghurt Mousse (3% chia seeds)	1	58	89	1.1	ND	ND
	4	46	167	1.5	ND	ND
	8	20	183	1.7	ND	ND

T.B.C: Total bacterial count.L.A.B.: Lactic acid bacteria.T.C.C. : Total coliform count

Table.6: Fatty Acids Composition of control yoghurt mousse and yoghurt mousse fortified by Chia seeds.

Fatty acids %	Yoghurt Mousse with gelatin	Yoghurt Mousse 3% Chia
Caproic Acid, C6:0	1.83	1.78
Caprilic Acid, C8:0	1.65	1.37
Capric Acid, C10:0	4.82	3.58
Lauric Acid, C12:0	2.65	2.37
Myristic acid (C14:0)	7.30	6.82
Pentadecanoic acid (C15:0)	1.86	1.36
Pentadecenóico acid (C15:1)	0.83	0.71
Palmitic Acid, C16:0	25.70	22.43
Palmitoleic acid (C16:1	0.67	0.60
Margaric acid (C17:0)	3.20	2.85
Stearic acid (C18:0)	14.67	14.26
Oleic acid (C18:1 – ω-9)	27.53	28.35
Linoleic acid(C18:2 – ω-6)	5.26	7.54
Linolenic acid (C18:3 – ω-3)	1.45	4.80
ΣSaturated acids	65.18	58.13
ΣUnsaturated acids	34.24	40.69
Σ PUFA ω-6/Σ PUFA ω-3	3.63	1.57

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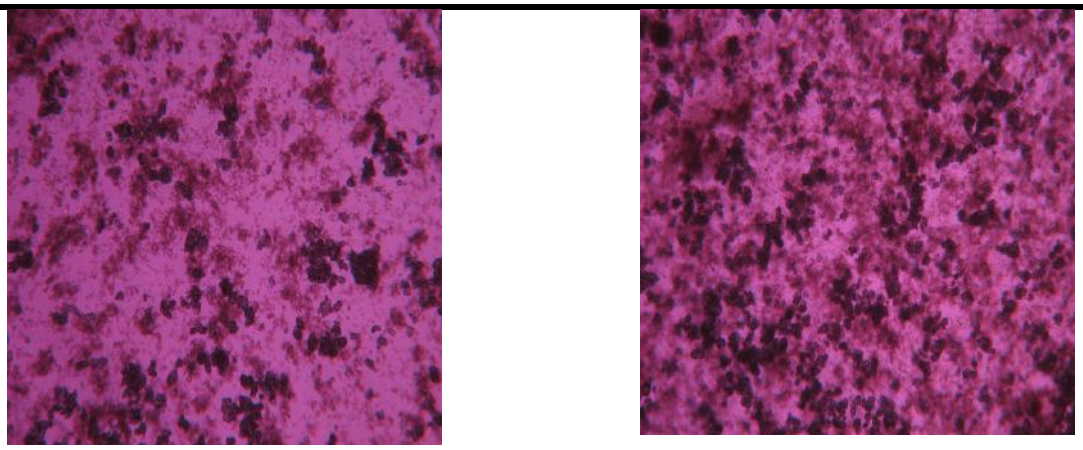

Yoghurt mousse (1.25%gelatin) **Yoghurt mousse (3% chia seeds)**

Fig.1: Microstructure of Yoghurt mousse fortified with chia seeds.

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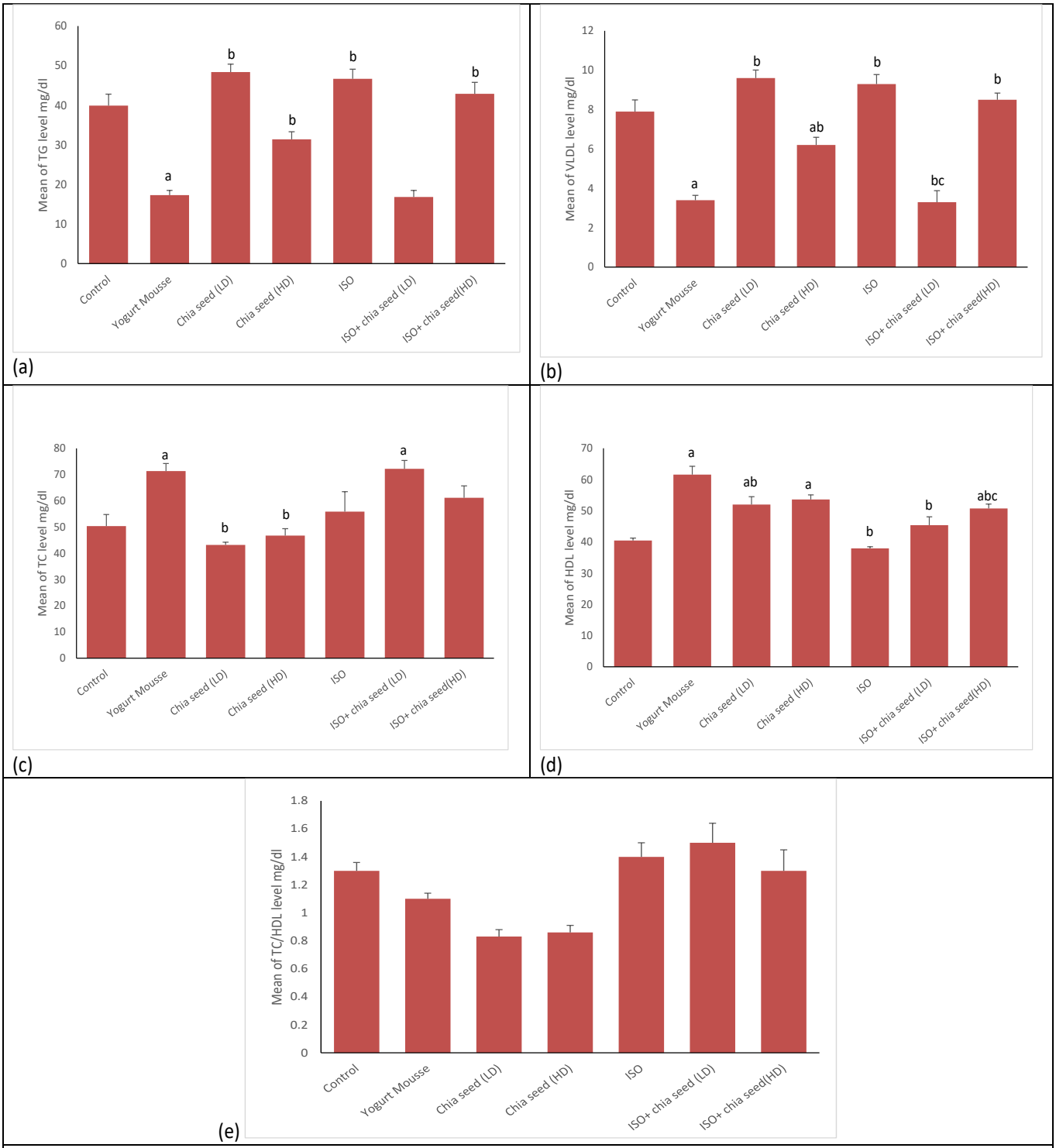

Fig.2 Effect of chia seeds on serum TAG (a), VLDL-C (b), TC (c), HDL-C (d) and TC/HDL-C (e) in ISO-induced rats. The data represent the means ± SEM.[a]: Significant difference vs. proper control. [b]: Significant difference vs. yogurt control. [c]: Significant difference vs. ISO-group values. P <0.05 is regarded as significant. SE M, standard error of the mean; ISO, isoproterenol;TG: triglycerides; vLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; LD, low dose; HD, high dose; vs, versus.

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Recent Developments in Goat Farming and Perspectives for a Sustainable Production in Western Africa

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Abstract**—** *The West African region has a great diversity of indigenous goat breeds that are well adapted to such environmental conditions. West African goat population was estimated to be around 150 million heads in 2014, representing 14.82% of the world goat population. Goats play an important role in the socio-economic, environmental and religious life of the farming communities in terms of income, payment of dowry and supplying food for the local population. The traditional keeping system based on natural rangeland use as feed resources and free roaming is the most common system in the regions. Although goat industries have significantly increased these last decades, they are subjected to a variety of factors limiting their development. For a sustainable development with the goals of meeting the increasing demand for goat products, a wide intensification of goat production policies should be implemented.*

Keywords**— Goat, Meat, Milk, Skin, West Africa.**

I. INTRODUCTION

Livestock especially goat production is a traditional activity practiced by 60-87% of the local populations either as the main activity or as a secondary activity [1, 2]. Goats play an important socio economic role in many West African local populations. African goat population represents 30% of Africa's ruminant livestock and produce about 17 and 12% of its meat and milk, respectively. According to the data of Food and Agriculture Organization of United Nation in 2014, the West African population of goats was approximately 150 million heads accounting for 14.82% of goat population in the world. Nigeria (48.34%), Mali (12.76%), Niger (9.93%) and Burkina Faso (9.27%) host a large number of goats [3]. Although it's crucial importance, goats still remain largely marginalized, even at the household level [4]. Therefore, there is the need for the understanding of the role of goat, the diversity of production systems, and the current production of goat products, and constraints that will contribute to establishing strategies to advance the development of this sector.

II. GOAT FARMING AND PRODUCTION IN WEST AFRICA

West African area involves a wide range of indigenous goat breeds well adapted to harsh environmental and precarious husbandry conditions but have low genetic potentials. The geographical distribution of the different SRs breeds in West Africa is almost exclusively determined by the presence or absence of the tsetse fly in the region. The vast majority of sheep and goats in the areas of high tsetse challenge are the West African dwarf trypanotolerant breeds. In the savannah and the semi-arid zone, the larger sized, long-legged Sahelian breeds thrive well [5; 6]. West African Dwarf goat is small size animals with a low meat yield and very low lactogenic productive potential [7, 8].

The West African Sahelian goat is a long leg breed mainly raised mainly for meat, skin and milk production. The Sahelian is large, long-legged goats mainly found in the semi-arid and arid of the Saharan and sub-Saharan region in distribution. It is a very rustic and butcher animal. In Liberia, there are considerable numbers of the Red Sokoto breed and crosses between the WAD and the Red Sokoto goat breeds [9]. Red Sokoto is also known as Maradi goat is used for the good quality skin production in Nigeria and Niger.

Additionally, some exotic breed such as Alpine goat (in Benin), Anglo-Nubian, Toggenburg, Boer, Saanen (in Nigeria) and Majorera goat (in Senegal) have been imported in order to increase the profitability of goat farming [8, 10, 11]. These breeds are currently being kept under an acclimatization research program and in some commercial private farm. In 2014, West African meat produced from indigenous goat, milk and skin from goat was, respectively, 39.7%, 24.5% and 36.4% of the African production [3]. The main indigenous meat, milk and skin producer countries in the region are shown in Table. Thus, countries like Mali, Mauritania, Niger, and

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Burkina Faso produce over 95 % of the whole fresh goat milk. Regarding indigenous meat, it is mainly produced in Nigeria (55.53%), Mali (14.85%), and Niger (8.87%), with 79.25% of the regional production [3].

The most spread meat processing methods of goat is the slaughtering procedures of animals. Indeed, according to the size, conformation and breed, animals are sold transported and slaughtered. Then, the cuts of fresh meat are collected and sold by retailer butchers to consumers on the local markets. Additionally, the fresh meat is processed and sold as fried or grilled meat directly consumed. After slaughtering, the skin is tanned to leather which is used in the textile industry or to make shoes.

III. GOATS FARMING SYSTEM

Goats are mainly kept in the traditional system. The traditional system is made up pastoral systems and agropastoral systems. However, some semi-intensive systems, ranching, and intensive dairy farming are encountered. These production systems differ from place to place due to socio-economic reasons.

3.1. Pastoral system

The pastoral system is the main traditional ruminant production systems and the widespread all across the region. It is a grassland-based system in which livestock include cattle, sheep, goats, and dromedaries. Pastoral systems are mainly encountered in the arid and semi-arid regions characterized by rainfall less than 600 mm per annum and 90 days of plant-growing period [12]. This system includes the nomadic pastoralism and transhumant pastoralism. The nomadic pastoralism is a pure pastoral system, characterized by little or no agriculture and by high mobility of people and animals in search of grazing and water. As for transhumant pastoralism, it is based on more or less regular seasonal migrations from a permanent homestead [9].

3.2. Agropastoral system:

it is a system in which livestock production is associated with dryland or rainfed cropping and animals range over short distances [12]. Agropastoralists are sedentary but sometimes practice transhumance. During the cropping season, animals are grazed on fallow lands and areas of natural vegetation. In the dry season, they are brought back to cultivated areas where they graze in swamps, rice fields and various areas which they cannot graze during the wet season [9].

3.3. Sedentary extensive system

In this system, goats are kept in free-roaming flocks or herds in villages and their environs, scavenging for feed. They have no benefit of prophylactic or curative medicinal treatment. Owners provide little or no supplementary feed. Good flock management practices are not applied and poor housing is provided to animals. Additionally, farmers having a small number of animals, and limited access to land tethered (in the compound/pens) free-roaming animals during the cropping season so as to avoid crop damage. Sometimes, animals may be tethered in areas where forages are available for in situ grazing. Cut-and-carried forage and waste from kitchen and crop processing are supplied in some instances to the animals [13, 9]. This is characterized by the absence of production target, limited food resource, low productivity and high losses due to accidents, diseases, and theft.

3.4. Semi-intensive and intensive systems

these systems are mainly encountered in urban and peri-urban areas where intensification of dairy production and small ruminants fattening are developed. Indeed, the increased demands for dairy products and meat of urban population led a gradual shift from extensive and unproductive livestock systems to more semi-intensive or intensive systems. Improved management practices such good housing of animals, supplementation of concentrate, appropriate breeding program, selection of best reproductive animals, and health monitoring of animals are applied.

Table.1: Goat population in West Africa

Country	Number (1000 Heads)	%	Meat (Tons)	%	Milk (Tons)	%	Skin (Tons)	%
Nigeria	72466.7	48.34	292650	55.53	-	-	46600	52.26
Mali	19126.8	12.76	78260	14.85	420102	41.02	11120	12.47
Niger	14883.6	9.93	46767	8.87	308099	30.08	8840	9.91
Burkina Faso	13891	9.27	26468	5.02	116086	11.33	6947	7.79
Mauritania	7040	4.70	18450	3.50	130975	12.79	1980	2.22
Ghana	6044	4.03	21469	4.07	-	-	4248	4.76
Senegal	5381.3	3.59	12937	2.45	13675	1.34	3905	4.38

[3] FAOSTAT, 2017.

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IV. FACTORS LIMITING GOAT PRODUCTION IN WEST AFRICA

4.1. Goat keeping and environmental issues

Climate change as a result of increased ambient temperature and concurrent changes in heat exchanges directly causes heat stress which influences growth, reproduction performance, milk production, wool production, animal health and welfare [14]. Heat stress caused by high temperature is a major constraint on animal productivity in the tropical belt and arid areas [15]. Research in West African Goat showed that the elevated temperature disturbs adversely the sexual activity, endocrine and testis functions, spermatogenesis and physical and chemical characteristics of buck semen [16]. Moreover, heat stress causes significant changes on behavioral, physiological and blood parameters via a reducing of feed intake, increasing of water consumption leading to weight loss [17, 18, 19, 20]. Also, the depression of feed intake and reduction of milk production are commonly observed in heat-stressed goats [21].

On other hands, drought is particularly detrimental to agriculture in sub-Saharan Africa, where it causes about 90 percent of the sector's production losses, which contributes on average to one-quarter of GDP, or even half, including agri-food [22]. Drought and shortage of rainy season as result of changes in climate and extreme weather conditions affect the quality and availability of feed resources notably pasture and of drinking water. The countries which are generally affected by these long droughts are generally the countries of the Sahel and the Sahara (Mali, Burkina Faso) [23].

4.2. Nutritional constraints

In West African goat management system, grazing and browsing on natural pastures, feeding with crop residues, agro-industrial by-product, and kitchen wastes are the main feed sources for animals. In most part of the region goat freely roam about scavenging for food over the day. This feeding system exposes animals to diseases risks, especially those related to feeding and nutritional disorders. Additionally, the inadequate feed supplying, the poor quality (inadequate levels of crude protein) of the available feed resources and inefficiency use of the available feed resources are the main nutritional problems in goat farming. The seasonal variation of fodder in term of nutritional value and quantity is most of the year due to the rainfall pattern. This is aggravated by inadequate management of feed resources such as overgrazing in the arid and semi-arid zones where rainfall is less than 600 mm and between 600-1000 mm per year, respectively [24].

4.3. Management and Diseases

The mismanagement and high prevalence of diseases and parasites are one of most limiting factors in the development of goat farming in West Africa. Diseases cause important direct and indirect losses of the high reproductive performance diminishing of benefits for farmers. The dominant pathologies within the goat population can be grouped into two categories: parasitic diseases and infectious diseases. Although some efforts have been made to eradicate PPR (Peste des Petit Ruminants) is still one of the main diseases affecting West African goat flocks. Due to the high mortalities rate for which they are responsible, pneumopathies and internal parasitoses (intestinal worms) are undoubtedly ones of the major constraints of goat farming in West Africa.

4.4. Difficulty in access to loans

For processing, farmers need to invest. Due to lack sufficient own resources, difficulties to access financial services (credit, savings, insurance), they are limited to primary production. These financial services and the supply of credit especially, remain failing in response to demand from farms. And then, farmers and agricultural organizations are particularly disadvantaged in access to credit [25].

V. STRATEGIES FOR A SUSTAINABLE DEVELOPMENT OF GOAT FARMING

In order to meet the increasing demand for livestock products especially goat, livestock development policies and strategies with an emphasis on the intensification of production methods and diseases control the introduction of exotic breeds to increase livestock production capacities.

5.1. Improving the productivity of native breeds

Genetic improvement through the selection or cross-breeding is very important to improve the genetic potential and livestock productivity. Thus, an effective breeding program including researchers, farmers and governmental institutions should be implemented to increase the meat, skin and milk yield of the indigenous goat by adopting within breed selection programs or controlled cross-breeding using exogenous breeds with high productivity.

5.2. Governmental policies for supporting the goat sector

The government through Ministries must have adequate resources, well-trained working staff to carry out activities and a modern and efficient internal functioning. It should support farmers by subsiding inputs costs (feed, drugs, fuel, machinery etc) and be regulating the animal products (milk, meat, and skin) prices on the markets. It also facilitates the access of farmers to credit services, the good animal reproducers. An effective animal health

inspection service should be implemented. In addition, regular extension service and veterinary services should be provided to famers. The implementation of modern slaughterhouses and subsidies for processing of animals should be considered in order to add value to the animal's products and improve livelihood of farmers.

5.3. Research and training

The goal of intensification and valorization of animal production can only be achieved with actors whose skills should be adapted to new production technologies. Governments should grant research and redirect its priorities to innovative action to enhance food self-sufficiency and reduction of poverty notably in the rural area. In order to be competitive and safety in term of biosecurity in future, the breeding must acquire appropriate skills and be able to adapt to changes. The Periodic training session should be set up to strengthen the research-extension link, the capacity and knowledge of breeders to improve the keeping and management practices of their business. Association of breeders and inputs and services suppliers involved in livestock value chains should be reorganized to defend the common interests and to adopt facilitation practices to be more competitive on markets.

VI. CONCLUSION

The main products from goat production in West Africa are meat, milk, and skin. Although many project and governmental actions have been taken to increase production of goat, it is still undeveloped. Constraints related feeding, health, management of flock, lack of structural and institutional organization are the major limiting the increase of the subsector. Therefore, strategic plan to increase animal productivity, the income of producers and the preservation of environment needs to be implemented. It should ensure the improvement of feeding, health care, the introduction of modern reproductive methods and management practices. Governments should also a) promote the access of farmers to the credit services, b) strengthen rural infrastructure through the rehabilitation or construction of pastoral hydraulic infrastructures, rural roads, slaughterhouses, meat, and milk processing industries and c) ensure sustainable management of natural resources through the regulation of their overusing by pastoralist.

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Potential of genomic approaches in conservation of plant and animal biodiversity in Africa: A review

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Abstract**—** *In Africa, status of biodiversity conservation of many plants and animals is questionable as this is considered to be caused by limited and lack of authentic information concerning genetic diversity. This has led to a considerable compromise of conservation decisions in Africa. As a result, lack of reliable information continues to cause a great effect on the long-term security of species of plants and animals. Current advancement in genomics has proved to play a vital role in conservation of plant and animal biodiversity. It produces genetic data that helps researchers to understand the interaction between ecosystem and organisms, also among organisms themselves. The information extracted from plants and animals via genomics techniques can be used to develop good approaches for biodiversity conservation. Despite its usefulness, there is a limited awareness on the application of potential genomics in plants and animals conservation in many developing countries, especially in Africa. The aim of this review is to raise awareness and catalyse the application of genomics techniques in rejuvenation and conservation of plants and animals in Africa. Precisely, the paper addresses the efficacy of potential genomics in plants and animals conservation; and seeks to show how Africa can benefit from genomics technology. About 62 peer-reviewed articles were reviewed. This current review has shown that genomics helps to identify good genes for fitness, and develops tools to monitor and conserve plants and animals biodiversity. The review recommends that regardless of the limitation of genomics application in biodiversity conservation in Africa, African researchers must consider using this technology for better conservation of plants and animals biodiversity.*

Keywords**— Biodiversity, Conservation genomics, Extinction genetic diversity, genetic tools, Natural variation.**

I. INTRODUCTION

Despite being rich in biodiversity, Africa is experiencing a considerable loss of its plant and animal biodiversity (Muhumuza and Balkwill, 2013). While most developed countries have adopted Genomic technologies in an effort to conserve biodiversity, their use elusive in Africa (Lyantagaye, 2013; Muhumuza and Balkwill, 2013). Climate change coupled with other human induced factors further threaten plant and animals species of which many are at risk of extinction (Thomsen and Willerslev, 2015; Yule et al. 2013). Anthropogenic activities such as pollution, habitat destruction of habitats, overexploitation and introduction of alien invasive species are among the factors causing loss of plant and animal biodiversity (Dirzo et al. 2014; Thomsen and Willerslev, 2015). Preventing this loss of plant and animal biodiversity is a challenge that many countries in Africa face (Dirzo et al. 2014). Several conservation policies, agreements, declarations and strategies have been implemented to stop the causal loss process of plant and animal species. Abascal et al. (2016) reported that, even if all above mentioned threats are eliminated, certain species may fail to survive because of accumulation of genetic deterioration — a process whereby an endangered animal and plant species with a limited gene pool shrinks more and some individuals from the living population even die before having a chance to breed with others in their endangered low population (Abascal et al. 2016). As a consequence, such deterioration leads to losses in genetic diversity (the raw materials required for adaptation by natural selection), poor fertility and health, and a great prevalence of genetically determined abnormalities and disorders. Additionally, genetic defects may reduce semen quality and cause several other abnormalities, hence affecting the population.

From an ecological and socio-economic perspective, conservation of natural variation of plant and animal species is important (Mazzotti, 2014). There are benefits

that may occur in some species as a result of natural variation, and other species can be valuable than others (Hoffmann et al. 2015). But anthropogenic pressure in the environment decreases natural variation of species, thus increasing life uncertainty of plants and animals in their habitats (De Vos et al. 2015; Godoy, 2016; Khan et al. 2016; Li et al. 2014). Anthropogenic disturbances in the environment are due to progress in technology and industrialization, increase in human population, global warming and other human related influences (Hoffmann et al. 2015; Yule et al. 2013). Prolonged exposure of organisms to anthropogenic activities causes loss of biodiversity (De Vos et al. 2015). Biodiversity conservation is the international political agreement agenda that emphasizes the management and conservation of plants and animals world-wide (Funk et al. 2012; Khan et al. 2016). However, the information about plants and animals biodiversity as well as genetic diversity in many places around the world is limited (De Cara et al. 2013; Hasbún et al. 2016).

The application of genomics for conservation of plant and animal population or biodiversity is known as conservation genomics (Garner et al. 2016; Grueber, 2015). It is the field of science that uses genomic data from thousands or tens of thousands of loci to address important questions for biodiversity conservation (Garner et al. 2016; Perry et al. 2012; Wamalwa et al. 2016). Compared to old conservation genetic methods that used 10-20 loci, conservation genomics is much more powerful (Gayral et al. 2013; McCormack et al. 2013; McMahon et al. 2014). The technique allows precise approximations of demographic parameters such as population size, variations in population size, and flow of gene (Du et al. 2016; Tian et al. 2017). It gives the opportunity to demonstrate adaptive genetic variation across real world (Hasbún et al. 2016). It is also possible to tell and describe the identity of plant and animal species, genetic diversity, hybridization level, effective population size and demographic history using genomic methods (Irizarry et al. 2016; Tian et al. 2017).

The total number of genetic characteristics in the genetic makeup of species refers to genetic diversity (Rao and Hodgkin, 2002), whereas, genetic variability is the variations of genetic characteristics in the population (Yazici and Bilir, 2017). Genetic diversity is very important for species existence because it helps populations to adapt to different environmental changes. Because of the development of genomic methods, it is possible to assess genetic variability (Hintzsche et al. 2016) and improve plant and animal conservation and restoration (Miller et al. 2012). Ecologists and biologists can understand the evolutionary tree of life (Hasbún et al. 2016) and provide measures for biodiversity conservation using genomic tools (Funk et al. 2012). They can solve biodiversity conservation and restoration difficulties using genomic techniques (Miller et al. 2012). Moreover, they can also influence conservation policy and strategies (Khan et al. 2016).

II. BIODIVERSITY, CONSERVATION AND GENOMICS

Genomics is defined as the branch of science in the field of molecular biology which deals with the function, evolution, structure and mapping of genomes (Kadakkuzha and Puthanveettil, 2013; Lyantagaye, 2013). It is concerned with the study of genomes and their interaction with the environment (Ekblom and Wolf, 2014; Reportlinker, 2013). Roderick (1986) defined the term genomics as a science discipline which refers to the mapping, sequencing, as well as analysis of the genome (Khan, 2016; Xu, 2012). A genome is whole set of deoxyribonucleic acid (DNA) within a single cell of an organism, or a complete set of chromosomes that decides an organism (Renaut et al. 2012; Khan et al. 2016). Genetic data generated from genomics study can help researchers and ecologists understand the interaction between ecosystem and organisms (Funk et al. 2012). Understanding this interaction is critical in developing a better approach for conservation (Hongbo et al. 2015; Lyantagaye, 2013; Toro et al. 2014). Furthermore, it may help finding out how living organisms differ between and within species as well as how they differ from each other (Reportlinker, 2013). Genomics potentially allows biologists or scientists to study genes over time and to test the genetic variability of any form of life, from prokaryotes to eukaryotes (Tian et al. 2017). One of the most evident findings of genomics is the ability to explain how much is shared between organisms (Xu, 2012). Different forms of life including diverse microorganisms, animals, plants, their ecosystems and the genes they contain on the earth is called biodiversity (Khan et al. 2016; McCarthy et al. 2012; Rawat and Agarwal, 2015). On the other hand, Rao and Hodgkin (2002) defined biodiversity as the variation existing in all species of animals and plants, their genetic material and the ecosystems in which they occur. Three levels of biodiversity are characterized within an area, biome or planet (Fig. 1). These are ecosystem diversity, genetic diversity and species diversity (Khan et al. 2016; Rawat and Agarwal, 2015; Nuijten et al. 2016). Ecosystem diversity means different habitats, ecological process and biotic communities within the biosphere (McMahon et al. 2014). Species diversity is defined as the variety of species within an ecosystem, while, genetic diversity refers to the variation within species and population (Nuijten et al. 2016) or variation in genes and genotype

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(Rao and Hodgkin, 2002). Despite the fact that genetic diversity is important for species adaptation and survival, it is also a major component in the ecosystem (Rawat and Agarwal, 2015). Existence and evolutionary success of many living organisms depend on the genetic diversity (Gülcü and Bilir, 2017; McMahon et al. 2014). Thus, biodiversity can also mean the variability within and between species, and between ecosystems (McCarthy et al. 2012: McMahon et al. 2014). In both genetics and genomics, diversity is recognized as one of the most fundamental levels of biodiversity together with ecosystem diversity, species diversity and community diversity (McMahon et al. 2014).

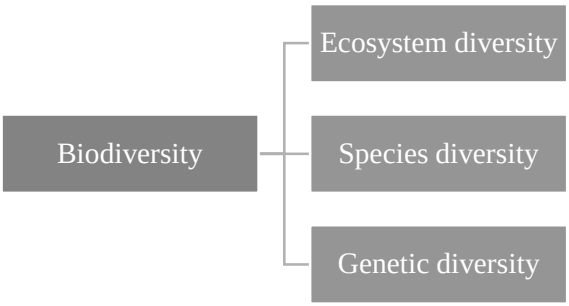

Fig.1: Three levels of biodiversity within a biome

There are several biotic and abiotic factors that have negative impacts on biodiversity (Bahrndorff et al. 2016). These factors are shown in Fig. 2, and include predation, parasitism, competition, diseases, and separation due to human actions, habitat alteration, climatic changes and natural catastrophes (Hoffmann et al. 2015; Khan et al. 2016), introduction of exotic species, destruction of natural habitat as well as killing of natural components of a population (IUCN, 2015; Mazzotti, 2014). These factors not only cause decrease in biodiversity of plants and animals, but also cause extinction of biodiversity of some species (De Vos et al. 2015). Most of these factors provoke displacement of species from their natural habitats, retreating, and completely vanish from the wilderness (Mazzotti, 2014). Because of these threats, several studies have suggested the use of genomic tools as effective methods for conservation of plants and animals biodiversity (Aravanopoulos, et al. 2015; De Vos et al. 2015; Khan et al. 2016; IUCN, 2015; Lyantagaye, 2013). The main concern of researchers is to maintain rare and endangered species of plants and animals via genomic methods (Aravanopoulos, et al. 2015; Nuijten et al. 2016; Shafer et al. 2015). Therefore, based on genomic studies there are genomic conservation tools developed in order to stop dwindling of biodiversity (Fig. 3).

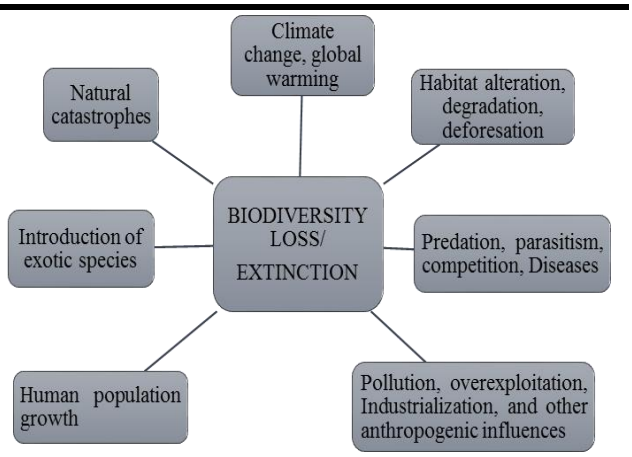

Fig. 2: Summarized factors that have negative impacts on biodiversity

Currently, the most used genetic tools in plants and animals include amplified fragment length polymorphism (AFLP), DNA and RNA sequence analysis, and DNA finger printing, microsatellites, minisatellites, and random amplification of polymorphic DNA (RAPD), random fragment length polymorphism (RFLP), single strand conformation polymorphism (SSCP) and single nucleotide polymorphisms (SNPs). Summary of these tools is shown in Fig. 3. Current individuals' DNA or historic DNA is used by these tools to analyse genetic variation in species or population (Khan et al. 2016). Because of the development in high throughput next generation sequencing, other tools are used in population and conservation genomic in forest and fruit tree. (Aravanopoulos et al. 2015). Numerous techniques and tools exist that use genomic methods to conserve plant and animal biodiversity. McCormack et al. (2013) emphasize that, if there is a need to apply genomics in conservation such as in endangered plant and animal species, the genomes of these species must be sampled at considerable densities and with extra markers.

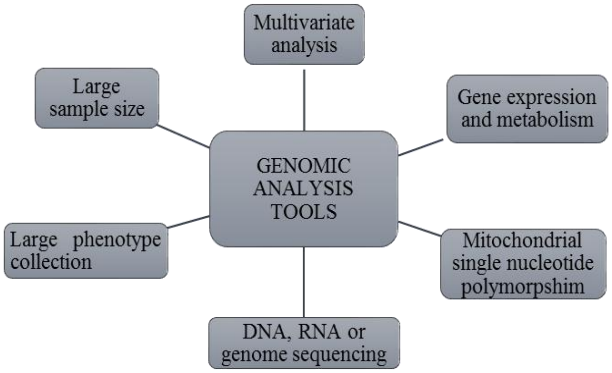

Fig. 3: Summary of genomic tools used to detect genetic variations in plants and animals

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III. WHY GENOMICS?

Understanding the genetic structure and composition of plants and animals in their habitat is important for developing successful management strategies for their conservation. Declining of global biodiversity of plants and animals attract attention of biologists and ecologists towards conservation (Aravanopoulos, et al. 2015; Nuijten et al. 2016; Rutledgea et al. 2012; Catchen et al. 2013; Hoffmann et al. 2015). In order to reduce loss of plant and animal biodiversity, researchers have been using genomic methods and techniques (McMahon et al. 2014). Genomic methods allow collection of extensive genetic information of phenotypic and ecological data from many species in numerous populations and individuals (Hohenlohe et al. 2012; Khan et al. 2016; Trinh et al. 2017). These genetic data are used to identify the signatures of selection and adaptive genetic variation on a complete genome scale (Catchen et al. 2013). The data can also be used to provide a possibility to differentiate nearly related but adaptively different populations (Hohenlohe et al. 2012). Further, Perry et al. (2012) claimed that, it is possible to gather huge amounts of data and sequence any species at moderate effort than previous due to advancement of genomics technology. Genomics plays an important role in conservation of plants and animals (Gardner et al. 2016; Godoy, 2016; Khan et al. 2016; McMahon et al. 2014). It aids to determine the genome segments responsible for adaptation, and improve our knowledge on microevolution through a better understanding of positive mutation, selection and recombination (Funk et al. 2012; Gülcü and Bilir, 2017; Trinh et al. 2017). It helps to identify essential genes for fitness and eventually develops modern monitoring tools for endangered plant and animal species (Godoy, 2016). Development of potential genomic tools has enabled studies of population structure, current demographic events and genetic variations in threatened species of plants and animals (Grueber, 2015). With advanced genomics tools it is possible to detect harmful mutations in the genes for metabolism, functions, immunity and in any part of living organism, plants and animals (Grueber, 2015; Khan et al. 2016). Techniques and tools of genomics are used to detect variations linked with conservation and population structure from the genome of various species (Khan et al. 2016; Mcmahon et al. 2014). Genomics analysis tools give researchers a deeper level of understanding the organisms in their environments (Funk et al. 2012; Gardner et al. 2016; Jones et al. 2013), and to track the movements of individual organisms (Simpson et al. 2017). For example, more than 3000 individual of humpback whales (*Megaptera novaeangliae*) have been distinguished using genetic fingerprints obtained from skin samples (WCS, 2017). This used genomics techniques by carefully comparing selected markers in the DNA of thousands of whales. According to WCS (2017), this technique is important for monitoring the movements of the whales from the South Atlantic to the Indian oceans, and conservation of the whale populations. Similarly, genomics tools like this can help to establish whether how and when the interaction of different species of the population occurs. Furthermore, a study on water voles in Scotland to understand if their survival is threatened by mink was done using DNA microsatellite markers, the DNA study showed that the mink is not a threat to the population of water voles because a result revealed high genetic mixing levels (Melis et al. 2013). In addition, the DNA studies on Pipistrelle bats in UK confirmed that there are two species but not a single species. Furthermore, Aravanopoulos et al. (2015) claimed that, advanced genomics accelerate the rate of conservation genomics in forest plants. This is because of the development in high throughput next generation sequencing capabilities.

In the absence of phenotypic information, the genomics has made possible to identify population with adaptive compatibilities on the basis of genetic data (Tian et al. 2017; Trinh et al. 2017). Ecologists and biologists have been trying to discover genes that support local adaptation in certain species in ecosystems. Understanding the genetic architecture of local adaptation is fundamental to defining conservation units, determine conservation priorities and design restoration programmes for threatened or endangered plant and animal species (Miller et al. 2012). Catchen et al. (2013) opined that, identification of genetic diversity is important for the adaptation of populations at their local habitats, and it can be used to design a biodiversity conservation framework. For example, the population with specific adaptive alleles can be identified and used to supplement the endangered population or reintroducing species into the habitat in which the natural population has vanished (Simpson et al. 2017). The loss of genetic diversity and inbreeding accumulation due to fragmentation and decrease of population may compromise the viability of population (Casas-Marce et al. 2013). An example of organism showing this is the Iberian lynx (*Lynx pardinus*) which is in the edge of extinction (Godoy, 2016). Mitochondrial sequences and 36 microsatellite markers were used to evaluate the current genetic status of the Iberian lynx and to assess the genetic signatures of its past history (Abascal et al. 2016; Casas-Marce et al. 2013). Species' mitochondrial diversity was found to be very low with only two haplotypes; furthermore, Abascal et al. (2016) and Godoy (2016) showed that the levels of genetic diversity at microsatellite markers were very low in both

remnant populations. Yet, genetic differentiation between the two populations was high. By using genomic tools, Abascal et al. (2016), Casas-Marce et al. (2013) and Godoy, (2016) concluded that, the present genetic patterns in the *L. pardinus* are because of the result of its recent decline and fragmentation. Therefore, conservation measures can be taken to stop further population fragmentation and decline. Although a recovery of endangered species of plants and animals is hindered by excessive population decline, genetic erosion can make it more badly (Godoy, 2016). In order to appreciate the patterns of genomic erosion and how this affect species viability of plants and animals we need to use genomics tools. This is because these tools help to conserve species, and contribute to save species from extinction (Aravanopoulos et al. 2015; Karolchik et al. 2014). Development of genomics has resulted into increase in the number of species with whole-genome sequence data (Ellegren, 2014; Grueber, 2015; Rutledgea et al. 2012). This has made availability of genome resources to most endangered plant and animal species (Ellegren, 2014; Grueber, 2015; Karolchik et al. 2014). Conservation of plants and animals is growing and promises to modernise the population genetics field due to the use of genome-wide single nucleotide polymorphisms (Rutledgea et al. 2012). In some species, a relationship between environmental characteristics and the distribution of genotypes can be detected using genomics technology, showing the importance of natural selection as the main source of differentiation (Hasbún et al. 2016).

Considering the species richness of Africa, including many endangered species, it is imperative to adopt usage of genomic methods for biodiversity conservatory purposes. Since genomic techniques and tools are becoming cheaper and more accessible (McCormack et al. 2013), biologists and researchers in Africa can use these tools to conserve and manage plant and animal biodiversity. Furthermore, the use of genomic analysis tools can be used to assess and track the distribution of threatened and endangered plants and animals (Bowden et al. 2012; Steiner et al. 2013). This makes conservation of plant and animal biodiversity very easier, and thus reducing the rate of biodiversity loss and even extinction from the wild (Hongbo et al. 2015). Genomics has enabled studies of how climate change has limited biodiversity by looking at DNA of ancient preserved specimens of plants and animals to understand, and how biodiversity has changed with time (Fitzpatrick et al. 2012; Johnson and Koepfli, 2014; McMahon et al. 2014; Miller et al. 2012). These techniques can further inform sound policy decisions for conservation and management of wildlife biodiversity against climate change effect. It is apparent, therefore that accurate usage of genomic tools can result into considerable conservation of Africa's biodiversity, to sustainably meet plant and animal species' demands for future generations.

IV. CONCLUSIONS

This review has demonstrated that biologists, conservationists, ecologists and researchers should appreciate the conservation benefit resulting from genomic methods especially for plants and animals. Advanced genomics plays a vital role in biodiversity conservation and produces genetic data that help researchers to know the interaction between ecosystem and organisms, and among organisms themselves. The information extracted from them through genomic techniques can be used to develop methods for biodiversity conservation. Moreover, the development of conservation genomic tools can enhance our understanding of the genetic variation and structure of plants and animals. Researchers in developing countries are highly encouraged to use the advanced genomic methods to improve biodiversity conservation sector and reduce loss of plants and animals. Genomic conservation is very crucial as this is key to understanding the genetic structure, relationships of phylogenetic, causes and reasons for loss of genetic diversity in plants and animals. Finally, if well utilized, genomic methods can guide decision making in our conservation strategies and policies in Africa.

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Isolation, Identification and Characterization of Keratin degrading microorganisms from Poultry soil and their Feather degradation Potential

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***Abstract*—** *Keratinolytic microorganisms have a great importance in poultry waste degradation and its bioconversion to compost or animal feed. The aim of this study was to isolate keratin degrading bacteria and fungi from poultry farm soil, and to study their ability to degrade chicken feathers. The poultry farm soil samples were added in basal medium with feathers as a source of carbon and nitrogen. Five bacterial cultures were isolated. Bacteria were grown in basal media with feathers meal and showed feather degrading capacity. Bacterial strains were identified as Aeromicrobium spp., Exiguobacter spp., Marinococcus spp. and Bacillus spp. 1 & Bacillus spp 2.These bacteria showed keratinolytic enzyme activity in the cell free culture supernatants. The highest biodegradation of feathers was obtained using Aeromicrobium (KD1-72.55%), among the isolated cultures. Two fungal cultures (F1 and F2) were also isolated by Hair Bait Technique, out of which F1 showed good keratinolytic activity. The good ability of selected microorganisms to degrade feathers can be utilized for their potential biotechnological application in processing of feather waste from poultry industry.*

Keywords*— *Feather degrading Bacteria,Characterization, Identification, Keratinase, Poultry waste.

I. INTRODUCTION

The day by day increase in consumption of meat received from chicken is causing harsh effect to environment, as the waste from the chicken birds, more particularly the feather are not properly treated. While in nature, the deterioration of feather is slow, generating sulphureous compounds, causing environmental problem. Feathers, which are almost pure keratin proteins, are produced in large amounts and constitute a waste by product at poultry processing plant. A total 5-7 % weight of mature chicken comprises of feathers. Feather waste is generated in large quantities as a byproduct of commercial poultry processing. Feathers are made up primarily of keratin which is resistance to common proteolytic enzyme such as pepsin, trypsin and papain [1] .World-wide poultry processing plants produce millions of tons of feathers as a waste product annually, which consists of approximately 90% keratin; the keratin is largely responsible for their high degree of recalcitrance if remain untreated.

Keratin is a major component of hair, feathers and wool and is the most complex of the cytoskeletal intermediate filament proteins of epithelial cells [2]. Keratin is an insoluble protein macromolecule with very high stability and low degradation rate. Keratin is mainly present in hair, feather, nails, wool and horns. High protein content of keratin waste can be used as a good source of protein and amino acids by systematic recycling. The prospective use of keratinases is in diverse applications where keratins should be hydrolyzed, such as the leather and detergent industries, textiles, waste bioconversion, medicine etc. Recycling of feathers can provide a cheap and alternative protein feed stuff. Further this can be used for animal feed and for many other purposes. However, poor digestibility of keratin is a problem in recycling.

Keratinase is an extracellular enzyme used for the bio degradation of keratin. Keratinase is produced only in the presence of keratin substrate. Keratinase attacks the disulfide bond of keratin to degrade it. Some microbes have been reported to produce keratinase in the presence of keratin substrate. Keratinase producing microorganisms have the ability to degrade chicken feather, hair, nails, wool etc. Keratinolytic enzymes are widespread in nature and are produced by several microorganisms including bacteria such as *Bacillus sp.*[3-8], *Fervidobacterium islandicum* [9], *Elizabethkingia meningoseptica* KB042 [10], *Pseudomonas aeruginosaKS1*[11] and Actinomycetes such as *Streptomyces sp*. [12-14] and fungi such as *Chrysosporium tropicum* [15], *Trichoderma atroviridae* [16], *Doratomyces*

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microsporus [17]; *Paecilomyces marquandii* [18]; *Scopulariopsis brevicaulis* [19]; *Alternaria, Paecilomyces, Penicillium, Curvularia* and several *Aspergillus sp*. [20]. Diversity of keratinolytic fungi in soils have been studied and reported [21, 22]

Keratinophilic fungi are generally considered as soil saprophytes. Soil that is rich in keratinous material is most conducive for the growth and occurrence of keratinophilic fungi. Keratin decomposition in soil leads to an increase in carbon, and nitrogen ratio in soil. They are therefore fast growing nonpathogenic keratinophilic fungi which can be utilized for the recycling of keratin in soil and may be exploited for their biotechnological potential in industry.

Keratinase which are produced by these keratinolytic organisms could be used to degrade feather waste and further the digested products could be an excellent material for producing animal feed, fertilizers or natural gas [23]. Use of keratinolytic organisms for feather degradation is an economical, environmentally friendly alternative. Keratinolytic proteases offer considerable opportunity for a low cost technology for biotechnology of poultry feather from pollutant to nutritionally upgraded protein feed for a livestock [24]. Most feather waste is land filled or burnt which involves expense and can cause contamination of air, soil and water. Utilizing poultry feathers as a fermentation substrate in conjunction with keratin degrading microorganisms and enzymatic degradation may be better alternative to improve nutritional value of poultry feathers and reduce environmental waste [25].It would also solve the waste disposal problem of poultry waste and recycling of keratinacious waste would be beneficial financially and environmentally.

Submerged fermentation of poultry waste by microorganism producing keratinase helps in the conversion of non-soluble keratin (feather) into soluble protein or polypeptide[26].These protein byproduct may be used as animal and livestock feed, and as leather filling agents[27]. Keratinase has also emerging application in de-hairing process in leather industry instead of sodium sulphides [28].

In view of above, the present study was aimed to isolate, identify and characterize the kearatin degrading microorganisms from poultry farm soil and study their feather degradation potential.

II. MATERIALS AND METHODS

2.1 Chemicals: All Chemicals required for experimental work were of analytical grade, pure and purchased from Hi-media laboratory.

2.2 Sample collection: Soil samples were collected from regular feather dumping site of poultry processing farms from outskirts of Pune, in sterilized sampling bottles. The samples were taken from 30 cm depth from the surface of the soil. The samples were brought to the laboratory and processed for isolation of microorganisms.

2.3 Processing of chicken feathers and Preparation of feather meal broth

Chicken feathers were washed thoroughly with tap water and dried. The dried feathers were defatted by soaking in diethyl ether for 24 hrs, and washed thoroughly with tap water and distilled water, air dried and cut into small pieces before autoclaving, the processed feathers referred as Feather meal. The medium used for keratinase production contained the following constituents (g/100ml.) - Feather meal 5gm, NaCl 0.005, K_2HPO_4 0.038, KH_2PO_4 0.04 $MgCl_2.6H_2O$ 0.02, Yeast extract 0.01, pH 7.5

2.4 Isolation and screening of keratinolytic bacteria

Soil suspensions were made with 0.9% saline and inoculated in feather meal broth and incubated on rotary shaker at room temperature. After visible turbidity was observed, serial dilutions of the culture suspensions were spread on skimmed milk agar plates for selection of protease producing bacteria, as per the method described earlier [29]. The petri plates were incubated at 30ºC for 24 hours. The isolated bacterial colonies showing zone of clearance on skimmed milk agar were selected for further studies. The isolates were characterized for colony characteristics, morphological characteristics and biochemical characteristics and identified with Bergey's manual of determinative bacteriology.

2.5 Isolation of keratinolytic fungi

The keratinophilic fungi were isolated using 'hair baiting techniques' [30]. In this technique sterile Petri plates were half filled with soil and short strand of sterilized chicken feathers were spread over the surface of soil. About 10-12 ml sterile water was added to Petri plates for the facilitation of fungal spores to germinate. Petri plates were incubated at 30˚C for 3-4 weeks. After 3-4 weeks the colonies were observed on surface of feathers, were picked up and grown on Potato Dextrose Agar, for purification and identification.

2.6 Production of keratinase Enzyme and feather degradation by bacterial and fungal isolates

Cultivation of the isolated cultures was performed using 250 ml Erlenmeyer flask containing 90 ml of Feather meal broth medium.10ml of overnight grown culture of each isolate were inoculated and incubated on rotary shaker at 150 rpm for 7 days. Control was feather meal broth without any inoculum. Growth was observed for visible turbidity

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and recorded a6 600 nm, degradation of feathers was visually observed.

2.6.1Extraction of Enzyme

The culture medium was filtered through WhatmannNo.1 Filter paper to remove un-degraded residues. The filtrate was then subjected to centrifugation at 10,000 rpm for 10 min to remove bacterial residue. After centrifugation keratinase activity was determined in supernatant.

2.6.2 Determination of feather degradation

The feather degradation was studied according to the method described by Kumar et. al[31]. The five bacterial isolates namely KD1, KD2, KD3, KD4 and KD5 were inoculated in feather meal broth with 1% feathers as the sole source of carbon and incubated on rotary shaker for one week. After one week the residual feathers remained was determined gravimetrically by filtering the culture broth and taking the weight of filter paper before and after filtration. Percent reduction of feathers was calculated from the difference in the initial weight and weight obtained after one week of incubation.

2.6.3 Assay for keratinase activity:

Keratinase activity was assayed according to the method of [32].Each culture filtrate was Centrifuged at 5000 rpm for 30 min.20 mg feather meal +3.8 Tris HCl buffer+0.2 ml supernatant of culture filtrate were taken ; control was kept where 0.2ml cultures supernatant was replaced with distilled water. the tubes were incubated at 30^0C for 1 hour then chilled in ice water for 10 minute, filtered and OD was taken at 280 nm.O.D. values were converted into enzyme unit/ ml Enzyme Units per ml was calculated by using following formula:

Enzyme Units per ml= Optical Density x 4 x dilution rate /0.01 x T

Where T = incubation time, 4= total volume used.

2.6.4 Effect of incubation time on growth of isolates

The effect of incubation time on growth of isolates was determined in feather meal broth for 7 days. Culture samples were added namely KD1, KD2, KD3, KD4, KD5, F1 and F2 and with one control flask which was without culture. Feather meal broth 100 ml with 1 gm feathers was added with 2 ml overnight culture in each flask ,incubated on rotary shaker 150 rpm. OD was taken at 600 nm, every 24 hours, up to 7 days for monitoring growth.

2.6.5 Effect of different temperatures on keratinase enzyme activity

The optimum temperature for keratinolytic protease activity was determined by performing the enzyme reaction at incubation temperatures between 30ºC to 90º C.40 mg feathers+7.5 ml Tris HCl buffer+0.4 ml supernatant of culture filtrate of each isolate were taken in sterile test tubes, and tubes were incubated at different temperatures (30ºC, 40ºC, 50ºC, 60ºC, 70ºC, 80ºC, and 90ºC) for 1 hour. After incubation at different temperatures all tubes were chilled in ice water for 10 minute. Filtered & OD was taken at 280 nm.O.D. values were converted to enzyme unit/ ml.

2.6.6 Effect of different pH on keratinase enzyme activity

Keratinolytic protease activity was studied in the pH range of 4 to 9 using 0.2 M Tris- HCl buffer. 40 mg feathers + 7.5 ml of bufferhaving different pH from 4-9, to which 0.4 ml supernatant of culture filtrate of each isolate was added. All tubes were incubated at 30^0C for 1 hour, and then chilled in ice water for 10 minute to stop the enzyme reaction, then filtered & OD was taken at 280 nm. O.D. values were converted to enzyme unit/ ml.

III. RESULTS AND DISCUSSION

Soil samples were inoculated in feather meal broth to obtain bacterial isolates which are feathers degrading and were capable of producing extra cellular keratinase, using feather (keratin) as sole carbon source. After a week of incubation the flasks showed turbidity and disintegration of feathers were observed, (fig 1).

Feather meal Broth

Growth observed

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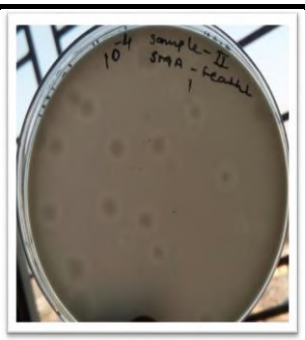

Bacterial Isolates with zone of Clearance on Skim Milk Agar

Fig 1: Growth on Feather meal broth and isolates on Skim milk agar

The same culture suspension was serially diluted up to 10^{-8} with normal saline and plated on skim milk agar for, selection of keratinolytic bacteria. The colonies showing zone of clearance were further selected and characterized. Fig 2 shows the fungal isolates by bait technique.

Feathers with poultry soil

Fungal Growth observed on feathers after 5 weeks

Fig 2: Bait Technique for isolation of keratinolytic Fungi

Colonies showing zone of clearance on skim milk agar were counted. Table 1 shows the bacterial counts of soil samples 1 and 2 on Nutrient agar plates and Skim milk agar plates. The colonies showing zone of clearance were selected and observed for colony characteristics, morphological characteristics and biochemical characteristics. Based on colony characteristics five types of bacterial isolates were obtained, these isolates were designated as KD1, KD2, KD3, KD4 and KD5.

Table.1: Bacterial Count on Nutrient Agar (NA) and Skim Milk Agar (SMA)

		NA Plates CFU/ml			**SMA plates CFU/ml**	
Dilutions	**10^{-4}**	**10^{-6}**	**10^{-8}**	**10^{-4}**	**10^{-6}**	**10^{-8}**
Bacterial Count, Soil sample1	**21 x 10^5**	**12x 10^6**	**4 x 10^8**	**4 x 10^4**	**1 x 10^6**	**1 x 10^8**
Bacterial Count, Soil sample 2	**14 x 10^5**	**8 x 10^6**	**2 x 10^8**	**3 x 10^5**	**2 x 10^6**	**1 x 10^8**

Table 2 shows the colony characteristics of the bacterial isolates. The morphological characteristics of the isolates are shown in Table 3.The results of biochemical characterization of the bacterial isolates are shown in Table 4.On the basis of colony, morphological and biochemical characteristics and reference to Bergey's manual of determinative bacteriology the five cultures KD1, KD2, KD3 KD4 and KD5 were identified up to genus level and were identified as *Aeromicrobium Sp*., *Exiguobacter*Sp., *MarinococcusSp*,*Bacillus Sp1 and Bacillus Sp2*.Most of the studies on isolation of keratinolytic organisms have resulted in isolation of Bacillus spp, however in the present study the isolated bacteria *Aeromicrobium*, *Exiguobacter* and *Marinococcus* have been reported for the first time.

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Table.2: Colony and Morphological Characteristics of the Bacterial Isolates

Colony characteristics	Shape	Size	Colour	Margin	Opacity	Consistency	Elevation
Isolates KD1	Irregular	Pin point	Cream	Irregular	Opaque	Sticky	Flat
KD2	Irregular	Pin point	Orange	Irregular	Opaque	Sticky	Flat
KD3	Circular	Pin point	Orange	Regular	Opaque	Sticky	Convex
KD4	Circular	2-4 mm	Cream	Regular	Opaque	Sticky	Flat
KD5	Circular	3-5 mm	White	Regular	Opaque	Sticky	Flat

Table.3: Morphological characteristics of the isolates

Morphological Characteristics of the isolates	KD1	KD2	KD3	KD4	KD5
Gram Reaction	**Gram + ve short rods**	**Gram + ve long rods**	**Gram +ve Coccobacilli**	**Gram +ve rods**	**Gram +ve rods**
Motility	**Non-motile**	**Motile**	**Actively motile**	**Actively motile**	**Motile**
Endospore Staining	**Non spore forming**	**Non spore forming**	**Non spore forming**	**Spore forming**	**Spore forming**

Table.4: Biochemical Characterization of the Bacterial Isolates

Sugar	KD1	KD2	KD3	KD4	KD5
Glucose	+	+	+	+	+
Cellulose	-	-	-	-	-
Fructose	-	+	+	+	+
Raffinose	-	-	-	-	-
Galactose	-	+	-	-	-
Maltose	+	+	+	+	+
Sucrose	-	+	-	+	+
Mannitol	-	-	-	-	-
Arabinose	-	-	-	-	-
Lactose	-	-	-	-	-
Xylose	-	-	-	-	-
Oxidase	+	-	-	+	+
Catalase	+	+	+	+	+
Citrate utilization	-	-	-	-	-
Gelatinase	-	+	-	-	-
Nitrate reduction	-	+	-	-	-
Caesin hydrolysis	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+
Identified organism	***Aeromicrobium* spp**	***Exiguobacter* spp**	***Marinococcus* spp.**	***Bacillus* spp 1**	***Bacillus* spp.2**

Out of five bacterial isolates, two isolates have been identified as *Bacillus spp*. as also reported by others[24,35]. In our study all the bacterial isolates have been found to be gram positive, whereas others have reported keratinolytic activity by gram negative bacteria [33]. Two fungal cultures, F1 and F2 were also isolated by bait technique and were found to grow on feather meal broth. However they could not be identified. Many researchers have worked on isolation and characterization of bacteria from poultry processing wastes and shown to degrade feathers, hair or

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wool, but the optimization for industrial production of keratinases still remains to be done.

Table 5 shows the results of feather degradation experiment.

The result shows that KD1 was the most effective and showed 72.55% feather degradation , it is followed by KD2- 70.59%, KD5-60%, KD4- 58.82% and KD3- 55%.

Table.5: Feather degradation by the isolated bacterial cultures

Culture sample inoculated in Feather Meal Broth (FMB)	Initial weight of filter paper with feathers(gm)	Final weight of filter paper with feathers(gm)	% reduction of feathers
KD1 (*Aeromicrobium* spp.)	1.02	0.28	72.55%
KD2 (*Exiguobacter* spp.)	1.02	0.3	70.59%
KD3 (*Marinococcus* spp.)	1.02	0.45	55.88%
KD4(*Bacillus spp. 1*)	1.02	0.42	58.82%
KD5(*Bacillus spp. 2*)	1.02	0.40	60.78%

Effect of incubation time on growth of microbial isolates is shown in figure 3. KD4 and KD5 isolates entered stationary phase after 48 hrs, KD3 and KD1 on 3rd day, while KD 2 on 4th day. After day 5 all isolated entered the decline phase. The highest keratinase activity was shown by KD1(18 U/ml), followed by KD 2, KD3, KD5, and KD4, as shown in fig 4.

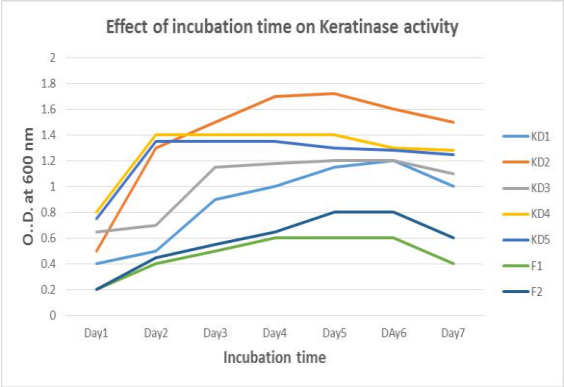

Fig.3: Effect of Incubation Time on Growth of Isolates

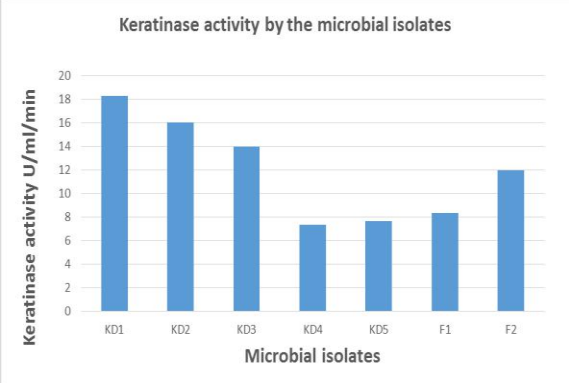

Fig.4: Keratinase Activity with The Isolated Cultures

Whereas other workers have reported maximum keratinase activity of 13.6U/ml and 8.8 U/ml[34]. F2 fungal isolate showed Keratinase activity of 12U/ml.

Fig.5 shows the result of the effect of different pĤ on keratinase activity, it was observed that the optimum pĤ for the bacterial isolates KD1, KD3, KD4, KD5 and fungal isolates F1& F2 was 9, whereas for KD2 it was 7.Most of the isolates in this study have shown higher activity at alkaline pH as also reported by Inamdar et.al [36]. Keratinase from the most of the bacteria, actinomycets and fungi have pH optima in neutral to alkaline range. Enzyme with optimum activity at alkaline pH has definite advantages in application, both in degradation of feathers as well as in leather industry.

The effect of temperature on keratinase activity is shown in figure 6. It was observed that the optimum temperature was 30°C for all the bacterial isolates, while others have reported optimum temperature of 40±2oC [14]. For F1 and F2 optimum temperature was found to be 50°C.

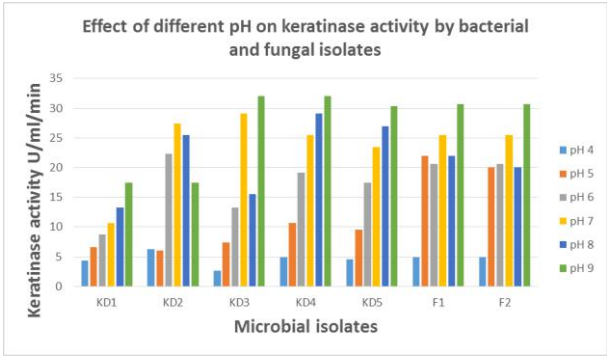

Fig.5: Effect of pH on Keratinase Activity

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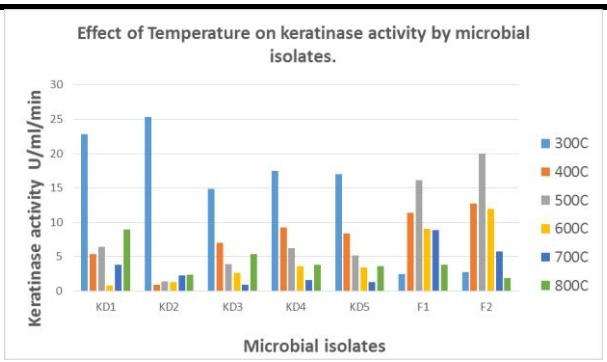

Fig.6: Effect of Temperature on Keratinase Activity of The Isolates

Feather degradation to the range of 70-72% by newly isolated *Aeromicrobium* and *Exiguobacter spp.* even under unoptimised condition can be further exploited for efficient degradation of feathers under optimised conditions.

In soil feathers are degraded by a consortium of bacteria and fungi, which act in synergy or compete for keratin.

Biodegradation by microorganisms possessing keratinolytic activity represents an alternative attractive method for improving the nutritional value for keratin wastes, as it offers cheap and mild reaction conditions for the production of valuable products there have been some reports on microorganism capable of degrading keratinous wastes.Further optimization of keratinase production and characterization of the keratinase would be helpful in application of keratinases on a large scale for degradation of keratin containing wastes.

IV. CONCLUSION

In the present study five bacterial cultures were isolated producing keratinase from habitats where keratin containing substrate were disposed in natural conditions. The five bacterial isolates were characterized and identified based on colony morphology, growth characteristics and biochemical characteristics. They were identified as belonging to genera *Aeromicrobium spp., Exiguobacter spp., Marinococcus spp., Bacillus spp1, Bacillus spp,* respectively. The isolate *Aeromicrobium spp*. (KD1) shows the highest feather degradation of 72.5%.The optimum temperature was 30°C for all the bacterial isolates, whereas for F1 and F2 it was 50°C.The optimum pH for bacterial and fungal isolates found to be 9except for KD2 it was 7.Feather degradation to the extent of 70-72% by newly isolated *Aeromicrobium* and *Exiguobacter spp.* even under unoptimised condition can be further exploited for efficient degradation of feathers under optimized conditions.

The ability of newly isolated bacteria to degrade feathers can be utilized for their potential biotechnological application in processing of feather waste from poultry industry. For the evaluation of biotechnological application of keratinase, however require more detailed understanding of the factors that enable this enzyme for complete degradation of native keratinase substrate. Therefore additional research will need to done for purification, characterization of keratinase, studying kinetics of enzymes, testing from various range of substrate, effect of inhibitors, and inducer on enzyme activity, submerged state fermentation for large scale production of keratinase. Further studies can be focused on whether consortium of bacteria and fungi can be utilized for feather degradation, rather than individual cultures for enhanced keratinolytic activity.

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Present Management of Common-pool Resource:Sinnakalapu Lagoon in Alayadivembu Pradeshiya Sabha, Ampara District, Sri Lanka

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Abstract— *In order to rectify common problems associated with common pool resource management different types of effective and efficient common pool resource management systems have been developed by experts though seldom published.Sinnakalapu lagoon is a brackish water ecosystem with low salinity and managed as a common pool resource of the five villages in Alayadivembu Pradeshiya Sabha. Flora and fauna present include35fin-fish species, four shrimp species, twelvebird species and a significant number of aquatic plants. These identified and biodiversity status of identified flora and fauna of Sinnakalapu was described under two-time periods before tsunami, (1990-2004) and after tsunami, (2005-2017).Ecological importance of Sinnakalapu is described based on biological, chemical and physical features of Sinnakalapu ecosystem. Mean annual income per year by fisheries resources in Sinnakalapu wasestimated. Based on this the economic value of Sinnakalapu was described. Mismanagement practices leading to the loss of biodiversity were identified and their impacts were analyzed. Numbers of fin-fishes, shrimps, insects, birds and aquatic plant species have reduced and some of the species have got extinct. Conservation and suitable management systems for sustainable management were identified concurring with the environmental policies and laws in Sri Lanka.*

Keywords— Alayadivembu Pradeshiya Sabha, Biodiversity, Common pool resource management, Sinnakalapu, Tsunami.

I. INTRODUCTION

Common property resource (CPR)is a private property of a group and the group may vary in size, nature and internal structure e.g. indigenous tribe, neighbours of village [1]. Common property resource includes fisheries, forests, irrigation systems, and pastures. Global CPR examples include the oceans and atmosphere. Major problems in CPR management is difficulty in excluding users, combined with a CPR's subtractability, creation management vulnerabilities that can result in resource degradation. The importance of such "tragedies" is evident in anecdotal examples, from the devastation of tropical rainforests to the depletion of local and regional fish stocks and also, atthe same time, many examples that suggest that people are capable of averting these tragedies and sustaining common pool resource [2].

Sinnakalapu is situated in Akkaraipattu south, south eastern coastal belt and very close to the Bay of Bengal. The river named as Thillaiaaru flows throughPanankadu bridge in this area. Sinnakalapu lagoonis located in coordinates 7°11' 31.6" N and 81°50' 54.9" E with an area of about 400 hectares [3]. Only during rainy season, this resource reaches the sea which makes it function as a lagoon.However due to continuous flowing of river water, salinity of water nears that of freshwater.

Sinnakalapu is surrounded by five villages viz., Kolavil, Panankadu, kannakipuram, Alayadivembu and Sinnamugathuwaram in Alayadivembu Pradeshiya Sabha. This lagoon supports the villages in various ways including e.g. economically, ecologically and culturally. Sinnakalapu lagoon is a home for diverse variety of aquatic plants, fishes, reptiles, insects, mollusks, crustaceans, etc. Fisheries resource is the main economically important resource in Sinnakalapu Lagoon. It contains both fresh water and brackish water fin-fish species, which includes both endemic and exotic fish species [Table 2] and also there are about four shrimp species identified in Sinnakalapu [Table-1]. These diverse variety of fish species and shrimps play an important economic role in Alayadivembu Pradeshiya Sabha area. This lagoon supports the villages directly and indirectly. Fisher men are the main stakeholders and the direct economical beneficiaries of Sinnakalapu resource. Farmers, especially paddy cultivators and local people are indirectly supported by cultivating paddy in lands close to river, which depends on this river water. Fish resources play an important role in the diet of local people in Alayadivembu Pradeshiya Sabha. Sinnakalapu lagoon provides fresh, protein rich, healthy and tasty fishes such as *Oreochromis niloticus*, *Channa striata*, *Anabas testudineus*, and cat fishes [4].

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Presently, there are several conflicts within the Fisheries Society and between Fisheries Society and Farmers' Society because of mismanagement practices including illegal fishing methods, land degradation and fragmentation of lands for paddy cultivation, human settlements and construction of hotels and timber mills which lead to loss of fish resources and biodiversity in Sinnakalapu lagoon [5,6,7]. There is a need to conserve the biodiversity of Sinnakalapu by identifying the ways to mitigate the mismanagement practices and proposing a well-defined plan of common pool resource management concurring with environmental policies and laws in Sri Lanka.

1.1 Objectives

The main objective of this project is to propose a sustainable common pool resource management plan for Sinnakalapu lagoon.

Specific objectives:

- To study the ecological and economic importance of Sinnakalapu.
- To identify the mismanagement practices which lead to the loss of biodiversity in Sinnakalapu.
- To identify and study the ways to mitigate the mismanagement practices in Sinnakalapu.
- To identify and implement the polices and laws in Sri Lanka to control the mismanagement practices in Sinnakalapu.
- To identify the shadow laws which can be implemented in Sinnakalapu

II. METHODOLOGY

Weekly field visits to Sinnakalapu lagoon for the data collection with respect observation of biodiversity, mismanagement practices, local fish marketing, and small-scale fish marketing to other areas by local people were performed duringthe period of December, 2017 to April, 2017. Photographs were taken to identify the fish, bird, shrimp and aquatic plant species found in Sinnakalapu with the help of Fishermen and other local people. Several meetings were conducted with the leaders and the members ofFisheries Societyand well as Grama Niladhari of Alayadivembu Pradeshiya Sabha to collect details about the mismanagement practices, biodiversity status, conservation and management plans.

III. RESULTS AND DISCUSSION

3.1 Biodiversity of Sinnakalapu

Time scale to explain the biodiversity of Sinnakalapu is divided as period before Tsunami (2004, December, 26) snd, after Tsunami. This is because there was a serious impact on biodiversity due to Tsunami and other development practices after Tsunami, which has led to the reduction of biodiversity in Sinnakalapu.

When the composition of fauna is considered, some needs the movement of the river water to survive, while some have to hold onto the rocks at the bottom in this moving water with the help of differently developed structures. Others thrive in stagnant waters. There are a variety of fin-fishes, birds,shrimps, insects, amphibians, and crustaceans that make Sinnakalapu lagoon as their home.With respect to flora, this lagoon supports diverse varieties of aquatic plants. Most of these plants float on the surface, while some attach themselves to underwater rocks, or even to the bottom. Cattails and watercress grow on the muddy banks, but because of their strong roots, they are not washed away by the water current.

"Field status" is used for the indication of number of the particular species in Sinnakalapu compared to the numberbefore Tsunami according to the observations of fishermen and local people [8,9]. "Very high", "High", "Common", "Less", "Very less", and "Not seen" terms used to explain the field status of identified species in Sinnakalapu.

Table.1: Shell-fish species in Sinnakalapu [10]

Common name/ Scientific name	field status
1.White shrimp/ *Penaeus monodon*	Common
2.King shrimp	Less
3.Lobster	Less
4.Small shrimp	Very Less

Except *Penaeus monodon* other three shrimp species density have become less, especially the Small shrimp species, which were abundant in Sinnakalapu before Tsunami; but found in very low numbers now.

Table.2: Fish species present in Sinnakalapu [10,11]

Fish species	Field status
1. *Anguilla nebulosa*	Less
2. *Anguilla bicolor*	Less
3. *Amblypharyngodonchulabhornae*	Less
4. *Dawkinsia singhala*	Less
5. *Devario pathirana*	Common
6. *Devario aequipinnatus*	Common
7. *Esomus thermoicos*	Less
8. *Gerres erythrourus*	Less
9. *Labeo dussumieri*	Less
10. *Oreochromis niloticus*	High
11. *Oreochromis mossambicus*	Less
12. *Cyprinus carpio*	Less
13. *Channa striata*	Less
14. *Channa gachua*	Less
15. *Elops hawaiensis*	Less

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16. *Mystus ankutta*	Less
17. *Mystus gulio*	High
18. *Mystus vittatus*	Very High
19. *Mugil cephalus*	Common
20. *Puntius bimaculatus*	Less
21. *Punitus vittatus*	Less
22. *Clarias brachysoma*	Common
23. *Etroplus suratensis*	Less
24. *Etroplus maculatus*	Not seen
25. *Anabas testudineus*	Less
26. *Ctenopharyngodon idella*	Less
27. Lady fish (Common name)	Less
28. *Gerres* sp.	Not seen
29. Emperor fish (Common name)	Not seen
30. Otti (Local tamil name)	Not seen
31. Vannathi (Local tamil name)	Not seen
32. Aaral (Local tamil name)	Not seen
33. Kaalai (Local tamil name)	Not seen
34. White mullet (Common name)	Not seen
35. Uumbakilaathi (Local tamil name)	Not seen

There were 19 fish species, which had become less in numbers including endemic and exotic fish species, 9 fish species which cannot be seen now, 4 fish species, which are found commonly and 3 invasive fish species, which became higher in numbers [12]. Especially most of the endemic fish species have become less in numbers.

Table.3: Bird species found in Sinnakalapu lagoon area [10]

Scientific name/ common name	Field Status
1.King fisher	Very less
2. *Tringa tetanus*	Very less
3. *Lanius*sp.	Very less
4. *Microcarbo niger*	Common
5. Pond heron/*Ardeola*	Common
6. Night heron	Common
7. Small Egret	Common
8. *Ciconia* sp.	Common
9. Mountain sparrow	Very less
10. *Passer domesticus*	Very less
11. *Eremopterix grisea*	Very less

There are only about 5 species of birds commonly found in Sinnakalapu and other 6 identified species have become significantly low in Sinnakalapu area.

Table 4: Aquatic plant species in Sinnakalapu [10]

Scientific name/ Tamil name (local name)	Field status
1. *Pistia* sp.	High
2. *Eichhornia* sp.	Very high
3. *Nymphaea lotus*	Common
4. *Nelumbo nucifera*	Common
5. *Hydrilla*	Less
6. Oolaivaal sallu (local name)	Not seen
7. Karukach sallu (local name)	Not seen
8. Poonaivaal sallu (local name)	Not seen
9. Veappilai sallu (local name)	Not seen

According the field status the numbers *Eichhornia* sp. and *Pistia* sp. have become higher in number but both were identified as exotic and invasive species in Sri Lanka [13]. Rooted submerged plants were also found in Sinnakalapu. Local people called it as Sallu which were the places where fishes laid eggs and the reproduction of fishes and other aquatic animals taken place. There are four types of Sallu were found in Sinnakalapubut now none of them can be seen.

3.2. Ecological importance of Sinnakalapu

This area supports diverse groups of plants, Algae and animals. This water body provides favourable conditions for the growth and development of floral and faunal species. Plants and algae found in Sinnakalapu are important to this aquatic ecosystem because they provide oxygen through photosynthesis, and food for animals in this biome such as Yum, delicious algae. In fact, the slimy scum found on the surface of waterbody is relished by many aquatic animals e.g. turtles and snakes. Some aquatic plants have strong roots that keep them anchored securely, while others have stems that bend easily with the movement of the water. Certain mosses are able to cling to the rocks. Plants, which grow in stagnant water of this water body have different adaptations. Water lilies, algae and duckweed float on the surface. Insects feed on fish larvae, while insects and fishes are providing foods forbirds. In addition, shrimps and fishes are consumed especially by the local people All these features have given a great importance to the ecosystem.

Sinnakalapu lagoon has become an important resource in Alayadivembu Pradeshiya Sabha because it provides transportation; recreation, like boating many employment opportunities in fisheries and research. One small dam also has been built across this river named Eaththaalakattu, which provides pollution-free energy, water for agricultural purposes and for aquaculture.

3.3. Economic value of Sinnakalapu

Sinnakalapu plays an important economic role for the surrounding villages. Stakeholders of Sinnakalapu get direct economic benefits and at the same time other

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village people are indirectly supported. There are about 7 291 families in Alayadivembu Pradeshiya Sabha which includes 800 of registered fisherman families. But, only about 400 families are continuously fishing and others are part time fishermen. Of the full-time fishermen, 5.5 % of the total families in Alayadivembu Pradeshiya Sabha depend on this waterbody.

There are four main Societies and eleven Sub-Societiesin Alayadivembu Pradeshiya Sabha.,Using their collected data least average annual income from Sinnakalapu fish resources was estimated [14,15]. Fish resources include only the fish and shrimp species, which were caught regularly and the average weight of fishes and shrimpsare calculated for 300 days in a year to estimate averageleast annual income of Sinnakalapu.Fishermen usually do not go for fishing, on Fridays. Hence, 300 days were considered for the estimation of least average income per year.

According to the estimated least average income per year from fish resources, the economic importance of Sinnakalapu in Alayadivembu Pradeshiya Sabah can be understood, which includes exotic and invasive fish species such as tilapia that had contributed more than the other fishes to the least average income of Sinnakalapu [Table-5].

Table.5: Least average annual income of Sinnakalapu from fisheries

Name of fish species	Average weight of fishes per year (kg)	Average income per year (Rs)
Tilapia sp.	120 000	36 000 000
Cat fishes	90 000	27 000 000
Mugil cephalus	105 000	31 500 000
Channa striata	3 600	1 800 000
Other fishes	45 000	6 750 000
Shrimp	900	90 000
Least averageannual income		103 140 000

3.4. Mismanagement practices has led to the loss of Biodiversity in Sinnakalapu

Land degradation and fragmentation are identified as main mismanagement practices in Sinnakalapu. Before Tsunami, the area of Sinnakalapu with Periyakalapu was 1100 ha (SWORD, 2003).However, according to a recent estimation, it is less than 850 ha [3]. Now the area of Sinnakalapuis only about 400 ha according to Google Earth Pro software. Land degradation is mainly due to the settlements of people in the area after Tsunami, filling of Sinnakalapu and using for the industrial purposes and agricultural purposes mainly for paddy cultivation. The land near to the villages are used by the local people illegally. Because of these activities the habitat of aquatic flora and fauna have been reduced. As the land degradation is still continuing, it is leading to the loss of certain fish and plant species in Sinnakalapu [5,6,7,10,13,16].

During the rainy season river over-flow passes through the Sinnamugathuwaram bridge which was built in 2015. Before this bridge was built, as it was a causeway over-flow of river passes only above the causeway but, after bridge was built, over-flowing river water has been increased. Because of that water carrying capacity has reduced and led to the drying up of river faster than before 2015. Nowadays most of the areas of Sinnakalapu has become dry. Other major problem is paddy cultivations close to the Sinnakalapu lagoon area. When the rainfall is high, farmers immediately cut-open the barmouth of lagoon to pass the water into the sea without taking permission from Fisheries Society to protect their paddy fields. As a result, even during the rainy season also the amount of retaining water is reduced and it speeds up the drying of river in the dry season.Prior to this in every two years fishermen caught large quantity of shrimps, but nowadays income of fishermen from shrimps is largely reduced due to this.

Illegal fishing is also problem. Though the use of monofilamentous nets for fishing is completely banned in Sri Lanka[17],majority of fishermen still used those instead of polyfilamentous nets. Monofilamentous nets are cheaper, freely available and more efficient. Other problem is the use of small-meshed gill nets for fishing, which results in catching large number of small fishes[17]. It leads to the depletion of fish species in this resource. Another important problem is illegal fishing by fishermen in other areas, without any permission.

Pollution is the next major threat to Sinnakalapu due to both point sources and non-point sources. Point sources are those directly contribute to pollution such as washing vehicles, oil containers, clothes, disposal of garbage, household wastes directed to the river [18].Nonpoint sources of pollution are a combination of pollutants from a large area rather than from specific identifiable sources such as discharge pipes. Runoff is generally associated with nonpoint source of pollution, as water is emptied into streams or rivers after accumulating contaminants from sources like gardens,paddy fields, parking lots or construction sites [18]. Sinnakalapu is surrounded by paddy fields.Thus, paddy field canals which contains water rich in pesticides, insecticides, weedicides, fertilizers and agricultural wastes are directed to the Sinnakalapu. Adding of weedicides into Sinnakalapu water body mainly resulted in the loss of aquatic plants [19] and adding of fertilizers into waterbody of Sinnakalapu has increased the phosphate and nitrate content, which has led to the algal blooming in the river

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[5,6,16,21]. Nowadays approximately about 40% of the water body is covered by the *Eichhornia* sp. [3], which indirectly affect fish in Sinnakalapu [21].

3.5. Need for Conservation

Most of the threatened freshwater fishes of Sri Lanka are found outside the Protected Area Network [10] and Sinnakalapu is also one of the lagoonswhich is found outside the protected area network. In addition, these habitats are under high human pressure. Therefore, they need to be protected; especially because their catchment areas will decide the water yield as well as the quality of water [10]. Any type of development affecting these habitats needs to be clearly assessed before granting approval. Further, species oriented conservation programs and habitat oriented conservation programs should be developed at least for the critically endangered species. As most of the species are foundoutside the protected areas local communities have to involve in conservation of these species. Conservation action plans should be drawn up for all identified threatened species. Ex-situ breeding programs should also be established with the aim of boosting dwindlingwild population. However, translocation or reintroduction programs should be planned with utmost care to prevent imbalances in the ecosystems and introduction of diseases to the population. Thus far, a number of translocations have been attempted in Sri Lanka with the aim of conserving threatened species. Some of these translocation programs have been highly successful while some have failed to achieve the desired objectives [10]. Introducing exotic but, commercially important fish species such as carpsand especially *Tilapia* sp. in Sinnakalapu has both positive and negative impacts. Economically it has a positive impact [Table -5] and ecologically it has a negative impact because it has been responsible for the depletion of some endemic fish species in Sinnakalapu [22,23]. Therefore, these programs should be carefully reviewed to document the lessons learnt before attempting further translocations and reintroductions.

3.6. Proposed management plans

3.6.1 Research gaps and research needs

The recent field surveys and phylogenetic studies by IUCN in Sri Lanka have demonstrated that there are still new species to be discovered in Sri Lanka [10]. Therefore, systematic surveys should be carried out to document the distribution and ecological conditions necessary for freshwater fishes in Sinnakalapu. The baseline data generated from such a survey can be used to make proper assessments of the conservation status of species as well as to draw up species conservation plans.

3.6.2. Education and awareness programs

It is important to educate people by organizing rational awareness programs with field visits to impart relevant knowledge about the mismanagement practices and their impacts on Sinnakalapu.

Implementation of different laws, (some of which are indicated below) by various institutes has made it impractical [22].

Law No 1.National Environmental Act No. 47 of 1980 (as amended by Acts No. 56 of 1988 and 53 of 2000) and the Regulations under the Act.

Law No 2. Fauna and Flora Protection Ordinance No. 2 of 1937 (as amended by Act Nos. 49 of 1993, 12 of 2005) and the Regulations under the Ordinance.

Law No 3. Water Resources Board Act No. 29 of 1964 (as amended).

Law No 4.Fisheries and Aquatic Resources Act No. 2 of 1996 (as amended).

Law No 5.Water Hyacinth Ordinance No. 4 of 1909.

Law No 6. State Lands Ordinance No. 8 of 1947. 54 and 83.

3.6.3. Managing the conflicts between Stakeholders.

Fishermen are the main stake holders of Sinnakalapu.There are several conflicts in between Fisheries Society and Farmer's Society becauseof the illegal fishing and other mismanagement practices in Sinnakalapu. It is better to form a Co-operative Society consisting of poor peopleof all different stakeholders. Educate and train the members about common pool resource management. Private sector can support the Society by paying for the different assistance provided by members to reduce pollution.Monthly meeting should be held to solve the problems as well as to get their opinions. Based on their opinions, new by-laws and rules which are accepted by all members could be framed for sustainable management of the resource.

IV. CONCLUSIONS

Sinnakalapu has a rich freshwater fish fauna with endemic fish species. However, nearly 19 freshwater fish species are listed as less in numbersand 9 fish species listed as "Not seen" in the Sinnakalapu based on the field status.Therefore, it is important to develop a conservation action plan for the endemic and threatened fish species in Sinnakalapu.Such an action plan should first a priority list of species as well as critical habitats of fishes that require immediate conservation action. This should be followed with preparation and implementation of species specific recovery plans. The implementation of such plans should be performed through co-management and the formed Society should be the nucleus in implementation process. Funds should be raised by the stakeholders through

membership fees and paying a reasonable share for using the resource in addition to what the polluters pay for the society for their assistance.

Therefore, it is essential to conserve the area of Sinnakalapu by reducing or preventing illegal settlements and agricultural practices in resource area by implementing available laws [Law No 1,3 and 6]. Necessary constructions to retain water during dry period should be done through the integrated wor ofline Ministries with the assistance of the Society. Society members should be used as workers, so that they get the feeling that this resource is theirs. Such constructions will lead to the formation of a healthy biome. Implementation of a polluter-pay system with the assistance of the bureaucrats and the Cooperative Society to control pollution of the resource would lead to develop a proper co-management strategy. Agrochemicals approved for use in Sri Lanka should be assessed for impact on non-target organisms and the environment in general, and the labeling of such products should include information on environmental safeguards.

Further, all future intentional release of exotic fishes should be preceded by an environmental impact assessment involving specific safeguards against invasiveness, and atthe same time a rule should be imposed to ban the importation of exotic fish species that are knownto be invasive in other countries. We can control the exportation and over exploitation of endemic and endangered fish species by the implementation of Law No 2 and Law No 4. and the aquatic invasive species *Eichhornia* sp. can be controlled by the implementation of Law No 5. Illegal fishing methods can be controlled by implementation of Law No 4.

Create a conservation model involving the Society members for the conservation of endangered freshwater fishes.Development of such a model as developed at Ibbankatuwa, Dambulla will lead to successful management of common pool resources of Sri Lanka. Fishes are renewable resources only if they are sustainably managed.Fresh water resources are most valuable treasures of our motherland.

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Evaluation of Maize Top Cross Hybrids for Grain Yield and Associated Traits in Three Agro-Ecological Zones in Ghana

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Abstract— *Maize (Zea mays L.) is an important food crop in Ghana, but its productivity in farmers" fields throughout the country is generally low. The low grain yields can be attributed partly to the use of traditional low-yielding open-pollinated varieties. In an attempt to increase maize productivity, 39 top cross hybrids were developed using 21 inbred lines and 3 open-pollinated varieties. The trial was set up in a randomized complete block design with two replications in three locations in southern Ghana. The overall objective of the study was to investigate traits which influence yields in top cross hybrids and to ascertain the yield potential of the hybrids in three agro-ecological zones in Ghana. The Genotypes were evaluated to determine agronomic performances and correlations between yield and yield component traits were calculated to assess the degrees of associations. Highly significant variations ($p<0.01$) were observed among the maize genotypes for grain yield, cob length, cob diameter and kernel row cob^{-1} and significant variations ($P<0.05$) for days to 50% tasseling, days to 50% silking and kernel row^{-1}. On the contrary, there were no significant differences among the genotypes for plant height and ear height. The significant ($P<0.01$) results for grain yield indicated the variable nature of the locations and differences in the performances of the genotypes evaluated. The mean grain yield was significantly ($p<0.01$) higher for the top cross hybrids than for the local checks.*

Keywords— Correlations, genotypes, grain yield, top cross hybrids, traits.

I. INTRODUCTION

Maize is the most extensively consumed cereal in Ghana with rising production since 1965 (FAO STAT., 2008; Morris et al., 1999). The per capita consumption of maize in Ghana in 2011 was predicted at 43.8 kg (MOFA-SRID, 2011a) and a predicted domestic consumption of 1,750,000 metric tons in 2011. Maize is considered a major source of protein ranking only behind meat, fish and legumes in terms of yearly protein production (Dasbak et al., 2008).

In order to meet the growing needs of farmers in Ghana, more than twenty-five (25) improved varieties comprising open pollinated and hybrid maize varieties of varying maturity periods have been developed and subsequently released by the CSIR-CRI (Badu-Apraku et al., 1992; Sallah et al., 1997; Twumasi Afriyie et al., 1997). These released varieties have been extensively adopted by maize farmers throughout the country (Dankyi et al., 1997; Morris et al., 1999).

In spite of this success, smallholder farmers continue to meet difficulties in accessing improved maize seeds. Moreover, productivity of maize in farmers" fields all over Ghana is low. The average grain yields of maize nationwide rests at 1.89 metric tons ha-1 (MOFA-SRID, 2011a). However, with the use of appropriate inputs together with the adoption of improved practices, yields of 4 or 5 tons ha^{-1} can be realized by farmers (MOFA-SRID, 2006).

The cause of low productivity has been ascribed partly to the use of traditional low yielding open-pollinated varieties (MOFA-SRID, 2006). At present, there is a growing demand for use of hybrid seeds especially early and extra-early drought resistant materials with high grain yield potential that can provide early harvest to bridge the hunger gap before the harvest of a full-season crop (Pswarayi and Vivek, 2007), ideal for off-season planting and suitable for minor rainfall season production which tend to be very short. Regrettably, the National Maize Program does not

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have any early maturing commercial hybrid. It is in view of this that the study was aimed at the development of top cross hybrids as the most viable solution. Therefore, the overall objective of this study was to investigate traits which influence yields in top cross hybrids and to ascertain the yield potential of the hybrids in three (3) agro-ecological zones of Ghana.

II. MATERIALS AND METHODS

2.1 Experimental sites

The study was conducted in three locations in Southern Ghana, namely; Fumesua, Ejura and Kpeve. Fumesua is situated in the Ashanti region (Latitude 6°43'N; Longitude 1°36'W), and falls within the Forest ecological zone of Ghana. Similarly, Ejura is situated in the Ashanti region at Latitude 7°24'N and Longitude 1°21'W with an elevation of 228.7m above sea level, and falls within the Forest-Savannah transition zone. Kpeve is situated in the Volta region (Latitude 6°41'N and Longitude 00°21'E) with an elevation of 513m, and falls within the Coastal Savanna transition zone. The three locations experience a bi-modal rainfall, with a major season stretching from April through July and minor from August to November.

2.2 Maize varieties and inbred lines used for the study

Twenty-seven genotypes including three open-pollinated varieties (normal OPV parents), three check varieties and 21 inbred lines (donor parents) were used for the study. The varieties were from the CSIR-CRI Maize Program while the tropical early maturing maize (TZEI white-endosperm) inbred lines were from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria Maize Improvement Program.

2.3 Experimental design and field layout

The trial was laid out in a randomized complete block design (RCBD) with two replications per location. Each experimental unit was a two-row plot of 5.0 m long, spaced 0.75 m by 0.45 m between and within rows, respectively, with 11 hills per row.

2.4 Development of top cross hybrids

A total of 39 top cross hybrids were formed using three OPVs and 21 inbred lines (testers). The generation of top cross hybrids was carried out between May to August 2012 at CRI. Controlled (hand) pollination was carried out approximately 60 days after planting at the CRI breeding nursery to prevent contamination. Prior to the appearance of the silk, developing ears were covered with a crystal clear plastic bag to make sure that emerging silks were not contaminated with undesirable pollen. At anthesis, pollen was collected from desirable plants in the individual inbred lines using brown tassel bags, bulked for each inbred line and used to pollinate agronomically good plants in the open-pollinated varieties which served as female parents.

A day prior to artificial crossing, the tassel of the male parent was covered with a brown tassel bag. This permitted fresh, uncontaminated pollen to be collected for use for the crosses the next morning. At harvest five clean cobs from each line with good husk cover were selected. These were de-husked, sun dried, shelled and put in separate envelopes and tagged.

2.5 Evaluation of top cross hybrids

Seeds collected from clean F^1 cobs were constituted into a trial and planted for evaluation in October 2012 in each of the three locations. A total of 42 entries comprising 39 F^1 hybrids and three early maturing elite varieties (Akposoe, Aburohemaa and Omankwa) used as checks were utilized in the trial. These elite OPVs were included as checks because the National Maize Program did not have any early maturing commercial hybrids. Two guard rows were planted at both sides of the experimental field to protect the trials.

2.6 Cultural/management practices

The trial site was carefully prepared by plowing and harrowing using tractor. This was carried out to manage weeds, provide good soil aeration and to obtain good seedling emergence and root penetration. Three seeds were sown in each hill (planting hole) for each set of genotypes and thinned to two plants per hill two weeks after emergence to give a final plant population density of approximately 60,000 plants per hectare. During the first three weeks of growth, the plants were irrigated using the sprinkler irrigation system at CRI. The trials were kept weed-free with the application of gramoxone and atrazine as pre- and post-emergence herbicides and manual hoeing. Fertilizer (NPK-15-15-15) was applied as basal after two weeks of planting and urea as top dressing after five weeks for optimum plant growth at each location. All trial management practices were based on the recommendations for each location.

2.7 Data collection

The following agronomic parameters were measured:

1) *Days to tasseling (DYTS)* - were recorded as number of days from planting to the time 50% of plant had fully

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emerged tassels; 2) *Days to silking (DYSK)* - were recorded as number of days from planting to the time 50% of plants had completely extruded silks; 3) *Plant height (PHT)*- the height of five randomly selected plants in centimeters were measured with a graduated measuring stick from soil surface to the last node; 4) *Ear height (EHT)* – the height of five plants in centimeters from the soil surface to the node on which the uppermost ear sits were measured from the same plant from which plant heights were recorded; 5) *Cob length (COL)* – the length of cobs in centimeters were measured using a caliper; 6) *Cob diameter (COD)* – the diameter of cobs in centimeters were also measured using a caliper; 7) *Kernel rows cob^{-1} (KRPC)* – the number of rows cob^{-1} of five cobs of each line was counted and the average recorded; 8) *Kernel row^{-1} (KPR)* – the number of kernels row^{-1} of five cobs of each line was counted and the average recorded; 9) *Grain Yield (GY)* – was determined by means of converting yields plot^{-1} into grain yield ha^{-1}. The formula used for the calculation of grain yield was:

$$\text{Grain yield (kg ha}^{-1)} = \frac{\text{F.W. (kg plot}^{-1}) \text{ (100-moisture \%) x S x 10,000}}{(85) \text{ x Harvested area (plot size)}}$$

Where,
F.W. = Fresh weight of ear in kg at harvest
Moisture percentage= Grain moisture content at harvest
85= moisture percentage used was 15%
S= Shelling co-efficient (0.80)

2.8 Statistical analysis
Data were analyzed using the Statistical Analysis System (SAS) version 9.2 (SAS Institute Incorporated, 2002). Data from each location were subjected to Analysis of Variance (ANOVA) individually to explore differences among entries for all traits and pooled across locations to determine G x E interactions. Means separation was carried out using least significant difference (lsd). Correlations among grain yield and yield contributing characters were examined. GGE biplot analysis (Yan, 2001) was used to assess yield stability among the maize varieties.

III. RESULTS AND DISCUSSION

3.1 Mean square analysis for agronomic traits
Highly significant (P<0.01) differences among locations were observed for the 42 genotypes for grain yield, days to 50% tasseling, days to 50% silking, plant height, ear height, cob length, cob diameter, kernel row cob^{-1} and kernel row^{-1} (Table 1). For genotypes, variations were highly significant (P<0.01) for grain yield, cob length, cob diameter and kernel row cob^{-1} and significant (P<0.05) for days to 50% tasseling, days to 50% silking and kernel row^{-1}. On the contrary, there were no significant differences among the genotypes for plant height and ear height. Apart from grain yield, differences due to the interaction (genotype x location) were not significant for all the traits studied. Yield differences could be due to differences in soil conditions and rainfall patterns at the different sites. Differences due to location influences were similarly noted by Sallah et al. (2004) when they studied genotype by environment interaction effects of three maturity groups of maize at the same sites. Differences among locations may also be due to the fact that the genotypes used were from parents of diverse genetic backgrounds.
The genotypic variations found were due to the diverse backgrounds from which the genotypes used in the study were developed. This result was in agreement with findings by Sallah et al. (2001) and Soza et al. (1996) and these authors also used open-pollinated varieties and hybrids. Genotype by location interaction for grain yield may be due to differences among the sites in soil fertility, relative humidity, season and temperature, all factors which affect performance. Similar findings were cited by Butron et al. (2002) and these imply that the genotypes should be partially released for locations where the performance was most favorable (Ogunbodede et al., 2001). Moreover, the observed lack of significant means squares for G x E of plant height, ear height, days to 50% silking, days to 50% tasseling amongst others showed that these parameters were stable across the three sites used for the study. Genotype x location interaction has, over the years, continued to cause setback for researchers which necessitate the need to carry out multi-location yield trials to enable plant breeders to categorize and select genotypes that are high yielding with specific or wide-ranging adaptation to diverse agro-ecological zones, prior to variety release. Information generated from these multi-location trials could be useful for state-run breeding program by identifying the appropriate breeding materials with advantageous agronomic qualities at test sites (Badu-Apraku et al., 2010).

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Table.1: Combined mean squares and degrees of freedom from ANOVA for agronomic traits of top cross hybrids and checks

		Mean Squares								
Source	**DF**	**GY**	**DYTS**	**DYSK**	**PHT**	**EHT**	**COL**	**COD**	**KRPC**	**KPR**
Replication	1	3520177.7	6.04	6.04	531.57	275.32	12.04	0.68	2.55	30.34
Location (L)	2	35750002.5**	1251.92**	660.49**	56160.72**	4366.56**	111.56**	3.73**	3.73**	145.12**
Genotype (G)	41	3030130.4**	5.34*	6.78*	261.18NS	181.12NS	3.47**	0.29**	2.64**	21.03*
G x L Interaction	82	902285*	2.90NS	3.77NS	167.83NS	172.93NS	1.32NS	0.11NS	0.6NS	8.16NS
Error	12 5	655462.3	3.00	3.76	233.10	151.91	1.03	0.12	0.49	9.37

*, **= Significant at 0.05 and 0.01 levels of probability, NS= Not significant, DF=Degree of freedom, GY=Grain yield, DYTS= Days to 50% tasseling, DYSK=Days to 50% silking, PHT=Plant height, EHT= Ear height, COL= Cob length, COD= Cob diameter, KRPC= Kernel row cob^{-1}, KPR= Kernel row^{-1}

3.2 Mean Performance of genotypes for grain yield

Nine quantitative traits (i.e. off-farm and on-farm) were investigated for the 42 genotypes used in the study including their entry numbers (Table 2) and observations recorded for mean performances across the three locations (Tables 3 & 4). Due to the number of genotypes involved, comparisons between yields and other traits were limited to the seven highest ranking top cross hybrids and the three checks.

The results showed that the seven highest ranking top cross hybrids TZEI W-POP DT STRC3 x TZEI-5, Fu 2080 DWFP x TZEI-4, Fu 2090 DWDP x TZEI-46, TZEI-W-POP DT STRC3 x TZEI-1, Fu 2090 DWDP x TZEI-30, TZEI-W-POP DT STRC3 x TZEI-39 and Fu 2080 DWDP x TZEI-1 were not significantly different from each other. The highest yielding genotypes averaging 5056.8 and 5001.0 kg ha^{-1} were TZEI-W-POP DT STRC3 x TZEI-5 and Fu 2080 DWFP x TZEI-4, respectively. The highest yielding check Omankwa had a mean grain yield of 4499.1 kg ha^{-1}. The seven highest ranking genotypes were not statistically different in yield from Omankwa, although TZEI-W-POP DT STRC3 x TZEI-5, Fu 2080 DWFP x TZEI-4, Fu 2090 DWDP x TZEI-46 and TZEI-W-POP DT STRC3 x TZEI-1 were statistically different from Aburohemaa and Akposoe. Among the checks themselves Omankwa was significantly different from Akposoe but not Aburohemaa.

Averaged across the three locations, TZEI-W-POP DT STRC3 x TZEI-5 was the highest mean grain yield of 5056.8 kg ha^{-1}. Fu 2080 DWFP x TZEI-19 was the lowest grain yield of 2062.3 kg ha^{-1}. Mean grain yield was significantly higher at Kpeve (4580.0 kg ha^{-1}) than Fumesua (3998.0 kg ha^{-1}) and Ejura (3277.3 kg ha^{-1}). Comparison of the results of the top cross hybrids and checks revealed that the highest yielding hybrid maize, TZEI-W-POP DT STRC3 x TZEI-5, had an average of 12.4% yield advantage over Omankwa, 30.6% over Aburohemaa and 66.1% over Akposoe (Table 3). The yield advantage of the different types of hybrids over the OPVs was outlined by Paliwal (2000) who observed yield advantages of 46% for single crosses, 30% for three way crosses, 23% for double crosses, 37% for double top crosses, 28% for top crosses, and 17% for variety crosses. The grain yield advantage of the top crosses could be due to the higher kernel number ear^{-1} (Correjado and Magulama, 2008), longer cobs and high number of rows. According to Asiedu et al. (2001), longer cobs and high number of rows are agronomic traits that plant breeders ought to look for at some stage in selecting high-yielding genotypes. This observation also supported findings of Kim et al. (1993) and Akande and Lamidi (2006) who confirmed that typical maize hybrids were found to be superior to other open pollinated maize varieties in yield potentials.

Also, majority of the hybrids evaluated in the study showed differential ranking in performance across the three locations with eight of the top cross hybrids (Fu 2080 DWDP x TZEI-1, Fu 2080 DWDP x TZEI-5, Fu 2080 DWFP x TZEI-19, Fu 2090 DWDP x TZEI-3, Fu 2090 DWDP x TZEI 36, Fu 2090 DWDP x TZEI-46, TZEI-W-POP DT STRC3 x TZEI-3 and TZEI-W-POP DT STRC3 x

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TZEI-47) performing likewise at either two of the locations. These dissimilar and similar rankings of top cross hybrids across the test locations are strong indications of possible existence of either crossover or non-crossover GEI and the existence of unstable genotypes. This means a closer evaluation of the top cross hybrids according to their interactions with the studied environments is indeed necessary. Differential performance of genotypes evaluated in a number of locations and in different years due to GEI was observed by Lin et al. (1986).

Table.2: List of the 42 genotypes / entries (i.e. 39 top cross hybrids and 3 checks)

Entry No.	Entry name	Entry No.	Entry name
1	Fu 2080 DWDP x TZEI-1	22	Fu 2090 DWDP x TZEI-42
2	Fu 2080 DWDP x TZEI-4	23	Fu 2090 DWDP x TZEI-45
3	Fu 2080 DWDP x TZEI-5	24	Fu 2090 DWDP x TZEI-46
4	Fu 2080 DWFP x TZEI-18	25	Fu 2090 DWDP x TZEI-47
5	Fu 2080 DWFP x TZEI-19	26	Fu 2090 DWDP x TZEI-48
6	Fu 2080 DWFP x TZEI-22	27	TZEI –W-POP DT STR C3 x TZEI-1
7	Fu 2080 DWFP x TZEI-41	28	TZEI –W-POP DT STR C3 x TZEI-2
8	Fu 2080 DWFP x TZEI-42	29	TZEI –W-POP DT STR C4 x TZEI-3
9	Fu 2080 DWFP x TZEI-43	30	TZEI –W-POP DT STR C3 x TZEI-4
10	Fu 2080 DWDP x TZEI-47	31	TZEI –W-POP DT STR C3 x TZEI-5
11	Fu 2080 DWDP x TZEI-48	32	TZEI –W-POP DT STR C3 x TZEI-34
12	Fu 2090 DWDP x TZEI-2	33	TZEI –W-POP DT STR C3 x TZEI-35
13	Fu 2090 DWDP x TZEI-3	34	TZEI –W-POP DT STR C3 x TZEI-36
14	Fu 2090 DWDP x TZEI-18	35	TZEI –W-POP DT STR C3 x TZEI-38
15	Fu 2090 DWDP x TZEI-19	36	TZEI –W-POP DT STR C3 x TZEI-39
16	Fu 2090 DWDP x TZEI-22	37	TZEI –W-POP DT STR C3 x TZEI-45
17	Fu 2090 DWDP x TZEI-30	38	TZEI –W-POP DT STR C3 x TZEI-46
18	Fu 2090 DWDP x TZEI-34	39	TZEI –W-POP DT STR C3 x TZEI-47
19	Fu 2090 DWDP x TZEI-36	40	Aburohemaa (check)
20	Fu 2090 DWDP x TZEI-38	41	Akposoe (check)
21	Fu 2090 DWDP x TZEI-39	42	Omankwa (check)

Table.3: Mean performance of 42 genotypes for off-farm agronomic traits across the three locations*

Entry No.	GY (kg ha^{-1})	COL (cm)	COD (cm)	KRPC	KPR
31	5056.8^{a}	12.6^{ji}	4.3^{ebdacf}	13.6^{ilkhjg}	27.6^{jihgf}
2	5001.0^{ba}	14.2^{cebd}	4.2^{ebdhgcf}	13.5^{milkhjg}	31.7^{bdac}
24	4938.2^{bac}	14.9^{b}	4.2^{ebdhagcf}	12.8^{mln}	32.0^{bac}
27	4849.5^{bdac}	13.4^{fjeihdg}	4.5^{ba}	14.4^{fbedc}	29.4^{ejbidhagcf}
17	4781.5^{ebdac}	13.1^{fjeihg}	4.4^{bdac}	13.7^{fikhjg}	28.7^{ejidhgcf}
36	4703.3^{ebdacf}	13.5^{fjeihdg}	4.1^{ebdhgcf}	13.5^{milkhjg}	30.4^{ebdhagcf}
1	4651.2^{ebdacf}	13.4^{fjeihdg}	4.3^{ebdagcf}	13.7^{fikhjg}	28.7^{ejidhgcf}
42	4499.1^{ebdagcf}	13.4^{fjeihdg}	4.2^{ebdhagcf}	13.8^{fiekhjg}	27.4^{jihgf}
10	4451.1^{ebdagcf}	14.8^{cb}	4.2^{ebdhagcf}	13.2^{mlkjn}	32.0^{bac}
14	4386.9^{ebdhagcf}	13.9^{fcebdg}	4.3^{ebdagcf}	13.7^{fikhjg}	31.3^{ebdac}

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33	4364.2^{ebdhagcf}	13.6^{fceihdg}	4.5^{bac}	14.6^{bedc}	27.5^{jihgf}
21	4341.3^{ebdhagcf}	12.7^{jih}	4.1^{ebdhgcf}	13.6^{ilkhjg}	27.0^{jih}
23	4338.5^{ebdhagcf}	13.2^{fjeihg}	4.1^{edhgf}	12.7^{mn}	29.5^{ejbidhagcf}
32	4326.7^{ebidhagcf}	13.2^{fjeihg}	4.5^{bac}	14.7^{bdc}	26.8^{ji}
29	4282.2^{ejbidhagcf}	12.7^{jih}	4.6^{a}	15.7^{a}	26.9^{ji}
8	4273.7^{kejbidhagcf}	13.4^{fjeihdg}	4.1^{ebdhgcf}	13.7^{fikhjg}	28.3^{ejidhgf}
3	4263.8^{kejbidhagcfl}	13.4^{fjeihdg}	4.2^{ebdhagcf}	13.5^{milkj}	30.7^{ebdacf}
26	4256.6^{kejbidhagcfl}	12.8^{jihg}	3.4^{i}	13.3^{mlkjn}	27.1^{3jihg}
34	4255.5^{kejbidhagcfl}	13.9^{fcebdg}	4.0^{hgf}	13.3^{mlkjn}	31.4^{ebdac}
30	4235.0^{kejbidhagcfl}	13.6^{fceihdg}	4.3^{ebdhagcf}	13.8^{fiekhjg}	28.9^{ejbidhgcf}
37	4225.8^{kejbidhagcfl}	14.4^{cbd}	4.0^{ehgf}	13.5^{milkhj}	30.6^{ebdagcf}
28	4101.4^{kejbidhgcfl}	13.3^{fjeihdg}	4.5^{bac}	15.1^{ba}	29.3^{ejbidhagcf}
16	4048.2^{kejidhmgcfl}	12.4^{j}	4.3^{ebdagcf}	13.3^{mlkjn}	29.6^{ebidhagcf}
11	3984.7^{kejidhmgfl}	13.8^{fcebdg}	3.9^{h}	13.0^{mlkn}	28.6^{ejidhgcf}
25	3892.5^{kejnihmgfl}	13.8^{fcebhdg}	4.1^{edhgcf}	12.8^{mln}	31.1^{ebdac}
6	3889.2^{kejnihmgfl}	13.6^{feihdg}	4.3^{ebdac}	13.7^{fikhjg}	30.6^{ebdagcf}
40	3872.3^{kejnihmgfl}	12.6^{ji}	4.4^{bdac}	14.3^{fbedcg}	26.0^{j}
18	3846.4^{kjnihmgfl}	13.4^{fjeihdg}	4.4^{ebdac}	13.8^{fikhjg}	30.7^{ebdacf}
12	3639.8^{kjnihmgol}	13.6^{fceihdg}	4.2^{ebdhagcf}	15.0^{bac}	31.7^{ebdac}
38	3616.5^{kjnihmgol}	13.9^{fcebdg}	4.1^{edhgcf}	13.5^{milkj}	29.0^{ejbidhgcf}
4	3605.9^{kjnihmgol}	13.9^{fcebdg}	4.2^{ebdhgcf}	13.5^{milkj}	30.8^{ebdacf}
35	3476.8^{kjnihmol}	14.0^{fcebd}	4.1^{ebdhgcf}	13.5^{milkj}	31.1^{ebdac}
15	3404.9^{kjnimol}	14.2^{cebd}	4.3^{ebdagcf}	13.6^{ilkhjg}	28.2^{ejihgf}
22	3376.4^{kjnmol}	13.5^{fjeihdg}	4.5^{bdac}	13.8^{fiekhjg}	30.4^{ebdhagcf}
20	3350.5^{knmol}	13.0^{fjeihg}	4.5^{bdac}	13.0^{mlkn}	27.6^{jihgf}
19	3346.3^{nmol}	13.9^{fcebdg}	4.1^{edhgcf}	12.6^{n}	32.3^{ba}
13	3173.1^{nmo}	12.9^{fjihg}	4.4^{bdac}	14.2^{fiedhcg}	31.3^{ebdac}
41	3043.7^{npo}	13.2^{fjeihg}	4.4^{ebdac}	14.9^{bac}	26.5^{ji}
7	2842.1^{qpo}	14.0^{fcebd}	4.1^{edhgcf}	13.4^{milkjn}	31.5^{ebdac}
9	2762.5^{qpo}	14.9^{b}	4.0^{hg}	13.5^{milkj}	32.2^{ba}
39	2150.5^{qp}	14.4^{cbd}	4.2^{ebdhgcf}	14.3^{fiedhjg}	29.7^{ebidhagcf}
5	2062.3^{q}	16.5^{a}	4.5^{bdac}	13.9^{fiedhjg}	32.6^{a}
Grand Mean	3951.6	13.6	4.2	13.7	29.6
Lsd (0.05)	925.1	1.2	0.4	0.8	3.5
CV (%)	20.5	7.4	8.1	5.1	10.3

*Means with the same letter (s) within the same column are not significantly different from each other at 5% level of probability.

Table.4: Mean performance of 42 genotypes for on-farm agronomic traits across the three locations*

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Entry No.	DYSK (days)	DYTS (days)	PHT (cm)	EHT (cm)
31	52.2^{ba}	49.3^{bac}	170.0^{ebdgcf}	84.3^{ebdfc}
2	49.7^{fdehg}	46.7^{fihg}	174.7^{ebdacf}	89.2^{ebdc}
24	49.7^{fdehg}	46.8^{fiehg}	183.8^{ba}	93.4^{ba}
27	50.8^{fbdec}	48.5^{fbdec}	182.6^{bac}	87.5^{ebdc}
17	48.0^{h}	45.8^{i}	181.2^{bdac}	80.4^{ebdfc}
36	51.8^{bdac}	49.2^{bdac}	175.4^{ebdacf}	87.4^{ebdc}
1	51.0^{bdec}	48.0^{fbdehcg}	163.9^{edgf}	82.0^{ebdfc}
42	49.7^{fdehg}	47.0^{fiehg}	187.8^{a}	103.4^{a}
10	49.0^{fehg}	46.7^{fihg}	165.5^{edgcf}	76.7^{ef}
14	49.0^{fehg}	46.7^{fihg}	172.0^{ebdagcf}	80.2^{ebdfc}
33	49.8^{fdehcg}	47.0^{fiehg}	169.8^{ebdgcf}	86.9^{ebdfc}
21	50.8^{fbdec}	48.2^{fbdecg}	170.7^{ebdagcf}	85.0^{ebdfc}
23	50.7^{fbdec}	47.5^{fdiehcg}	169.3^{ebdgcf}	91.0^{bdac}
32	51.2^{bdec}	47.7^{fbdiehcg}	164.8^{edgf}	83.8^{ebdfc}
29	51.0^{bdec}	48.5^{fbdec}	166.9^{ebdgcf}	82.1^{ebdfc}
8	49.2^{fehg}	47.2^{fiehg}	161.3^{gf}	77.3^{edf}
3	49.7^{fdehg}	47.2^{fiehg}	171.7^{ebdagcf}	79.3^{edfc}
26	50.3^{fbdecg}	48.0^{fbdehcg}	174.0^{ebdacf}	92.6^{bac}
34	50.0^{fbdehcg}	47.5^{fdiehcg}	169.4^{ebdgcf}	83.7^{ebdfc}
30	50.7^{fbdec}	48.2^{fbdecg}	172.7^{ebdagcf}	84.7^{ebdfc}
37	51.0^{bdec}	48.7^{bdec}	169.0^{ebdgcf}	87.1^{ebdc}
28	50.5^{fbdec}	48.2^{fbdecg}	166.3^{edgcf}	79.5^{ebdfc}
16	49.2^{fehg}	47.2^{fiehg}	169.6^{ebdgcf}	81.5^{ebdfc}
11	48.2^{hg}	46.2^{ih}	165.7^{edgcf}	88.8^{ebdc}
25	50.8^{fbdec}	48.0^{fbdehcg}	171.4^{ebdagcf}	82.0^{ebdfc}
6	50.2^{fbdehcg}	47.2^{fiehg}	173.2^{ebdagcf}	85.2^{ebdfc}
40	49.5^{fehg}	47.0^{fiehg}	168.1^{ebdgcf}	81.2^{ebdfc}
18	50.7^{fbdec}	47.7^{fbdiehcg}	175.1^{ebdacf}	86.2^{ebdfc}
12	49.5^{fehg}	47.2^{fiehg}	179.8^{ebdac}	86.9^{ebdfc}
38	52.0^{bac}	49.5^{ba}	167.8^{ebdgcf}	81.8^{ebdfc}
4	49.8^{fdehcg}	47.3^{fdiehg}	162.4^{egf}	85.2^{ebdfc}
35	50.7^{fbdec}	48.2^{fbdecg}	164.9^{edgf}	83.7^{ebdfc}
15	50.0^{fbdehcg}	47.2^{fiehg}	177.8^{ebdacf}	89.5^{ebdac}
22	53.5a	50.7a	166.4^{ebdgcf}	91.5^{bac}
20	49.7^{fdehg}	47.0^{fiehg}	167.5^{ebdgcf}	83.2^{ebdfc}
19	48.7^{fhg}	46.5^{ihg}	175.1^{ebdacf}	78.6^{edfc}
13	50.8^{fbdec}	47.3^{fdiehg}	164.5^{edgf}	82.0^{ebdfc}
41	50.7^{fbdec}	47.3^{fdiehg}	164.5^{edgf}	82.0^{ebdfc}
7	49.3^{fehg}	47.2^{fiehg}	156.0^{g}	72.8^{f}
9	49.8^{fdehcg}	47.3^{fdiehg}	166.8^{ebdgcf}	83.1^{ebdfc}

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	39	50.0^{fbdehcg}	47.8^{fbdehcg}	167.7^{ebdgcf}	79.0^{edfc}
	5	50.2^{fbdehcg}	48.0^{fbdehcg}	182.6^{bac}	87.2^{ebdc}
Grand Mean	50.2	47.6	170.8	84.4	
Lsd (0.05)	2.2	2.0	17.4	14.1	
CV (%)	3.9	3.6	8.9	14.6	

*Means with the same letter (s) within the same column are not significantly different from each other at 5% level of probability.

3.3 The GGE biplot analysis

The GGE biplots data analysis conducted in this study showed the 'which won where' pattern (Fig. 1), mean performance and stability of tested genotypes and rankings (Fig. 2) as well as the discriminating ability and representativeness of the genotypes (Fig. 3). Fig. 1 & 3 were based on environment-focused singular value partitioning (SVP=2) suitable for studying the relationships among locations, while Fig. 2 was based on genotype-focused singular value partitioning (SVP=1) suitable for genotype evaluation. The biplots data was not transformed ("Transform=0"), although it was standardized ("scale =1") and environment-centered ("centering =2"). Analysis of Fig. 1, 2 & 3 revealed that Principal Components PC1 and PC2 for Model 3 jointly explained 84.7% of total variation in grain yield of the entries due to combined location, genotype and genotype by location interaction effects.

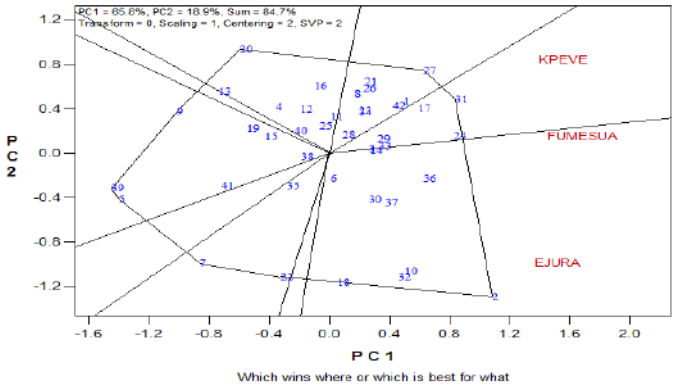

Fig.1: A 'which-won-where' view of the GGE biplot of grain yield for 42 genotypes evaluated in three locations in Ghana.

In Fig. 1, the perpendicular lines are equality lines connecting closest entries on the polygon, which make easy visual similarity of them. The equality lines split the biplot into sectors, and the winning entry for each sector was the one situated on the individual vertex (Yan and Tinker, 2006). The shape of the polygon is determined by the pointers linking the different entries that are distance away from the biplot source such that all other entries are enclosed in the polygon (Yan 2002). Hence, entries 31 (TZEI-W-POP DT $STRC_3$ x TZEI-5) was the winner in Kpeve, 24 (Fu 2090 DWDP x TZEI-46) in Fumesua and 2 (Fu 2080 DWFP x TZEI-4) in Ejura.

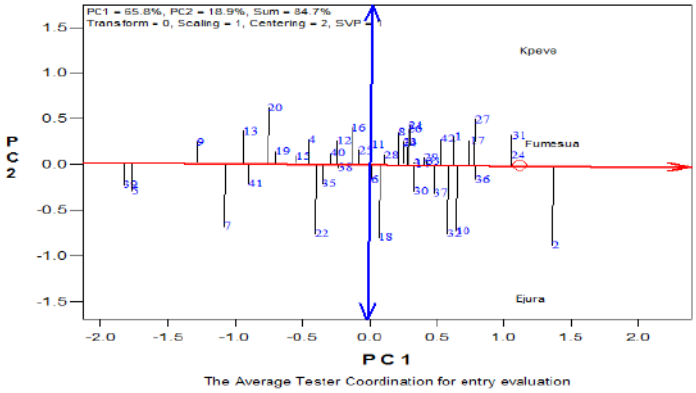

Fig.2: The "mean vs. stability" view of the GGE biplot of grain yield for 42 genotypes evaluated in three locations in Ghana.

In Fig. 2, the biplot is divided into four sectors by the single-arrowed line (AEC abscissa or x-axis) and the double-arrowed line (AEC ordinate or y-axis). Entries on the left side of the vertical line had lower than the average yield, while those on the right had higher than average yield. The AEC abscissa points to higher mean grain yield across locations. The red circle on the AEC abscissa is referred to as the average tester yield. Hence, entry 2 (Fu 2080 DWFP x TZEI-4) had the highest mean yield, followed by entries 31 (TZEI-W-POP DT $STRC_3$ x TZEI-5), 24 (Fu 2090 DWDP x TZEI-46), 27 (TZEI-W-POP DT $STRC_3$ x TZEI-1), 17 (Fu 2090 DWDP x TZEI-30), 36 (TZEI-W-POP DT $STRC_3$ x TZEI-39) and 1 (Fu 2080 DWDP x TZEI-1). The stability of a genotype is determined by their projection against the y-axis, therefore the shorter the projection of the genotype the more stable it is (Yan *et*

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al., 2007). Thus, entry 24 (Fu 2090 DWDP x TZEI-46) was identified as highly stable among the seven highest yielding genotypes, followed by entries 31 (TZEI-W-POP DT $STRC_3$ x TZEI-5), 17 (Fu 2090 DWDP x TZEI-30), 36 (TZEI-W-POP DT $STRC_3$ x TZEI-39) and 1 (Fu 2080 DWDP x TZEI-1). The checks Omankwa, Aburohemaa and Akposoe were also stable.

An interesting observation from this study was that the mean vs. stability GGE biplot identified Fu 2090 DWDP x TZEI-46 as the most stable genotype. It also ranked Fu 2090 DWDP x TZEI-46 as the highest in yield across the locations. On the contrary, the combined ANOVA ranked TZEI-W-POP DT $STRC_3$ x TZEI-5 as the highest across the three locations, although the yield difference between the two hybrids was not significant. This may be due to the scaling methods used for the construction of the biplot. A similar observation was made by Yan (2002) when he reported that the choice of scaling may affect the ranking of the genotypes depending on mean performance and stability. ANOVA is usually concerned with means but GGE biplot considers both mean and variability.

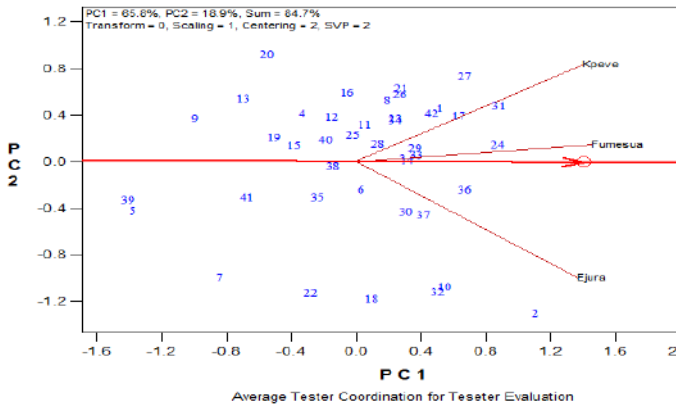

Fig. 3: The ranking of trial locations based on both discriminating ability and representativeness GGE biplot of grain yield for 42 genotypes evaluated in three locations in Ghana.

In Fig. 3, the GGE biplot presented the ideal trial location for the 42 genotypes studied across the three locations. An ideal trial location may be defined as one with high genotype discriminating ability and more representative of the broad mean of the location. While it is true that such an ideal location might not exist in actuality, it can be used as a reference for genotype selection in the multi-location yield trials. It is represented by the tiny red circle with an arrow pointing to it (Yan *et al.*, 2007). A trial location that has the slighter angle of deviation with the AEC abscissa is more representative when compared with other trial locations. Hence, Fumesua was identified as the most ideal trial location.

3.4 Correlation between grain yield and traits of agronomic importance

The phenotypic correlation coefficients between yield and yield attributes are presented in Table 6. Grain yield exhibited positive and significant ($p<0.01$ or 0.05) correlation with plant height (0.550), ear height (0.458), days to 50% tasseling (0.207), days to 50% silking (0.124), cob length (0.181), cob diameter (0.246) and kernel row^{-1}; while a non-significant association was exhibited with kernel row cob^{-1}. The highest correlation was recorded between days to 50% silking and days to 50% tasseling (0.943). While medium values were recorded between plant height and grain yield (0.550), ear height and plant height (0.675), days to 50% tasseling and plant height (0.536), cob length and plant height (0.567) and kernel row^{-1} and cob length (0.678). The lowest correlation was recorded between days to 50% silking and grain yield (0.124). Non-significant correlations were recorded between kernel row cob^{-1} and grain yield, days to 50% silking and ear height, kernel row cob^{-1} and days to 50% tasseling, kernel row cob^{-1} and days to 50% silking, kernel row^{-1} and days to 50% silking, kernel row cob^{-1} and cob length. A negative correlation was recorded between kernel row^{-1} and kernel row cob^{-1}. It is important to note that whenever two traits are correlated, selecting for one would ensure selection for the other trait, therefore selecting for the best of the traits that correlated with yield in this study would result in increased yields. Association between grain yield and plant height, ear height, days to 50% silking, days to 50% tasseling, cob diameter, cob length, kernel row^{-1} and kernel row cob^{-1} was also reported by Annapurna *et al*. (1998), Manivannan (1998) and Burak and Magoja, (1991).

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Table.6: Correlations between grain yield and other agronomic traits

Traits	PHT	EHT	DYTS	DYSK	COL	COD	KRPC	KPR	GY
PHT	1								
EHT	0.675**	1							
DYTS	0.536**	0.169*	1						
DYSK	0.374**	0.111ns	0.943**	1					
COL	0.567**	0.283**	0.423**	0.309**	1				
COD	0.385**	0.168*	0.238*	0.188*	0.350**	1			
KRPC	0.179*	0.134*	0.048ns	0.024ns	0.088ns	0.393**	1		
KPR	0.333**	0.173*	0.161*	0.080ns	0.678**	0.139*	-0.018ns	1	
GY	0.550**	0.458**	0.207*	0.124*	0.181*	0.246**	0.107ns	0.139*	1

**Highly significant (P<0.01), *Significant (P<0.05), NS=Non significant

GY=grain yield, PHT=plant height, EHT=ear height, DYTS=days to tasselling, DYSK=days to silking, COL=cob length, COD=cob diameter, KRPC=kernel rows per cobs, KPR=kernel per row,

IV. CONCLUSION

1. Traits possessing highest correlations with grain yield such as plant height and ear height can be chosen as superior characters to help improve maize grain yield. It is important to identify a variety of traits largely correlated to grain yield, which is the ultimate goal in most breeding programs.
2. The study clearly identified seven promising top cross hybrids, with the highest yielding top cross hybrid (TZEI-W-POP DT $STRC_3$ x TZEI-5) having a 12.4% yield advantage over the highest yielding check (Omankwa). The above results support the notion that moving from OPVs to top cross hybrids will enhance the productivity and production of maize.
3. The results from this study brought into focus the general opinion held by many stakeholders that use of hybrids hold the future of Ghanaian agriculture and that serious efforts must be made to encourage the adoption and use of superior hybrid maize varieties in Ghana as means of increasing maize productivity and production in the country.
4. Finally, the GGE biplot analysis used in this study could assist breeders to make better decisions on what genotypes should be recommended for release in Ghana.

ACKNOWLEDGEMENT

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Spatio Temporal Land Use Land Cover Change Mapping of Malete Elemere: Implication on Development Planning of Emerging Communities

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Abstract— *The use of Ecosystem and Biodiversity mapping, land use land cover change detection has been advocated in preparation of developmental master plan in towns and cities. Noticeable changes have been observed within Malete Elemere community since the establishment of Kwara State University Malete, yet its spatial pattern and socio ecological implication have not been investigated. This work seek to determine and produce land cover land use change map of Malete Elemere over the last 10 years and post 15 year periods through change detection techniques so as to evaluate the impact of the establishment of Kwara State university on the settlement spatial development. Landsat 7 Enhanced Thematic Mapper Plus (ETM+) satellite images of 2005, 2010 and 2015 of the study area were acquired from USGS at spatial resolution of 30 m. Radiometric correction were applied to all the images using radiance modules in Idrisi32 with radiance spectral value set at DN 0 (Lmin) and 255 (Lmax). An unsupervised classification was carried out on the composite images of bands 4,3,2,1 for all the selected years to identify possible maximum spectral reflectance classes, this was followed by supervised classification using training sample from the field survey from which image to image spatio-temporal changes statistics were extracted. To generate a prediction of LULC changes for 2025, Cellular Automata-Markovian transition estimator (CA-Markov) in Idrisi32 was used. Various Kappa statistics was used to evaluate the performance of prediction with an average K statistics of above 0.83 recorded. The result shows that built up area gained an astronomical increase (180%) between 2005 and 2015 while forest lost significantly (34%) within the same periods, with most of the gains occurring in 2010 and 2015 after the establishment of KWASU. By 2025, two Major growth pole centres will emerge along Malete Elemere Axis and one minor in Jenkunu Omoni Axis which will exert a great stress on infrastructural facilities and may create a chaotic condition if left unattended to.*

Keywords— Land use land cover (LULC) change, Spatio temporal, prediction, developmental planning.

I. INTRODUCTION AND LITERATURE REVIEW

Ecosystem and Biodiversity Mapping (EBM) has been a veritable tool being used by environmental managers and scientists for sustainable land use and planning of natural resources (Fuller et al 2014, Barthlot*het al.*, 1999). EBM does not only provide information on spatial distribution of species across the landscape but also serve as vital source of information on species natural habitat, species values and functions, the level and magnitude of any disturbance in the ecosystem (land cover land use change) all of which have great implication on developmental planning (Hegazy and Kaloop, 2015). Given the rate of deforestation and loss of biodiversity especially in developing countries through carelessness, poor planning and high level of poverty which has put undue pressure on natural resources, it is practically challenging to attain sustainable development without adequate information on the ecosystem and the biotic and abiotic composition (Gladstone and Thomas, 1990). The use of ecosystem land use land cover change detection and biodiversity mapping have been advocated in preparation of developmental master plan in towns and cities. This could help development planners in identifying protected areas, open space and designing of zoning (BRC, 2013). The Biodiversity Resources Centre, New York United States

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had undertaken the habitat (biodiversity) mapping project for over ten towns in Hudsonia developed areas as a tool for town and country planning.

Geographical Information System and Remote Sensing (GIS/RS) have proven to be very useful for large scale mapping of ecosystem and land cover (Trisurat*et al*., 2000; Foody 2002; Lu and Weng, 2007). These approaches are faster and enable wider geographic coverage within limited time frame (USGS/GAP, 2002; Lowry *et al*., 2005). Many studies on land cover and vegetation/ecosystem mapping have used data from Advanced Very High Resolution Radiometer AVHRR (Defries and Townsend, 2002), Multispectral Moderate Resolution Imaging Spectra Radiometer-MODIS (Xiao *et al*., 2002), and Landsat Enhanced Thematic Mapper Plus-ETM+ (Lu and Weng, 2007; Yuan *et al*., 2006; Yang and Lo, 2002). While, AVHRR was originally designed for meteorological service and has only two spectral bands-red and near infrared which although sufficient for basic vegetation study, MODIS though has low spatial resolution yet has more spectral bands including short wave infrared (SWIR) which can be used for obtaining greater details and advanced vegetation analysis such as leaf moisture, soil moisture, canopy water contents among others (Boles*et al*., 2006;Caccet*oet al*., 2002a, b)

Malete and its adjoining settlements were and still are rural communities with the establishment of Kwara State University Malete (KWASU) campus in 2009. It has since witnessed significant physical infrastructural development many of which are done with little or no consideration for its ecological implication, now that the development is still at its early stage and given the vision of KWASU to prepare a Development Master Plan.

1.1 Aim

To assess the landscape dynamics prior and since the establishment of KWASU and predict the socio economic and ecological implication on the adjoining community.

1.1.1 Objectives:

- To assess the landscape dynamics prior to and since the establishment of KWASU and predict the socio economic and ecological implication on the adjoining community.
- To determine biodiversity loss/gain over 15 year periods through change detection techniques and highlight its implication on developmental Planning.
- To predict possible land use pattern in the next 15 years and the relevant planning strategies to adopt.

II. MATERIALS AND METHOD

2.1 Study Area

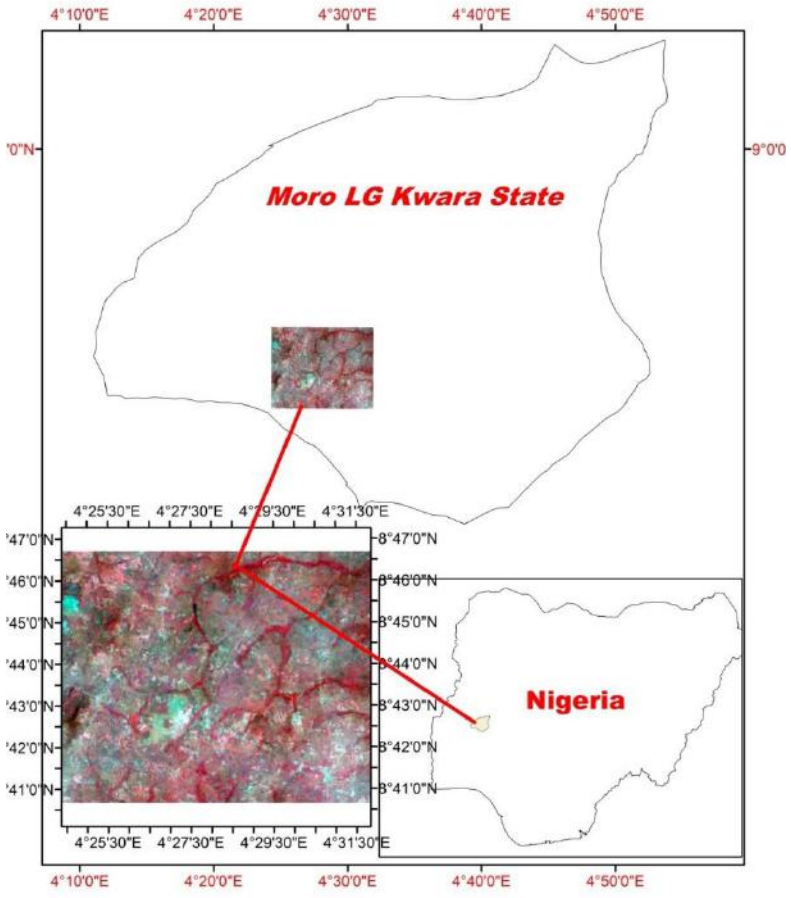

Fig.1: The study area with Nigeria map inset

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The study area is located in Moro Local Government Area of Kwara State and lies within latitude 8.6563°N to 8.8136°N and longitudes 4.2359°E to 4.5410°E. It comprises of Malete, Elemere, KWASU Campus, and the adjoining communities covering an area of about (157,701 Hectares) of land.

The study area is about 25 km North of Ilorin, the Kwara State capital though a relatively virgin area, it is highly vulnerable to unplanned expansions due to its proximity to the state capital and recently the siting of KWASU campus.

2.2 Methodology Flow Chart

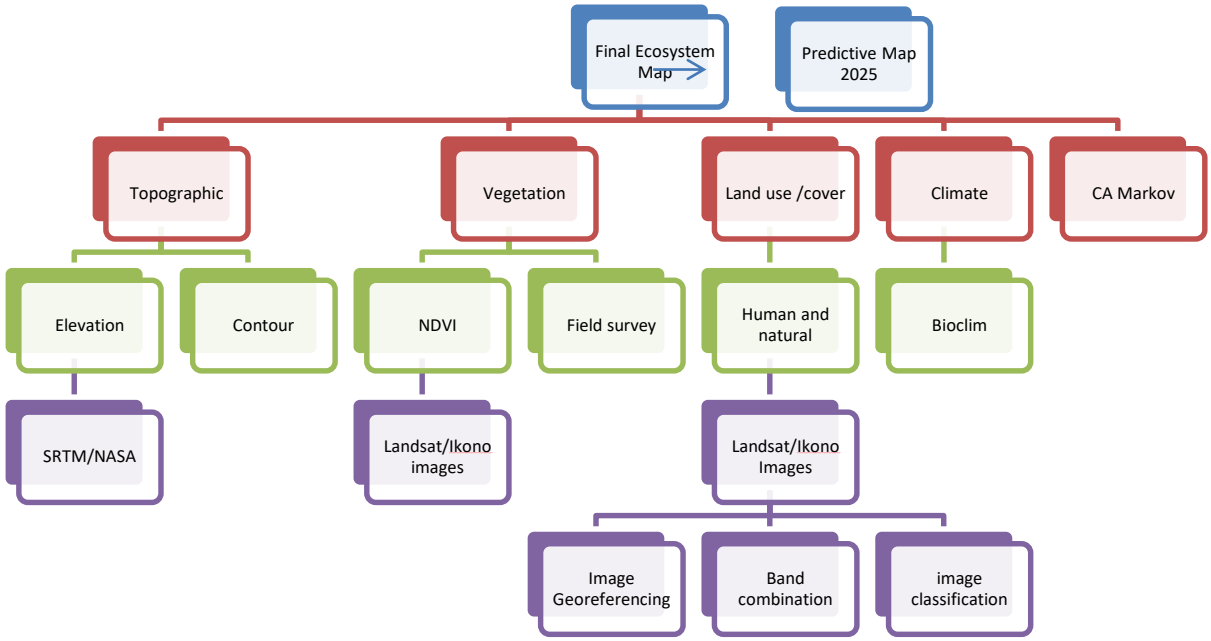

Fig.2: Methodology flow chart

2.3 Materials

The geographic extent of the study area was first determined and the shapefile prepared in Arc GIS 10.2. All the satellite images were co registered to the same study area shapefile to give similar spatial dimension. Archive Landsat 7 Enhanced Thematic Mapper Pus (ETM+) satellite images of 2005, 2010 and 2015 of the study area were acquired from USGS at spatial resolution of 30*m*.

Table.1: Summary of data acquired and used

S/N	Data Type	Years of Acquisition	Resolution / Scale	Source	Application
1	Landsat ETM+ Images	2005, 2010 2015	30m	GLCF/USGS	Land cover Mapping (NDVI)
2	Digital Elevation Model (DEM)	2000	90m	SRTM/NASA	Physiographic Mapping
3	GeoEye-1	2016	40cm	TerraServer	Image Validation/Classification
4	Climatic Data	1900-2000 2000 – 2013	0.86km²	WorldClim / URBDA	Bioclimatic classification
5	Species Occurrence	2005	Nil	GPS/GBIF Google Earth Field work	Suitability index
6	Soil Data	2007	250m	UNEP	Physiographic Mapping
7	Vegetation data	2013	25m×4m	Field Work	Veg/Classification

Acronyms:
Enhanced Thematic Mapper Plus = **ETM+**

Global land Cover facility = **GLCF**
Shuttle Radar Topographic Mission = **SRTM**
Global Biodiversity Information Facility = **GBIF**
Upper Benue River Basin Development Authority = **URBDA**
National Aeronautics and Space Administration = **NASA**
United Nation Environmental Programme = **UNEP**

2.4 Image Pre-Processing

Radiometric corrections were applied to all the images using radiance modules in Idrisi 32 with radiance spectral value set at DN 0 (Lmin) and 255 (Lmax). All images were collected in the months of July respectively being the possible periods of rainy seasons for effective measurement of plant vigour. Attempt was made to collect cloud free images in all the time series. Images band combinations were performed on bands 3, 2, 1 and 4, 3, 2 for classification (urban, water bodies and agriculture) and vegetation differencing (forest and grassland) respectively.

2.5 Image Processing

The unsupervised classification was carried out on the composite images of bands 4,3,2,1 based on pixel spectral characteristics/signatures of various land cover. An iterative ISODATA (Maximum Likelihood Classifier) algorithm was used in ArcView GIS (version 10.2) and Multispec (2013 version). This grouped similar pixels in the image into clusters or categories, and help us in determining maximum spectral classes in the images. There was no significant class change after eight spectral classes thus provided a good idea on possible classes for our classification.

To enhance our classification and identify the green index or plant cover in the study area, the Normalized Difference Vegetation Index (NDVI) was performed to compliment the earlier unsupervised classification. NDVI is a remote sensing /GIS techniques used over the years by scientists to quantitatively and qualitatively evaluate the vegetation covers of an area (Neelima T.L et al 2013). NDVI as proposed by Rouse, et al (1974) is mathematically defined as:

$$NDVI = \frac{NIR - R}{NIR + R}$$

Where, NIR and R are the reflectance in the near infrared and red regions respectively. It is the algebraic combination of red and near infrared bands to represent the amount of green vegetation in the image. In the NDVI, the values for a given pixel value is always in a number that ranges from -1 to +1. A zero means no vegetation and close to 1 indicates the highest possibility of green leaves (Biehl, 2010).

Field survey/ground truthing of 120 points were conducted on 20-30th July 2016 and was used as our training sites. This were overlaid on unsupervised land cover classes and combined with GeoEye-1 images of 2016 to prepare the supervised classification (Salako *et al*., 2016). The result of supervised classification produced the following classes of land use land cover in the study area: Forest, Mixed forest, Grassland, Farmland, Adjoining built up and Built up.

2.6 Land use land covers projection: CA Markov Techniques

To generate a prediction LULC changes for 2025, the Markovian transition estimator in Idrisi32 was used. 2005 land cover image was input as earlier image while 2015 land cover image was used as later or second image with the number of time periods between the first (earlier image) and second (later image) was 10 while the number of time for projection from the second image was also set at 10 years that is 2025. Equal probability was assigned to the entire pixel under estimation. Based on this the following estimation was generated: the probability transition matrix (Table 2), the transition area matrix (Table 3) and conditional probability image. To add the spatial dimension to our prediction the cellular automation (CA) was combined with Markov transition estimation with 2015 land cover image used as basis for projection and the earlier generated transition area matrix. The cellular automation was set at 10 to project for 2025.

2.7 Model Validation

An important stage in the development of any predictive change model is validation. Typically, one gauges one understanding of the process, and the power of the model by using it to predict some period of time when the landcover conditions are known. This is then used as a test for validation. IDRISI supplies a pair of modules to assist in the validation process. The first is called VALIDATE, and provides a comparative analysis on the basis of the Kappa Index of Agreement. Kappa is essentially a statement of proportional accuracy, adjusted for chance agreement. However, unlike the traditional Kappa statistic, VALIDATE breaks the validation down into several components, each with a special form of Kappa or associated statistic based on the work of Pontius (2000): Kappa for no information = Kno · Kappa for location = Klocation · Kappa for quantity = Kquantity · Kappa standard = Kstandard · Value of Perfect

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Information of Location = VPIL · Value of Perfect Information of Quantity = VPIQ With such a breakdown, for example, it is possible to assess the success with which one is able to specify the location of change versus the quantity of change. The accuracy of prediction is measured by the performance of various K statistics, the higher the value the better the prediction.

III. RESULT AND DISCUSSION

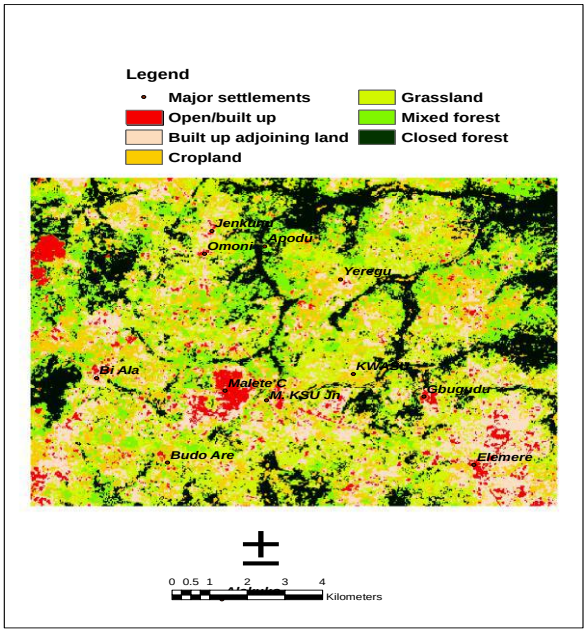
3.1 LULC 2005 – 2015

In 2005 forest cover (mixed and closed) constituted over 32 % of total LULC with built up covering only 876 ha of land representing 6% (Fig.3). While, open but adjoining built up constituted about 11%. Five years later, in 2010, part of adjoining built up had changed to cropland thus cropland increased from 19% in 2005 to 25% due to Fadama project established at the period (Fig. 4). The Built up was almost at stable point remaining at 5%. By 2015 the effect of siting KWASU campus in Malete has become obvious, the built up had increased from 5% in 2010 to 15% in 2015 covering 2400.03 ha. of land from 803 ha in 2010 (fig.5). Many estate developers and private builders sought for land within KWASU campus and Malete . However forest cover has been worst hit It fell from 17% in 2005 to about 11% in 2015. This was noticeable in Western Bi Ala where about 150 ha of forest land changed to shrubby forest and grassland in 2010 and by 2015 reduced to a narrow strip of forest of less than 45 ha (fig. 5)

Percentage of change analysis between 2005 and 2015 revealed that adjoining lowland and forest cover were the top losers with about 66% of adjoining lowland lost to either cropland and or built up (Fig. 6). This was followed by forest cover which lost about 34% of their total land (1500 ha). The top gainer was the built up area which recorded an astronomical increase of 180 % totalling over 1600 hectares of land (Table 2). This was noticeable at the major settlements of Malete, Elemere, and KWASU campus with several residential buildings used either for student hostels or private residences within 500 m radius of the campus. Smaller settlements like Apodu, Jenkunu and Gbugudu increased by 25% between 2009 and 2015.

Table.2: LULC change analysis 2005- 2015

Classes	Hectares 2005	%	Hectares 2010	%	Hectares 2015	%
Open /Built up	876.42	5.5	803.25	5.2	2400.03	15.4
Adjoining built up	1695.96	10.9	105.57	0.7	568.17	3.7
Cropland	2986.83	19.2	3796.74	24.6	4529.16	29.1
Grassland	4879.71	31.4	3782.43	24.5	3210.12	20.6
Mixed forest	2529.09	16.3	4862.97	31.5	3142.08	20.2
Closed forest	2591.73	16.7	2075.22	13.5	1710.18	11
Total	**15559.74**	**100**	**15426.18**	**100**	**15559.74**	**100**



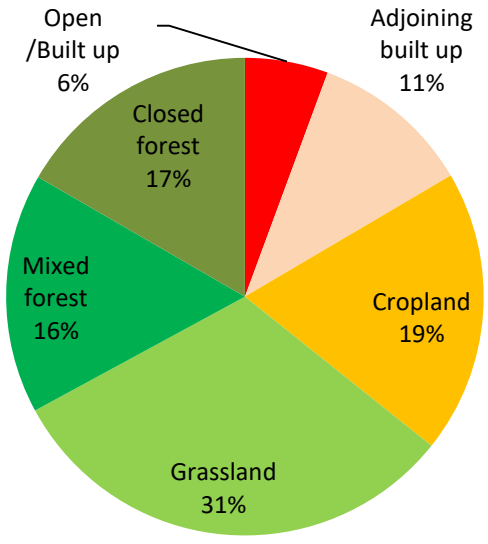


Fig.3: LULC 2005

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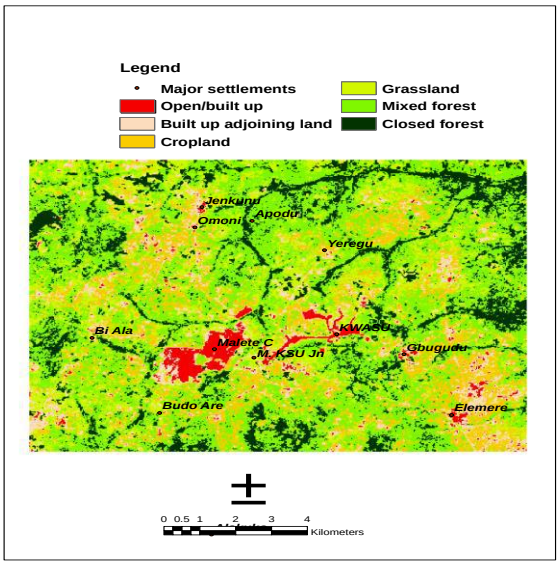


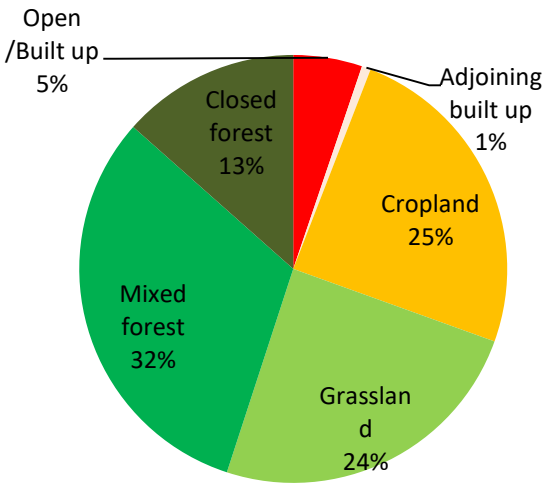
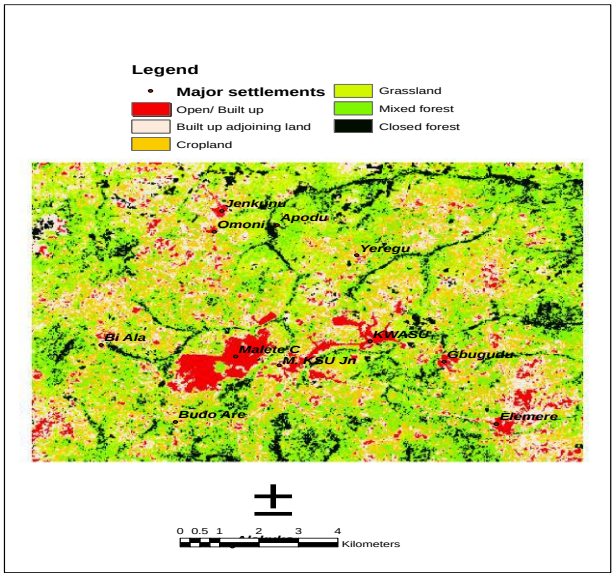


Fig.4: LULC 2010



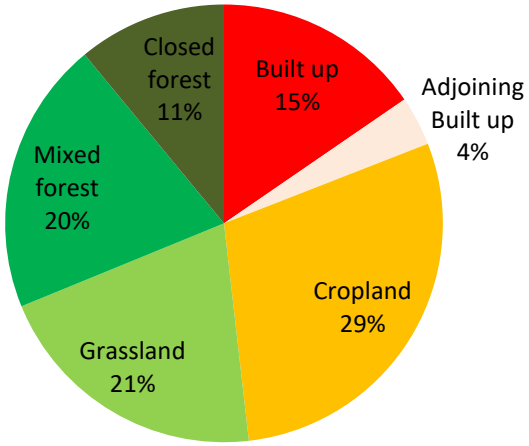


Fig.5: LULC 2015

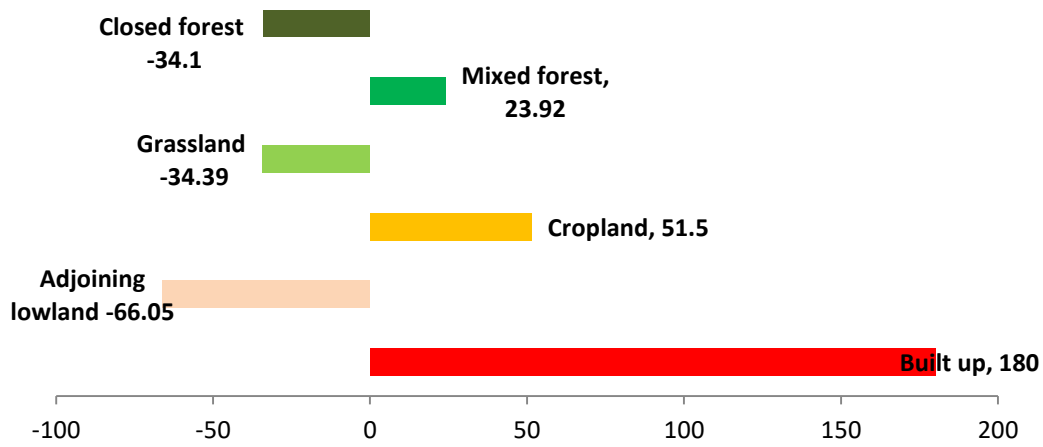


Fig.6: LULC 2005- 2015chart

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3.2 Probability transition matrix for 2025

Probability matrix of land use land cover change for 2025 was generated using CA Markov model (Table 3). Most land use tend to transit to cropland and Mixed forest especially in Elemere segment, the two LU had the highest transition probability matrix of 0.25 as compared to the overall average of 0.13 The predicted land use changes are as follows: Open adjacent to Built up, Forest to Mixed Forest, Grassland to Cropland.

Table.3: Probability matrix 2015 and 2025

		2025					
		OPB	**ADJ**	**CRP**	**GRS**	**MF**	**FR**
2015	**OPB**	0.0192	0.0871	0.2082	0.1733	0.2853	0.2269
	ADJ	0.0312	0.1136	0.2331	0.1836	0.2547	0.1838
	CRP	0.0473	0.1449	0.2556	0.1911	0.2197	0.1413
	GRS	0.0646	0.1737	0.2703	0.1941	0.1887	0.1086
	MF	0.0859	0.2022	0.2760	0.1922	0.1597	0.0840
	FR	0.1250	0.2416	0.2702	0.1836	0.1216	0.0581

OPB= Open and Built up area
ADJ= Land adjacent to Built up
CRP= Crop/farmland
GRS= Grassland
MF= Shrubby/mixed forest
FR= Closed/dense forest

3.3 Land Use Land Cover Projection 2025

Land use Land cover change for 2025 was done using CA Markov model. This explains the probability transition matrix and area change calculated from ArcGIS and Multispec. The pattern observed between 2005 and 2015 persisted in the projection with built up predicted to be having the higher percentage gain in land cover land use statistics of about 32% by closing up the adjoining open land (Fig. 8) while crop land especially around Elemere had a gain of over 4 % Mixed forest equally rose close to 5% by 2025 and this was probably due to forest degradation which would be losing over 17% of its 2015 hectares of land (1710.2) to about 1408.14 in 2025 (Table 4).

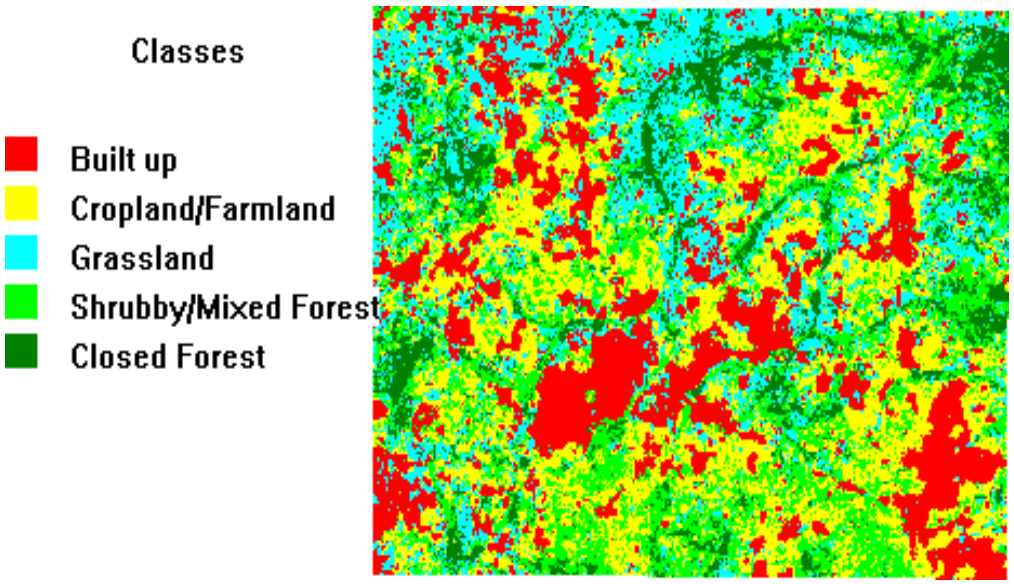

Fig.7: Projected LULC 2025

Table.4: Percentage change analysis of LULC 2015 2025

Class	**2025**		**2015**		**2015/2025**
	Area (Ha)	**%**	**Area**	**%**	**Δ %**
Forest	1408.14	9.1	1710.2	11	-17.2
Mixed Forest	3268.71	21.2	3142.4	20.2	4.95
Grassland	2572.02	16.7	3210.1	20.6	-18.91

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Crop/Farmland	4683.63	30.4	4528.3	29.1	4.46
Adj Built up	520.69	2.3	567.5	3.7	-37.83
Built up	3066.11	20.3	2400.1	15.4	31.82
Total	**15519.3**	**100**	**15558.6**	**100**	

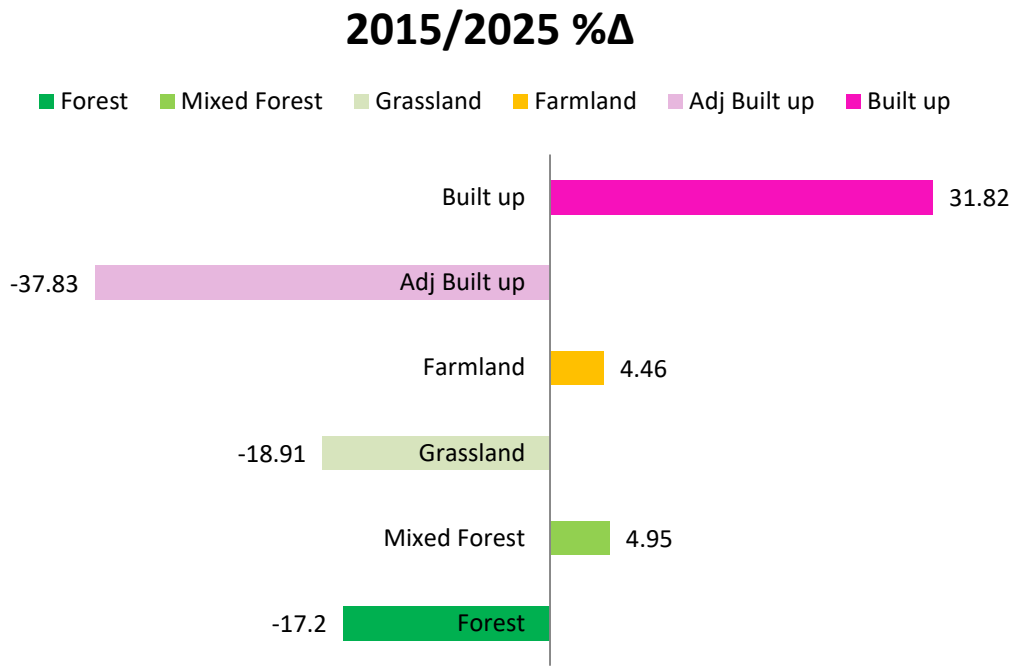

Fig.8: LULC Changes 2015 and 2025

3.4 Model Evaluation

For validation of Markov CA model using various Kappa statistics, 2015 observed image was used as referenced and was compared with the simulated image of 2015 to see the similarity between the actual and projected land use land cover map the following Kappa statistics was generated (Table 4). The result shows high performance of the model and its prediction for 2025 and 2030 since most K Statistics (K standatd value of about 0.893 and Klocation of 0.922 Kno -0.8937) were above 80% (Praveen-Subedi*et al*., 2013).

Table.5: Validation of projected 2015 LULC map with actual 2015 LUC map

		Ability to Specify Quantity		
		No[n]	Medium[m]	Perfect[p]
Ability to Specify Location	Perfect[P(x)]	P(n) = 0.9007	P(m) = 0.9754	P(p) = 1.0000
	Medium[M(x)]	M(n) = 0.8461	M(m) = 0.9150	M(p) = 0.9381
	No[N(x)]	N(n) = 0.2000	N(m) = 0.1996	N(p) = 0.2053

CorrectChance = 0.1996 CorrectQuantity = 0.0000 CorrectLocation = 0.7154 ErrorLocation = 0.0604 ErrorQuantity = 0.0246 PerfectChance = 0.2000 PerfectLocation = 0.7947 PerfectQuantity = 0.0053 VPIL = 0.0604 VPIQ = 0.0231 Kno = 0.8937 Klocation = 0.9221 Kquantity = 0.7488 Kstandard = 0.8938

IV. CONCLUSION AND RECOMMENDATION

- The adjoining built up will be closed up and fully merged with the built up area for developmental project by 2030.
- Malete-Elemere growth pole axis is at risk of chaotic urban growth if action is not taken now.
- BialaBudo Are forest will be lost and transit to shrubby forest and perhaps grassland thus deplete the area of high biodiversity values.
- The North East section is potentially forest reserve zone and could be designated as conservation area.

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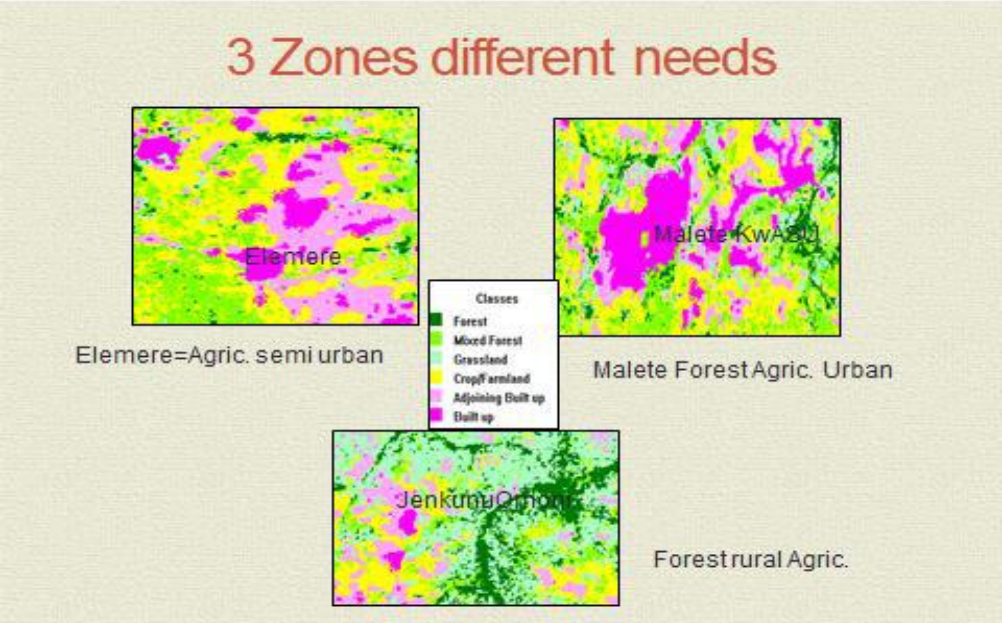
- Sustainable land use plan should give high priority to enhancement of community/land biodiversity value.
- Full community participation and input in land use planning.
- High level of adaptability in response to change with emphasis on bottom top approach to planning.
- Planning based on up-to-date data and full integration of geospatial data.
- Functional government agency to regulate, administer and implement plan policy e.g. Malete Elemere Development Area Commission.

Recommendation: Three zones of different land use planning are recommended



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Investigation of the proteolytic activity of liver trematodes in goats of Khizi-Khachmaz zone of Azerbaijan

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***Abstract*—** *The article presents experimental data on the detection of proteolytic activity of liver trematodes in the goats of Khizi-Khachmaz zone of Azerbaijan in different seasons of the year.*

Determination of the enzymatic activity was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena).

The maximum peak of intensity of proteolytic activity of trematodes isolated from goat liver was detected. The maximum value of the enzyme activity was reached in March equal to 170 μg of tyrosine per gram of wet weight of the helminth, and the minimum in June reaching 70 μg of tyrosine per gram of wet weight of the helminth.

***Keywords*—proteolytic activity, goats, trematodes.**

I. INTRODUCTION

Proteolytic enzymes play an important role in the study of nutrition of some trematodes and mainly in the study of feeding tapeworms [6].

One of the important factors determining the degree of spread and intensity of invasions is the time of year and the climatic conditions of farms.

In the literature, data are given on the extent of the invasion, depending on climatic conditions. The difference in invasiveness is explained by unequal conditions of keeping, the degree of contamination of keeping and feeding areas of animals. The isolation of invasive elements in their opinion is dependent on the condition of the host organism, feeding, habitat conditions and abiotic factors. All these factors affect the viability of helminthes in the external environment and the host organism [1, 2]

It is noted that the increase in the physiological activity of parasites and the mass maturation of most of them occur in the spring and summer and in a lesser degree in the autumn. In this case, the sexual activity of helminthes in a temperate climate begins 1.5-2 months before the growing season and the pasture of animals on the pasture. It should be noted that the time of the year is an important factor determining the effectiveness of diagnostics and establishing the intensity of infestations. All this is due to the biological cycle of helminthes in the host organism and in the environment, the nature of the feeding of the animal, the phenomena of latent invasion and the increase or suppression of the helminthic sexual activity in the host organism [3, 4, 5].

It should be noted that the pathogenesis of helminthiases is a complex phenomenon and has various aspects. The primary pathogenic factors include mechanical and toxic effects of helminthes on the organs and tissues of hosts. The mechanical action is carried out by various morpho-physiological and endoecological features of helminthes, which is manifested by traumas, destruction and tissue rupture in the host by special structural elements of parasites (oral capsule, cones, outgrowths, etc.). The toxic effect on the host's organism turns out to be the products of the vital activity and decay of helminthes, toxins that produce in the process of habitation, as well as larvae and products of their vital activity during migration. In the opinion of the authors, in the case of moniosis of lambs, the increase in body weight is reduced by 1.8-3.0 kg, from which received wool gets less, on average, by 700 g, with low tonnage [7,9,10,11].

Proceeding from the foregoing, the purpose of our studies was to study the dynamics of enzymatic activity of trematodes in biomaterial taken from the liver of killed goats of Khizi-Khachmaz zone of Azerbaijan in different seasons of the year.

II. MATERİAL AND METHODS

The object of the study were goats from the districts of Khizi and Khachmaz. The material for the study was the liver of goats slaughtered in winter (January, February), spring (March, April) and summer periods of the year (June and July).

Determination of the enzymatic activity was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena).

We have developed a modified method for the determination of enzymatic activity, using a casein

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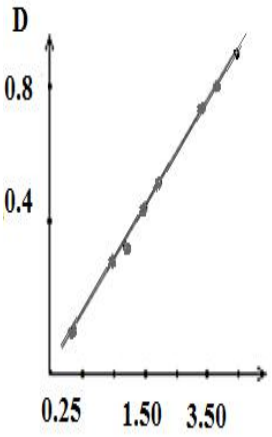
substrate, based on the determination of the rate of enzymatic substrate hydrolysis reaction under the influence of the proteolytic enzymes contained in the biomaterial under analysis.

The reaction rate corresponds to the amount of amino acids (tyrosine and tryptophan formed) that were determined spectrophotometrically with Folin reagent. This method was used to determine the studied amino acids in the free and bound state. At the same time, the amount of tyrosine and tryptophan contained in the hydrolyzate was used to determine the amount of protein converted during the enzymatic reaction, based on the protein content of 5% tyrosine and 1.5% tryptophan.

For a unit of proteolytic activity, the amount of enzyme catalyzing 30 min hydrolysis of 1 g of protein not precipitated with trichloroacetic acid was taken. In this case, 1 g was 25% of the protein taken for the enzymatic reaction.

Figure 1 shows a plot of the optical density versus the amount of protein converted during the enzymatic activity.

Figure 2 shows the data of the dependence of the optical density on the number of units of activity of proteolytic enzymes.



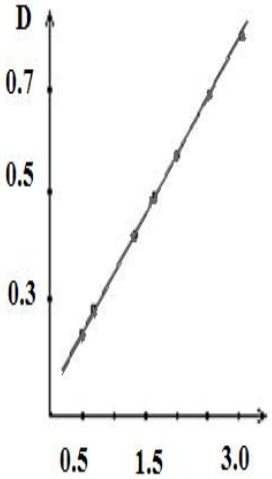


Fig.1. Dependence of the optical density of the test substance on the amount of protein converted during the enzymatic activity	Fig.2. Dependence of the optical density of the test substance on the number of units of activity of proteolytic enzymes.

III. THE PROTEIN CONTENT IN MG UNIT OF ACTIVITY

Proteolytic activity is characterized by the number of units of activity of the enzyme contained in the 1-gram of the biomaterial. This method makes possible to determine the enzymatic activity of the substances under study.

The results of the study and their discussion

Helminthes were extracted from the liver of slaughtered goats in the winter, spring and summer periods, carefully washed with 0.9% sodium chloride solution, then dried with filter paper, followed by grinding and homogenization with three volumes of 0.025N HCl at room temperature. The homogenizer was placed in an ice vessel. As a substrate, casein was used.

Proteolytic activity was determined by the method of Kunitz and Anson in the modification of Orekhovich [8].

1 ml of homogenate of worms was added to a solution of 1 ml of casein. The mixture was incubated for 1 hour in a thermostat at 370 ° C, then 3 ml of a 5% solution of trichloroacetic acid was added. Samples were left for 1 hour to form a precipitate, followed by centrifugation. Further, 1 ml of a centrifuge was taken, 2 ml of 0.5 M NaOH and 0.9 ml of Folin solution were added. Previously, the Folin solution was diluted three times with distilled water. The prepared samples were left for 10 minutes before the development of a stable color.

The extinction measurements were carried out on a spectrophotometer at a wavelength of 750 nm. As controls, samples were taken into which trichloroacetic acid was added together with the filtrate. The activity of proteolytic enzymes was expressed in 1 µg of tyrosine. The results were recalculated for 1 gram of green worm weight.

The activity of proteolytic enzymes was determined by the calibration curve. To construct a calibration curve, solutions of tyrosine containing from 1 to 100 µg of tyrosine in 1 ml were prepared.

Studying proteolytic activity in homogenates of liver tissues of goats in all experimental groups of samples revealed an increase in the quantitative indices of tyrosine in comparison with the control samples. This indicated the presence of proteolytic activity in the studied homogenates.

Quantitative data on the determination of the proteolytic activity of helminth enzymes isolated from liver tissues of goats in winter, spring and summer are given in Table 1.

Table 1.

Proteolytic activity in µkg tyrosine					
Months					
Winter season		Spring season		Summer season	
February	140	April	110	June	70
March	170	May	120	July	80

The seasonal dependence of the proteolytic activity of helminth enzymes in goat liver homogenates (in µg tyrosine per gram wet weight of helminths)

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According to the results of our organoleptic studies, the main changes in trematodes were detected in the liver in goats. At fascioliasis (medium invasion from 16 to 31 specimens), the liver of infected goats was increased, the capsule tense, of a dense consistency, brownish brown (40% of cases) or light brown in color.

In one animal (20%) at monoinvasation during palpation, the presence of parenchyma heterogeneity, granularity, was palpable. The liver was brown, the body consistency during palpation was uniform, without foci of compaction, the capsule was not strained. Only in one animal (20%) the color of the organ was changed, had a pronounced light brown hue. With an average degree of invasion, the liver of infected goats was light brown in color,was increased, the capsule tense, of a dense consistency

In the infected animals, the percentage of the liver increased by 0.03-0.77%. Thatmeans, there is an increase in the organ, which is a consequence of inflammatory processes and intoxication of the animal's body and is accompanied by hyperfunction and compensatory increase in the size of organs (Fig.3) 1

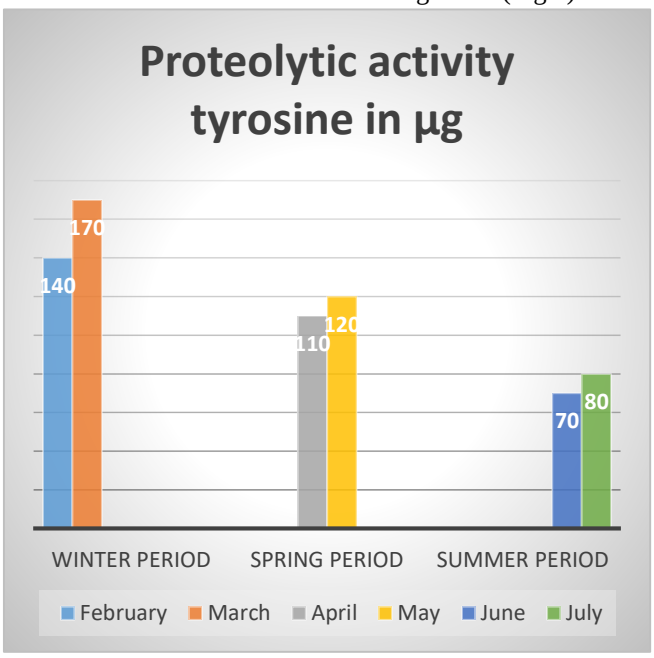

Fig.3. Diagram of seasonal dependence of proteolytic activity of helminth enzymes in goat liver homogenates (in µkg of tyrosine per gram of wet weight of helminths)

At trematodes, the decrease in the quality and nutritional value of meat, especially protein, is recorded to varying degrees, which is accompanied by a decrease in calorie content by 6.7-21.9%. At the same time, the protein-to-fat ratio for monoinvasions is significantly lower than the control group. This may be due to intoxication of the animal's organism and violation of protein and fat metabolism [12].

Subordinate to the general physiological patterns, immunity in helminthiases has its own characteristics, which depends on parasitic host relationships, physiological and ecological characteristics. There are no parasites that cause only local reactions in the host's body. The changes occurring in helminthiases in organs and tissues serve as an indicator of metabolic disturbances, the presence of dystrophic processes, allergic and immunomorphological reactions, that means, they are the response of the organism to the pathogenic action of the helminth.

E.S. Leikina [13] analyzed the domestic and foreign literature on the mechanism of immunity in helminthiases, which showed that parasites can have a double effect on the host's organism. So, on the one hand, they stimulate the immune response, as a result of which a number of phenomena of the cellular and humoral response are observed, and on the other hand - inhibit the functional and proliferative activity of cells of the lymphoid tissue, which leads to the development of secondary immune deficiencies. This contributes to a sharp change in the nature of the relationship in the host-parasite system and helps the survival of the host in the host organism [14-20]

Thus, comparing the average values of proteolytic activity in tissue homogenates of non-modems isolated from goat liver in different seasons of the year, it should be noted that their difference is significant. In conclusion, it should be noted that proteolytic activity is non-modal, in goat liver tissues reaches its maximum value in the spring season and is characterized by the highest rates in March, and the lowest in June reaching 170 and 70 µkg, respectively, in terms of µg tyrosine per gram of green worm weight.

IV. CONCLUSIONS

Thus, the carried out experimental studies revealed the presence of goat proteolytic activity in the homogenates of the liver studied.

Based on the obtained data, it can be stated that the season of the year has a significant effect on the enzymatic activity of trematodes in goat homogenates.

The maximum peak intensity of proteolytic activity of trematodes isolated from goat liver was detected. The maximum value of the enzyme activity was reached in spring in March equal to 170 µkg of tyrosine per gram of wet weight of the helminth, and minimal in the summer season in June reaching 70 µkg of tyrosine per gram of wet weight of the helminth.

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Study of the Activity of Phospholipase A2 in Venom of the Transcaucasian Macrovipera Lebetina Obtusa

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Abstract**—** *The activity of phospholipase A2 in the venom of the Transcaucasian Macrovipera lebetina obtusa, collected from viperas inhabiting in different regions of Azerbaijan in terms of pollution, was studied.*

*The lowest (*30,2 *IU / mg) was detected in the venom of Viperas collected in Sabirabad district, Karatuga village and the highest was detected in the venom of Viperas collected in Baku, s. Bina (*38,5*IU / mg).*

As a result of experimental studies it follows that the activity of phospholipase in the venom collected in the vicinity of the Sabirabad district Karatuga village and Agsu district Garagoyunlu village, Gobustan district Childag village, Bina and Sumgait is 30.2 ± 1.1 IU */ mg, 32.6 ± 0.9* IU */ mg, 34.5 ± 0.8* IU */ mg, 38.5 ± 0.2* IU */ mg and 36.1 ± 0 , 8* IU */ mg, respectively.*

Thus, the activity of phospholipase A2 in the venom of the Transcaucasian Viperas Macrovipera lebetina obtusa collected from snakes inhabiting different in the degree of contamination of the regions of Azerbaijan was studied.

The results of the experimental data can be used to determine the biological activity of the venom samples, including, for identification and standardization of the venom of the vipera.

***Keywords— phospholipase A2, venom, snake, Macrovipera lebetina obtusa, heavy metals,* enzyme activity.**

I. INTRODUCTION

Among the huge number of biologically active substances of natural origin, one of the central places is occupied by animal poisons. Toxicity and enzymatic activity are the main characteristics of the biological activity of poisonous secretions. Venom toxicity is an integral characteristic and reflects the general effect of a toxin on a living organism, while snake venom enzymes have specific application points and mechanisms of action [1,2,3].

Venomous snakes of genus Bungarus from family Elapidae are generally neurotoxin, but toxicity strongly depends on the particular species and regional origin of snakes. It should be noted that no proteomic data for B. multicinctus venom existed so far. In this venom, almost half (45%) of the proteins by weight was represented by β-bungarotoxins, followed by three finger toxins (28%) and phospholipases A2 (16%), other proteins being present at the level of 1-3%. In B. fasciatus venom, phospholipase A2 was the main component (71%), followed by oxidase of l-amino acids (8%), acetyl cholinesterase (5%) and metalloproteinases (4%). Unexpectedly, extremely low amount of three finger toxins (1%) was found in this venom. . Interestingly, the presence of complement depleting factor was observed in both venoms.

This concerns especially B.fasciatus venom with predominant content of phospholipases A2 and very low amount of three finger toxins [10].

Toxins from snake venom (among them the PLA2 and myotoxins) are neutralized by various compounds, such as antibodies and proteins purified from animal blood. Venomous and nonvenomous snakes have PLA2 inhibitory proteins, called PLIs, in their blood serum. One hypothesis that could explain the presence of these PLIs in the serum of venomous snakes would be self-protection against the enzymes of their own venom, which eventually could reach the circulatory system. However, the presence of PLIs in non-venomous snakes suggests that their physiological role might not be restricted to protection against PLA2 toxins, but could be extended to other functions, as in the innate immune system and local regulation of PLA2s [11].

Neuro- and myotoxicological signs and symptoms are significant clinical features of envenoming snakebites in many parts of the world. The toxins primarily responsible for the neuro and myotoxicity fall into one of two

categories—those that bind to and block the post-synaptic acetylcholine receptors (AChR) at the neuromuscular junction and neurotoxic phospholipases A2 (PLAs) that bind to and hydrolyse membrane phospholipids of the motor nerve terminal to cause degeneration of the nerve terminal and skeletal muscle. The rationale behind the experimental studies on the pharmacology and toxicology of the venoms and isolated PLAs in the venoms is discussed, with particular reference to the way these studies allow one to understand the biological basis of the clinical syndrome. The review also introduces the involvement of PLAs in inflammatory and degenerative disorders of the central nervous system (CNS) and their commercial use in the food industry. It concludes with an introduction to the problems associated with the use of antivenoms in the treatment of neuro-myotoxic snakebite and the search for alternative treatments [12].

More proof to influence ¬ of factors of an environment there are venoms only after drying over steams chloride calcium or after liofils drying. Venom of a cobra, at storage on a cold in the soldered ampoule has kept toxicity more than 20 years. Snake venoms are thermostabilite and in the sour environment maintain heating to 120°C without loss of activity. The analysis of the biological activity of new proteins is a rather complex task due to a wide range of possible effects. Snake venoms that exhibit pronounced biological effects are complex protein mixtures. Well established targets at the organism or molecular levels are sufficient for a number of components of snake venoms, but the mechanisms of their action at the cellular level are far from understanding even for such well-studied proteins as alpha-neurotoxins or phospholipase A2. Proteins and peptides of snake venoms can influence all key processes of cell life. The components of venoms acting on transformed cells can prove to be valuable tools for the study of tumors, and can also be used to develop new diagnostic and medicinal products [4].

Phospholipase A2 (FLA2) is one of the main toxic components of venom of snakes and as a rule has various physiological properties, including neuro-, myo- and cardiotoxic. The biological activity of snake phospholipases is extremely diverse and depends on both the structure of the enzyme and the type of cells to which they affect. The content of this review is focused on the structural and functional features of the three types of components that predominate in snake venoms: trituration toxins, metalloproteinases and phospholipases A_2.

Phospholipase A2 (FLA2) is a broad and heterogeneous family of enzymes that hydrolyze the ester bond of glycerophospholipids in the Sn2 position to form lysophospholipids and free fatty acids. Both these components participate in the generation of physiologically important secondary messengers. Arachidonic acid (AA) can serve as a precursor of eicosanoids (prostaglandins and leukotrienes), and lysophospholipids can be converted to lysophosphatidic acid or acetylated to form a platelet-activating factor (PAF) [5].

Phospholipase A2 is one of the main toxic components of venom of snakes, their biological properties are well studied. In contrast to mammalian phospholipase A2, many of them are toxic and exhibit a wide range of pharmacological effects [6, 7].

Experimental studies of the influence of the level of environmental pollution (heavy metals and radionuclides) on the enzymatic activity of the venom of the Transcaucasian vipera (Macrovipera lebetina obtusa Düigubsky, 1832) in the Absheron peninsula of Azerbaijan is undoubtedly an important and necessary step in the identification and standardization of zootoxins.

Phospholipase A2 is widespread in nature and exists in a secreted and intracellular form. Secreted phospholipases A2 include enzymes of venoms of reptiles, arthropods and coelenterates, digestive enzymes of mammals [8]. Phospholipase A2 is the most studied enzyme from the group of phospholipases. It is allotted in its pure form from the venom of snakes, bees and from the tissue of the pancreas of animals.

Snake venoms are a complex mixture of components, and more than 90% of their dry weight consists of proteins with a large variety of enzymes, and a non-protein portion comprising carbohydrates, lipids, metals, free amino acids, nucleotides and others [13].

Phospholipases are a superfamily of enzymes that act on phospholipids in the cell membrane leading to their cleavage in fatty acids and lysophospholipids. Phospholipases A_2 (PLA_2) (EC 3.1.1.4) were the first phospholipases to be known and their discovery was based on observation of the action of pancreatic fluid of mammals and snake venom in the hydrolysis of phosphatidylcholine [14].

Interestingly, despite having no catalytic activity, the homologous PLA_2s Lys49 have a wide variety of pharmacological and/or toxic effects, including myotoxicity, cytotoxicity, antibacterial, antifungal, muscle necrotic and anticoagulant activities [15, 16, 17]. According to some authors, the main structural domain responsible for the toxic effect, particularly cytotoxic, in homologous Lys49-PLA_2 is the C-terminal region (amino acids 115–129) [18].

Currently, antiserum composed of specific immunoglobulins is the only treatment for snake envenomation, but there are ongoing issues with availability, effectiveness and dosing [19, 20]. These antivenoms neutralize the toxicity and lethality of specific

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venoms, but their administration is often related with significant clinical side effects [21, 22]. Additionally, the production of antivenoms is associated with high costs related to animal maintenance and also comes across animal welfare concerns, which instigates the search for innovative products for snakebite therapy [23, 24].

The study of the activity of phospholipase A_2 is of great importance in determining the quality of snake venom and the standardization of zootoxin.

The study of biochemical, physic-chemical properties of snake venom depending on the degree of contamination of the territories of Azerbaijan is in great interest.

The purpose of this work was to study the activity of phospholipase A_2 in the venom of Transcaucasian Macrovipera lebetina obtusa, collected from snakes in different territories of Azerbaijan depending on degree of contamination.

II. MATERIALS AND METHODS

The material of the research was samples of the poison of the viperas collected from snakes in different territories of Azerbaijan depending on the degree of contamination : from the vicinity of the Gobustan district Childag village, Baku city Bina settlement, Sumgait city, Sabirabad district Karatuga village and Agsu district Garagoyunlu village.

We have conducted summer field researches in areas of Azerbaijan. During the expedition catching of Viperas has been spent with a capture of venom. A part of venom of snake has been subjected to the analysis of heavy metals by a method of atom-absorption spectrometry (AAS-300 Perkin Elmer, USA).

Determination of the content of metal ions in snake venom samples collected from snakes from the studied territories of Azerbaijan was performed by atomic absorption spectroscopy with subsequent determination of enzymatic activity. The technique of study of viper venom by atomic absorption spectrometry consists in the following. An exact amount of snake venom in quantity of 20 mg was placed in centrifuge tube, 10 ml of solution HCl (1:1) was added and further a solution left in the thermostat at 400 C at 1 hour. After that 2 ml of 20 % solutions CCl3COOH was added, with the subsequent keeping during of 1st hour at room temperature and centrifuged during 10 minutes at 1500 rpm. Fe, Cr, Cu, Cd was detected in the filtrate. It is necessary to consider that fact that standard solutions should contain 5 % trichloroacetic acid. Thus, we pick up optimum conditions for detecting Fe, Cr, Cu, Cd, Zn from trichloroacetic acid filtrate. For qualitative determination of concentration of investigated metals in bioobjects we constructed the graduated diagrams of determination of standard metals in coordinate's A-C. Under the graduated diagrams in coordinate's A-C concentration of detected elements was determined. Construction of graduated diagrams for detection of standard metals. For construction of graduated diagrams working standard solutions were entered serially into an air-acetylene flame of a burner, beginning from a solution with the minimum content of a detected element not less than four concentration, including the concentration close, to that which is expected in an analyzed solution. Each measurement repeated twice (not less than 2 times), at diagram construction average value was taken. The method of atom-absorption spectrophotometry (AAS-300, Perkin-Elmer) in vipera venom, caught from ecologically polluted sites of Absheron, defines the maintenance of heavy metals-pollutants.

The PLA2s activity in the venom was determined according to the titration method [5]. In the test tubes were placed 1 ml solution (0.05 g poison+ 0.9% KCl + water), reagent 1 (1ml) (0.1g albumin + 80 mg 0,05M Tris buffer solution pH 8.0 + 2 ml 0.05 M Trilon B solution + 0.4 ml of 50% solution of calcium chloride and the volume of solution was adjusted by 0.05 M Tris buffer to 100 ml) and 1 ml L-Alpha-lecithin in absolute ethanol. Test tubes were placed in the thermostat at a temperature of 37C for 30 min. Then 7 ml of mixture was added into all tubes, shaken and stored at 20°C for 1 hour. Then 3 ml of the upper layer from each tube was placed in 25 ml conical flasks, added 5 drops of 0,2% thymol blue in 95% ethanol and used micro burette for titration with 0.01 M of potassium hydroxide solution until the color of the solution changes from yellow to blue. The parallel control experiment was carried out, where water was taken instead of venom and then was done as described previously.

III. RESULTS AND ITS DISCUSSION

By using the method of atomic absorption spectroscopy, the content of metal ions in samples of snake venom collected from snakes from the studied territories of Azerbaijan was determined (Table 1) with the subsequent determination of enzymatic activity (Table 2).

Table.1: Data on the content of metal ions in the investigated samples of the venom of vipera, collected from snakes caught from the territory of Absheron region of Azerbaijan

Territory	Concentration of heavy metals, mg / kg (M ± m)			
	Cr	Pb	Cd	Zn
Gobustan district, Childag village	-	13.39±0.033	1.9 ± 0.200	266.9±0.034

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Sabirabad district, Karatugai village	87.0 ±0.049	8.70±0.030	-	269.0±0.076
Agsu district Garagoyunlu, village.	103.1 ± 2.793	8.13±6.560	2.42 ± 0.985	250.0±3.063
Baku city, s.Bina	-	13.86±2.36	-	354.7±8.604
Sumgait city	-	19,0±1.321	-	377.6±8.402

It has been established by method of atom-absorbing spectrometry that the maintenance of heavy metals in venom of snake changes depending on degree of impurity of district of dwelling and corresponds: Cr(87.0-103.1), Pb (8.13-19,0), Zn (250.0- 377.6), Cd (1.9-2.42) mg/kg.

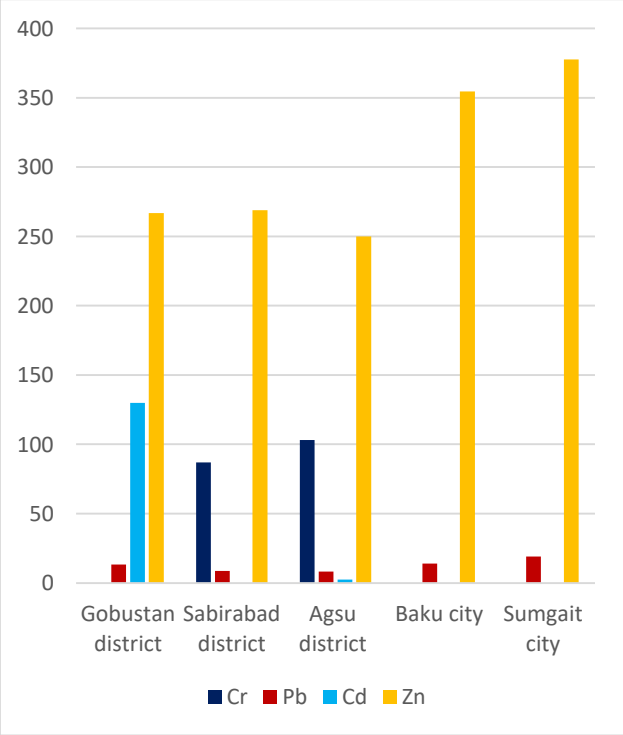

Fig.1. Dynamics of heavy metals of venom of the vipera, in the different researches district.

The results of the experimental data on the activity of phospholipase A2 in the samples of the venom of the vipera collected from snakes from different in the degree of contamination of the territories of Azerbaijan are shown in Table 2.

Table.2: The activity of phospholipase A2 in samples of the venom of vipera

(IU / mg)

The territory of Azerbaijan	Enzyme activity (IU / mg)
Gobustan district, Childag village	34,5±0,8
Sabirabad district, Karatuga village	30,2±1,1
Agsu district, Garagoyunlu village	32,6±0,9
Baku, s. Bina	38,5±0.2
Sumgait city	36,1±0,8

It can be seen from the experimental data that the activity of phospholipase A2 in samples of venom collected from snakes from the village Childag of Gobustan district is 34.5 ± 0.8 IU / mg.

It follows from the table that the activity of phospholipase in the venom collected in the vicinity of the Sabirabad area Karatuga village and the Agsu district, Garagoyunlu village is 30.2 ± 1.1 IU / mg and 32.6 ± 0.9 IU / mg, respectively. The activity of phospholipase A2 in the venom collected from snakes from the Childag village of Gobustan district is 34.5 ± 0.8 IU / mg. In the venom collected from snakes from the territory of Baku, s. Bina and Sumgait, the activity of the enzyme is 38.5 ± 0.2 IU / mg, and 36.1 ± 0.8 IU / mg, respectively.

Thus, it can be stated that the lowest enzymatic activity was established in the samples of the vipera venom from the territory where the chromium and lead ions predominated, and the largest, where chromium and cadmium ions were absent at the same time.

At the same time, the activity of the enzyme in the venom collected in the vicinity of the Sabirabad area Karatuga village and Garagoyunlu village of Agsu district was 8.3, 5.9 and 5.9, 3.5 IU less than those in the samples collected from snakes from the territory of Baku, Bina and Sumgait. The enzymatic activity of phospholipase A2 in the venom of viperas caught in the vicinity of the Gobustan district Childag village is slightly lower, of the order of 4.0 and 1.6 IU than in the samples of the venom collected from snakes from the territory of Baku, Bina and Sumgait. This fact is explained by the presence of cadmium ions in samples of the poison of viperas caught in the vicinity of the Gobustan district Childag village.

From the foregoing and as a result of experimental studies it follows that the activity of phospholipase in the venom collected in the vicinity of the Sabirabad district Karatuga village and Agsu district Garagoyunlu village, Gobustan district Childag village, Bina and Sumgait is 30.2 ± 1.1 IU / mg, 32.6 ± 0.9 IU / mg, 34.5 ± 0.8 IU / mg, 38.5 ± 0.2 IU / mg and 36.1 ± 0 , 8 IU / mg, respectively.

In conclusion, therefore, the PLA2s activity is one of the criteria for the quality of snake venom. The PLA2s is an indicator of the biological activity of snake venom. For

this indicator, the venom from the surrounding area Sabirabad district, Karatuga village and Agsu district, Garagoyunlu village has a low biological activity.

The venom collected on apiaries of Baku, s. Bina, Sumgait city and Gobustan district, Childag village has a high biological activity. The results of the experimental data can be used for identification, standardization and determining the biological activity of honeybee venom.

Thus, the activity of phospholipase A2 in the venom of the Transcaucasian Vipera Macrovipera lebetina obtusa collected from snakes inhabiting different in the degree of contamination of the regions of Azerbaijan was studied (Zagatala, Gobustan district, Childag village, Sabirabad district, Karatugai village, Agsu district Garagoyunlu, village, Baku city , s.Bina, Sumgait city)

IV. CONCLUSION

Analyzing the presented results of our research we come to following generalizations. The PLA2s activity is one of the criteria for the quality of snake venom. The PLA2s is an indicator of the biological activity of snake venom.

1. It has been established by method of atom-absorbing spectrometry that the maintenance of heavy metals in venom of snake corresponds: Pb (49.13-134.9), Zn (360.8-863.6), Cd (1.6-1.9) mg/kg.
2. The lowest enzymatic activity was found in the samples of the vipera venom from the territory where the chromium and lead ions predominated, and the largest, where chromium and cadmium ions were absent, were found.
3. The negative influence of metal ions on the enzymatic activity of phospholipase A2 of vipera venom was revealed.
4. The PLA2s activity is one of the criteria for the quality of snake venom.
5. The PLA2s is an indicator of the biological activity of snake venom.
6. The venom from the surrounding area Sabirabad and Agsu (30.2 ± 1.1 IU/mg) and (32.6 ± 0.9 IU/mg), Bina, Sumgait and Gobustan, has 38,5±0.2 IU / mg, 36.1 ± 0,8 IU / mg and 34,5±0,8 IU / mg, biological activity.
7. The venom collected on aria of Bina (38.5 IU/mg) has a high and Sabirabad (30,2 IU/mg) low biological activity.
8. The results of the experimental data can be applied in the identification, standardization and determination of the biological activity of the venom of the Transcaucasian vipera

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Cress Seed (*Lepidium sativum*) Role in the healthy Processed Spread Cheese and Its Anti-Diabetic Activity

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***Abstract*—** *The present study dealt with utilization of cress seeds (Lepidium sativum) in the manufacture of processed spread cheese, instead of emulsifying salt. Cress seed have also health promoting properties especially lowering glucose ratios. Cress seeds powder were prepared and added with the ingredients during manufacture of processed spread cheese at levels of six ratios (0.05, 1.5, 2.5, 3.5, 4.5 and 5.5%) compared with control (3% commercial emulsifying salt). The chemical, physical, microbiology and organoleptic properties of resultant samples were evaluated. Data revealed that processed spread cheese sample fortified with 3.5% cress seeds was the best either when fresh or during storage (8±2ºC for 3 months) and they had acceptable properties. Microstructure of processed cheese spread samples were also conducted. From nutritional view, processed cheese spread samples fortified with 3.5% cress seeds were used for feeding Adult male albino rats to study their effect on plasma glucose level. Obtained data indicated that the glucose level in plasma was significantly decreased (P<0.01) compared with the diabetic group. Data revealed that using of 3.5% cress seeds as a natural emulsifier agent succeed in prepare acceptable processed spread cheese sample and led to decrease plasma glucose level in Adult male albino rats.*

Keywords*— *Cress seeds, Lepidium sativum, Processed spread cheese, Rheological properties, Type-2 diabetes.

I. INTRODUCTION

The Nutritional and potential therapeutic value of food is a key characteristic in the development of new value-added products that are manufactured for health-conscious consumers [1][2]. Functional foods refer to foods or food ingredients that provide specific physiological beneficial effects and/or reduce the risk of chronic disease beyond basic Nutritional functions [3]. Processed cheese products are widely consumed as retail products (as spreads or slices) or as an ingredient in cheese-based dishes, including sandwiches, hamburgers, pizza, and lasagna. Their popularity as products may be attributed to several factors including inter alias, the diversity they offer in flavor, texture, and cooking properties; easy customization to cheese ingredient applications, adaptability to fast food trade; and their attractive packaging into convenient formats and shapes. Such diversity is controlled by changes in formulation, processing conditions and composition [4].

Processed cheese is produced by blending shredded natural cheeses of different types and degrees of maturity with melting salts, followed by heating the blend under a partial vacuum with a constant agitation until homogenous mass is obtained. In addition to natural cheeses other ingredients of both dairy and non-dairy origin may be included in the blend [5] [6]. Unripen cheeses such as White or Ricotta cheeses made by coagulating hot milk with an acid may also be used as ingredients in the process cheese blends. The use of these products in process cheese would be economically advantageous because (a) unripen cheese may be used directly from the manufacture without aging, (b) the yield is higher due to the recovery of both casein and whey proteins, and (c) unripen cheeses have very low bacterial counts at the time of manufacture [7]. Cheese whey has high biological and chemical oxygen demands, which make its disposal is a problem [8]. However, the use of cheese whey in processed cheese blends could potentially alter quality of the product. Various researchers studied the influence of incorporation of whey proteins on the functionality of processed cheese [9] [10].

Function of emulsifiers in food processing is to keep oil and water bound together. Unfortunately, some compounds used as emulsifiers are known to have potential health risks. Sodium phosphate is one such substance you'll occasionally find listed as an ingredient in processed cheese products which can damage the kidneys. Examples of other harmful emulsifiers are potassium phosphate, which may trigger allergic reactions, and tartrate which may cause diarrhea [11]. [6] revealed that use of tri-sodium citrate and sodium polyphosphate in cheese processing increased the oxidative stress in male mice that increased the toxicity

response on genetic materials, liver and kidney functions. So, an urgent demand for searching new materials used as emulsifying agents is still need.

Mucilages are polysaccharides complexes formed from sugar and uronic acid units. They form slimy masses in water are typically heterogeneous in composition. Mucilages are obtained mainly from seeds or other plant parts. Some are obtained from marine algae and from selected microorganisms [11]. Plant mucilages have been widely explored as pharmaceutical excipients [13] and have been known, since ancient times for their medical uses [14]. They are widely used in the industry as thickeners, water-retention agents, emulsion stabilizers, gelling agents, suspending agents, binders, film formers and sustained-release agents [15] [16] one of these seeds is cress.

Cress seed (*Lepidium sativum*) is a fast-growing annual herb belonging to the Brassicaceae family that are native to Egypt and west Asia, are rich source of proteins, dietary fiber, minerals and essential amino acids. They contain phenolic compounds which might be responsible for its strong antioxidant capacity. Toxicology studies of cress seeds revealed that cress seeds can be considered as non-toxic and safe. Previous studies have recommended cress seed in the treatment of hypertension, renal disease and diabetes which help to control glucose level [17]. It also shows many medicinal properties such as hypocholesterolemic, antidiarrheal, antispasmodic and laxative activities. It also has fracture healing hepatoprotective, diuretic, nephrocurative, nephroprtective, galactogogue, anti-inflammatory and antipyretic [18] [19].

Management of diabetes is being a tough task with the organic medicines which often associated with many side effects including hypoglycemic episodes and gastrointestinal disorders [20]. Recently, the world health organization (WHO) as encouraged the use of medicinal plants and their bioactive components to treat diabetic patients, as they are frequently considered to be less toxic than the synthetic ones, especially in countries where conventional treatment of diabetes seems insufficient [21].

Diabetes mellitus is a chronic metabolic disorder that is characterized by a relative or absolute lack of insulin, resulting in hyperglycemia. International Diabetes Federation has estimated the incidence of diabetes projection for the year 2015 to be 419 million adults and is expected to be 642 million by 2040 [22]. There are several forms of diabetes mellitus, the main are: type-1 and type-2. Type-2 (noninsulin-dependent diabetes, NIDDM) is a heterogeneous disorder of insulin resistance and pancreatic β-cell dysfunction [23] [24]. The rates of type-2 diabetes have increased markedly since 1960 as of 2015 there were approximately 392 million people diagnosed with type-2 diabetes compared to around 30 million in 1985, making up About 90% of all diabetes cases, which is equivalent to about 6% of the world's population [25]. In addition to serious health complications that associated with diabetes, an enormous economic cost is referred to diabetes. If untreated, it can lead to destructive conditions including metabolic problems, infection, macrovascular complications such as hypertension and stroke, microvascular complications as retinopathy, and diabetic foot disorders [26] [27].

Physico-chemical properties of cress seeds mucilage's were widely studied[28]. However, there are few controversial reports in the literature for using cress seeds mucilage's as dairy food supplements [29] [30]. Therefore, the present study describes the utilization of cress seeds instead of emulsifying salt in processed cheeses speared and the effect of this additive on the chemical, microbiological, rheological properties and organoleptic evaluation of the obtained processed cheese. The second objective of this study is to evaluate the modulatory effect of processed cheese spreads with 3.5% cress seed on the glucose level in diabetic rats.

II. MATERIALS AND METHODS

2.1. Materials:

- Fresh Buffalo's milk was obtained from Animal Production Research Institute, Ministry of Agriculture, Egypt.
- Ras cheese was obtained from Arabic Food Industrial Co. (Domty), 6th October City, Egypt. Commercial emulsifying salt (JOHA S9), special recommended for spreadable processed cheese making, was obtained from BK Ladenburg corp., GmbH, Germany.
- Citric acid was obtained from local market.
- Streptozotocin (STZ) (C8H15N3O7; molecular weight 265.22 Da) and nicotinamide (C6H6N2O; molecular weight 122.12 Da) were purchased from Sigma-Aldrich (St Louis, MO, USA).
- Citrate buffer (pH 4.5) was purchased from Bio-diagnostic (Dokki, Giza).
- Pure Crees seed (*Lepidium sativum*) were obtained from Suttons, Woodview Road, Paignton Devon TQ4 7NG Registered in England and Wales.

2.2. Methods:

2.2.1. Processing of cress seeds:

The seeds were grinded to powder of -100 mesh size and were stored in tightly capped glass bottles until used. The composition of the cress seeds varies widely between varieties and environmental conditions. Therefore, cress seed used in the present study was analyzed to explore its chemical composition of the ingredients used in the

manufacturing of processed cheese is presented in Table (1).

2.2.2. Manufacture of soft acid curd (cheese base):
Buffalo milk is an ideal raw material for manufacture of good quality cheese. Buffalo milk is standardized to a fat level of 6.0 %. The standardized milk was heated to 90ºC without holding. Thereafter, the temperature of milk was brought down to 70ºC and was coagulated at this temperature using 1 % citric acid solution. Citric acid solution was added with continuous stirring till clear whey separated out. After complete coagulation, the stirring was stopped and the coagulated mass (curd) was allowed to settle down for about 5 minutes. The whey was then drained through stainless steel strainer. The temperature of the content was not allowed to drop below 63ºC until this stage. The curd was collected and was filled in hoops (with holes on all its sides to facilitates the expulsion of whey) lined with clean fine cloth. The hoops containing curd was pressed for about 10-20 minutes [7]. Thereafter, the pressed block of curd was removed, cut into pieces. Reservation of whey product for re-use in processed cheese manufacturing process.

2.2.3. Manufacture of processed spread cheese:
Processed spread cheese samples were manufactured as mentioned by [6]. Seven different experimental batches of processed spread cheese were manufactured by mixing Ras cheese, soft acid which made as in previous and cheese whey. Commercial emulsifying salt (3%) was added as control. Cress seed was instead of emulsifying salt at six levels 0.5, 1.5, 2.5, 3.5, 4.5, 5.5% addition as well as control treatment**.** All treatments were processed in double jacket ban at 90-98 ºC/8-12 min, then placed in containers (100-120 g) and rapidly cooled to 10-14 ºC/30 min. All containers were stored in refrigerator at 8±2ºC for 3 months. Three replicates of each treatment were prepared.
Representative samples were taken for physico-chemical, rheological, microbial analysis and organoleptic assessment along storage period at zero time, 30, 60 and90 days. The formulations of processed cheese are presented in Table (2).

2.2.4. Experimental procedure:
Preliminary experiments showed that addition of 0.5 % cress seed gave very weak body & texture as well as flat flavor. On the other hand, 4.5, 5.5 % cress seed resulted in more viscous and hard consistency so, led to refuse these ratios.

2.2.5. Physico-chemical analysis:
Cress seed, Ras cheese, soft cheese, cheese whey and processed spread cheese were analyzed for moisture, fat, crude protein, ash and fiber according to the method of [31]. Total carbohydrate was calculated by difference of fat, protein and ash contents from the total solids of cress seeds. Minerals were determined using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICPAES) according to [32] procedure. pH value was measured by type-digital pH meter (model HANNA HI9321).

2.2.6. Physical analysis:
Penetrometer readings, oiling off and melting index were also determined to reflect the physical properties of the processed cheeses. Melltability was measured using the meltability test apparatus as described by [33] and modified by [34]. Oil separation of processed cheese was determined according to [35]. The processed cheeses penetrometer was measured using a penetrometer (Kochler Instrument Co. Inc., USA) as described [36].

2.2.7. Microbiological tests:
The technique according to the [37] was used. Samples were examined when fresh and monthly along the storage period for total bacterial count, moulds & yeasts count, aerobic spore forming bacterial count and coliform bacterial count according to [38].

2.2.8. Organoleptic properties:
Organoleptic properties were carried out according to the Scheme of [39]. Sensory attributes of processed spread cheese product samples were evaluated by the staff members, Animal Production Research Institute, Ministry of Agriculture, Egypt.

2.2.9. Experimental Animals:
Adult male Wistar albino rats (*Rattus norvegicus*), weighting between 130 and 140 g. They were housed in suitable cages, and acclimatized to laboratory conditions for 1 week before the commencement of the experiments. The animals were provided with fresh tap water and standard rodent food pellets; and were humanely treated in accordance with the WHO guideline for animal care. The study design was approved by the Ain Shams University Research Ethics Committee.

2.2.10. Induction of type-2 diabetes:
Type-2 diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of 100 mg/kg body weight (b.w) of STZ, 15 min after a single i.p. injection of 110 mg/kg b.w of nicotinamide. STZ and nicotinamide were dissolved in citrate buffer (pH 4.5) and physiological saline, respectively, and

hyperglycaemia was confirmed by the determination of fasting blood glucose level after 3 days of STZ injection.

2.2.11. Animal grouping and treatments:

After one week of acclimation, the animals were randomized and divided into four groups and treated for 14 days as follow: **Group I** (normal control): the animals housed in suitable cages in the same conditions of the treated groups. **Group II** (Cress): the animals were orally and daily received processed cheese spreads with 3.5% cress seed as one diet via gavage. **Group III** (diabetic control): after induction of type-2 diabetes, the animals were housed in suitable cages in the same conditions of the treated groups and served as a reference group for the diabetic-treated group. **Group IV** (diabetic + cress): diabetic rats were administered processed cheese spreads with 3.5% cress seed as one diet orally and daily via gavage for 14 days from the beginning of the experiment.

At the end of the experiment, the weight of each animal was recorded and the animals were subjected to light diethyl ether anesthesia before sacrificing. The blood was collected into clean centrifuge tubes with anticoagulant, ethylene diamine tetra-acetic acid (EDTA), then was centrifuged in a cooling centrifuge (IEC centra-4R, International Equipment Co., Needham Heights, MA, USA) for 15 minutes at 3000 rpm and 4oC to obtain plasma.

2.2.12. Determination of the glucose level:

Blood glucose level was colorimetrically determined in the plasma according to the method of [40] using Diamond Diagnostic kit (dp International, Cairo, Egypt).

2.2.13. Statistical analysis:

Statistical evaluation was conducted with Instat Program GraphPad. Software, Inc, San Digeo, USA, version3. 6, Copyright©1992-2003 Results were expressed as mean ± SEM. The results were analyzed for statistical significance by One-Way ANOVA followed by Tukey-Kramer multiple comparisons post-test. Values of $P<0.05$ were regarded as significant [41].

III. RESULTS AND DISCUSSION

3.1Physico-chemical analysis:

The results presented in Table (3) shows the physico-chemical composition of processed spread cheese. Addition of cress seeds in the processed cheese slightly increased the total solids and decreased the moisture contents with the increase ratio of cress seeds. these changes were significant ($P<0.05$). Moisture variation can also affect the rheological properties, shelf life and sensorial characteristics [42].

In the same table, the fat and protein content of processed spread cheese gradually increased with the increased ratio of cress seeds. The differences between the control and treatments in fat and protein content were significant ($P<0.05$). This can be attributed to the fat content and protein in cress seeds [43] [44]. Calculating the fat/DM recorded significant differences ($P<0.05$) between the control and all treatments due to differences in the TS and fat contents of processed spread cheese. Wide variation in the ash content of processed spread cheese were reported by [45] [46]. due to differences in the NaCl content, emulsifying salt percentage and the use of different ingredients in different studies. The control cheese had the highest ash content due to the addition of percentage commercial emulsifying salt. On the other hand, the fibers content of processed cheese samples increased with the increased of the ratios of cress seeds in the product which may be attributed to the presence of fiber content in cress seed [43]. Fiber are linked to less cardiovascular disease and plays a role in gut health [47].

Minerals content of processed spread cheese samples was given in Table (3). The iron, potassium, phosphorus and zinc contents of processed spread cheese increased gradually with addition of cress seeds and differences were found significant ($P<0.05$) due to cress seeds contained high of iron, potassium, phosphorus and zinc content [43] [44]. The highest of iron, potassium, phosphorus and zinc contents were observed in Tr_3. However, control of processed spread cheese was lowest of minerals content in all processed spread cheese treatments. The pH values of processed spread cheese are shown in Table (3). Values were 5.73, 5.59, 5.55 and 5.51 for control, Tr_1, Tr_2 and Tr_3, respectively. Therefore, control cheese had the highest pH and it is decreased with increasing the ratios of cress seed so Tr_3 was the lowest one. Statistical analysis showed that both the percentages of cress seed had a significant effect ($P<0.05$) on the pH value of processed spread cheese.

3.2. Physical analysis:

3.2.1. Penetration values

Fig. (1) indicated the change of penetration values on fresh and during storage (8±2°C). It showed that the penetration values get similar score in Tr_3 and control, while higher values can be observed in Tr_1 and Tr_2. This mean that the curds of Tr_1 and Tr_2 were weaker and softer textures than control and Tr_3, due to different between the impact of cress seed powder and commercial emulsifying salt in terms of the ability to bind water and emulsifying.

Penetration values can be affected by addition whey during processing steps. It can bind more water as a denaturation effect which leads to increase the emulsification of the fat globules [48] [49]. At the same fig. it can be showed gradually decreased in penetration

values till 3 months in all treatments. This could be related to the interaction between cress seed and state of protein network as well as the changes in chemical composition during storage [50] [51].

3.2.2. Meltability

Meltability is an important character, which determines to a great extent, the quality of processed cheese. Fig. (2) showed that melting index of processed spread cheese containing of cress seeds increased gradually with increasing the ratio of cress seeds, while control was the lowest when fresh and during storage.

The melting index of all treatments even control tended to decrease as the storage period progressed, [46] [52] [53] reported that the changes in meltability values of stored samples could be due to the changes occurred in chemical properties of processed spread cheese such as pH, protein state, emulsifying salts and the slight decrease of moisture content. Data are in agreement with that of the analysis of variance showed that the meltability was significantly affected (P<0.05) by the percentages of cress seeds and storage period.

3.2.3. Oil separation index

Oil separation defect in the process cheese can arise due to variety of reasons as low or too high level of emulsifying salts [39]. As shown in Fig. (3), cress seeds showed a pronounced effect on the oil separation of processed spread cheese. Gradual decrease of oil separation can be observed with the increasing ratios of cress seeds.

This is could be due to the emulsion effect of this seeds; while oil separation index got similar score in Tr_3 and control. Oil separation index of stored samples increased with prolonging the storage period. Data are agreed with [54,55]. Analysis of variance showed that oil separation and storage period had significantly affected (P<0.05).

3.3. Microbial content

The microbiological analysis, including total bacterial counts, coliform counts, yeast and mold counts and aerobic spore forming bacteria of processed spread cheese samples was done to determine the effect of adding of cress seed instead of emulsifying salts. Total bacterial count for treatments were 27 $x10^2$, 24 x 10^2, 21 x 10^2 and 18 x 10^2 cfu/g for control, Tr_1, Tr_2 and Tr_3, respectively, the total bacterial counts of control and treatments were lower than that reported by [56] namely: 60-5000 cfu/g, and by [57] namely: 42-87 x 10^2 cfu/g in market processed cheese samples. In the overall results observed a decrease in the number of tested microorganisms in all processed spread cheese treatments in comparison to that obtained in cheese control. These results were similar to what found by [58]. A slightly increased in the total bacterial count was observed along storage period. Total bacterial count recorded $29x10^2$, $25x10^2$, $22x10^2$, and $19x10^2$ cfu/g for control, Tr_1, Tr_2 and Tr_3, respectively. Moulds and yeasts began to appear after 2 months in control cheese (data not shown), however, these results were disagreement with what was found by Mohamed (2004), who found that processed cheese were free from yeasts and moulds during storage at 5°C or 25°C. While Moulds and yeasts were not detected in processed spread cheese containing of cress seeds in fresh and through the storage period. This is due to the presence of cress seeds which contain several compounds as possess antimicrobial against food spoilage organisms [59] Coliforms and aerobic spore forming bacteria were not detected in all cheese treatments either when fresh or during the storage period. This may be due to the high hygienic condition during the preparation.

3.4. Organoleptic evaluation

One of the most important things that were kept in view was the consumer acceptability of the final product. The assessment was done by studying the characteristics like flavor, body, texture, color, appearance and overall acceptability of processed spread cheese and result are presented in Table (4). The obtained degrees recorded that flavor was improved with addition of cress seed powder. Control sample gained 35 score while fortified samples gained 37, 38 and 39 for 1.5, 2.5, and 3.5 % cress seed powder, respectively; The flavor score between control and the differences treatments were significant. Control sample gained the lowest score due to the addition immature cheese base (acid curd) at the highest level (70%) and small amounts of extra-mature cheese which lead to weak and flat flavor in the final product. This observation was agreed with the results obtained by other researchers who used immature raw materials for processing cheese [60].

Addition of cress seed was improved the flavor; [61] reported that cress seed is peppery, tangy flavor and aroma. [62] Reported that cress have been used primarily to add flavor to simple soups and in return get the health benefits. Body and texture of cheese was composite sensory attribute resulting from a combination of physical properties that are perceived by the senses of touch (tactile texture) and sight (visual texture) during consumption. In the same table, body and texture score of the different processed spread cheese prepared using different amounts of cress seed powder were 36±2.1, 35±1.2, 37±0.2.3 and 39±0.91 for control, Tr_1, Tr_2 and Tr_3, respectively. Statistical analysis showed that there was a significant difference (P<0.05) between them. Physico-chemical properties cress seeds mucilage's are

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used to improve body and texture in the processed spread cheese as thickeners, water-retention agents, emulsion stabilizers, gelling agents, suspending agents [15] [16]. The appearance got similar score in Tr_3 and control, while Tr_1 and Tr_2 showed lower score. As a result of the decrease in rate of cress seed which did not give the required appearance, strength, and structure. Also, Table (4) shows that the scores for colour attribute decreased significantly ($P<0.05$) with the increased addition of cress seed in the processed spread cheese.

Although, differences in colour scores between control and Tr_1 were not significant, statistical analysis showed that there was a significant difference ($P<0.05$) between treatments. The organoleptic evaluations showed that the sample prepared with cress seed powder at 3.5% (w/v) had highest overall acceptability and cress seed instead of emulsifying salt is an excellent; it also helps to create a smooth texture and a shiny appearance. Fig. (4) indicated acceptability of all processed spread cheese was reduced with the progress of storage period. Values of total score were 76, 79, 82 and 86 for control, Tr_1, Tr_2 and Tr_3, respectively. These results are in agreement with those obtained by Aly *et al*. (1995) [55]

3.5. Microstructure of processed cheese spreads:

Microstructure of cheese represents the spatial distribution of the compositional components (casein, minerals, fat, moisture and dissolved solutes such as lactose, lactic acid, soluble salts and peptides) [64]. Surprising, they are only a few systematic studies on the effect of the major solid components (fat and protein) and emulsifying salt types and concentrations on the microstructure of processed spread cheese. Studies on the cress seed instead of emulsifying salt on processed spread cheese as shown in Fig. (5).

Processed cheese with 3.5% cress seed (Tr_3) seems to be better than processed cheese (control) with a good dispersion fat particulate highly scatter in the hydrated wrapped casein mixture. Due to increasing dry mater content and the fat globules in cheese, it could be found a uniform structure of casein indicating that the moisture in processed spread cheese was mainly bound water combined with the fat globule and hydrated casein; it appears having much fat globule. Mixture formulation of (Tr_3) with the highest level of cress seeds improve the structure form, this is may be due to the excess of protein and fiber in cress seeds contents, which consciously increase the penetration values [42] and increase the meltability of the cheese [36]. Regarding the microscopic investigation, it was founded that processed spread cheese with 3.5% cress seed (Tr_3) is more effective on microstructure and in appearance than processed spread cheese (control).

3.6. Effect of processed cheese spreads with 3.5% cress seed on plasma glucose level (mg/dL):

As shown in Table (5) and Fig. (6), Plasma glucose level did not significantly change ($P>0.05$) in the group that received processed cheese spreads with 3.5% cress seed for 14 consecutive days as compared with the normal control group. However, the diabetic group showed a significant increase ($P<0.001$) in the plasma glucose level compared with the normal control one. Oral treatment of diabetic groups with processed spread cheese with 3.5% cress seed showed a hypoglycaemic effect as it significantly decreased ($P<0.01$) the plasma glucose level as compared with the diabetic group.

Medicinal plants provide health benefits. Recently, several studies have investigated the hypoglycaemic property of cress seed in diabetic animal models. STZ affected β-cells of islets of Langerhans by release of toxic radicals leading to β-cell death, as they are particularly sensitive to damage by NOx and free radicals because of their low levels of free radical scavenging enzymes [65,66,27]. This caused insufficient production of insulin and consequently the elevation of blood glucose level. In the present study, induction of type-2 diabetes with STZ/nicotinamide significantly increased the glucose level and this hyperglycaemic action of STZ was confirmed by many previous studies [24,67,68,69,70,71,72]

In the present study, treatment of diabetic groups with processed cheese spreads with 3.5% cress seed showed powerful antidiabetic properties, as it decreased blood glucose level. In a similar manner, the anti-hyperglycemic effect of cress seed was investigated in STZ-induced diabetic rats that were orally administrated cress seed aqueous extract for 16 days [73]. The hypoglycaemic action of the processed cheese spreads with 3.5% cress seed may be referred to the presence of linolenic acids, high concentrations of tocopherols, flavonoids, glycosides triterpenes, sterols and many alkaloids that present in the cress seed. These compounds have been demonstrated to be able to stimulate the β-cells of pancreas to release more insulin and to enhance glucose metabolism [73-75,). Moreover, the cress seed extract has been demonstrated to affect glucose homeostasis through several pathways as the inhibition of glucose production in the liver [76], the inhibition of renal glucose reabsorption [77] increasing both glucose uptake [78] and glucose metabolism in the muscle and adipose tissues [79] or the inhibition of intestinal glucose absorption [80].

IV. CONCLUSION

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It could be concluded that cress seeds powder can be consider as an excellent natural emulsifier could be replacement by commercial emulsifier in the manufacture of processed spread cheese at the level of 3.5% (w/v) which gain the highest overall acceptability. Also, it could be concluded that processed spread cheese with 3.5% cress seed as one diet showed hypoglycemic activity and promise a new natural antidiabetic product.

Table.1: Chemical composition of all ingredients used in manufacture of processed spread cheese samples.

Composition %	Ras cheese	Cheese base (Acid curd)	Fresh whey	Cress seed powder
Total Solids	61.83	32.8	6.88	95.89
Fat	28.11	14.5	0.6	26.68
Total protein	26.70	13.0	0.8	23.27
Total carbohydrates	1.22	3.0	4.8	34.24
Fiber	--	--	---	7.3
Ash	5.8	2.3	0.68	4.4

Table.2: Formulations of processed spread cheese containing different contents of Crees seed.

Ingredients (%)	Control	Tr	Tr_1	Tr_2	Tr_3	Tr_4	Tr_5
Soft cheese	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Ras cheese	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Emulsifying salt	3.0		-	-	-	-	-
Cress seed	-	0.5	1.5	2.5	3.5	4.5	5.5
Cheese whey	18.5	21.0	20.0	19.0	18.0	17.0	16.0
Total	100	100	100	100	100	100	100

control: processed spread cheese with commercial emulsifying salts (3%).
Tr : processed spread cheese with Cress seeds (0.5%).
Tr_1: processed spread cheese with Cress seeds (1.5%).
Tr_2: processed spread cheese with Cress seeds (2.5%).
Tr_3: processed spread cheese with Cress seeds (3.5%).
Tr_4: processed spread cheese with Cress seeds (45%).
Tr_5: processed spread cheese with Cress seeds (5.5%).

Table.3: Physico-chemical analysis of processed spread cheese containing different percentages of Cress seed Mean ± standard deviation.

Composition	Control	Tr_1	Tr_2	Tr_3
Moisture %	57.59 ± 2.31^{a}	59.88 ± 1.34^{a}	58.76 ± 2.12^{b}	57.50 ± 3.52^{c}
Total Solid %	42.41 ± 1.42^{a}	40.12 ± 2.71^{c}	41.24 ± 2.33^{b}	42.50 ± 1.62^{a}
Fat %	16.57 ± 0.11^{b}	16.97 ± 0.07^{b}	17.68 ± 0.04^{a}	17.84 ± 0.05^{a}
Fat/DM %	39.10± 2.52	42.30± 2.21	42.87± 1.21	42.98± 3.01
Total Protein %	14.59 ± 0.01^{b}	14.98 ± 0.11^{b}	15.34 ± 0.12^{ab}	15.87 ± 0.06^{a}
Ash %	4.08 ± 0.04^{a}	2.97 ± 0.01^{c}	3.04 ± 0.06^{b}	3.13 ± 0.08^{b}
Fiber %	ND	0.11 ± 0.13^{c}	0.18 ± 0.07^{b}	0.25 ± 0.01^{a}
Iron%	ND	0.11 ± 0.01^{c}	0.18 ± 0.01^{b}	0.23 ± 0.03^{a}
Potassium%	97.73 ± 0.12^{d}	115.63 ± 1.0^{c}	127.58 ± 1.1^{b}	135.43 ± 1.13^{a}
Phosphorus%	512.12 ± 0.23^{d}	521.41 ± 0.12^{c}	527.62 ± 0.15^{b}	531.08 ± 0.3^{a}
Zinc%	4.1 ± 1.0^{d}	4.18 ± 1.3^{c}	4.23 ± 0.05^{b}	4.28 ± 1.3^{a}
pH	5.73± 0.08	5.59± 0.14	5.55± 0.03	5.51± 0.06

Means with the same small letters are not significantly ($p \leq 0.05$)
control: processed cheese spread with commercial emulsifying salts (3%).
Tr1: processed cheese spread with Cress seeds (1.5%).
Tr_2: processed cheese spread with Cress seeds (2.5%).
Tr_3: processed cheese spread with Cress seeds (3.5%).

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Table.4: Organoleptic evaluation of processed cheese spreads containing different percentages of cress seed Mean ± standard deviation

Organoleptic properties	Control	Tr_1	Tr_2	Tr_3
Flavor (40)	35±1.1d	37±2.3c	38±1.6b	39±1.4a
Body &Texture (40)	36±2.1c	35±1.2d	37±2.3b	39±0.91a
Appearance (10)	9±1.0a	5±2.1c	6±1.8b	9±2.4a
Color (10)	9±0.5a	8±1.1ab	7±0.9b	6±0.7c
Total (100)	89±1.9b	85±1.3c	88±1.6b	93±1.5a

Means with the same small letters are not significantly ($p \leq 0.05$)

control: processed cheese spreads with commercial emulsifying salts (3%).

Tr_1: processed cheese spread with Cress seeds (1.5%).

Tr_2: processed cheese spread with Cress seeds (2.5%).

Tr_3: processed cheese spread with Cress seeds (3.5%).

Table.5: The Mean values ± SEM of plasma glucose level (mg/dL) of control and diabetic rats treated with cress seeds.

	Control	Diabetes	Cress	Diabetes+Cress
Glucose (mg/dL)	90.125±4.44	160.9±2.39***	102.7±7.74^{ns}	127.65±4.50##

ns: non-significant with the control group; ***$P<$ 0.001 and ## $P<$ 0.01 significantly different from the control and diabetic group, respectively.

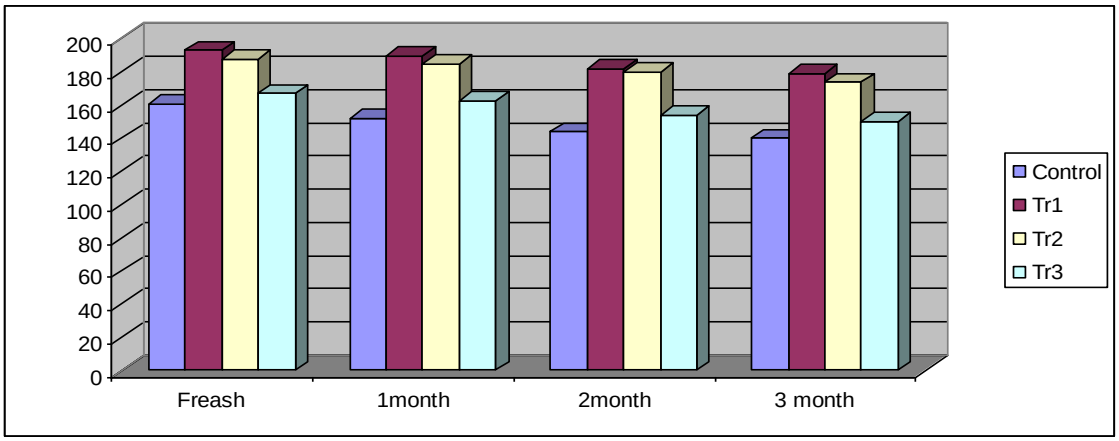

Fig. 1: Penetration values of processed spread cheese containing different percentages of cress seeds during storage period.

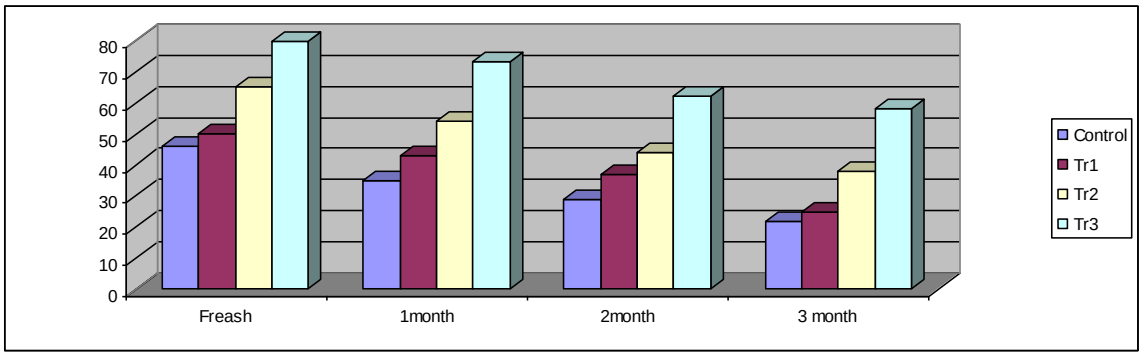

Fig.2: Meltability of processed cheese spreads containing different percentages of cress seeds during storage period.

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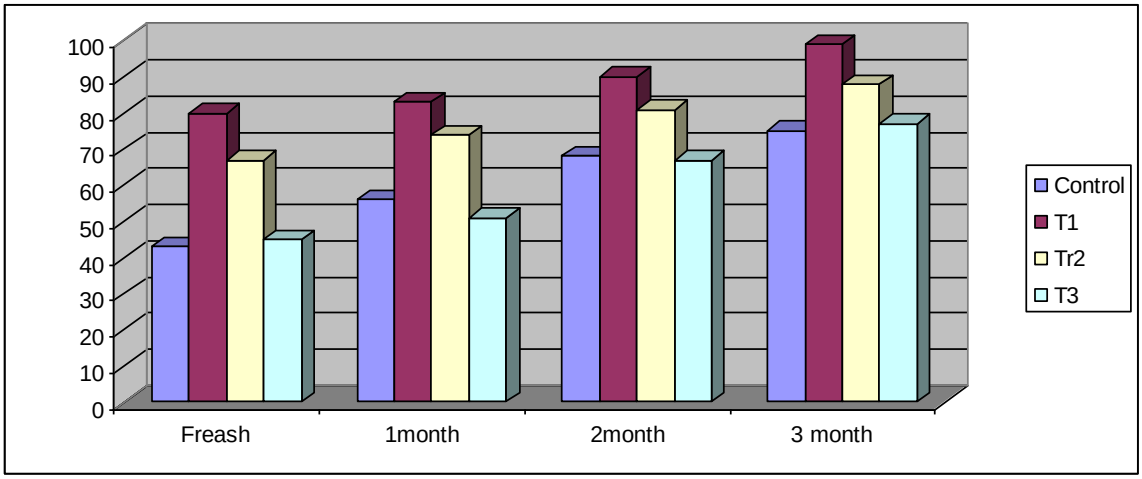


Fig.3: Oil separation index of processed cheese spreads containing different percentages of cress seeds during storage period.

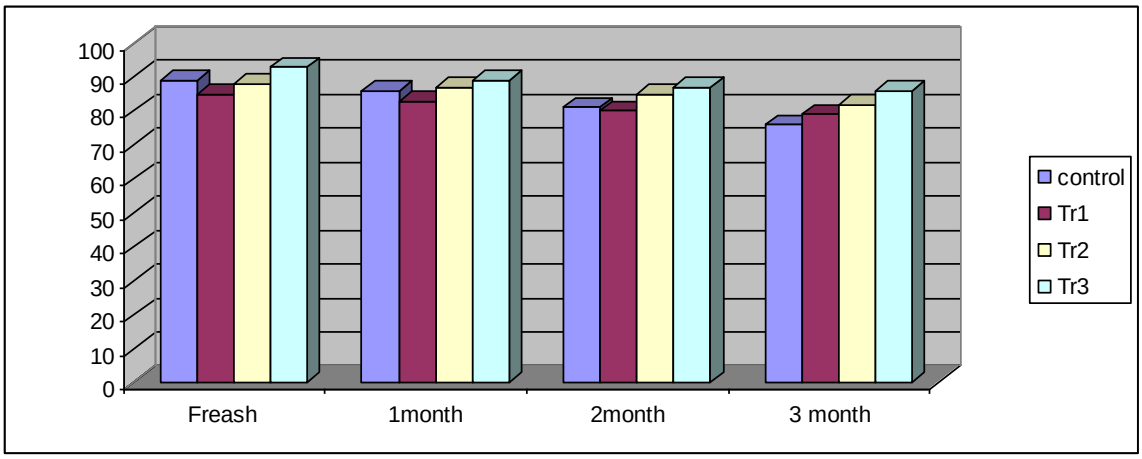
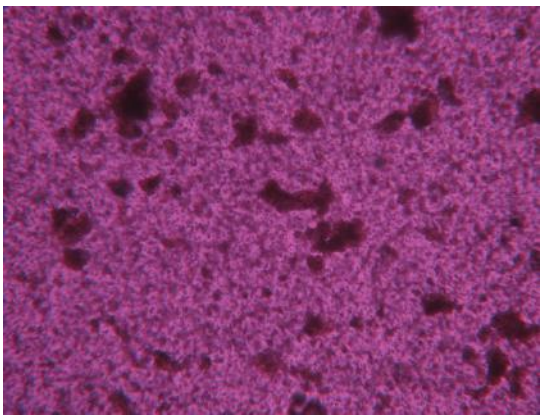
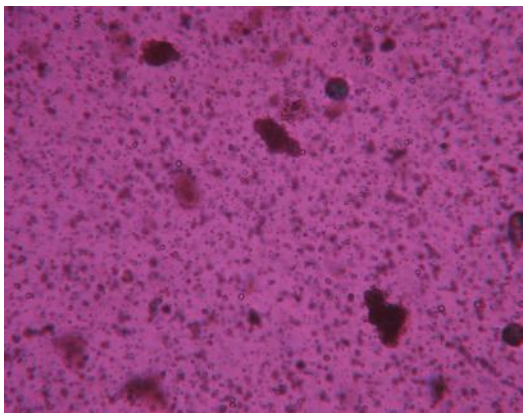


Fig.4: Overall acceptability of processed cheese spreads samples containing different percentages of cress seed during storage period.



processed spread cheese with Cress seed (3.5%).



processed spread cheese with commercial emulsifying salts.

Fig.5: Microstructure of processed cheese.

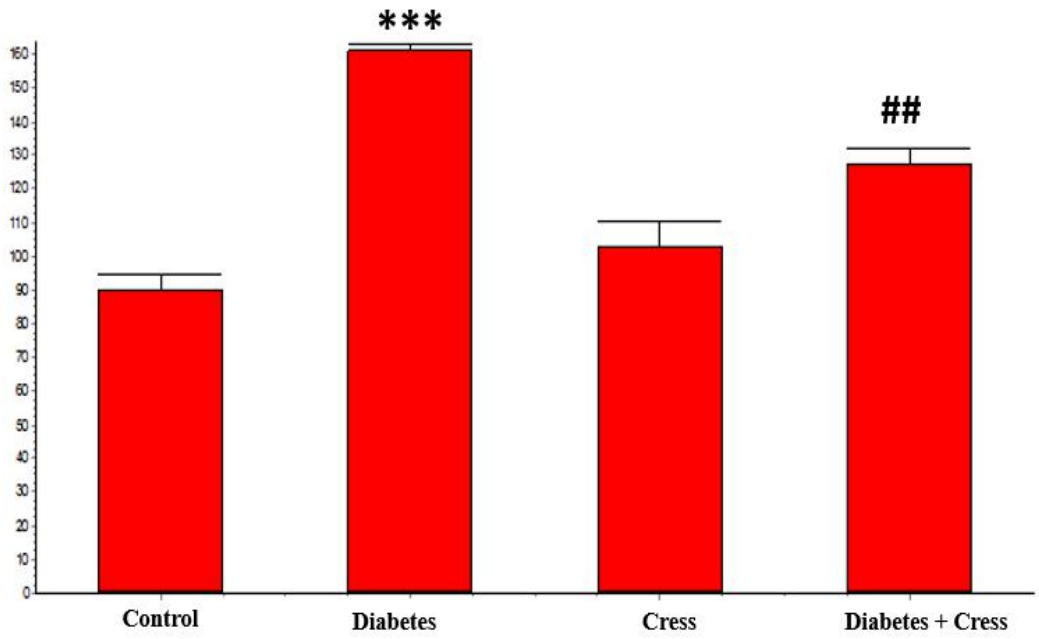


*Fig.6: Plasma glucose level (mg/dL) in control and diabetic male albino rats treated with processed cheese spreads with 3.5% cress seed. The data represent the means ± SEM.***P<0.001 and ## P<0.01 significantly different from the control and diabetic group, respectively.*

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Mosquitos' species of Diyala province, Iraq

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Abstract— *In the present study; the electrical mosquito's killer collection method was used for adult mosquitoes. Two different stations for fixing electricalmosquito's killer were chosen in the study area of veterinary college of medicine of Diyala University,Baquba, Al-muradia. Total number of 553 insects collected ; total number of mosquitoes n = 70 ;Two genera Anopheles n=21 and Culex n= 49 adults were recorded with no significance difference P-Value = 0.565; Three spp. were identified and classified as followsCx. (Cux.) pipiens Linnaeus 1758 , An. (Cel.) stephensi Liston 1901 and An. (Ano.) sacharovi Favre 1903.*

There was significant difference p=0.010 between monthly distribution in favor of March 2017 , were the total number of the insects (n=507)and the number of mosquitoes(n=64) , and lowest number were in January and February (n=0)reported ; Results revealed alsono significant difference p=0.248 between the monthly total number of mosquitoes and other insects and monthly total number of mosquitoes; Mosquitoes were captured indoors more Anopheles than outdoors; Culex recorded in November, December and March, while anopheline reported in March only in time of study.

Our present study entomological data calls the health authority to conduct further survey for Mosquito species in the province to its great role as vector of malaria.

Keywords— Mosquito, Anopheles, Culex, Diyala, Iraq.

I. INTRODUCTION

There are 3,500 named species of mosquito, of which only a couple of hundred bite or bother humans.[1]. Mosquitoes are one of the deadliest animals in the world. Their ability to carry and spread disease to humans causes millions of deaths every year. In 2015 malaria alonecaused 438 000 deaths. The worldwide incidence of dengue has risen 30-fold in the past 30 years, and more countries are reporting their first outbreaks of the disease. Zika, dengue, chikungunya, and yellow fever are all transmitted to humans by the *Aedesaegypti* mosquito. More than half of the world's population lives in areas where this mosquito species is present [2].

Not only can mosquitoes carry diseases that afflict humans, they also transmit several diseases and parasites that dogs and horses are very susceptible to. These include dog heartworm, West Nile virus and Eastern equine encephalitis. In addition, mosquito bites can cause severe skin irritation through an allergic reaction to the mosquito's saliva - this is what causes the red bump and itching[3].

Iraqi Culicidaemosquitoes had been studied by many workers since 1920 ,[4]wrote on some Culicidaeof soutren Iraq ; then in 1921 by Christopher and Shortt[5]; [6,7] have been recorded from Iraq *genusAnopheles* : *algeriensis,marten, claviger, sacharovi, maculipennis* (typical form), *hyrcanus, dthali, fluviatilis, multicolor* (the inclusion of *multicolor* rests on the reputed capture of the an adult at Sedat-al-Hindiyeh in May 1943), *superpictus, stephensi, pulcherrimus*;[8]write an list of Culicinein the central region including Baghdad during August to November ,1954; [9] found *Aedesaegypti* in Baghdad; [10], [11]and [12]worked on keys for Iraqi culicine larvae in general.

The previous authors belive that the culicine mosquitoes are still improperly sutided; only 12 species (*Culex theileri ,C. pusillus, C. tritaeiniorhynchus ,C.pipinespipines , C. pipinesfatigans ,C. torrentium , Aedescaspius , A. dorsalis , Theobaldialongiareolata, Th.subochrea ,Th.annulata and Urantaeniaunguiculate*)have been reported from Iraq and half that number from Baghdad .

[13]Provided some notes on the bionomics of *An. Maculipennis*and *An. sacharovi*from Iran and Iraq and examined the distribution of the two species in central and northern areasof Iran. [14]Recorded 15 species of *Anopheles* from Iran and provided a key for the identification of these species in both Iran and Iraq.

Of the almost 16 anopheline species so far recorded in Iraq [5, 14, 7, 15] only 3, *Anopheles stephensi*Liston, *An. sacharoui*Favre and *An. superpictus*Grassi are proven to be vectors of malaria. *An. p-ulchcrrimus*Theobald has been suspected of being a vector in Najaf Province[16] .

Mosquitoes records in Iraq shows Variation of species number reported; In12 Iraqi provinces werecollected and speciated. Four *Anopheles* (*An. pulcherrimus, An. stephensi, An. superpictus,* and *An. sacharovi*) and one *Culex (Cx.*

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pipiens) species were identified. *Anopheles pulcherrimus* was found in 11 provinces, *An. stephensi*in7, *An. superpictus*in 2and *An. sacharovi*in one province, while *Cx. pipiens*was found in all the 12 provinces. Two peaks of mosquito density were found: the first from April–June and the other from September–October[17]; while 10 speciesup to37 species belong for 4 gerera (*Anopheles*,*Culelx*, *Aedes and Culiseta)* as shown in table (1),[18].

Three species belong to three genera of Culicidae were identified,*Aedescaspius*(Pallas),*Culex pipiens*(Linnaeus) and *Culisetalongiareolata* (Macquarrt) in Al Kut city recorded by[19].

[20]Wrote aboutA parasitological survey carried in 2002 were they identified no malaria cases butan entomological survey found both *Anopheles stephensi* and *A. pulcherrimus* in high densities.

Modified Table.1: Updated checklists of mosquito species from Afghanistan and Iraq (after Rueda et al.2008).

Species	Iraq
Aedes (Aedemorphus) vexans (Meigen 1830)	R
Ae. (Ochlerotatus) caspius (Pallas1771)	A1, K, X
Ae. (Och.) dorsalis (Meigen1830)	I, K
Anopheles (Anopheles) algeriensis Theobald1903	A2, G, P
An. (Ano.) clavigerMeigen1804	A2, G, P
An. (Ano.) hyrcanus (Pallas) 1771	A2, G, K, P
An. (Ano.) maculipennisMeigen1818	A2, G, K, P
An. (Ano.) marteriSenevet and Prunnelle1927	A2, G, K, P
An. (Ano.) melanoon Hackett	G
An. (Ano.) sacharoviFavre 1903	A2, G, K, P
An. (Cel.) apoci Marsh	A2, G, K
An. (Cel.) culicifacies Giles	A2, G, K
An. (Cel.) dthaliPatton 1905	A2, G, K, P
An. (Cel.) fluviatilisJames 1902	A2, G, K, P
An. (Cel.) multicolor Cambouliu1902	A2, G, K, P
An. (Cel.) pulcherrimusTheobald 1902	A2, G, K, P, X
An. (Cel.) sergentii (Theobald) 1907	A2, G, K, X
An. (Cel.) stephensi Liston 1901	A2, G, K, P, X
An. (Cel.) superpictusGrassi1899	A2, G, K, P
An. (Cel.) turkhudi Liston	A2, G
Culex (Barradius) modestusFicalbi	A1, K, X
Cx. (Bar.) pusillusMacquart	A1, I, K
Cx. (Culex) mimeticusNoe	A1, I, H1, H2
Cx. (Cux.) perexiguus Theobald	H2, X H1, H2
Cx. (Cux.) pipiens Linnaeus	A1, I, H1, H2, K, X
Cx. (Cux.) pseudovishnuiColless	H2
Cx. (Cux.) quinquefasciatus Say	H2, I, K, X
Cx. (Cux.) theileri Theobald	A1, H1, H2, I, K, X
Cx. (Cux.) tritaeniorhynchus Giles	A1, H1, H2, I, K, X
Cx.(Mailloitia) deserticola Kirkpatrick	H1, I
Cx. (Mai.) hortensisFicalbi	A1, I
Cx. (Neoculex) territans Walker	A1
Culiseta (Allotheobaldia) longiareolata (Macquart)	AI, I, K, X
Cs. (Culicella) fumipennis (Stephens)	U
Cs. (Culiseta) annulata (Schrank)	I, K
Cs. (Cus.) subochrea (Edwards)	A1, I, K
Uranotaenia (Pseudoficalbia) unguiculata Edwards	A1, K
Total number of species	37

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*References: A1 (Abul-hab 1968), A2 (Abul-hab and Al-Kassal 1986), G (Glick 1992) ,H1 (Harbach 1985), H2 (Harbach 1988), I (Ibrahim et al. 1983), K (Khalaf 1962), P (Pringle1954), R (Reinert 1973), U (WRBU 2001), X (Rueda et al.2008).

The aim of present study is to provide an up-to date list of mosquitoes collected from internal girl's residence and animal farm of veterinary college of medicine of Diyala University.

II. MATERIALS AND METHODS

In the present study; The electrical mosquitoes killer collection method was used for outdoor and semi-indoor resting mosquitoes. For the present entomological survey, 2 fixed stations of electrical mosquitoes killer were put in the internalgirl's residence and animal farm of veterinary college of medicine of Diyala universityarea, Al-muradia, and theywere visited weeklyto collect mosquitoesvector and other insects killed. The study time conducted from November 2016-March 2017.The vectors were monitored at adult stages from various habitats. Specimens were identified to species using keys and descriptions from pertinentliterature (e.g., [24, 22,28]).

Fig.1: Electrical mosquitoes killer

Fig.2 : Petri dish used for collection of insects killed.

III. RESULTS

Total of553as shown in table (2); Two genera Anopheles n=21 and Culex n= 49adult species of mosquitoes were recorded with no significance differencebetween total number of genraP-Value = 0.565 ,table (2) , fig.(2, ; their spp. were identified and classified as follows:

Table.2: Monthly distribution total adult mosquitoes.

Month	**Total number of genera**		**Total number of mosq.**	**Total number of insects**
	Cul. mosq.	Ano. mosq.		
November 2016	4	0	4	12
December 2016	2	0	2	34
January 2017	0	0	0	0
February 2017	0	0	0	0
March 2017	43	21	64	507
total	49	21	70	553

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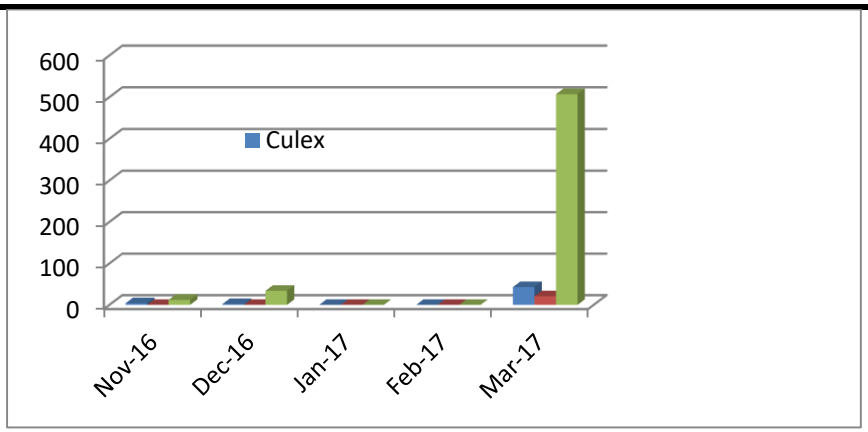

Fig.3: Monthly distribution of Mosq. and other insect

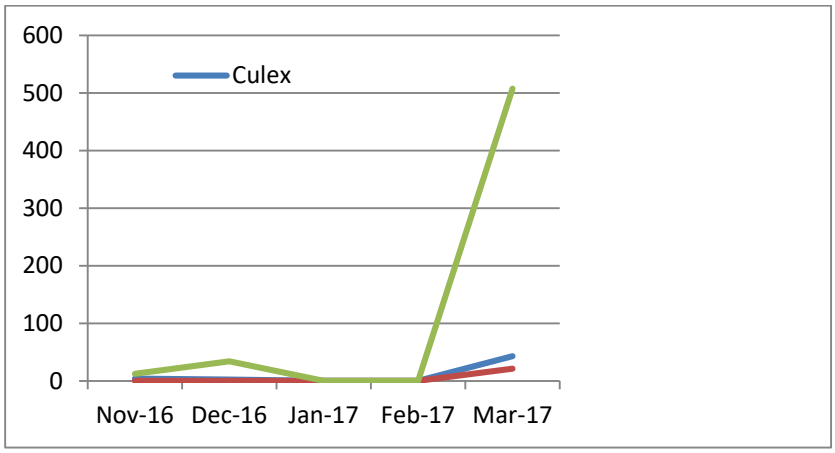

Fig.4: Monthly distribution of Mosq. and other insecta

Order Diptera
Family Culicidae

i. Sub family culicinae
Cx. (Cux.) pipiens Linnaeus 1758
ii. Sub family Anophelinae
An. (Cel.) stephensi Liston 1901
*An. (Ano.) sacharovi*Favre 1903

Fig.5: Cx. (Cux.) pipiens Linnaeus 1758 .

Fig.6: An. (Cel.) stephensiListon 1901.

Fig. 7: Anopheles An. (Ano.) sacharovi Favre 1903 .

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Fig.8: Anopheles An. (Ano.) sacharoviFavre 1903.

There was significant difference between monthly distribution in favor of March month p=0.010;Results revealed that the highest number of mosquitoes reported in March 2017 (n=70) and lowest in January and February (n=0) ; Results revealed also that the highest number of mosquitoes and other insect reported in March 2017 (n=507) and lowest in January and February (n=0) but with no significant difference p= 0.565 ; mosquitoes were captured indoors more Anopheles than indoors ; Culex recorded all over the time outdoors more than indoors resting places.

IV. DISCUSSION

There is growing evidence that the northern house mosquito, *Culex pipiens* (Diptera: Culicidae), is a major vector of avian malaria in the northern hemisphere. This mosquito, which can act as a vector of several other infectious diseases such as arboviruses[29].

Mosquitoes of*Culexpipiens* prevailing in November December and March both indoors and outdoors, this result agree with[30] inthat , it is a highly adapted to all the different types of environments; the adults of *C. pipiens* group are thought to appear throughout the year[31].

The study shows that 2 species Anopheles,*An. (Ano.) sacharovi* Favre 1903 ;*An. (Cel.) stephensi* Liston 1901 the proven vectors of malaria were encountered in Diyala area.

The findings revealed that A.*stephensi*and*A.saccharovi*only present during March 2017 in indoors resting disagrees with(20) who found that *A. stephensi* adults were present during all months of the year except January and also disagree with[17] who recorded the presence of *An. Pulcherrimus*and *An. Superpictus*only in Diyala province, but our finding of *C.pipiens*identificationagree with previously author.

Both the critical and normal thresholds were determined from the entomological data before, during and after the epidemic which is an important signal in malaria epidemiology and mosquitoes control.

In Iraq, increased *Anopheles* densities are not always associated with an epidemic disease but could be used as an indicator of epidemic risk. *A.stephensi*is the major malaria vector in the central and southern regions of Iraq. Indoor resting *A. stephensi* density was used as an indicator of epidemic risk when its density exceeded the critical level. [32].

V. CONCLUSION

Our present study entomological data calls the health authority to conduct further survey for Mosquito species in the province to its great role as vector of malaria.

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Adoption of Urea Deep Placement Recommended Practices among Rice Farmers in Niger State, Nigeria

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***Abstract*—** *This study assessed the factors influencing adoption of UDP recommended practices by rice farmers in Niger State, Nigeria. A total of 86 rice farmers were selected for the study using multi-stage sampling procedure. Structured questionnaire was used for data collection. Descriptive statistics and multiple regressions were used for data analysis. Results revealed that majority (60.5%) of the rice farmers were male and the respondents' mean age was 49 years. The mean household size was 7 people while less than half (48.9%) of the respondents had no formal education. The average farm size cultivated by the rice farmers was 0.8ha and all (100.0%) the respondents had no access to credit facilitates2015/2016 cropping season. The significant variables were sex, education, rice farming experience, farm size, paddy output, training and complexity. Constraints hindering adoption of UDP recommended practices were lack of credit facilities, labourious nature of line transplanting and USG application as well as limited USG supply, time spent on application of USG, inadequate training and health status of the rice farmers. It was recommended that government should ensure that credit facilities are made available and accessible to rice farmers in the study area. Also, trainings on UDP recommended practices should be a focal point for the government, non-governmental organizations and private sector actors to deal with the inadequate knowledge of UDP recommended practices among the rice farmers.*

***Keywords*— adoption, urea deep placement (UDP) recommended practices, rice farmers, Niger State.**

I. INTRODUCTION

Urea is the most widely used source of Nitrogen (N) fertilizer globally including Nigeria, both for irrigated and rain-fed rice cultivation. Nitrogen (N) use efficiency in rice production which is expected to increase yield can be achieved by the adoption of UDP recommended practices by rice farmers. Field demonstrations in several sites across Africa and Asia have indicated significant potential benefits of the UDP technology (IFDC, 2011) and (IFDC, 2013). IFDC promoted UDP technology adoption in Benin, Burkina Faso, Mali, Niger, Nigeria, Senegal and Togo to increase paddy grain yield and nutrient efficiency (IFDC, 2011)

According to IFDC (2012), UDP technology consists of two key components. The first is a fertilizer 'briquette' produced by compacting commercially available urea fertilizer (e.g., which is then known as Urea Super Granules (USG) weighing roughly 1-3 grams per briquette). The second key component of UDP is the placement of urea briquettes (USG) below the soil surface. When used to fertilize irrigated rice, the briquettes are centred between four plants at a depth of 7-10 centimetres within seven days after transplanting. Placement can be done either by hand or with a mechanical applicator.

USG is said to increase nitrogen use efficiency on rice fields because more urea nitrogen stays in the soil, close to the plant roots where it is absorbed more effectively (Adjornon and Liverpool-Tasie, 2014), thereby reducing greenhouse gas (GHG) emissions. In addition, the environment is protected as urea fertilizer broadcasting under the ordinary application, leach into streams and rivers rendering it unfriendly to the environment. The application of USG gradually releases Nitrogen (N) gradually in to the soli, thereby coinciding with rice crop's requirements during the growing season (IFDC, 2012). In this production process N fertilizer is required to be applied only once for the entire crop season unlike the conventional urea production process when 3-4 applications are required (mainly broadcasting first and then top-dressing subsequently).

Small granular urea is the most commonly used nitrogenous fertilizer for rice cultivation in Nigeria. In view of the fact that rice farmers yield is directly related to efficient use of urea fertilizer, the federal government of Nigeria through Federal Ministry of Agriculture and Rural Development (FMARD), Notore Chemical Industries Limited, IFDC and Maximizing Agricultural Revenue and Key Enterprises in Targeted Sites II (MARKETS II), a USAID funded project began collaborating on expanding the supply and demand of USG in targeted Nigerian rice production regions since 2012 (Tarfa and Kiger,

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2013). Likewise, FMARD approved the introduction of USG fertilizers as one of the agro-inputs distributed under the 2014 Growth Enhancement Support Scheme (GESS), in Niger, Kano, Kebbi, Jigawa, and Sokoto States on a pilot base (Fertilizer Suppliers Association of Nigeria (FEPSAN, 2014). The introduction of the USG into the GES scheme was aimed at increasing the adoption of UDP technology among rice farmers in the selected States.

Despite these interventions on UDP technology, adoption of recommended practices by rice farmers in Niger State is low. Studies carried out to examine the factors influencing adoption of UDP recommended practices are inadequate and very scanty in Niger State. The broad objective of the study is to examine the adoption of UDP recommended practices among rice farmers in Nigeria State, Nigeria. The specific objectives were to describe the socio-economic characteristics of rice farmers in the study area; determine the factors influencing the adoption of UDP recommended practices among the rice farmers, and identify constraints limiting adoption of UDP recommended practices among respondents. This study hypothesized that there is no significant relationship between the rice farmers' socio-economic characteristics and the adoption of UDP recommended practices.

II. METHODOLOGY

The study was carried out in Niger State, the state falls in the guinea savannah zone and has a climate and ecological conditions that favors agricultural production. It has an annual rainfall of between 1100mm – 1600mm and has an average temperature of 35^0C (Koloche *et al.*, 2016; Gbako, 1991). Based on the 2006 National Population Census, the state has a total population of 4,250,429 in 2006. The majority tribes are Nupe, Gwari, Hausa and Kambari with about 85% of this population practicing agriculture specifically, growing rice, sorghum, cowpea, yam and maize in large quantity.

Fig.1: Map of Niger State

The target population for this study included of rice farmers registered under the MARKETS II Project in Niger State. Multi-stage sampling procedure was used to draw samples for the study. The first stage involved a purposive sampling

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of all the intervention local government areas (LGAs) in the State as follows: Bida, Edati, Gbako, Lavun and Wushishi. This was followed by random selection of networked cooperative societies from the participating LGAs using a proportionate sampling technique, thus given 76 cooperative societies were selected from 228 cooperative societies. Lastly, since membership of the cooperative societies was not the same a proportionate sampling technique was also applied to randomly select the rice farmers, hence; a total 86 rice farmers were selected for this study.

A well-structured questionnaire was used to collect primary data for the study. Enumerators were used to administer the questionnaire to the rice farmers. Both descriptive and inferential statistics were employed in data analysis. Objectives i and iii were achieved using descriptive statistics which included frequency distribution, percentage and ranking while multiple regression analysis was used for objective ii. Multiple regression was also used to test the stated hypothesis. The functional form is expressed in the explicit form below.

$Y = a + b_1X_1 + b_2X2 + b_3X_3 \ldots\ldots\ldots\ldots\ldots\ldots b_{14}X_{14} + U$

Where:

Y = Adoption of UDP technology (UDPT)

X_1 = Sex (male=1, female= 0)

X_2 = Farmers age (years)

X_3 = Household size (number)

X_4 = Education (years)

X_5 = Rice farming experience (years)

X_6 = Extension agents visit (Yes=1, No=0)

X_7 = Access to credit (Yes=1, No=0)

X_8 = Rice farm size (hectares)

X_9 = Paddy output (Kg)

X_{10} = USG source (number of sources)

X_{11} = Training (number of trainings received)

X_{12} = Affordability of UDP technology (Expensive to use=1, Otherwise= 0)

X_{13} = Compatibility of UDP technology (Meet needs with existing values=1, Otherwise= 0)

X_{14} = Complexity of UDP technology (Difficult to use=1, Otherwise= 0)

b_1 - b_{14} = Regression coefficient

a = constant term

$X_1 – X_{14}$ = Independent variables

U= error term

III. RESULTS AND DISCUSSIONS

Table 1 shows that majority (60.5%) of the rice farmers were males. This is consistent with the findings Chekene and Chancellor (2015) who found that majority of rice farmers in Borno State, Nigeria were males. Also, the average age of the sampled farmers was 49 years. This implies that majority of the sampled farmers were within the active productive ages. This means that rice farmers are physically fit and mentally alert to embrace new techniques of rice production, such as UDP recommended practices. This agrees with Jamiu *et al.*, (2016) that the average age of rice farmers was estimated to be between 40 and 49 years in Kogi State, Nigeria.

It was further found in Table 1 that the average household size of the farmers was 7 persons. This implies that rice farmers in the area have considerable family, labour thereby reducing the cost of labour needed in adopting UDP recommended practices prescribed practices. The average rice farming experience in the area was 30 years. This implies that the respondents had long years of rice farming experience. Long farming experience is an advantage for increased rice production since it may encourage rapid adoption of improved rice recommended practices (Onyeneke, 2017).

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Table.1: Distribution of respondents on socio-economic variables

Variables	Niger State (n=86)	Mean
Sex		
Male	52(60.5)	
Female	34(39.5)	
Age (years)		
20-30	1(1.2)	49
31-40	7(8.1)	
41-50	49(57.0)	
51- 60	23(26.7)	
>60	6(7.0)	
Household size		
1 – 5	4(4.7)	7
6 – 10	11(12.8)	
11- 15	71(82.6)	
16 – 20	-	
Educational level		
No formal education	25(29.1)	
Adult education	11(12.8)	
Quranic education	6(7.0)	
Primary education	34(39.5)	
Secondary education	10(11.6)	
Tertiary education	-	
Rice farming experience		
1 – 5 years	-	30
6-10 years	-	
11-15 years	4(4.7)	
16-20 years	24(27.9)	
21-25 years	11(12.8)	
>25 years	47(54.7)	
Farm size		
< 1.0 ha	70(81.4)	0.8
1.0-1.5 ha	6(7.0)	
1.6- 2.0 ha	9(10.5)	
2.1-2.5 ha	1(1.2)	
2.6-3.0 ha	-	
>3.0 ha	-	
Access to credit		
Yes	-	
No	86	

Source: Field survey, 2016

Table 1 also indicates that the mean farm size of the respondents in the study area was 0.8 hectares which implies that majority of the respondents were in small-scale farming. The lesson to be drawn here is that, a situation where a large percentage of farmers have access only to small pockets of land does not promote agricultural production beyond subsistence level. This agrees with the findings of Musa *et al.*, (2015) in Gombe State, Nigeria. All (100%) the respondents did not have access to credit facilities in 2015/2016 cropping season, implying that the rice farmers in the study area used their personal savings as their major source of fund. This is in accord with Jamiu *et al.*, (2016). This might hinder the adoption of UDP recommended practices in the State. According to Ndagi *et al.*, (2016), lack

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of credit facilities limited the adoption of lowland rice technologies in Niger State, Nigeria.

The result of the regression model showed the factors influencing the adoption of UDP recommended practices in the study area as presented in Table 2. The regression result showed R^2 value of 0.90 which implies that 90% variation in the adoption of UDP recommended practices in the study area was explained by the independent variables included in the model. The coefficient of sex was negatively related to the adoption of UDP recommended practices and statistically significant at 10%. This is an indication that women rice farmers in the study area were more positively disposed to the use of UDP recommended practices. This result is in line with the findings of Omorogbee and Onemolease (2007) in Edo State, Nigeria where female Fadama farmers were more positively inclined to the use of recommended practices. The coefficient of household size was also positive and statistically significant at 10%. This shows that as household size increased the probability of adoption of UDP recommended practices because the use of UDP recommended practices require additional labour from the rice farmers, which could be provided by household members. The finding agrees with the report Gasarah and Aye (2015) in Kwande Benue State, Nigeria.

Table.2: Factors influencing the adoption of UDP recommended practices

Variables	Coefficient	Standard Error	T-value
Sex	-0.895	2.263	- 1.78*
Age	- 0.017	0.071	- 1.16
Household size	0.154	0.398	1.84*
Education	0.252	0.285	4.22***
Rice farming experience	- 0.021	0.055	- 1.85*
Farm size	0.364	0.672	2.58**
Access to credit	(Omitted)	-	(Omitted)
Extension visit	0.001	0.062	0.08
Paddy output	0.002	0.002	3.38***
USG source	0.815	3.070	1.26
Training	1.185	1.310	4.32***
Affordability	0.911	4.135	1.05
Compatibility	0.586	4.451	0.63
Complexity	3.008	2.209	6.48***
Constant	4.414	7.203	2.92**
F-Value			137.45
R-squared			0.90

Source: Field survey, 2016 *** = significant at 1%, ** = significant at 5%, *= significant at 10%

Table 2 also reveals that the coefficient of education was positive and significant at 1%. This indicates a positive relationship between respondents' education level and adoption of UDP recommended practices among the rice farmers in the study area. This finding corroborates that of Ehinmowo and Fatuase (2016) who reported significance influence of education on adoption of cassava processing technologies by women entrepreneurs into South-West, Nigeria. As against *a priori* expectation, the coefficient of number of years of farming experience by the farmers was negative and significant at 10%. This signifies that the experience the rice farmers have gained over the years made it difficult for them to switch to UDP recommended rice practices, no matter the perceived benefits. This finding concurred with Ume and Ochiaka (2016) in Ebonyi State, Nigeria. A positive and significant relationship was found between rice farmers' farm size and adoption of UDP recommended rice practices with coefficient at 5% probability level. Farmers with bigger farm size were better adopters, a result consistent with the findings of Kagbu *et al.*, (2016), who found a positive and significant relationship between women rice farmers' farm size and adoption of recommended rice production practices in Nasarawa State, Nigeria.

Furthermore, the coefficient of paddy output was positive significant at 5%. This implies that the output of the respondents increased with the adoption of UDP recommended practices. This agrees with Tsado (2013) who maintained that adoption of improved technology led to increased output of paddy rice in Niger and Kwara States, Nigeria. Likewise, training was positive and significant at 1% level of probability, indicating that training led to

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increase in adoption of UDP recommended practices among the rice farmers. This is line with Adjornon, and Liverpool-Tasie (2014) who opined that training focused on a new technology to a specific group is likely to increase the adoption. In addition, the coefficient of complexity was positive and significant at 1% level of probability. This suggests that the more complex the UDP recommended practices, the more the farmers sought for alternative to the recommended practices. Complexity was also found to be positive and significant to the adoption of special rice project technology package by farmers under the rice value chain in Niger State, Nigeria by Mohammed *et al.,* (2015).

Additionally, the null hypothesis which stated that "There is no significant relationship between the rice farmers' socio-economic characteristics and the adoption of UDP recommended practices." was rejected because adoption was significantly affected by the rice farmers' sex, household size, education, rice farming experience and farm size.

Table.3: Constraints to adoption of UDP recommended practices

Constraints	Percentage*	Rank
Lack of credit facilities	100.0	1st
Line transplanting is labour intensive	82.5	2nd
Limited supply USG	75.6	3rd
USG application is time consuming	75.6	3rd
Inadequate training	67.4	4th
USG application is labour intensive	60.4	5th
Health Status	58.2	6th
Lack of improved rice seeds	39.5	7th
Nursery establishment is labour intensive	34.9	8th

Source: Field survey, 2016
*Multiple responses

Table 3 presents the result of constraints to adoption of UDP recommended practices by rice farmers in the study area. Lack of credit facilities (100.0%), labourious nature of line transplanting (82.5%), limited USG supply and time spent on application of USG (75.6%) as well as inadequate training (67.4%) were the major constraints to adoption of UDP recommended practices. Other constraints were the labourious nature of USG application (60.4%), and the health status of the respondents (58.6%). This result implies that the rice farmers were faced with myriads of constraints which limited their adoption of UDP recommended practices. The implication of lack of credit facilities could be that the rice farmers could not have the purchasing power for necessary farm inputs such as USG, which could reduce the level of adoption of UDP recommended practices among the rice farmers. Mustapha *et al.,* (2012) found similar result in Borno State, Nigeria. Also, the laborious nature of USG application and transplanting as well as time spent during line transplanting and USG application deters the adoption of UDP recommended practices among the rice farmer in the study area. This agrees with Rahman and Barmon (2015), who reported that the utilization of UDP technology is labour intensive. Likewise, Tarfa and Kiger (2013) presented that that lack of access to USG is major problem hindering rice farmers from adopting UDP technology in Nigeria. Additionally, Ajibola *et al.,* (2015); Adesiji and Komolafe (2013) observed that good health affects agricultural activities by boosting farmer's capacity for intensive work and thus increases output.

IV. CONCLUSION AND RECOMMENDATIONS

Adoption of UDP recommended practices was significantly influenced by sex, education, rice farming experience, farm size, paddy output, training and complexity. Constraints hindering adoption of UDP recommended practices were lack of credit facilities, labourious nature of line transplanting, limited USG supply, time spent on application of USG, inadequate training, the labourious nature of USG application and health status of the rice farmers were the major constraint to adoption of UDP recommended practices in the study area. Relevant agencies should make credit facilities accessible to rice farmers through removal of stringent conditions attached to credit assessment. Also, to better MARKETS II Nigeria Project UDP technology dissemination in the State, more trainings on UDP recommended practices should be a focal point for the government, NGOs, private sector actors. This will go a long way to improving the adoption of UDP recommended practices among the rice farmers. More so, the private sector actors that produce USG should develop a model that would make the input more accessible to rice

farmers; this is strategic to improving the adoption of the UDP recommended practices in Niger State.

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Socio-Economic Determinants of Entrepreneurship Decision among Yam Agribusiness Entrepreneurs in Benue State, Nigeria.

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Abstract— *The socio-economic determinants of entrepreneurship decision among yam agribusiness entrepreneurs in Benue State of Nigeria were examined. The specific objective was to identify and analyse the determinants of entrepreneurship decision among yam agribusiness entrepreneurs. Data were collected from 288 yam agribusiness entrepreneurs in six local government areas and 24 wards using a multi-stage sampling technique. Structured interview schedule was used to collect the data. Data collected were analysed using logit model. The finding indicates that age, educational status, years of experience, profit motive, financial independence, place to retire, and household entrepreneurial history significantly influence entrepreneurship decision of yam agribusiness entrepreneurs. It was recommended that workshops aimed at educating yam entrepreneurs on yam processing equipment and various processed forms of yams be encouraged; campaigns geared towards promoting the financial benefits of these various food forms from yam be encouraged; and credit facilities should be made available and accessible to these yam entrepreneurs to enable them adopt and utilize these yam processing equipments.*

Keywords— Benue State, decision, determinants, entrepreneurship, yam entrepreneurs.

I. INTRODUCTION

Post-harvest losses have continued to limit the growth and development of the agricultural sector in Nigeria. For instance, a report from [15] revealed that though 92% of the world's production of yam comes from West Africa, with Nigeria alone producing 65% (i.e. more than 37 million tons), however, 20-30% of the yams produce go to waste annually due to poor post-harvest management.

Similarly, [3] revealed that the pre and post-harvest food crop loss among African countries was estimated at about 10% which is higher than the global average. According to [12], the post-harvest technological scenario in tubers, roots etc of Nigerians present a dismal picture and are mostly comprised of traditional techniques practiced by growers, traders and the processor thus resulting in considerable deterioration of physical and nutritional qualities of harvested crops.

Losses associated with these food crops limit the potential income of farmers, threatens food security, and exacerbates conditions of poverty among rural households whose income stream depends on the ability to store excess farm produce for a later date [11]. Similarly, [18] pointed out that not only are these losses a waste of food, but they also represent a similar waste of human efforts, farm inputs, livelihoods, investments and scarce resources such as water. Furthermore, [7] reported that owing to these post-harvest losses, yam in the country is becoming expensive and relatively unaffordable in urban areas as production has not kept pace with population growth leading to demand exceeding supply. Similarly, yam farmers sell their produce just after harvest to avoid losses thereby leading to low income or reduced profits as well as food insecurity particularly in the lean season [2].

In order to reduce these post-harvest losses and increase the profitability and productivity of yam agribusiness entrepreneurs, there is need to invest in yam processing equipments that produce varieties of product such as yam flour, flakes, starch, chips which tend to have longer shelf-life. According to [13], there are many export opportunities for Nigerian products (i.e. processed yam of various forms) to countries in the West African sub-region. Similarly, [16] revealed that yam flour is one of the Nigerian food products

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which could be exported if produced and displayed in a more hygienic condition.

Having recognized the importance of processing yam into various food forms to the growth and development of the yam agribusiness sector of the country as well as the consequences of poor post-harvest management of yam tubers, it becomes imperative to examine what determine the entrepreneurship decision of yam entrepreneurs in an effort to encourage investment in these yam processing equipments that are often exorbitant.

The main objective of this study was to examine the socio-economic determinants of entrepreneurship decision among yam agribusiness entrepreneurs in Benue State. Specifically, the study sought to identify and analyse the determinants of entrepreneurship decision among respondents.

II. METHODOLOGY

2.1 Study area

The study was conducted in Benue State, Nigeria. The state lies between latitudes $6^0 25^/N$ and $8^0 8^/N$ and longitudes $7^0 47^/E$ and $10^0 E$. Benue State is the nation's acclaimed food basket because of the abundance of its agricultural resources. The state is a major producer of food and cash crops [4]. Yam agribusiness entrepreneurs who are engaged in yam production, distribution/marketing of yam, yam chips production, and yam flour production abound in the state.

2.2 Sampling technique and data collection

The population for the study consisted of yam agribusiness entrepreneurs in the state. As a result of the enormity of the population for the study, a sample of 288 yam entrepreneurs from six local government area and 24 wards known for yam production was selected using multi-stage sampling technique.

The data for the study were collected using a well-structured interview schedule.

2.3 Data analysis

Logit model was used to realize the determinants of entrepreneurship decision among respondents.

2.4 Model specification

The model for the determinants of entrepreneurship decision was explicitly expressed as follows:

$$P(Y = 1) = \frac{exp^{(a+b1x1+b2x2+b3x3+\cdots+b9x9+\mu)}}{1+exp^{(a+b1x1+b2x2+b3x3+\cdots+b9x9+\mu)}}$$ where:

P (Y = 1) = the probability that a respondent deliberately seek other investment opportunities

exp = the base of natural logarithm

a = the constant of the equation

b_1- b_9 = the coefficients of the predictor variables

x_1 = age (years)

x_2 = educational status (years)

x_3 = years of experience (years)

x_4 = profit motive (quest for profit = 1; no quest for profit = 0)

x_5 = financial independence (quest for independence = 1; no quest for independence = 0)

x_6 = place to retire (quest for retirement place = 1; no quest for retirement place = 0)

x_7 = household entrepreneurial history (lineage entrepreneur = 1; lineage not entrepreneur = 0)

x_8 = household size

x_9 = marital status (married = 1; single = 0)

μ = stochastic error term

The *a priori* expectation was that the coefficients of age, marital status, and household size will be negative while those educational status, years of experience, household entrepreneurial history, profit motive, financial independence, and place to retire will be positive.

III. RESULTS AND DISCUSSION

3.1 Determinants of entrepreneurship decision

The logit model was used to investigate the effect of socio-economic characteristics of agribusiness entrepreneurs on their decision to seek other investment opportunities. The estimated relationship is presented in Table 1.

Table.1: Determinants of entrepreneurship decision

Variables	B	Sig	S.E	Wald	Exp (β)
Age	-0.192***	0.001	0.059	10.511	0.826
Educational status	0.118**	0.025	0.052	5.017	1.125
Years of experience	0.080**	0.040	0.039	4.238	1.083
Profit motive (1)	1.724***	0.002			

			0.570	9.161	5.606
Financial independence (1)	2.183***	0.000			
Place to retire (1)	2.105***	0.006	0.550	15.741	8.875
			0.768	7.505	8.208
Household entrepreneurship history (1)	-1.413**	0.013	0.571	6.136	0.243
Household size	-0.016^{NS}	0.736			
			0.047	0.114	0.984
Marital status (1)	-0.192^{NS}	0.864			
			1.119	0.029	0.825
Constant	3.436^{NS}	0.137			
			2.310	3.213	31.064
Model Chi-square	123.179***	0.000			
Nagelkerke R square	0.676				
Percentage correct	87.5				

Source: Field survey, 2015. * Significant at 10.0% level; ** Significant at 5.0% level; * Significant at 1.0% level; NS = Not significant.**

From the analysis, the model chi-square was 123.179 which were significant 1% thus rejecting the null hypothesis that there is no difference between the model with only a constant and the model with independent variables. In other words, the existence of a relationship between the socio-economic characteristics of yam agribusiness entrepreneurs and their entrepreneurship decision was supported.

The Nagelkerke R square was 0.676 thus indicating a strong relationship of 67.6% between the predictors and the predictions. The analysis also revealed that none of the independent variables had a standard error (S.E) greater than 2.0 thus confirming the absence of numerical problem such as multicollinearity among the independent variables.

The prediction success overall was 87.5% (71.4% for does not deliberately seek other investment opportunities and 94.1% for deliberately seek other investment opportunities) which was substantially higher than the accuracy attainable by chance alone (73.3%). Thus, the independent variables could be characterized as useful predictors distinguishing survey respondents who have deliberately sought other investment opportunities from survey respondents who have not deliberately sought other investment opportunities.

Analysis of the result reveals that the coefficient of age was significant at 1% and negatively related to entrepreneurship decision. The negative sign of the coefficient is in consonance with the *a priori* expectation, implying that as the age of yam agribusiness entrepreneur increases, they are 0.826 times less likely to seek other investment opportunities. As these yam agribusiness entrepreneurs advances in age, the likelihood of them entering into riskier agricultural projects will decline owing to the vigorous nature of such farm business and their desire for leisure. This finding is corroborated by [8] as cited in [5] who posited that the propensity to become an entrepreneur decreases with age as old people will prefer activities with immediate payoffs such as waged labour than activities requiring a time commitment before becoming income producing such as a new firm.

The coefficient of educational status was significant at 5% and positively related to decision to become entrepreneur. The positive sign of the coefficient conforms to the *a priori* expectation, implying that as the educational level of yam agribusiness entrepreneurs increases, they are 1.125 times more likely to seek other investment opportunities. Education influences entrepreneurship through providing people with the necessary skills and information to start up a business in addition to stimulating entrepreneurial values such as creativity, independence and risk taking. This is corroborated by [10] who reported that as individual increases his educational attainment, his entrepreneurial quest and skill increases as well as his knowledge base which makes him alert to new opportunities.

The coefficient of experience in years was significant at 5% and positively related to entrepreneurship decision. The positive sign of the coefficient agrees with the *a priori* expectation, implying that as the experience of yam

agribusiness entrepreneurs increases, they are 1.083 times more likely to seek other investment opportunities. Training and learning are very significant in increasing one's entrepreneurial experience. Thus, as yam agribusiness entrepreneurs increases their entrepreneurial experience through training and learning, their quest to seek other investment opportunities increases. This confirms the finding of [6] who deduced that having had experience in the same sector or business increases the probabilities of becoming entrepreneur of self owned business. Also, [14] in a study on experience and entrepreneurship, reported that accumulation of experience brings about the accumulation of wealth and this rising wealth thus makes entrepreneurship more flexible with age.

The coefficient of profit motive was significant at 1% and positively related to entrepreneurship decision. The positive sign of the coefficient conforms to the *a priori* expectation, implying that as the quest to make profit by yam agribusiness entrepreneurs increases, they are 5.606 times more likely to seek other investment opportunities. The perceived certainty of sufficient return in a venture triggers entrepreneurship in that it influences the individual to mobilize his idle resources to take advantage of such opportunity. This is supported by [17] who reported that people that avoid uncertainty are likely to avoid entrepreneurship, as this occupational option often involves high risk. This is also corroborated by [1] who reported that financial benefit is a major inducement for a good number of persons who venture into entrepreneurship as salaries and wages are considered irregular or inadequate to meet the demands of fairly well standard of living.

The coefficient of financial independence was significant at 1% and positively related to decision to become an entrepreneur. The positive sign of the coefficient is in consonance with the *a priori* expectation, implying that as the quest by yam agribusiness entrepreneurs to become financially independent increases, they are 8.875 times more likely to seek other investment opportunities. The quest to become independent financially creates a sense of dissatisfaction with one's income which further drives the individual into self-employment. This is in consonance with [1] who revealed that people perceived entrepreneurship as a means of running away from subordination and also an opportunity to become masters of their own.

The coefficient of place to retire was significant at 1% and positively related to entrepreneurship decision. The positive sign of the coefficient conforms to the *a priori* expectation, implying that as the quest for a retirement place by agribusiness entrepreneurs increases, they are 8.208 times more likely to seek other investment opportunities. The desire to maintain an already established standard of living even at old age will drive people to start building up entrepreneurial ventures as a support or fallback position on retirement. This is in conformity with [9] who posited that retirement is believed to be the last stage of life and as such a farmer will be pleased to save and invest so as to maintain the already established standard of living.

The coefficient of household entrepreneurial history was significant at 5% and negatively related to entrepreneurship decision. The negative sign of the coefficient is at variance with the *a priori* expectation, implying that if a yam agribusiness entrepreneur comes from a home where one of the lineages is an entrepreneur, he is 0.243 times less likely to seek other investment opportunities. The sense of security inherited family business creates tends to restrain agribusiness entrepreneurs from venturing into other investment opportunities. This finding is supported by [1] who pointed out that when family businesses of good standing are inherited, people are tempted to stay back and run such business in order to keep safe the inherited family wealth. This finding however, is at variance with that of [10] who revealed that individuals that came from a family of entrepreneurs will tend to aspire to be entrepreneur than those from non-entrepreneurial families.

IV. CONCLUSION AND POLICY IMPLICATIONS

Evidence from the study indicates that age, educational status, years of experience, profit motive, financial independence, place to retire, and household entrepreneurial history significantly influence entrepreneurship decision of yam agribusiness entrepreneurs.

On the basis of this finding, the following recommendations were made:

- Workshops should be organized in these yam producing areas to inform and educate these entrepreneurs on the various food forms that can be obtained in processing yam as well as how to use these yam processing equipments.
- Campaigns aimed at promoting the potential financial benefits of processing yam tubers into various food forms should be encouraged in the localities of these entrepreneurs.
- Credit facilities should be made available and accessible to these entrepreneurs in order to encourage them to adopt and utilize these modern yam processing equipments.

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Statistical optimization of α-amylase production by *Escherichia coli*using extruded bean as nitrogen and carbon source

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***Abstract*—** *Response surface methodology based on mixture design was employed for statistical optimization of medium components for the growth and production of α-amylase by Escherichia coli pAC92. The combined effects of media constituents (peptone, yeast extract and extruded beans) were analyzed using a cubic model, which was developed by3-factor simplex lattice mixture design in predicting the optimum yield of growth and α-amylase activity. Results evidenced that extruded common bean was more effective as a nutrient source for E.colipAC92 growth. On the other hand, the completely substituted medium with extruded common bean resulted in 68% of increase in the growth of Escherichia coli pAC92. In addition, the culture medium containing 0.5% of extruded bean and 0.5% of peptone reached a α-amylase activity of 44.59 U. The optimal medium composition was determined by a numerical method based on desirability function, by which the optimal composition for maximum optical density and enzyme activity was found using 0.5% peptone and 0.5% extruded common bean as media constituents. Therefore, these results evidenced that extruded common bean can be successfully used as substitute of peptone and yeast extract in culture media for production of α-amylase byE.colipAC92.*

***Keywords*— extruded bean; mixture design; α-amylase production; culture medium.**

I. INTRODUCTION

Amylases are enzymes widely used in biotechnology processes, constituting a class of industrial enzymes having ¼ of the world enzyme market. α-Amylases have potential application in food, fermentation, textile, paper, detergent, and pharmaceutical industries. In addition, with the advances in biotechnology, the amylase application has expanded in many fields such as clinical, medicinal and analytical chemistry, as well as their widespread application in starch saccharification and in the brewing and distilling industries(Souza and Magalhães, 2010; Gashtasbi et al., 2014).

In the recent decades, the demand for industrial α-amylase has been on the rise because of their economic and environmental benefits(Hellmuth and Van-Den-Brink, 2013; Kim et al., 2014). In addition, due to rising prices of nitrogen and carbon sources, the bio-based enzymes production has been emerged as a promissory technology in biotechnology field. It is known that the development of a bio-based industrial enzymes production requires a low-cost medium and a versatile producing organism able to utilize a wide range of low-cost feedstock(Carneiro et al., 2013). In this scenario, hardened beans, an agro-industrial by-product that contains high amount of carbohydrates and proteins could be a promising nutrient source for α-amylase production.

However, the microbial production of amylases can be affected by certain factors, including microbial strain, culture medium formula and physicochemical conditions(Hii et al., 2012). Considering the composition of culture medium, the nature of nitrogen and carbon source could influence the rate of amylase production and, therefore the enhancement of target enzyme productivity in any fermentation system could be achieved through the improvement of the culture medium composition(Ye et al., 2010; Hortsch and Weuster-Botz, 2011; Boumba et al., 2013; Carneiro et al., 2013).

Conventional change one-factor-at-a-time approach has been applied to optimize the enzyme production using different medium constituents. However, this technique is time-consuming, expensive, and often leads to misinterpretationof results, once the interactions among parametersare not taken into account(Hii et al., 2012). An alternative to overcome this technical limitation is the use of statistical designs coupled to response surface methodology, which allows the study of several factors

influencing the responses by varying them simultaneously and carrying out a limited number of experiments(Ye et al., 2010; Carneiro et al., 2013).

The objective of this study was to optimize the α-amylase production by *Escherichia coli* pAC92 using extruded bean as nitrogen and carbon source. A 3-factor simplex-lattice design coupled to response surface methodology (RSM) was applied to evaluate the combined effects of medium components on biomass and enzyme production. The information gathered was used to develop a mathematical correlation in searching of the optimum conditions for the growth and α-amylase production by the strain.

II. MATERIAL AND METHODS

2.1. Bacterial strain and inoculum preparation

The bacterium *Escherichia coli*pCA92 was used in this study. The strain was stored at -80 ºC in 15% (v/v) glycerol. For inoculum preparation, a single colony was picked up from the Luria-Bertani agar (enriched with 100 mcg/mLampicillin) and subcultured in 250-mL Erlenmeyer flasks containing 50 mL of Luria-Bertani (LB) broth and incubated at 37 ºC for 24 h to obtain an initial cell concentration with optical density (600nm) ranging from 0.4 to 0.6. The inoculum that consisted of 10% (v/v) of culture was used in all experimental runs.

2.2. Flour bean preparation and extrusion

The hardened beans (*Phaseolus vulgaris*and *Vigna unguiculata*) utilized for the extrusion process were provided by EMBRAPA Arroz e Feijão, Santo Antônio de Goiás, Goiás, Brazil. The grains were grounded in a Tecnal mill-grinder, sifted in a screen 0.42 mm and then extruded according to methodology described by Batista et al. (2010a). The extrudates were ground, sealed in plastic bags and refrigerated at 4 ºC until use.

2.3. Experimental design for medium formulation

A 3-factor simplex-lattice design with axial points and overall centroid was used to evaluate the effect of substitution of peptone and yeast extract by extruded flours from common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*). The mixture design allows studying the relationship between the proportion of the different nutrient sources and their respective responses in the optical density and α-amylase production by *Escherichia coli*pAC92. The design was implemented using Statistica 7.0 software (StatSoft Inc. Tulsa, OK, USA) and the factors defined as independent variables were peptone, yeast extract and extruded bean (Table 1).

2.4. Growth profile of *Escherichia coli*pAC92

The growth kinetic of *E.coli* pAC92 in the experimental media was compared with its growth in a commercial LB broth. For this, 5 mL of inoculumprepared in LB mediumwere centrifuged at 5000 g for 10 min. The medium-free cells were transferred to 0.15 mol/L sterile saline solution for reaching an absorbance of 0.5 at 600nm. Onemilliliter of cell suspension was transferred to 50 mL of the different media, incubated at 37 °C for 24 h under shaking (80 rpm). Aliquots of 1 mL were withdrawn every 2 h for determination of optical density and α-amylase activity. Collected aliquots were centrifuged at 5000 g for 10 min and the cell mass was re-suspended in 1 mL of 0.15 mol/L saline solution. The absorbance was determined at 600 nm by using a UV–VIS Spectrophotometer.

2.5. Determination of α-amylase activity

The enzymatic activity was evaluated from aliquots withdrawn during the growth kinetic experiment. The collected aliquots were centrifuged at 5000 g for 10 min and the cellular mass was washed twice with 0.15 mol/Lsaline solution. Then, the bacterial cellular wall was broken by using a saturated sucrose solution. The α-amylase activity was determined according to Bernfeld (1955), and the content of produced reducing sugar was determined following the methodology described by Miller (1959), using glucose as standard. One unit of enzyme was determined as the content in micrograms of reducing sugar released per milliliter of sample per minute of reaction.

2.6. Statistical analysis

The statistical analysis of the experimental mixture design was performed by multivariate analysis. The model was simplified to exclude terms that were not considered statistically significant ($p>0.05$) by analysis of variance (ANOVA). The quality of the polynomial model equation was judged by using the coefficient of determination adj-r^2. The mixture design and all subsequent tests were conducted in triplicate and the level of significance was 95%. All analyses were carried out using the software Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA).

III. RESULTS AND DISCUSSION

3.1. Model establishment

The composition of a culture medium is one of the most important parameters to be analyzed in biotechnological processes with industrial purposes, because around 30-40% of the production costs were estimated to be accounted for the cost of the growth medium (Hajii et al., 2008; Batista et al., 2013). In addition, the cellular growth and protein expression by different microorganisms are greatly influenced by the media components, especially carbon and nitrogen sources(Ye et al., 2010; Rughoonundun et al., 2012).

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In this scenario, to study the feasibility of using extruded bean flours as a low cost nutrient source in substitution of peptone and yeast extract for growth of *E.coli*pAC92, a 3-factor simplex-lattice mixture design was used. The results of optical density of *E.coli*pAC92 using *Phaseolus vulgaris* and *Vigna unguiculata* as media constituents are shown in Table 1. As can be observed, the culture medium containing extruded common bean (*Phaseolus vulgaris*) presented values of optical density higher than those observed for cowpea (*Vigna unguiculata*).

Regarding to the effect of extruded common bean inclusion on the *E.coli* growth, the results from multivariate analysis evidenced that only the effect of interaction between the proportion of peptone and yeast extract (X_1X_2) did not affect the optical density of *E.coli*pAC92. On the other hand, the variable that most affected the response was the content of extruded bean (X_3), being observed a strong positive correlation with the optical density (r=0.93). The regression analysis showed an adequate fit of experimental values to the first-order polynomial model as a function of significant factors (adj-r^2=0.958). The mathematical model is represented in following equation:

$$OD_{600}(Phaseolus) = 0.20X_1 + 0.43X_2 + 1.35X_3 + 1.34X_1X_3 + 1.42X_2X_3 - 13.65X_1X_2X_3 - 1.84X_1X_2(X_1 - X_2) + 3.65X_1X_3(X_1 - X_3)$$

where X_1, X_2 and X_3 denotes peptone, yeast extract and extruded bean, respectively. The value of adjusted r^2 indicates that the proposed experimental model can determine 95.8% of the response variability.

Results of the multivariate analysis for the media using cowpea as nitrogen source evidenced that although the interaction between peptone and extruded bean (X_1X_3) had no effect on the response, all other variables significantly affected the growth of *E.coli*pAC92. In addition, the variable that most influenced the response was the content of extruded cowpea, which presented a positive correlation with the bacterial growth(r=0.78).

Using regression analysis, the polynomial equation that describes the correlation between the response and the significant variables can be represented by the following equation (adj-r^2=0.979):

$$OD_{600}(Vigna) = 0.19X_1 + 0.41X_2 + 0.74X_3 - 0.39X_1X_2 - 0.65X_2X_3 + 5.43X_1X_2X_3 - 3.69X_1X_2(X_1 - X_2) + 7.86X_1X_3(X_1 - X_3)$$

where X_1, X_2 and X_3 denotes peptone, yeast extract and extruded bean, respectively. The fitness of the model was expressed by the adj-r^2 value, which indicates that 97.9% of the variability in the response can be explained by the model. This suggested that the model accurately represents the data in the experimental region.

3.2. Response surface analysis

Multivariate design of experimentscoupled to response surface methodologyis a widely used method in optimization processes, once it consumes less time, requires a smaller set of experimental procedures and resources, allows the obtainment of large quantities of data in a single step process and enables the discoverof the most desirable conditions, or desirability(Yin et al., 2009; Mohamad et al., 2011; Boumba et al., 2013; Cheng et al., 2013).Hence, a response surface methodology (RSM) was used to determine the influence of nutrient sources (peptone, yeast extract and both extruded bean) in the *E.coli* growth.

Despite the good results of optical density in the media containing common bean and yeast extract or peptone in equivalent proportions, the surface response confirms the highest growth profile using extruded common beanas sole nitrogen source(Figure 1a).The projection evidenced a negative effect of peptone (run 1) and yeast extract (run 2) as isolated sources, while common bean shows a trend to a further growth by increasing its concentration.In this scenario, the mathematical prediction of an optimum composition is presented as 0.25% yeast extract and 0.75% of extruded bean. With this formulation, is expected to achieve an optimal density of 1.385 at 600 nm, a value very close to that previously observed in the culture medium containing extruded bean as unique nitrogen source (run 3).

This fact can be explained in terms of the nutritional requirements of the bacteria under study,which needs beyond the protein from peptone, also minerals and vitamins present in yeast extract. Thus, the improvement is probably due toa better nutritional balance betweenthe extruded bean and the yeast extract, encompassing not only a more complete source of nitrogen, but also essential vitamins and active compounds(Watson, 1976; Pepler, 1982; Batista et al., 2013).

The effect of inter-relations and interactions of the proportion of peptone, yeast extract and extruded cowpea on *E.coli*growth are depicted in Figure 1b. The response

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surface plot describes the variation on the response as a function of mixture composition. Unlike the extruded common bean, contradictory results were obtained from the medium containing extruded cowpea. The bacteria presented a greatest predicted value for optical density at the medium containing 0.75% peptone, 0.05% yeast extract and 0.20% of cowpea extruded, with values of OD_{600} of 0.897. However, in the tested compositions, results evidenced that the media containing extruded cowpea as unique nitrogen source (run 3) presented the highest optical density. In addition, the presence of extruded cowpea in low quantities positively affected the media containing peptone (run 7) and yeast extract (run 8) as main nitrogen source.

As can be observed in the surface plots depicted in Figure 1, there is a different influence of each bean specie over the *E.coli* growth, once the values ofoptical densitywere significantly different in each medium containing extruded bean. These results may be explained by the different intrinsic composition of each bean specie, especially regarding to the content of proteins and amino acid composition, as well as polysaccharide quantity and quality (Batista et al., 2010a,b). In this sense, the extrusion process interfered differently in each studied bean, modifying the biochemical proprieties and anti-nutritional components singularly.

Also, the lower values of optical density using cowpea as nutrient source can be due to the fact that cowpea presents storage proteins (vicilins) with biocidal activity(Ribeiro et al., 2007). It is possible that the extrusion process was not effective to inactivate these proteins, contributing to the lower efficiency of cowpea as nutrient source.

3.3. Growth profile of *Escherichia coli*AC92

Due to the constant modification in microbial genetics, new recombinant species and strains are frequently discovered and created. This demand requires new media formulation and optimization in industrial scale, continuously. However, this is a delicate process, since culture media formulation involves several variables and a large flow of data, which increase the difficult, especially when more than one variable changes at a time(Ye et al., 2010; Cofré et al., 2012; Delabona et al., 2013).In addition, an optimized medium has to be more profitable and efficient than traditional media. Thus, the growth profile of *E. coli* on media with higher optical density was compared to the growth profile in Luria-Bertanimedium (Figure 2).

As can be seen in Figure 2a, there was a clear improvement over the cellular growth in media containing extruded common bean as exclusive nutrient source (run 3), being observed an increase of 68% in the growth when extruded common bean was used as sole nutrient source and 20% when mixed with yeast extract (run 6).

Previous studies showed that LB medium contains large quantities of all of the essential inorganic compounds, necessary for the *E.coli*pAC92culture. Nevertheless, the carbon source is a probable limitation to a further growth(Sezonov et al., 2007). Considering that during the extrusion process of common bean, extremely high temperatures and pressure are reached, the modification of some polypeptide chains and the higher interaction between the molecules during the process, enhance the bioavailability of essential nutrients, such as carbon and nitrogen. This may be the reason for the improved efficiency of extruded common bean flouras *E.coli* pAC92 growth medium and acorroborationfor the successfully replacement of LB medium by this medium.

Despite the similar growth profile between cowpea bean medium and the commercial medium, the bacteria reached the saturation at an OD 18% inferior than LB medium, when cowpea was used as unique nitrogen source (Figure 2b). This may be due to a smaller amount of essential inorganic molecules available in the extruded cowpea, which causes a faster depletion of nitrogen and carbon, reaching the saturation earlier and limiting a further growth.

Once the culture medium containing extruded common bean was more effective than extruded cowpea for *E.coli* growth, the optimization tests for production of α-amylase were performed using extruded common bean as medium constituent.

3.4. α-amilase expression

It is known that high values of growth profile do not necessarily mean a proportional protein expression. Despite the culture medium containing extruded common bean had increased the growth of *E. coli*, changes in medium composition can interfere with the protein expression profile(Potvin et al., 2012; Carneiro et al., 2013). Aiming to verify the effectiveness of α-amylase production by *E.coli*pAC92in different composition media, tests were performed and the results are demonstrated in Table 2.

The results of the multivariate analysis evidenced that the effect of ternary interaction $X_1X_2X_3$ and the effects of binary interaction between the proportion of peptone and yeast extract (X_1X_2) and yeast extract and extruded bean (X_2X_3), did not affected significantly the production of α-amylase by *E. coli*(Figure 3a). In addition, the binary interaction between peptone and extruded common bean (X_1X_3) had the most pronounced effect on the response, presenting a strongly positive correlation with the α-amylase activity(r=0.92).

The regression analysis showed an adequate fit of experimental data to the full-cubic model as a function of significant variable. Thus, the polynomial equation that describes the correlation between the optical density and

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the media constituents is represented below (adj-r^2=0.993):

$$\alpha\text{-}amilase activity(U) = 2.16X_1 + 4.17X_2 + 3.58X_3 + 165.06X_1X_3 + 45.23X_1X_2(X_1 - X_2) - 318.27X_1X_3(X_1 - X_3)$$

This equation is based on the production of α-amylase as a function of the three different variables, concentration of peptone (X_1), concentration of yeast extract (X_2) and concentration of extruded common bean (X_3).The fitness of the model was expressed by the adj-r^2 value, which indicates that 99.3% of the variability in the response can be explained by the model.

In order to obtain the best condition for protein expression, diagrams of response surface were designed, presenting the effects of inter-relations and interactions of the different nutrient sources on theα-amylase expressionby *E.coli*pAC92 in media containing extruded common bean (Figure 3b).

The surface plot forα-amylase productionpresented an overall convex curvilinear profile. The mathematical prediction described the optimal medium as containing 0.30% of peptone and 0.70% of extruded bean, with maximal enzyme activity of 64.38 U.The optical density results on the other hand, shows a better condition in the medium composed exclusively by extruded bean (Table 1, run 3). These results show two different compositions of optimal media: one for cultivation and accumulation of biomass and other for protein production. However, the production of an optimized culture medium requires a unique formulation, efficient for cellular growth and protein expression, once is impracticable for industrial scale to manufacture two different media with the same components.

In this sense, a desirability test was performed aiming to obtain the values of the experimental variables that maximize both responses.The desirability function approach is one of the most widely used methods in industry for the optimization of multiple response processes, simultaneously. Desirability consists of an optimization method by combining all variables into a single objective function, which represents the relationship of all responses being optimized(Jeong and Kim, 2009; Costa et al., 2011). Figure 4 shows the diagrams describing the variation on the desirable response as a function of the mixture composition. In order to establish the most desirable media formulation for *E.coli* culture, the two main parameters were analyzed, optical density and α-amylase activity.

Results evidenced that the function D would be maximized by using a culture medium containing 0.5% of peptoneand 0.5% of extruded bean (run 5). In this condition,the mathematically predicted values for optical density and α-amylase activity were 1.111 and 44.60 U, respectively. These results were very close to those obtained in the experiments from the mixture design (Tables 1 and 2). Despite this medium did not present the highest optical density in first place, the growth profile was not significantly lower than in run 3, which presented the highest values of optical density.In view of the importance of an efficient nutrient supply for α-amylase expression in an optimized *E.coli* culture medium, the decrease of optical density can be disregarded.

Through desirability test, it was possible to reach a maximal optimization of this medium, encompassing production of cell mass and α-amylase expression in a one-step process. Once the production cost of extruded beans is cheaper than the production processes of yeast extract and peptone, this medium has an enormous potential, being extremely attractive for industrial and biotechnological applications.

IV. CONCLUSION

The mixture design and response surface analysis were found useful in locating the optimum level of the most significant factors that contribute to the maximum growth and α-amylase production by *Eschericia coli* pAC92. This study evidenced that the extruded common bean may be successfully used as substitute of peptone and yeast extract in culture media. The use of extruded bean improved the growth of*Escherichia coli*pAC92 as well as occasioned a good production of α-amylase. Therefore, considering the lower costs of extruded bean as nitrogen source, this substrate is financially attractive substitute of peptone and/or yeast extract in culture media formulation for the expression of α-amylase by*E.coli*pAC92 in an industrial scale production.

ACKNOWLEDGMENTS

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Table.1: Mixture design employed for optimization of E.coli PAC92 growth using extruded beans as nutrient source.

Run	Peptone (X_1)	Yeast Extract (X_2)	Extruded Bean (X_3)	Optical Density of *E.coli*	
				Common bean	Cowpea bean
1	1% (1.0)	0% (0.0)	0% (0.0)	0.190	0.190
2	0% (0.0)	1% (1.0)	0% (0.0)	0.414	0.414
3	0% (0.0)	0% (0.0)	1% (1.0)	1.333	0.746
4	0.5% (0.5)	0.5% (0.5)	0% (0.0)	0.205	0.205
5	0.5% (0.5)	0% (0.0)	0.5% (0.5)	1.082	0.496
6	0% (0.0)	0.5% (0.5)	0.5% (0.5)	1.215	0.420
7	0.66% (0.67)	0.17% (0.17)	0.17% (0.17)	0.521	0.589
8	0.17% (0.17)	0.66% (0.67)	0.17% (0.17)	0.641	0.612
9	0.17% (0.17)	0.17% (0.17)	0.66% (0.67)	0.934	0.178
10	0.33% (0.33)	0.33% (0.33)	0.34% (0.33)	0.294	0.561

Values in bracket correspond to the coded variable level.

Table.2: Mixture design matrix used for optimization of α-amylase production by E.coli pAC92 using extruded common bean as nutrient source.

Run	Peptone (X_1)	Yeast Extract (X_2)	Extruded Bean (X_3)	α-amylase activity (U)	
				Observed	Predicted
1	1% (1.0)	0% (0.0)	0% (0.0)	2.39	2.16
2	0% (0.0)	1% (1.0)	0% (0.0)	4.39	4.17
3	0% (0.0)	0% (0.0)	1% (1.0)	3.81	3.58
4	0.5% (0.5)	0.5% (0.5)	0% (0.0)	2.99	2.53
5	0.5% (0.5)	0% (0.0)	0.5% (0.5)	44.59	44.13
6	0% (0.0)	0.5% (0.5)	0.5% (0.5)	3.83	3.37
7	0.66% (0.67)	0.17% (0.17)	0.17% (0.17)	5.04	6.41
8	0.17% (0.17)	0.66% (0.67)	0.17% (0.17)	4.77	6.14
9	0.17% (0.17)	0.17% (0.17)	0.66% (0.67)	38.64	40.01
10	0.33% (0.33)	0.33% (0.33)	0.34% (0.33)	24.87	22.82

Values in bracket correspond to the coded variable level.

Figure captions

Figure 1. Mixture contour maps showing the effect of three variables on the optical density of *E.coli* pCA92. (a) yeast extract, peptone and extruded common bean or (b) yeast extract, peptone and extruded cowpea.

Figure 2. Effect of different nitrogen sources in the growth of *Escherichia coli*: (a) mixture design using common bean as nitrogen source; (b) mixture design using extruded cowpea as nitrogen source. As the control, the microorganism was grown in a commercial Luria-Bertani medium. Results are means ± standard deviation of triplicate samples.

Figure 3. Pareto chart (a) and mixture contour map (b) for the α-amylase activity in the mixture design experiments. In the Pareto chart, the horizontal bar represents the ratio between the effects of variables and their respective standard error. All tests were conducted in triplicate.

Figure 4. Response surface plot of desirability as a function of the proportion of yeast extract, peptone and extruded common bean.

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Figure 1

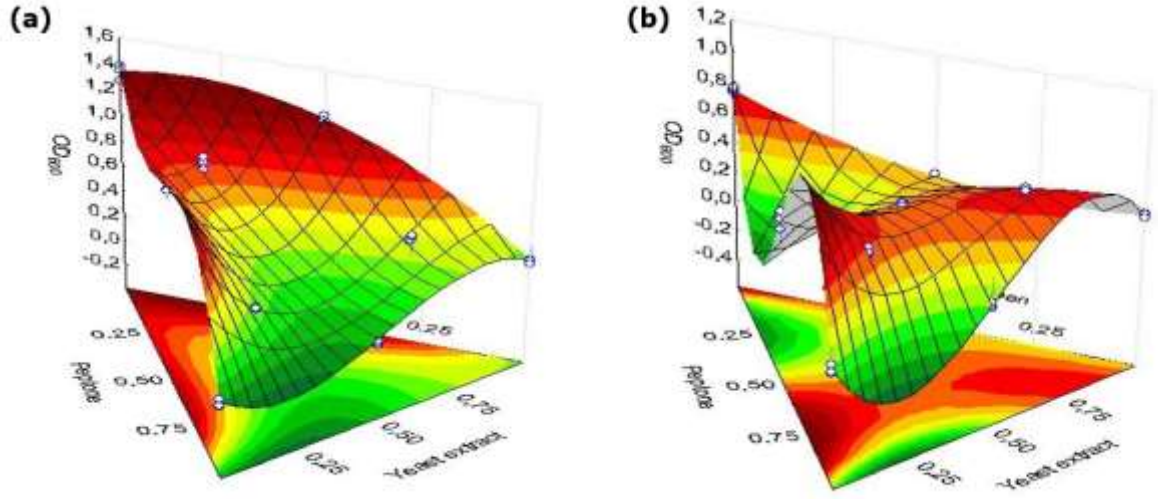

Figure 2

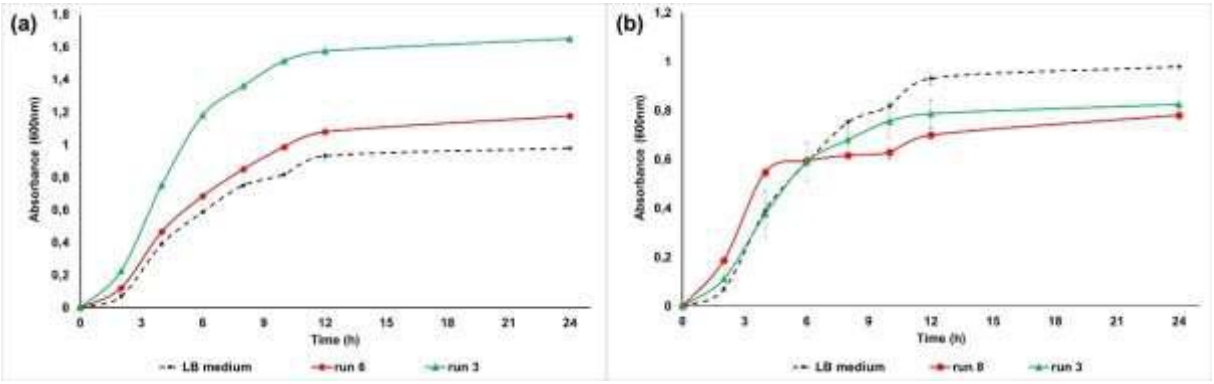

Figure 3

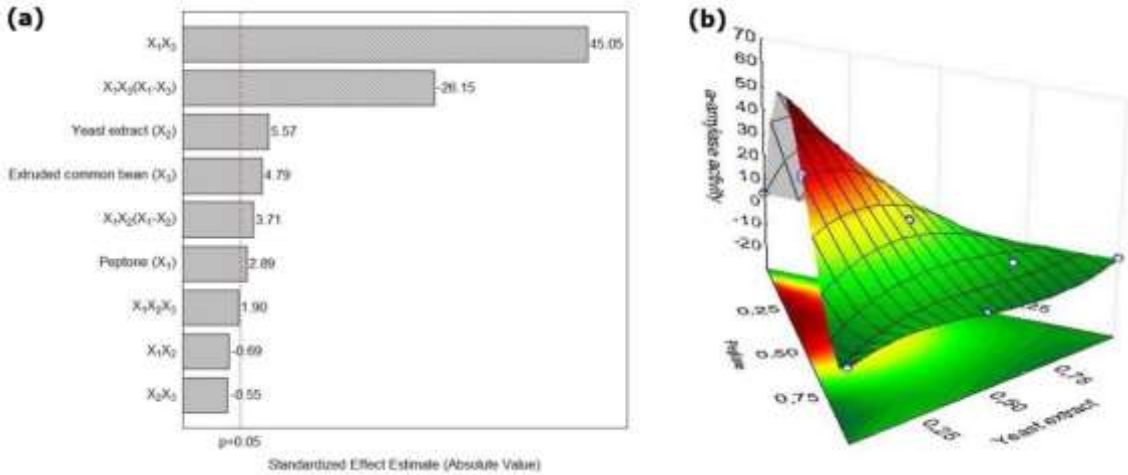

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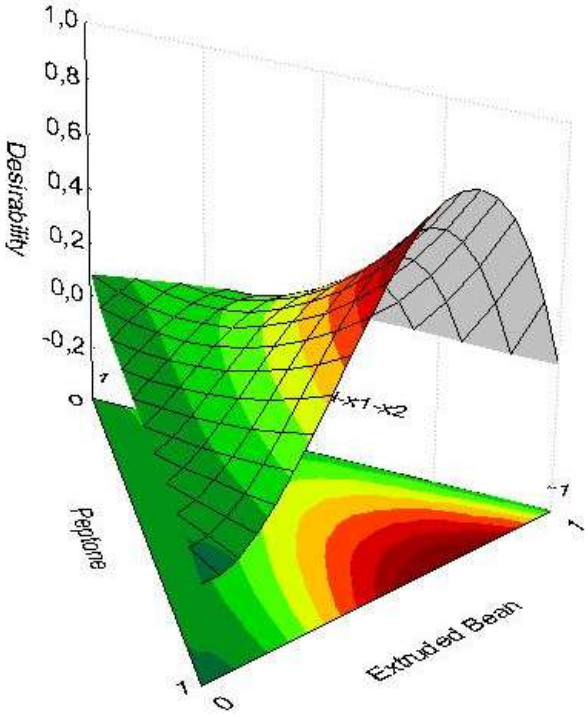
Figure 4



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Assessment of the Benefits of National Fadama III Agricultural Project among Participants through her Activities (Indicators) in Bayelsa State

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Abstract— *The study assessed the benefits of national fadama III agricultural project among participants through her activities in bayelsa state. Objective one (1) assessed the benefits of National Fadama III agricultural project through her activities, while objective two (2) Identified problems encountered by the participants in the cause of participation in the study area. Purposive sampling technique was used to select 150 farmers that participated, and 150 staff from the delivery agency. Data were collected with a structured questionnaire. Both objectives were analyzed using descriptive statistics such as frequency count, percentage, and mean ($\bar{X}$). The finding showed that Farmers did not benefit commendably from Fadama III Project. No access to Fadama project personnel's when the need arise, lack of fund, non-regular training, poor communication channel and delays in being attended to by the delivery agency were the top constraints faced by farmers in National Fadama III Project. The study therefore recommended that National Fadama III Project should be properly funded and its activities should be reorganized to be of great benefit to the farmers, the study recommends that proper training in all the activities should be made available for effective participation, empower the participants to improve their income level, and to move them away from poverty by improving their standard of living.*

Keywords— Activities, Agricultural, Bayelsa State, Benefits, Fadama III, Participants.

I. INTRODUCTION

One of the major problems confronting Nigeria today is how to improve the quality of life in the rural areas, reduce the level of poverty and contribute to economic growth. Fadama III Development Project through Agriculture contributes immensely to the Nigerian economy in many ways, namely; in the provision of food for the increasing population; supply of adequate raw materials to a growing industrial sector; a major source of employment generation, foreign exchange earnings; and, provision of a market for the products of the industrial sector [1], [2], [3].

The inability of this sector to expand was due to inadequate financing to improve on the situation that is, facilitating Agricultural credit. Also, the problem of rapid Agricultural Development in Nigeria indicates that efforts directed at achieving expanded economic base of the rural farmers were frustrated by the scarcity of and restrictive access to loan fund. One of the reasons for the decline in the contribution of agriculture to the economy is lack of formal National credit policy and paucity of credit institutions which can assist farmers, [4].

Fadama III project provides rural finance through the Nigerian Agricultural Co-operative and Rural Development Bank and as well develops the interests of the private sector in Agriculture by contracting private organizations to support farmers with advisory and technical services.

II. THE SPECIFIC OBJECTIVES WERE:

i.) assess the benefits of the National Fadama III agricultural project among empowering participants through her activities in the study area;
ii.) Identify problems encountered by the participants in the study area.

III. METHODOLOGY

The study was carried-out in Bayelsa State; the State is made up of eight Local Government Areas, namely: Brass, Ekeremor, Kolokuma/Opokuma, Nembe, Ogbia, Sagbama, Southern Ijaw and Yenagoa Local Government Areas respectively. Each of this L.G.As is known as Agricultural districts. The major occupation of the people are farming

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and fishing [5]. Purposive sampling technique was used to select the communities that participated in Fadama III Project; one (1) Local Government Area was used to represent each of the three (3) Agricultural zones, five Fadama communities were used to represent each of the selected Local Government Areas, the Local Government Areas were Kolokuma/Opokuma, Ogbia and Sagbama respectively, ten (10) participants belonging to a Fadama User Group were selected from each of the communities which gave us a sample size of One-Hundred and fifty (150) respondents.

3.1 Method of data analysis

Data collected from the survey for both objectives were analyzed using descriptive statistics such as: mean frequency and percentages.

IV. RESULTS AND DISCUSSION

4.1 Benefits of national fadama III agricultural project among participants through her activities in bayelsa state

TABLE.1: Benefits of National Fadama III Agricultural Project among Participants through Her Activities in Bayelsa State

S/N	Activities farmers benefited from in Fadama III agricultural projects in Bayelsa state.	So much	Much	Moderate	Little	Almost nothing	Mean Score $\bar{X}$
1	Adequate and timely supply of agro chemicals	-	-	139(92.7)	6(4.0)	5(3.3)	2.9
2	Access to improved planting materials	-	-	132(88.0)	6(4.0)	12(8.0)	2.8
3	Access to improved stock livestock, fisheries	-	-	122(81.3)	17(11.3)	11(7.3)	2.7
4	Provision of essential farm (hoes, cutlass etc)	-	-	133(88.7)	9(6.0)	8(5.3)	2.9
5	Support for provision of high quality livestock/fish feed	-	1(0.7)	127(84.7)	13(8.7)	9(6.0)	2.8
6	Procurement of irrigation equipment (pump, pipes)	-	1(0.7)	135(90.0)	6(4.0)	8(5.3)	2.9
7	Training on modern farming techniques	2(1.3)	-	86(57.3)	51(34.0)	11(7.3)	2.5
8	Advisory services on various farm enterprises	1(0.7)	-	74(49.3)	69(46.0)	6(4.0)	2.5
9	Provision of loans/credits	-	-	113(75.3)	33(22.0)	4(2.7)	2.7
10	Marketing for my produce	-	-	129(86.0)	16(10.7)	5(3.3)	2.8
11	Income for my enterprise	-	-	124(82.7)	19(12.7)	7(4.7)	2.8
12	Procurement of agro-processing equipment	-	-	132(88.0)	7(4.7)	11(7.3)	2.8
13	Infrastructure facilities	-	-	126(84.0)	17(11.3)	7(4.7)	2.8
14	Acquisition of farm management skills	-	-	117(78.0)	30(20.0)	3(2.0)	2.8
15	Availability of food for my household	-	-	93(62.0)	51(34.0)	7(4.7)	2.6
16	Employment opportunities for household members	-	-	76(50.7)	70(46.7)	4(2.7)	2.5
17	Improved living standard	-	-	100(66.7)	44(29.3)	6(4.0)	2.6
	Overall mean score						2.7
	Bench mark mean score						3.00
	Number of respondents						150

Source: Computed by the author from field survey data, 2016

The result in Table 1 showed that all the farmers had pooled mean rating of below the mean cut off point of 3.0 in all the seventeen (17) items bordering on the benefits of national fadama III agricultural project among participants through her activities in Bayelsa state. The pooled mean score of the responses of the respondents was 2.7.

This implies that the respondents do not think that they benefited that much from Fadama III Agricultural project in

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Bayelsa state. The result showed that in terms of adequate and timely supplies of agrochemicals, majority of the respondents (139 participants) representing 92.7% of the entire sampled farmers for the study benefited moderately from these activities of Fadama III Agricultural project in the study area. This implies that farmers do not benefit commendably from Fadama III Agricultural project in Bayelsa state in terms of adequate and timely supply of agrochemicals. This will hamper the ability of farmers to cope with the intricacies of pest and disease outbreak in their farmers which may translate to low output.

In terms of access to improved planting materials, the result in Table 1 showed that larger proportion (132 farmers representing 88% of the entire sampled farmers) had only but a moderate level of access to improved planting materials under Fadama III Agricultural projects in the study area. This implies also that access to improve planting materials in Fadama III Agricultural project in Bayelsa is relatively low. This suggest that most of the participants of Fadama III Agricultural project in the study area still uses the unimproved planting materials in planting in the study area. This will affect production and ability to easily transform the farmers from subsistence orientation to market orientation in the study area. [6] Suggested that the fastest means of transforming rural farmers from subsistence orientation to market orientation is by provision of improved planting materials and production technologies. Furthermore, larger proportion of the respondents (122 farmers representing 81.3% of the entire farmers) had moderate access to improved stock (livestock fisheries). This implies that access to improved breeding stock for livestock and fisheries production is poor in Bayelsa state under Fadama III Agricultural project. This will discourage sustainable livestock and fisheries production in the study area.

Table 1 showed that 133 participants representing 88.7% of the entire participants; 127 participants representing 84.7% of the entire sampled respondents; 135 participants representing 90% of the participants; 86 participants representing 57.3% of the entire respondents; 74 participants representing 49.3% of the entire respondents; 113 participants representing 49.3% of the entire respondents; 113 participants representing 75.3% of the entire respondents; 129 participants representing 86% of the entire respondents; 124 participants representing 82.7% of the entire respondents; 132 farmers representing 88% of the entire respondents; 126 farmers representing 84%; 117 farmers representing 78%; 93 farmers representing 62%; 76 farmers representing 50.7% and 100 farmers representing 66.7% of the entire sampled farmers had moderate provision of essential farm implements, support for provision of high quality livestock/fish feed, procurement of irrigation equipment, training on modern farming techniques, advisory services on various farm enterprises, provision of loan/credits, marketing for their produce, income for their enterprise, procurement of agro-processing equipment, infrastructural facilities, acquisition of farming management skills, availability of food for their household, employment opportunity for household members and improve living standard respectively.

This implies that provision of essential farm implements, support for provision of high quality livestock/fish feed, procurement of irrigation equipment, training on modern farming techniques, advisory services on various farm enterprises, provision of loans/credits, marketing produce, income for enterprises, procurement of agro-processing equipment, infrastructural facilities, acquisition of farm management skills, availability of food for farmer's household, employment opportunity for household members and improved living standard is poor under Fadama III Agricultural projects in Bayelsa state. Such conditions is expected to affect the performance of the farmers (participants) and may not be able to create a shard difference between their farming activities and that of non-participants in Fadama III Agricultural projects in the area of income generation and welfare of the farmers. [7].

Figure 1 below shows a bar chart representation of the variations in the mean ratings of the responses of the respondent the benefits of the National Fadama III Agricultural project in empowering the participants in Bayelsa state. The bar chart showed that the various responses of the respondents were below the cut point of 3.00. This suggested that the farmers (participants) seem not to benefit from Fadama III Agricultural project in Bayelsa state. The bar charts were presented alongside the error bar with standard deviation. The error bar with standard deviation displays the extent of deviation from the mean scores of the responses of the respondents with one standard deviation.

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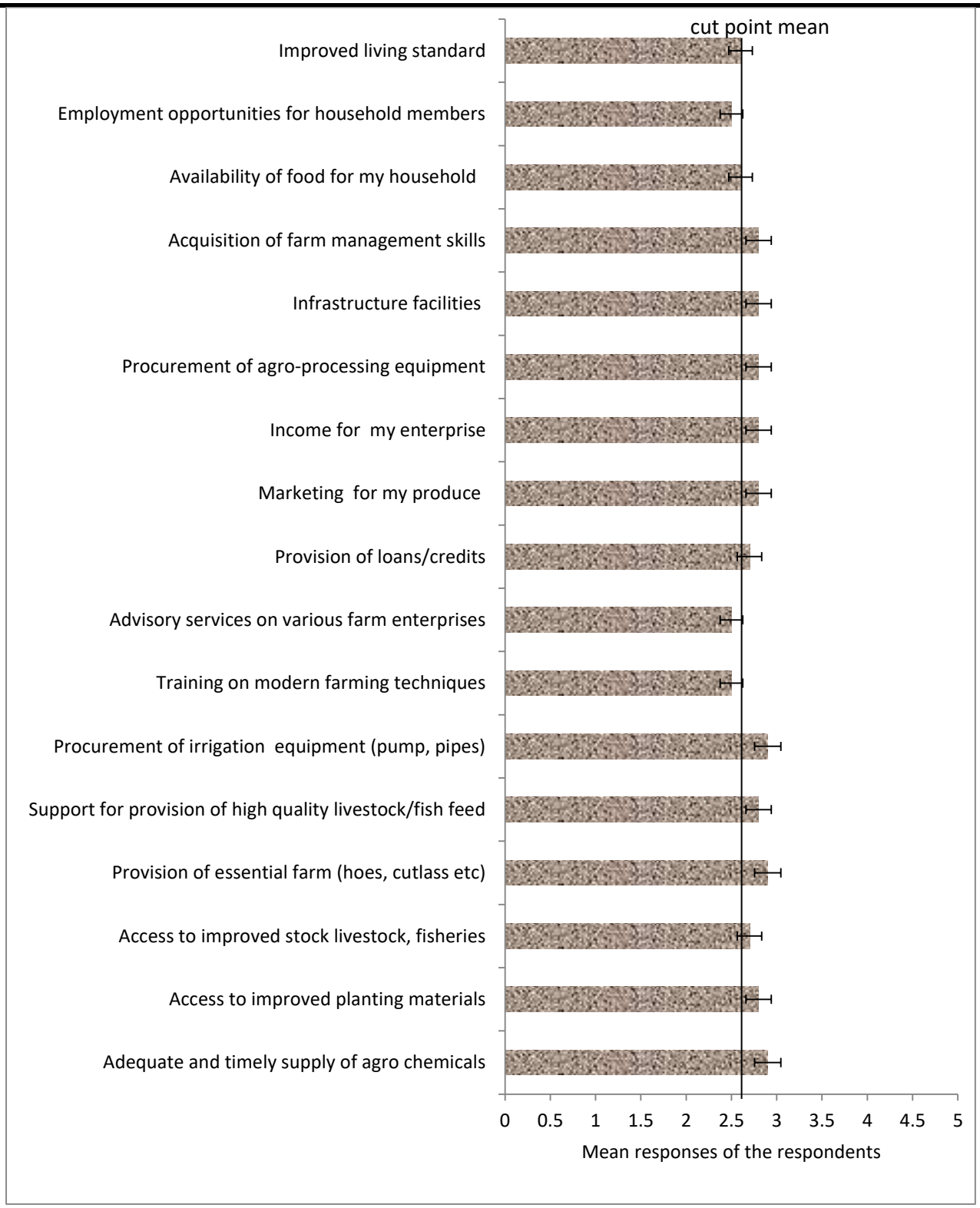

Fig. 1: variations in the mean ratings of the responses of the respondents of the benefits of national fadama iii agricultural project among participants through her activities in bayelsa state.

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4.2 Distribution of problems encountered by the participants in national fadama III agricultural project in bayelsa state.

Table.2: Problems Encountered by the Participants in the Study Area

Problems Encountered by the Participants	Frequency	%	Rank
No access to Fadama project personals when the need arise	144	96.0	1st
Lack of fund	132	88.0	2nd
Non regular training	129	86.0	3rd
Poor communication channel	119	79.3	4th
Delay in being attended to by the delivery agency	105	70.0	5th
No regular meeting to achieve effective participation	91	60.7	6th
Lack of farm land	86	57.3	7th
Poor infrastructure	83	55.3	8th
Distance to training centre	80	53.3	9th
Lack of farm input supply	77	51.3	10th
Lack of storage facilities	77	51.3	10th
Bad roads	76	50.7	11th

Source: Computed by the author from field survey data, 2016

The result in table 2 showed that the most challenging problem faced by farmers in National Fadama III Agricultural project in Bayelsa state were the problems of no access to Fadama project personal when the need arises (96%) ranked 1st, lack of fund (88%) ranked 2nd, non regular training (86%) ranked 3rd, poor communication channel (79.3%) ranked 4th and delay in being attended to by the delivery agency (70%) ranked 5th.

This was followed by other problems as no regular meetings to achieve effective participation (60.7%) ranked 6th, lack of farm land (57.3%) ranked 7th, poor infrastructure (55.3%) ranked 8th Distance to training centre (53.3%) ranked 9th, lack of farm input supply (51.3%) and lack of storage facilities (51.3%) ranked 10th, and bad roads (50.7%) ranked 11th. These problems affected farmer's level of commitment and adoption of new technology being presented to them through Fadama III Agricultural project in the study area. This situation would also affect the rate of development of farmers through Fadama III Agricultural project in the study area.

No access to Fadama project personals when the need arise will limit the ability of a farmer to tackle his immediate challenges so as to increase his level of production. No access to Fadama project personals may be due to poor ratio of Fadama project personals –to- farmers in Bayelsa state. According to [8] shortage of Fadama staff will affect the rate of response of farmers to any challenge they face in trying to adopt new farming technologies introduced to them through Fadama project and will translate into low productivity on the part of the farmers. Lack of fund will hinder most farmers from making huge investment in their farming business or to adopt costly technologies. This will also affect most farmers from purchasing sufficient raw-materials and to finance their business. [9] Noted that the larger the farm size, the larger the scale of farming operation and hence the higher the demand for funds to meet up with the scale of operation. This assertion was supported by [10] who noted that lack of fund affect the willingness of an individual to make investment and/or set up any enterprise that is much fund demanding. This will hinder the farmers from expanding their business away from subsistence level.

Non regular training will demoralize a farmer from continuous participation in National Fadama III Agricultural project and at times cause them to forget any training they have received. This will also affect the level of participation of farmers in the project in the study area. Poor communication channel will affect the easiness with which a farmer would relate its problems to the project personnel and get expected feedback as timely as possible. The inability of a farmer to communicate his/her problems effectively will translate to low productivity. Delay in being attended to by the delivery agency will discourage the willingness of a farmer to relate his/her problems to the project personnel. This will affect the performance of the farmers and make most Fadama III Agricultural project as not an ideal project for amelioration of their farming challenges.

No regular meeting to achieve effective participation will affect the ability of a farmer to learn new farming technologies and to brainstorm for solution of the most pressing needs of the farmer. This will affect their productivity. According to [7], irregular meeting of farmers with Fadama II project personnel affect the rate of exchange of ideas among the farmers and between the farmers and the

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project personnel for increasing the productive aptitude of the farmers.

Lack of farm land will affect the ability of the farmers to acquire production knowledge and to produce beyond their available resources. The quantity of crops planted by a farm firm depends on the quantity of land available to it [10]. According to [11], limited access to land limits the size and scale of the farm business. Crop planted is likely to decrease as the area of land available to a farmer decreases. As the area planted of crop decreased, crop output decreased. Poor infrastructure will affect the level of participation of farmers in National Fadama III Agricultural project especially for those technologies that required electricity. This will affect the level of performance of the farmers.

Distance to training centre when far will discourage most financially incapacitated farmers from participating. Lack of farm input supply will also discourage many farmers from participating actively in National Fadama III Agricultural project. Farm input supply to the farmers serves as an incentive to the farmers. Farmers will participate more when they are sure of being supplied of farm input after participating in the programme. Lack of storage facilities will affect the ability of the farmers to store their excess products during its peak season for sale during the off season for higher profit will discourage most farmers from participating in Fadama III project that is capable of increasing the output level of the farmers. Bad roads will discourage farmers from participating in Fadama III Agricultural projects as expected since the possibility of going to Fadama III Agricultural project demonstration farm site and exporting their produce is limited by bad nature of roads in Bayelsa state especially in the rural areas.

A bar chart representation of the Variations in the percentage responses of the respondent on the problems encountered by the participants of Fadama III project in Bayelsa state,

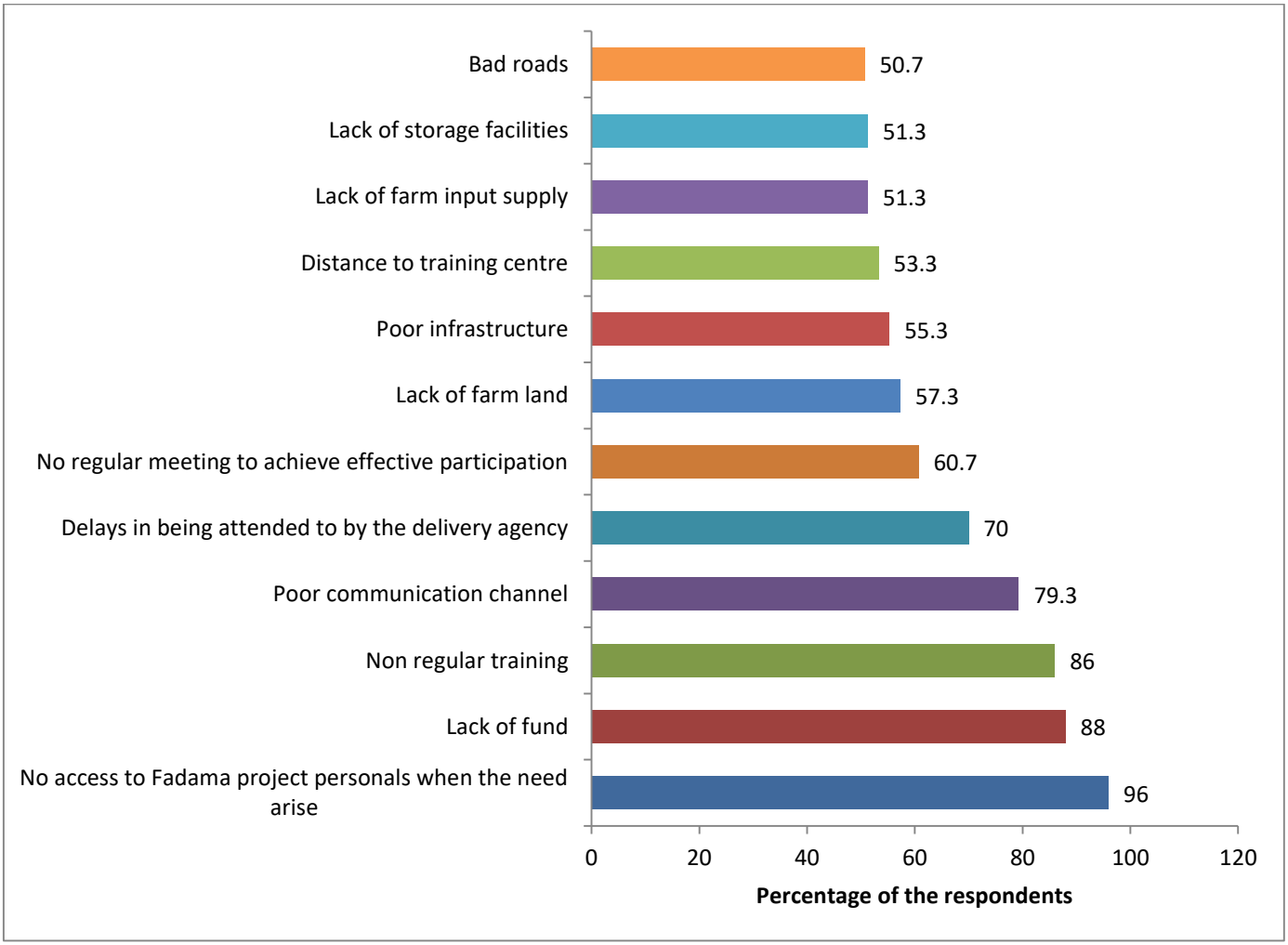

Fig.2: variations in the percentage responses of the respondent on the problems encountered by the participants of fadama III project in the cause of participation in bayelsa state.

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V. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Farmers did not benefit commendably from Fadama III Agricultural Project in Bayelsa State in terms of adequate and timely supply of agrochemicals, access to improved planting materials, access to improved livestock, fisheries, provision of essential farm (hoes, cutlass etc), support for provision of high quality livestock/fish feed, procurement of irrigation equipment (pump, pipes), training on modern farming techniques, advisory services on various farm enterprises, provision of loans/credits, marketing for my produce, income for my enterprise, procurement of agro-processing equipment, infrastructure facilities, acquisition of farm management skills, availability of food for their household, employment opportunities for household members and improved living standard.

5.2 Recommendations

Cordial relationship with project communities and introducing the principles of comparative advantage, by the provision of credit facilities to the comparative group in Bayelsa State, only for those businesses that earned them the highest income should be encouraged.

Problems of no access to Fadama project personals when the need arise, lack of fund, non regular training, poor communication channel and delays in being attended to by the delivery agency etc., which mostly constrained farmers in National Fadama III Agricultural Project in Bayelsa State should be given serious attention and ensure its immediate resolution.

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Changes in the Mineralisation of Nutrients and Sunflower Biomass in Soil Irrigated with Water from Oil Exploration in a Semi-Arid Environment

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Abstract*—Wastewater from oil fields may be an option for irrigation, especially in regions which have low rainfall with high variability. The aim was to evaluate the composition and decomposition of shoot biomass from sunflower plants irrigated with water from oil wells, which had been subjected to filtering (FPW) and reverse osmosis (OPW), using groundwater (UGW) as a reference. Two tests were then carried out to evaluate decomposition of the residue. In the first test, residues produced with FPW, OPW and UGW were incubated in soil and irrigated with groundwater (UGW). In the second test, residues from plants irrigated with UGW were also incubated, but irrigated with FPW, OPW and UGW. Significant differences were seen in the levels of Na, Mg and lignin in the residues with the use of FPW, showing greater levels for Na, and lower levels for Mg and lignin. The loss in biomass of the incubated residues was not significant in either test; this was not seen in the Mg e N with smaller losses than the biomass, or the Na, K e S with greater losses, especially when produced with FPW and OPW respectively. In the residue produced with UGW, differences were identified for Ca and Na, with the order of losses for type of water being UGW=FPW> OPW and OPW=UGW>FPW respectively. Irrigation using water from oil extraction alters the chemical characteristics of the soil and the composition of cultivated plants at a level sufficient to influence the rate of decomposition of the organic residue.*

Keywords*— *Sunflower residue, Produced water, Wastewater management, Mineralisation, Substrate quality.

I. INTRODUCTION

The use of wastewater has become an acceptable agronomic practice [1, 2], being of considerable interest to the oil industry as it removes the problem of disposing of produced water, helps to conserve water resources, and improves nutrient recycling [3, 4]. However, the produced water which is generated in oil wells, may contain heavy metals, organic and inorganic compounds [5], salts, and additives used during extraction, such as anticorrosives and biocides [6], which can pose risks to the environment. A large volume of produced water is generated in an oil field, and may be an option in the irrigation of crops grown for fuel. Irrigation with produced water can be particularly effective in areas with poor rainfall distribution and a shortage of drinking water. However, this can alter the chemical properties of the soil [3, 7-9] and as a consequence, the chemical composition of plants [7, 9-11].

The decomposition of organic matter is an important stage in nutrient cycles, and is affected by the chemical composition of the plant residue, as nutrient levels and the energy available to decomposers determine the efficiency of the mineralisation of organic residues [12, 13]. Studies into decomposition have shown a reduction in the mineralisation of organic residue for levels of Mg, while the opposite was seen for Ca [14] and P [15, 16]. Talbot and Treseder [17] reported that initial levels of N in organic residue increased mineralisation, while Birouste et al. [15] were unable to confirm this observation. On the other hand, initial concentrations of lignin were seen to negatively influence losses in biomass [17, 18] but had no effect on the release of N [17].

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Changes in soil properties, in particular increases in the concentration of toxic minerals, can also affect the decomposition of organic residue, since they affect the structure and activity of microbial communities in the soil [19, 20]. The physical and chemical properties of the soil are known to influence microbial communities [21]. This effect can be modified by salinity, sodicity and alkalinity, which can reduce biomass and microbial activity [22], and inhibit respiration [23].

It is probable that irrigation with produced water from oil extraction causes changes in the chemical properties of the soil and the chemical composition of plants. Information is available on the effects of chemical composition, both of the soil and of organic residue, on rates of decomposition [17, 18, 24], but no study has evaluated the effects of water quality on the chemical composition of plants, and the consequent changes in the mineralisation of nutrients and the decomposition of biomass, cellulose and lignin. Crop residues are important for the nutrient-cycling process in systems of agricultural production; it is therefore essential to evaluate any possible changes in the decomposition of plants irrigated with produced water. In the present study, decomposition rates for the residues of sunflower shoots (*Helianthus annuus* L. cv. BRS 321), irrigated with produced water subjected to filtration and reverse osmosis, and with groundwater captured in the Açu aquifer, were studied. The aim was to determine whether produced water submitted to two different treatments (filtering and reverse osmosis) alters the chemical characteristics of plants and influences decomposition of organic residue.

II. MATERIALS AND METHODS

The study area was an experimental field of the Brazilian oil company, Petrobras, located on the Belém Farm, in Aracati, in the State of Ceará, in the semi-arid region of Brazil, (4°43'6" S, 37°32'48" W). Average annual temperature and rainfall in the region are 28°C and 949.2 mm respectively, with the greatest concentration from March to May. Profiles were described for the study area, and the class of soil identified as a Haplic Arenosol [25].

Two crop cycles of the sunflower *Helianthus annuus* L., cv. BRS 321 were conducted in an experimental design of randomised blocks, with three replications in plots of 400m². In the first growing period, the crop cycle ran from July to Octoberof 2012, and in the second, from March to June of 2013. The plots were irrigated with wastewater from oil production, which were subjected to two pre-treatments after extraction of the oil. For the first pre-treatment, the water was initially filtered through sand filters, and then passed through a cation-resin filter to remove residue of the caustic soda used in the oil-water separation process (FPW). In the second pre-treatment, the FPW was subjected to nanofiltration and reverse osmosis (OPW). The control treatment used groundwater captured from wells at a depth of 250 m in the Açu aquifer (UGW). The chemical characteristics of the irrigation water are shown in Table 1.

Table.1: Principal chemical characteristics of the waters used for irrigation and the soil after irrigation.

Characteristic		Type of water						Soil‡ (0.0-0.1 m)		
		OPW		FPW		UGW		OPW	FPW	UGW
		n=4†	n=6††	n=4†	n=6††	n=4†	n=6††	n=3	n=3	n=3
EC	dS m^{-1}	0.62	0.38	2.51	1.95	0.65	0.66	2.05	5.34	1.96
pH	-	7.35	7.52	8.84	9.21	8.24	8.34	8.15	8.53	8.53
Ca^{2+}		0.01	0.11	0.18	0.11	0.21	0.21	4.50	2.31	2.66
Mg^{2+}		0.03	0.07	0.65	0.16	0.10	0.11	4.26	1.98	2.20
Na^{+}		3.75	2.95	24.15	18.15	7.10	6.23	7.80	40.47	14.53
K^{+}	$mmol_c$ L^{-1}	0.11	0.05	0.68	0.09	0.09	0.08	3.63	1.77	0.82
Cl^{+}		2.89	1.21	13.74	12.7	2.06	2.41	24.10	59.45	20.28
CO_3^{2-}		0.00	0.07	1.73	1.07	0.5	0.17	-	-	-
HCO_3^{-}		0.59	1.95	3.00	3.55	3.00	3.74	3.90	5.50	4.60

†first growing period, when the residue was obtained, ††second growing period, when the residue was incubated; ‡Soil chemical attributes at the time of incubation and decomposition of the residue.

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A drip irrigation system was used, with the emitters distributed along the crop rows, at a spacing of0.30 m and with a flow of 1 L h^{-1}. In order to meet the water requirement of the crop, the amount of water applied to the soil was up to 4.5 L m^{-2} day^{-1}, calculated based on the evapotranspiration of the sunflower crop and water loss through drainage, employing columns of mini-lysimeters in the experimental plots. During the first growing period, on average 271 L m^{-2}OPW, 365 L m^{-2}FPW, and 393 L m^{-2}UGWwere applied for plant irrigation. During thesecond period, 395 L m^{-2} OPW, 353 L m^{-2} FPW, and 260 L m^{-2} UGW were applied. During the experiment, the maximum mean temperature was 33ºC,with a minimumof 23ºC,and a precipitationof 483 mm (L m^{-2})in the second growing period.Based on the soil analysis prior to planting, it was necessary to correct the soil to meet the nutritional requirements of the crop. The soil was therefore corrected with organic fertiliser, 7.5 kg/lm before the first crop, and 2.5 kg/lm before the second crop. Also, in each cycle, doses of 80 kg/ha P_2O_5 and 40 kg/ha K_2O were incorporated into the soil before planting, as well as 50 kg/ha N close to the flowering stage.

Samples of shoot residue from sunflower plants produced in the first crop cycle, and irrigated with OPW, FPW, and UGW, were incubated with UGW in the irrigated plots (Test 1); and plant residue from plots where only UGW was used was incubated in the plots irrigated with OPW, FPW and UGW (Test 2).

For incubation, 30 g of air-dried shoot residue with a maximum size of 0.05 m, were placed into 0.14 m by 0.15 m nylon bags of anti-aphid mesh [26] and arranged horizontally in the soil at a depth of 0.05 to 0.10 m, near the drippers, avoiding direct contact with the plant roots. The chemical characteristics of the 0 to 0.1 m layer of soil (which corresponds to the depth of the incubated residue) after irrigation with the different types of water, are shown in Table 1. The bags were collected after 14, 28, 41, 55 and 69 days of incubation, and the residue dried at 65°C, weighed to determine the biomass, and stored for further chemical analysis. Sub-samples of the crop residue were used to determine the dry weight (65°C) and chemical characteristics at the start of the experiment (t = 0). For each trial, 45 nylon bags containing residue were incubated in the soil, considering three treatments (OPW, OPF and UGW), five collection periods (14, 28, 41, 55 and 69 days of incubation) and three replications (n = 3).

The collected residue was submitted to nitro-perchloric digestion (3:1 v/v) and the levels of Ca and Mg determined by atomic absorption spectrophotometry (Analyst 400, PerkinElmer), the Na and K content was determined by flame photometry (DM-62, Digimed), and S and P determined using spectrophotometry (Femto 600 Plus). Levels of $N\text{-}NH_4$ were determined by Kjeldahl distillation [27], and TOC was quantified by wet digestion with potassium dichromate and H_2SO_4 while heating [28]. Lignin and cellulose levels were also determined, using the method of acid detergent fibre (ADF) [29]. All the levels were calculated by multiplying the above concentrations by the weight of the collected residue.

At the end of the 69 day incubation period, losses were estimated for the biomass, minerals, lignin and cellulose for each situation under study, the half-life (t) for each of these being determined with the equation t = ln(2)/k [30], where k is the decay constant obtained from the equation $Xt\text{-}X0.e^{KT}$ [26].

The data were subjected to the Shapiro-Wilk test for normality, and Bartlett's test for the homogeneity of variances, to verify that the assumptions of the variance analyses were met. After noting the normal distribution of the variables, analysis of variance (ANOVA) was used to determine the statistical differences ($P<0.05$) between the mean values for the loss of biomass, nutrients (Ca, Mg, Na, K, S, P, and N), C, cellulose and lignin at the end of the 69 day incubation period. Mean values for data displaying any variation were compared by Tukey's test at a level of 5%.

To identify the relationship between the chemical characteristics of the residue and the loss of biomass, nutrients, lignin and cellulose, multivariate analysis of variance (MANOVA) was performed. To identify which variables (chemical characteristics of the residue) were more important in controlling decomposition, the value for Wilks's lambda was calculated; this allows evaluation, for each variable, of the statistical differences for the mean values between groups. The value for Wilks's lambda varies between 0 and 1; the smaller this value, the greater the discriminatory power between sets of variables. Due to interference from the chemical composition of the residue, and the combination and proportions of the different constituents of the decomposing material, the predictor variables used in the model were the initial values for Ca, N, P, K, Na, S, Mg, C, lignin and cellulose, and the ratios of C:N, cellulose:Ca, cellulose:N, cellulose:Pcellulose:K, cellulose:Na, cellulose:S, cellulose:Mg, lignin:Ca, lignin:N, lignin:P, lignin:K, lignin:Na and lignin:Mg. The response variables were the weight-loss percentage (%) for Ca, Mg, Na, N, P, K, S, C, cellulose, lignin and biomass in organic residue seen at the end of the incubation period (69 days). Statistical analysis was carried out using the R software [31].

III. RESULTS

3.1 Chemical composition of the plant residue

The nutrient concentrations and compounds from the biomass of the sunflower shoots exhibited distinct

behaviours when irrigated with the different types of water. Significant differences (P<0.05) were found in the levels of Na, Mg and lignin, with different behaviour when using FPW, the highest levels being seen for Na, while Mg and lignin displayed the lowest values. S and P were significantly lower, while lignin levels were higher compared to UGW when OPW was used. For the other elements and compounds (Ca, K, N, C and cellulose) there was no effect from the different types of water used, and no significant statistical differences were noted (P<0.05) (Table 2).

Table.2: Chemical composition of sunflower shoot residues irrigated with reverse-osmosis produced water (OPW), filtered produced water (FPW) and underground water (UGW).

Type of Water	Chemical composition, g kg^{-1}									
	Ca	Mg	Na	K	S	P	N	C	Cellulose	Lignin
OPW	29.9a	7.0a	1.8b	61.9a	3.6 b	5.2b	10.5a	330a	34.7a	8.0a
FPW	31.2a	5.5b	10.3a	63.3a	3.8ab	5.9ab	13.4a	294a	27.2a	6.8c
UGW	28.1a	7.5a	2.8b	69.7a	4.7 a	7.7a	13.3a	308a	30.5a	7.4b

Different letters in a column indicate differences between mean values by Tukey's test at 5% probability.

3.2 Decomposition of shoot residue from sunflowers irrigated with different types of water (OPW, FPW and UGW), and incubated in soil irrigated with UGW (Test 1)

The loss percentage for biomass in the residues produced with OPW, FPW and UGW was around 73%, with no significant difference (P<0.05) when irrigated with UGW (Table 3). The same behaviour was also seen for P, Ca, C, cellulose and lignin, with overall mean values of 52, 50, 80, 80 and 44% respectively. However, this lack of significance in the differences was not found when evaluating the remaining nutrients, identifying loss percentages which were smaller (Mg, P and N) and greater (Na, K and S) than the mean for biomass (the main reference, due to being composed of these nutrients), and highlighting the significant statistical differences for the losses of Na, Mg and K. The losses were higher for Na and Mg when the residues were produced with FPW and OPW respectively. K had the greatest losses among all the elements studied, on average 98%, with similar losses whether the residue was irrigated with FPW or UGW, these losses being greater than for OPW. S and N also showed significant losses, but in the following order for the type of water used for irrigation: UGW>OPW=FPW and FPW>OPW=UGW respectively.

It was found that generally the greatest losses correspond to the smallest values for half-life. Again, this assertion can be made considering the biomass as reference, as was done with the loss of nutrients. Elements with losses greater than the average seen for biomass, had the lowest values for half-life, while those with smaller losses, had the longest half-life.

There are statistical differences between the values for half-life of the residues when irrigated with the different types of water (Table 3); here, the use of OPW is highlighted, since the half-life was longer using that type of water, as was the case with Na, K, S, N and the biomass. Some cases showed similar results when using the other types of water (UGW and FPW), however no general trend was seen. In the case of Mg, the use of FPW and UGW gave the greatest value for half-life, while the half-life of P was greater in residue produced with UGW.

Table.3: Loss of mass and the half-life of biomass and nutrients in differing sunflower shoot residues irrigated with reverse-osmosis produced water (OPW), filtered produced water (FPW) and underground water (UGW) after 69 days of incubation in soil irrigated with UGW (Test 1).

Residue	Biomass	Chemical Constituent									
		Ca	Mg	Na	K	S	P	N	C	Celluose	Lignin
		Loss of mass, %									
OPW	71.9a	52a	64.8a	66.2b	96.6b	73.8b	58.3a	52.8b	83a	79a	41a
FPW	74.6a	49a	44.6b	94.0a	98.4a	78.8ab	52.6a	66.7a	79a	82a	44a
UGW	72.8a	52a	38.9b	77.5b	97.5a	82.2[a]	45.1a	61.7ab	81a	82a	49a
		Half-life, *t*-days									

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OPW	35.3a	66a	49.3b	29.7a	14.3a	40.3a	49.0b	44.0a	27a	ND	ND
FPW	30.3b	74a	74.7a	12.3b	9.0b	30.3b	48.0b	30.7a	30a	ND	ND
UGW	31.0ab	65a	77.3a	23.7a	13.0a	29.7b	69.3a	37.3a	28a	ND	ND

Different lowercase letters in a column indicate differences between mean values by Tukey's test at 5% probability. ND = Not determined.

3.3 Decomposition of shoot residue from sunflowers in the area of UGW, and incubated in soil irrigated with different types of produced water (OPW, FPW and UGW) (Test 2)

The loss of biomass in this test (73%) was similar to the previous test (72%) with no statistical differences for type of water used for irrigation in the residue produced with UGW (Table 4). The same trend was seen for some nutrients and compounds of the biomass, for example Mg, S, P, K, C and N, cellulose and lignin. The nutrients which exhibited statistical difference were Ca and Na, however no similarity was seen between their behaviour, with the order of losses for type of water used for irrigation being: UGW=FPW>OPW and OPW=UGW>FPW respectively.

Half-life was more sensitive in indicating variations in decomposition, as there were significant statistical differences for Ca, Mg, Na and biomass (Table 4). There was statistical similarity between FPW and UGW for half-life in Ca and Mg, however FPW gave a greater half-life for Na with a shorter half-life for biomass. OPW and UGW showed similarity between Na and biomass for half-life, with OPW giving a shorter half-life for Mg and a longer half-life for Ca.

Table.4: Loss of mass and the half-life of biomass and nutrients in sunflower shoot residues irrigated with UGW after 69 days of incubation in soil irrigated with reverse-osmosis produced water (OPW), filtered produced water (FPW) and underground water (UGW) (Test 2).

Type of water	Biomass	Chemical Constituent									
		Ca	Mg	Na	K	S	P	N	C	Cellulose	Lignin
		Loss of mass, %									
OPW	72.3a	41.1b	51.1a	85.5a	97a	80.6a	49a	62a	76a	84a	58a
FPW	74.8a	48.7a	47.8a	43.8b	100a	82.6a	41a	68a	84a	81a	60a
UGW	72.8a	52.4a	38.9a	77.5a	98a	82.2a	45a	62a	81a	82a	49a
		Half-life, *t*-days									
OPW	36a	91a	67b	20.0b	13a	29a	73a	50a	33a	ND	ND
FPW	35b	72b	75ab	44.3a	8a	27a	91a	43a	26a	ND	ND
UGW	37a	64b	97a	23.7b	13a	27a	80a	50a	28a	ND	ND

Different lowercase letters in a column indicate differences between mean values by Tukey's test at 5% probability. ND = Not determined.

3.4 Influence of the chemical composition of the residue on decomposition and the loss of biomass and nutrients

The loss of nutrients and organic compounds was influenced by the chemical properties of the residue under decomposition (MANOVA, F=12.85, R^2=0.85, P<0.001). Considering the effect of the predictor variables group (residue composition) on the variable response group, the C:N ratio displayed the greatest control over the loss of nutrients and organic components, as indicated by the lower lambda value (Table 5), followed by the cellulose:Mg ratio. The C:N ratio influenced the loss of Mg, Na, N, S, biomass, lignin and cellulose, while the cellulose:Mg ratio affected the loss of Mg, Na, biomass and cellulose. The loss of Na and cellulose, and of N and S, were also influenced by the ratios of cellulose:N and cellulose:S, respectively.

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Table.5: F-test probability and Wilks's lambda for the initial chemical characteristics of sunflower shoot residue produced with reverse-osmosis produced water (OPW), filtered produced water (FPW) and underground water (UGW) on mineralisation, in soils irrigated with UGW after 69 days incubation.

Initial chemical characteristics[§]	Loss of nutrients/organic constituent									Wilks's lambda	
	Lignin	Mg	Na	N	K	S	Biomass	Cellulose	C		
	F-test probabilility									Value	P
C:N	<0.01	<0.01	<0.01	<0.001	0.15	<0.001	<0.001	<0.01	<0.10	0.014	<0.01
Ca	0.16	0.16	0.15	0.25	0.88	<0.10	0.24	<0.05	<0.10	0.10	0.11
Cellulose	0.81	0.19	0.46	0.32	0.55	0.15	<0.10	0.62	0.67	0.13	0.16
Cellulose:Ca	0.87	0.69	0.41	0.31	0.62	0.42	0.27	<0.10	0.35	0.27	0.47
Cellulose:Mg	0.55	<0.05	<0.05	0.36	0.64	0.97	<0.10	<0.05	0.74	0.05	<0.05
Cellulose:N	0.16	0.84	<0.05	0.24	0.34	0.17	0.44	<0.05	0.87	0.07	<0.10
Cellulose:K	0.21	0.50	0.88	0.70	0.79	<0.10	0.42	<0.05	0.32	0.16	0.21
Cellulose:S	0.22	0.86	0.99	<0.01	0.77	<0.05	0.64	0.27	0.35	0.10	<0.10
Carbon	<0.05	0.53	0.82	0.26	0.41	0.15	0.18	<0.10	0.99	0.23	0.37

[§]The remaining constituents (N, P, K, Na, S, Mg, lignin e cellulose, and the ratios of cellulose:P, cellulose:Na, lignin:Ca, lignin:N, lignin:P, lignin:K, lignin:Na, and lignin:Mg) did not affect mineralisation, according to the MANOVA test.

IV. DISCUSSION

The composition of the treated water (FPW or OPW), may be related to changes in the levels of the same elements when also evaluated in the soil, i.e. reductions or increases in the levels of these elements in the water correspond to similar behaviour in the soil (Table 1). The change in soil properties (salinity, for example) may be associated with changes nutrients found in sunflowers tissues. Despite these variations in the soil being relatively wide for the type of water/treatment, only the levels of Na, Mg and lignin showed significant changes in the tissue. Variations in the levels of Na in the different types of water used for irrigation may be associated with the different levels of Na [32], Mg [33] and lignin [34] found in the sunflower tissue. These findings also underline the efficiency of the adopted treatments, as regards the presence of elements and the effect on the soil and plants, with a clear advantage seen with OPW, where their composition displays a reduction in levels. Similar results for the influence of the type of water on the soil, and the efficiency of the wastewater treatments [35].

However, the rates for loss of mass and half-life in the residue, when compared to the overall average biomass in both tests under study, were very similar, which demonstrates that if evaluated using only biomass, control of residue decomposition should be attributed to the environment and its conditions (humidity, wind, sunlight, microbial activity, etc.). This association will be real; but it is also necessary to consider variations in the composition of plant tissue and the type of water that was used in producing the residue, since, in the two situations under study (Tests 1 and 2), there was an effect on the loss (%) and half-lives (t) of the nutrients, and on the differences in the loss of total biomass for the residue in decomposition. These results are consistent with results obtained in previous studies [14, 18, 24], in which those authors observed that the chemical composition of the residue resulted in variations in its mineralisation.

When considering the reference conditions (UGW), changes in the residue produced with FPW were enough to increase the loss of Na and K, whereas the changes that occurred in the residue from plots irrigated with OPW were enough to increase the loss of Mg and P, and reduce loss of S. Compared to the residue produced with OPW, the loss of Na, K, S, N, and biomass was greater in residue obtained with FPW. It can therefore be demonstrated that irrigating with produced water alters the chemical composition of the sunflower, and subsequently influences mineralisation of the plant residue.

Previous studies have demonstrated the isolated effects of the initial chemical composition of plant residue on the decomposition of such components of organic residue as C and lignin [17, 18], and Mg, P, and Na [16, 36], or on the ratios of C:N, lignin:N and lignin:P [37, 38]. In the present study, the loss of total biomass, Na, Mg, S, N, C, cellulose and lignin was mainly influenced by the C:N ratio. However, the ratios of cellulose:Mg, cellulose:N and cellulose:S also influenced decomposition of the sunflower residue (Table 5), possibly due to the decomposer organisms in the soil used the cellulose as a C source [39]. For decomposition of structural components such as cellulose, high levels of nutrients are required [17], which may explain the results found in this study, since the ratio of cellulose to nutrients affected the rate of decomposition, which did not occur when cellulose was considered in isolation.

As demonstrated by the MANOVA analysis, the cellulose:Mg ratio was the most important in controlling the rate of decomposition of the sunflower residue than the ratios of cellulose:S and cellulose:N, as it showed the lowest value for lambda ($\lambda = 0.053$, $P<0.05$; Table 5). It is possible that the chemical fertilisation of the soil during preparation of the area met the needs for N, P and K of the microorganisms in the soil, thus not depending on the nutrient content of the residue during decomposition of the organic matter. However, this relationship is not yet clear; new studies could therefore consider residues with different cellulose to nutrient ratios, in evaluating the rate of decomposition.

In contrast to results obtained in other studies [17, 18] there was no effect from lignin content on the loss of nutrients, C, cellulose or total biomass. It is possible that the incubation period of the residue (69 days) was not sufficient for the lignin to affect decomposition, or it can be considered that interference by the lignin in the rate of decomposition only occurs after depletion of the more labile fractions of the organic residue [38].

There were similar variations in the loss of some residue components in soils irrigated with UGW and with produced water (OPW and FPW). However, irrigating the soil with OPW or FPW favoured the loss of Ca and Mg respectively. High levels of salts affect the decomposition of organic residue by reducing the size and diversity of the microbial community in the soil [22, 40-42] as well as its activity [23, 43]. But in this study, the highest values seen for Na^+, Cl^-, HCO_{3-} and EC with FPW (Table 1) did not reduce the loss of nutrients (except for Na), biomass or other constituents of the sunflower residue. Under these conditions, it can be associate the capacity of soil microorganisms for rapid response with changes in soil salinity [21, 43]; this can be attributed to adaptation to the new conditions. Also to be considered are the joint changes in the microbial structure of the soil due to salinity and alkalinity [22] in arid soils, resulting in selection of the most efficient species for promoting decomposition of the residue. In order to clarify this issue, further studies are needed into microbial communities involved in the decomposition of residue in soils irrigated with FPW.

V. CONCLUSIONS

Irrigation with produced water changes the chemical characteristics of the soil and the composition of cultivated plants at a sufficient level to influence the rate of decomposition of the organic residue, these effects being variable and dependent on the type of pre-treatment used. The produced water treated by filtration favoured greater the decomposition of sunflower residue than that by reverse osmosis.

It is necessary to test new ways of treating produced water to be used in the irrigation of crops, especially processes where there is no addition of biocides, in the case of treatment by reverse osmosis, and which are effective in the removal of salts, in the case of treatment by filtration. Studies are also necessary to evaluate the cumulative effect of successive irrigation with produced water on the decomposition of residue and on the soil microbiota, as well as on the accumulation of toxic minerals in the soil.

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In vitro Propagation of Malaysian Cassava (*Manihot esculenta* Crantz) Variety through Low Cost Tissue Culture Media

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Abstract*—Cassava (*Manihot esculenta *Crantz) is a perennial woody plant belongs to Euphorbiaceae family and listed as one of the most important source of carbohydrates around the world. In Malaysia, Cassava is an important industrial crop for starch processing and food industries. Hence, an* in vitro *propagation technique is needed to produce these highly demand industrial crop. In this study, the Malaysian cassava variety which was Putih variety was cultured onto low cost tissue culture media by using nodal explants. The low cost media were tested using locally available ingredients which were 2 ml/L Maxigreen50 liquid fertilizer as the substitute to MS salt, 3%(w/v) table sugar as the substitute to sucrose, agar-agar strip (14 g/L), corn flour (20 g/L), and tapioca flour (20 g/L) as the substitute to Phytagel powder. The low cost media were supplemented with young coconut water of Matag variety at the concentration of 0, 25, 50, 75, and 100 mL/L. The results showed that the best low cost media for the induction of shoot multiplication, height and number of leaves was the low cost media supplemented with 2 mL/L Maxigreen50 liquid fertilizer, 14 g/L agar-agar strip + 20 g/L corn flour, and 100 mL coconut water for cassava Putih variety.*

Keywords— In vitro, Manihot esculenta, low cost, shoot multiplication.

I. INTRODUCTION

Manihot esculenta or also known as Cassava, Tapioca, Manioc, Yuca (Spanish), and Ubi Kayu (Malay) is a perennial woody shrub from Euphorbiaceae family which is native to Central and South America (FAO, 2000). Cassava is categorized as an important source of carbohydrate after rice and corn which provide important component of diet to more than 800 million of people around the world (Richardson, 2013). In Malaysia, Cassava is mainly cultivated for the large scale industrial purposes for starch processing industries (Lian and Idris, 2000). According to Department of Agriculture (DOA) Sarawak (2015), the Cassava industry for production of chip and snack production has been increasingly in demand and been a source of income generation for the small scale farmer (DOA Sarawak, 2015). Hence, tissue culture propagation technique is needed for the rapid production of plantlets which have uniform genetic characteristics and free from diseases. However, the high cost of the chemical ingredients for the preparation of tissue culture media becomes one of the problems for the application of tissue culture technology (IAEA, 2004). Thus this research will investigate the suitable low cost tissue culture media which use available and cheap materials as the substitute to the high cost of chemical used in conventional tissue culture media in order to reduce the cost of cassava plant production.

II. MATERIALS AND METHODS

The vegetative germplasms of local Cassava (*Manihot esculenta* Crantz) variety in Sarawak which was Putih variety was obtained from Agriculture Research Centre (ARC) Semongok. The nodal cuttings of three months old's cassava Putih variety which consists of node number 2 to node number 4 from the shoot tip were excised into 1.0-1.5 cm length before surface sterilized by immersing in 70% ethanol for 1 minute followed by agitation in 25% Clorox (active ingredients: 5.25% Sodium Hypochlorite (NaOCl)) for 10 minutes with two drops of Tween-20 before rinsed with sterile distilled water for five minutes. The sterile nodal explants were cultured onto the control media which was full strength MS media with 7 g/L Phytagel, 30 g/L sucrose, and Benzylaminopurine (BAP) at 1.0 mg/L and 1-Naphthaleneacetic acid (NAA) at 0.01 mg/L, whereas in the low cost tissue culture media, the materials used for the low cost media were 2 mL/L Maxigreen50 liquid fertilizer (22-16-12+2MgO+TE) as the substitutes to MS salt, 30 g/L table sugar as the substitutes to sucrose, 14 g/L agar-agar strip with or without combination with 20 g/L corn flour and 20 g/L tapioca flour as the substitute to Phytagel powder, and young coconut water of Matag variety at the concentrations of 0 mL, 25 mL, 50 mL, 75 mL, and 100 mL as the substitutes to BAP and NAA. There were six replicates on each

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treatment and five explants in each replicate. The observation on growth parameters such as number of shoots, plant height, and number of leaves were observed on each four weeks interval until week 12. One way analysis of variance (ANOVA) was used for analyzed the data and comparison of mean by using Tukey test ($p<0.05$).

III. RESULTS AND DISCUSSION

The Effect of Different Concentrations of Coconut Water and Different Types of Gelling Agents on the Mean Number of Shoots of Cassava in Low Cost Media

For the number of shoots, there was a significant difference ($p<0.05$) on the mean number of shoots produced from the nodal explants where the highest mean number of shoots was obtained from control treatment, T1 (1.0 mg/L BAP + 0.01 mg/L NAA) which was 3.77±0.43 in cassava Putih variety (Table 1). For the low cost media, there were no shoot multiplication recorded in which only one shoot per nodal explant was grown from T4 (agar-agar strip with 50 mL coconut water), T5 (agar-agar strip with 75 mL coconut water), T6 (agar-agar strip + corn flour with 100 mL coconut water), T8 (agar-agar strip + corn flour with 25 mL coconut water), T9 (agar-agar strip with 50 mL coconut water), T10 (agar-agar strip + corn flour with 75 mL coconut water), and T11 (agar-agar strip + corn flour with 100 mL coconut water) which were 1.00±0.00 (Table 1).

Table.1: Mean number of shoots, heights and leaves of cassava Putih variety on low cost media of different concentrations of coconut water and different types of low cost gelling agents after 12 weeks cultures

Treatment	Gelling agent	Plant growth regulator	Mean no. of shoots	Mean shoot heights	Mean no. of leaves
T1	Phytagel	1 mg/L BAP + 0.01 mg/L NAA	3.77 ± 0.43^{c}	6.97 ± 0.13^{g}	26.37 ± 0.61^{f}
T2	Agar-agar strip	0 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T3	Agar-agar strip	25 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T4	Agar-agar strip	50 mL/L coconut water	1.00 ± 0.00^{b}	3.65 ± 0.08^{b}	2.90 ± 0.41^{b}
T5	Agar-agar strip	75 mL/L coconut water	1.00 ± 0.00^{b}	4.45 ± 0.10^{c}	0.00 ± 0.00^{a}
T6	Agar-agar strip	100 mL/L coconut water	1.00 ± 0.00^{b}	5.59 ± 0.09^{d}	3.20 ± 0.41^{b}
T7	Agar-agar strip + corn flour	0 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	3.67 ± 0.48^{c}
T8	Agar-agar strip + corn flour	25 mL/L coconut water	1.00 ± 0.00^{b}	3.69 ± 0.13^{b}	4.70 ± 0.47^{e}
T9	Agar-agar strip + corn flour	50 mL/L coconut water	1.00 ± 0.00^{b}	5.56 ± 0.13^{dz}	0.00 ± 0.00^{ax}
T10	Agar-agar strip + corn flour	75 mL/L coconut water	1.00 ± 0.00^{b}	6.23 ± 0.10^{e}	3.53 ± 0.51^{c}
T11	Agar-agar strip + corn flour	100 mL/L coconut water	1.00 ± 0.00^{b}	6.87 ± 0.10^{fz}	5.77 ± 0.43^{gy}
T12	Agar-agar strip + tapioca flour	0 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T13	Agar-agar strip + tapioca flour	25 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T14	Agar-agar strip + tapioca flour	50 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T15	Agar-agar strip + tapioca flour	75 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
T16	Agar-agar strip + tapioca flour	100 mL/L coconut water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

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These findings were in agreement with the findings obtained by Daud *et al.* (2011) on *in vitro* culture of Celosia spp. using low cost media in which there was a shoot regeneration recorded on the low cost media supplemented with 70 mL/L of young coconut water plus corn flour, rice flour, cassava flour, and potato starch without addition of MS although the low cost media supplemented with ½ MS showed more effects on shoots regeneration compared to the medium without MS (Daud *et al.*, 2011). However, this experiment showed no shoot multiplication even at the highest concentration of coconut water of Matag variety which is 100 mL/L and could be due to the presence of other precipitates in the coconut water which can hindered the action of endogenous cytokinin in the coconut water.

The Effect of Different Concentrations of Coconut Water and Different Types of Gelling Agents on the Mean Shoot Height of Cassava in Low Cost Media

For the mean shoot heights of cassava Putih variety, there was a significant difference ($p<0.05$) on the mean shoot height produced where the highest mean shoot height was obtained from the control treatment, T1 (1.0 mg/L BAP + 0.01 mg/L NAA) which was 6.97±0.13 (Table 1).

For the low cost media, the highest mean shoot height was recorded on the low cost media supplemented with the highest concentration of coconut water which was T11 (agar-agar strip + corn flour with 100 mL coconut water) which produced the mean shoot heights of 6.87±0.10 (Table 1). This findings was in agreement with the findings of Buah and Agu-Asare (2014) on their study of using coconut water from fresh and dry fruit as an alternative to BAP on Dwarf Cavendish Banana where the plant cultured on medium supplemented with fresh coconut water produced the highest shoot height compared to the plant cultured on medium supplemented with BAP. Throughout all treatments of low cost media, the highest mean shoot height was recorded on low cost media supplemented with 20 g/L corn flour. This indicates that the presence of coconut water at the highest concentration on the low cost media with the addition of agar-agar strip with corn flour can produce the best shoot height. This result was in agreement with the finding obtained by Mohamed et al. (2010) on the uses of corn starch and potato starch as an agar alternative to Solanum tuberosum in which the uses of corn starch and potato starch has no significant effect in plantlet height but produce the significant effect on the number of shoots over the control treatment with agar. The use of commercial starch or flour as the alternative gelling agents in tissue culture media are due to high amount of starch, vitamin C and carbon sources and low amount of other minerals (Daud *et al.*, 2011).

The Effect of Different Concentrations of Coconut Water and Different Types of Gelling Agents on the Mean Number of Leaves of Cassava in Low Cost Media

For the number of leaves of cassava Putih variety, there was a significant difference ($p<0.05$) on the mean number of leaves produced where the highest mean number of leaves was obtained from the control treatment, T1 (1.0 mg/L BAP + 0.01 mg/L NAA) which was 16.23±0.73 (Table 1). For the low cost media, the highest mean number of leaves was recorded on T11 (agar-agar strip + corn flour with 100 mL coconut water) which produced the mean number of leaves of 5.77±0.43 (Table 1). The result was in agreement with the findings obtained from Lalitha *et al.* (2013) on the effect of plant derived gelling agents on micropopagation of mulberry. From their experiment, the highest number of nodes and number of leaves were obtained from the MS medium gelled with corn flour (22 g/L) in combination with 3.5 g/L agar compared to other plant derived gelling agents.

From this experiment, the lowest mean shoot height, mean number of nodes and mean number of leaves were recorded from the low cost media of 14 g/L agar-agar strip only or 14 g/L agar-agar strip with combination of 20 g/L corn flour without addition of coconut water (Table 1). This showed that coconut water is needed in the growth of shoot and the use of Maxigreen50 liquid fertilizer only cannot induce the shoot growth in the low cost media. This could be due to absence of important vitamins in Maxigreen50 liquid fertilizer needed for plant growth in vitro. Thus, the addition of coconut water can help to supply the vitamins or nutrient sources for the growth of plantlets as the use of young coconut water can acts as a plant growth regulators that gives a better response on plant tissue culture (Daud *et al.*, 2011).

IV. CONCLUSION

The best low cost media for the induction of shoot height and number of leaves for cassava Putih variety was the low cost media of 2 mL/L Maxigreen50 liquid fertilizer, 30 g/L table sugar, 14 g/L agar-agar strip + 20 g/L corn flour supplemented with 100 mL coconut water. However, there was no shoot multiplication recorded on the low cost media of 2 mL/L Maxigreen50 liquid fertilizer, 30 g/L table sugar, 14 g/L agar-agar strip + 20 g/L corn flour supplemented with 100 mL coconut water.

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Assessment of Factors that Influence Participants Level of Participation in Fadama III Agricultural Project in Bayelsa State, Nigeria

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Abstract— *The study assessed factors that influenced participant's level of participation of fadama III agricultural project in bayelsa state. Objectives were the participant's socioeconomic characteristics, and the factors that influenced participant's level of participation. Purposive sampling technique was used to select 150 farmers that participated, and 150 staff from the delivery agency. Data were collected with a structured questionnaire. Objective one was analyzed using descriptive statistics, while inferential statistics such as ordinary least square (OLS) regression analysis was used. The finding showed that farmers were averagely 42.6 years old, 54.7% of the farmers were females while 45.4% were males, 72.1% of the farmers were married. The farmers farm averagely 0.9 hectares and had averagely 7 persons per household. The farmers were literate, experienced in farming with mean monthly estimated income from Fadama of ₦44, 133.83. Extension visit to farmers was low with 38.7 percent being the largest proportion of the entire sampled farmers. Age of respondents, food security status, household size, and income level, and poverty status, leadership propensity, farming experience, educational level and distance to Fadama III training centre influenced participant's level of participation in Fadama III agricultural project in Bayelsa state. The study concludes that agricultural and rural development projects such as fadama III are fundamental to nation building and the agricultural sector is what can fast track the challenges faced by rural dwellers in terms of agricultural development in most of the developing countries. The study recommends that funds meant for agricultural projects be properly disbursed to farmers to enable them participate actively as farmer's income level motivate them to participate in agricultural and rural development projects.*

Keywords— Agricultural, Bayelsa State, Fadama III, Influence, Participation.

I. INTRODUCTION

The National Fadama Development Project was introduced as a strategy to tackle rural development problems. There are quite a number of studies on rural development in general and fadama project in particular. These studies have been carried out in different parts of Nigeria and on different aspect of the impact analysis of the National Fadama Development Project.

Fadama areas are typically waterlogged in the rainy season but retain moisture during the dry seasons. Fadama areas are considered to be of high potential for economic development through appropriate investments in productive assets, rural infrastructure and technical assistance. The desire to harness the verse potentials of Fadama in Nigeria culminated in the design of National Fadama Development Project I, II and III. Fadama I (Phase I of the National Fadama Development Project) was implemented during the 1993-1999 period. While, Fadama I focused mainly on crop production, downstream activities such as processing, preservation and marketing were largely neglected. The design did not take into cognizance of need for spatial integration of the markets (creating of physical and market infrastructure). It also failed to take into consideration other Fadama resource users such as livestock producers, fishing folks, pastoralists, hunters etc. The project did not also support post-harvest technology, which manifested in reduced crop prices and increased storage losses during the period, [1].

Some of the lessons learnt in Fadama I informed the birth of Fadama II. Fadama II was targeted at dry season farming agro-processing, preservation and marketing. It also allowed for acquisition of productive assets, provision of

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rural infrastructure to ensure the efficient transportation of farm output to markets as well as marketing activities. The project development objective was to sustainably increase the incomes of the beneficiaries through empowering communities to take charge of their own development agenda through Community Drive Development (CDD) approach in project implementation in a socially inclusive manner. Fadama II also provides special preferences to groups of youths, women (especially widows), physically challenged, the elderly and people with HIV/AIDs, [2].

Fadama III project is a follow-up to the Fadama II project which was assessed to have impacted the lives of rural farmers, raising their incomes by 63 percent. The project like Fadama II takes the CDD approach, which places beneficiaries in driver's seat. Local community members under the umbrella of Fadama Community Associations (FCAs and Fadama Users Groups (FUGs), oversee the design and implementation of the project and are empowered through skills and capacity building to improve their livelihoods by increasing income generating activities.

II. THE SPECIFIC OBJECTIVES WERE:

i) describe the socio-economic characteristics of the participants in the study area,

ii) determine the factors that influence the level of participation of the respondents in Fadama III agricultural project in the study area.

III. METHODOLOGY

The study was carried-out in Bayelsa State; the State is made up of eight Local Government Areas, namely: Brass, Ekeremor, Kolokuma/Opokuma, Nembe, Ogbia, Sagbama, Southern Ijaw and Yenagoa Local Government Areas respectively. Each of this L.G.As is known as Agricultural districts. The major occupation of the people is farming and fishing, [3]. Purposive sampling technique was used to select the communities that participated in Fadama III Project; one (1) Local Government Area was used to represent each of the three (3) Agricultural zones, five Fadama communities were used to represent each of the selected Local Government Areas, the Local Government Areas were Kolokuma/Opokuma, Ogbia and Sagbama respectively, ten (10) participants belonging to a Fadama User Group were selected from each of the communities which gave us a sample size of One-Hundred and fifty (150) respondents.

3.1 Method of Data Analysis

Data collected from the survey were analyzed using descriptive statistics such as frequency, percentages and mean for objective one (1) while Objective two (2) was analyzed using ordinary least square multiple regression analysis technique.

3.2 Model Specification for Ordinary Least Square Regression Analysis

The ordinary least square regression model used to estimate the factors that influenced the level of participation of farmers in Fadama III Agricultural project in Bayelsa state is given in implicit form as:

$$PFFAP = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, e) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where,

PFFAP = Participation in Fadama III agricultural project (mean response of the respondent on a 4 point likert type rating);

X_1 = Age of respondents (years);

X_2 = Gender (Dummy variable: 1= male; 0 = female);

X_3 = Food security ($\frac{\text{Per capita Food expenditure for the i}^{\text{th}}\text{ household}}{\text{2/3 mean per capita food expenditure of all households}}$);

When Fi ≥ 1 = food secure i^{th} household and when Fi ≤ 1= food insecure i^{th} household.

X_4 = Household size (Counts of people living in the same home and feeding from the same pot);

X_5 = Income level (Naira);

X_6 = Poverty status of the respondents (Measured as Mean per Capita Household Expenditure (MCHE));

X_7 = Marital status (Married =1; Unmarried =0);

X_8 = Farm size (Hectares)

X_9 = Leadership style (Supportive = 1; Unsupportive = 0)

X_{10} = Farming experience (years)

X_{11} = Educational level (years spent in schooling)

X_{12} = Distance to Fadama III training centre (kilometers);

X_{13} = Cooperative membership (Yes =1; No =0)

e = error term.

The logit regression analysis used to estimate the significant factors that influenced the effectiveness of Fadama III project in empowering the participants in the study area is given in implicit form as:

$$EFAEP = f(Z_1, Z_2, Z_3, Z_4, Z_5, Z_6, Z_7, Z_8, Z_9, Z_{10}, Z_{11}, \ldots\ldots, Z_{21}, Z_{22}, Z_{23}, e) \ldots\ldots\ldots\ldots (2)$$

Where,

EFAEP = Latent dummy variable indexing effectiveness of Fadama III project in empowering the participants (Effective =1; Not effective = 0);

Z_1 = Delay in provision of advisory services (Yes =1; No = 0)

Z_2 = Unavailability of funds for loan services (Yes =1; No = 0)

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Z_3 = Quality of technical advice (Good =1; Poor = 0)

Z_4 = Relationship with project communities (Cordial =1; Hostile = 0)
Z_5 = Types of enterprise farmers participate in (Crop production =1; livestock production = 2; Apiculture =3; Fish production = 4; Snail production =5)
Z_6 = Attitude to work of the Fadama delivery personnel (Good =1; Poor = 0)
Z_7 = Resourcefulness of the Fadama delivery personnel (Resourceful =1; Non- resourceful = 0)
Z_8 = Leadership style of the Fadama delivery personnel (Supportive=1; Unsupportive = 0)
Z_9 = Number of Fadama delivery personnel (Abundant manpower=1; Few manpower = 0)
Z_{10} = Inadequate funding (Yes =1; No = 0)
Z_{11} = Redundancy of Fadama delivery personnel (Yes =1; No = 0)
Z_{12} = Delay in input delivery (Yes =1; No = 0)
Z_{13} = Educational competency of delivery personnel (Competent =1; Not competent = 0)
Z_{14} = Distrust of delivery agent personnel by farmers (Yes =1; No = 0)
Z_{15} = Unnecessary bureaucracy (Yes =1; No = 0)
Z_{16} = Inadequate availability of operational logistics (Yes =1; No = 0)
Z_{17} = Supply of unviable/insufficient farm inputs (Yes =1; No = 0)
Z_{18} = Poor monitoring of project activities (Yes =1; No = 0)
Z_{19} = High cost of management of project activities (Yes =1; No = 0)
Z_{20} = Farmers unwillingness to participate (Yes =1; No = 0)
Z_{21} = Limited Information on improved technologies (Unlimited =1; Limited = 0)
Z_{22} = Climatic uncertainties/flooding (Yes =1; No = 0)
Z_{23} = Low adoption of technology (Yes =1; No = 0)
e = error term.

IV. RESULTS AND DISCUSSION

4.1 Fadama III Agricultural Project Participants Socioeconomic Characteristics in Bayelsa State

4.1.1 Age of respondents

The distribution of the respondents by their age shows that majority (41.3%) of the farmers who participated in Fadama III Agricultural project in Bayelsa State were within the age bracket of 41-50 years old while the least (8.7%) of the farmers who participated in Fadama III Agricultural project in Bayelsa State were within the age bracket of 21-30 years old. The mean age of the respondents was 42.6years old. This implies that most of the farmers that participated in Fadama III Agricultural project in Bayelsa State were still in their active stage in life and can be effective in utilizing any training they received from Fadama III Agricultural project in Bayelsa State to better their income generating capacity and better their standard of living. [4] And [5] succinctly observed that farmers within the active age brackets have more innovative ability and capacity to do manual work than farmers in their inactive age. The pie chart representation of the percentage variations in the ages of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 1 below.

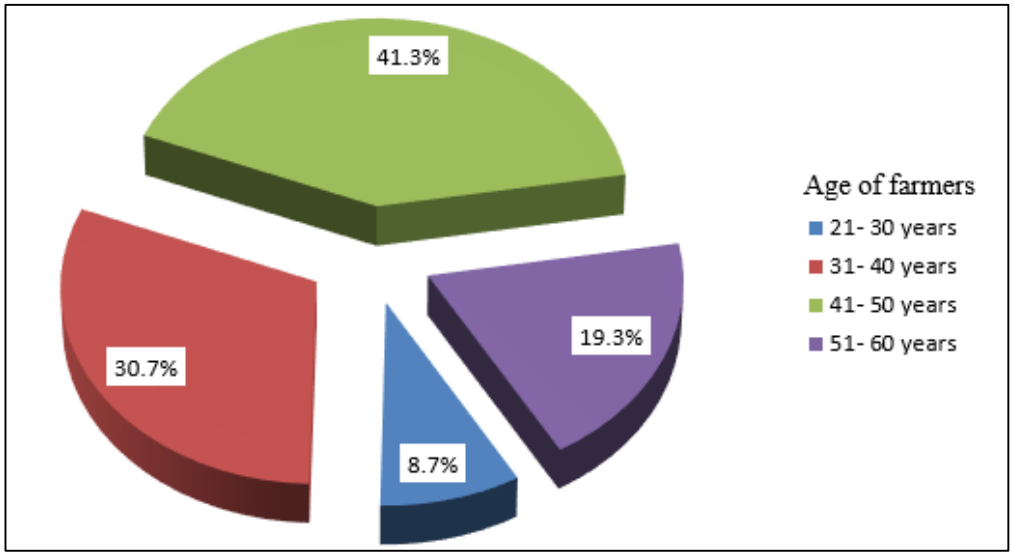

Fig. 1: pie chart representation of the percentage variations in the ages of the sampled farmers participating in fadama iii agricultural projects in bayelsa state.

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4.1.2 Gender of respondents

The distribution of respondents by gender shows that, 82 farmers representing 54.7% of the entire sampled farmers were females while 68 farmers representing 45.4% of the entire sampled farmers were males. This indicates that female were more involved in Fadama III Agriculture project activities in the area studied than their male counterparts. This finding is consistent with [6], [7] and [8] who noted that female farmers often engaged in Agricultural activities than male farmers in their various studies with a relevant observation that men especially the youths that are suppose to embrace farming are neglecting Agriculture and probably migrating from the rural villages to the cites in search of white collar jobs, while the women still remain and engaged in agriculture despite their dual roles as farmers and mothers. This assertion was further confirmed by [9] who reported that women constitute the major actors in all aspects of life. [10] in their study noted that the role women play and their position in meeting the challenges of Agricultural production and Development are quite dominant and prominent. Their relevance and significance in Agriculture, therefore, cannot be overemphasized [11]; [12]. Findings from a study financed by the United Nations Development Programme (UNDP) revealed that women make up some 60 to 80% of Agricultural labour force in Nigeria [13], depending on the region and they produce two-third of the food crops. Yet, in spite of these, widespread assumption that men - and not women - make the key farm management decisions has prevailed. Sadly, female farmers in the country are among the voiceless, especially with respect to influencing Agricultural policies. The bar chart representation of the percentage variations in the gender of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 2 below.

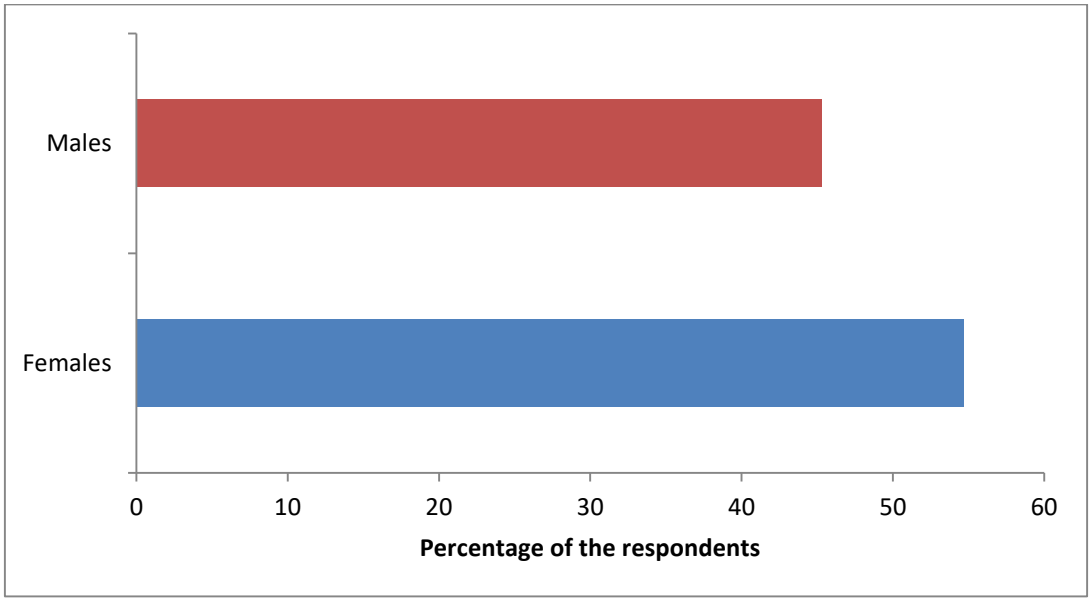

Fig.2: bar chart representation of the percentage variations in the gender of the sampled farmers participating in fadama iii agricultural project in bayelsa state.

4.1.3 Marital Status

For marital status, larger proportion (72.1%) of the respondens was married while 9.3 percent were single, 3.3 percent were divorced. 11.3 percent were widows and 4.0 percent were widowers. This implies that married individuals dominated among the sampled farmers that participated in Fadama III Agricultural project. The plethora of married people has huge implication for family labour supply, [14]. Marriage predisposes an Individual to become more responsible than even being since they must cater for their family needs. The high percentage of the married individuals in Agriculture is consistent with [15] who reported that getting married is highly cherished among farming families in rural areas of Nigeria due to their relevance in boosting family labour supply. The bar chart representation of the percentage variations in the marital status of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 3 below.

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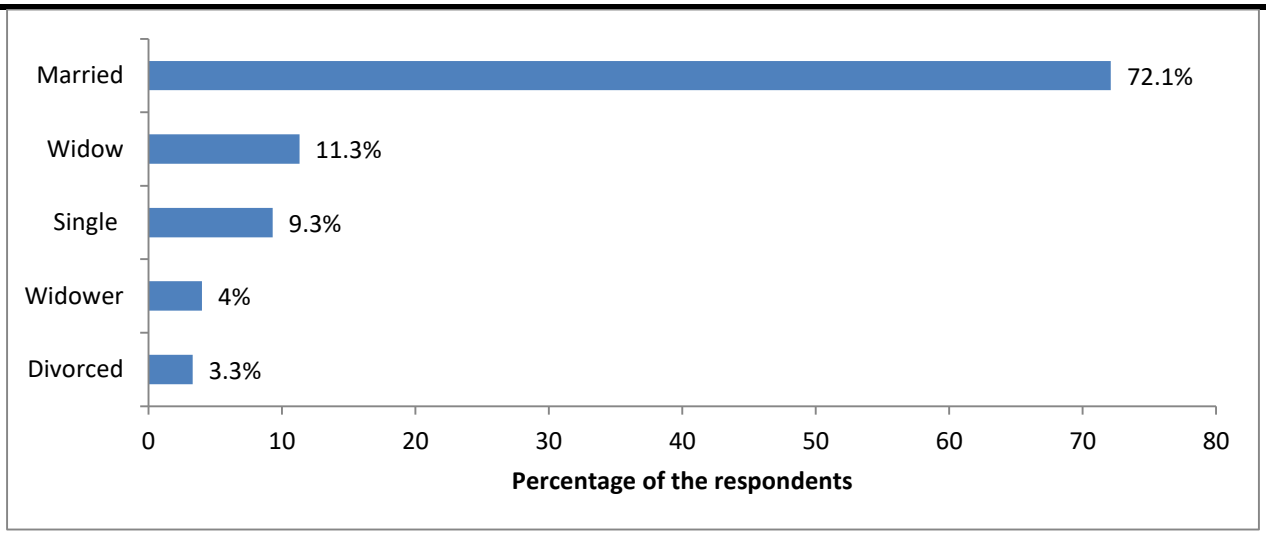

Fig. 3: bar chart representation of the percentage variations in the marital status of the sampled farmers in fadama iii agricultural projects in bayelsa state

4.1.4 Farm size

The result with respect to farm size showed a larger proportion of the respondents 78.7 percent had farmed sizes of at most one (1) hectare. This was followed by 10.7 percent of the respondents with farm sizes of at most two (2) hectares. The least proportion of the respondents 0.7 percent had farm sizes of at most five (5) hectares. The mean farm size of the respondents in the study area was 0.9 hectares. This implies that most of the farmers that participated in Fadama III Agricultural project in Bayelsa state were small scale farmers who are subsistent in nature. [16] noted that farmers that have small farm size produce for their family consumption. The implication of the finding shows that rural farmers had only little land to cultivate their arable crops because of the geographical location of their domain and this means that access to land is limited in the study area. This may be due to the scarcity of land and constant fragmentation of available land in the study area which was necessitated by constant oil spillage in most parts of the state. According to [17], the small farm sizes cultivated by farmers may be due to land fragmentation most common in rural areas and such smaller farm size would lead to smaller output and smaller income for the owner of such farms. The series of fragmentation of farm lands in the rural area is because most land is gotten from heritage [18]. This finding is consistent with [19] who succinctly observed that the size of farm cultivated is a function of population pressure, family size and financial capacity of the farmers; and with [20] who averred that the quantity of crops planted by a farm firm depends on the quantity of land available to it. The study is also consistent with [21], who asserted that limited access to land limits the size and scale of the farm business. The column chart representation of the percentage variations of farm sizes (hectares) of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 4 below.

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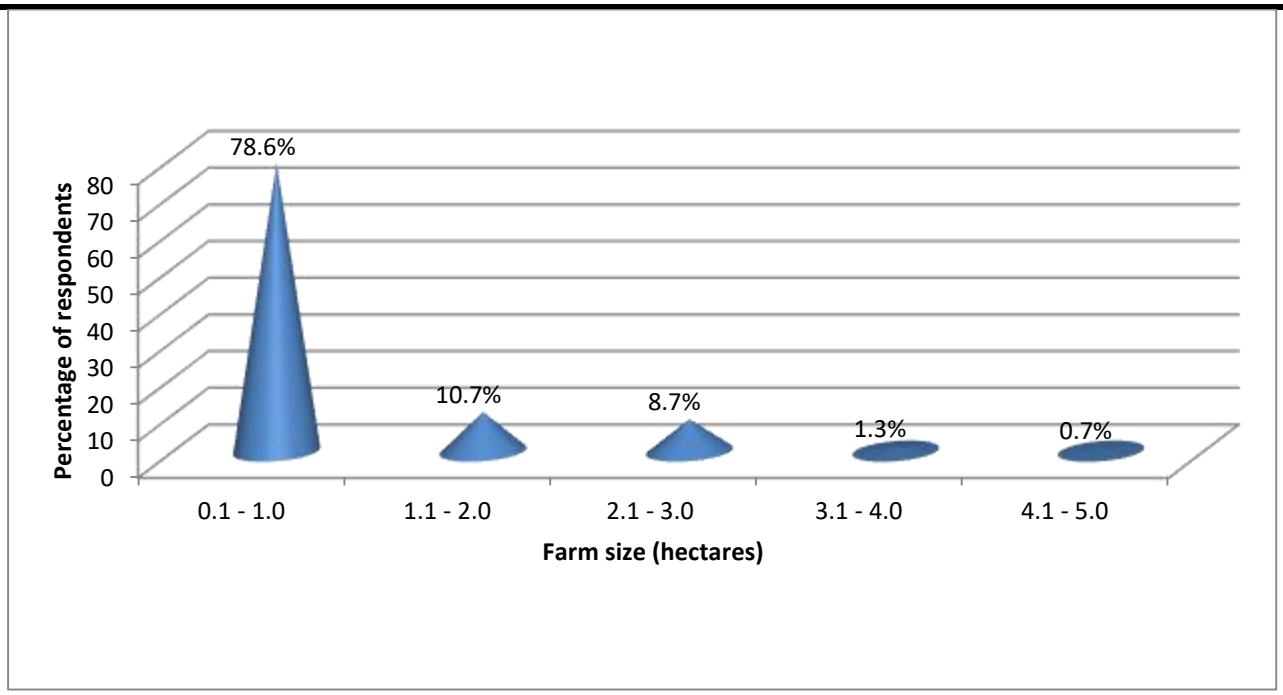

Fig. 4: column chart representation of the percentage variations of farm size of the sampled farmers in fadama iii agricultural projects in bayelsa state.

4.1.5 Household size

[22] Define household size as the number of people eating from one pot. It implies that the consumption unit is also the production unit. Family composition is an important variable in Agricultural production [23]. The finding shows that larger proportion (50.7 percent) of the farmers that participated in Fadama III Agricultural project in Bayelsa state had a household size of between 6 and 10 persons per household while a fewer proportion (17.3 percent) of them had a household size of at most 15 persons per household. The mean household size of the respondents was 7 persons per household. This implies that the farmers that participated in Fadama III Agricultural project in Bayelsa state had relatively large household size. This large household size may have positive implications for these rural farming households since it has been found that most rural households depend on their family members to provide labour on the farm [24] and [25]. The study is also in line with [26] who reported that farmers are committed in whatever they do because they have a large household size that depends on them for food, shelter and clothing. The larger the size of a household the more it could provide farm labour and the lower will be their expenses on hired labour. However, large household sizes have been noted to have correlation with food insecurity and poverty especially when the household head is engaged in Agriculture as the main source of livelihood and income, [27]. [28] Also assert that household size has a significant impact on aggregate food expenditure especially when there are more young children in the household who do not contribute to household income generation. The column chart representation of the percentage variations in the household size of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 5 below.

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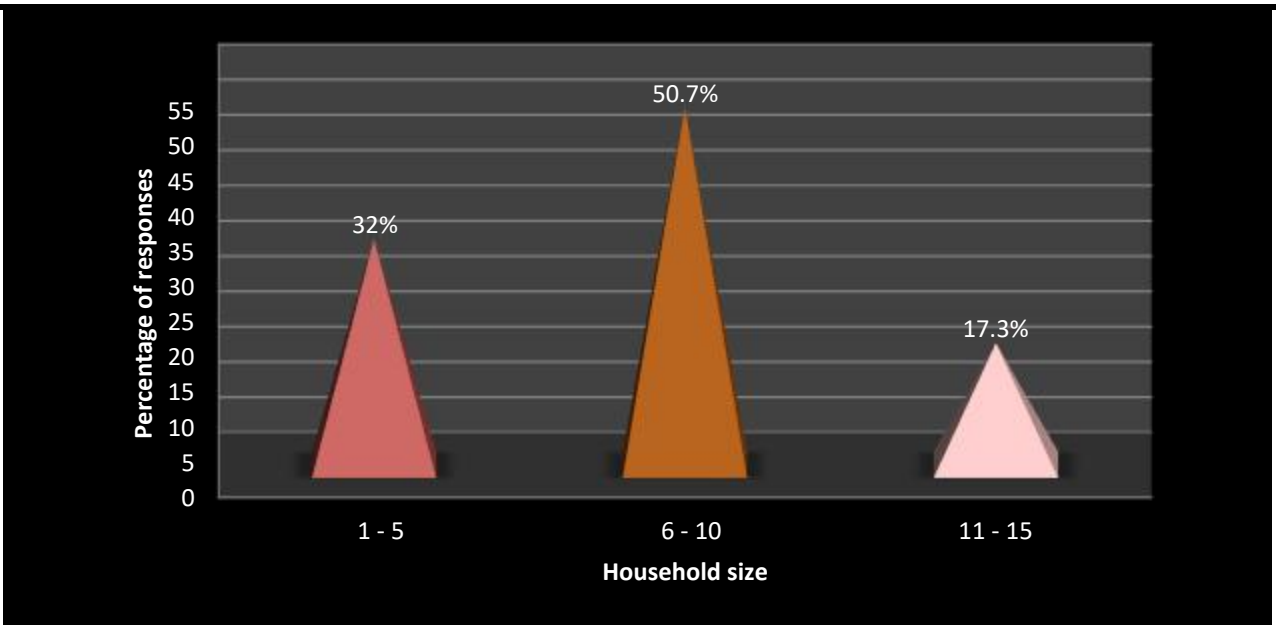

Fig. 5: column chart representation of the percentage variations in the household size of the sampled farmers in fadama iii agricultural project in bayelsa state.

4.1.6 Mode of farming involvement

The result for mode of farming involvement showed that larger proportion of the respondents (62.7 percent) of the sampled farmers for the study were into farming on a full-time basis while fewer proportion of the respondents (37.3%) were part-time farmers. This implies that most of the participants in Fadama III Agricultural projects in the study area are devoted farmers who take farming as their major business and means of generating income of their well being and survival. This is consistent with the findings of [14] who succinctly observed that full time farmers are more adoptive to new farming systems and new or improved innovations than part-time farmers since the former depend so much on the outcome of their farming activities for their survival. The pie chart representation of the percentage variations in the mode of farming involvement of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 6 below.

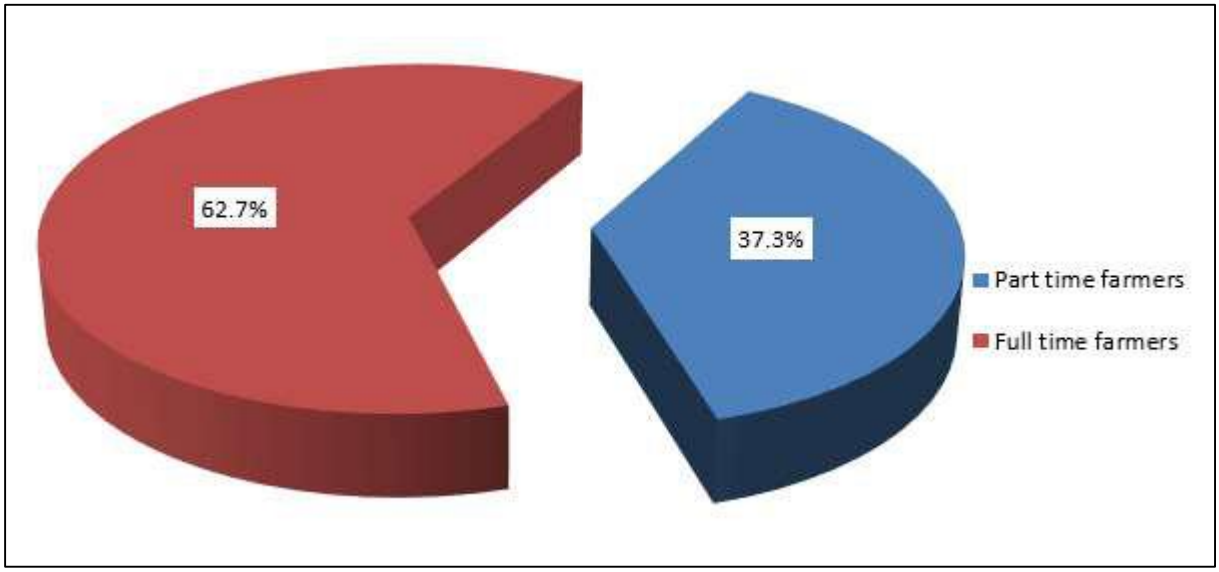

Fig. 6: Percentage variations in the mode of farming involvement of the sampled farmers in fadama III agricultural project in Bayelsa state

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1.7 Monthly estimated income from fadama (₦)

The distribution of the respondents by monthly estimated income from Fadama shown in the result revealed that larger proportions (57.3 percent) of the sampled farmers for the study earned at least ₦50, 000 per month from their farming activities while fewer proportions (4.7 percent) of them earned at most ₦10, 000 per month from their farming activities. The mean monthly estimated income from Fadama of the sampled farmers in the study area was ₦44, 133.83. This implies that the monthly income of the farmers in the study area is quite low and points to the fact that Fadama project in Bayelsa State may not have improved on the financial status of its participants so much as expected. [29] and [30] have noted that Fadama farming has led to increased productivity and output, and thus increased income among the participating farmers. The increased income provides more funds for capital investment especially since personal fund is a major source of credit for the respondents. It should also translate into better standard of living for this group of farmers because as noted by [31], there is an assumption by economists that a person with higher income is deemed to enjoy a higher living standard. This result agrees with the findings of [32] who reported higher farm incomes for Fadama beneficiaries than the non beneficiaries in their study areas. The assertion is in line with the studies of [33] and [34], whose study were conducted in Ogun and Gombe States respectively, and showed that Fadama project had no significant impact on participant's income, assets and/or poverty status. According to [35], income from farming activities is very low and there is general poverty amongst small holder farmers in Bayelsa state. The pie chart representation of the percentage variations in the monthly estimated income from Fadama of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 7 below.

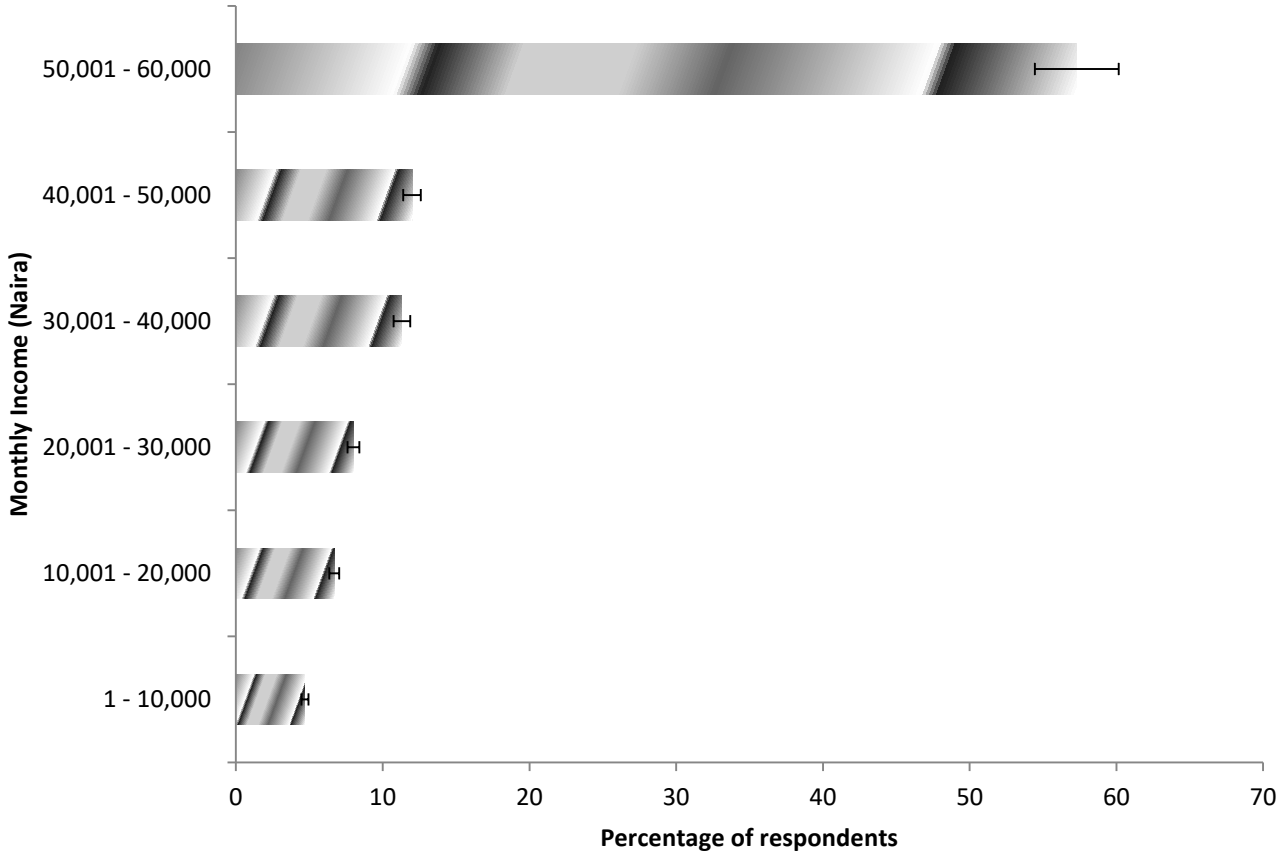

Fig.7: percentage variations in the monthly estimated income from fadama of the sampled farmers in fadama iii agricultural project in bayelsa state.

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4.1.8 Educational qualification

The distribution of respondents by educational status shows that larger proportions (67.3 percent) of the sampled respondents for the study had tertiary education while fewer proportions (2.7 percent) of them had no formal education. In all, 97.3 percent of the respondents had one form of formal education or another. This implies that most of the farmers that participated in Fadama III Agricultural projects were literate. The implication of this is that these households are better positioned to take advantage of new techniques and technologies that could lead to increased Agricultural output. This is imperative as it will enable the farmers to be able to understand and communicate basic principles guiding each of the activities made available to farmers through Fadama III Agricultural project and will also affect their performance. The finding was consistent with [36] and [37], noted that education will likely enhance the adoption of modern adaptation strategies, thereby sustaining a virile farming population. In the same vein, the finding was consistent with [22], who posited that education is important for socio – awareness, perception, reception and the adoption of innovation that can bring about increase in Agricultural production. The finding was consistent with [38] who posits that educated individuals and households are better positioned to take advantage of new farming techniques and technologies that could lead to increased Agricultural output. [39] noted that education exposes an individual to the right methods of utilizing resources. This is more so for the beneficiaries whose high educational status enhances their ability to understand and derive necessary benefits accruing from the project. [18] Also asserts that higher levels of literacy increase the ability of farmers to cope with the complexities of new technologies and the intricacies of new product and factor markets. [40] Posit that participants of Agricultural projects benefit more when they have basic education such that they can appreciate the importance of these projects and the benefits they would derive from them. The bar chart representation of the percentage variations in the level of education of the sampled farmers in Fadama III Agricultural project in Bayelsa state is presented in figure 8 below.

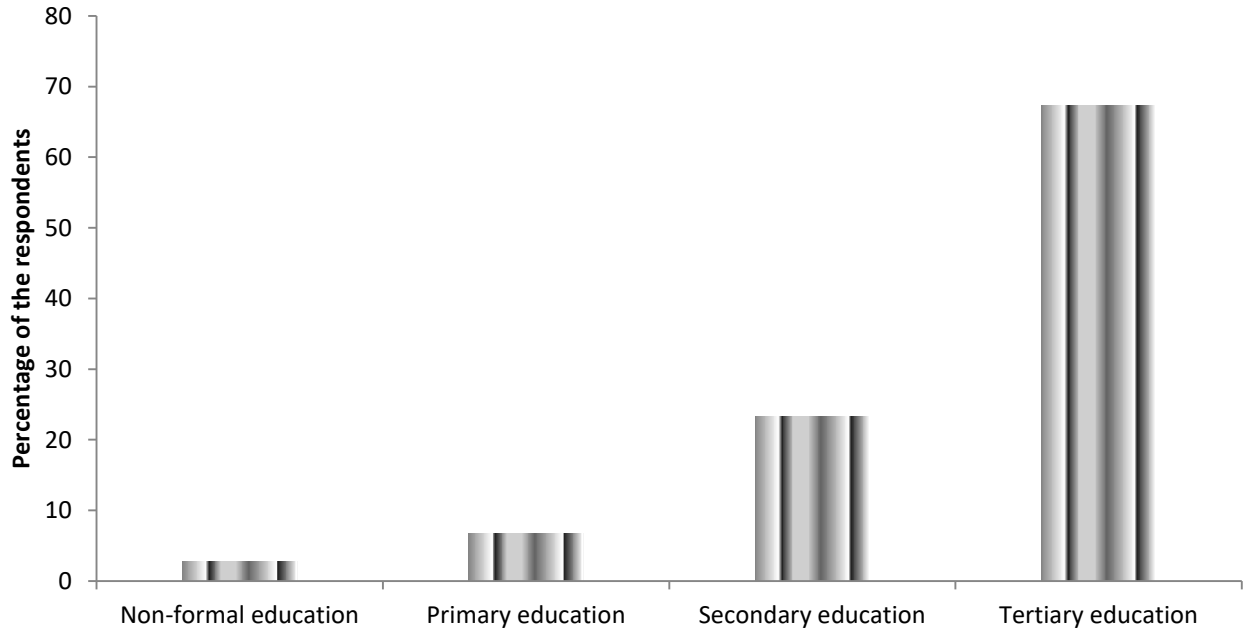

Fig. 8: percentage variations in the level of education of the sampled farmers in fadama iii agricultural projects in bayelsa state

4.1.9 Farming experience

The findings also showed that larger proportion (55.3% of farmers in Fadama III Agricultural project in the study area had farming experience of at least 11 years. The mean years of farming experience of the farmers was twelve (12) years and one (1) months. This implies that the farmers in Fadama III Agricultural project in Bayelsa state have been into farming for several years and may be considered quite experienced. As managers of the farm firm, farmers farming experience is an important factor for a successful farming business. Farming experience affects the income of farmers. This according to [25] may be due to the fact that farmers

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rely a lot on their farming experience for increased productivity. This study is consistent with [24] who agrees with this position, adding that the number of years a farmer has spent in the farming business may give an indication of practical knowledge he has acquired on how he could \overcome certain inherent farm production challenges. This study is consistent with [41] succinctly observed that farming experience enhances the participation and adoption of improved farming techniques, thereby increasing output. This study is also consistent with [4] who asserted that experience in a business would enable a business operator to set realistic cost and time targets, allocate and utilized resources efficiently and identify production risks. The bar chart representation of the percentage variations in the farming experience of the sampled farmers in Fadama III Agricultural projects in Bayelsa state is presented in figure 9 below.

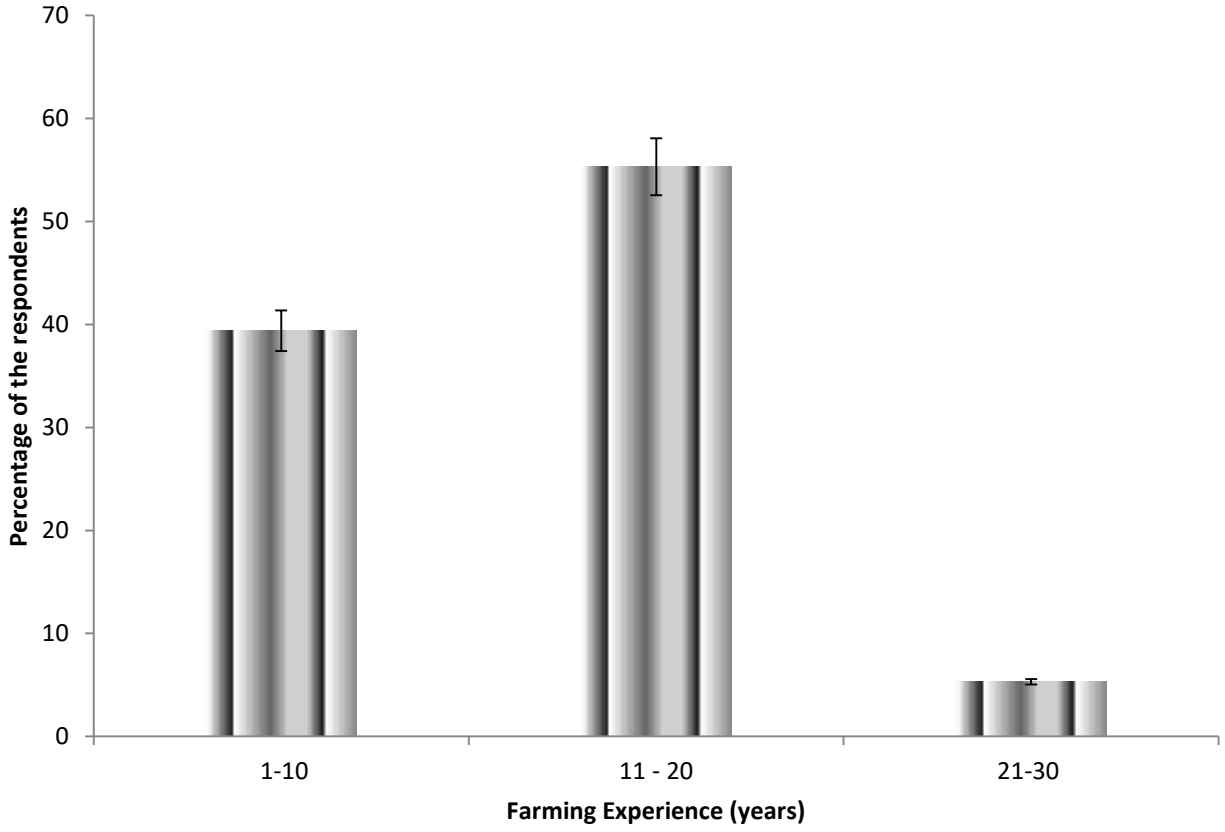

Fig. 9: percentage variations in the farming experience of the sampled farmers in fadama iii agricultural projects in bayelsa state.

4.1.10 Years of active participation

The findings showed that larger proportion (60.7 percent) of the sampled farmers in Fadama III Agricultural project in Bayelsa state have participated actively in Fadama III Agricultural project in Bayelsa state for at least three (3) years old while fewer proportion (39.3 percent) participated for at most two (2) years old. This implies that most of the participants in Fadama III Agricultural project in Bayelsa state have been in the project for long time and can to a high extent explain what is happening in the project in the state and how it has influenced their income status. The finding is consistent with [42] who posited that without participation; there are obviously no partnerships, no developments, and no program. Thus, no Agricultural project can successfully achieve its policy objective without active participation of the expected individual in such project. The pie chart representation of the percentage variations in the year of participation of the sampled farmers in Fadama III Agricultural project in Bayelsa state is presented in figure 10 below.

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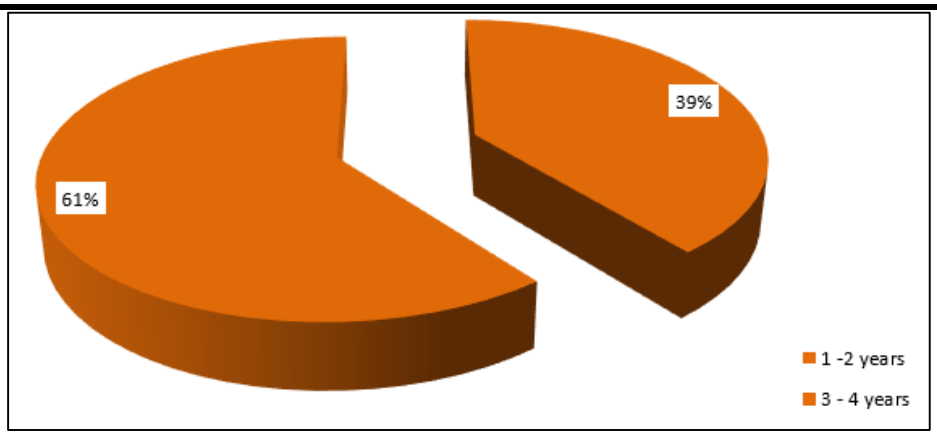

Fig. 10: percentage variations in the year of participation of the sampled farmers in fadama iii agricultural projects in bayelsa state

4.1.11 Fadama extension agent visit

The number of times extension agent visit participants of National Fadama III Agricultural project in Bayelsa State, larger proportion (38.7 percent) of the entire sampled farmers were visited by Extension Agent twice while fewer proportion (8.7 percent) was visited thrice. However, 53 participants which constitute 35.3 percent were not visited by Extension Agent(s) at all. This implies that extension visit to the farmers in the study area is very poor. Agricultural Extension services has been identified to be relevant in rapid increase in Agricultural production that aims to involve a shift from traditional resources based method to science based method which involves varieties of new cultural practices like use of fertilizer, organic manure, pesticides and capital investment inputs which farmers must learn how to use through the education role of extension workers [43]. Thus Agricultural Extension services aims at changing the rural people and train them to make independent decisions and make use of available local resource [44]. This suggested that the farmers in Fadama III Agricultural project were not receiving the needed encouragement from extension agents for their farming business and are not always communicated of new innovations and better farming system which may translate to higher output for the farmers. The bar chart representation of the percentage variations in the number of time extension agent visit participants of National Fadama III Agricultural project in Bayelsa State is presented in figure 11 below.

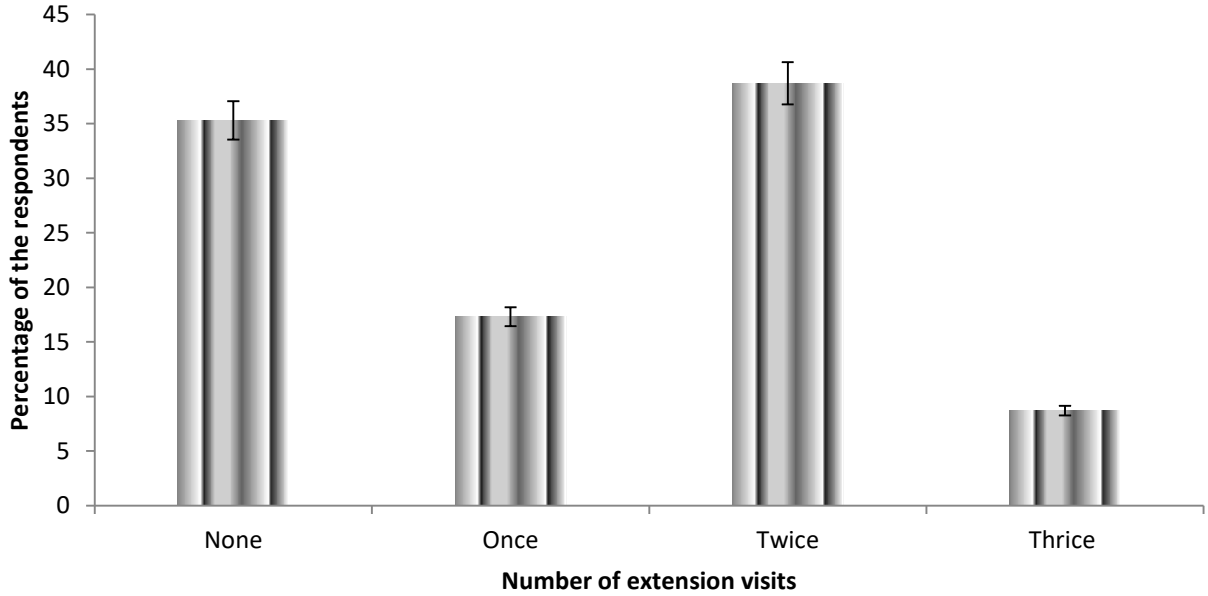

Fig. 11: number of time extension agent visit participants of national fadama iii agricultural project in bayelsa state.

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4.2.1 Factors that influence participants level of participation in fadama iii agricultural project in bayelsa state

The ordinary least square (OLS) regression result of the factors that influenced participant's level of participation in Fadama III agricultural project in the study area is presented in table 1.

Table 1: Regression Result of the Factors that Influenced Participant's Level of Participation in Fadama III Agricultural Project in Bayelsa State

Variable	Linear	Exponential	Double-log^{L}	Semi-log
Constant	732.517	6.881	27.908	23509.001
	(8.184)***	(23.775)***	(6.297)***	(6.591)***
Age of respondent	6.788	0.019	-1.753	2278.497
	(8.399)***	(7.409)***	(-4.497)***	(7.656)***
Gender	7.037	0.032	-0.176	110.088
	(0.698)	(0.981)	(-0.670)	(0.007)
Food security status	1.197	-1.02E-05	-2.797	-125.879
	(0.736)	(-0.304)	(-3.358)***	(-2.598)**
Household size	2.434	0.007	1.227	341.971
	(1.138)	(0.972)	(2.358)**	(0.821)
Income level	-6.287	0.001	1.249	-242.530
	(-5.216)***	(2.337)**	(3.713)***	(-0.427)
Poverty status	6.497	1.024	-2.255	614.087
	(3.319)***	(3.753)***	(-3.375)***	(2.410)**
Marital status	4.001	-1.08E-06	-0.147	171.187
	(4.408)***	(0.874)	(-1.313)	(0.185)
Farm size	1.712	-0.028	0.068	-213.337
	(1.121)	(1.865)*	(0.918)	(-1.931)*
Leadership propensity	65.938	0.353	1.887	109.409
	(3.656)**	(4.062)***	(2.460)**	(6.410)***
Farming experience	2.005	1.01E-05	1.029	-205.082
	(1.466)***	(2.409)**	(3.071)***	(-3.106)***
Educational level	6.788	0.019	1.413	2278.497
	(8.399)***	(7.409)***	(3.914)***	(7.656)***
Distance to Fadama III	-4.250	-0.068	-1.793	-166.542
training centre	(-3.042)***	(-2.729)***	(-2.393)**	(-3.720)***
Cooperative membership	0.118	0.017	0.049	1320.227
	(1.520)	(0.732)	(1.065)	(4.396)***
R^2	0.928	0.910	0.938	0.892
Adj.R^2	0.904	0.899	0.917	0.871
F-statistics	101.980***	98.341***	114.841***	88.568***

Source: Computed by the author from field survey data, 2016

*** Significant at 1%; ** Significant at 5% and * Significant at 10%. Figures in parenthesis are t-values. L= means lead equation.

The double-log functional form was chosen as the lead equation based on the number of significant independent variable, magnitude of the coefficient of multiple determinations and conformity of the signs of the significant regression coefficient to *a priori* expectation. The overall goodness of fit of the equation as indicated by the coefficients of multiple determinations (R^2 = 0.938) indicates that the explanatory variables included in the model explained about 93.8% of the variation in the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. The F- statistics of the lead model

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was significant at 1% and confirms the significance of the entire model.

Age of respondents, food security status, household size, and income level, and poverty status, leadership propensity, farming experience, educational level and distance to Fadama III training centre were the significant factors that influenced the level of participation of the respondents in Fadama III agricultural project in Bayelsa state.

The regression coefficient for age of respondents was negative and significant at 1% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This indicated an inverse relationship between level of participation and their age in years. This implies that the level of participation in Fadama III agricultural project in Bayelsa state decreases with increases in the age of the respondents. This suggests that younger farmers are more willing to participate in Fadama III agricultural project than their older counterparts. Therefore, level of participation in Fadama III agricultural project in Bayelsa state by the respondents is age dependent. This finding is in consonance with [45] who stated that younger farmers tend to be more willing to participate in agricultural development projects than their older counterparts. This result also agrees with the findings of [46]; [14]; [47] whose studies shows the dominance of middle aged farmers in agricultural activities and/or projects.

The regression coefficient for food security status of the respondents was negative and significant at 1% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This indicated an inverse relationship between level of participation and food security status of the respondents. This implies that the level of participation in Fadama III agricultural project in Bayelsa state increases with the respondents being food insecure (below the food security line). Food insecurity will cause most persons to participate more in any programme that will help to become food secured. The interest in getting over one's current food insecurity status will compel an individual to put in his/her best commitment to such programme so as to be well empowered to combat the menace of food insecurity. Therefore, level of participation by the respondents is dependent on their food security status. This assertion was supported by [38] who noted that the involvement of many farmers in Imo state in Fadama II Agricultural project was to improve upon their standard of living and food security through Agricultural commercialization; and by [48] whose report showed that for the sake of attaining food security, many farmers from developing economies participates in agricultural development projects and schemes.

The regression coefficient for household size was positive and significant at 5% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This indicated a direct relationship between level of participation and their household size. This implies that the level of participation in Fadama III agricultural project in Bayelsa state increases with increases in the household size of the respondents. This suggests that farmers with large household members participate in Fadama III agricultural project than those with small household members. Therefore, level of participation in Fadama III agricultural project in Bayelsa state by the respondents is dependent on their household size. This finding is in consonance with [38] and [49] whose studies shows that farmers with large household size participates more in agricultural projects that will enable them purvey their family needs.

The regression coefficient income level of the farmers was positive and significant at 1% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This indicated a direct relationship between level of participation by the respondents and their income level. This implies that the level of participation in Fadama III agricultural project in Bayelsa state increases with increases in the income level of the respondents. Level of income affects the standard of living of a farmer, as well as hinders the ability of a farmer to expand his/her farming business away from subsistence level. Farmers will be more willing to participate in Fadama project if the project promises to positively impact economically on their level of income [32]. This suggests that farmers whose income level improves due to participation in Fadama III agricultural projects will continue to participate in such agricultural projects. Therefore, level of participation in Fadama III agricultural project in Bayelsa state by the respondents is dependent on their level of income. This finding is in consonance with [38]; [49],[29] and [30] whose studies revealed that farmer's higher income level from agricultural projects motivates their continuous participation in agricultural projects.

The regression coefficient for poverty status of the respondents was negative and significant at 1% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This indicated an inverse relationship between level of participation and poverty status of the respondents. This implies that the level of participation in Fadama III agricultural project in Bayelsa

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state increases with the respondents being poor (where the mean household expenditure was used as poverty line). This suggests that farmers who are poor will strive to participate in Fadama III agricultural projects in lieu to better their poverty status. High level of poverty will influence a farming household to participate actively in National Fadama III Agricultural project as a means of poverty alleviation strategy. However, a farmer will not participate actively if there is no improvement in his/her poverty status after participating at certain level in the project. Level of participation in Fadama III agricultural project in Bayelsa state by the respondents is dependent on their poverty status. This finding is in line with [50], [38]; [51] and [52] showed that poor farmers mostly participated in Fadama projects in Nigeria.

The regression coefficient for leadership propensity of the Fadama III agricultural project agents was positive and significant at 5% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This indicated a direct relationship between level of participation by the respondents and leadership propensity of the Fadama III agricultural project agents. This implies that supportive leadership propensity of the Fadama III agricultural project agent's leads to more participation in Fadama III agricultural project in Bayelsa state by the respondents. Leadership propensity and time management in Fadama III Agricultural projects will positively influence the level of participation of farmers by making them to get committed more than usual in the projects and will also reflect in the rate of adoption of new farming technologies showcase by the project by the farmers. Therefore, level of participation of farmers in Fadama III agricultural project is dependent on the nature of the leadership propensity of the delivery agency.

The regression coefficient for farming experience was positive and significant at 1% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This implies that the level of participation in Fadama III agricultural project in Bayelsa state increases with increases in the farming experience of the farmers. This suggests that experienced farmers are more willing to participate in Fadama III agricultural project due to the benefits the derived from such agricultural projects than their inexperience counterparts. Therefore, level of participation in Fadama III agricultural project in Bayelsa state by the respondents is farming experience dependent. This finding is in consonance with [46]; [53]; [47]; and [52] whose studies shows the dominance of long experienced farmers in agricultural activities and/or projects than beginners in farming activities.

The regression coefficient for educational level of the farmers was positive and significant at 1% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This implies that the level of participation in Fadama III agricultural project in Bayelsa state increases with increases in the educational level of the farmers. This suggests that well educated farmers are more willing to participate in Fadama III agricultural project due to the benefits the derived from such agricultural projects than those with low level of education. Therefore, level of participation in Fadama III agricultural project in Bayelsa state by the respondents is dependent on the educational level of the farmers. This finding is in consonance with [53], [47], [52], and [51] whose studies shows the dominance of educated farmers in agricultural activities and/or projects in their various study areas.

The regression coefficient for distance to Fadama III agricultural project training centre was negative and significant at 5% as it relates to the level of participation of the respondents in Fadama III agricultural project in Bayelsa state. This implies that the level of participation in Fadama III agricultural project in Bayelsa state decreases with increases in the distance to Fadama III agricultural project training centre. This suggests that farmers are more willing to participate in Fadama III agricultural project with training centre closer to them than when the training centre is far and costs those more to transport themselves to the centre. Therefore, level of participation in Fadama III agricultural project in Bayelsa state by the respondents is dependent on the distance to Fadama III agricultural project training centre.

V. CONCLUSION AND RECOMMENDATIONS

Agricultural and rural development programs such as fadama III and others are fundamental to nation building and the agricultural sector is what can fast track the challenges faced by rural dwellers in terms of agricultural development in most of the developing countries, participants participated and are still willing to participate in such activities irrespective of their socioeconomic characteristics.

The study therefore recommend's that participants of younger age especially the youths should be encouraged in agricultural and rural development programs as age determines one's ability and willingness to participate in such programs effectively, also, programs of this nature

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should be of continues basis in other to overcome problems of food security in Nigeria and other developing countries.

Also, funds that are meant for agricultural projects should be properly disbursed to farmers on time to enable them participate actively as farmer's income levels motivate them to participate in agricultural and rural development programs. Scientist/extension training personnel's should frequently visit farmers to know their immediate challenges in time of program life cycle, this will always motivate participants to be more focused while participating and will make them adopt any new technology easily been made available for them.

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Imposing Criminal Liability on Government officials for Haze in South Kalimantan, Indonesia

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***Abstract*— *The background of this research is based on the Haze that happens almost every year in South Kalimantan. Society are questioning the Government's Responsibility of this annual disaster in South Kalimantan. The purpose of this research is to analyze the reasons underlying the possibility to Impose Criminal Liability for Haze in South Kalimantan to the Government Officials, and to describe the form of criminal liability of the Government as environmental policy stakeholders on haze in South Kalimantan. This research is a normative legal research using statute approach and conceptual approach. The results of this study are: **First**, as a legal/law subject, the Government of South Kalimantan Province also has rights and obligations. Therefore, the Government is classified as a legal/law subject that can also be asked for its criminal liability. In addition, the negligence of the Government in South Kalimantan related to Haze could be criminalized based on the criminalization theory. **Second,** according to the Act Number 32/2009 on the Environmental Protection and Management, it has not been regulated about criminal sanction for the negligent government which resulted a repeated environmental pollution that harms the society. Thus, if then formulated in the Act Number 32/2009 on Environmental Protection and Management, then a criminal act that can be imposed is a material crime committed by the Government in the form of an impure passive criminal action with imprisonment and fine as sanction.*

Keywords*— *Criminal Liability, Haze, Criminalization, Environment.

I. INTRODUCTION

A. Background

A good and healthy environment is the basic right of every Indonesian citizen. It is mandated in the Constitution of the Republic of Indonesia Year 1945, in Article 28H Paragraph (1) which states; *"Everyone has the right to live a prosperous and spiritual life, to live, and to get a good and healthy environment and have the right to receive health services".*

Thus the importance of the role of the State in this matter is represented by the Government to maintain and ensure that the environment remains good and sustainable. The developed countries incorporated in The Organization for Economic Cooperation and Development (COECD) also gave a big focus for the development of institutional authority of environmental management. It stated that "*institutional frame work is an interest part of the environmental management system. It facilitates and supports the environment policy making process, and ensures the implementation and enforcement of policies ".*[1] It can be concluded that the environmental management system can facilitate and support the formulation of environmental policies and ensure the implementation and enforcement of the policy.

Nowadays, most countries in the world have poured environmental policies in the constitution, albeit to varying degrees. In 2004-2005 over 100 countries have contained environmental provisions in the Constitution. [2] In this case, Indonesia is also one of the countries in the world that have incorporated human rights related to the good environment in the Indonesian constitution.

The concept of an ideal environmental directs these changes toward the improvement of the quality of the system. Such changes are in addition important to maintain the stability of the system process, in a practical order as well as to maintain the stability of life as well as its sustainability. Changes in the quality of ecosystem components, such as air, water, soil,

[1] Magda Lovei and Charless Weiss, Jr, *Environmental Management and Institution in COECD Countries: Lessons (*Washington DC *: World Bank Techinique paper* No 391, 1998) , 31-32

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forests etc. individually / group will degenerate the quality of the environment, the life process and ultimately life itself. [3]

In everyday life, many of us encounter legal cases related to the environment either licensing issues, waste management, or environmental pollution due to certain efforts. As Findley states in his Environmental Law; "*Almost all environment litigation involves disputes between government parties, rather than disputes between private parties, such litigation typically takes one of two persons*".[4] Which means, it is related to the issue of environmental law disputes always involve the government, rather than with the private sectors

According Koesasi Hardjosoemantri, the material of the field of the environment is very broad that concerns on the terms of space to the bowels of the earth and the seabed, and includes human resources, biological resources, non-physical resources, artificial resources, it is not possible that all the material is regulated completely in a single law.[5] In Indonesia, many legal instruments related to the management and conservation of the environment. And the latest as the revision of the previous act is The Act Number 32 Year 2009 on The Environmental Protection and Management.

From many environmental law cases, the author is interested in researching the haze occurring annually in South Kalimantan.

Data from LAPAN (National Aeronautics and Space Agency) and National Disaster Management Agency (BNPB) mentioned that 148,194 hectares of land in South Kalimantan has burned. A total of 18,665 hectares of burned land is peatland. While Walhi (The Indonesian Spacecraft Environment) monitors in 2015 there are 2.418 hotspots in South Kalimantan. A total of 1,830 hotspots were located in the peat swamp area. A total of 771 hotspots are located in the permit area of the plantation company. According to Walhi Kalsel, there are 12 companies whose territory suffered land fires. Uniquely, some oil palm plantation companies that have seized and damaged peat swamps in South Kalimantan are untouched by the law enforcement. The latest is the ruling of the Rantau District Court released by the Supreme Court on July 28th, 2016. Rantau District Court acquitted PT Platindo Agro Subur (PAS) from accusation for committed the environmental crimes. According to Walhi Kalsel analysis, the destruction of peat swamp in Banua is supported by regulation such as South Kalimantan RTRWP Number 9 / Year 2015 paragraph (1) letter (b) stating that about 1,255,721 hectares is for plantations spread over five districts which are also national peat swamp areas Prone to fire. South Kalimantan provincial government should change the existing governance model of development into a policy that interests the people, guarding the landscape of South Kalimantan by no longer giving permission for coal mining activities and oil palm plantations. [6]

Haze in South Kalimantan is a form of smog contaminated the environment. According to The Act Number 32 of 2009 on The Environmental Protection and Management Article 1 number (14) "*Environmental pollution is the entry or inclusion of living things, substances, energies, and / or other components into the environment by human activities so that it exceeds the quality standard of the environment. "*

The haze that occurred in South Kalimantan has occurred since the 1990s until 2015. As a result of the haze, South Kalimantan Provincial Health Office noted, people with Upper Respiratory Tract Infection (ISPA) in South Kalimantan is already at the level of dangerous. The city of Banjarmasin is the most common case of ARI. In the period January - September 2015, people with ISPA in South Kalimantan as many as 289,334 inhabitants. Banjarmasin contributed 73,693 people, Banjar District 37,020 people, and Barito Kuala District 32,656 inhabitants. Children under five who suffered from ISPA were 125,942 people and more than 5 years old were 163,392 people. Meanwhile, when compared with the number of patients with ISPA in 2014, it is still below it, which is 404,863 inhabitants. [7]

According to Nugroho, haze occurs because of the primary air pollutant, the air pollutant component covers 90% of the total air pollutant components. The shape and composition are similar to those emitted, for example Carbon Monoxide (CO), Nitrogen Oxide (NO), Hydrocarbons (HC), Sulfur Dioxide (SO), as well as various particles. The toxicity of the five groups of pollutants varies. The most harmful pollutants for health are the particles, followed respectively by NO, SO, Hydrocarbons and the lowest toxicity is CO. [8]

When tracing the cause of the haze, More than 50 percent of the smog is caused by the negligence of palm oil and rubber plantation companies. A total of 2,682 hectares of burning land is the area of oil palm and rubber plantations, of a total of 5,622 hectares of land fires. Apparently there are two

[3] Ida Bagus, *The Institutional Law of Environment* (*B*andung:Rafika Aditama., 2003) 3

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areas that become the main source of this haze. These areas are Tapin and Banjar Subdistrict. These two areas have large amounts of palm and rubber plantations, and due to the company's negligence caused 2,682 hectares of burning land. As a result of a thick haze occurs in South Kalimantan. Until now the efforts that can be done by the government is limited to efforts to extinguish in the area of land and forest fires. The efforts taken include bringing a water bombing helicopter from Russia, with pilots and a technician also from the country was named the Soviet Union. Artificial rain was not effective because several times goes to the wrong target. So, It cannot dispel the land and forest fires quickly. [9]

In 2016, South Kalimantan Governor, H Sahbirin Noor began to take a stand in preventing and responding to the haze disaster in South Kalimantan. The Governor focuses on the prevention of haze which pays attention to mobilize all elements such as society and government agencies, private sector including Indonesian National Army and Indonesian Republic Police in various action activities of environment care. The real action in rescuing the environment due to the haze is also a follow-up of President Joko Widodo's direct order of the joint movement to tackle the smog. If the year 2015 and the spreading point of fire according to the Regional Disaster Management Agency (BPBD) of South Kalimantan Province as many as 1,291 hotspots, then in 2016 successfully derived and only recorded 56 hotspots. Another factor in the reduction of fires in South Kalimantan throughout 2016 is also due to the factor of normal weather relatively, in addition the increased of public awareness and measured prevention socialization.[10]

The interesting point to be examined here is on whether or not the Government is accountable for the haze that occurs annually in South Kalimantan according to criminal law. If we observe that civic responsibility has become common thing in cases of environmental pollution when it is associated with the Government. As an example of the decision from the Palangkaraya District Court Number 118 / Pdt.G / LH / 2016 / PN-Plk which provides decisions related to the class action of the public against the Government including the President of the Republic of Indonesia. The authors wish to examine further whether in this case the government can also be held criminally liable in connection with the issue of environmental pollution in this case haze in South Kalimantan. The civil responsibility of the Government has become common thing in environmental cases, but related to criminal liability, it should be explored more deeply.

According to the description of the case above, the authors are interested to conduct a research related to how the Government criminal liability of the haze that occurred in South Kalimantan is.

B. Research Questions

The problems were studied in this paper are as follows:

1. What is the underlying reason that the Government is punishable for the Haze occurs in South Kalimantan according to The Act Number 32 Year 2009 on The Environmental Protection and Management?
2. What is the form of criminal liability for the Government as an environmental policy stakeholder over haze in South Kalimantan?

C. Resarch Methods

The type of research used in this study is the normative legal research which collecting and analyzing the legal materials related to the problems studied. According to Peter Mahmud Marzuki legal research is a process to find the rule of law, legal principles, and legal doctrines in order to answer the legal issues faced. This corresponds to the prescriptive character of jurisprudence. Legal research is conducted to generate arguments, theories or new concepts as prescriptions in solving the problems encountered. In legal research is rigth, appropriate, inappropriate, or wrong. It can thus be said that the results obtained in legal research already contain values.

This study uses statutory approach (approach statute) and conceptual approach. The legislation approach is used to analyze how the policy in The Act Number 32 Year 2009 on Environmental Protection and Management relates to the extent of government responsibility in pollution and environmental damage. While to examine the rationale of imposing the government for a criminal liability on haze in South Kalimantan is using the conceptual approach.

This study uses a prescriptive analysis. The nature of prescriptions in the field of legal scholarship is trying to study and seek answers about what should be of every problem. In this case It will be examined to what extent the government accountability of haze in South Kalimantan. Prescriptive analysis can also be interpreted as a research

[9] "Oil Palm Company Causes the Haze", 2014, Accessed July 10th 2017, http://www.beritabanjarmasin.com/2014/12/perusahaan-sawit-dalang-kabut-asap.html

[10] "South Kalimantan Overcome the Haze Succesfully", 2016, Accessed July 10th 2017 http://kalsel.antaranews.com/berita/43050/kalsel-sukses-tanggulangi-kabut-asap

aimed to obtain suggestions as to what should be done to solve a particular problem. [11]

The legal material used is Primary Legal Material. Covering the 1945 Constitution of the State of the Republic of Indonesia, The Act Number 32 Year 2009 on Environmental Protection and Management. The legal materials are all publications about law that are not official documents. Legal publications include textbooks, legal dictionaries, legal journals, and comments on court decisions. [12]

The method of analysis used is the method of interpretation with reference to the theories used. The interpretive method used is grammatical interpretation[13] (interpretation by language). Language interpretation is used because a sentence in the text of the Act plays an important role in determining the meaning of a Undnag-Unndag provision. In this case, the provisions in Law Number 32 Year 2009 on Environmental Protection and Management will be explained according to everyday common language.

II. LITERATURE REVIEW

A. The Criminal Liability on Environmental Law

Criminal law is essentially a law followed by sanction whose function is to regulate and establish public order in society, ensuring state security and safety. Criminal law is a means of coercion to protect citizens against harmful acts or that cause suffering on the other side. (In this case environmental pollution). [14]

The term environmental law is a novel conception of law, it grows in tandem along with the growing awareness of the environment. With the growth of understanding and awareness to protect and preserve this environment then grow legal attention to it. Thus, It grows and develops of a branch of law called as the environmental law. [15]

The pure environmental law is that an administrative law which has the support of civil and criminal law. Therefore, in the world of law, environmental law has a complex problems that sometimes must be solved not only in one legal domain only.

In Indonesia, The Act Number 32 Year 2009 as the main provision accommodates environmental policy in Indonesia. The responsibility for environmental management rests with the government in the sense that it is not submitted to individual citizens or become civil law. The responsibility of environmental management lies with the government which has consequences for the institution and authority for the government to manage the environment[16]

As an administrative law with its instrumental character, the prominent function in administrative environmental law is preventive form of pollution and / or environmental damage. [17]

The application of criminal law in environmental cases must be addressed carefully. Van De Bunt in his paper at a meeting of environmental law associations in the Netherlands argued that there were some signs in choosing the application of administrative instruments and criminal law instruments or both at once with several criteria. The criteria are: [18]

a The Normative Criteria holds that criminal law can only be applied to offenses which have very negative and very socially discouraging negative ethical values.
b Instrumental Criteria that are pragmatic more oriented to the detention of suspects, or the restoration of the situation or the repair of damage. In this case, the administrative instrument is better applied. However, if it is felt that the application of administrative law will go through a very long procedure, then applying the penal law will be better.
c Opportunistic criteria may be included if the application of administrative instruments cannot work, for example administrative coercion or forced money (*dwangsom*) cannot be imposed because the violator has been bankrupt, then it is better to apply a criminal law instrument.

In the opinion of Andi Hamzah, the criminal law is as *ultimum remedium*, means there are three kinds, the first is the criminal law as *ultimum remedium* because the application of the criminal law can only be done against people who violate the heavy law ethically, the second is the criminal law as *ultimum Remedium* because criminal penalties are heavier and tougher than other sanctions, and the third is criminal law as an ultimum remedium because the administrative official knows in advance that the offense is actually prioritized in taking steps and actions rather than law enforcement. [19]

The formulation of environmental offenses is always associated with criminal sanctions (threats), because theoretically this criminal sanction aims to run the norms of environmental law. This criminal sanction arose in response

[11] Soerjono Soekanto, *The Introduction to the Legal Research,* (Jakarta: UI Press, 2012) 10
[12] Soekanto, *Legal Research,* 10
[13] Sudikno Mertokusumo, *The Invention of Law, (Y*ogyakarta: Liberty, 2007) 56
[14] Ninien Suparni, *The Conservation, Management, and Law Enforcement of Environmental Law*, (Jakarta: Sinar Grafika, 1994) 191
[15] M. Hadin Muhjad, The *Introduction to the Environmental Law in Indonesia*, *(*Yogyakarta: Genta Publishing, 2015) 1
[16] Hadin, *The Introduction*, 36
[17] Hadin, *The Introduction*, 36
[18] Hadin, *The Introduction*, 215
[19] Hadin, *The Introduction*, 222-223

to disobedience to environmental law norms, as Gustaaf Biezeveld puts it in his Criminal Enforcement of Environmental Law: General Introduction, Investigation, and Prosecution. [20]

Criminal law enforcement in environmental cases cannot be regarded as the final law in an environmental case if other areas of the law cannot solve it, since this criminal law only resolves unilaterally yet reaches the affected party / affected group in the form of recovery to its original state.

The criminal law does not stand alone as an instrument of law enforcement. It depends on the administrative law imposed by administrative officials. No criminal law will be imposed if an irreparable or recoverable damage, such as tree felling or the killing of protected animals / birds, including irreparability. The repair or recovery of the problem cannot be done physically. [21]

In a foreign language, criminal liability is referred to as "*Toerekenbaarheid"*, "criminal responbility", "criminal liability". That criminal responsibility is intended to determine whether a suspect / defendant is held liable for a crime committed or not. If he is convicted, it should be found that the act is unlawful and the defendant is responsible. This capability shows the errors of a deliberate act or *omission*. This means that the actions are reprehensible accused of aware of the actions taken. [22]

A person who commits a crime, is not always punishable. It depends on whether the person in doing the crime has an error or not. Because to be able to impose a criminal against a person is not enough with only a criminal act, but other than that there must also be a mistake or according to Moeljatno disgraceful attitude of heart. Who makes mistakes, then he is responsible. In this case is known as a principle "no crime without error" (*qeen straf zonder shuld*). [23]

In the idea of *mens rea*, it must be proved first the inner attitude or the mental ability of the perpetrator whether the perpetrator committed a criminal act in the form of intentional or negligent. The difference between intentional and negligent is the deliberate attitude of one's inner self is violated, whereas in the neglect of the attitude of this person only ignores the prohibition of the law so as not to be careful in doing an act that leads to prohibited circumstances.[24] As a form of error in criminal law, they differ only "graduil" or only in quality. [25]

There is a passive criminal act called *delicta omissionis*. The omissionist offender (*delicta omissionis*) is divided into actual (pure) omission deliberations, commonly called *delicta omissionis* and impure omissionist offenses, often called *delicta commissists per omissionem commissa*. Delicta omissionis (delict omissionis pure), is the offense, criminal offense or criminal acts that is formulated by the lawmakers that can only be realized by passive action, do not do or ignore the legal obligation, where he should be actively do so . While the pure omission offense, commonly called *oneigenlijke omissidelicten* or delicta commissionis per omissionem commissa, is a delict that can be realized by active deeds or passive deeds in other words can occur due to deed (handeling) or neglect (nalaten). [26]

Van Hamel states that: a person who does not act, he can not be considered to cause a result, if he has no legal obligation to do (*als de dader de rechtsplicht heft om te doen*). The legal obligation of a person who at a certain time and circumstances is required by law must do so. If by law a person is obliged to do, and then he does not do that cause a consequence, then the cause of that result lies in the possession of that legal obligation[27].

B. The Theory of Identification *(Alter Ego Theory)*

According to E. Utrecht, a legal entity (*rechtpersoon*), is a body which, according to the law of authority, becomes the supporter of rights, it is further explained that the legal entity is any supporter of the right that is not soulless or more precisely non-human. [28]

Legal entities consist of public legal entities and private legal entities. Private legal entities consist of Limited Liability Company (PT), Foundation and Cooperative. While public legal entities may take the form of State, Province, Regency, State Institution, State Bank, etc. [29]

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[20] Dr. Muhammad Akib, *Environmental Law on Global and National Persepctive,* (Jakarta:Raja Grafindo, 2014)

[21] Prof. Dr. Jur. Andi Hamzah, *The Environmental Law Enforcement*, (Jakarta: Sinar Grafika, 2005)

[22] Roeslan Saleh, *The Main Idea of Criminal Liability*, *(*Jakarta: Ghalia Indonesia, 1982) 250

[23] Moeljatno, *The Principle of Criminal Law*, *(*Jakarta: PT Rineka Cipta, 1993) 153

[24] In general, jurists agree that there is no substantial difference between "intentional" and "negligence". Both show the inner connection between the "actor" and his "deed" in such a way that it can cause "reproach". *The Basics of Indonesian Criminal Law in The Reformation of Law Perspective,* (Malang: UMM Press, 2012) 244

[25] Theoretically, if the form of error is sorted from the form of the most severe quality errors to the form of error of the lightest quality, then the hierarchy of qualitative order of the form of error is as follows: 1). The deliberate as a purpose, 2). Purpose as a certainty, 3). Deliberation as a possibility, 4). A conscious omission, and 5). Unconscious neglect. It is also stated that between intent and omission is very close, especially Deliberate as a possibility with conscious omission". So the thought arises to include the conscious neglect as deliberate. Tongat, *The Basic*s 244

[26]Zainal Abidin Farid, *Criminal Law Volume I, (*Jakarta: Sinar Grafika, 2010) 213-214

[27] Adami Chazawi, *Criminal Law Part 2, (*Jakarta: PT RajaGrafindo Persada, 2011) 228.

[28] Chidir Ali, *The Legal Entity, (*Alumni, Bandung, 1999) 18-19.

[29] Chidir, *The Legal, 18*

Theory of Identification or *Alter Ego Theory* (Instrumental Rule) states that a criminal offense is committed by a corporation if it is committed by persons who have a functional position in the structure of a corporate organization, acting for and on behalf of a corporation or for the benefit of a corporation based on employment or other relations, Within the scope of the corporation itself both individually and collectively. [30]

The behavior of the corporation is actually an attribute or identification or instrument of the will of those who have such a functional position. In the case of a crime perpetrated by the corporation, the corporation is represented by the board, who may be able to appoint others. The judge in this case may also order that the caretaker be brought to court. The indictment for the corporation is separate from the indictment against the board. [31]

The theoretical shifts in corporate criminal liability as well as those occurring in some countries are due to the growing awareness of the dangers posed by corporate crime, both to the state (eg tax evasion, terrorism and corruption), rival firms (unfair competition), consumer protection, (Environmental pollution), shareholders (due to business closure sanctions), as well as to labor (lack of job security protection), due to corporate-oriented anomic behavior that is only profit-oriented and overriding the principles of good corporate governance and business ethics. [32]

Governments can be categorized as a legal subjects. The subject of law is a party based on the law of having rights, a certain duty and power over something. In accordance with the definition expressed by Algra, [33] the subject of law is any person who has rights and obligations, which gives rise to legal authority (*rechtsbevoegheid*), while the notion of the authority of the law itself is the authority to be the subject of rights. Thus, the Government as the stakeholders of the environment-related policy also has obligations and responsibilities that must be implemented.

Direct liablity doctrine for legal entities, for example officials / errors of senior officials identified as corporate actions / errors. Also referred to as the *alter ego theory* or organ theory. The officials referred to in this case are the ones who control the company, both alone and together, for example the"directors and managers".

According to the theory of common law, that everyone including the Government must be held accountable for any action, whether by mistake or without a strict liability. From this theory further emerged a legal responsibility in the form of criminal liability, civil, and state administration. The legal liability of such a government is done in court. [34]

According to Jimly Asshiddiqie[35], accountability has two personal or personal responsibilities and institutional or occupational responsibilities. It further said that if an official in carrying out his duties and authorities in accordance with applicable norms or rules of law, then his actions are accounted for (institutional). On the contrary, if an official performs his duties and authority violates the applicable norms or rules of law, then the exercise of his actions is personally accountable or personal liability.

According to Brautigam[36], government liability can be divided into 3 (three) types, namely; First: political liability, second: legal liability, and third: economic liability. Legal liability implies that both the government and the local government in administering the government that harms the interests of the people or other parties must be accountable and accept the lawsuit for his actions. Legal responsibility by the government can be done through 3 (three) means, namely through administrative law, through civil law, and criminal law. Based on the legal instrument, it is known that there is administrative responsibility, civil liability, and criminal liability.

In relation to legal liability, Hadjon said that the actions of officials must be observed, whether the action includes the responsibility of institutional or personal responsibility. [37] Basically every government official in the conduct of the government is charged with the responsibilities of private positions and responsibilities. The distinction between the responsibilities of institutional and personal responsibility for the actions of the government brings consequences related to criminal liability, civil liability, and administrative responsibility. Legal accountability of government / local government in the administration of governance, can be done at any time without having to wait for the end of his term of position.

[30] Chidir, *The Legal,* 152
[31] Chidir, *The Legal, 152*
[32] Chidir, *The Legal, 152*
[33] Zainal Asikin, *The Introduction to Law*, (Jakarta: Raja Grafindo Persada, 2012) 12-13

[34] Munir Fuadi. *The Theory of a Modern Law State*, (Bandung:Refika Aditama, 2009) 147-148
[35] Jimly Asshiddiqie, *"Islam and Its Tradition in Constitusional Country"* (The paper is presented at the annual meeting of Indonesian-Malaysia Seminar, Uin/IAIN Padang, 2010)
[36] Anis Zakaria Kama, " *The Essence of Government Accountability in the Governance"* (PhD diss, University of Indonesian Islam, Makassar, 2012) 258.
[37] Philipus M. Hadjon, *The Governance According to Law*, (Surabaya: Universitas Airlangga, 1992) 6.

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C. The Theory of Criminalization

The definition of criminalization can be seen from various literatures, such as the meaning of Criminalization in Indonesian Big Dictionary (KBBI) is defined as a process that shows behavior that was not originally considered as a crime, but later classified as a crime by the public. [38]

Based on this it can be understood that criminalization is a process undertaken by the forming of a criminal law norm to make an action categorized as a criminal action with rational foundations arranged in such a way as to combat crime.

The policy of criminalization is a policy in determining an act which was not originally a criminal act into a criminal offense (criminal grievances). Thus, in essence, criminalization policy is part of criminal policy by means of criminal law (penal), and therefore includes from (criminal law policy), especially formulation policy. [39]

Sudarto said that in the process of criminalizing an action, we must pay attention to four things. First; The use of criminal law should pay attention to national development objectives, namely to create a just and prosperous society that is equally material and spiritual based on Pancasila; In relation to this, the aims using the criminal law is to tackle crime and to reduce the countermeasures themselves, for the welfare and protection of society. Second; Acts attempted to be prevented or dealt with by criminal law shall be "undesirable deeds," for example acts that bring harm (material and or spiritual) to the citizens. Third; The use of criminal law should also take into account the principle of cost benefit principle. Fourth; The use of criminal law should also take into account the level of capacity or the ability of the work of law enforcement agencies that do not let the *overbelasting* task overload. [40]

Salman Luthan argues that the criminalization theories that have developed so far in criminal law include morals and individualistic liberal theory, Feinberg theory, theoretical *strafrecht* theory, and joint theory. First, the moral theory put forward by Lord Devlin. This theory states that criminalization stems from the opinion that the act that should be viewed as criminality is any act that is destructive or moral action. This is because common morality has a sensible role to defend society. If the moral bonds that bind the society are lost, the community will experience disintegration. Therefore, society has the right to invite morality that can guarantee its unity. If society is entitled to do so, then there is a practical limit on the maximum number of individual freedoms that correspond to community integration. But if individual freedom goes beyond the permissible limits, then immoral acts that generate noise, anger, aggravation and disgust are worth receiving arrangements with various instruments of the criminal law. [41]

Second, individualistic liberal theory. The starting point of this theory is the antithesis of moral theory is the principle of loss. It is said by Jhon Stuart Mill that the power of the state to govern society is limited by the freedom of citizens. States may only interfere with the private life of the citizen, if the citizen concerned harms the interests of others. If one's actions do not harm others, then there can be no restriction on his freedom. Based on this opinion, a particular act is prohibited because the act is harmful to others. A certain act is prohibited as long as it is harmful to others. As long as a certain act does not harm others, the state has no right to interfere with the lives of citizens in the life of society. [42]

Third, the theory of *paternalism*. This theory is a reaction to the weakness of individualistic liberal theory that cannot provide protection to groups of people who have physical, mental, and mental weaknesses such as children and drug users. The main task of the theory of *paternalism* is the protection of not harming oneself. Criminal law legitimizes the prohibition of the actions of a person who can harm himself. [43]

Fourth, Feinberg's theory put forward by Joel Feinberg. This theory is not merely adding to the principle of John Stuart Mill, but also clarifying the concept of loss as the basis for criminalizing an act of being forbidden. If Mill establishes that the sole justification of criminalization is the act of a person harming others, Feinberg proposes two reasons as a basis for criminalization, first is to prevent or reduce harm to others and second is to prevent serious attacks against others. [44]

In relation to the principle of assault, Feinberg gives an example of a person's actions in public that are very offensive to others and which cannot easily be avoided by the person being attacked. The act is related to lewd acts by two passengers on a bus, which is quite attacking even though the perpetrators are married couples. In this case, criminalization is done on the basis of assault rather than on the basis of show immorality, and will not change if the act will become immoral if the show is private. Beyond the context of loss

[38] The Minister of Cultural and Arts Republic of Indonesia, *Indonesian Big Dictionary Online, Accessed on August, 17th 2017, http://bahasa.kemdiknas.go.id/kbbi/index.php*.

[39] Barda Nawawi Arief, *The Criminal Law Reform from Comparative Law Perspective,* (Bandung : PT. Aditya Bakti, 2011) 126.

[40] Mahrus Ali, *The Basics of Criminal Law* (Jakarta : Sinar Grafika, 2012), 240.

[41] *Mahrus, The Basics, 240-241*

[42] *Mahrus, The Basics, 240-241*

[43] *Mahrus, The Basics,* 242 - 243.

[44] *Mahrus, The Basics,* 243.

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and assault, the individual must be left free by the state to pursue its own goals and what the priorities of its desires. [45]

Fifth, the theory of *strafrecht ordenings* put forward by Roling and Jesseren d'Oliveira-Prakkan. According to this theory criminal law is a tool or instrument of government policy. The use of criminal punishment as an instrument of government policy is a new trend in the development of modern criminal law. There are three main premises of the theory of strafrecht's ordenings. First, the criminal law is not shown to the free individual, nor to the act of law seen socially and psychologically, but rather directed against the human being as the player of certain roles, which is required to confirm himself with the forms of action appropriate to his role. Second, the determination of criminalization is not what the people perceive as a criminal act such as in the fields of economy, health, environmental protection and traffic. Thirdly, the criminal law is no longer a criminal law concerning an act, or a criminal law concerning his actions, but a criminal law concerning the rules. [46]

The sixth is the *combined theory*. Combined theory is not the name of a theory but a term to describe two theories combined into one to form a new theory of criminalization. The idea of merging the two theories is grounded by the weaknesses in each period of the criminalization theory in searching for justification to justify an action. [47]

III. ANALYSIS AND DISCUSSION

A. The reasons for the Government to be punished because of The Haze that Occurs in South Kalimantan according to The Act Number 32 Year 2009 on the Environmental Protection and Management

By realizing the Government's crucial role in the protection and management of the environment, the Government should respond more quickly in handling a pollution case or environmental damage. The government not only serves as the supervisor but also as a responsible person for the activities that have an impact on the environment both done by the Government itself, as well as by the public and private.

In the framework of the implementation of the mandate from the Constitution of the Republic of Indonesia 1945, Article 28H Paragraph (1) which states "*Everyone has the right to live a prosperous and spiritual life, to live and to get a good and healthy living environment and the right to receive a health services*" It is the government's duty to work harder in fulfilling the Indonesian people's right to a good and healthy environment.

As mentioned earlier, there are so many government duties and responsibilities for the environment as regulated in The Act Number 32 Year 2009 on Environmental Protection and Management such as;

1. Pollution control and / or environmental damage shall be carried out by the Government, regional government, and the party responsible for the business and / or activities in accordance with their respective authorities, roles and responsibilities. (Article 13)
2. In the protection and management of the environment, the Government has the duty and authority to establish national policies; Establishing norms, standards, procedures, and criteria; Coordinate and implement pollution control and / or environmental damage; Conducting guidance and supervision on the implementation of national policies, regional regulations, and regulations of regional heads; Conducting guidance and supervision of the compliance of the party responsible for the business andlor activity on the provision of environmental licensing and legislation; Develop and apply environmental instruments; Develop and implement public complaints management policies. (Article 63 paragraph (1))
3. In the protection and management of the environment, the provincial government is tasked and authorized: establish provincial policies; Establish and implement SEA at the provincial level; Establish and implement policies on provincial RPPLH; Coordinate and implement control of pollution and / or environmental damage across districts / municipalities; Conduct guidance and supervision on the implementation of policies, regional regulations and regulations of regency / municipality heads; Conduct guidance and supervision of the compliance of the party responsible for the business andlor activity on the provision of environmental licensing and legislation in the field of environmental protection and management; Develop and apply environmental instruments; Enforce environmental law at the provincial level. (Article 63 paragraph (2))
4. The minister, governor, or regent / mayor in accordance with his / her authority shall supervise the compliance of the party responsible for the business andlor activity on the provisions stipulated

[45] *Mahrus, The Basics,*. 243-245.
[46] *Mahrus, The Basics,*. 245.
[47] *Mahrus, The Basics, 245*

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in the legislation in the field of environmental protection and management. (Article 71 paragraph (1))

Of the many duties and responsibilities of the government mentioned above, related to the haze in South Kalimantan, the government should participate in the control of environmental pollution of haze by forming norms and policies related to the problem. If you look at the cause of the haze, then there are two main causes of natural forest fire due to very hot climate, and the burning of forests / lands conducted by several companies or individuals.

In relation to the government's duty and authority over haze in South Kalimantan, the Government referred to the Regional Government, more specifically the Provincial Government based on the principle of Regional Autonomy, namely clarity on the distribution of central and regional authorities. Unfortunately, the haze occurrs in South Kalimantan is not a one-time haze, but it has become an annual problem that has not only affected South Kalimantan but also national and even international problems (since the haze often reaches neighboring countries).

From some articles related to haze in South Kalimantan we can see that the government tends to be less care and less focus in regulating and managing this sector. The fragmentation of functions associated with haze prevention and control in many institutions that cause disorganization becomes one of the indicators. The government should integrate the functions of the Office of the Environment, Regional Disaster Management Agency, Indonesian National Army, The Police of The Republic of Indonesia and the community.

The inclusion of regulation on criminal liability in The Act Number 32 Year 2009 on The Environmental Protection and Management is as *ultimum remedium* and *premium remedium*. The enforcement of criminal law in this Act introduces the threat of minimum penalties in addition to the maximum, expansion of evidence, criminalization for quality offenses, integration of criminal law enforcement, and corporate criminal arrangements. Enforcement of environmental criminal law is concerned with the principle of *ultimum remedium* which requires the application of criminal law enforcement as a last resort after the implementation of administrative law enforcement is considered unsuccessful. The application of the *ultimum remedium* principle applies only to certain formal criminal acts, namely punishment for violations of waste water quality standards, emissions, and disruptions. While the *primum remedium* principle is applied as a preventive business before a result occurs, for example punishment for the person making the Analysis of Environmental Impact but not competent or not being certified.

In environmental criminal liability related to the government duties and responsibilities for pollution and environmental damage has not been regulated. What is regulated is only the obligation of supervision over the permits granted to the company related to activities that affect the environment. So far, the regulation on government accountability as responsible for the people who are victims of environmental pollution (in this case because of the haze) has not been regulated in the Act.

According to the theory of common law, that everyone including the government must be held accountable for any action, whether by mistake or without strict liability. From this theory further emerges the legal responsibility of criminal, civil, and state administration. The legal liability of such a government is done before the court. [48]

To achieve legal objectives such as the objectives of law according to Sudikno above, it is necessary to be connected with the theory of legal ideals by Gustav Radbruch, where there are 3 basic values which should ideally serve the basis in operating the law in Indonesia, namely: [49]

1. The value of justice;
2. The value of benefit; and
3. The value of legal certainty.

This theory teaches that there is a scale of priorities that must be done in the law enforcement. With the request of government criminal liability for haze in South Kalimantan, for example in the form of a fine, the benefit and justice for society can also be fulfilled. The fines can be used for the recovery of environmental and social conditions from haze. On the other side, the deterrent effect of a punishment can be felt by the government so that in the future it can prevent the occurrence of haze repeatedly.

In Article 112 relating to the mention of government as a person who can be convicted in the case of intentionally not supervising the company in the implementation of its environmental permit is one example of the recognition of the Act on the status of government which is also one of the legal subjects. The subject of law is a party which by law has certain rights, obligations, and powers over something. According to the definition described by Algra the subject of law is that every person who has rights and obligations, which gives rise to legal authority (*rechtsbevoegheid*), while the definition of the law authority itself is Authority to be the subject of rights. Thus, as a legal subject, the government

[48] Munir, The Theory, 147-148

[49] Marwan Mas, *The Introduction to the Science of Law*, (Jakarta: Ghalia Indonesia., 1997) 73-74

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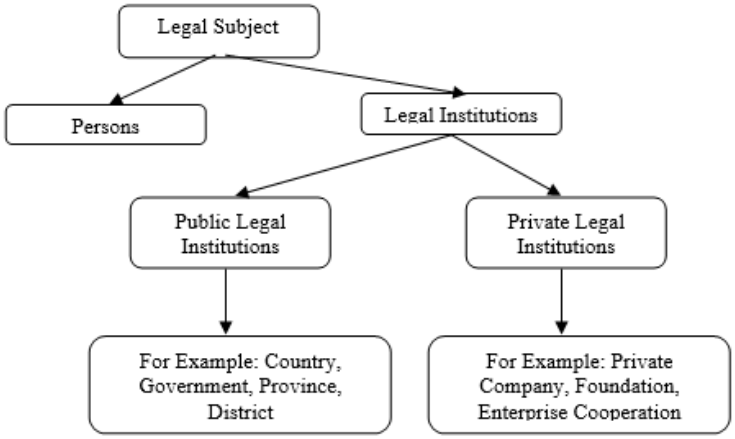
also has obligations and responsibilities that must be implemented.

For further explanation for the position of government as a legal subject can be seen in the following figure:



By understanding the status of the government as one of the criminal law-abiding subjects, the author will attempt to establish a criminal law policy analysis in the case of criminalization. Attempts to criminalize the actions of the government in relation to its duties and responsibilities for pollution and environmental damage that have not been used as a form of criminal offense shall be based on rational and objective considerations. Because of that It will be presented some theoretical foundation that can be used as a basis in criminalizing the government that does not carry out its duties and responsibilities for pollution and environmental damage.

As a basic theoretical foundation, we must first understand that the criminalization process should at least pay attention to four basic things. As Sudarto puts it in Mahrus Ali, that the first criminalization must consider the use of criminal law should take account of the national development goal of creating a just and prosperous society equally material and spiritual based on Pancasila. Second, it is noticed that the act which is attempted to be prevented or overcome by criminal law must be "undesirable deed," which is the act of bringing harm (material and or spiritual) to the people. Third, considering the use of criminal law must also take into account the principle of cost benefit principle. And fourth, considering the use of criminal law should also take into account the level of capacity or the ability of the work of law enforcement agencies that do not let there is an *overbelasting* task overload. 50

Based on the four basic elements that must be considered before criminalizing an act then can be described some argumentation. First, a healthy and good environment is the fundamental guarantee contained in the constitution and part of the national goal. Therefore, efforts to criminalize irresponsible governments and carry out their functions to safeguard the environment from pollution and destruction become an important part of legal policy to achieve national goals.

Second, pollution and environmental degradation are things that bring harm to society. Disadvantages due to pollution and environmental damage make the community will be distracted to do activities or even experience health disruption, for example due to haze that can cause respiratory infection. In order to ensure pollution and environmental damage the government must be fully responsible, therefore if the government deliberately and neglected to its related duties and functions then the government as one of the legal subjects can be criminalized.

Thirdly, the costs incurred to carry out the action against the irresponsible parties and not perform their functions if compare to the damage and pollution of the environment are certainly still within the rational limitations. Therefore, the principle of cost and benefit in the criminalization process will be maintained in this case.

Fourth, the capacity of law enforcement agencies to carry out actions that will be criminalized will not experience an overbelation of tasks or overbelasting. This is because, as long as this law enforcement agencies have the ability to run the prosecution of violations of criminal provisions in the law of the environment. Therefore, basically law enforcement apparatus has had an understanding in environmental criminal prosecution.

In the literature review section, several criminalization theories have been described. So, in the context of this study, one of the theories of criminalization that can be used is Feinberg theory proposed by Joel Feinberg. This theory suggests that the reason for an action to be criminalized is that it harms others. It is also to prevent or reduce harm to others and to prevent serious attacks against others. Based on this theory, to prevent the emergence of losses due to pollution and environmental damage as well as the emergence of various tensions of society who are disadvantaged against the government and have a negative impact on the life of the nation and state, it is necessary to create a conception of criminal law policy that accommodates criminal sanctions for governments that do not perform the duties and Its function in maintaining the environment clean and healthy. The forms of criminal sanctions that w can give such as fines and imprisonment.

[50]Mahrus Ali, *The Basics,* 240.

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B. The Government's Criminal Liability for Haze in South Kalimantan

The enforcement of criminal law in The Act Number 32 Year 2009 on The Environmental Protection and Management introduces the threat of minimum penalties in addition to the maximum, extension of evidence, criminalization for quality violations, integration of criminal law enforcement, and corporate criminal law. Enforcement of environmental criminal law still observes the principle of *ultimum remedium* which requires the application of criminal law enforcement as a last resort after the implementation of administrative law enforcement when it is considered unsuccessful. However, the application of the *ultimum remedium* principle applies only to certain formal criminal acts, namely the prosecution of violations of waste water quality standards, emissions, and disturbances.

The government liability concerning the environment is actually already regulated in The Act Number 32 Year 2009 on The Environmental Protection and Management. The existence of the principle of government liability is actually one of the balancer in positioning between the position of government and society in running the wheel of the state organization. The government has the authority to regulate, collect taxes, enforce the law, impose sanctions, and so on, which is a series of "powers" in achieving the goals of living the state. On the other hand, the community also has the right to obtain legal protection from various government actions that may cause harm to the community. Therefore, the existence of this responsibility actually provides enough space for the emergence of community participation that is needed by the democratic government.

The implementation of the principle of responsibility should be consistent, will also increase the dignity of the government in the eyes of its people. This is because if the government is willing to uphold this principle then at least some important things will be achieved: (a) enforcement of the principles of the rule of law, the rule of law, the supremacy of law and equality before the law in the administration of government, because the government is not only respected but also Law-abiding; (b) considering that in general the people of this nation are still adhering to paternalistic culture then the existence of accountability of this government encourages the emergence of voluntary compliance of public law; (c) strengthening reform commitments to achieve good governance in line with the strengthening of civil society; (d) to strengthen government accountability in order to ensure legal certainty, justice and legal protection, it is necessary to think about establishing a State Liability Act (called the State Liability Act 1981 in Japan called the Government Liability Act, 1946). In addition, the Act on Compensation (in Korea is called the National Compensation Act, the Administrative Compensation for Injury and the Administrative Compensation for Loss).

In general, the definition of Governmental Responsibility is a compulsory compliance obligation of a state or government or a government official or other official performing a governmental function as a result of an objection, a lawsuit, a judicial review, filed by a person, a public, Through the settlement of the courts or outside the court for the fulfillment of: (a) payment of money (subsidies, compensation, benefits, etc); (b) issue or cancel / revoke a decision or regulation, and; (c) other acts which are the fulfillment of its obligations, for example to conduct more effective and efficient oversight, to prevent any harm to humans or the environment, to protect the property of the people, to manage and maintain public facilities and infrastructure, to impose sanctions on an offense etc. .

The definition is clear that governmental liability is more emphasized on civic and administrative responsibility, whereas criminal liability is attached to the personal actions of the official concerned, such as corruption, murder, adultery, etc., in accordance with criminal provisions. In the context of governmental liability, in the field of civilization is generally based on an act against the law done by the authorities (*onrechmatige overheidsdaad* or unlawful acts of the government) as specified in Article 1365 of the Civil Code. The settlement of this civil action can be done through court or out of court through the ADR mechanism (inter alia: mediation and arbitration).

One of the duties and responsibilities of the government of South Kalimantan Province to the environment is to seek the Protection and Management of the Environment in South Kalimantan. One of the most perennial environmental cases in South Kalimantan is the haze. In carrying out its duties and responsibilities, the provincial government of South Kalimantan should be able to analyze the causes and minimize the impacts as much as possible. So, in every year there is progress made by the government and preventing haze in South Kalimantan.

The duties and responsibilities of the Regional Government on the environment-related policies in their respective regions are not yet formulated into the form of criminal offense if the government fails to do so. Among the many duties and responsibilities of the government to the environment such as the control, prevention and restoration of environmental pollution as stipulated in The Act Number 32 Year 2009 on Environmental Protection and Management, have not provided criminal sanctions if the government neglects or intentionally does not do so. Right now, civil action is the only way available.

If it is associated with the idea of *mens rea*, it must first be proved in the attitude of the soul or the soul's ability of the government of South Kalimantan Province whether the government is not immediately take action to the annual haze that occurred in South Kalimantan, whether in the form of intentional or negligent. The difference between intentional and negligent is the deliberate attitudes of one's inner person is indeed violating, whereas in the neglect of the inner attitude of this person simply does not heed the legal prohibition so as not to be careful in doing an act that causes a prohibited circumstance.[51] As a form of error in criminal law, they differ only "graduil" or only in quality. [52]

As the provincial government of South Kalimantan responsible for the good and healthy environmental feasibility, the environmental conditions must meet the standards of hygiene and environmental health. Therefore, the provincial government of South Kalimantan that does not immediately overcome the haze in South Kalimantan almost every year can be said to be negligent in carrying out its duty to guarantee human rights to a good and healthy environment.

Based on the opinions of Van Hamel and Bertens, the government of South Kalimantan Province has the ability to be responsible for:

- The South Kalimantan Provincial Government is able to understand and be aware of the true intentions of what it does, for example; not immediately overcome the haze or preventing the recurrence of haze in South Kalimantan and it cannot be justified by the community.
- The Provincial Government of South Kalimantan is able to determine the will if He does not immediately Overcome the haze or prevent the recurrence of haze in South Kalimantan so that he is responsible for what caused it.

Thus, in the case of haze in South Kalimantan, the Government of South Kalimantan Province has the ability to be responsible, this is because the Provincial Government of South Kalimantan is negligent in terms of:

- Not supervising the companies or people who burn the forests without permits
- Not trying to analyze the occurrence of haze in South Kalimantan that almost happens every year so that no efforts to prevent the occurrence of haze repeatedly
- Not providing haze-related preventive solutions in South Kalimantan
- Not conducting environmental maintenance and recovery of haze that occurs quickly, causing a lot of people who are affected by their health and activities due to haze
- No law enforcement related to the company or people suspected to be the cause of the haze.

According to Jimly Asshiddiqie, [53] accountability has two personal responsibilities and institutional or occupational responsibilities. It further said that if an official in carrying out his duties and authorities in accordance with applicable norms or rules of law, then his actions are accounted for (institutional). On the other hand, if an official performs his duties and authority violates the prevailing norms or rules of law, the exercise of his or her actions is personally accountable or personally liable.

According to Brautigam,[54] government accountability can be divided into 3 (three) types, namely; First: political accountability, second: legal accountability, and third: economic accountability. Legal liability implies that both the government and the local government in administering the government that harms the interests of the people or other parties must be accountable and accept the lawsuit for his actions. Legal responsibility by the government can be done through 3 (three) means, namely through administrative law, through civil law, and criminal law. Based on the legal instrument, it is known that there is administrative responsibility, civil liability, and criminal liability

In relation to legal liability, Hadjon said that the actions of officials must be observed, whether the action includes the responsibility of office or personal responsibility. Basically, every government official in the conduct of the government is charged with the responsibilities of private positions and responsibilities. [55] The distinction between the responsibilities of office and personal responsibility for the actions of the government brings consequences relating to criminal liability, civil liability, and administrative responsibility. Legal accountability of government / local government in the administration of government can be done at any time without having to wait for the end of his term of office.

Basically the responsibility attached to the government / local government in performing governmental acts is a limited responsibility. This means that the responsibility depends on the actions of the government / local government performed on the basis of his position, thus raising the responsibility of office. Or, on the contrary, the act in fact has used its authority with other purposes as defined in its basic rules of arbitrary action or the misuse

[51] Tongat. *The Basics,* 244
[52] Tongat, *The Basics*, 244
[53] Jimly Asshiddiqie*, "Islam and Its Tradition in Constitusional Country"* (The paper is presented at the annual meeting of Indonesian-Malaysia Seminar, Uin/IAIN Padang, 2010)
[54] Anis Zakaria Kama, The Essence"
[55] Philipus, The Governance, 6

of authority, then the responsibility arising is personal responsibility.

From the above description can be concluded that, civil and administrative responsibility is the responsibility of position. Whereas criminal liability is the personal responsibility of the official concerned in accordance with criminal provisions. The criminal liability of the South Kalimantan Provincial Government of haze in South Kalimantan is a form of legal liability for violation of legal obligations set forth in The Act Number 32 Year 2009 on The Environmental Protection and Management, so that such violations may be punishable by criminal sanctions.

The existence of shifting paradigm punishment, where previously criminal sanction is the last alternative (*ultimum remedium*), but now criminal sanctions serve as the main effort (*primum remedium*). Criminal sanctions imposed if an act is considered really harm the interests of both the State and the people According to the applicable law as well as the sociological feelings of the community, the criminal sanction becomes the main choice (*primum remedium*).

According to the theory of identification or *alter ego theory* (instrumental rule), that all the actions of the Government of South Kalimantan Province is always manifested through the actions of its leader, in this case related to the environment is the Governor of South Kalimantan that can be represented by the Head of Environmental Agency of South Kalimantan Province. Because of its position carrying out all legal obligations as stipulated in The Act Number 32 Year 2009 on the Environmental Protection and Management, can be charged with criminal liability if negligent in carrying out duties and obligations.

It should be remembered that based on the theory of identification, the actions and attitudes of the body of law that can be accounted for in the criminal law is the actions and attitudes of the people who are identified or equalized with a legal entity called directing mind of legal entity. Directing mind of legal entity is people who have authority and ability to influence policy in a legal entity. In the case of the accountability of the South Kalimantan Provincial Government above, He is the directing mind of the Environmental Agency of South Kalimantan Province. Therefore, both the Governor and the Head of the Environment Agency are the highest authority in South Kalimantan regarding to the policy of haze in South Kalimantan.

The act of the Provincial Government of South Kalimantan that is not immediate and appropriate to tackle and prevent the annual haze in South Kalimantan is a passive act, so it includes a passive criminal act called *delicta omissionis*. The omissionist offender (*delicta omissionis*) is divided into actual (pure) omission deliberations, commonly called *delicta omissionis* and *impure omissionist* offenses, often called *delicta commissists per omissionem commissa.* Delicta omissionis (*delict omissionis pure*), is the offense, criminal offense or criminal acts that by the lawmakers formulated so in other words declared can only be realized by passive action, do not do or ignore the legal obligation, where he should be doing . While the pure omission offense, commonly called *oneigenlijke omissidelicten or delicta commissionis per omissionem commissa*, is a delict that can be realized by active deeds or passive deeds in other words can occur due to deed (handeling) or neglect (*nalaten*). [56]

Based on the above description, it can be said that the act of South Kalimantan Provincial Government which is not immediately and deserves to overcome and prevent the occurrence of annual haze in South Kalimantan is the act of neglect (*nalaten*) to his obligation protecting and managing the environment in South Kalimantan. Thus, the actions of the Provincial Government of South Kalimantan is categorized as the deliberate omission offense (*delicta commissionis per omissionem commissa*).

If then we want to formulate the criminal provision related to the government's responsibility for environmental pollution, then the possible criminal form is a form of material penalty. In the case of the material realization of material offenses, three essential conditions are required:

a The realization of behavior;
b The realization of the consequences (due to constitutive or *constitutief gevolg*);
c There is a causal relationship (causal verband) between behavioral forms and the constitutive consequences.

Van Hamel states that: a person who does not act, he cannot be considered to cause a result, if he has no legal obligation to do so (*als de dader de rechtsplicht heft om te doen*). The legal obligation of a person to do something a certain time and in certain circumstances is required by law. If the law is obliged a person to do something, and then he does not do any cause, then the cause of that result lies in his or her legal obligation. [57]

The legal obligations held by the Provincial Government of South Kalimantan are the obligations established by law, namely obligations under The Act Number 32 of 2009 on the Environmental Protection and Management. This means that the Provincial Government of South

[56]Zainal, *Criminal Law,* 213-214

[57] Adami Chazawi, *Criminal Law Volume 2,* (Jakarta: PT RajaGrafindo Persada, 2011) 228

Kalimantan has an immediate and deserved obligation to recover from the haze in South Kalimantan and analyzes and assesses the causes of haze in South Kalimantan as a form of preventive effort. Thus, the legal obligation of the South Kalimantan Provincial Government is the cause of the repeated haze in South Kalimantan.

However, in formulating the provision to impose criminal liability to the Provincial Government of South Kalimantan, it is necessary to note that there should be justification reasons that can eliminate the criminal characteristic. Where if the Provincial Government of South Kalimantan has made a proper and quick effort in tackling the haze that occurred in South Kalimantan as evidenced by the small number of victims of haze as well as has been taken the action to the company / people who burn the land without permission.

Therefore, based on the above description in case of haze in South Kalimantan, the Governor of South Kalimantan or the Head of Environment Agency of South Kalimantan Province must be punishable (could be an imprisonment or fine) due to pollution of the environment, the fall of victims of haze, The disruption of the public activities and these are all the responsibility of the Provincial Government of South Kalimantan in The Act Number 32 Year 2009 on The Environmental Protection and Management. And This is also to fulfill the public sense of justice.

IV. CLOSING

A. Conclusion

The conclusions in this paper are as follows:

1. The underlying reason to impose criminal liability on Haze in South Kalimantan to the Government officials is according to The Act Number 32 Year 2009 on The Environmental Protection and Management, the South Kalimantan Provincial Government meets the qualifications as a legal subject, and the Government of South Kalimantan Province has a national responsibility to the environmental management as stipulated in The Act Number 32 Year 2009 on The Environmental Protection and Management.
2. The form of criminal liability that can be imposed to The government as the environmental policy stakeholder on haze in South Kalimantan is in the form of imprisonment and/or fine.

B. Suggestions

The writer's suggestion related to the criminal provision formula on the criminal liability on haze to the government officials based on the case study in South Kalimantan is as follows:

"The government official who is not immediately coping the environmental pollution that occurs repeatedly or annualy, causing the victim suffering from diseases caused by the contamination of the pollution should be imprisoned for a maximum of 5 years or a minimum fine of 1 billion rupiah".

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Analysis of Physical and Chemical Composition of Sweet Orange (*Citrus sinensis*) Peels

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Abstract**—** *Sweet orange is one of the most common fruits in the World. The waste generated from the fruits needs to be put into a beneficial use. In this study some physical and chemical properties of the primary waste of sweet orange was investigated. The result showed sweet orange rinds (peels) as the major waste and contains 45-50% of the total mass of sweet orange fruits. The chemical analysis showed sweet orange rinds to be rich in protein of 7.15% and crude fibre of 12.79% which can be used as ingredients in processed food. These uses will promote sustainable disposal of orange rinds.*

Keywords**— Orange rinds, proximate analysis, protein, crude fibre.**

I. INTRODUCTION

Citrus (*citrus spp*) is one of the most abundant fruit crops with World production estimated at 115 million tons per year. Citrus is a large family whose dominant members include sweet oranges (*Citrus sinensis*), tangerines/mandarin (*Citrus reticulata*), lemon (*Citrus limon*), limes (several species) and grape fruits (*Citrus paradis*). Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids contained in the rinds (Manthey, 2004). Also, citrus fruits and juices are important sources of bioactive materials including antioxidants such as ascorbic acid, flavonoids and phenolic compounds that are important to human nutrition (Kamran *et al*., 2009).Citrus fruits are good source of folic acid, vitamin B (thiamine), potassium, phosphorus, calcium, iron, magnesium, sodium and sulphur (Nagy *et al.*, 2007).

The endocarp is rich in soluble sugar and contains significant amounts of vitamin C, pectin, fibres, different organic acids and potassium salt which give the fruits its characteristic "citrus flavour" (Ezejiofor*et al*., 2011).

Africa produces 3,741,000 tonnes of varieties of citrus fruits of which Nigeria contributes 3,240,000 tonnes (FAO, 2004). Nigeria produces 3% of fresh citrus in the World (FAO, 2004). The rinds (peels) obtained from the pericarp of these fresh fruits are available in large quantities during the citrus season thereby constituting environmental problems since it is not being put into any productive use. The production of citrus fruits in Nigeria is significant, with heavy direct consumption due to few and small capacity processing industries to convert the fruit to juice, concentrate and canned fruit.

The inability of the few and small capacity processing industries to convert the fruit juice and concentrate has led to the generation of wastes. Waste is anything in a ruined or devastated condition (merriamwebster). It can also be defined as any unavoidable material resulting from an activity, which has no immediate economic demand and which must be disposed of (NISP, 2003). Physical properties are important in many problems associated with the design of machines and the analysis of the behaviour of the product during agricultural processing such as extraction of phytochemicals. Physical characteristics of agricultural products are the most important parameters for determination of proper standards to design of grading, conveying, processing, and packaging systems (Tabatabaeefar, and Rajabipour, 2005, Karimi*et al.*, 2009). The food industry has shown a special interest in finding uses for citrus industry by-products. Hence the need to know the physical and nutrient component of the peels.

II. MATERIALS AND METHODS

2.1 Raw material characterization

Harvested sweet orange was obtained from a fruit and vegetable market centre from a local market in Akure, Ondo State, Nigeria. The samples were selected manually from unripe and overripe fruits, thus providing a uniform samples or fruits of the relatively same ripeness stage. The ripe fruits were processed at the Crop Processing Laboratory of Agricultural and Environmental Engineering, Federal University of Technology Akure. These fruits were washed to remove dirt's and foreign materials from the epicarp. The fruits were peeled with knife to remove epicarp or rind or flavedo or shell.

2.1.1 Physical Properties of Citrus species

a. Weight determination

The weights of the selected agricultural materials were determined using a method described by Varnamkhasti*et al.* (2007). The materials were randomly selected from each sample into flat plates which were carefully weighed using an electronic balance to an accuracy of 0.01g. The

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procedure is replicated three times for each sample, and average value was taken and recorded.

b. Volume determination

The volume of the fruits and peels were determined by filling a 250 ml measuring cylinder with 150 ml of water. The whole fruits and peels of each variety (separately) were immersed in the water. The amount of displacement in water was recorded. The procedure is replicated three times and volume was calculated as:

$$Final\ volume - Initial\ volume = Volume\ of\ water\ displaced \quad (1)$$

$$Average\ volume = \frac{Volume\ of\ water\ displaced}{Total\ number\ of\ fruits/peels} \quad (2)$$

c. Oven dried

The peeled epicarp were dried in an oven with forced air circulation (Marconi MA03515, Piracicaba, BR) at 50ºC for 54h. The dried peeled were then crushed in knife mill type (Marconi M340 Piracicaba, BR) and kept in sealed plastic bags in a frost free freezer (BVR 28 GBBNA BRASTEMP, Joinville, BR) at -22ºC until its use.

d. Moisture content determination

Thermal drying method was used in the determination of moisture content of the samples.100g of sample were placed in oven at 105± 3^{0}C and allowed to dry to a constant weight for 24 hours (Lagha-Benamrouche, S. and Madani, K., 2013). The moisture content (MC) was calculated by expressing the weight loss upon drying a fraction of the initial weight of sample used. The moisture content of the seeds was determined by gravimetric method which determines the mass loss from the sample by drying to constant weight (ASABE STANDARDS, 1993 and AOAC, 2000).

$$DM(\%) = \frac{W_3 - W_0}{W_1 - W_0} * 100 \quad (3)$$

$$\%MC_{db} = 100 - DM\% \quad (4)$$

Where W_o is weight of empty crucible

$W_1$is weight of crucible plus sample before drying

$W_3$is weight of crucible plus sample after drying

DM is dry matter and

MC is the moisture content

e. Bulk density

The bulk density was determined using the mass to volume ratio. The volume was determined by water displacement method as described by Archimmedes law of floatation. True or real density is the mass of the sample divided by volume and can be calculated using the equation (5):

$$P_r = \left(\frac{M}{V}\right)(kg/m^3) \quad (5)$$

Where,

P_r(kg/m^3) = True density

M (kg) =dry specimen mass 0f 300g

V(m^3) =Volume of sample = Volume of water displaced by 300g when immersed in water (Archimedes Principle of Floatation.)

f. Relative density

The relative density was determined for the dried sample of sweet orange. A fixed bed of 300 cm^3(extractant) was used as standard volume of the container and the mass was measured in a previously weighed beaker. No separate manual compaction of sample was done. The bulk density was then calculated from the mass of the sweet orange peels and the volume of the container (Sacilik*et al*., 2003).

g. Particle Size analysis of the milled Sweet orange peels

Particle size analysis was carried out on the milled peels product from the knife edge mill after comminution. 200g sample was put into a stack employing a seven-frills set of the standard tyler series of size 10-65 mesh and un shaker screens magnetic type (Bertel, Caieiras, Brazil) which help to promote sufficient granulometric distribution of particles. Mass retained (MR) on each sieve sequel to shaking were retrieved. MR (g) is % of material retained

$$MR(D)(\%) = \frac{mmr}{ms} x\ 100 \quad (6)$$

Where MR (D) is mass of material retained in percent

mmr is mass of material retained

The milled peels size (200 g) was determined in a vertical vibrator sieve shaker Tyler series system (Model 1868 Bertel, Caieiras, BR) with sequential openings of 10, 14, 20, 28, 35, 48, and 65 mesh. The mean particle diameter was determined using the method of ASAE S319.4 (ASAE, 2008) for granulometry.

The diameter measurement of the particles of Orange peels was determined in terms of geometric mean diameter (or median size), geometric standard deviation of log-normal distribution by mass in ten- based logarithm, and geometric standard deviation by mass (ASABE STANDARDS, 2008), A mass retained in cadapeneira was weighed in semi-analytical balance (the 5500 Mars, Sao Paulo, Brazil) and the average diameter of the particles was calculated by the equation

$$d_{gw} = log^{-1}\left[\frac{\sum_{i=1}^{n}(W_i log d_i)}{\sum_{i=1}^{n} W_i}\right] \quad (7)$$

$$S_{log} = \left[\frac{\sum_{i=1}^{n} W_i(log d_i - log d_{gw})^2}{\sum_{i=1}^{n} W_i}\right]^{1/2} = \frac{S_{in}}{2.3} \quad (8)$$

$$S_{gw} \approx \frac{1}{2} d_{gw}[log^{-1} S_{log} - \left(log^{-1} S_{log}\right)^{-1}] \quad (9)$$

Where d_i is nominal sieve aperture size of the ith sieve, (mm)

d_{i+1} is nominal sieve aperture size in next larger than ith sieve (just above the set), (mm)

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d_{gw} is geometric mean diameter or median size of particles by mass, (mm) , or is geometric mean diameter or median size of particles on sieve, (mm) or is $((d_i X d_{i+1})^{1/2}$, which is d_i

$S_{log}$is geometric standard deviation of log-normal distribution by mass in ten-based logarithm, (dimensionless)

$S_{in}$is geometric standard deviation of log-normal distribution by mass in natural logarithm, (dimensionless)

$S_{gw}$is geometric standard deviation of particle by mass ,(mm)

$w_i$mass of material retained on the i-th sieve (g)

N is number of sieves +1 (pan

h. Proximate analysis

The proximate compositions of the dried sample were determined using standard methods to know the nutritive properties. All measurements were done in duplicates and values were presented in percentage.

i. Ash content determination

$$\% \, ash \; content = \frac{weight \; of \; ash}{weight \; of \; sample} x100 \quad (10)$$

ii. Fat content determination

$$\% \, fat \; content = \frac{weight \; of \; ether \; soluble \; material}{weight \; of \; sample} x100 \quad (11)$$

iii. Fibre content determination

$$\% \, Crude \; fiber = \frac{loss \; of \; weight}{weight \; of \; sample} x100 \quad (12)$$

iv. Protein determination

Protein content of the sample was determined using the Kjeldahl method

v. Carbohydrate determination

$$\% \, CHO = 100 - (\% fat + \% \, ash + \% \, fiber + \% \, protein) \quad (13)$$

III. RESULTS AND DISCUSSIONS

3.1 Physical Properties of the Citrus Species

Physical properties are important in many problems associated with the design of machines and the analysis of the behaviour of the product during agricultural processing operations such as extraction of phytochemicals. The summary of the result for all the physical parameters measured were, collated, analysed and presented (Table 1).

Table.1: Descriptive statistics of sweet orange from Nigeria.

Citrus species	Sweet orange Nig.		
Property	Max	Min	mean ± St.dev
Total mass (g)	313	202.72	250.05±27.10
Mass of fruit (g)	280.18	177.26	213.10±22.26
Mass of peel(g)	55.23	73.96	32.69±7.90
Total volume (cm^3)	240	85	149.43±39.30
volume of fruit (cm^3)	190	64	112.25±31.56
Volume of peels (cm^3)	65	12	37.18±13.88
Bulk density of peel (gcm^{-3})	2.24	0.085	0.99±0.38
Relative density of dried peels (gcm^{-3})			0.4007±0.0023

The mass of orange ranges from 202.72 - 3132 g with average mass of 250.05 g Mass of orange peels ranges from 73.96 - 55.23 g with average mass of 32.69 g for Nigeria orange. Citrus fruits contains pulp, peels, internal tissues and seeds. Citrus pulp is the solid residue that remains after fresh fruits are squeezed for their juice. It amounts to 50 – 70% of the fresh weight of original fruit, contains peels 30 – 45%, internal tissues of 20 – 35% and seeds 0 – 10%. The result obtained from the peels were lower than the result reported by Sharma *et al*, (2017) of 50% of citrus peels.

The bulk density was determined using the mass to volume. Natural bulk density of sweet orange was 0.99 gcm^{-3} and mean relative density obtained was 0.4007 ± 0.0023 gcm^{-3} for the dried milled sweet orange peels.

The rinds (peels) obtained from the pericarp of citrus is the primary waste and is available in large quantities during the citrus season thereby constituting environmental hazard and pollution to the environment. There is therefore an urgent need to put it into productive use. Figure 1 shows the result of dried orange milled peels from sweet orange.

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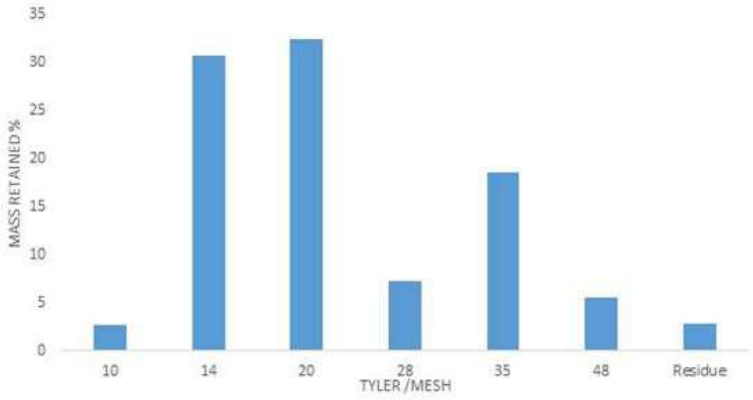

Fig.1: Mass Ground sweet orange peels retained in the sieves

3.2 Granulometry.

The milled peels size were determined in a vertical vibrator with six series tyler sieve shaker Tyler series system (Model 1868 Bertel, Caieiras, BR) with sequential openings of 10, 14, 20, 28, 35, 48, and 65 mesh with retained percentage mass of 2.62, 30.73, 32.53, 7.21, 18.59, 5.50 and 2.82 % respectively. The calculated average particle diameter is 0.84 mm using equation (7).

3.3 Nutrient composition of sweet orange (*Citrus Sinensis*) rinds

The result of Nutrient composition of dried milled sweet orange peels using proximate analysis is shown in Figure 2.The nutritive composition of sweet orange peels shows crude protein of 7.15% and crude fibre of 12.7% (Figure 2). The result of crude protein indicating that they could serves as very high protein supplements in addition to contributing to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance of body protein.

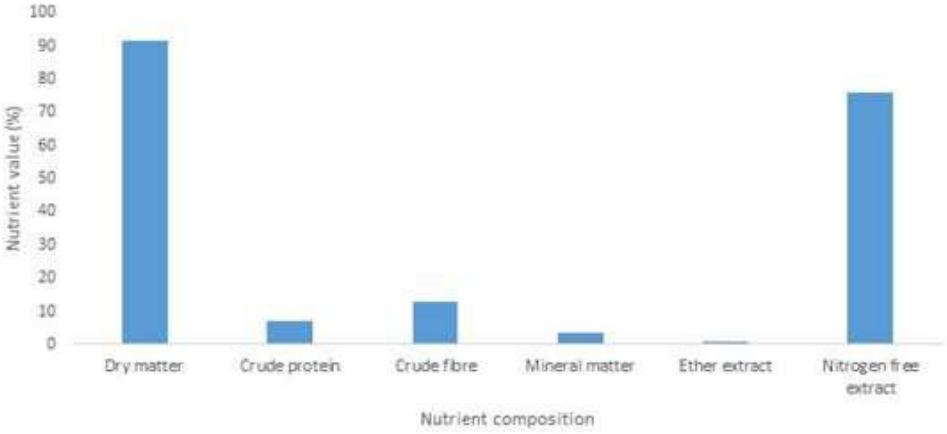

Fig.2: Nutrient composition of sweet ornage (Citrus Sinensis) rind (dry matter basis)

The crude fibre was 12.79 % which also have high value of dietary fibre and higher than the value of water leaf (Talinumtriangulare) of 12% as reported by Aja *et al*, 2010. It is also higher than the crude fibre of red bell pepper of 7.4% as reported by Odewole and Olaniyan, (2016). The result of the crude protein of 7.4% is higher than the crude protein reported by Awogbemi and Ogunleye (2009) of Fluted pumpkin (*Telfairiaoccidentalis*) of 2.3%. The result from the nutritive composition showed sweet orange as a promising source of proteins and crude fibre. The average daily requirement of dietary fibre is 21–25 g per day for women and 30–38 g per day for men (Food and Nutrition Board, Institute of Medicine, 2001).Nassar*et a*l. (2008) suggested that 15 % of orange peel and pulp could be incorporated as an ingredient in making biscuits, as they are a suitable source of dietary fibre with associated bioactive compounds (flavonoids, carotenoids etc.).It also contains a variety of other nutrients such as proteins, crude fibre and some minerals.

IV. CONCLUSION

In this study some physical properties and chemical characteristics of sweet orange peels were determined. It showed sweet orange rinds as a primary waste. The study

provided relevant data which are required for the design of processing equipment for sweet orange rinds.

Based on the chemical composition of sweet orange rinds, the crude fibre and protein of sweet orange rinds can serve as non-caloric bulking agents. They are also capable of offering significant low-cost nutritional dietary supplement to livestock and human beings. Sweet orange rinds could be incorporated as an ingredient in processing of foods and in livestock feeds. These uses will promote sustainable disposal of sweet orange rinds residues.

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Traditional Techniques of oil extraction from Kapok (*Ceiba pentandra* Gaertn.), Mahogany (*Khaya senegalensis*) and Neem (*Azadirach indica A.* Juss.) Seeds from the Far-North Region of cameroon

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Abstract—*An investigation was carried out in four localities of the Far-North of Cameroon (Maroua, Mokolo, Kaele and Yagoua in order to improve endogenous methods of oil extraction from kapok (Ceiba pentandra Gaertn.), mahogany (Hhaya senegalensis) and neem (Azadirachta indica A. Juss.) seed. The questionnaire administered to 75 traditional producers permitted us to note that extraction of oil from kapok is scarce. The traditional extraction processes from these oilseeds vary. But two principal techniques are predominant: the kneading process and the heated paste process. Husking, pounding and extraction make up the bottleneck. The yields are low, averagely six pans (of 1L capacity) are used to obtain one litre of oil. Amelioration of these methods through the introduction of grinders and pressers will not only help reduce strenuousness, but also increase the capacity to treat the yields and oil quality*.

Keywords— Azadirach indica A. Juss., Ceiba pentandra Gaertn., Far-Nord Cameroon, Khaya senegalensis, oil extraction method.

I. INTRODUCTION

Kapok (*Ceiba pentandra* Gaertn.), mahogany (*Khaya senegalensis*), and neem (*Melia azadirach L.* Inde) oils are known to possess nutritional, medicinal and cultural properties. They are used as a mixture or individually. Locally, these oils serve in the treatment of various diseases such as constipation, diarrhea, malaria, typhoid, worms and hemorrhoids [1, 2]. Neem oil is equally applied as a pomade to treat "getti getti" [3, 4]. Concerning bio-control in grain storage, they function as insecticides, protecting crops [4]. Kapok oil is mostly used during massages, to treat rheumatism and wounds [5]. It acts as an engine lubricant, as a raw material for soap production and as fuel for lamps [6]. All these virtues make these oils to become highly demanded and income generating. Hawkers sell these oils in town. But, the requests essentially come mainly from the meridian part of Cameroon and from neighbouring countries.

One constraint to this sector seems to be the artisanal extraction of these oils whose yield is low and cannot meet up with increasing demands. In fact, the raw materials are truly available, at least during the production season. Knowledge of the extraction processes becomes necessary in order to understand the mentioned techniques and the constraints that can be taken away so as to ameliorate extraction yield and extracted oil quality. To the best of our knowledge, studies on the description of traditional methods of extraction of these oils are almost no-existent. [5] Presented two extraction processes

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of kapok oil in Garoua (North) and in Mindif (Far-North). But this research was not focused on the entire Far-North.
The aim of this research is to investigate the different methods of extraction of oil from these three oilseeds in the Far-North in order to know their production and quality constraints.

II. METHODS

A preliminarily tested questionnaire was used to obtain information on the description of the methods of oil extraction. The questionnaire was administered to selected producers in the localities of Maroua, Mokolo, Kaele and Yagoua (table 1). These localities correspond to the administrative centres of Diamare, Maya Tsanaga, Mayo Kani and Mayo Danay divisions, respectively. The choice of these localities was drawn from locally obtained information and from preliminary tests which present these localities as areas of high production of these oils. In each locality, at least 10 persons were randomly chosen and interviewed.
The questionnaire comprised general information on the respondent (age, sex, region, division and village), methods of fruit treatments and information on the oil from the seeds (extraction techniques, uses and appreciation).
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Table 1: *Number of interviews per area*

Region	Town / village	Number of interviews
Far North	Maroua	11
	Kaélé	22
	Mokolo	20
	Yagoua	22
Total	**4**	**75**

III. RESULTS AND DISCUSSION

The processes for traditional oil extraction from kapok, mahogany and neem are numerous and diverse (fig. 1 to 17). We notice from the present investigations that the methods of extraction of oil from kapok show some subtlety (fig. 17) as compared to those obtained by [5]. Thus, after paste heating, the oil filtration step has disappeared in the actual process. Moreover, the scarceness of traditional methods of transformation kapok grains into oil is justified on one hand by the fact that this practice has been abandoned by the producers and on the other hand by local beliefs. In effect, in Kaele, for example, the producers say these trees harbour spirits that should not be disturbed.
These oils are generally extracted in the dry season and the process varies from one division to another, village to another, tribe to another. However, we can summarise them into two principal processes: the kneading process and the paste heating process.
The first technique uses hot or cold water as an extraction vector. In spite of a few hues, it can be summarised into the following operations: seed picking, husking, pounding, kneading and boiling of the oil. Fig. 1 to 8 present the charts applied in the towns of Maroua, Mokolo, Kaélé and Yagoua.
The second technique consists of heating the paste made out of the seed in such a way that the overlying fatty material can be recovered. It comprises the following principal operations: seeds picking, roasting, husking, pounding and paste heating. Fig. 9 to à 17 present the charts applied in the study area.

a. Seeds picking

Due to their heights, the grains are picked up. For the kapok tree, the fruits fall by themselves during the dry season then the grains can be selected and used. The same goes for mahogany where the dry grains are picked up. The neem grains are picked up fresh or dry. This step can be done either in open spaces or besides houses where these trees grow.

b. Roasting

Roasting is done by burning out the picked grains in order to facilitate husking and to make the oil-producing cells to become fragile. To this, a worn out metal sheet can be used or even dry straw from trees can be used. In this way, the grains are placed on a sheet under which a fire blazes. The crackling nature the grains develop indicates the end of the operation. The grains can also be wrapped up in dry straw and the whole thing burnt up. The roasted grains can then be husked.
However, the roasting conditions (temperature) may have negative effects on the variability of the organoleptic and sensorial qualities of the oil.

c. Husking

The shells of the seeds are removed to obtain kernels. This operation is done manually. Husking is done by passing a stone or stick over a bag containing the roasted grains. In all the cases, the sand and ash residues are separated from the seeds by winnowing.
In some processes, the seeds obtained (neem) are washed and dried before grinding takes place. Sometimes, the seeds are simple dried in order to facilitate the next step which is pounding. Drying is by exposing the seeds to sun for a duration that depends on the sun's intensity without exceeding one day.

d. Pounding

This is the step where the seeds are fragmented with the aim to facilitate the next steps. The seeds are pounded in a mortar with a little pestle. The final product is relatively crude. The granulation of the seeds is as reliable as dry.

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When the granulation of the seeds is not satisfactory, certain variants give a fine texture. That is, pounding is done a second time to obtain a finer powder. This facilitates the bursting of the oil-producing cells and prepares the way for extraction. This operation is very laborious and requires much muscular strength from the operator. The powder obtained is ready for the extraction step.

e. Extraction proper

This can be carried out either by kneading or by hearting the paste.

During kneading the finely ground kernels are placed in a clay pot in which water is progressively added. The water can be cold, warm or hot. The operation takes place at room temperature and during the dry season. Water is thus added as time goes by while stirring until an oil/water emulsion is obtained. The fatty matter is collected at the surface. This operation goes on until the paste becomes whitish. The quantity of water is not specified. Make sure during kneading, the paste is neither too thick nor too light.

Paste hating consists of heating the powder obtained mixed with water. The amount of water added is fundamental because the paste must remain thick, adding about one litre per pan (of 1L capacity). One of the additives used in the process is Rock-salt commonly called in the area "*Dalang*". This additive is said to be the agent that causes the oil to be liberated. This additive is only used to extract mahogany oil. As for neem oil, things like garlic are added in an attempt to take away the pungent odour. Oil is collected by hand exudation. The oil that becomes limpid is removed and allowed to settle. Opposite to the oil obtained by kneading, it is the rest of the paste that is made void of its oil by adding water and cooling. In this method, temperature is higher.

f. Boiling the oil

This operation is done by boiling the oil obtained in a clay pot or a pot in order to evaporate water. Certain variants go further to do an additional decantation which helps separate the oil from residual impurities. Oil is collected in recipients and is ready for conditioning. The methods of conditioning are numerous and diverse. We generally see conditioning in plastic bottles (35 millilitres and 1litre). But the extracted oil is immediately sold because generally, bookings for the oil are made way before production.

Considering the exploitation scale, the yields are very low. The instruments used for measuring raw materials (pans, calabashes) are local instruments. Globally, six pans of grains permit that one litre of mahogany and neem oil be obtained. In order to have one litre of mahogany oil in Kaele, three 10 kg (rice bags) need to be treated. Some producers use ten calabashes for one litre. In Yagoua, three calabashes of neem seeds and ten calabashes of mahogany seeds give one litre of the corresponding oil. In Mokolo, most of the women use three calabashes of neem and mahogany seeds to obtain one litre of oil. This is the same thing for Maroua. These yields are not far from those found in literature. In fact, 30 kg of neem fruits provide 13.6 kg of seeds, giving 3.75 litres of oil obtained by local pressing methods [4].

The Price for one litre of oil from these oilseeds varies in the market between 3000 and 6000FCFA. In the meantime, this price can rise to 12000FCFA in the rainy season or when the production of the grains has been low.

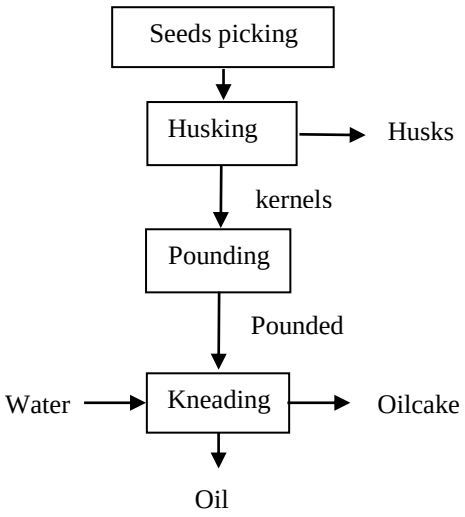

Fig.1: *Chart 1 for the extraction of neem oil in*

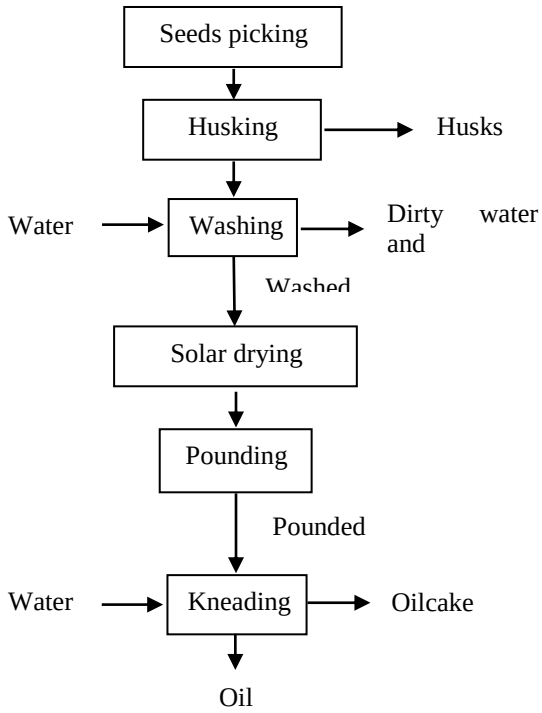

Fig.2: *Chart 1 for the extraction of neem oil in Mokolo*

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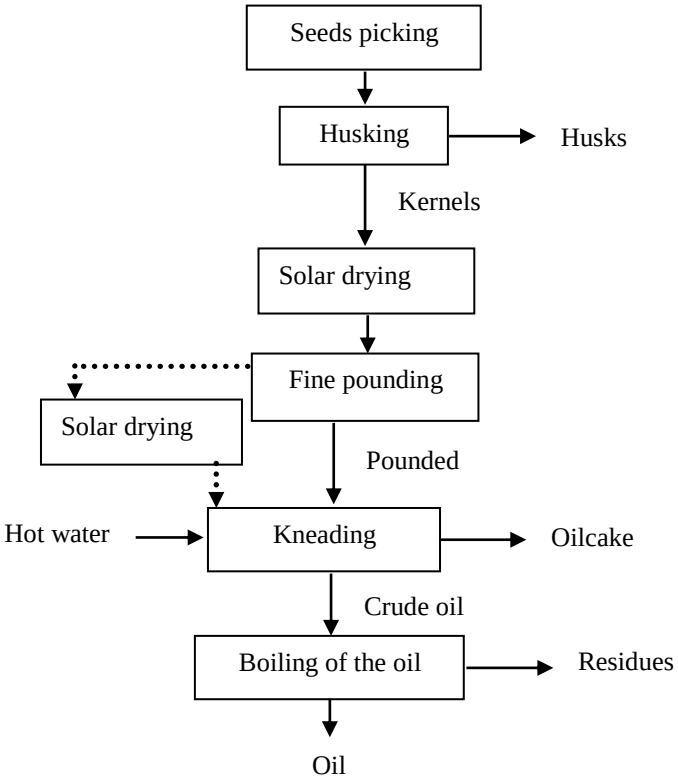

Fig.3: *Chart 1 for the extraction of neem oil in Kaele*

If the powder is not dry enough

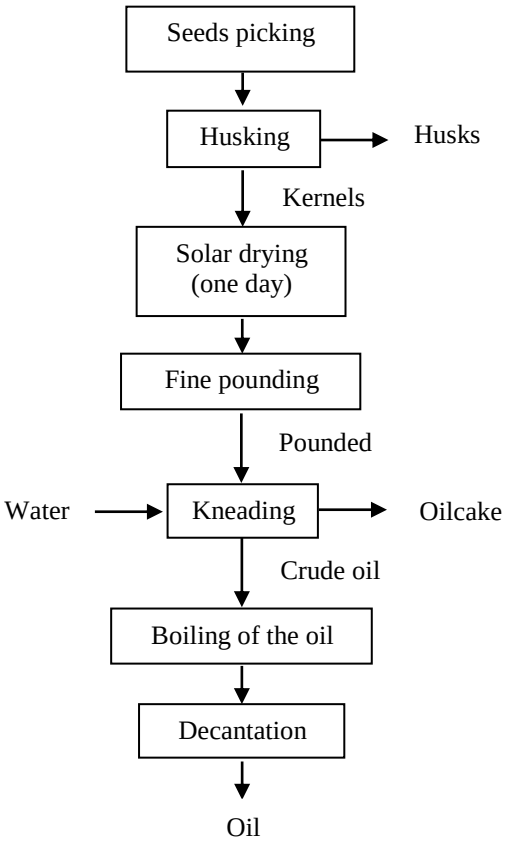

Fig.5: *Chart 3 for the extraction of neem oil in Kaele*

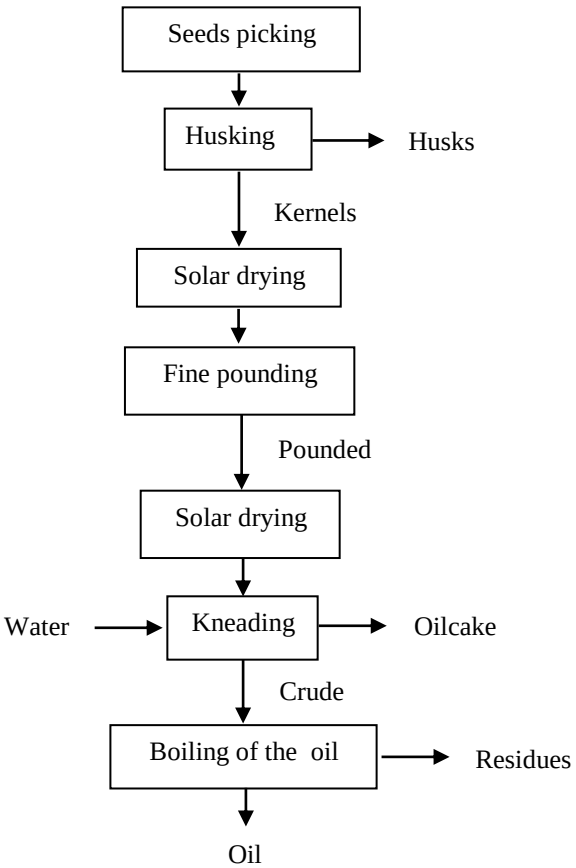

Fig.4: *Chart 2 for the extraction of neem oil in Kaele*

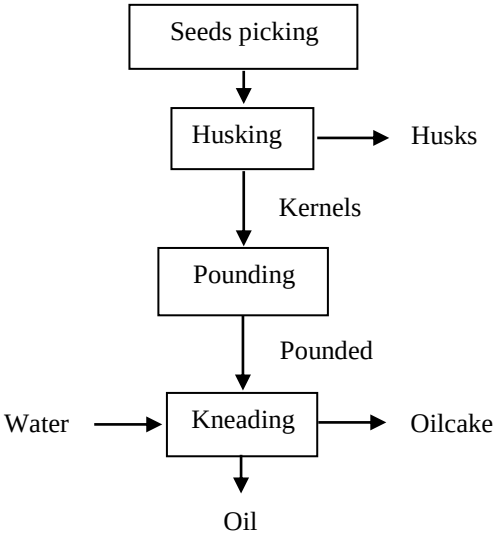

Fig.6: *Chart 1 for the extraction of neem oil in Yagoua*

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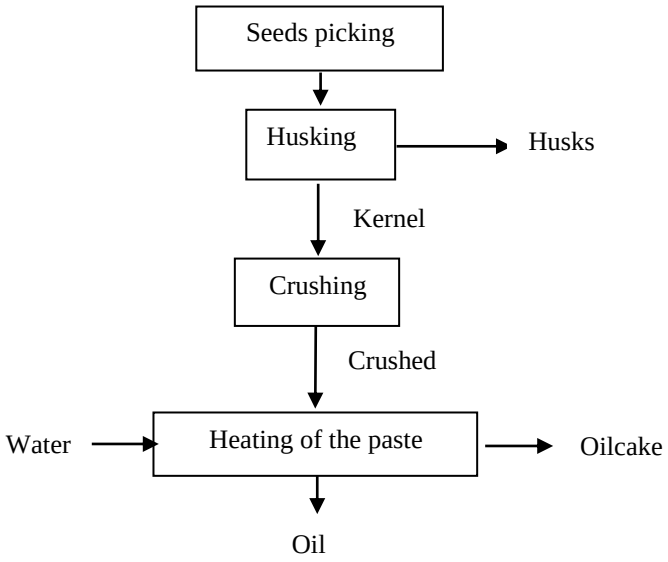

Fig.9: *Chart 2 for the extraction of neem oil in Maroua*

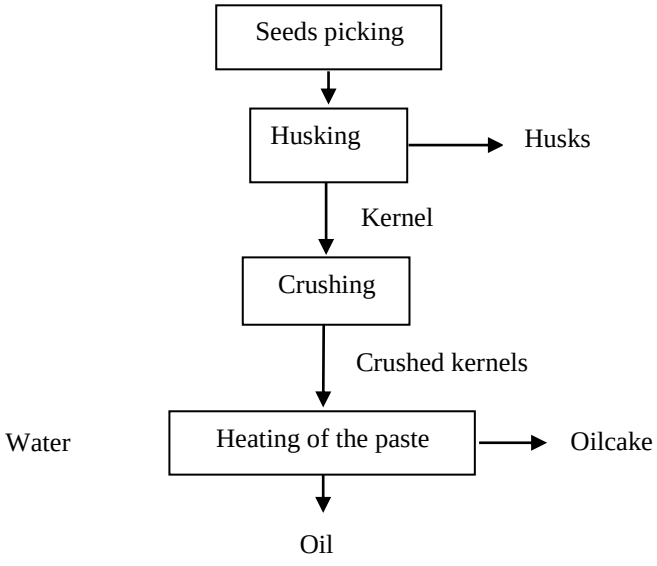

Fig.10: *Chart for the extraction of mahogany oil in Maroua*

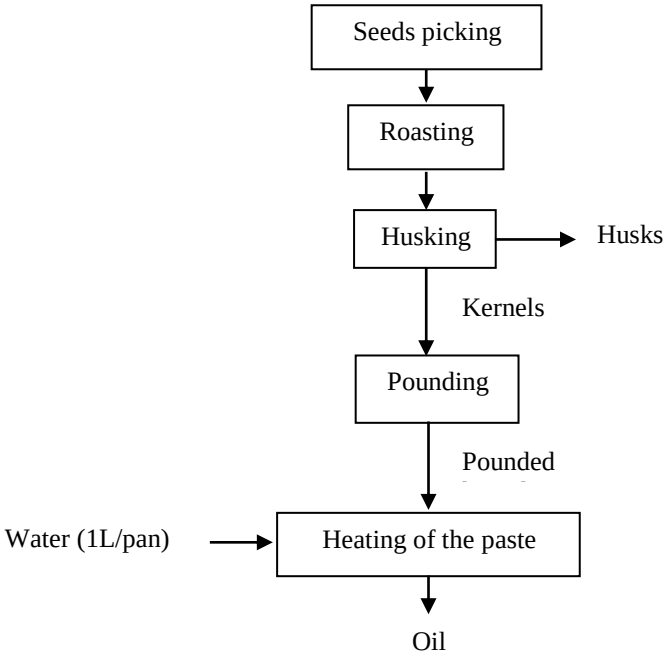

Fig.11: *Chart 2 for the extraction of neem oil in Mokolo*

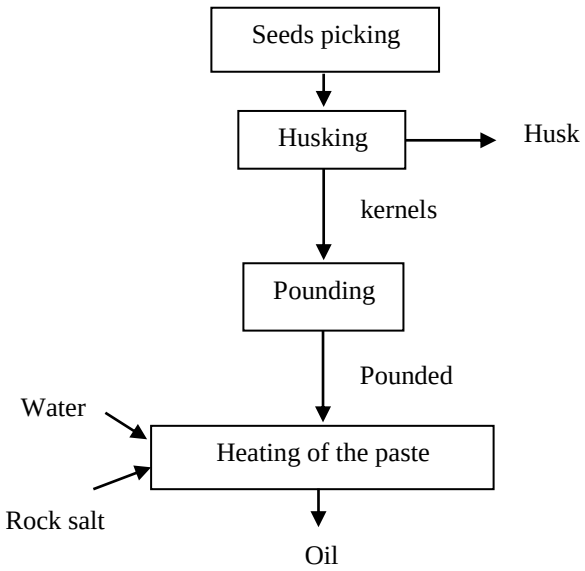

Fig.12: *Chart for the extraction of mahogany oil in Mokolo*

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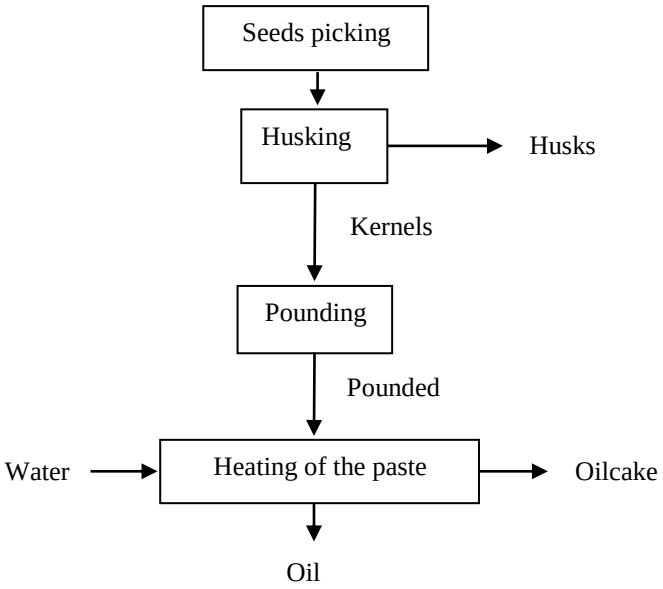

Fig.13: *Chart 3 for the extraction of neem oil in Kaele*

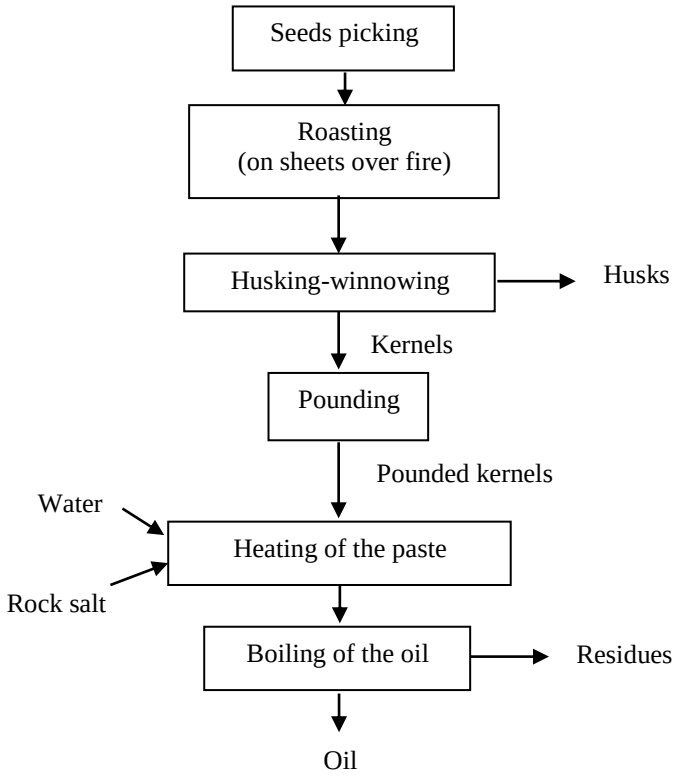

Fig.14: *Chart 1 for the extraction of mahogany oil in Kaele*

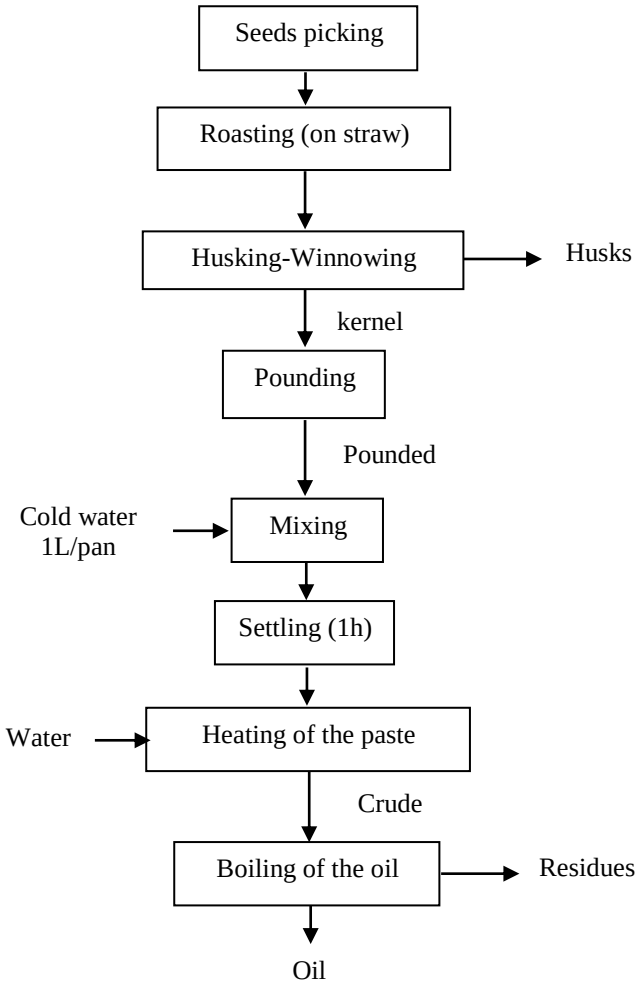

Fig.15: *Chart 2 for the extraction of mahogany oil in Kaele*

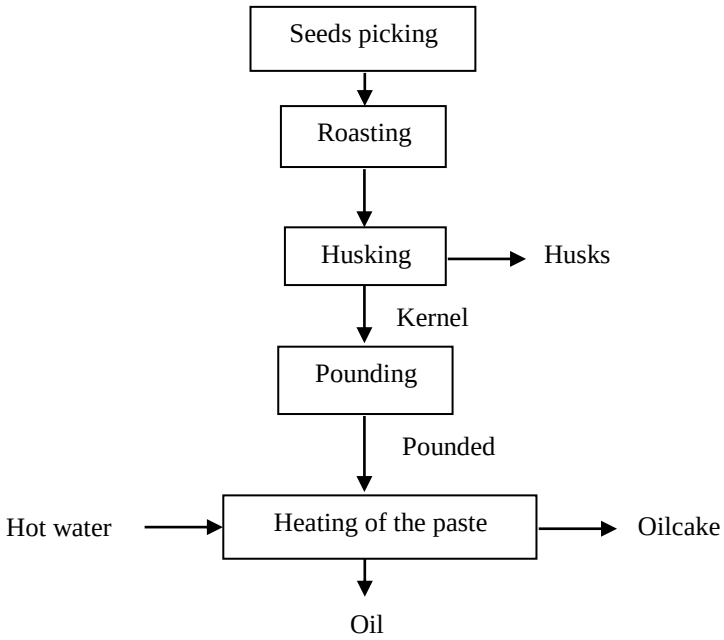

Fig.16: *Chart 1 for the extraction of mahogany oil in Yagoua*

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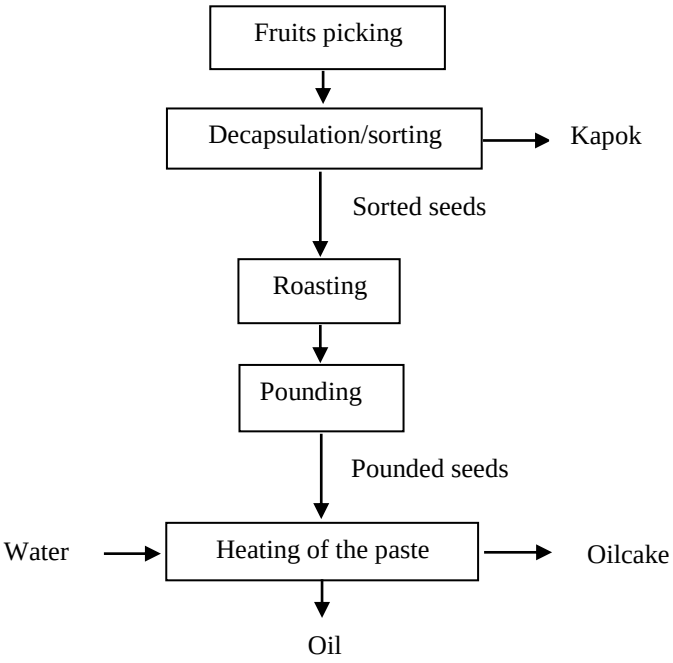

Fig.17: *Chart for the extraction of kapok oil in Maroua*

IV. CONCLUSION

A questionnaire administered to traditional producers of oil from kapok, neem and mahogany in four towns of the Far-North permitted the description of the traditional extraction processes of these oils. The processing of kapok grains into oil is scarce. The processes for traditional extraction of these oils are numerous and vary. Two principal techniques are found in all the investigated towns. That is the kneading process and the paste heating process. Husking, Pounding and extraction are the most tedious steps. Despite the increase in the demand for these oils, the yields are still low, averagely six pans of grains to produce one litre of oil. These extraction methods can be ameliorated by introducing crushers and pressers. This will help reduce tediousness, increase the capacity to treat, the yields and the quality of the oils.

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Local uses of kapok (*Ceiba pentandra* Gaertn.) Tree from the Northern Part of Cameroon

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Abstract**—** *An investigation was carried out in the Adamawa, North and Far-North Regions, in order to gather information about the actual uses of Ceiba pentandra* Gaertn*. a fruit tree widely domesticated by the population in this part of Cameroon. Data were collected from a sample of 300 persons from different localities of these regions. The results showed that almost all parts of Ceiba are used in curing many diseases such as sexually transmitted illnesses (syphilis, gonococci), fever and skin or eyes infections. Men use these trees as antibiotic or aphrodisiac. Generally, leaves (55%), roots and bark (28%) are the most used part of the tree. Oil was extracted from the seeds. The fact that the local population masters the know-how of the methods of extracting traditional oil needs to be exploited. These results are important data for the valorization of this tree in Cameroon.*

Keywords**— *Ceiba pentandra* Gaertn., *extraction, oil, Northern Cameroon regions, uses*.**

I. INTRODUCTION

The northern part of Cameroon is full of many non-conventional oilseeds. More studies have been done on some of these oilseeds, especially shea tree [1, 2, 3]. Notwithstanding, little has done researched on the kapok (*Ceiba pentandra* Gaertn.), neem (*Melia azadirach L.* Inde), mahogany (*Khaya senegalensis*) and baobab (*Andasonia digitata*) trees. This motivated the researcher to take an interest in the non-conventional and neglected oilseeds of the northern part of Cameroon.

The first phase of the investigations was aimed at examining the fruits from the kapok tree, a species of the Bombaceae family which natural habitat is equatorial and tropical [4, 5]. It has a height of 40 to 60 m [6]. Its smooth trunk is covered with large conical spines and as time passes, it bears enormous thorny buttresses. Flowering occurs early in the year between January and February. The fruit is an elliptic, woody, pendulous capsule of 10 to 30 cm long. It opens with 5 valves and reveals a whitish cotton like fluff called kapok and brown seeds. The kapok tree is a multipurpose tree. It is used as food, medicine and source of income [4, 7, 8]. Its seeds produce 11 to 28% of oil [9, 10]. The main fatty acids are palmitic acid (10-16%), stearic acid (2-9%), oleic acid (49-53%) and linoleic acid (26-29%) [10]. Despite this richness, this oilseed has lost interest in the inhabitants of the northern regions of Cameroon. This loss of interest follows the exploitation and processing of conventional oilseeds in the region, including cotton and maize. Indeed, with the creation of "Société de Développement du Coton (SODECOTON)" in 1974, the local inhabitants almost diverted from the exploitation of this plant, especially of its fruits whose oil was an integral part of their diet.

To the best of our knowledge, previous studies on the kapok tree have been done mainly in other countries. These works centred on its barks [11], wood [4, 12], the kapok itself [13, 14], its oil [9, 10] or on leaves and seeds [8], have provided a lot of information about the constitution of the parts of the tree. The studies of [15] concerned the antimicrobial activities of the parts of the kapok tree. However, given the variability of the properties of species based on a natural environment, the objective of this work is to contribute to the growing knowledge of the local uses of kapok tree in the Grand North of Cameroon.

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II. MATERIAL AND METHODS

2.1. Preparation and administration of questionnaires

A questionnaire was drafted and administered in the three northern regions (Adamawa, North and Far-North). In each region, five localities were chosen (table 1). The choice of the localities was guided by local information and data from the preliminary survey, which point out that there exists an abundance of these trees in the said localities. A translator was engaged in case of any communication difficulties with local population. In each locality, about 20 persons were chosen at random and questioned.

The questionnaire included general information about respondent (name, age, gender, region, division and village), general knowledge of the tree (abundance areas in the village, period and harvesting technique, edibility, uses of different parts of the tree), processing of fruits and knowledge of oil (extraction technique, use and appreciation).

Table 1: *Number of interviews per area*

Region	Localities	Number of interviews
Adamawa	Mbé	20
	Meyganga	17
	Wack	21
	Tibati	18
	Ngaoundéré	22
North	Pitoa	21
	Dourbey	19
	Garoua	22
	Guider	20
	Sackdjié	20
Far North	Maroua	22
	Mora	18
	Mindif	18
	Mokolo	20
	Limani	22
Total	**15**	**300**

2.2. Data analysis and processing

The SPSS (Statistical Package Social Sciences version 16.0) software [16] was used for data analysis and processing.

III. RESULTS AND DISCUSSION

3.1. Location

In the Adamawa Region, it was reported that the kapok tree is abundant in the localities of Mbe, Wack and Sackdjie. In the North, these trees are found in Pitoa and Dourbey. As for the Far-North Region, these abound in the towns of Maroua and Mokolo. Generally, this tree is found throughout the three northern regions.

3.2 Different names of kapok tree

The results show that women are mostly involved in kapok tree exploitation. In the three regions, 89.2% of the population claim to know kapok tree. This proportion is predominantly female (about 60%). This is due to the fact that women are the most involved in fruit harvesting activities, especially activities involved in local oilseed farms. More than 52% of the inhabitants call the tree *Bantaïdjé*. This is so because Fulfulde is the commercial language to all the three regions under study. Several other names are given to this tree (fig. 1 to 9) according to local languages (table 2).

Table 2: *Local names of the kapok tree in the Grand North of Cameroon*

Country	Local languages	Names
Cameroon	Fulfulde	*Bantaïdjé/ Bantaï/biguelbantaï*
	Duru	*Doukdouk*
	Mundang	*koumi*
	Mbum	*Ta'amoul*
	Guidar	*Kosso'onbana*
	Falli	*Boudjou*
	Tupuri	*Mougna'h*
	Mafa	*Koukouwaï*
	Arab shoa	*Roum*
	Laka	*Bikora*
	Massa	*Gounoura*
	Guiziga	*Kilmbana*
	Hausa	*Rimi/ Dan rimi*
	Kanuri	*Toum*
Cameroon/Chad	Kablaï	*Mania*

Fig.1: *Trunk of an adult C. pentandra*

Fig.2: *Trunk of a young C. pentandra*

Fig.3: *Buttresses of a young C. pentandra*

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Fig.4 : *Leaves of C. pentandra*

Fig.5: *Flowers of C. pentandra*

Fig.6: *Fresh fruits of C. pentandra*

Fig.7: *Dried fruits of C. pentandra*

Fig.8 : *Kapok of C. pentandra*

Fig.9: *Seeds of C. pentandra*

3.3. Uses of different parts

The fruits of the kapok tree are harvested during the months of June and at the beginning of July. According to [17], this period is convenient after that of flowering as described by. Indeed, fruits cannot be harvested during this period. The fruits are therefore allowed for the dry season and they fall down by themselves. The inhabitants can then simply pick them up. But before the fruit, other parts are continually exploited for various purposes.

3.3.1. Leaves

They are the most used part of the plant (55%). The leaves are cherished vegetables for majority of inhabitants of Nigeria and of a small group of bordered Cameroonians in the Far-North. This use is marked among the Kanuri and the Hausa people in the dry seasons during which vegetables are scarce. Softened on fire, crushed and then mixed with palm oil, the young leaves are taken in decoction to resolve some heart problems. The Kanuri (Limani) and the Mundang (Mora) also use this decoction, but without this oil, to cure diarrhea. Crushed fresh leaves are used for wound dressing, bandaging of tumours or abscesses, and control of skin infections by the Tupuri of Mindif. A decoction of young leaves mixed with palm kernel oil is taken by women as antibiotics when they have just been delivered to the Duru, Fali and Mundang tribes in Mora (Far-North). Northern Fali use young flowers to treat gonorrhea and syphilis. These uses corroborate with those mentioned by [8, 18].

3.3.2. Roots and barks

The barks are the second most used part of the tree (28%). However, among the Fali, in the North region, the roots are used as much as the barks. The Mundang of the North and Far-North regions, mix powdered roots and, dried and crushed barks with palm kernel oil and anointed on new-borns to drive out evil spirits. The barks and roots also have an aphrodisiac power. Indeed, the Fali men in the North and the Tupuri in the Far-North constantly chew the barks and roots to keep their manhood. These tribes also use the roots to cure dysentery. These therapeutic properties corroborate well those mentioned by [8].

3.3.3. Wood

In the three regions, *C. pentandra* wood is only used locally, for the manufacture of objects such as spatulas, mortars, musical instruments (drums), furniture, doors, and canoes. In the Far-North and Adamawa, about 65% of the population exploit the wood for the manufacture of utensils. Unlike in the Far-North and Adamawa regions, in the North region (Guider and Garoua), it is more solicited for the manufacture of canoes as mentioned by [12]. Generally, less than 7% of the respondents use it as firewood because it is a very bad wood that emits a lot of smoke when burned. This explains why it is more solicited as craftwork and industrial wood.

3.3.4. Kapok

Given that it cannot be hand-spun like cotton, kapok remains to the local inhabitants as a simple padding material used for the manufacture of cushions, pillows and handmade mattresses. It is the main padding material in all villages in the Grand North. More than 80% of the respondents say it is used for these purposes. The ashes of the kapok are used in the North by the Guidar (Guider) and the Fali (Garoua) people to treat cough. These ethnic groups take crushed mosses and mix them with palm kernel oil to cure syphilis and gonorrhea. This false cotton was used for a long time by the inhabitants (Fulani shepherds) of the North and the Far-North. A small sample of kapok placed on a so-called special stone and struck vigorously with a piece of iron for rubbing,

produces fire following a spark between the stone and the piece of iron. This traditional match has long been used by these shepherds, even in the rainy season due to the impermeable nature of the kapok.

3.3.5. Seeds

The seeds contained in the fruits of *C. pentandra* are also useful for the inhabitants. Roasted with salt, seeds are consumed as groundnuts (appetizers) by villagers, especially children in the North and Far-North. This use has already been reported by [19]. Once reduced in the North and Far-North regions, seed exploitation for oil production has almost disappeared with the creation of "SODECOTON". In the North, crushed seeds are used as feeds for livestock. This corroborates the statements of [20].

3.3.6. Traditional oil extraction techniques

The Mundang tribe of Mindif (Far-North) and Fali tribe of Garoua (North) each have a technique for extracting this oil. These processes differ only in a few steps. They have in common the use of local materials: pot, spatula, crushed stone or millstone and fine fabric. The operations of the processes are summarised in the collection of the fruits, the sorting, the roasting, the grinding of seeds, the mixing and the extraction proper.

3.3.6.1. Roasting

After picking up the fruits, the seeds are manually separated from the kapok. The roasting step helps to make the oil-producing cells to become fragile and prepares the extraction, while reducing the water content of the seeds. The seeds obtained are then roasted over low heat before being ground.

3.3.6.2. Pounding

This is the seed fragmentation step in order to facilitate subsequent operations. The seeds are crushed with two stones or crushed in a mortar using a pestle. The final product is relatively thin.

3.3.6.3. Kneading

This operation is very tedious, and requires a lot of muscular force from the operators. Water is added to the powder obtained and the mixture is kneaded. At this level, the difference lies in the amount of water added in each process. A small amount of water is added to have just a paste which will then be mixed for at least half an hour. Finally, the supernatant oil is collected with a hollow spatula (fig.11).

The extraction yield is low. Indeed, a mature tree produces 300 to 400 fruits, each containing 220 to 250 seeds, from which 1.5 to 2 litres of oil are extracted. This oil was used by the Mundang Fali, Massa and Laka tribes of the North and Far-North, mainly for massages and very little for fries and table oils. It also served as an antibiotic for wounds and injuries. This low use as table oil is indeed the one mentioned by [21].

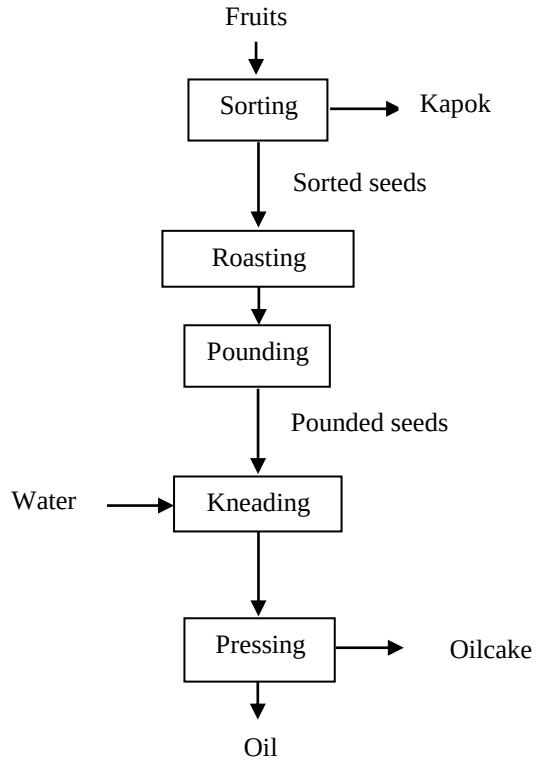

Fig.10: *Extraction process of C. pentandra oil in Garoua in the North Region*

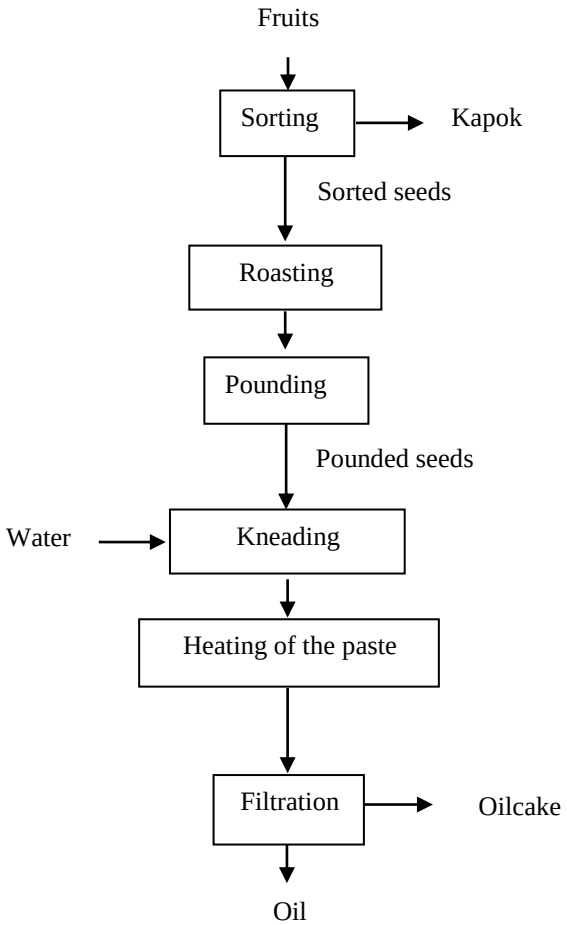

Fig.11: *Extraction process of C. pentandra oil in Mindif in the Far-North Region*

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IV. CONCLUSION

The investigations carried out in the three northern regions of Cameroon and on a sample of 300 people showed observing that the local inhabitants are quite aware of the existence and different use of the kapok tree. It has multiples uses and all parts of the tree are exploited. Leaves, roots, barks, wood, seeds and oil are used for food, craft and medicinal purposes. However, the leaves (55%), roots and barks (28%) constitute the most solicited parts. In spite of the little interest it brings today, the kapok tree has a strong therapeutic, food and industrial potential that can contribute to the economic development of Cameroon in general and in particular to the localities in which it is grown.

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Microbial and Physicochemical Qualities of River Owena Sediments

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***Abstract*—** *Microbial quality, physicochemical parameters and heavy metals determination of sediment samples from River Owena, Oriade local government area, Owena, Nigeria. For period of dry and wet seasons. The pH of the sediment samples ranged from 6.44 to 8.00±0.01, organic matter ranged from 17.15 to 35.31%; water holding capacity ranged from 0.323 to 1.779±0.01 ml/g; composition of sand: clay: silt were 75:12:13 %, 33:17:50 %, 62:18:20 % and 50:20:30 %; 82:2:16 %, 48:22:30 %, 32:25:43 % and 43:27:30 % wet and dry seasons respectively indicated more of loamy sand, clay loam, silt clay and loam. Mean concentrations of heavy metals measured in the sediment samples included iron, zinc, manganese, lead, chromium, cadmium, nickel and copper, iron had highest values of 1.89 to 4.1±0.01 mg/kg and cadmium lowest values of 0.01 to 0.12±0.01 mg/kg. A total of fifteen bacterial species were isolated from River Owena sediments, which included Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enteritidis, Bacillus subtilis, Serratia marcescens, Shigella sonnei, Bacillus cereus, Micrococcus luteus, Micrococcus varians, Pseudomonas aeruginosa, Proteus vulgaris and Erwinia amylovora. A total of ten fungal species were isolated, which included Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium chrysogenum, Cladosporium herbarum, Mucor mucedo, Mucor plumbeus, Fusarium oxysporum, Rhizopus stolonifer and Rhizopus oryzae. The total bacterial count of the sediment ranged from 4.1 x10^3 to 3.02 x 10^3 cfu/g while the total fungal count of the sediment ranged from 4 x 10^3 to 7.6 x 10^3 cfu/g. However, the pollution level due to the presence of some pathogenic microorganisms which are of public health significance, but it could be improved upon with appropriate treatment and sanitation.*

***Keywords*— Microbial, Physicochemical, Heavy metals, Sediment, Wet and Dry season, Health.**

I. INTRODUCTION

Sediment is any material that settles to the bottom of a lake, river or ocean and are composed of dead and living organisms, dust from the air, soil eroded from the continents and chemical solids as well as water and nutrients (Alexopolous, 1983, Carla,1997, Robert and Stanley, 2000). Sediments differ from soil but they both provide environments for microorganisms and play different roles in the ecosystem of a wetland. Sediments near urban areas commonly contain high levels of contaminants (Lamberson *et al*., 1992; Cook and Wells, 1996). This constitutes a major environmental problem faced by many human impacted aquatic environments (Magalhaes *et al*., 2007).

The contamination of sediments with heavy metals leads to serious environmental problem (Loizidou *et al*., 1992). Today there is trace contamination not only of surface water but also river sediments, which are susceptible to leaching from waste dumps, mine tailings and industrial production sites (Moore *et al*., 1998). Organic manure, municipal waste and some fungicides often contain fairly high concentration of heavy metals. Soils receiving repeated applications of organic manures, fungicides and pesticides have exhibited high concentration of extractable heavy metals, thereby increase their concentration in runoff (Moore *et al*., 1998),

The composition of sediments is dependent on natural factors (geological, topographical, meteorological, hydrological and biological) in the drainage basin and varies with seasonal difference in runoff volumes, weather conditions and water levels. Importantly, sediments can also be contaminated by naturally occurring sources (Muller, 2001).Heavy metals may adsorb onto sediments or be accumulated by the benthic organisms; their bioavailability and toxicity depend upon the various forms and amount bound to the sediment matrices (Chukwujindu *et al*., 2007). Additionally, pollutants released to surface water from industrial and municipal discharges, atmospheric deposition and run off from agricultural, urban and mining areas accumulate to harmful levels in sediments (Chukwujindu *et al*., 2007).

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The soil pH, organic content and water are the main factors affecting the bacterial and fungal population and diversity. The organic carbon, nitrogen, phosphorus, potassium are important for fungi. In the absence of any of these the growth and sporulation of moulds as well as other microorganisms are hampered a lot. It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high. Also environmental factors such as pH, moisture, temperature, organic carbon, organic nitrogen play an important role in the distribution of mycoflora (Muller, 2001).

Soil texture can have a profound effect on many other properties and is considered among the most important physical properties. Texture is the proportion of three mineral particles, sand, silt and clay, in a soil. These particles are distinguished by size, and make up the fine mineral fraction. Particles over-2 mm in diameter (Olaitan and Lombin 1984).

River Owena is a good source of domestic water to Owena people, during collection of water from this river, sediments sometimes get into the water. Therefore there is need to study the level of pollution by determining the microbial and physicochemical qualities of sediments because of its potential health hazard.

II. MATERIALS AND METHODS

The study area

River Owena is located about four kilometers from Joseph Ayobabalola University Ikeji Arakeji along Ilesha-Akure express way in Oriade local government area of Osun State, Nigeria on latitude N 7.403135 and longitude E 5.014589. It is a fresh water and free-flowing during raining season but slow-moving at the onset of dry season.

Study design

The analysis cover a period of six months, from July 2015 to January 2016 covering wet and dry season, sediment samples were collected at four sampling points (two each at both side of the bridge). The human activities around the river and the sampling points were evaluated and noted on monthly basis.

Sampling points

The four sampling points along the longitudinal course of River Owena (Figure 2), were river water flowing across the right-side of the bridge along Akure-Ilesha express-way at N 7^{o} 24′ 11″ E 5^{o} 0′ 52″, this point was about 64 cm deep (point One); Two- This point was across the river about one hundred metres away from sampling point One, It is about one hundred and thirty metres from the palm plantation at N 7^{o} 24′ 1″ E 5^{o} 0′ 49″, this point was about 80 cm deep; Three- This point was one hundred and fifty metres away from sampling point two. It was close to the farmland along the bank of the river at N 7 24′ 12″ E 5^{o} 0′ 48″, this point was about 50 cm deep; and four- This point was one hundred metres away from sampling point three at N 7^{o} 24′ 12″ E 5^{o} 0′ 46″, this point which was about 90 cm deep used to be the site where domestic water was collected. Water from this river is still collected for domestic purposes at the peak of dry season especially by the people living near and close to the river. Prior to this study, water from sampling point four was used for domestic purposes. Human activities on the river recently before the conclusion of this research included use of the water for cement block-moulding, car wash, commercial water supply to people, along the bank of the river are farms with crops such as maize, sugar cane and vegetables such as spinach and pepper plantation. The river also serves as recreational swimming pool for small children from nearby primary and secondary schools. Various types of birds and egrets were seen on the river.

Sample collection

Sediment samples were collected during wet and dry season from the four points. Sterile bottles of 500 ml were used for sample collection. Samples for microbial analysis were collected aseptically, labeled and stored in ice packed plastic coolers and transported to the laboratory where analysis within 24 hours of collection was carried out (Dubey and Maheshwari, 2004).

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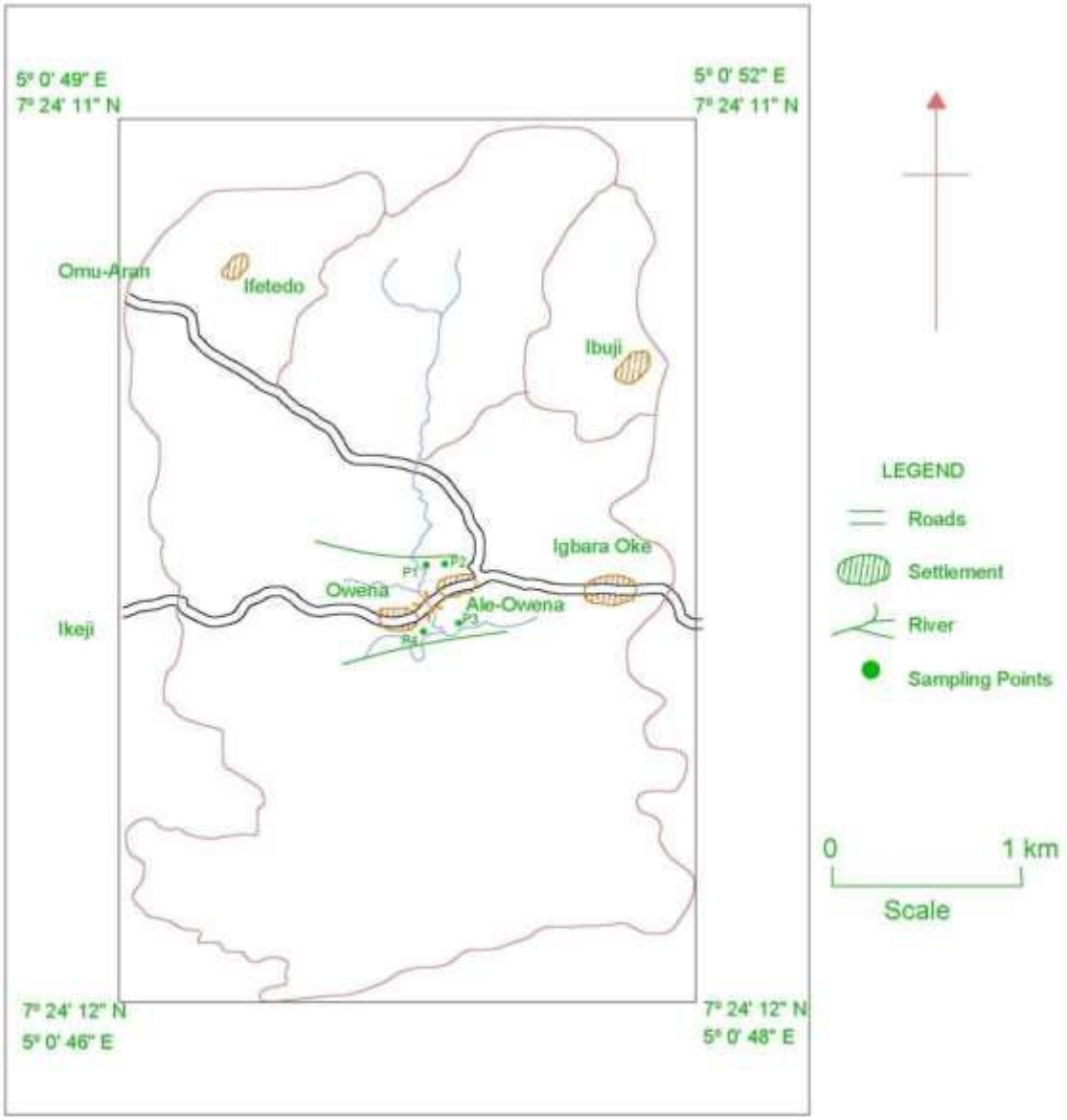

Fig.1: Map of River Owena sampling sites

Isolation of fungi from sediment samples

Pour plate method was used to isolate fungi associated with the sediment samples and the plates were incubated at 25°C for 48-72 hours for growth. The fungal counts were determined according to the methods of (Olayemi, 1990; APHA, 2002; Dubey and Maheshwari, 2004).

Purification, characterization and identification of fungal isolates

Pure cultures were obtained by sub-culturing different and distinct colonies onto sterile sabouraud dextrose agar plates containing 1% streptomycin. The fungal isolates were incubated and examined macroscopically and microscopically using lacto-phenol cotton blue to determine their colonial and morphological characteristics respectively. The isolates were identified according to the keys of Onions *et al*. (1981), Alabi (1994), Fawole and Oso (2001) and Dubey and Maheshwari (2004).

Isolation of bacteria from sediment samples

Pour plate method was used to isolate bacteria from sediment samples, during which the plates were incubated at 37°C for 24-48 hours. The bacterial counts were determined according to the methods of (Olayemi, 1990; APHA, 2002; Dubey and Maheshwari, 2004).

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Purification, characterization and identification of bacterial isolates

Pure cultures were obtained by streaking different and distinct colonies onto sterile nutrient agar plates. The pure cultures obtained were then transferred onto agar slants in McCartney bottles and incubated at 37ºC for 24 hours. The characterization and identification of bacterial isolates were based on the colonial morphology and biochemical tests carried out on pure culture of the isolates (Buchanan and Gibbons, 1974; Fawole and Oso, 2001; Dubey and Maheshwari, 2004; Garrity *et al*., 2004).

Trace and heavy metals

Trace and heavy metals determination was carried out using standard of Ravera *et al*. (2003) and Aiyesanmi (2006). This technique permits the measurement of a series of elements at the same time, the sediments were analyzed for zinc, manganese, cadmium, lead and iron, chromium, copper, nickel and their concentrations.

Determination of physicochemical characteristics of River Owena sediments

The pH, organic matter content and water holding capacity were determined using the method of Pramer and Schmidt (1984). Determination of sediments texture was done using the method of Awolumate, (1977); Olaitan and Lombin, (1984). Appropriate textural classification was then referred to get the soil texture.

Statistical analysis

All the data obtained in this study were subjected to analysis of variance (ANOVA) Followed by Duncans New multiple range test was used to separate means using SPSS 11.09 for windows and significance level was set at $p<0.05$ (Norusis, 2006).

III. RESULTS AND DISCUSSION

The pH, total alkalinity, organic matter content, nitrate and phosphate of the sediment samples ranged from 6.44 to 7.00 ±0.01, 19.40 to 138.16±0.01 mg/l $CaCo_3$, (% dry wt) 17.16 to 20.04%, 0.42 to 1.03±0.01mg/kg, and 28.87 to 30.00±0.01mg/kg respectively during wet season (Table 1). While pH, total alkalinity, organic matter content, nitrate and phosphate ranged from 6.98 to 8.00±0.01, 119.40 to 138.16±0.01 mg/l $CaCo_3$, (% dry wt) 27.26 to 35.32%, 1.55 to 2.30±0.01 mg/kg, 0.40 to 1.30±0.01 mg/kg respectively during dry season (Table 2).The pH of the sediments was slightly acidic during wet season and slightly alkaline during dry season which could be as a result of seasonal variation. This is similar to the results obtained by Tamaki *et al*.(2005) and Ekeanyanwu *et al*.(2010)with a pH of 6.4, that variation in pH depend on the season of the year.The total alkalinity, and phosphate values of the soil sediment were higher during dry season compare to wet season, while nitrate values was higher during wet season compare to dry season. Discharge and subsequent sedimentation of suspended particulates from phosphate and nitrogen fertilizers, and domestic wastes discharged into the river as a result of rainfall might have also contributed to the increase in sediment nitrate and phosphate contents. The findings in this study were consistent with those reported by (Tukura *et al*., 2005) and (Ekeanyanwu *et al*., 2010).

Table.1: Physicochemical properties of River Owena sediment (wet season)

Parameters	P1	P2	P3	P4
pH	6.76±0.01^{c}	7.01±0.01^{d}	6.67±0.01^{b}	6.45±0.01^{a}
P.Alkal. (mg/L $CaCo_3$)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T.Alkal. (mg/L $CaCo_3$)	73.81±0.01^{c}	80.21±0.01^{d}	67.42±0.01^{b}	63.35±0.01^{a}
NO_3^- (mg/kg)	2.02±0.01^{a}	2.95±0.01^{d}	2.66±0.01^{c}	2.42±0.01^{b}
PO_4^{3-} (mg/kg)	0.21±0.01^{b}	0.41±0.01^{c}	0.10±0.01^{a}	0.86±0.01^{d}
Organic matter (%)	17.15±0.01^{a}	19.35±0.01^{c}	20.03±0.01^{d}	17.76±0.01^{b}

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Table.2: Physicochemical properties of River Owena sediment (dry season)

Parameters	P1	P2	P3	P4
pH	8.01±0.01^{d}	7.67±0.01^{b}	7.71±0.01^{c}	6.97±0.01^{a}
P.Alkal. (mg/L $CaCo_3$)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

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T.Alkal. (mg/L $CaCo_3$)	119.14±0.01[b]	138.16±0.01[d]	122.31±0.01[c]	113.01±0.01[a]
NO_3^- (mg/kg)	1.87±0.01[b]	2.31±0.01[d]	1.98±0.01[c]	1.56±0.01[a]
PO_4^{3-} (mg/kg)	0.42±0.01[a]	1.01±0.01c	0.56±0.01[b]	1.31±0.01[d]
Organic matter (%)	30.31±0.0[c]	27.27±0.01[a]	35.31±0.01[d]	28.11±0.01[b]

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Texture and water holding capacity of River Owena sediment (wet and dry season)

The texture of the sediments of River Owena during wet season was composed of sand, clay and silt however, the percentage composition of sand to silt to clay in sediment samples points one, two, three and four was 75:12:13 %, 33:17:50 %, 62:18:20 % and 50:20:30 % respectively (Figure 2). The texture of the sediments of River Owena during dry season was composed of sand, clay and silt however, the percentage composition of sand to silt to clay in sediment samples points one, two, three and four was 82:2:16 %, 48:22:30 %, 32:25:43 % and 43:27:30% respectively (Figure 3). This was similar to the findings of Morgan (2010), which stated that the texture of a soil is not readily subject to change, so it is considered a basic property of a soil. The water holding capacity (ml/g) of sediment from River Owena from July 2015 to Jan. 2016 is shown in (Table 3). During wet season the values ranged from 0.323 ml/g in sample point one being lowest in September to a highest point of 0.738 ml/g in sample point two in July 2015.The water holding capacity ml/g of the sediment during dry season ranged from 0.381 ml/g in sample point three being lowest in Nov. and 1.779 ml/g in sample point two being highest in Dec. 2015., these variation may be due to the nature of the sediment, high rate of sedimentation and decomposition of foliage and other vegetative remains in the sediment Adegunwa (2003), who obtained 0.4 to 0.2 ml/g in his research.

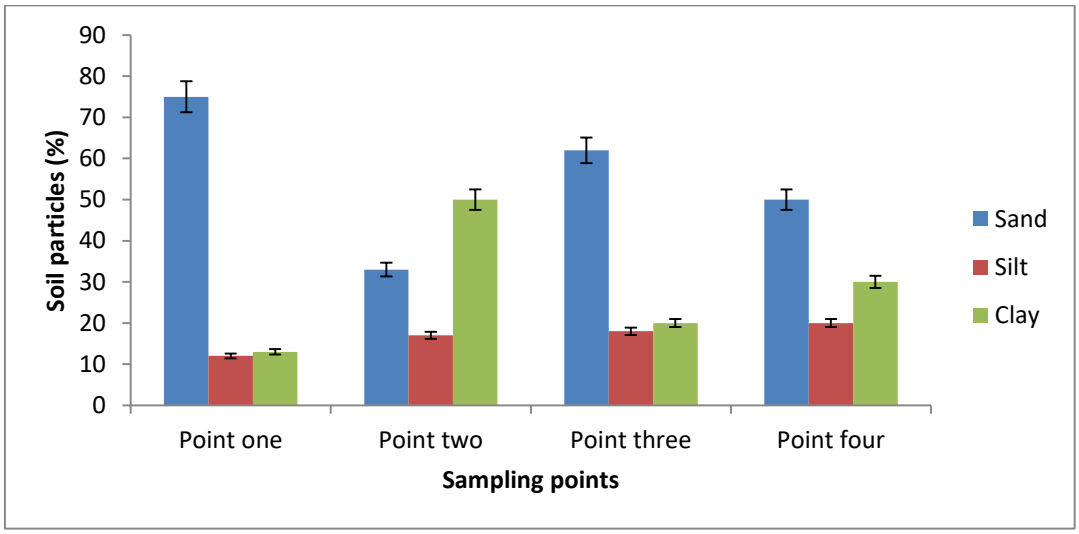

Fig.2: Wet season texture of River Owena sediments

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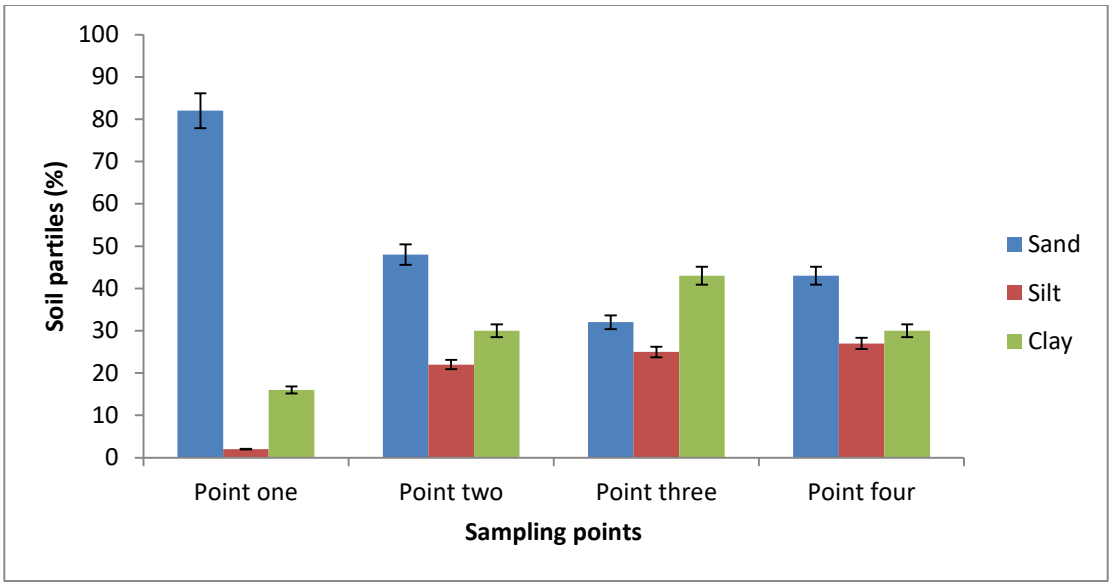

Fig.3: Dry season texture of River Owena sediments

Table.3: Water holding capacity (ml/g) of River Owena sediments

Months	Point One	Point Two	Point Three	Point Four
July. 2015	0.387±0.01^{a}	0.738±0.01^{d}	0.386±0.01^{b}	0.503±0.01^{c}
Aug. 2015	0.354±0.01^{a}	0.596±0.01^{d}	0.548±0.01^{c}	0.482±0.01^{b}
Sept. 2015	0.323±0.01^{d}	0.617±0.01^{c}	0.497±0.01^{b}	0.476±0.01^{a}
Oct. 2015	0.700±0.01^{c}	0.889±0.01^{d}	0.383±0.01^{a}	0.561±0.01^{b}
Nov. 2016	0.722±0.01^{c}	0.863±0.01^{d}	0.381±0.01^{a}	0.512±0.01^{b}
Dec. 2015	0.960±0.01^{a}	1.779±0.01^{d}	1.415±0.01^{c}	1.350±0.01^{b}
Jan. 2015	0.989±0.01^{a}	1.709±0.01^{d}	1.333±0.01^{c}	1.305±0.01^{b}

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Heavy metals of River Owena sediments (dry and wet season)

The mean concentrations of heavy metals (mg/kg) of sediments samples determined during wet season indicated iron has the highest values ranged from 1.89 to 3.00±0.01 mg/kg, while cadmium had the least values of 0.01 to 0.04±0.01 mg/kg (Figure 4). Similarly during dry season iron had the highest values ranged from 2.38 to 4.10±0.01 mg/kg while, cadmium had the least values of 0.04 to 0.12±0.01 mg/kg (Figure 5).This result is similar to the one obtained by Dosumu *et al*. (2003); Ravera *et al*. (2003) and Adefemi and Awokunmi (2010), they stated that increase of heavy metals could be due to the precipitation of these elements in this aquatic environment.

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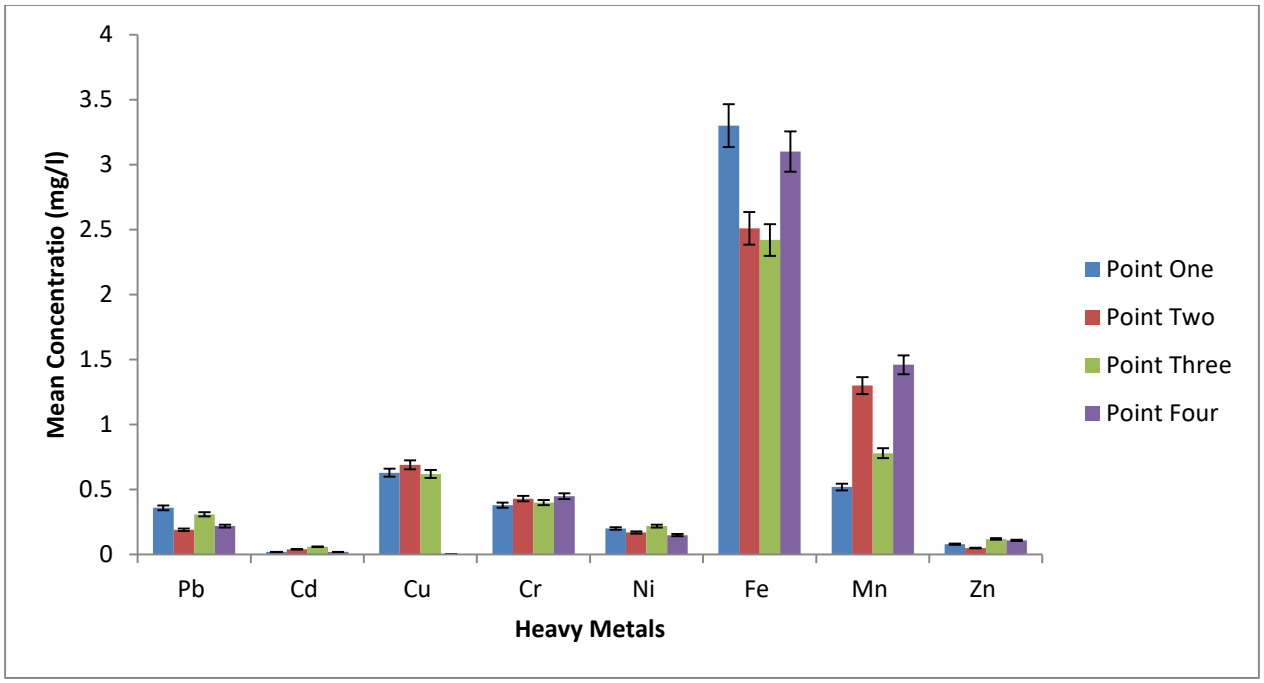

Fig.4: Wet season mean concentration of heavy metals from River Owena sediments

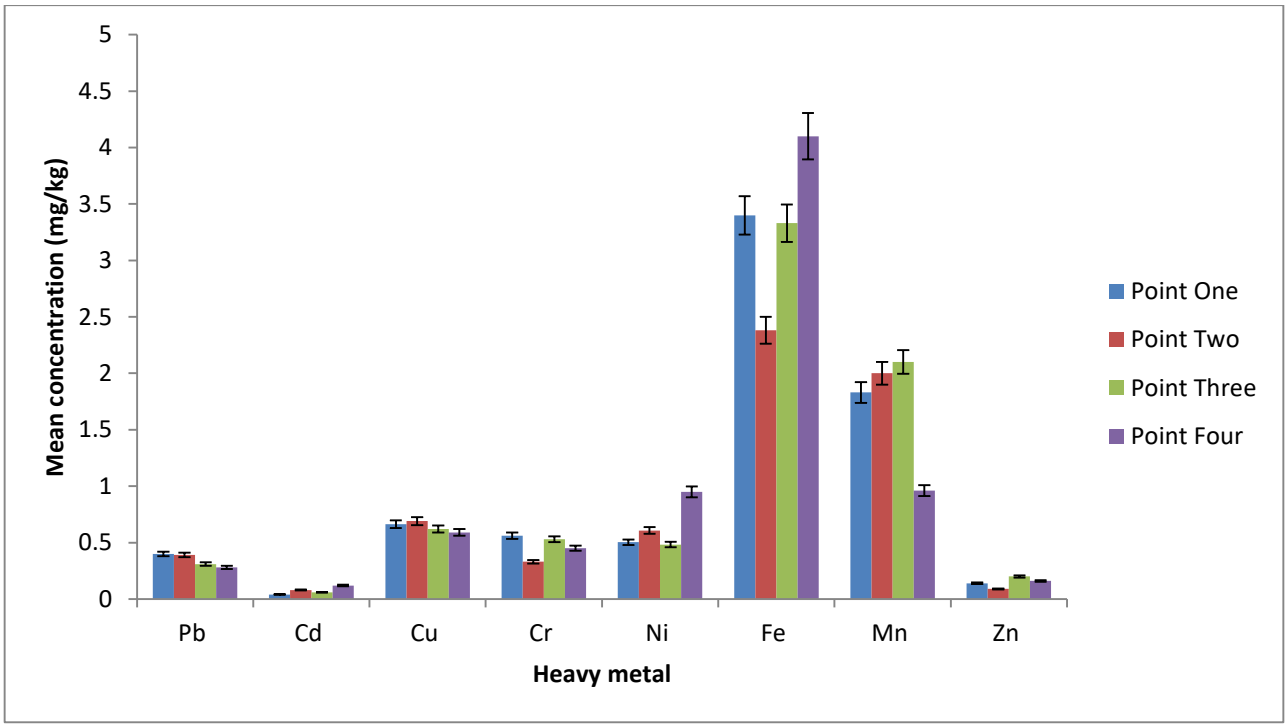

Fig.5: Dry season mean concentration of heavy metals from River Owena sediment

The total bacterial counts (cfu/g) of River Owena sediments

The total bacterial counts for the four samples points of sediments determined from July 2015 to Jan. 2016 covering (wet and dry seasons) ranged from 4.1 x 10^3 to 3.02 x 10^3±0.01 cfu/g (Table 4). The lowest count occurred in sample point two in August, 2015, while, the highest count occurred in sample point one in July, 2015. Wet season recorded a range of 3.02 x 10^3 cfu/g in July, 2015 to 4.1 x 10^3±0.01 cfu/g in August, 2015, but dry season had a range

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of 1.23 x 10^3±0.01 cfu/g in Nov., 2015 to 3.0x10^3±0.01 cfu/g in December. Total bacterial counts of the four samples points of sediments determined illustrated that wet season had higher count compared to dry season. Sediment is novel in the sense that the organism is found in fecal contaminated water and its presence in sediment too is reported by few researchers. Erah *et al*. (2011) in their study of quality of underground water in Benin found *Escherichia coli* and *Streptococcus faecalis* to be the major contaminants at abnormal levels.

Table.4: Total bacterial counts of River Owena sediments

Months	Point One	Point Two	Point Three	Point Four
July.2015	3.02 x10^3±0.01^d	1.51 x10^3±0.01^c	6.4 x10^3±0.01^a	7.6 x10^3±0.01^b
Aug. 2015	5.5 x10^3±0.01^b	4.1 x10^3±0.01^a	7.5 x10^3±0.01^c	5.5 x10^3±0.01
Sept. 2015	5.0 x10^3±0.01^a	5.1 x10^3±0.01^b	6.7 x10^3±0.01^d	6.2 x10^3±0.01^c
Oct. 2015	1.10 x10^3±0.01^c	6.3x10^3±0.01^a	8.6 x10^3±0.01^b	1.20 x10^3±0.01^d
Nov. 2015	1.23 x10^3±0.01^d	9.9 x10^3±0.01^b	9.1 x10^3±0.01^a	9.9 x10^3±0.01^b
Dec. 2015	4.3 x10^3±0.01^c	3.7 x10^3±0.01^b	6.2 x10^3±0.01^d	3.0 x10^3±0.01^a
Jan. 2016	5.8 x10^3±0.01^b	6.2 x10^3±0.01^c	6.2 x10^3±0.01^c	3.8 x10^3±0.01^a

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Bacterial isolates of River Owena sediments

A total of fifteen bacterial species were isolated from sediments which included *Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcu saureus, S. epidermidis, Salmonella enteritidis, Bacillus subtilis, Serratia marcescens, Shigella sonnei, Bacillus cereus , Micrococcus luteus, Micrococcus varians, Pseudomonas aeruginosa, P. vulgaris* and *Erwinia amylovora* (Table 5). The frequencies of occurrence of bacterial isolates ranged from 1.8-15.0% in sediments from River Owena *Enterobacter aerogenes* occurred most frequently (15%), while *M. luteus, B. subtilis, Proteus vulgaris*, occurred least (1.8%).The finding was similar to that of Adegunwa (2003) in Oyun River, Ishii *et al*., (2006) and Erah *et al*., (2011).

Table.5: Bacterial frequency distribution of River Owena sediments

Isolates	% Frequency of Occurrence
Escherichia coli	14.0
Enterobacter aerogenes	15.0
Klebsiella pneumoniae	4.8
Staphylococcus aureus	5.7
Micrococcus luteus	1.8
M. varians	8.5
Pseudomonas aeruginosa	5.8
Bacillus subtilis	1.8
Salmonella enteritidis	8.4
Proteus vulgaris	1.8
S. epidermidis	9.3
Erwinia amylovora	3.8
B. cereus	6.7
Shigella sonnei	6.3
Serratia marcescens	6.3
	Total 100

Values represents means ± Standard error of means.

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Total fungal counts (sfu/g) of River Owena sediments

Total fungal counts for the four samples points of sediments determined from July 2015 to Jan. 2016 covering (wet and dry seasons) ranged from 4 x 10^3 to 7.6 x 10^3±0.01 sfu/g (Table 6). The lowest count occurred in sample point four in Dec., 2015.While, the highest count occurred in sample point one in Aug., 2015.Wet season had a range of 2.3 x 10^3±0.01 sfu/g in Sept., 2015 to 7.6 x 10^3±0.01sfu/g in Aug., 2015. Dry season had a range of 4 x 10^3±0.01 sfu/g in Dec., 2015 to 4.9 x10^3±0.01sfu/g in Nov. This result is similar to the result obtained by Alabi, (1994).

Table.6: Total fungal counts of River Owena sediments

Months	Point One	Point Two	Point Three	Point Four
July. 2015	3.3x10^3±0.01^c	7.1x10^3±0.01^d	3.2x10^3±0.01^a	4.3 x10^3±0.01^b
Aug.2015	7.6 x10^3±0.01^d	5.1 x10^3±0.01^c	2.9 x10^3±0.01^a	5.0 x10^3±0.01^b
Sept. 2015	2.3 x10^3±0.01^a	2.8 x10^3±0.01^b	3.0 x10^3±0.01^c	6.5 x10^3±0.01^d
Oct. 2015	4.6 x10^3±0.01^b	5.3 x10^3±0.01^c	3.7 x10^3±0.01^a	5.3 x10^3±0.01^c
Nov.2015	3.2 x10^3±0.01^a	3.9 x10^3±0.01^b	4.9 x10^3±0.01^d	4.4 x10^3±0.01^c
Dec.2015	6 x10^3±0.01^b	1.0x10^3± 0.01^d	7 x10^3±0.01^c	4 x10^3±0.01^a
Jan. 2016	2.7 x10^3±0.01^b	4.0x10^3±0.01^d	2.3 x10^3±0.01^a	3.0 x10^3±0.01^c

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Fungal isolates of River Owena sediments

The ten fungal species isolated from the sediments included *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Mucor mucedo*, *M. plumbeus*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Rhizopus oryzae* (Table 7). *Aspergillus niger* was the most frequently isolated fungus occurring in all the samples, with a frequency of occurrence of 22.2% and *Penicillium chrysogenum* occurred less abundantly in the samples which is similar to those obtained by Alabi, (1994)in a similar study on Oyun River and Akpata and Ekundayo, (1983).

Table.7: Fungal frequency distribution of River Owena sediments

Isolates	Frequency of Occurrence
Aspergillus niger	22.2
Cladosporium herbarum	7.8
A. flavus	12.5
Mucor mucedo	4.4
A. fumigatus	10.5
Fusarium oxysporum	6.5
M. plumbeus	12.5
Penicillium chrysogenum	3.4
Rhizopus stolonifer	10.7
R. oryzae	9.5
	Total 100

Values represents means ± Standard error of means

Fungi and bacteria are part of the group of living organisms found in sediments, they may be found in the surface of decaying plant or animals materials in river. Human activities such as farming, swimming, grazing of animals and car wash park in and around the river bank which will increase the contamination level along-side run-offs should be reduced or totally discouraged.

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Microbial and Physicochemical Qualities of River Owena Water: An Important Source of Domestic Water in Owena Metropolis

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***Abstract*—** *Microbial quality, physicochemical parameters and heavy metals determination of water samples from River Owena, Oriade local government area, Owena, Nigeria. For period of dry and wet seasons. The pH of water ranged from 6.72 to 7.52±0.01, dissolved oxygen ranged from 5.95 to 7.63±0.01 mg/l, biological oxygen demand ranged from 3.20 to 11.47±0.01 mg/l. Other physicochemical parameters measured for the water included alkalinity, turbidity total solids, suspended solids, dissolved solid, total hardness, calcium hardness, magnesium hardness, temperature and conductivity which were of higher values during dry season compared to wet season. The MPN index ranged from 7 to1100 cfu/100 ml, mean concentrations of heavy metals in water such as iron, zinc, manganese, lead, chromium, cadmium, nickel and copper, iron had the highest values of 1.1 to 3.3±0.01 mg/l and cadmium had lowest values of 0.01 to 0.06±0.01 mg/l. A total of ten bacterial species were isolated from River Owena water, which included Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Staphylococcus aureus, S. epidermidis, Salmonella enteritidis, Micrococcus luteus, Pseudomonas aeruginosa, Proteus vulgaris and Shigella sonnei. A total of eight fungal species were isolated from water of River Owena, which included Aspergillus niger, A. flavus, A. fumigatus, Cladosporium herbarum, Mucor mucedo, M. plumbeus, Fusarium oxysporum and Rhizopus oryzae. The total bacterial count of the water ranged from 2.2 x 10^3 to 2.25 x 10^3 ±0.01 cfu/ml. The total fungal count of the water ranged from 6 x 10^3 to 1.22 x 10^3±0.01 sfu/ml. However, the quality of the water from this river did not conform to the drinking standard of world health organization (1984) hence poor due to the presence of some pathogenic microorganisms and heavy metals which are of public health significance, but it could be improved upon with appropriate treatment.*

***Keywords*— Microbial, Physicochemical, Heavy metals, Water, Wet and Dry season, Drinking, Health.**

I. INTRODUCTION

The current concern with regards to environmental quality is partly focused on water because of its importance in maintaining the human health and health of the ecosystem. Freshwater reservoirs play an important role in the livelihood of human populations. They are used as a source of domestic water supply, irrigation, fishery development, hydropower generation and flood control (Kitur, 2009). Additional benefits of the reservoirs are tourist attraction and opening up of new areas for development (Kitur, 2009). According to Junk (2002) and Dudgeon (2006), freshwater ecosystems are vulnerable to human impacts, hence they are likely to be influenced by reservoir catchment activities. This is because terrestrial ecosystems have linkages with aquatic ecosystems (UNEP, 2000). Contamination of aquatic ecosystems with a wide range of pollutants has become a matter of concern over the past few decades (Dirilgen, 2001; Vutukuru, 2005).

FAO (1992) noted that the contamination of water supplies from both natural and anthropogenic sources has impacted on the health and economic status of populations. Human activities cause pollutants such as heavy metals, pesticides and herbicides to enter aquatic ecosystems. Thus, heavy metal pollution is growing at an alarming rate and has become an important worldwide problem (Malik *et al.*, 2010). Increase in population, urbanization, industrialization and agricultural practices as well as lack of environmental regulations have further aggravated the situation (Gupta *et al.*, 2009).

Heavy metals cannot be degraded, but they are deposited, assimilated or incorporated in water, sediments and aquatic biota causing heavy metal pollution in water bodies (Linnik and Zubenko, 2000; Malik *et al.*, 2010). Heavy metals in water can originate both from natural sources, industrial, agricultural and domestic activities in the drainage basin of a water system. As the metal levels in many aquatic ecosystems increase due to anthropogenic activities, they

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raise the concern on metal bioaccumulation through the food chain and related human health hazards (Wright and Welbourn, 2002; Indrajith *et al.*, 2008; Agah *et al*., 2009).

Raw influent includes household waste liquid from toilets, baths, showers, kitchens, sinks, and so on and so forth that is disposed off via sewers. In many areas, sewage also includes liquid waste from industry and commerce. As rainfall runs over the surface of roofs and the ground, it may pick up various contaminants including soil particles and other sediment, heavy metals, organic compounds animal waste and oil and grease. These contaminants eventually get into the water bodies where they negatively impacts their influence. Since all natural waterways contain bacteria and nutrients, almost any waste compounds introduced into such waterways will initiate biochemical reactions (Gupta *et al.,* 2009). The measurement of dissolved oxygen (DO) can be used to indicate the degree of pollution by organic matter, the destruction of organic substances and the level of self-purification of the water. Its determination is also used in the measurement of biochemical oxygen demand (BOD) (Eniola, 2005; Okonko *et al.,* 2008). From a physical point of view, the suspended solids can lead to the development of sludge deposits and anaerobic conditions when discharged into the receiving environment (Maiti 2004). Chemically, wastewater is composed of organic and inorganic compounds as well as various gases. Organic components may consist of carbohydrates, proteins, fats and greases, surfactants, oils, pesticides, phenols and so on and so forth (Tchobanoglous *et al*. 2003., Maiti, 2004). Drinking water treatment efforts can become weighed down when water resources are heavily polluted by wastewater microorganism species.

Water is of great importance to all living organisms and it is essential to life. It is one of the most demanded of all urban and rural amenities and it is indispensable for human's activities. The importance of River Owena in Ifedore Local Government, Ondo State, and Oriade local government area of Osun state to Owena town and local native populace of some communities near the river in term of access to domestic water and small scale farming around the river inspired the research. Therefore, this work focused on the microbial and physicochemical qualities of River Owena water with the aim of ascertaining its qualities.

II. MATERIALS AND METHODS

The study area

River Owena is located about four kilometers from Joseph Ayobabalola University Ikeji Arakeji along Ilesha-Akure express way in Oriade local government area of Osun State, Nigeria on latitude N 7.403135 and longitude E 5.014589. It is a fresh water and free-flowing during raining season but slow-moving at the onset of dry season. Figure 1 showed the study area and point of samples collection

Study design

The analysis cover a period of six months, from July 2015 to January 2016 covering wet and dry season, water samples were collected at four sampling points (two each at both side of the bridge) once a month. The human activities around the river and the sampling points were evaluated and noted on monthly basis.

Sample collection

Water samples were collected during wet and dry season from four sampling points. Sterile bottles of 500 ml were used for sample collection. All containers were rinsed at least three times with water that is to be analyzed. Samples for microbial analysis were collected aseptically, labeled and stored in ice packed plastic coolers and transported to the laboratory where analysis was done within 24 hours of collection (Dubey and Maheshwari, 2004).

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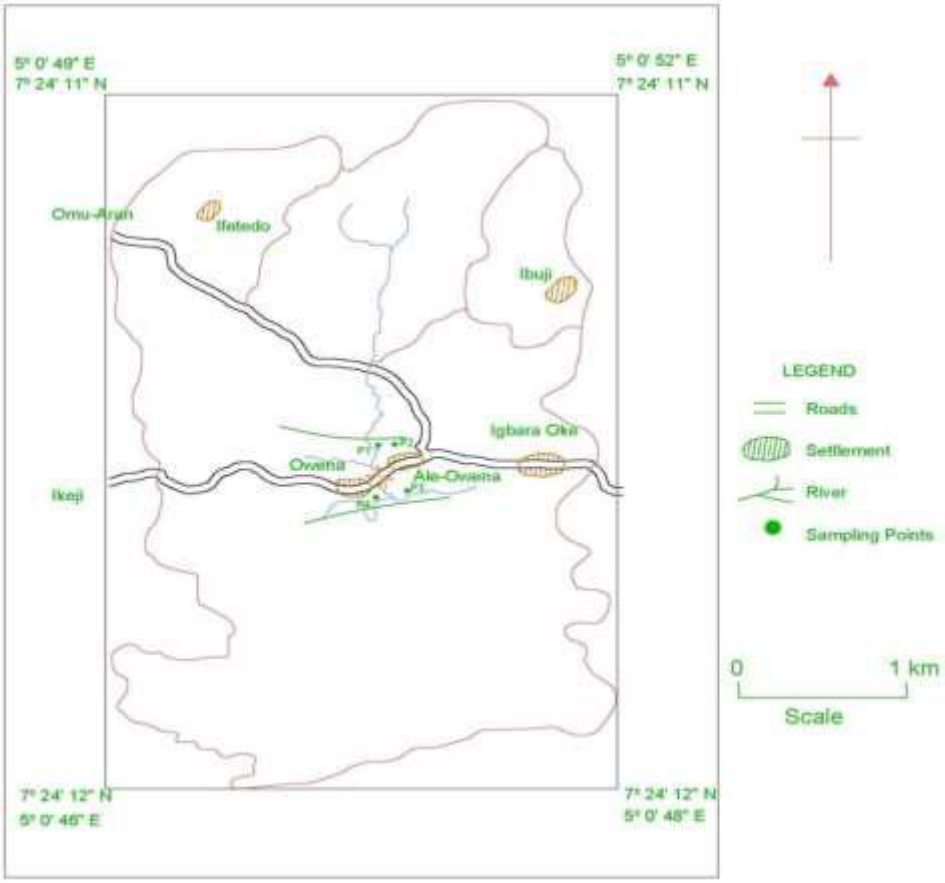

Fig.1: Map of River Owena sampling sites

Test for water coliform

This was carried out in three stages which were presumptive test, confirmatory test and completed test, using standard method of (APHA, 2002)

Isolation of bacteria from water

Pour plate method was used to isolate bacteria from water samples and bacterial count determined using standard method of APHA, (2002); Dubey and Maheshwari, (2004).

Purification, characterization and identification of bacterial isolates

Pure cultures were obtained by streaking different and distinct colonies onto sterile nutrient agar plates and incubated for growth. Characterization and identification of bacterial isolates were based on the colonial morphology and biochemical tests carried out on pure culture of the isolates (Buchanan and Gibbons, 1974; Fawole and Oso, 2001; Dubey and Maheshwari, 2004; Garrity *et al*., 2004).

Isolation of fungi from water samples

Pour plate method was used to isolate fungi from water samples, one milliliter of the water from the different points were serial diluted to obtain 10^{-1} to 10^{-9} dilutions and pour plate method was done. The plates were incubated at 28±2°C for 48-72 hours and fungal counts was determined (Olayemi, 1990; APHA, 2002; Dubey and Maheshwari, 2004).

Purification, characterization and identification of fungal isolates

Pure cultures were obtained, by subculturing the grown colonies and culture of the fungal isolates were examined macroscopically and microscopically using lacto-phenol cotton blue to determine their colonial and morphological characteristics respectively. The isolates were identified according to the keys of Onions *et al*. (1981), Alabi (1994), Fawole and Oso (2001) and Dubey and Maheshwari (2004).

Determination of physicochemical characteristics of water samples

Physicochemical characteristics of the water samples from four sampling points were determined once a month. The physicochemical characteristics such as pH, turbidity, total solids, suspended solids, dissolved solids, colour, total hardness, alkalinity, temperature, conductivity, dissolved oxygen, biological oxygen demand, nitrate, phosphate and

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chloride, were determined using standard method of (Anon, 2002), trace and heavy metals determination was carried out following the method of Ravera *et al*. (2003) and Aiyesanmi (2006).

III. RESULTS AND DISCUSSION

The physicochemical characteristics of River Owena water during wet and dry season are illustrated in table 1 and 2.

The temperature obtained from the water samples from River Owena ranged from 25.98 to 27.11±0.01 ºC, the colour values ranged from 31.00 to 34.10±0.01 (Pt/Co unit), turbidity values ranged from 7.98 to 8.78±0.01 NTU, conductivity values ranged from 111.03 to 112.37±0.01 µS/cm, total dissolved solid values ranged from 54.37 to 56.76±0.01 mg/l, total suspended solid values ranged from 2.03 to 3.12±0.01 mg/l, total solid values ranged from 57.49 to 58.79±0.01 mg/l during wet season (Table 1). Also, the pH of water samples from River Owena ranges from 6.72 to 6.86±0.01, which was slightly neutral, the total alkalinity values ranged from 59.33 to 66.33±0.01 mg/l $CaCo_3$, total hardiness values ranged from 66.00 to 71.00±0.01 mg/l $CaCo_3$, calcium hardiness values ranged from 14.53 to 16.93±0.01 mg/l, magnesium hardiness had values ranging from 6.52 to 8.21±0.01 mg/l, sodium hardiness values ranging from 11.33 to 18.66±0.01 mg/l, potassium values ranged from 15.00 to 2.33±0.01 mg/l, dissolved oxygen (DO) ranged from 6.73 to 7.63±0.01 mg/l, biochemical oxygen demand (BOD) values ranged from 10.70 to 11.47±0.01 mg/l. chemical oxygen demand (COD) values ranged from 18.50 to 19.80 mg/l, chloride values ranged from 18.17 to 20.00±0.01 mg/l, nitrate values of ranged from 1.03 to 1.30±0.01 mg/l, and phosphate values ranged from 0.08 to 0.17±0.01 mg/l during wet season (Table 1).

However the temperature obtained from the water samples from River Owena ranged from 31.90 to 32.20±0.01 ºC, this was higher compare to that of wet season which may be due to the high intensity of the sun during dry season. Temperature is an important factor in the survival of microorganisms in surface water as it affects the metabolic activities, water activity of microbial cells, Ogunnusi and Olanipekun (2010) and Ogbonna *et al*. (2010). Colour values ranged from 25.67 to 29.33±0.01 (Pt Co unit) turbidity values ranged from 5.88 to 7.83±0.01 NTU, High turbidity levels were obtained in all the samples with the lowest turbidity value obtained during dry season, while in December which was harmattan period and no rain had the lowest level hence, its clear nature. Wet season had higher values and the water colour of the river turns brownish. Only sample point three in dry season had a value that fall within the permissible limit of WHO (1984), the rest samples for all the months showed pronounced deviations from WHO standard of 5.0 NTU. The values in all the samples during the period of wet season were high when run-off from land enters the river which is similar to the results obtained by Akpata and Ekundayo (1983). Conductivity values ranged from 138.90 to 148.10±0.01 µS/cm, total dissolved solid values ranged from 57.42 to 70.76±0.01 mg/l, total suspended solid values ranged from 1.34 to 2.01±0.01 mg/l, and total solid values ranged from 58.76 to 78.28±0.01 mg/l during dry season (Table 2). In addition, the pH of water samples from River Owena ranged from 6.94 to 7.52±0.01. Sample point four had the highest value of 7.52±0.01 which was slightly alkaline and sample point one had the lowest of 6.94±0.01 which was neutral slightly, the pH of water samples from River Owena was observed to be higher during dry season compare to wet season which was similar to that obtained by Ogunnusi and Olanipekun (2010) in a similar work in Oyo town with their own range of 6.39 to 8.95. This was also obtained by Olayemi (1994) and Ogbonna *et al*. (2010) of various water samples, variations in the pH is related to seasonal changes of rain and dryness. Total alkalinity values ranged from 98.23 to 107.20±0.01 mg/l $CaCo_3$, total hardiness values ranged from 75.20 to 90.67±0.01 mg/l $CaCo_3$, calcium hardiness values ranged from 14.98 to 17.96±0.01 mg/l, magnesium hardiness values ranged from 8.39 to 11.72±0.01 mg/l, sodium hardiness values ranged from 13.77 to 18.67±0.01 mg/l, potassium values ranged from 20.43 to 24.97±0.01 mg/l, these values were higher during dry season than wet season and were within the permissible limit of NSDQW (2007) which is 150 mg/l, similar to that obtained by Akpata and Ekundayo (1983), Ogunnusi and Olanipekun (2010) and Ogbonna *et al*. (2010). Dissolved oxygen (DO) ranged from 5.95 to 6.23±0.01 mg/l biochemical oxygen demand (BOD) values ranged from 3.20 to 5.00±0.01 mg/l. Chemical oxygen demand (COD) values ranged from 13.57 to 14.40±0.01 mg/l, chloride values ranged from 24.90 to 34.03±0.01, nitrate values of ranged from 0.21 to 0.39±0.01 mg/l, and phosphate values ranged from 0.18 to 0.23±0.01 mg/l during dry season (Table 2), Chloride values of the water was higher during dry season than wet season, the presence of chloride ions in the water bodies is an indication that chloride ion is higher in this environment, at elevated levels. Chloride can inhibit plant growth, slow reproduction and reduce diversity of aquatic life, this is similar to results obtained by Odeyemi *et al*., (2009). Nitrate values of the water had a lower range during dry season than wet season. The presence of *Proteus*

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specie as part of the isolates in this study could have a contributory factor to the levels of nitrate obtained. Since *Proteus* specie live in the soil and water, and organic detritus consists of large amounts of protein and nucleic acids from dead organisms and nitrogenous animal wastes such as urea and uric acid. This is similar to results obtained by Odeyemi *et al*., (2009). Phosphate values were higher during dry season compare to wet, this is similar to results obtained by Odeyemi *et al*., (2009).

Table.1: Physicochemical properties of River Owena water (wet season)

Parameters	**P1**	**P2**	**P3**	**P4**
Temp (°C)	27.11±0.01^{c}	25.98±0.01^{a}	27.10±0.01^{c}	26.50±0.01^{b}
Colour (Pt/Co unit)	34.09±0.01^{d}	32.46±0.01^{b}	31.00±0.01^{a}	33.27 ±0.01^{c}
Turbidity (NTU)	8.51±0.01^{b}	7.95±0.01^{a}	8.78±0.01^{d}	8.61±0.01^{c}
Cond. (µS/cm)	111.01±0.00^{c}	108.73±0.01^{a}	109.01±0.00^{b}	112.38±0.01^{c}
TD.Solid (mg/L)	54.87±0.01^{a}	54.37±0.01^{c}	54.49±0.01^{d}	56.76±0.01^{b}
TS.Solid (mg/L)	2.92±0.60^{b}	3.12±0.00^{d}	3.02±0.01^{c}	2.03± 0.60^{a}
T.Solid (mg/L)	57.79±0.01^{b}	57.49±0.01^{a}	57.51±0.01^{a}	58.79±0.01^{c}
pH	6.85 ±0.01^{b}	6.88±0.01^{c}	6.88±0.01^{c}	6.71 ±0.01^{a}
P.Alkal. (mg/L $CaCo_3$)	00.00	00.00	00.00	00.00
T.Alkal. (mg/L $CaCo_3$)	66.32±0.01^{d}	65.32±0.01^{c}	59.32±0.01^{a}	60.01±0.01^{b}
T.Hard. (mg/L $CaCo_3$)	66.67±0.01^{b}	68.67±0.01^{c}	66.01±0.01^{a}	71.02±0.01^{d}
Ca^{2+} (mg/L)	15.12±0.01^{b}	14.52±0.01^{a}	16.92±0.01^{c}	14.41±0.01^{a}
Mg^{2+} (mg/L)	7.33 ±0.01^{b}	6.52±0.01^{a}	7.35±0.01^{c}	8.21±0.01^{d}
Na^{+} (mg/L)	18.66±0.01^{a}	11.33±0.01^{c}	18.66±0.01^{a}	16.32±0.01^{b}
K^{+} (mg/L)	16.32±0.01^{a}	15.01±0.01^{b}	20.32±0.01^{d}	16.66±0.01^{c}
DO (mg/L)	7.62±0.01^{a}	7.62±0.01^{a}	6.72±0.01^{c}	7.26±0.01^{b}
BOD_5 (mg/L)	10.72±0.01^{a}	10.86±0.01^{b}	10.72±0.01^{a}	11.46±0.01^{c}
COD (mg/L)	18.51±0.01^{a}	19.81±0.01^{b}	19.61±0.01^{c}	19.02±0.01^{d}
Cl^{-} (mg/L)	20.01±0.01^{b}	18.66±0.01^{b}	16.66±0.01^{a}	19.32±0.01^{b}
NO_3^{-} (mg/L)	1.31±0.01^{d}	1.02±0.01^{a}	1.04±0.01^{b}	1.21±0.01^{c}
PO_4^{3-}(mg/L)	0.06±0.01^{a}	0.16±0.01^{c}	0.15±0.01^{c}	0.12±0.01^{b}

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at p<0.0

Table.2: Physicochemical properties of River Owena water (dry season)

Parameters	**P1**	**P2**	**P3**	**P4**
Temp (°C)	32.11±0.01^{c}	31.97±0.01^{b}	31.91±0.01^{a}	32.21±0.01^{d}
Colour (Pt/Co unit)	29.33±0.01^{d}	26.01±0.01^{b}	25.68±0.01^{a}	27.05±0.01^{c}
Turbidity (NTU)	7.82±0.01^{d}	6.36±0.01^{b}	5.87±0.01^{a}	7.01±0.01^{c}
Cond. (µS/cm)	138.91±0.01^{a}	139.02±0.01^{a}	141.65±0.01^{a}	145.77±0.01^{b}
TD.Solid (mg/L)	68.92±0.01^{c}	57.42±0.01^{a}	60.62±0.01^{b}	70.76±0.01^{d}
TS.Solid (mg/L)	1.97±0.01^{c}	1.34±0.01^{a}	1.52±0.01^{b}	2.01±0.01^{d}
T.Solid (mg/L)	70.89±0.01^{c}	58.76±0.01^{a}	62.14±0.01^{b}	78.28±0.01^{d}
pH	6.94±0.01^{a}	7.22±0.01^{c}	7.12±0.01^{b}	7.52±0.01^{d}
P.Alkal. (mg/L $CaCo_3$)	00.00	00.00	00.00	00.00
T.Alkal. (mg/L $CaCo_3$)	98.22±0.01^{a}	100.55±0.01^{b}	111.33±0.01^{d}	107.21±0.01^{c}
T.Hard. (mg/L $CaCo_3$)	89.76±0.01^{c}	88.52±0.01^{b}	75.21±0.01^{a}	90.66±0.01^{d}
Ca^{2+} (mg/L)	14.98±0.01^{a}	17.96±0.01^{b}	17.01±0.01^{b}	14.97±0.01^{a}
Mg^{2+} (mg/L)	11.72±0.01^{d}	9.57±0.01^{b}	8.38±0.01^{a}	10.32±0.01^{c}
Na^{+} (mg/L)	17.01±0.01^{c}	13.76±0.01^{a}	18.66±0.01^{d}	14.62±0.01^{b}
K^{+} (mg/L)	21.96±0.01^{b}	22.12±0.01^{c}	24.96±0.01^{d}	20.42±0.01^{a}

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DO (mg/L)	7.28±0.01^{d}	5.94±0.01^{a}	5.98±0.01^{b}	6.23±0.01^{c}
BOD_5 (mg/L)	4.42±0.01^{c}	3.91±0.01^{b}	3.21±0.01^{a}	5.01±0.01^{d}
COD (mg/L)	13.81±0.01^{b}	14.41±0.01^{c}	13.81±0.01^{b}	13.56±0.01^{a}
Cl^- (mg/L)	34.02±0.01^{d}	25.93±0.01^{b}	25.97±0.01^{c}	24.91±0.01^{a}
NO_3^- (mg/L)	0.35±0.01^{b}	0.34±0.01^{b}	0.38±0.01^{d}	0.22±0.01^{a}
PO_4^{3-}(mg/L)	0.15±0.01^{a}	0.21±0.01^{b}	0.16±0.01^{a}	0.22±0.01^{b}

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Water purity assessment of River Owena (dry and wet season)

Most Probable Number index (MPN) of water samples from River Owena in July 2015 to Jan. 2016 illustrated that wet season had the highest MPN of 1100 cfu/100 ml in August and September, point three and point four, lowest value of 73 cfu/100 ml in September, point three (Figure 3). The water sample had highest MPN of 150 cfu/100 ml in Oct., point one and lowest value of 7 cfu/100 ml in November, point two during dry season (Figure 4). The result indicated that the river was contaminated with feaces, thus contain pathogens that can cause gastroenteritis, hence is neither potable nor fit for domestic use and recreational activities. The higher microbial counts obtained during the wet season was due to increased nutrients and aeration which enhanced decomposition of organic matter, hence increase in bacterial populations as supported by Gareth (1973) and Olayemi (1990).

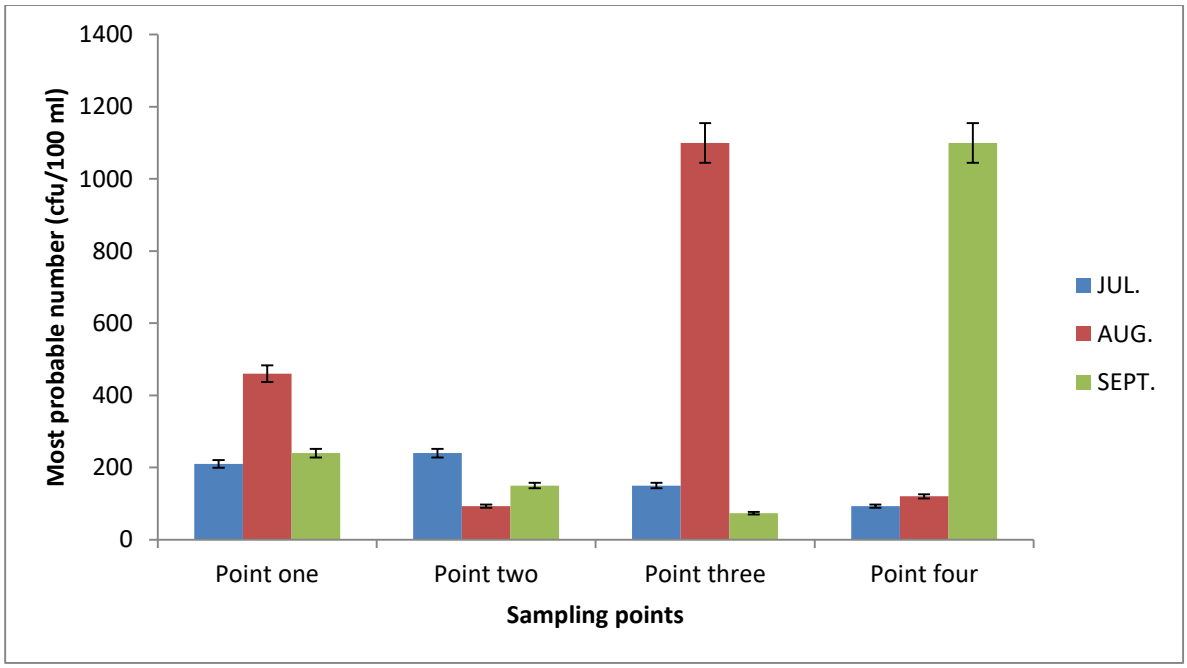

Fig.3: Wet season most probable number index values of River Owena water

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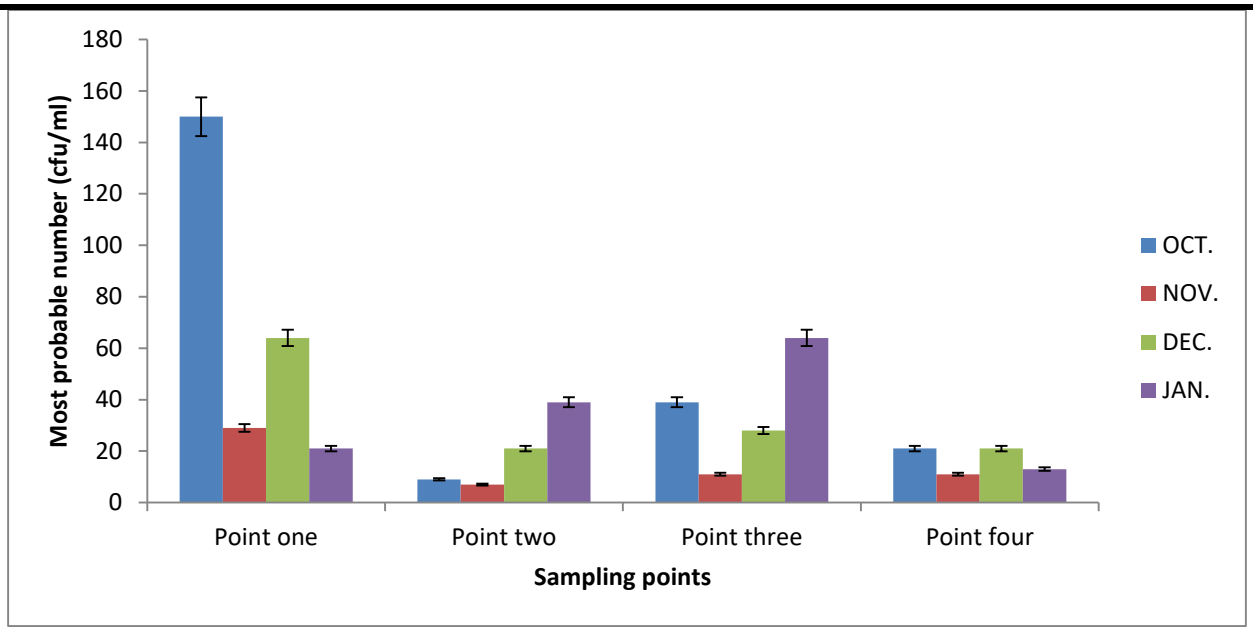

Fig.4: Dry season most probable number index values of River Owena water

Heavy metals of River Owena water (dry and wet season)
The mean concentrations of heavy metals (mg/l) of water samples determined during the wet season showed that iron has the highest values ranged from 1.1 to 1.21±0.01 mg/l, while cadmium had the least values of 0.01 to 0.02±0.01 mg/l (Figure 5). During dry season iron had the highest values ranged from 2.42 to 3.30±0.01 mg/l, while cadmium had the least values of 0.02 to 0.06±0.01 mg/l (Figure 6). The mean concentrations of heavy metals (mg/l) of water samples was found to be higher during dry season compare to wet season. This result is similar to the one obtained by Dosumu *et al*. (2003) and Adefemi and Awokunmi (2010).

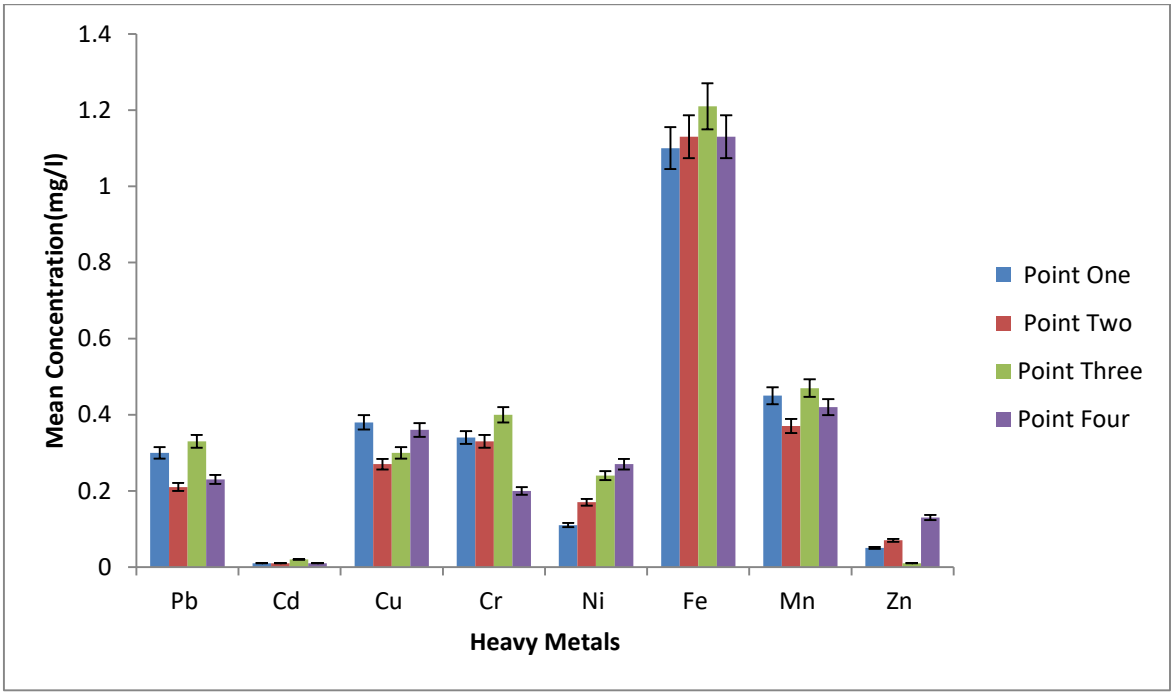

Fig.5: Wet season mean concentration of heavy metals of River Owena water

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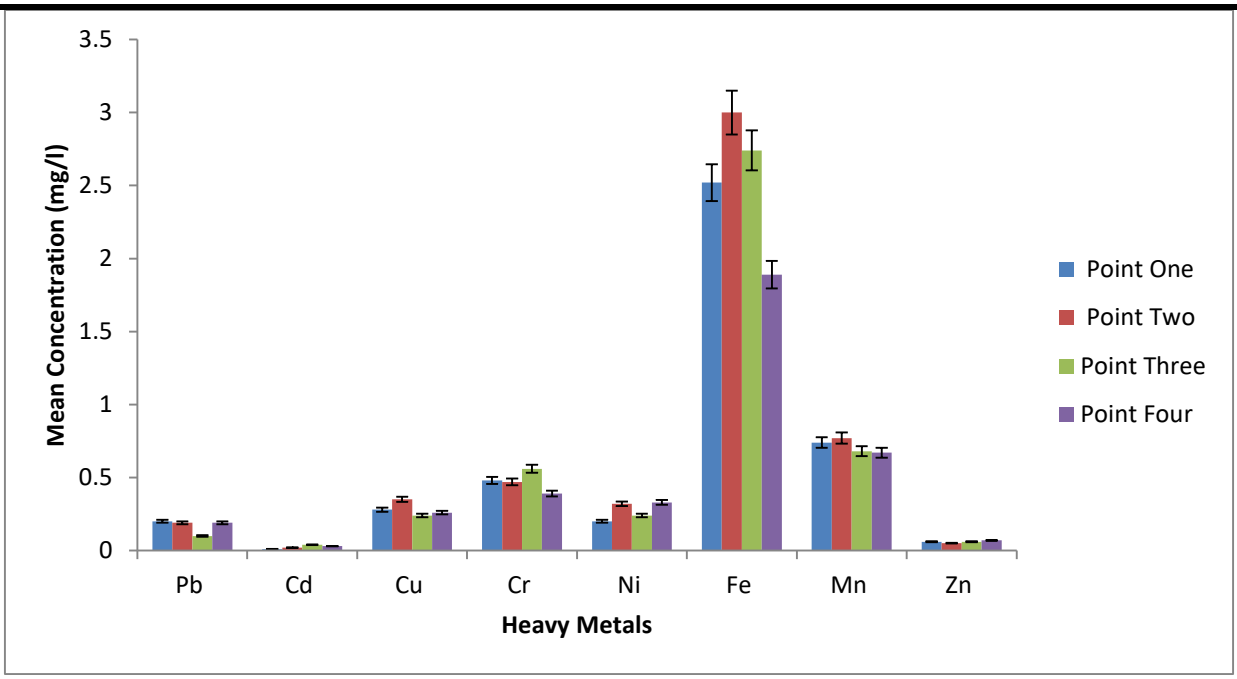

Fig.6: Dry season mean concentration of heavy metals of River Owena water

The total bacterial counts (cfu/ml) of River Owena water

The total bacterial counts for the four samples points of water determined from July 2015 to Jan. 2016 covering (wet and dry seasons) ranged from 2.2 x 10^3 to 2.25 x 10^3±0.01 cfu/ml (Table 3). The lowest count occurred in sample point four in August, 2015, while the highest count occurred in sample point one in July, 2015. Wet season ranged 2.25 x 10^3±0.01 cfu/ml in July, 2015 to 2.2 x 10^3±0.01 cfu/ml in August, 2015, dry season ranged 1.42 x 10^3±0.01 cfu/ml in Nov., 2015 to 5.5 x10^3±0.01 cfu/ml in Dec., illustrated that wet season had a higher bacterial count compared dry season, the increase in bacterial load during wet season could be due to run-off.

Table.3: Total bacterial counts of River Owena water

Months	Point One	Point Two	Point Three	Point Four
July.2015	2.25x10^3±0.01^d	1.23 x10^3±0.01^c	9.8 x10^3±0.01^a	1.20 x10^3±0.01^b
Aug. 2015	3.3 x10^3±0.01^b	6.0 x10^3±0.01^d	4.9 x10^3±0.01^c	2.2 x10^3±0.01^a
Sept. 2015	1.10 x10^3±0.01^b	6.6 x10^3±0.01^a	1.15 x10^3±0.01^c	1.23 x10^3±0.01^d
Oct. 2015	4.3 x10^3±0.01^a	5.2 x10^3±0.01^b	1.31 x10^3±0.01^d	8.8 x10^3±0.01^c
Nov. 2015	1.42 x10^3±0.01^d	1.21 x10^3±0.01^c	1.08 x10^3±0.01^b	7.4 x10^3±0.01^a
Dec. 2015	6.5 x10^3±0.01^a	5.5 x10^3±0.01^b	6.4 x10^3±0.01^c	7.7 x10^3±0.01^d
Jan. 2016	1.17 x10^3±0.01^d	9.4 x10^3±0.01^b	7.4 x10^3±0.01^a	1.01 x10^3±0.01^c

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Bacterial isolates of River Owena water

A total of ten bacterial species were isolated from River Owena water samples. The bacteria included *Enterobacter aerogenes*, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Shigella sonnei*, *Klebsiella pneumoniae* and *Salmonella enteritidis.* The frequency of occurrence of each isolate is shown in (Table 4). Both *Escherichia coli* and *Enterobacter aerogenes* had highest frequency of occurrence of 20.1 %, while *Micrococcus luteus* had the lowest of 1.8% and *Shigella sonnei*, *Salmonella enteritidis* had 4.3 % frequency of occurrence

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for bacterial isolates. this is similar to the work of Ekpo *et al*. (2010). Some of the isolates found in River Owena also occurred in findings of other researchers of freshwater in different locations in Nigeria which include that of Ogunnusi and Olanipekun (2010) and Ajayi (2010).

Table.4: Bacterial frequency distribution of River Owena water

Isolates	% Frequency of Occurrence
Enterobacter aerogenes	20.1
Proteus vulgaris	8.7
Escherichia coli	20.1
Staphylococcus epidermidis	12.2
S. aureus	11.1
Pseudomonas aeruginosa	8.7
Micrococcus luteus	1.8
Klebsiella pneumoniae	8.7
Shigella sonnei	4.3
Salmonella enteritiditis	4.3
	Total 100

Values represents means ± Standard error of means.

Fungal count (sfu/ml) of River Owena water

Total fungal counts for the four samples points of water determined from July 2015 to Jan. 2016 covering (wet and dry seasons) ranged from 6 x 10^3 to 1.22 x 10^3±0.01 sfu/ml (Table 5). The lowest count occurred in sample point one in Jan., 2016, while the highest count occurred in sample point two in Sept., 2015. Wet season ranged 7 x 10^3±0.01 sfu/ml in Aug., 2015 to 1.22 x 10^3±0.01 sfu/ml in Sept., 2015. Dry season ranged 6 x 10^3±0.01 sfu/ml in Jan., 2016 to 8.3 x 10^3±0.01 sfu/ml in Nov. Total fungal counts of the four samples points of water determined illustrated that wet season had higher count compared to dry season.

Table.5: Total fungal counts of River Owena water

Months	Point One	Point Two	Point Three	Point Four
July 2015	8 x10^3±0.01^a	9 x10^3±0.01^b	1.3x10^3±0.01^c	2.9x10^3±0.01^d
Aug. 2015	9x10^3±0.01^b	1.7 x10^3±0.01^a	7 x10^3±0.01^a	1.3 x10^3±0.01^c
Sept. 2015	1.22x10^3±0.01^c	1.05x10^3±0.01^b	1.03x10^3±0.01^a	1.11x10^3±0.01^b
Oct. 2015	2.2x10^3±0.01^c	2.5x10^3±0.01^d	8x10^3±0.01^a	1.0x10^3±0.01^b
Nov. 2015	8.3 x10^3±0.01^d	2.1 x10^3±0.01^b	6.2 x10^3±0.01^c	1.5 x10^3±0.01^a
Dec. 2015	5.3 x10^3±0.01^b	5.1x10^3±0.01^d	4.9x10^3±0.01^c	4.2x10^3±0.01^a
Jan.. 2016	6 x10^3±0.01^a	9 x10^3±0.01^b	2.1 x10^3±0.01^c	9 x10^3±0.01^b

*Data are presented as Mean ± S.E (n = 3). Values in the same row followed by the same superscript letters are not significantly different using Duncan's multiple range test at $p<0.05$

Fungal isolates of River Owena water

A total of eight fungal species isolated from River Owena water were *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Mucor plumbeus*, *M. mucedo*, *Cladosporium herbarum*, *Fusarium oxysporum* and *Rhizopus oryzae*. The frequency of occurrence of each isolate in (Table 6). *Aspergillus niger* had the highest occurrence with 21 % frequency of occurrence for fungal isolates, while *Fusarium oxysporum* had the least occurrence with 7.9 % frequency of occurrence which is similar to those obtained by Alabi, (1994). Akpata and Ekundayo (1983) found that occurrence of fungi varied with season with higher populations observed during the wet season in Lagos lagoon which is similar to this finding.

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Table.6: Fungal frequency distribution of River Owena water

Isolates	% Frequency of Occurrence
Aspergillus niger	21.0
Mucor plumbeus	15.0
A. flavus	12.5
Cladosporium herbarum	9.5
Rhizopus oryzae	10.3
M. mucedo	10.3
Fusarium oxysporum	7.9
A. fumigatus	13.5
	Total 100

Values represents means ± Standard error of means.

Sanitary surveillance

Along the bank of the river are farms with crops such as banana, guinea corn, sugar cane and vegetables (spinach), Human activities at the bank of the river were car wash park, washing of clothes, washing of farm implements and so on and so forth. The river also serves as recreational swimming pool for small children, various types of birds including ducks, pigeons and egrets were seen on the river. There are also well-constructed drainages leading into the water which was recently constructed in 2014. Solid waste dump include both biodegradable and non-biodegradable waste matters. Cow dung and human excreta along the bank of the river and inside the shallow areas of the river are all biodegradable, and these droppings were noticed at sampling points three and four. These activities requires monitoring to reduce the pollution level as observed by Environment Canada, (2010) and Figueras *et al*., (2000). The distribution pattern of wastes noticed at the sampling points (Table 7).

Table.7: Sanitary surveillance at the bank of River Owena

Type of wastes	**Point One**	**Point Two**	**Point Three**	**Point Four**
Solid Waste Dump	+	-	+	+
Cow Dump	-	-	+	+
Domestic Waste Droppings	+	-	+	-

The River Owena water was found to favour the growth of bacteria and fungi, which makes it unsafe for consumption, because of the presence of pathogenic microbes. River Owena water therefore needs treatment prior to use for both domestic and recreational purpose.

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Determination of Slope of Enugu for Erosion Models

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Abstract— *Slope in soil erosion models (soil loss equations) refer to slope components known as the LS factor. Generating the LS values poses the largest problem in using the Universal Soil Loss Equation (USLE). This work gives the practical solution to slope determination using two methods; the direct field measurement and the topomap techniques. From direct field measurement technique, slope was ascertained by excavation of the study area and measurement of the selected vertical distance (rise) over a selected horizontal distance (run), which was used for the determination of the slope length and gradient (angle) using the general slope equations. Using the topomap method, point A and B were identified on the map sheet, the elevations of point A and B were obtained from the contour lines while the distance of the two points were obtained from the map using a meter rule and the map scale. The values of their elevations (rise) over their distances (run) were used to compute slope length which was useful in obtaining slope angle. Either way, the values of L and S were multiplied together to generate a single value known as the LS factor which is applicable in the Universal soil loss equation and other erosion models to be developed in the area in future years. These models can be used to calculate and predict soil erosion occurrence in the study area; Enugu State of Nigeria. Slope management techniques to minimize erosion were also prescribed as a prevention strategy rather than remediation of eroded soil.*

Keywords— soil erosion, slope, gradient, universal soil loss, Enugu State.

I. INTRODUCTION

A slope is the measure of steepness or degree of inclination of a feature relative to the horizontal plane. It has been demonstrated that increase in slope length and slope steepness can produce higher overland flow velocities and correspondingly higher erosion [5]. While slope measures the degree of inclination, it also exist as a declination which it also a measure of significant. Climbing from the foot of a hill toward the top is a rising slope and vice versa (towards downhill) is a falling slope. These can be seen by the directions of the two arrows in fig. 1. An escarpment is a steep slope or long cliff that forms as an effect of faulting or erosion thereby separating two relatively level areas of differing elevations. Enugu's hill at the extreme is about 1,000 meters (3,300ft) in elevation. From the satellite map below, (fig. 2) Enugu escarpment can be seen on the left where it has a lighter colour.

Fig. 1: A rising and a falling slope (FAO, 1995). (NASA, 2007).

Fig. 2: Satellite map of Enugu, Nigeria (NASA, 2007).

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It is necessary to understand the various shapes of slope because slope shape determines whether water is dispersed or concentrated. Convex slope, flat or straight slope and concave slope are the three main types of slope shapes. Convex slopes roll from less steep to steeper terrain. Flat slopes otherwise known as straight or parallel slopes areas are never strictly horizontal, there are gentle slopes in a seemingly flat area, they are often hardly noticeable to the naked eye, and they demand an accurate survey of land to be identified. Concave slopes go from steeper to gentler terrain with movement down slope. Slope plays an important role in soil erosion models like; USLE, RUSLE, AGNPS, ANSWERS, WEEPS, e.t.c. It is represented as LS factor which include the slope length and slope angle. The L and S factor are commonly combined as LS and are referred as the slope factor [6]; [9]. This slope factor can be obtained either by field measurement or by using the topomap method. Therefore, the objective of this research is to determine the slope of Enugu for erosion models.

THE STUDY AREA:

The study area; Enugu State of Nigeria, West Africa, derived its name "Enugu" from the two Igbo words "enu" and "ugwu" meaning hill top prior to its hilly geography. It lies approximately within latitude 6º31''N and 7º16''N and longitude 7º20''E and 7º41''E. Its capital city, Enugu, lies approximately within latitude 6º 20''N and 7º 30''N and longitude 7º 20''E and 7º30''E. Enugu is bounded by several other states; in the North by Benue and Kogi States, in the South by Abia and Imo States, while in the West and East by Anambra and Ebonyi States respectively. Minerals mined in Enugu State includes; coal, iron ore, fine clay, silica sand, lime stone, and marble [14]. The climate is tropical hinterland in nature and is comparatively congenial, characterized by high temperature, high humidity and substantial rainfall which is entirely seasoned, most of it falling between May and October [14]. These locations are described in the map below;

Fig. 3*: Map of Nigeria with Enugu)* (Wikipedia, 2013, Atlas, 2016)

Fig. 4: *Map of Africa with Nigeria (Wikipedia,2013, Atlas, 2016*

II. MATERIALS AND METHODS

The major materials used in the field measurement techniques are; the excavator machine and the measuring tape. Excavator machine, this is a heavy duty equipment used in earthwork operations. $Cat_{(R)}$ 336EH hydraulic excavator was used to dig through the soil to a desired length of cut (vertical distance of 7.2m or 24 feet and horizontal distance of 29.1m or 97 feet) so as to take the run and rise measurements. The machine has net flywheel Power of 286.0 hp, Engine model of $Cat_{(R)}$ C9.3 (ATAAC), Bores at 0.1151m, Operating weight of 37194.58kg (minimum operating weight of 37013.14kg and maximum operating weight of 37194.57kg), Maximum travel speed of 3.0 mph, Maximum drawback pull of 30081.79kg, Swing system maximum flow of 73.0 gal/min, Heavy lift maximum pressure of 5366.0 psi, Fuel tank capacity of

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163.8 gal, Sound performance (Operating noise) of 71.0 dB. This excavator machine is shown in figure 6. The measuring tape is a measuring instrument used in taking reading in different units like inch, meter, and feet, convertible to other units of measurement. The Heng Feng®, POWERLOCK 19mm measuring tape was used to measure the topographic run and rise distances on the field (26.50m and 5.55m, horizontally and vertically respectively) necessary for slope determination. This measuring tape is shown in figure 7. Also, the major materials used in topomap technique are; the topomap and the meter rule. The topomap (topographic map with contour readings) was used to obtain the elevation and distance of Enugu State. An example of this map is shown in figure 9. The second apparatus used is the Meter rule, this is a calibrated plastic, glass, wood, e.t.c., that measures readings as low as in centimeter (cm), this was used to measure the distances on the topomap before converting the unit prior the map scale. The slope of a field is expressed as a ratio of its vertical distance (rise) or difference in height between two points in a field, divided by the horizontal distance (run). In Enugu metropolis, slope was calculated from direct field measurement by; excavation of the soil (vertical distance of 7.2m or 24 feet and horizontal distance of 29.1m or 97 feet), measurement of selected run and rise distances (26.50m horizontally and 5.55m vertically), and determination of slope length, slope angle and LS factor using the following slope equation 1;

Fig. 5: Hydraulic Excavator.

Fig. 6: Measuring Tape.

$$\mathbf{S = \frac{V}{h}} \quad \text{- -} \quad \mathbf{(1)}$$

Where; S = slope length (expressed as a ratio); V = Vertical distance in topography (rise). S.I unit in meter (m); h = Horizontal distance in topography (run). S.I unit in meter (m).

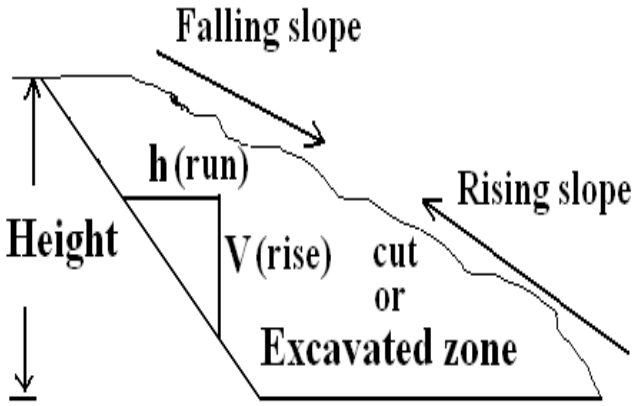

Fig.7: Run and Rise distances of a slope.

Slope Percent was determined was determined using the equation 2;

$$\textbf{\textit{Slope percent, }} S\,\% = \frac{V}{h}\, x\, \frac{100}{1} \quad \text{-} \quad \mathbf{(2)}$$

The Gradient was determined using equation 3;

$$\textbf{\textit{Gradient, }} \alpha = arc\ tan\ S \quad \text{- - -} \quad \mathbf{(3)}$$

Soil Loss Factor was determined using equation 4;

$$\textbf{\textit{LS factor}} = S \times \alpha. \quad \text{- - --} \quad \mathbf{(4)}$$

Using the topomap method, LS factor of Enugu State of Nigeria was obtained from a topomap sheet of Enugu State as provided in figure 8. A ruler was used to measure the scale bar of the map at the bottom left corner as 2cm on the map which equals 5000m (5km) in real world (this also

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means one may adjust the computer screen size by zooming in or out till the map scale line at the bottom left of the topomap is equal to 2cm when measured with a ruler). Point A was chosen within the topomap, and a line was drawn to a run direction in horizontal distance to point B. The distance of both points in the topomap would be identified as shown in figure 8.

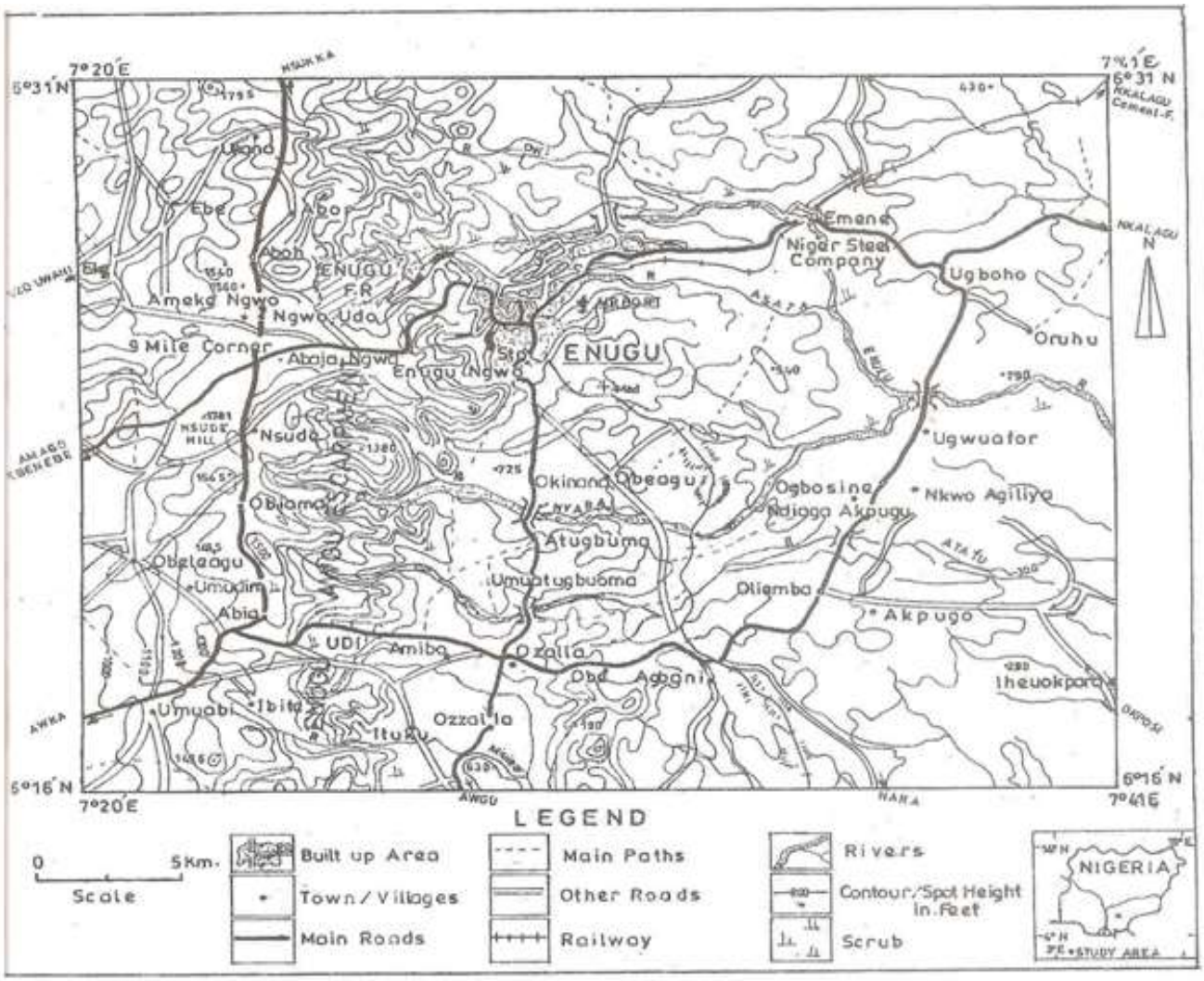

Fig.8: Topomap of Enugu (Scanned from the Ministry of Land and Survey, GRA, Enugu, Enugu State).

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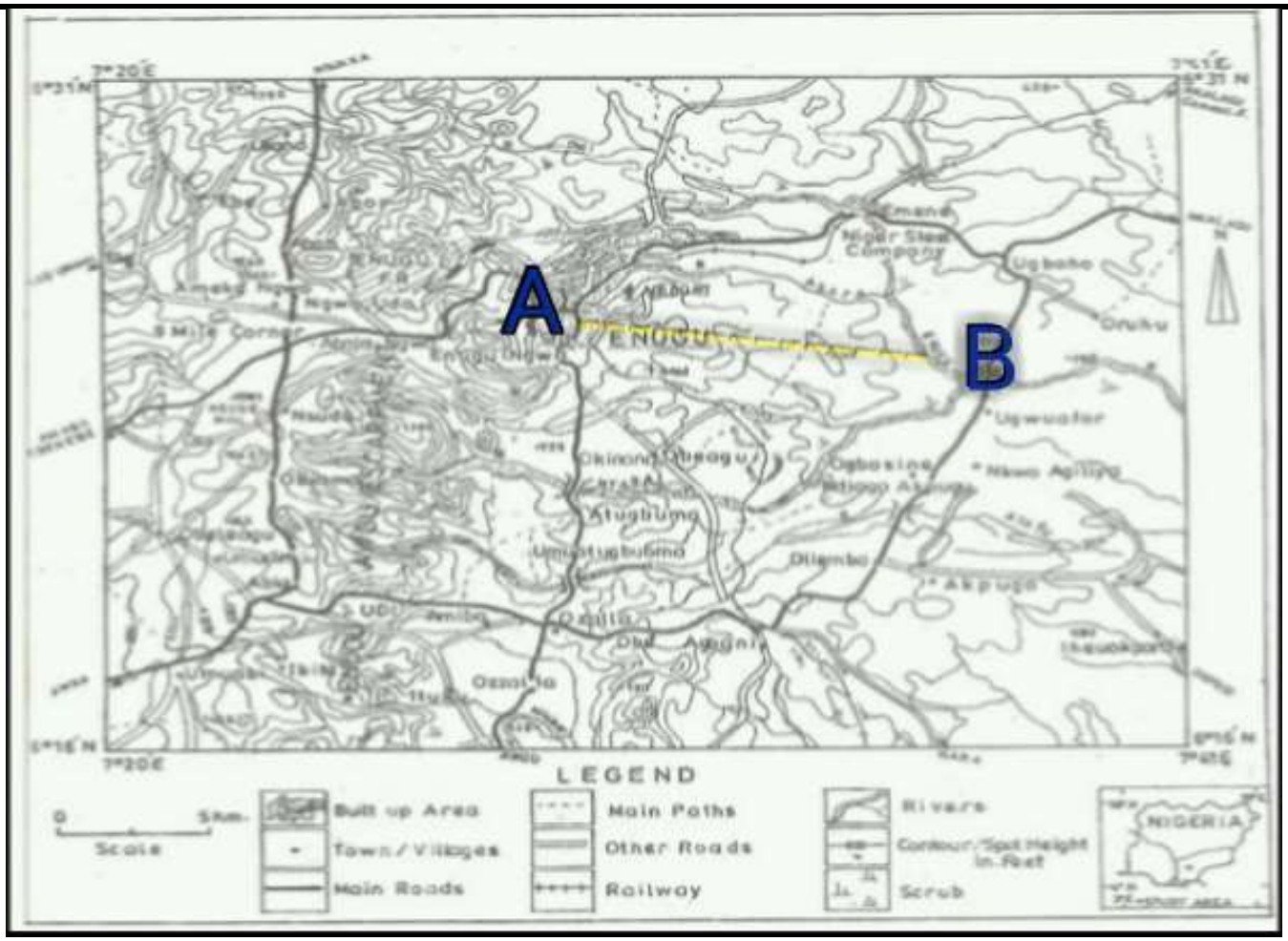

Fig.9: Topomap of Enugu State with point A and B (Edited from scanned copy from Ministry of Land and Survey, GRA, Enugu).

III. RESULTS AND DISCUSSIONS

Results are as tabulated below;

Table.1: Slope variables and LS factor for Enugu State and its capital city

LOCATION	RISE (Vertical Distance, V) M	RUN (Horizontal Distance, h) m	SLOPE LENGTH, S =V/h	SLOPE PERCENT (%)	SLOPE ANGLE, $\alpha=\tan^{-1} S$	LS FACTOR (S × α)
ENUGU CITY	5.55	26.50	0.2094	20.9434	11.8268	2.4765
ENUGU STATE	1195	11500	0.1039	10.39	5.9317	0.6163

The topographic factor otherwise known as the LS factor for Enugu State and its city gave; 0.6 and 2.5 respectively, this can be related to the value range in [14] universal soil loss equation;

$$\boldsymbol{A = E = R\,x\,K\,x\,SL\,x\,C\,x\,P} \quad - \quad \textbf{(5)}$$

Where, ***A*** = Annual soil loss (metric tons x m/ha/cm of rainfall); ***E*** = Kinetic energy of rainfall (in metric tons x m/ha/cm of rainfall); ***R*** = Rainfall erosivity index (kinetic energy of rainfall, ***E*** x maximum intensity of rainfall in 30 minutes, I_{30}); ***K*** = soil erodibility = 0.7 for fragile soil or 0.01 for stable soil (Depends on organic matter and texture of the soil, permeability and profile structure; ***SL*** = Topographic factor = 0.1 to 5 in most frequent farming contexts in West Africa and may reach 20 in mountainous areas depending on both length and gradient of slope; ***C*** = Plant cover factor = 0.001 to 1 for forest region, 0.01 to 1 for grass lands and cover plants, 0.9 to 1 for root and tuber crops; ***P*** = Specific erosion practices = From1 (for soil with no erosion control) to 0.1 (for soil with tied ridging on a gentle slope).

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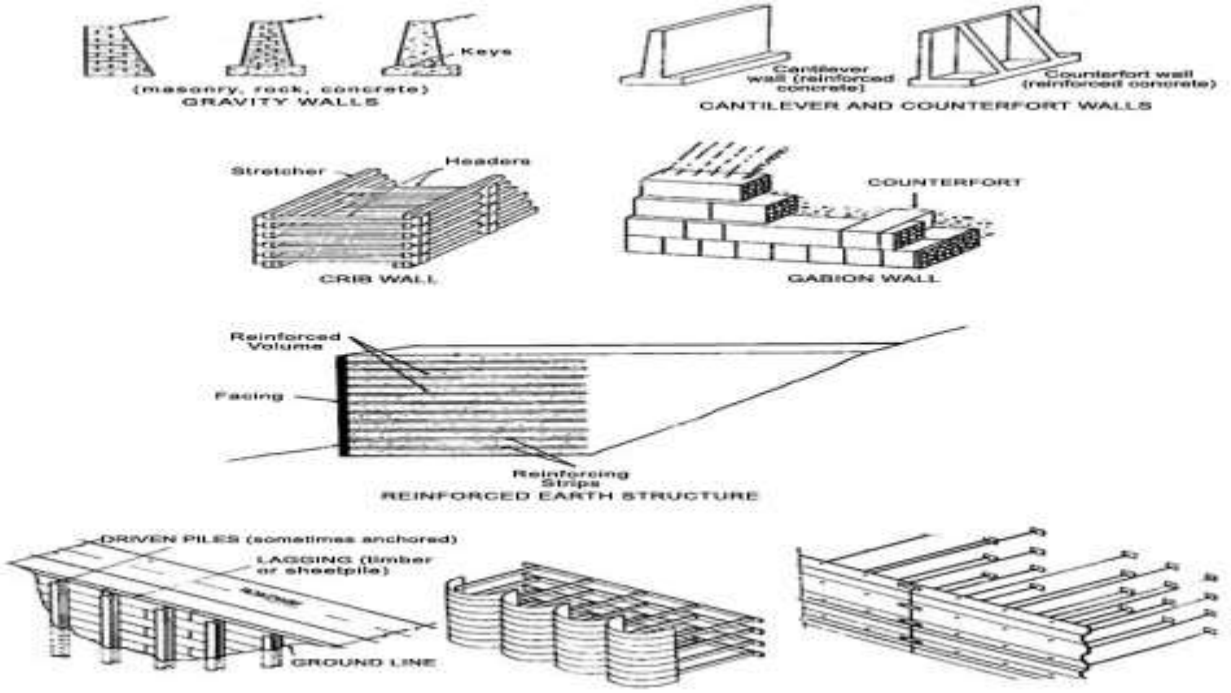

Fig. 10: USDA, Forest Service Technical guide, erosion prevention and control on timber sales areas, intermountain region

Also, from the erosion equation 5, erosion is the multiplication of its factors. Thus, if one factor of erosion is minimize to zero, erosion becomes equal to zero thereby not occurring at all. To manage slope and erosion cases related to it requires applications of some techniques like vegetation, reinforcement, etc. Vegetation provides cover to slope surfaces against erosion forces like rain drop splash. The root systems of live plants act in several ways to increase slope stability and serve as a binder for individual soil particles. Also, retaining structures maybe use to minimize erosion prior slope. In a case of a fall line, slope demands a more careful examination before calculating. A fall line (or fall zone) is the geomorphologic break between an upland region of relatively hard crystalline basement rock and a coastal plain of softer sedimentary rock. It is typically prominent when crossed by a river or other flowing water bodies, for there will often be rapids or waterfalls which creates room for erosion within the contact area of coastal plain (flatlands with relatively low relief) and Piedmont (region of foothills of mountain range) with high elevation.

IV. CONCLUSSION

This work indeed provides practical solutions to ***LF*** factor using the two techniques as well provide means of minimizing erosion using slope. The ***LS*** factor for Enugu State and its capital city are 0.6 and 2.5 respectively.

RECOMMENDATION

More effort should be put in controlling the effects of slope and other erosion factors as they are the keys to preventing water erosion.

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Material Losses and Garri Recovery Rate during the Processing of Varieties of Cassava into Garri

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Abstract**—** *Materials losses and garri yield during garri processing on different cassava varieties; TMS/92/0057, TMS/30572, TME/419 and Vitamin A: 01/1368 were conducted. The results showed that there were variations within the different processing unit as well as the cassava varieties during garri processing. Losses were recorded highest at grating, dewatering and fermentation processes with the values of 9kg from TMS/92/0057 recording the highest loss, 8.5kg from Vitamin A: 01/1368, 7.7kg from TMS/30572 and 7kg from TME/419. On roasting processes, TMS/30572 had 3.2kg loss, Vitamin A: 01/1368, had 2.5kg loss, while TME/419 and TMS/92/0057 had 2kg loss respectively. Material losses at peeling showed that Vitamin A: 01/1368 recorded 4.5kg loss being the highest, TMS/92/0057 and TME/419 recorded 3kg loss each, while TMS/30572 recorded 2.5kg loss. Sifting losses indicated that vitamin A: 01/1368 and TME/419 had 1kg loss each, whereas TMS/30572 had 0.8kg loss and TMS/92/0057 had 0.5kg loss. Overall material loss for each cassava variety based on fresh weight of 20kg and maturity age of 14 weeks was determined, which vitamin A: 01/1368 recorded the highest loss of 16.5kg, TMS/92/0057 had 14.5kg loss, TMS/30572 had 14.2kg loss and TME/419 recorded 13kg loss. The total garri yield from each variety was also determined with TME/419 having 7kg yield, TMS/30572 had 5.8kg yield, TMS/92/0057 had 5.5kg yield and Vitamin A: 01/1368 recorded 3.5kg yield. Percentage losses from the tested varieties showed that vitamin A: 01/1368 had the highest percentage of 82.5% loss, TMS/92/0057 had 72.5% loss, TMS/30572 had 71% loss and TME/419 recorded 65% loss. Percentage yield of garri was also determined on the cassava varieties, this showed that TME/419 had 35% yield being the maximum, TMS/30572 had 29% yield, TMS/92/0057 had 27.5% yield and Vitamin A: 01/1368 recorded 17.5% yield.*

Keywords**— Garri processing, Garri loss, grating, roasting, yield.**

I. INTRODUCTION

Cassava (*Manihot Esculenta Crantz*) is a staple food in most tropical regions, and is grown over a range of climate and altitudes and on a wide variety of soils (Tivana 2012). Cassava is one of the most important crops in Nigeria and Africa as a whole (Amadi et al 2011, Nweke et al 2002). Cassava is tolerant to drought; it is productive in poor soil where other staple crops cannot grow without intensive inputs (Tivana 2012, Bradbury and Holloway 1988, and Leihner 2002). Cassava has special attributes which include ability to make return of root yield even at extreme stress conditions, high tolerance to unfavorable conditions, all year round availability, highly suitable to various farming and food system in Africa as well as efficient production of food energy. (Amadi et al 2011, Beeching et al 2000, Awa and Tumanteh, 2001). However, cassava has certain drawbacks, its tissues contain toxic cyanogenic compounds, it has a very low protein content (1-2% dw) and a very short shelf life in fresh form of 1-3 days (Booth et al 1974, Rickard, 1985, Westby, 2002). The roots and leaves which contain various amounts of cyanide at high levels are toxic to both humans and animals. Therefore after harvest cassava has to be quickly converted into suitable forms of low cyanide with longer and stable shelf life (Asiedu 1989, Opara 1999). The processing of cassava into various forms that combine the advantages of diversity, nutritional value and convenience of use is further means of promoting its consumption among different strata of the society (Oduro and Ellis, 2000). The various derivatives into which fresh cassava roots can be processed are unlimited. By far its processing into a fermented dried, granular food product called garri is more popular in Nigeria and as well as in sub-saharan Africa than other derivatives (Asiedu 1989, Opara 1999). Improved processing techniques which significantly reduced drudgeries and difficulties associated with traditional methods transformed garri as one of the fore most Nigeria staple. More about garri is that it is convenient, ready to eat, storable and easily processed to conform to the organoleptic preferences of the consumers (Sani et al 1994). Garri processing is becoming a fast expanding enterprise, providing employment and income generation opportunities for farmers and commercially oriented individuals in the rural economy. Over the years

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and by indigenous practices, processing of cassava roots into such major products like fufu, abacha (african salad), starch, and garri were based on local preferences and feeding patterns. Fufu otherwise called wet paste was the most popular cassava products in Nigeria especial in the eastern and south south zones as it was mostly preferred and consumed by many farm house holds due to its perceived attributes of providing instant vigor for physical labor as well as thickening a man's bones (Amadi et al 2011). But on the other hand, lacked of storage quality for use in famine period, cannot be consumed instantly commands low market demand and still not easily portable. Garri soon became popular choice of consumers owing to its long storability and ready to eat attributes. Therefore, the purpose of this study was to compare garri yielding amount among cassava varieties, material loss within each processing limit and determined the amount of garri that can be produced from any quantifying amount of raw cassava tubers maturity based on varieties; TMS 9210057, TMS 30572, TME 419 and vitamin A: 011368.

II. MATERIAL AND METHODS

CASSAVA TUBER OR CASSAVA ROOTS:

The cassava tubers or roots used in the research work were harvested from the Cross River Basin Development Authority farm, at the maturity age of 14 months. The varieties were of improved type commonly planted by the farmers within the farm, Akwa Ibom and Cross River State. The varieties include TMS/92/0057, TMS/30572, TME/419 and Vitamin A: 01/1368.

EQUIPMENT

The following equipment were used to estimate the material losses and the garri yield from the various cassava varieties. These include; enamel basin, sack (bags)**,** fire wood, water, palm oil, calabash (for tossing the particles during frying), Jute sack (for storing or marketing), peeling knives, cassava grater (powered by 5hp diesel engine), a double screw press, a rectangle wooden box sifter, an insulated – walled chimney stove with an open iron pan on the fire box and weighing balance, this was used to obtain the weight of the processed roots from each unit operation. It has an accuracy of + 0.05kg.

III. EXPERIMENTAL DESIGN AND PROCEDURES

The research study centre is located at Abak Irrigation Project of the Cross River Basin Development Authority Calabar, Nigeria, which lies within latitude 4°58' and longitude 7°48' with an elevation of 30 meter above sea level. The material losses from each cassava variety were based on the following processing units and the equation that followed based on measured weight after each operation.

Peeling Losses *(LP)*

If **wf** is the initial weight of fresh cassava tubers in kilogram, and ***wp*** is the weight of peeled tubers. Then ***wf – wp*** represents the peeling losses.

Peeling looses $(\boldsymbol{LP}) = \boldsymbol{wf - wp}$ - - - **(i)**

Grating/Dewatering/Fermentation Losses *(LGD)*

Let **wgd** is weight of dough after grating/fermentation and dewatering.

Then grating/dewatering/fermentation losses $(\boldsymbol{LGD}) = \boldsymbol{wp - wgd}$ - **(ii)**

Sifting Losses *(LS)*

Material losses during sifting are mainly due to spillage, the residual fiber and un-grated masses that are retained over the sifter. If **ws** is the weight after sifting, then $\boldsymbol{wgd - ws}$ represents the sifting loss ***(LS)***

Sifting losses $(\boldsymbol{LS}) = \boldsymbol{wgd - ws}$ -- - **(iii)**

Roasting Losses *(RL)*

Material losses encountered at the roasting stage include evaporation of moisture into the atmosphere as well as spillage of particles as the operator stirs through with a portion of calabash. Let ***wr*** be the weight of roasted flour (garri) then $\boldsymbol{ws - wr}$ represents the roasting losses. Therefore;

Roasting losses $(\boldsymbol{RL}) = \boldsymbol{ws - wr}$ - - **(iv)**

Similarly the percentage losses for each processing unit on each cassava variety can be obtained from the following equations

Percentage Peeling Loss $(\boldsymbol{LPP}) = \frac{\boldsymbol{wf-wp}}{\boldsymbol{wf}} \boldsymbol{x} \frac{\mathbf{100}}{\mathbf{1}}$ **(v)**

Where; LPP = percentage peeling loss (%), wf = fresh cassava root weight (kg), wp = weight after peeled or weight of peeled tubers (kg).

Equally equation (v) can be written as; $\boldsymbol{LPP} = \frac{\boldsymbol{LP}}{\boldsymbol{wf}} \boldsymbol{x} \frac{\mathbf{100}}{\mathbf{1}}$ - - **(vi)**

Where; LPP = percentage peeling losses (%), wf = fresh cassava root weight (kg)

Percentage grating/dewatering/fermentation losses $(\boldsymbol{LGDP}) = \frac{\boldsymbol{lgd}}{\boldsymbol{wf}} \boldsymbol{x} \frac{\mathbf{100}}{\mathbf{1}}$ - **(vii)**

Where; Lgdp = percentage grating/dewatering/fermentation losses (%),

Ldg = grating/dewatering/fermentation losses**,** wf = fresh cassava root weight (kg)

Percentage Sifting Losses $(\boldsymbol{LSP}) = \frac{\boldsymbol{ls}}{\boldsymbol{wf}} \boldsymbol{x} \frac{\mathbf{100}}{\mathbf{1}}$ **(viii)**

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Where; LSP = percentage sifting losses (%), ls = sifting losses, wf = fresh cassava root weight (kg).

Percentage Roasting Losses $(R_{LP}) = \frac{R_L}{wf} \; x \; \frac{100}{1}$ **(ix)**

Where R_{LP} = percentage roasting losses (%), R_L = roasting losses, wf = fresh cassava root weight (kg).

IV. DETERMINATION OF MATERIAL LOSSES

The total loss for each cassava variety was obtained by adding all the losses in equation i to iv as applicable to each variety.

GARRI YIELD DETERMINATION

To obtain the yield of garri from each cassava variety, the fresh weight of each variety minus the material loss from each variety gives the garri yield.

PERCENTAGE LOSSES DETERMINATION

The percentage losses for each cassava variety was obtained by adding all the processing unit losses of each variety together and divide by the fresh weight times 100.

PERCENTAGE YIELD OF GARRI DETERMINATION

The percentage yield of garri from each cassava variety obtained by subtracting losses percentage of each variety from 100.

V. RESULT AND DISCUSSION

The material losses in (kg) for different unit operations on each cassava variety at the same age of maturity is shown in Table 1 below. The TMS/92/0057 variety has the following material losses in each unit processing; peeling recorded 3kg, grating/dewatering/fermentation was 9kg, sifting was 0.5kg and roasting recorded 2kg (table1), while TMS/30572 recorded 2.5kg on peeling, 7.7kg on grating/dewatering/fermentation, 0.8kg on sifting and 3.2kg on roasting (table1). TME/419 variety recorded 3.0kg on peeling, grating/dewatering/fermentation was 7kg, sifting was 1kg and roasting 2kg, while Vitamin A01/1368 on peeling had 4.5kg, grating dewatering/fermentation 8.5kg, sifting 1kg and roasting 2.5kg.

Table.1: Material Losses in kg for the different unit operations for TMS/92/0057, TMS/30572, TME/419 and Vitamin A: 01/1368.

Cassava Variety	Age at Harvest (kg)	Fresh Weight of Root (kg)	Peeling Losses (kg)	Grating/ Dewatering/ Fermentation (kg)	Sifting Losses (kg)	Roasting Losses (kg)	Total Losses (kg)	Yield of Garri in (kg)
TMS/92/0057	14	20	3.0	9	0.5	2	14.5	5.5
TMS/30572	14	20	2.5	7.7	0.8	3.2	14.2	5.8
TME/419	14	20	3.0	7	1	2	13	7
Vitamin A:01/1368	14	20	4.5	8.5	1	2.5	16.5	3.5

Percentage materials losses for the different unit operations for different cassava varieties were also obtained (Table 2) for TMS/92/0057, the peeling loss percentage was 15%, grating/dewatering/fermentation had on record 45%, sifting was 2.5% , 10% on roasting and total loss percentage for TMS/92/0057 was 72.5%. TMS/30572 with peeling loss percent was 12.5%, grating/dewatering/fermentation 38.5%, sifting 4.0%, roasting 16.0% and a total of 71% losses was obtained. TME/419 had on peeling 15%, grating/dewatering/fermentation 35%, sifting 5%, roasting 10% and had a total of 65% losses (table 2). Vitamin A: 01/1368 had on its processing units as follows; peeling 22.5%, grating/sifting/dewatering 42.5%, sifting 5%, roasting 12.5%, and with total percentage losses of 82.5% (table 2).

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Table.2: Percentage Material Losses for the different unit operations for different cassava varieties TMS/92/0057, TMS/30572, TME/419 and Vitamin A: 01/1368.

Cassava Variety	Age at Harvest (months) (%)	Peeling Losses (%)	Grating/ Dewatering/ Fermentation Losses (%)	Sifting Losses (%)	Roasting Losses (%)	Total Losses (%)
TMS92/0057	14	15	45	2.5	10	72.5
TMS30572	14	12.5	38.5	4.0	16.0	71
TME419	14	15	35	5	10	65
Vitamin A:01/1368	14	22.5	42.5	5	12.5	82.5

PERCENTAGE YIELD OF GARRI

The percentage garri yield was also obtained. TMS/92/0057 had a yield percentage of 27.5%. While TMS/30572 had 29%, TME/419 had 35% and Vitamin A:01/1368, 17.5%, see table 3.

Table.3: Percentage Yield of Garri from the different Cassava Varieties

Cassava varieties	Percentage yield
TMS/92/0057	27.5
TMS/30572	29
TME/419	35
Vitamin A:01/1368	17.5

The material losses in kg at different stages of processing and the final garri yield from the different cassava varieties harvested at the same age (14 months) of maturity are shown in table 1. Peeling loss was highest on vitamin A: 01/1368 with 4.5kg. While TMS/92/0057 and TME/419 has 3kg losses respectively and TMS/30572 with a loss of 2.5kg. From records on grating/dewatering/fermentation; Vitamin A: 01/1368 had a loss of 8.5kg, TMS/92/0057 had 9kg, TMS/30572 had 7.7kg and TME/419 had 7kg. This indicates that Vitamin A:01/1368 had the greater loss on grating/dewatering/fermentation. 1kg was obtained from sifting for both vitamin A:01\1368 and TME/419. TMS/30572 got 0.8 and TMS/92/0057 had 0.5. Roasting losses had the highest losses of 3.2kg on TMS/30572 and followed by Vitamin A:01/1368 of point 2.5kg losses while TME/419 and TMS/92/0057 had 2kg respectively. The highest total loss was recorded on vitamin A: 01/1368 of 16.5kg from initial weight of the fresh tubers see table 4.1 while the highest garri yield was recorded from TME/419 of 7kg (table 1). The percentage material losses for the different unit operations were also obtained. From each cassava variety. Peeling percentage loss evaluated, the results showed that Vitamin A: 01/1368 had the highest value of 22.5%, following by TME/419 and TMS/92/0057 of 15% respectively and TMS/30572 with 12.5%. Grating/dewatering/fermentation recorded 45% from TMS/92/0057, followed by 42.5% from Vitamin A: 01/1368. 38.5% and 35% were obtained from TMS/30572 and TME/419 respectively. Sifting losses records showed that Vitamin A: 01/1368 and TME/419 had the same value of 5% respectively, while TMS/30572 had 4.0% and TMS/92/0057 2.5%. Roasting percentage losses showed 16.0% from TMS/30572, 12.5% on Vitamin A:01/1368 and 10% each from TME/419 and TMS/92/0057. The total percentage losses were recorded as follows, Vitamin A:01/1368; 82.5%, TMS/92/0057; 72.5%, TMS/30572; 71% and TME419; 65% having the least value. See table 2. Also the percentage losses of the four cassava varieties is shown in Fig. 1.

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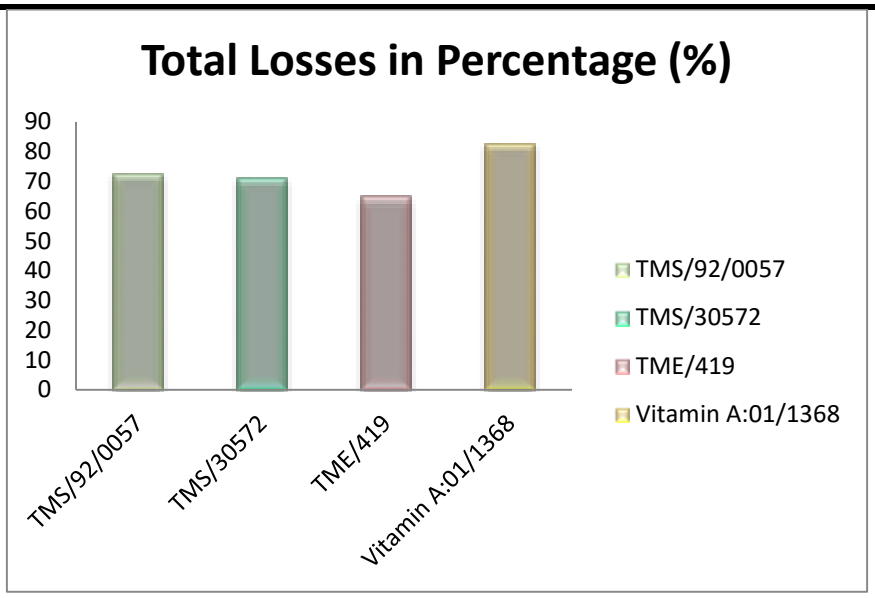

Fig.1: Total losses of four cassava varieties in percentage during garri processing.

Percentage yield of garri was determined based on the initial fresh tuber weight of the different cassava varieties (table 3). The highest value of garri yield was obtained from TME/419 of 35%, TME/30572 recorded 29% yield and Vitamin A: 01/1368 had the least value of 17.5% (table 3). Fig 2 also shows the percentage yield of garri from the four cassava varieties.

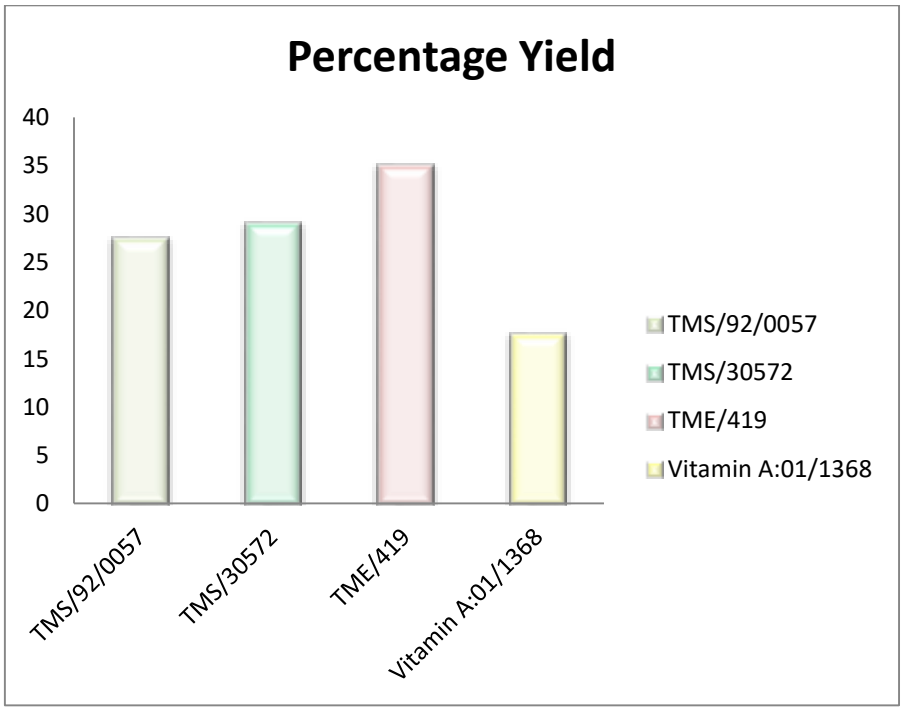

Fig. 2: Percentage yield of four cassava varieties during garri processing.

VI. CONCLUSION

It has been established from this study that different cassava varieties have their different varietal characteristics and that these account for material losses which consequently affect the garri yield from any given set of processing equipment and method.

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The Impact of Drought: A Study Based on Anuradhapra District in Sri Lanka

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Abstract*— *Anuradhapura District being one of the paddy providers in Sri Lanka highly affected due to the drought disaster. The trend and cause for the drought should be identified for future remedial measures. Thus this study is conducted based on the following objective. The primary objective is that 'identifying the impact of drought in Anuradhapura District' and the secondary objective are 'finding the direct and indirect factors causing drought and the influence of drought in agriculture in the study area and proposing suggestions to lessen the impact of drought in the study area. To attain these objectives data from 1900 to 2014 were collected. All the data were analysed and the trend of drought, condition of drought and the impact of drought were identified. Many suggestions have been provided in the suggestion part.

***Keywords*— drought, disaster, agriculture, impact.**

I. INTRODUCTION

Drought is an extended period of unusually dry weather when there is not enough rain. The lack of precipitation can cause a variety of problems for local communities, including damage to crops and a shortage of drinking water. These effects can lead to devastating economic and social disasters, such as famine, forced migration away from drought-stricken areas, and conflict over remaining resources (National Geography, 2017).

The drought disaster, frequently strike Sri Lanka is a serious problem to the nation. Most of the districts are being experienced by drought in Sri Lanka. Loss of agriculture and economy are evidences for the strike of drought in Sri Lanka. Many Districts in Sri Lanka are facing drought problems. Anuradhapura District, called as "the heart of Sri Lanka's rice bowl" one of the paddy providers to the nation is being severely affected due to the drought occurrence.

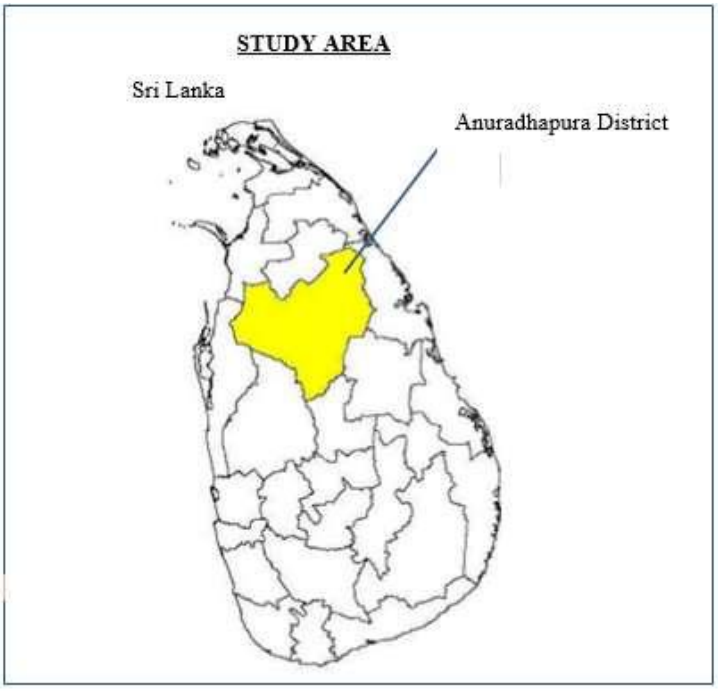
Anuradhapura District receives rainfall from northeast monsoon. The annual rainfall ranges from 1250mm – 2000mm from northeast monsoon. Increasing evaporation causes many dryland in Anuradhapura District. Small ponds, streams and other small-scale water bodies are highly affected where much amount of paddy cultivated using these water bodies.

Due to the severe drought many places 14 Divisional secretaries are highly affected due to the drought disaster in Anuradhapura Districts: Horovapothana, Ipolagama, Nuwaragampalatha, Rambewa, Thirappana, Nachchathuwa, Palugaswewa, Kekirawa, Kahalkasthikiliya, Thambuthegama, Pathaviya, Madavachchi and Kepatikollawa are the Divisional Secretariats, highly affected.

The impact of the drought occurrence should be controlled to pave a way for the agriculture and for the socio economic development of inhabitants in Anuradhapura.

II. STUDY AREA

Anuradhpura District is situated in the dry zone of Sri Lanka in the north central province of Sri Lanka. It has 22 Divisional Secretariat Divisions combined by 694 GramaNiladhary Division. It has 886945 total population of 2387769 families. The boundaries of Anuradhapura are in north Vavuniya District, in east Trincomalee District, in South Mattale District and in the west Puttalam District. Paddy is one of the major incomes of the inhabitants of Anuradhapura District. Fishery, industries, business activities and service sectors are other sources for the income.



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OBJECTIVES

Primary objective

- Identifying the impact of drought in Anuradhapura District

Secondary objectives

- Finding the direct and indirect factors causing drought and the influence of drought in agriculture in the study area
- Recommendingsuggestions to lessen the impact of drought in the study area.

III. MATERIALS AND METHODS

Both primary data secondary data were used for this study.

Primary data

Using simple random sampling technique, 40 percent of experience farmers were selected and questionnaires were distributed among them.

- Conversation
- Meeting with farmers
- 02 officials from Department of irrigation in Anuradhapura
- 02 officials from Agrarian Service Centre
- 01 official from Meteorological Department

Secondary data

Reports from Divisional Secretariat, District Secretariats, meteorological department, disaster management centre, newspapers, books, published researches and internet sources were used as secondary data.

IV. RESULTS AND DISCUSSION

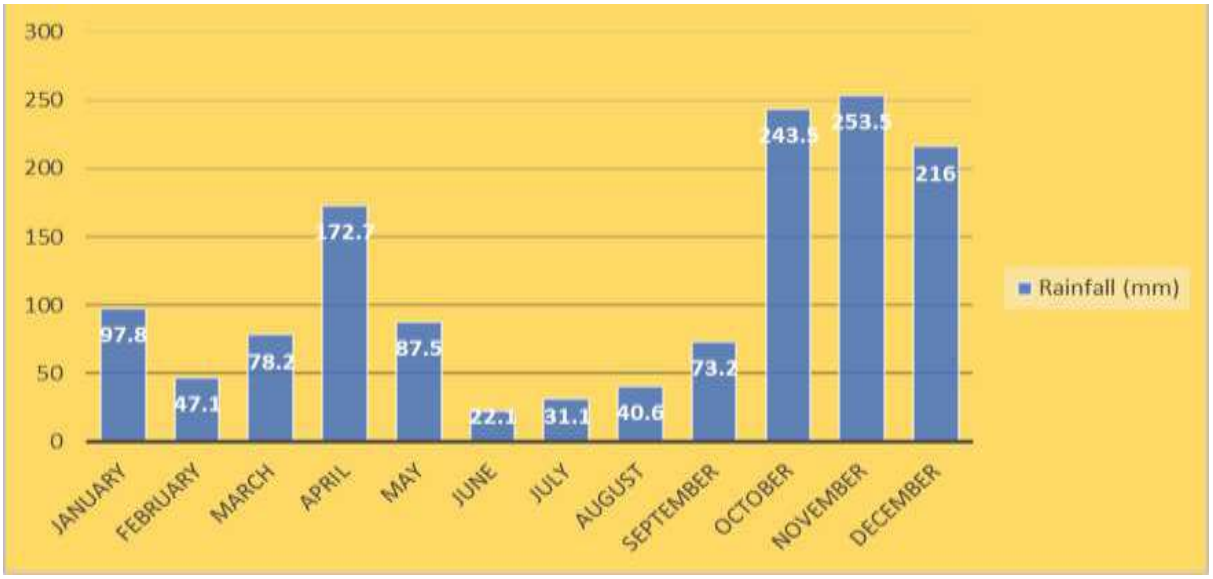

Fig.1: Monthly Rainfall of Anuradhapura District 1970 - 2009

Source: Meteriological department, colombo

Figure no: 01 shows the monthly rainfall of Anuradhapura District. Accordingly, Anuradhapura District receives more rainfall from October to December. The drought disaster affects in Anuradhapura in June, July and August. The mid part of the year experience the drought highly. Thus, there is a need to be a preparedness in the middle of the month in Anuradhapura District.

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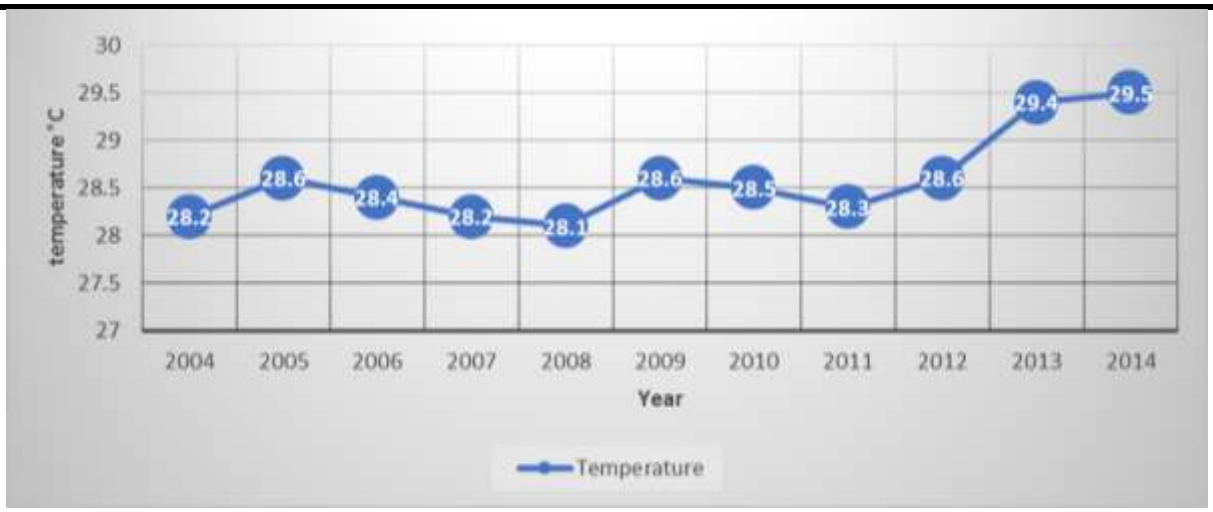

Fig.2: Annual Temperature of Anuradhapura District 2004 - 2014
Source: meteorological Department, Colombo

Figure: 02 shows the temperature trend of the Anuradhapura District. Temperature in 2004 has been registered as 28.2 °C but in 2014 it has been registered as 29.5. It is evident that, the rate at which temperature had been increasing is a cause for the drought in Anuradhapura. Increasing temperature has caused the evaporation. Evaporation leaded to the increasing drylands in the study area.

Fig.3: monthly water balance of Anuradhapura District - 2012
Source: meteorological department, Colombo

Figure: 03 shows the average water balance of Anuradhapura District. Decreasing influx of water has been registered from May to August. At the same time, the evaporation rate is high during this period. And also, increasing influx of water registered from October to December but evaporation rate is low. These are the periods of water availability in Anuradhapura District.

Moving average curve

With the help of MINITAB software, moving average curve and residual mass curve have been created using collected data. To figure out the condition of drought, 11 and 21 years' moving averages have been calculated.

To explain the condition of the drought in Anuradhapura District, 150 years rainfall data from selected stations were used. The highest rainfall has been registered as 2428 mm in 1957 at the same time, 741 mm rainfall in 1956 as a lowest amount of rainfall has been registered.

- 11 wet seasons
- 09 dry seasons
- 35 dry seasons
- 06 wet seasons
- 12 dry seasons

Comparatively, Anuradhapra District has experience more dry seasons than wet seasons.

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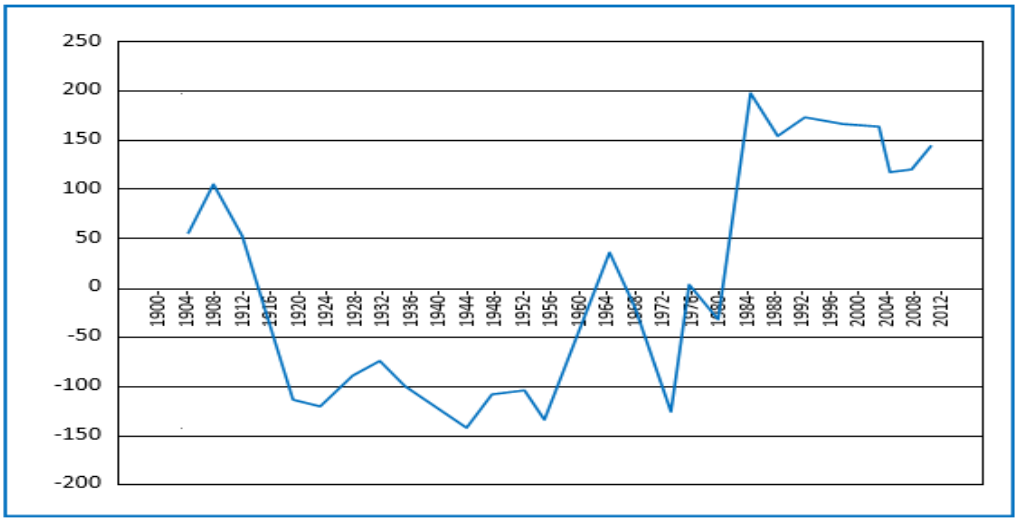
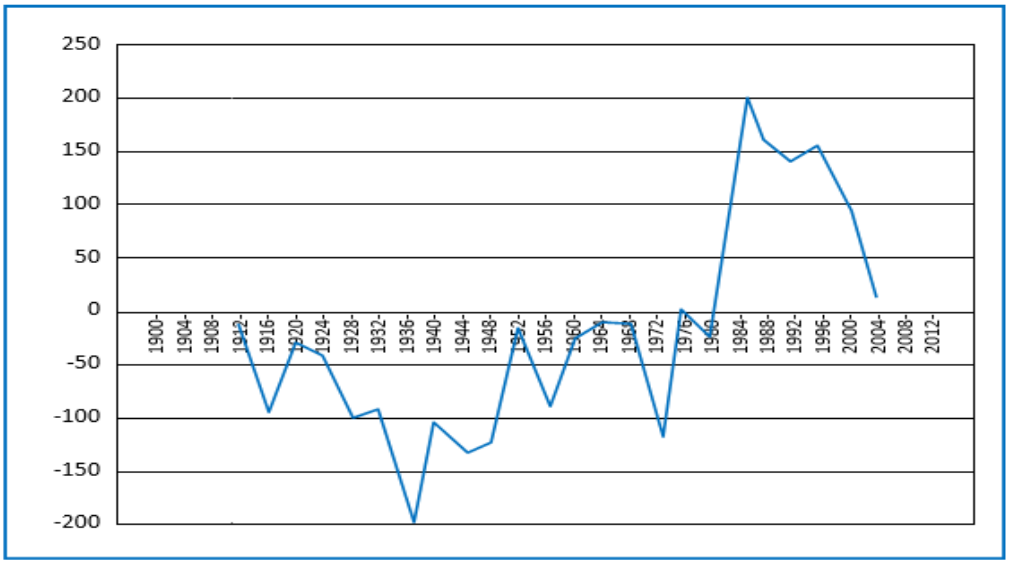
11 years' moving average curve



Source: meteorological Department, Colombo

21 years' moving average curve



Source: meteorological Department, Colombo

The impacts cause by drought disaster have been listed as follow.

- Environmental impact
- Economic impact
- Social impact

Environmental impact

- Loss of soil fertility
- Loss of flora and fauna
- Drying water bodies
- Threats to the biodiversity
- Collapsing food chain
- Loss of ground water quantity

Economic impact

- Industrial activities have been affected in many areas such as brick production and fishing
- Increasing state coast. Due to the drought the government has to spend a lot of money for the rebuilding of the inhabitants.
- Due to the drought the income of the people by the tourist have been affected.

Social impacts

- Waterscarcity for drinking and agricultural purposes
- Dwindling freshwater fishery
- Diseases. Particularly, water borne diseases such as cholera and diarrhoea
- Poverty
- Loss of animal farming

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V. RECOMMENDATIONS AND CONCLUSION

Recommendations

Many recommendations have been suggested to control the impact of the drought in the study area.

Before drought

- Adopting rainwater harvesting systems during the rainy season
- To cut the water wastages
- Introducing water storing techniques among the inhabitants
- Using surface and groundwater in sustainable manner
- Controlling the deforestation
- Using seawater by desalination with the help of government
- Introducing waste water purification system

During the drought

- Supplying drinking water for the victims by bowsers. In 2014, clean drinking water was supplied to the inhabitants of Kepatikollawa
- Granting subsidies for the victims
- Changing the land use pattern in the study area viable to keep the water
- Assisting for the victims with the help of NGOs and government organizations such as local governments

Post drought period

- Project for the rehabilitation of hand pump tube wells in Anuradhapura and Pollonnaruwa.
- Taking actions to reduce the impact of drought in the study area
- Adopting cascade system for the water management
- Conserving the crop cover in the study area
- Educatingstudents to conserve water for the future generation
- Changing the crops viable to grow in less amount of water
- Identify the yearly drought prone areas in the study area
- Taking actions to construct small scale ponds to collect water.
- Proving awareness among people in the study area

VI. CONCLUSION

According to the analysis, Anuradhapura District has been highly affected by the drought. The increasing temperature and decreasing rainfall caused the drought in Anuradhapura District. 114 years data clearly show the condition of the drought in Anuradhapura District. The agriculture of Anuradhapura District hasalso been affected due to the drought and the national paddy supply would be reducing if this condition continue.

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Effect of Seed Priming on Seed Germination and Vigour in Fresh and Aged Seeds of Cucumber.

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Abstracts*—Seed priming (seed invigouration) has gained a lot of importance in recent times as it emerged as a most promising area of seed quality enhancement technique. Priming is a pre-sowing treatment, that involves exposure of seeds to a low external water potential that limits hydration,(controlled hydration of seed) to a level that permits pre-germinative metabolic activity to proceed, but prevents actual emergence of the radical. Fresh seeds of cucumber was artificially aged as per ISTA standards to obtain low vigour seed lots. Both high vigour (unaged) and low vigour (aged) seeds were subjected to seed priming with various chemicals (water, KH_2PO_4, ,K_2HPO_4,oxalic acid,gibberellic acid,KNO3,calcium chloride, salicylic acid) and results were compared to identify the best priming treatments. Changes in physiological (per cent germination, total seedling length, total seedling dry weight, seedling vigour index I and II). In cucumber seed priming improved seed germination and vigour significantly over unprimed. The response of low vigour (aged) seeds to seed priming was much higher when compared to high vigour (unaged) seeds. In cucumber, among various priming chemicals tested, seed priming with KH_2PO_4 10-^{3}M, $K_2HPO_4$10-^{3}M, KNO_3 0.5% were found best as there was 8, 8 and 8 % more germination in high vigour seeds and 15, 15 & 10 % higher germination in low vigour seeds over unprimed (control).Priming with these chemicals also showed better germination per cent in both high and low vigour seeds, over hydropriming, in both the crops. Besides germination, marked increase in vigour as evident from seedling vigour index I and II was noticed due to seed priming in both the crops when compared to unprimed. In cucumber, there was 56 and 53 % higher SVI in less vigour seeds due to priming with KH_2PO_4 10-^{3}M and $K_2HPO_4$10-^{3}M,respectively,compared to unprimed, with respect to SVII, the values were 73 and 62 % more due to priming with KH_2PO_4 10-^{3}M and $K_2HPO_4$10-^{3}M, respectively, compared to unprimed in cucumber. Priming with chemicals appears beneficial as there was high per cent vigour when compared to priming with water (hydropriming). In cucumber, priming with KH_2PO_4 10-^{3}M increased SVI by 39 and 36.7 % and SVII by 35 and 26.6 % over hydropriming.*

Keywords:-seed quality, priming, hydropriming, seed ageing.

I. INTRODUCTION

Today's competitive agricultural development environment demands that growers produce high yield of good quality seeds to meet the market demand. In this scenario vegetables play a vital role in the health and nutritional security of human beings in addition to improve the economy of the farmers. Although India is the second largest producer of vegetables in the world next to China, the productivity is very less (<14 t/ha). Hence, there is a great need to enhance the productivity of vegetables gradually to boost up the production. The present seed requirement under vegetables crops is estimated to be 49.1 thousand tones . In crop production, stand establishment determines plant density, uniformity, and management options. For expensive hybrid vegetable seeds, it is particularly important that seeds germinate rapidly and uniformly, tolerate adverse germination conditions, and produce normal seedlings. Seed vigor has been proved to be the primary factor governing seed quality, in the context of successful stand establishment. Hence seed invigouration/enhancement of seed vigor has been a major area of interest for researchers, owing to its high industrial and economical implications. Seed invigouration is a post harvest, pre-sowing technique for improvement of seedling emergence and stand establishment. The most promising invigouration technique for improving the rate and uniformity of plant stand is seed priming.

Priming has practical implications in improving performance of vegetable crops under stressed environmental conditions such as salinity, drought, low and higher temperature (M. Piri 2009). Poor quality seeds

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generally show decline in their ability to germinate and emerge into vigourous seedlings, leading to problems for successful crop production. Seed priming is a pre-sowing treatment that involves controlled hydration of seeds, sufficient to allow pre germinative metabolic events to take place and to restrict radical protrusion through the seed coat (Heydecker *et al*., 1973). This technique has been used in some vegetables seeds including cucumber to augment the germination rate, total germination and seedling uniformity etc., mainly under unfavorable environmental conditions. It is a useful technique to exploit seed potential in arid and desert ecosystem. Also the knowledge gained on the repair mechanisms that take place upon various priming treatments has been used in many crops of seed industry. Hydro priming is nothing but soaking seeds in water for a precise time followed by re-drying. Osmopriming involves soaking seeds in aerated water potential of different osmotica (Polyethylene glycol, mannitol, sorbitol, glycerol, etc.,) to control the amount of water imbibed by seeds. Chemopriming is soaking seeds in various inorganic salt solutions like KCl, KNO_3, $CaCl_2$, KH_2PO_4, etc.the objectives of this research is To identify suitable priming chemicals for enhancement of vigour in cucumber.and the objective of this project is to identify suitable priming chemicals for enhancement of vigour in cucumber.

II. MATERIAL AND METHODS

The present investigation on seed invigouration aspects of cucumber was conducted in the Section of Seed Science and Technology, Indian Institute of Horticultural Research, Hesaraghatta, Bangalore- 560089. The details of the materials used and methods adopted for the conduct of various experiments on seed invigouration are described hereunder:

Source of seeds

Freshly harvested and graded tomato seeds of cv. Arka meghali were obtained from the seed production unit of IIHR, Hessaraghatta, Bangalore and cucumber seeds of cv. Green long were obtained from the seed market, Avenue Road, Bangalore.

Fresh cucumber (> 95% germination) seeds was subjected to accelerated ageing as per ISTA procedure to obtain low vigour (< 60 % germination) seed lot for use in the experiment. These low and high vigour seeds were subjected to different invigouration treatments to standardize suitable chemicals. Among these treatments, best treatments were identified for further in-depth study to know the physiological changes in relation to seed quality enhancement in cucumber.

Treatment details

Vigour levels (V) : V1- Fresh seeds - high vigour (>87 %)
V2- low vigour (<50%)
Socking time:- 24 hours(tomato), 48 hours(cucumber)
Soaking temperature (T): Ambient (28 ± 1^0C)
Treatments:
T_1- Control, T_2 Hydro priming, T_3-$CaCl_2$ (0.001M), T_4-Oxalic acid (0.01M), T_5 - GA3 (500 ppm), T_6-KNO_3(0.5%), T_7-K_2HPO_4 (0.001M) T_8- KH_2PO_4 (0.001M), T_9 -SALYCILIC ACID (0.5%)
Total treatment combinations: = 9
Replications: 4

Observations recorded

The different observations recorded were

I. Final count germination (%)
II. Total seedling length (cm)
III. Total seedling dry weight (mg)
IV. Seedling vigour index –I (Germination percentage X Seedling length)
V. Seedling vigour index –II (Germination percentage X Seedling dry weight)

III. RESULT & DISCUSSION

In cucumber seed priming improved seed germination and vigour significantly over unprimed. The response of low vigour (aged) seeds to seed priming was much higher when compared to high vigour (unaged) seeds. In cucumber, among various priming chemicals tested, seed priming with KH_2PO_4 10^{-3}M, $K_2HPO_4 10^{-3}$ M, KNO^3 0.5% were found best as there was 8, 8 and 8 % more germination in high vigour seeds and 15, 15 & 10 % higher germination in low vigour seeds over unprimed (control). Priming with these chemicals also showed better germination per cent in both high and low vigour seeds, over hydropriming, in the crops. Besides germination, marked increase in vigour as evident from seedling vigour index I and II was noticed due to seed priming in both the crops when compared to unprimed. In cucumber, there was 56 and 53 % higher SVI in less vigour seeds due to priming with KH_2PO_4 10^{-3} M and $K_2HPO_4 10^{-3}$ M respectively, compared to unprimed. Similarly, with respect to SVII, the values were 73 and 62 % more due to priming with KH_2PO_4 10^{-3} M and $K_2HPO_4 10^{-3}$M, respectively, compared to un-primed in cucumber. Priming with chemicals appears beneficial as there was high per cent vigour when compared to priming with water (hydropriming). In cucumber, priming with KH_2PO_4 10^{-3} M

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increased SVI by 39 and 36.7 % and SVII by 35 and 26.6 % over hydropriming. **(Table 1).**

IV. CONCLUSION

Fresh seeds of cucumber was artificially aged as per ISTA standards to obtain low vigour seed lots. Both high vigour (unaged) and low vigour (aged) seeds were subjected to seed priming with various chemicals (water, KH_2PO_4, ,K_2HPO_4,oxalic acid,gibberellic acid,KNO3,calcium chloride, salicylic acid) and results were compared to identify the best priming treatments. Changes in physiological (per cent germination, total seedling length, total seedling dry weight, seedling vigour index I and II) and biochemical parameters(Electric conductivity, total soluble sugars and proteins, dehydrogenase activity, amylase activity, catalase activity,protein profiles,isozyme profiles) in relation to enhancement in viability and vigour upon priming were identified. The germination ability of primed seeds was compared with unprimed seeds under various abiotic stress conditions.

Table.1: Effect of seed priming on seed germination and vigour in fresh and aged seeds of cucumber.

TREATMENTS	Germination Percentage		Total Seedling Length (cm)		Total Dry Weight (g)		Seedling Vigour Index I		Seedling Vigour Index II	
	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged	Fresh	Aged
Control	87.0	50.0	29.5	25.0	0.21	0.12	2566	1250	18.27	6.00
Hydropriming	90.0	55.0	30.8	25.5	0.23	0.14	2772	1402	20.70	7.70
$CaCl_2$ (10^{-3})	83.0	60.0	31.7	25.9	0.23	0.14	2631	1554	19.09	8.40
Oxalic Acid (10^{-3})	82.0	46.0	31.2	27.3	0.25	0.14	2558	1255	20.50	6.44
GA3 500ppm	87.0	60.0	32.2	26.2	0.21	0.16	2801	1572	18.27	9.60
KNO3 0.5%	95.0	55.0	29.7	28.3	0.26	0.16	2821	1556	24.70	8.80
K2HPO4 (10-3)	95.0	65.0	32.7	29.5	0.28	0.15	3106	1917	26.60	9.75
KH2PO4 (10-3)	95.0	65.0	34.2	30.0	0.28	0.16	3249	1950	26.60	10.40
Salicylic Acid 0.1%	92.0	50.0	33.2	30.0	0.20	0.13	3054	1500	18.40	6.50
CD@ 1% (means-main factor)	F:- 2.1 A-3.3				F-0.8 A-0.5		F-0.02 A-0.02		F-184 A-190	
	F-0.07 A-0.06									

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Effect of the use of Potassium Fertilizer on the Resistance and Growth of Tomato to Bacterial Wilt caused by *Ralstonia solanacearum*

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Abstract*—The research aims to study the effect of sources and doses of potassium fertilizer on the resistance and growth of tomato to bacterial wilt caused by Ralstoniasolanacearum. We conduct experiment in a screen house in Faculty of Agriculture, Islamic University Malang. The research is conducted experimentally using completely randomized block design (RAK) arranged in factorial with three repetition. There are six combinations of treatment. Factor I: source of potassium fertilizer, consists of two levels: KCl and K_2SO_4. Factor II:dose of K_2O, consists of three levels: 50 kg ha^{-1}, 100 kg ha^{-1} and 200 kg ha^{-1}. The inoculation of Ralstonia solanacearum is conducted a week after transplanting. There is significant effect on the use of different sources and doses of potassium fertilizer. As whole, the use of potassium fertilizer originated from K_2SO_4 is better than that of KCl and the magnitude of the increase depends on dose applied. The best result indicates by treatment of the use of K_2SO_4 with dose of 200 kg ha^{-1} K_2O that able to extend the incubation period of 6,27 days, decrease the attack level of 73,15%, increase the uptake of potassium and leaf chlorophyll of 4,58% and 7,17%, respectively,and increase root lignin of 3%, whereas total phenol is decreased of 27,27% compare to the use of KCl in the same dose.*

Keywords*— *Potassium fertilizer, Source, Dose, Plant resistance, Ralstonia solanacearum.

I. INTRODUCTION

Tomato is one of superior horticultural commodities in Indonesia and has a promising economic prospect. Therefore, it needs serious handling, especially, in terms of increasing its yield and fruit quality. The projection of national tomato demand for 2014-2019 is around 970.499 – 1,107,168 ton, whereas tomato product by 2013 was only 922,780 ton with average productivity of 16.61 $t.ha^{-1}$ (Bureau of Statistics and Directorate General of Horticulture, 2014).

One of obstacles in the low production of tomato is the occurrence of wilt disease caused by *Ralstonia solanacearum* bacteria, which is a threat for hot climate areas or areas with warm rainy season. Result of field observation shows that the disease has caused the loss of fresh fruit in approximately 7.1 – 63.7% (Rosyidah *et al.*, 2014). It is unfavorable for farmers since the investment for production cost is high.

Various efforts of controlling the disease have been conducted, such as the use of organic material from chicken manure (Rosyidah, A., 2012), the use of cabbage family as bio-fumigant, and the use of resistance variety (Rosyidah *et al*., 2014). Another effort is the use of potassium fertilizer with appropriate source and dose. This effort is another alternative to increase plant resistance against environmentally friendly disease.

Tomato plant absorbs large amount of potassium element in approximately 1-5% of plant's dry weight (Chen and Gabelman, 2000). Potassium plays important role in plant metabolism (Farhad *et al*., 2010), helps in the formation of protein, carbohydrate, enzyme activity, regulation of osmotic, water use efficiency, translocation of photosynthate (McKenzie, 2001), stimulate the development of root and increase the size of fruit (Marsono and Sigit, 2001), and increase the transportation of sugar and acid to storage organ (Bernardi *et al.*, 2013). The application of potassium could increase the formation of thick lignin compound; therefore, wall cell will be stronger and able to protect plant from pathogen interference (Fageria *et al*., 2009).

Potassium fertilizer mostly used in Indonesia is muriate of potash (KCl) containing about 60% of K_2O. Recently, however, there is a development in the use of potassium sulphate (K_2SO_4). Some research found that the incident of disease is higher when potassium fertilizer used is originated from KCl, whereas potassium sulphate is proven to improve some characteristics of quality of various vegetable products (Gunadi, 2009). At present, the role of potassium in increasing plant resistance, especially in tomato, has not been studied.

The aim of the research is to study the effect of combination of source and dose of potassium fertilizer on

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the resistance and growth of tomato toward bacteria wilt caused by *Ralstonia solanacearum*.

II. MATERIALS AND METHODS

The research was conducted experimentally in a screen house in Faculty of Agriculture, Islamic University Malang in April – July, 2016. The altitude of the location was 460 above sea level. Type of soil is loam. Air temperature is around 22.5 ^{0}C – 25.5 ^{0}C with air humidity of 80% - 86%. The research was conducted experimentally using completely randomized block design (RAK) arranged in factorial with three repetition. There were six combinations of treatment. Factor I: source of potassium fertilizer, consisted of two levels: KCl and K_2SO_4. Factor II was dose of K_2O, consisted of three levels: 50 kg ha^{-1}, 100 kg ha^{-1}and 200 kg ha^{-1}. Each treatment consisted of ten sample plants.

Tomato seeds from Lentana variety were planted in seeding basin with media of soil + sand + compost that previously sterilized with hot steam for 3 hours with ratio of 1:1:1. At the age of 10 days, plant's seedlings were transferred to seedling glass, one seedling per glass.

Growing media used weresoil:sand:organic material of chicken manure (C/N = 12) (ratio of 2:1:1) that previously sterilized with hot steam for 3 hours. Growing media of 8 kg was put into a polybag. The transplanting of tomato seedling was conducted when the seedling has height of 10 cm and 4 leaves.

Inorganic fertilizers applied were SP-36 and it was applied on 3 days after transplanting with dose of 150 kg/Ha and Urea on 7 days after transplanting with dose of 150 kg Ha^{-1}. The application of KCl and K_2SO_4 fertilizers was conducted at the age of 7 days after transplanting with dose in accordance with the treatment.

The isolate of *R. solanacearum* used was the result of isolation of tomato plant attacked by *R. solanacearum* in Donowarih Village, Karangploso, Malang. The purification isolation and propagation of *R. solanacearum* was conducted using media of TZC (2,3,5-triphenyl tetrazolium chloride). Population density for inoculation was 2.78x10^8 cfu.mL^{-1} measured with spectrophotometer in OD 600. Plants at the age of 1 week after transplanting were inoculated with *R. solanacearum* with concentration of 10^8 cfu/ml of 20 ml by wounding the plant roots using scalpel.

Observation was conducted on: incubation period of the disease, level of disease attack (Sinaga, 2003), potassium uptake in leaves (through extraction using NH_4Oac), root lignin (Acid detergent fiber method), total phenol (Folin-Denis method), level of leaf chlorophyll (SPAD) and plant height.

In order to see the influence of treatments on observation conducted, data of observation result was statistically analyzed based on analysis of variance (ANOVA) and followed by Least Significance Difference test in confidence level of 95% to see their significances.

III. RESULT ANDDISCUSSION

The status of soil fertility used in the experiment is presented in Table 1. The content of carbon (C), nitrogen (N), and C/N ratio is classified as low. Phosphor (P) and potassium (K) are classified as medium and low, respectively, whereas, cation exchange capacity (KTK) is classified as medium.

Incubation period and level of attack of R. solanacearum disease

Based on research result (Table 2), it can be seen that there was a significant interaction ($p < 0.05$) in the treatment of the use of potassium fertilizer sources and doses on incubation period of the disease and the attack level of bacterial wilt disease.

Based on Table 2, it is known that the use of different potassium fertilizer sources and doses resulted in different disease incubation period and level of attack. The incubation period of the disease was ranged from 16.21 to 25.74 days after inoculation. Level of attack of the disease was ranged from 6.22% to 12.21% at the age of 35 days after transplanting. The application of potassium fertilizer of K_2SO_4 is better than those of KCl. It is estimated that it is due to the content of SO_4 in potassium sulphate fertilizer since one of the functions of sulfur (S) is to reduce the attack of the disease (Tisdale *et al.*, 1990). The higher the dose of potassium fertilizer (200 kg ha^{-1} K_2O) applied, the longer the incubation period and the lower the attack level caused by *R. solanacearum* pathogen. It happens because the initial content of K element in soil used for the experiment was low. With the addition of potassium of 200 kg ha^{-1} K_2O in the treatment gives sufficient nutrient and good plant resistance. One of the function of K element is to improve plant resistance by strengthening plant tissues and thickening epidermic wall. Nurhayati (2008) stated that potassium in plant plays role in the formation of protein and carbohydrate as well as in the increasing of resistance against pathogen.

Potassium uptake, root lignin and total phenol

Research result shows that there was significant interaction ($p < 0.05$) in the treatment of the use of potassium fertilizer sources and doses on potassium uptake, root lignin and total phenol (Tabel 3).

Based on Table 3, it is known that the use of different potassium fertilizer sources and doses resulted in different potassium uptake, root lignin level and total phenol level of plant. The application of potassium fertilizer K_2SO_4 was better than that of KCl. In addition, the higher the dose of potassium fertilizer (200 kg ha^{-1} K_2O) applied,

the higher the leaf potassium intake and root lignin level and the lower the level of total phenol.

Result of observation on the level of potassium uptake in leaves shows that bigger potassium uptake in leaves will increase availability status of potassium in plant organs. The sufficiency of potassium has function in increasing the status of plant defense to improve damage caused by pathogen since plant is able to increase the strength of its cell wall. Hardter, R (2003) and Pervez, H *et al*., (2007) add that the sufficient level of potassium in plant could increase the strength of paddy's stem and stalk due to the increase of its resistance. It is also explained that plant stomata and lenticel work well if sufficient potassium is exist. When pathogen invaded the plant, stomata and lenticel have the ability to close quickly. Potassium is also able to improve the work of enzyme for plant metabolism. The sufficiency of potassium in plant will increase the synthesis of molecular compounds with high molecular weight (protein, starch, cellulose) thus decreasing the synthesis of molecular compounds with low molecular weight, such as: organic acid, amino acid, and amide in plant tissues. It is the decrease of the synthesis of compounds with low molecular weight that able to increase plant resistance against pathogen infection (Marschner, P., 2012; Mengel, K., 2001). Potassium element also plays role in lignification of sclerenchyma tissue (Fageria *et al*., 2009). Therefore, the sufficiency of potassium could increase the formation of thicker lignin compound; thus, cell wall is stronger and able to protect plant from external disturbance.

Observation on total phenol shows that the increasing of potassium dose applied will decrease the level of total phenol. In other words, the lower the doses of potassium fertilizer applied, the bigger the level of attack; therefore, a tendency of the increase in phenol compound level. The increase in phenol compound is the reaction of plant toward infection of *R. Solanacearum* pathogen and root wounding before pathogen inoculation. Pieterse *et al*., (2009) stated that the increase of plant resistance through SAR (Systemic Acquired Resistance) occurs after local pathogen infection in plant; the infected plant, then, activates genes that play role in the resistance to produce chemical compounds for plant resistance, such as salicylate. When the plant has the resistance, it will be able to protect itself if another pathogen exists; thus, pathogen infection will not be developed. According to Goodman *et al.,* (1986), plant tissue infected by pathogen indicates a change in metabolic pattern, including, activating peroxide and other phenoloxidase enzymes. It is in line with Matern *et al*. (1995) and De Ascensao *et al.* (2003) stated that great phenol synthesis will occur if plant is attacked by pathogen. Agrios (2005) stated that pathogen microorganism causing mechanical and chemical damages will stimulate plant to produce toxin compound against pathogen (phytoalexin). Plants need peroxide enzyme to produce resistance compounds, such as lignin, chitin, and various compounds that build cell wall (Hallman, 2001). Further, Bruce et al. (1989) stated that peroxide is another component in the initial response of plant to pathogen attack and plays key role in the biosynthesis of lignin that limit the area of pathogen distribution.

Leaf chlorophyll and plant height

Research result shows that there was significant interaction ($p<0.05$) on the treatment of the use of potassium fertilizer sources and doses on the content of leaf chlorophyll. Regarding observation on final plant height, it shows no interaction between potassium fertilizer sources and doses tested (Figure 1A and 1B).

Treatment of the application of K_2SO_4shows more chlorophyll content than that of KCl. It is likely due to the content of sulfur in K_2SO_4 fertilizer. Sulfur is the main element in the formation of leaf chlorophyll that closely related to photosynthesis process and takes part in various metabolism reactions, such as carbohydrate, fat and protein (Tisdale *et al.,* 1990). The increase in potassium fertilizer dose applied will increase chlorophyll content. It is due to the sufficiency of potassium in the plant that will increase the work of enzymes thus increasing the activation of plastid in leaf, synthesis of protein, photosynthesis and stomata movement. It results in the increase in the production of leaf chlorophyll. The optimum availability of potassium in leaf will make the leaf to be more efficient in the photosynthesis and plant will be more resistance and tolerance.

IV. CONCLUSIONS

In the research, we report the effect of the use of potassium fertilizer on the resistance and growth of tomato to bacterial wilt caused by *R. solanacearum*. The result can be summarized as follow: the effect of the interaction is significant in the component of resistance but not in the observation on plant height. Treatment of K_2SO_4 and doses of potassium fertilizer (200 kg ha^{-1} K_2O) is the best as indicated by longer incubation period of 6.27 days, decrease in the level of attack of 73.15%, increase in potassium uptake and chlorophyll in leaf of 4.58% and 7.17%, respectively and the increase in root lignin of 3% and decrease in phenol of 27.27% compare to the use of KCl in the same dose.

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Table.1: Status of soil fertility used as growing media

pH	C	N	C/N	P	K	KTK
(H2O)	....%			(Bray)	me/100g	me/100g
5,4	0,98	0,13	8	417,1	0,29	22,38

Source: Laboratory of Soil, Faculty of Agriculture, University of Brawijaya, 2016

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Table.2: Incubation period and attack level of the disease due to the interaction of potassium fertilizer sources and doses

Treatments	Incubation period (days)	Disease incidence (%)
KCl		
50 kg /ha K2O	16,21 a	12,21 e
100 kg /ha K2O	17,58 b	11,10 d
200 kg /ha K2O	19,47 c	10,77 d
K2SO4		
50 kg /ha K2O	22,23 e	9,25 c
100 kg /ha K2O	21,13 d	8,80 b
200 kg /ha K2O	25,74 f	6,22 a
LSD 5%	0,60	0,41

Note: Numbers with different letters in the same column shows significantly different in Least Significance Difference test with level of 5%

Table.3: Potassium uptake, root lignin and total phenol due to the interaction of potassium fertilizer sources and doses

Treatments	Potassium uptake (%)	Root lignin (%)	Total phenol (mg/g)
KCl			
50 kg/ha K2O	1,087 a	15,01 a	1,393 a
100 kg/ha K2O	1,114 b	15,96 b	1,430 ab
200 kg/ha K2O	1,141 c	16,03 c	1,453 ab
K2SO4			
50 kg/ha K2O	1,114 b	15,98 b	1,513 c
100 kg/ha K2O	1,157 d	16,06 c	1,593 d
200 kg/ha K2O	1,165 e	16,46 d	1,833 e
LSD 5%	0,0087	0,03	0,056

Note: Numbers with different letters in the same column shows significantly different in Least Significance Different Test in level of 5%

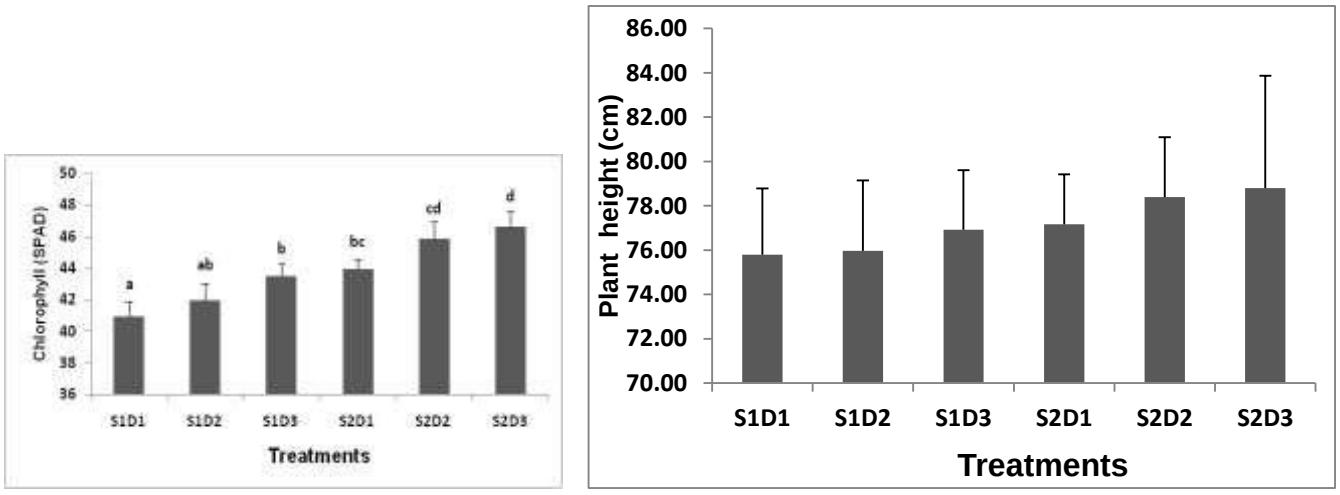

Fig.1: A and B. The interaction of the effect of potassium fertilizer sources and doses on leaf chlorophyll (A) and plant height (B).S1D1= KCl 40 kg ha^{-1} K_2O, S1D2= KCl 80 kg ha^{-1} K_2O, S1D3= KCl 160 kg ha^{-1} K_2O, S2D1 = K_2SO_4 40 kg ha^{-1} K_2O, S2D2 = K_2SO_4 80 kg ha^{-1} K_2O, S2D3 = K_2SO_4 160 kg ha^{-1} K_2O

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Use of Nanotechnology in Food Industry: A review

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Abstract— *Food science is emerging in a fast way with collaboration of nanotechnology. The food market demands technologies, which are essential to keep market leadership in the food processing industry to produce fresh authentic, convenient and flavorful food products and nanotechnology is the answer to it. Nano particles are used as nano inside as additives and nano outside as packaging. The packaged food products are proving more health beneficial and hygiene with the help of nanotechnology. Nano particles are using as food additives makes food to stay away from microbial contamination hence lengthening the lifespan. Nanoscale food additives may for example be used to influence product shelf life, texture, flavor, nutrient composition, or even detect food pathogens and provide functions as food quality indicators. Nanotechnology providesa vast range of opportunities for the development of new products and applications in food system. Functional foods, nutraceuticals, bioactives, pharmafoods, etc. are very recentexample of it. Lowering of the cost of food additives is a milestone of using nano food additives.*

Keywords— Nano Foods, Nano Emulsion, Nanoencapsulation.

I. INTRODUCTION

Nanotechnology is the field deals with the materials of nanoscale. The National Nanotechnology Initiative calls it "nanotechnology" if only, "the research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1-100 nanometer range, creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size and ability to control or manipulate on the atomic scale" [Ozimek et. al 2010]. Nano Particles are typically results in greater chemical activity, biological activity and catalytic behavior compared to large particles of the same composition. In food nano particles are used as additives such like preservatives, flavoring agent, antimicrobial sensors etc. and packaging substances. Nanotechnology provides a vast range of opportunities for the development of new products and applications in food system. Functional foods, nutraceuticals, bioactives, pharmafoods, etc. are very recentexample of it.Nano particles of Titanium dioxide, Silver, Zinc, Zinc Oxide, Silicon dioxide, Platinum, Gold are use vastly in food industry in different forms [Alfadul&Elneshwy2010]. In human food processing, nanocapsuleshave been used as nano-sized ingredients,additives, nutritional supplements, and infunctional foods reported thatnanoencapsulation of food ingredients andadditives have been carried out to provideprotective barriers, flavor and taste masking,controlled release, and better dispensabilityfor water-insoluble food ingredients andadditives [Mahmoud 2015].They are also aiming to develop improved tastes, reduce the amount of salt, sugar, fat and preservatives, address food-related illnesses (e.g. obesity and diabetes), develop targeted nutrition for different lifestyles and aging population, and maintain sustainability of food production, processing and food safety [Chaudhry et al. 2008].Nanoparticles and Nano capsules containing several foods are currently available for purchase, though without being required to indicate the presence of these Nano-materials on their packaging [Paul&Dewangan 2016].

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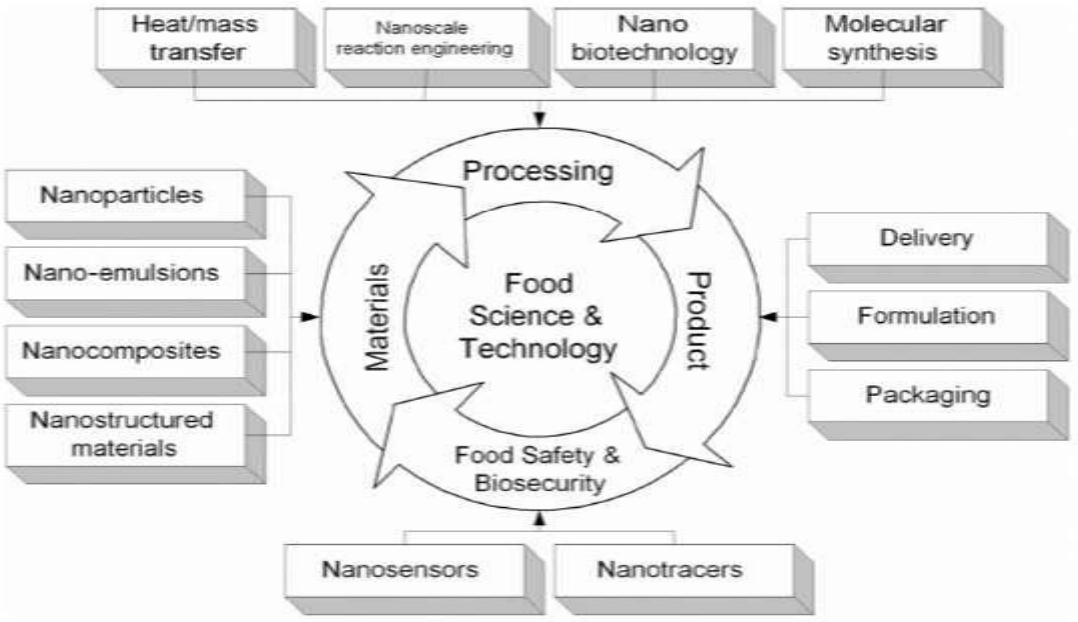

Fig.1: Application matrix of nanotechnology in food science and technology [GuhanNath et al. 2014].

Nanotechnology is contributing to the development of innovative packaging materials that can improve the safety and shelf life of products by providing barrier materials or detect foodborne pathogens.As example,Synthetic amorphous silica (SAS) is used for surface coating of packaging materials, for the clearing of beverages, and mostly as a free-flow and anti-caking agent in many powdered food items (E551) [Aschberger et al. 2015].Some food materials packaging are equipped with nano sensors is designed to track either the internal or the external conditions of the food products, pellets and containers throughout the supply chain. Such packaging can monitor temperature or humidity over time and then provide relevant information on these conditions [Prasanna&Shanmuganathan 2011]. Also, Nanotechnology is used these days to increase the bioavailability of many vitamins as well astheir precursors. The vitamins and precursors which are insoluble in water can be solubilized by a nanoparticleformulation [Prakash et al. 2013].The unique properties of these nanostructures and nanomaterials including physical, chemical, and biological properties are considerably different from their bulk counterparts alter the understanding of biological and physical occurrence in food systems. Several recent reports and reviews have identified potential applications of nanotechnology for the food sector to improve food safety, to enhance packaging and lead to improved processing and nutrition[Pathakoti et al. 2017].

The next wave of food innovation will require a shift of focus from macroscopic properties to those on the meso- and Nano-scales, as these subsequently control the hierarchical structures in food and food functionality [Paul &Dewangan 2016]. Though the urge of use of nanotechnology in food is increasing day by day the potential (eco) toxicological effects and impacts of nano particles have so far received little attention [Bouwmeester et al. 2009]. Very often the physicochemical characterization of the nano particles is very poor and was reported in less than

15% of the records concerning (eco)toxicity and risk assessment of nano particles. The most tested toxicity endpoints include genotoxicity, acute toxicity, cytotoxicity and repeated dose toxicity [Aschberger et al. 2015]. Before applying nano particles in consumer based products a good understanding of its potential negative impact on biological system is needed.

Nano Particles in Food Processing:

Food processing is the practice to preserve the food by different methods and techniques to transform food to a consumable state and these techniques are designed in such a way that the flavor and quality of the food are kept intact but they are also protected from micro-organisms.The food market demands technologies, which are essential to keep market leadership in the food processing industry to produce fresh authentic, convenient and flavorful food

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products and nanotechnology is the answer to it [Chaudhry 2009]. Food processing methods like incorporation nutraceuticals, mineral and vitamin fortification, gelation and viscosifying agents, nutrient delivery and nanoencapsulation of flavors use nanomaterials in their contents [Pradhan et al. 2015]. Nanoparticles are added to many foods to improve flow properties, colour and stability during processing, or to increase shelf life. For example, aluminosilicate materials are commonly used as anticaking agents in granular or powdered processed foods, while anatase titanium dioxide is a common food whitener and brightener additive, used in confectionery, some cheeses and sauces [Alfadul&Elneshwy2010].

Nanoencapsulation:

Nanoencapsulation provide several benefits such as enhance stability, ease of handling, retention of volatile ingredients, protection against oxidation, p^H and moisture triggered controlled release, taste making, consecutive delivery of multiple active ingredients, change in flavor character, long lasting organoleptic perception and enhanced bioavailability and efficacy [Marsh &Bugusu 2007]. Nanocapsules are prepared basically in six ways named as nanoprecipitation, emulsion-diffusion, double emulsification, emulsion-coacervation, polymer coating and layer-by-layer [Maynard et al. 2006]. Nanocapsules are used to deliver lipophilic health supplements such as vitamins and minerals in the food, fatty acids and growth hormones, increasing the nutrient content of the food [Dreher 2004].Patented "Nano drop" delivery systems is in the form of encapsulated materials, such as vitamins. It is administered transmucosally, rather than through conventional delivery systems such as pills, liquids, or capsules [Paul &Dewangan 2016].

That contains a natural biopolymer from yeast cell walls that is intended to bind mycotoxins to protect animals against mycotoxicosis is an example of a food additives. The potential use of an aflatoxin-binding nano-additive for animal feed, which is derived from modified Nano clay, has also been suggested. An NP that adheres to E. coli, comprising a polystyrene base, polyethylene glycol linker and mannose-targeting biomolecule has been developed by scientists. These NPs are designed to be administered through feed to remove food-borne pathogens in the GI tracts of livestock (FAO/WHO, 2010).Nanoparticles of Silica has no nutritional value. In food applications, it is mainly used as a technical additive by encapsulatingfor food processing. Silicas are especially used as free flowing agents (e.g. tomato powder, table salt and spices). They are also used as input and dispersion aids in vitamin additives, for example. In powder- type foods, synthetic amorphous silicas prevent clumping and maintain the pouring properties [8].

Some major nano-encapsulation techniques used in food processing are

1. Introduction of novel encapsulation techniques based on cold-set gelation for delivering heatsensitive bioactives including probiotics [GuhanNath et al. 2014].
2. Harnessing the casein micelle, a natural Nano - vehicle of nutrients, for delivering hydrophobic bioactives.
3. Developments and use of Maillard reaction based conjugates of milk proteins and polysaccharides for encapsulating bioactives [Livney 2009].
4. Discovering unique nanotubes based on enzymatic hydrolysis of α-lactalbumin.
5. Introduction of β-lactoglobulin–pectin nanocomplexes for delivery of hydrophobic nutraceuticals in clear acid beverages [GuhanNath et al. 2014].
6. Fatty acid-coated bovine serum albumin nanoparticles for intestinal delivery, and Maillard conjugates of casein and resistant starch for colon targeting [Livney 2009].
7. Development of core-shell nanoparticles made of heat- aggregated -lactoglobulin, nanocoated by beet-pectin, for bioactive delivery [Augustin &Sanguansri 2015].

Nanoemulsions:

Nanoemulsion production for delivery of functional compounds is one of the emerging fields of nanotechnology applied to food industry. Nanoemulsions consist of oil droplets in the nano-ranged size, between 10 and 100 nm dispersed within an aqueous continuous phase, with each oil droplet surrounded by surfactant molecules [Acosta 2009,McClements et. al 2007, 2009]. Nanoemulsions can protect flavor compounds from manufacturing conditions and throughout the beverage's shelf-life. NutraLease, a technology start-up company established by a scientific team, is working to improve the bioavailability of functional compounds. It is claimed that Nanoemulsions can capture the flavor and protect it from temperature, oxidation, enzymatic reactions and hydrolysis and are thermodynamically stable at a wide range of pH values [NutraLease 2011c].Unilever has made ice cream healthier without compromising on taste through the application of

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Nanoemulsions. The objective is to produce ice cream with lower fat content, achieving a fat reduction from the actual 16% to 1% . Nestlé has a patent in water-in-oil emulsions (10–500 nm), aiming at achieving quicker and simpler thawing through the addition of polysorbates and other micelle-forming substances; these are claimed to contribute to a uniform thawing of frozen foods in the microwave [Silva et al. 2012].

Nano Particles in Food Packaging:

Food packaging continues to evolve in response to the advancement of material science andtechnology, as well as the changing consumers' lifestyle. In today's global economy, packagingnot only is essential to enable effective distribution and preservation of food and other consumerproducts, but also to facilitate their end-use convenience and communication at the consumerlevels. With these important functions, packaging has become the third largest industry in the world and it represents about 2% of Gross National Product (GNP) in developed countries (Han, 2005; Robertson, 2005). Packaging provides containment and protects food products during distribution andstorage from external and internal unfavorable conditions, such as water vapour, microorganism,gases, orders, dust, and mechanical shock and vibrations. Due to consumers' complex and busy life-style in the modern society, food producers are striving to develop functional packagingsystems with enhanced end use convenience features. Besides all these functions, it providesessential product information to consumer to facilitate the promotion and advertisement of theproduct. In advanced packaging systems,these functions are augmented through interactive mechanisms driven by physical, chemical and/or biological processes. Here, intelligent packaging systems are those that possess enhancedfunction with respect to communication and marketing functions, such as to provide dynamicfeedback to the consumer on the actual quality of the product. On the other hand, activepackaging is focused on providing protection and preservation of the food through somemechanism activated by intrinsic and/or extrinsic factors (Lim, 2011).Nanotechnology is a powerful interdisciplinary tool for the development 47 of innovative products. It has been predicted that nanotechnology will impact at least $3 trillion across the global economy by 2020, creating a demand of 6 million employers in various industries (Duncan, 2011). In 2008, the global nanotechnology-related food packaging was US$4.13 billion, which has been projected to at about 12% compound annual growth rate (" Nano-enabled Packaging for the Food and Beverage Industry – A Global Technology, Industry and Market Analysis 2009," 2009). With this global trend, it is expected that nanotechnology will provide an important technology push in the food packaging industry to develop advanced packagingapplications for fulfilling consumer's needs. Nanotechnology is highly interdisciplinary involving the exploitation of materials with one or more dimensions that are less than 100 nm. Typical nanomaterials can be classified intothree main classes: 1) particulates; 2) platelets; and 3) fibers (Schmidt et al., 2002; Thostenson et al., 2005). Due to their nano-sized dimensions, these materials possess very large surface-to-volume ratio and surface activity. When added to compatible polymers, the nanomaterials can dramatically enhance the material properties of the resulting nanocomposites, such as improved mechanical strength, enhanced thermal stability, increased electrical conductivity, and so on (Uskokovic, 2007). Thus, nanomaterials are promising for improving the mechanical and barrier properties of food packages, as well as the development of advanced structures for active and intelligent applications.

A literature review of the safety and regulation of nanotechnologies in food packaging,specifically, the aim of the review was to:

I. Identify types of nanotechnologies currently used in food contact packaging with an aim to identify those that may result in migration of Nanomaterials from the packaging into food.
II. Where possible, identify publically available evidence that the nanotechnologies identified in the previous task are applied in Australia and/or New Zealand, either in domestically produced or imported products.
III. Ascertain if there is reasonable scientific evidence that the application of nanotechnologies to food packaging materials may potentially pose a risk to public health and safety, due to the migration of Nanomaterials into food and its subsequent ingestion.
IV. Include a brief synopsis of international regulations currently in place, or in development, which deal with the use of nanotechnologies in food contact packaging.
V. Use case studies, based on data, to place the above tasks into context and assist to identify data gaps that may hinder formal risk assessment of a novel Nanomaterials intended for food packaging.

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Nanoparticles in this report are defined as an engineered form of matter having at least one dimension in the nanometer scale (<100 nm). Similarly, Nanomaterials are materials with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.

Numerous applications for Nanomaterials in food packaging have been proposed. Their purpose includes conveying antimicrobial and barrier properties to prevent food spoilage, enhancing film mechanical properties such as emulsification, foaming and water binding capacity, or enhancing other chemical-physical properties of polymers used in food packaging such as thermal stability and crystalline (Aresta et al. 2013, Beltran et al. 2014).

Although many Nanomaterials have been proposed for use in food packaging, this report focuses on those currently used in foods. The focus is also on food packaging per se(e.g. food containers, food wraps and films), rather than food contact materials (e.g. fridges, cutting boards, cutlery). Identifies the functions and types of Nanomaterials proposed for use in food packaging, and identifies those for which there is evidence of their use in world.Nanotechnology-enabled food packaging can generally be divided into three main following categories (Silvestre et al. 2011; Duncan 2011).

Improved packaging:

Whereby Nanomaterials are mixed into the polymer matrix to improve the gas barrier properties, as well as temperature and humidity resistance of the packaging.

Active packaging:

Illustrated by the use of Nanomaterials to interact directly with the food or the environment to allow better protection of the product. For example, silver nanoparticles and silver coatings can provide anti-microbial properties, with other materials being used as oxygen or UV scavengers(Chaudhry et al. 2008, Bott et al. 2014b, Dainelli et al. 2008, FoE 2008, Kuorwel et al. 2015).

Intelligent/smart packaging:

Designed for sensing biochemical or microbial changes in the food(de Azeredo et al. 2013, Emamifar et al. 2010, Fortunati et al. 2013, Llorens et al. 2012, Valipoor et al. 2013), for example detecting specific pathogens developing in the food, or specific gases from food spoiling. Some "smart" packaging has also been developed to be used as a tracking device for food safety or to avoid counterfeit.

Nanotechnologies used in food packaging

The information gathered indicated nanotechnology applications in the food sector are increasing worldwide, and many international food companies are exploring their potential applications. Among the nanotechnology applications for the food sector, nanotechnology-derived food contact materials make up the largest share of the current and short-term predicted market; a range of these are already available in some countries, and it is widely expected they will become increasingly available worldwide in the next few years (Chaudhry et al. 2008). In 2008 the global nano-enabled food and beverage packaging market was 4.13 billion US dollars and was projected to grow to 7.3 billion US dollars by 2014 (Duncan 2011, iRAP 2009).

Current uses

Among several thermoplastics, polyolefin are the most used plastics materials in the food packaging sector. Polypropylene (a type of polyolefin) films are often used because of their transparency, brilliance, low specific weight and chemical inertness. However, polypropylene (PP) (like other polyolefin's and other polymers is also characterized by low barrier properties (i.e. an inherentpermeability to gases and other small molecules), which results in poor protection of packaged foods. One of the methods to improve PP and other plastics' barrier deficiencies is to add a second component such as a polymer blend or multilayer, filler, etc(Avella et al. 2007, Duncan 2011, Fabra et al. 2013, Han et al. 2011, Manikantan and Varadharaju 2011, NanosafePACK 2012, Tang et al. 2008). Polymer-based nanocomposites are reported to achieve the same or better barrier properties than their conventional composite counterparts (Avella et al. 2005, 2007; Bott et al. 2014a, Mihindukulasuriya and Lim 2014).

Such nanocomposites are reinforced with small quantities (typically up to 5% by weight) of nanoparticles4, which have very high aspect ratios (L/h>300) (Chaudhry et al. 2008, FAO/WHO 2009, NanosafePACK 2012). They are incorporated in addition to the traditional fillers and additives. Nanomaterials have large aspect ratios which, when incorporated as fillers into the walls of packaging, creates an obstacle for gas and moisture passing through packaging walls by increasing the path that the gas/moisture must travel (Hannon et al. 2015).

Apart from conferring barrier properties to extend the shelf life of food (e.g. through an antimicrobial function or an oxygen- or water vapour- permeability barrier), other nanocomposites confer various physical characteristics to make the packaging more tensile, durable, or thermally stable (Beltran et al. 2014, Duncan 2011, NanosafePACK 2012). Examples of other nanocomposites include UV

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absorbers (e.g. nano-titanium dioxide, iron oxides, silica, alumina) to prevent UV degradation of plastic polymers, titanium nitride (TiN) used to improve strength of packaging materials, nano-calcium carbonate-polymer composites, nano-chitosan-polymer composites, biodegradable nanoclay composites of starch and polylactic acid5, biodegradable cellulose nano-whiskers, and other gas-barrier coatings (e.g. nano-silica) (Reig et al. 2014, Sanchez-Garcia et al. 2010, Siracusa et al. 2008, Smolander and Chaudhry 2010).

A well-known example of one of the first nanocomposites to be explored for use in food packaging to enhance barrier properties is nano-clay, which has been incorporated with nylons, polyolefins, copolymers, epoxy resins, polyurethane, polyethylene terephthalate, etc. Some materials are already commercially available, and used by beverage companies in certain countries. Nano-clay is presented in this report as a case study. Formulation of nanobiocomposites combining nanosilver with nano-clay or other nanomaterials (e.g. titanium dioxide) to enhance both barrier and antimicrobial properties has also been studied for potential future application in food packaging (Busolo et al. 2010, Cozmuta et al. 2014). Other examples of nanomaterials potentially used in food packaging6 include alumina (e.g. wheel-shaped alumina platelets used as fillers for plastic materials), nano-precipitated calcium carbonate (to improve mechanical properties, heat resistance and printing quality of polyethylene), polyhedral oligomericsilsesquioxane (POSS) nanoclay (to improve barrier properties), zinc oxide calcium alginate nanofilms (used as a food preservative), and silica/polymer hybrids (to improve oxygen-diffusion barriers for plastics) (Smolander and Chaudhry 2010, Bajpai et al. 2012).

II. CONCLUSION

In conclusion, nanotechnology will serve as an important tool to overcome existingchallenges that are associated with packaging materials. This advancement will positively affectthe shelf-life, quality, safety, and security of foods, which will ultimately benefit both the producers and consumers. However, precautionary principles should be applied and moreresearch is needed, especially on the migration behaviors of Nanomaterials in food and their potential impacts on health/safety, as well as the environment. A sustainable packaging solution can be achieved only if it is socially responsible, economically viable, and environmentallysound.

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