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Editorial Board

FOREWORD

I am pleased to put into the hands of readers Volume-2; Issue-6: Nov-Dec 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
Date: Dec, 2017

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Analysis of Variation of Water Quality in Kelani River, Sri Lanka

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Abstract— *This research attempted to analyze the variation of surface water quality in Kelani River and finally to create a mathematical model for the Water Quality Index (WQI) by using the data that have been obtained. Kelani River is the second largest river in Sri Lanka while being the most polluted river in the country. It is one of the main sources of drinking water for human beings while being the home for many aquatic species. The ecological aspects of Kelani River are threatened due to the increasing number of industries along both sides of the watershed. The present study is conducted to analyze the water quality in Kelani River using six parameters including pH, Turbidity, Temperature, Total phosphate, Nitrates, Dissolved Oxygen (DO), and ultimately finding the Water Quality Index (WQI) by collecting 5 sets of samples from seven locations from March 2017 to August 2017. The highest WQI (72.90) was reported near the Water Treatment Plant in Biyagama, while the lowest WQI (62.98) was achieved near the Water Board Pump in Ambathale. Water quality of five of the samples were in good quality range and the other two samples were in medium quality range. A mathematical model for WQI was created using the above mentioned six water quality parameters.*

Keywords— Kelani River, Surface Water, Water Quality Index.

I. INTRODUCTION

Water is one of the main sources on which our existence and settlement are built upon. Water sources can be found in the forms of rivers, glaciers, rain water, ground water etc. Degradation of these natural water resources and management of available fresh water is becoming more challenging due to various reasons such as Climate change, Geology, topography and soil type. Other than the natural characteristics of these natural water resources, the main factor which disturbs the water quality and the management of these water resources is the anthropogenic influence. As a result it has made the water quality and available fresh water management task more challenging for the water service providers, especially in developing countries. Somehow these activities generate contaminants which finally end up in rivers, streams and oceans through runoff and disposal of effluents. Climate change and land use can be identified as the factors that will directly affect the water bodies available for human and agricultural purposes. Improper land use will lead to soil erosion increasing the mobilization of suspended solids in receiving waters through runoff [1,2,5].

There are 103 natural river basins in Sri Lanka, with a total length of about 4,500km. However, the increasing demand of urban activities due to the rapid growth of population has been affecting the water quality of these river bodies. It has been identified by several researchers that the decreasing of water quality in rivers is due to point source of pollution such as the direct discharges from various land use types including residential, industrial and agricultural, and also non-point source of pollutants such as urban storm water run-off.

Kelani River is one of the main rivers in Sri Lanka which is considered as the most important river as it covers 80% of the water supply to Colombo. The starting point of the Kelani River is the Sri Pada mountain range and it travels along the hill country and finally flows in to the ocean at Colombo. Apart from being one of the main sources for potable water, Kelani River is used for transportation, hydropower generation, fisheries, irrigation, sewage disposal and sand extraction. The flow of the river varies between 800-1500 m^3/s during the monsoon and 20-25 m^3/s in the dry season, depending on the operation of 3 reservoirs in the catchment. The annual sand extraction is 600-800,000 m^3 per year. The sand is mined exclusively by hand. The sand mining causes the river bed to sink by some 10 cm per year [8].

However the Environmental Foundation Limited (EFL) has stated that Kelani River is the most polluted and threatened river in Sri Lanka due to agricultural runoff and domestic and municipal effluents and the effluents that have been discharged in to the river by the increasing number of industries which are located in the close vicinity of the Kelani River [3].

It has being identified that Kelani River is being polluted day by day due the increasing amount of pollutants that are released into the river. As a result it may reach a point in

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the future where it will no longer be obtainable for the purpose of supplying drinking water. This can be a huge problem as Kelani River plays a vital role in providing drinking water to the Colombo city. So understanding the current status of water quality in Kelani River will be useful for the future.

The aim of this study is to find out how the water quality parameters and the Water Quality Index has been varying along the Kelani River with the influence of the waste disposed by different land use types and finally to create a mathematical model for the Water Quality Index using the collected water quality data.

II. METHODOLOGY

2.1 Study Area

Samples were collected from seven sampling sites on a stretch of 8 km along the Kelani River. Fig. 1 shows the selected sampling sites namely,

1 Hela Textiles
2 Water board pump division
3 Pattivila
4 Biyagama Water Treatment Plant
5 Kaduwela Expressway Entrance
6 Lion Brew Factory
7 Malwana

Fig.1: Sampling sites

Five sets of surface water samples were collected at monthly intervals from March, 2017 to August, 2017. Poly ethylene terephthalate (PET) bottles were used to collect the water samples and they were transported to laboratory within 24 hours in a cooler box containing ice.

2.2 Measurement of Parameters

Temperature and Dissolved Oxygen (DO) were measured using a EUTECH DO meter, pH value was measured by a HANNA Combined pH/TEMP/MV meter, Total phosphate and Nitrate was measured using a UV visible spectrophotometer and Turbidity was measured by a HACH Turbidity meter. Model and the serial numbers of these apparatus are given in Table 1.

Table.1: Specifications of apparatus

Apparatus	Model	Serial Number
EUTECH DO Meter	DO600	S/N965992
HANNA Combined Digital PH/TEMP/MV Meter	HI2211-02	S/N08702309
UV Visible Spectrophotometer	DR6000	S/N1569293
HACH Turbidity Meter	2100N	S/N14010C031037

2.3 Data Analysis

Microsoft excel 2013 was used to calculate the water quality index value and to interpret the variations of the water quality parameters along the Kelani river.

Water quality Index can be found by using the equation shown in Fig. 2.

$$Water\ Quality\ Index = \frac{\sum(Q\ value\ x\ Weighting\ factor)}{\sum weighning\ factors}$$

Fig.2: WQI equation

Here, the corresponding q values for the raw data must be taken from the standard q value graphs. Each of the water quality factors is assigned a weighting factor which signifies its importance [6].

A mathematical model for the Water Quality Index was created using The R Project for Statistical Computing with the use of collected water quality data.

III. RESULTS AND DISCUSSION

The final results obtained for each and every seven sampling sites are given in Table 2.

3.1 Dissolved Oxygen

The dissolved oxygen (DO) is the amount of oxygen that is dissolved in water. The Dissolved Oxygen (% sat) values for this study varied between 19.63 and 41.45. The oxygen dissolved in lakes, rivers, and oceans is very important when it comes to the living of the organisms and creatures in it. The quality of water can be affected, as the amount of dissolved oxygen drops below normal levels in water bodies. As a result the organisms and creatures living in it may die. Moving water contains a large amount of dissolved oxygen while stagnant water has less amount of dissolved oxygen. If the DO concentration is high, then the quality of water can be considered as good [7]. The maximum DO value (41.45) was recorded near the Biyagama Water Treatment Plant while the minimum DO value (19.63) was recorded near the Hela Textiles Company. The standard value for DO (% sat) is given as 40 or more and here, only one sample has passed that

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value. The variation of DO along the selected area of the Kelani River is given in Fig. 3.

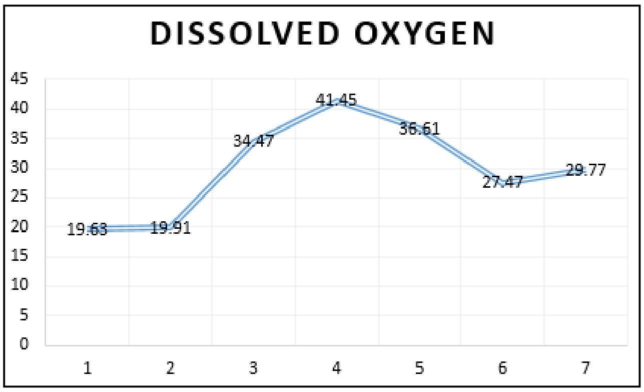

Fig.3: Variation of DO (%sat)

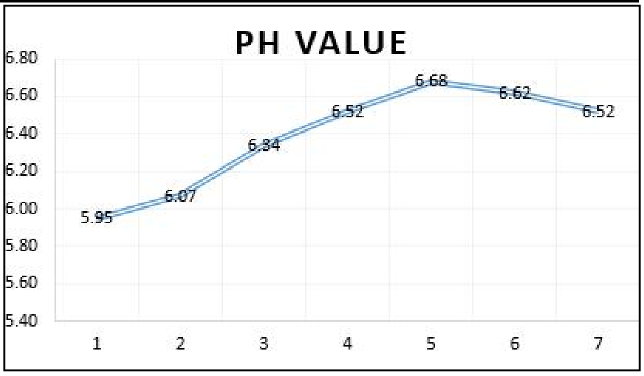

Fig.4: Variation of pH (units)

3.2 pH value

pH is an important parameter for water quality measurement. It is a measure of the acidity or alkalinity of the water. It can also be identified as the measure of hydrogen ion concentration. The pH scale ranges from 0 to 14. The highest pH value was recorded near the Kaduwela Expressway Entrance while the lowest pH value was recorded near the Hela Textiles Company.

Table.2: The average value of each parameter

Parameter	1	2	3	4	5	6	7
DO (% sat)	19.63	19.91	34.47	41.45	36.61	27.47	29.77
pH	5.95	6.07	6.34	6.52	6.68	6.62	6.52
Turbidity (NTU)	8.13	4.54	9.27	9.41	6.16	11.84	9.31
Temperature (^{0}C)	28.32	28.28	28.44	28.36	26.14	27.96	26.66
Total Phosphate (mg/L)	0.09	0.36	0.08	0.17	0.46	0.14	0.09
Nitrate (mg/L)	0.70	1.28	0.58	0.60	0.46	0.34	0.56
WQI	65.24	62.98	70.40	72.90	70.22	70.03	70.37

The normal range of pH for surface water is 6.5 to 8.5 [7]. Here, four of the samples are in the normal range. The variation of pH values is given in Fig. 4.

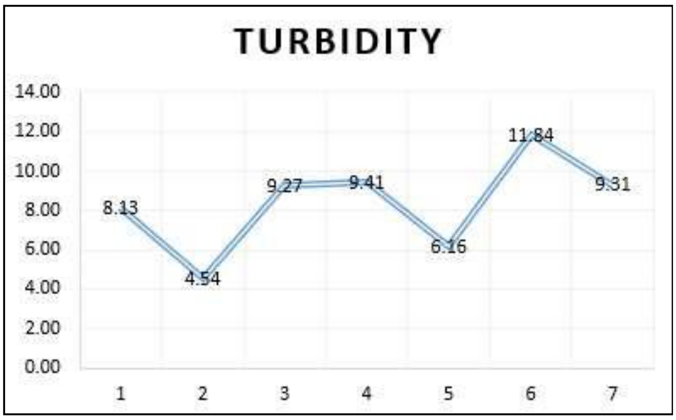

Fig.5: Variation of Turbidity (NTU)

3.3 Turbidity

Turbidity is the amount of cloudiness in the water. Water with high turbidity is cloudy, while water with low turbidity is clear. When the turbidity is high, water will be less aesthetically pleasing and also sunlight will warm it more. As a result temperature of the water will be increased. Turbidity is measured by Nephelometric Turbidity Units (NTU). The turbidity of surface water is usually between 1 and 50 [7]. The maximum turbidity value (11.84 NTU) was recorded near the Lion Brew Factory and the minimum turbidity value (4.54 NTU) was recorded near the Water Board Pump Division. The variation of turbidity of the samples is shown in Fig. 5.

3.4 Temperature

Temperature is a critical water quality and environmental parameter. It impacts the dissolved oxygen levels, chemical processes, biological process, water density and stratification of a river. The temperature of surface water is usually between 0ºC and 30ºC and all the values which were obtained are in this range [7]. The highest temperature (28.44 ^{0}C) was recorded near Pattivila area while the lowest temperature (26.14 ^{0}C) was recorded near the Kaduwela Expressway Entrance. Normally the temperature difference of the upstream and the downstream of the river is used for the WQI calculation process. Here it was assumed that the upstream and downstream temperature values are equal, hence there is no temperature difference. The variation of temperature is shown in Fig. 6.

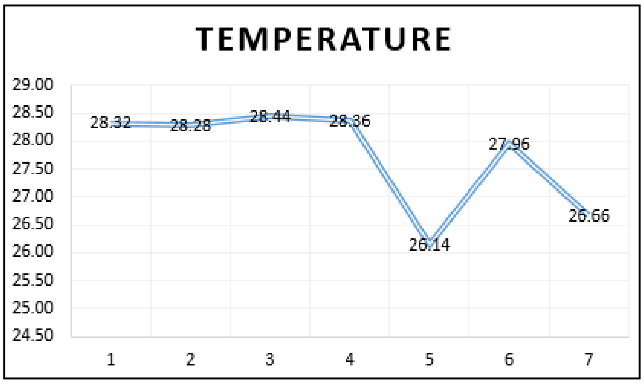

Fig.6: Variation of Temperature (^0C)

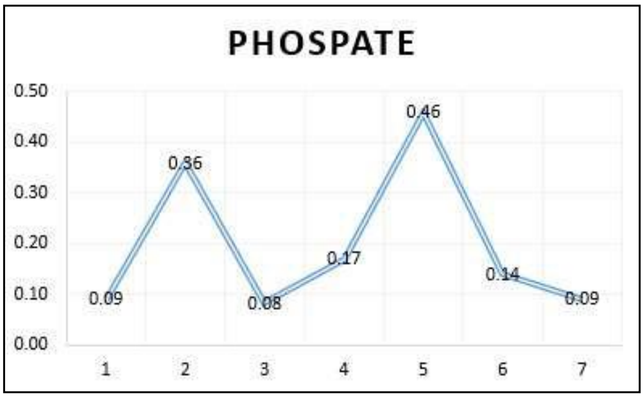

Fig.7: Variation of Total Phosphate (mg/L)

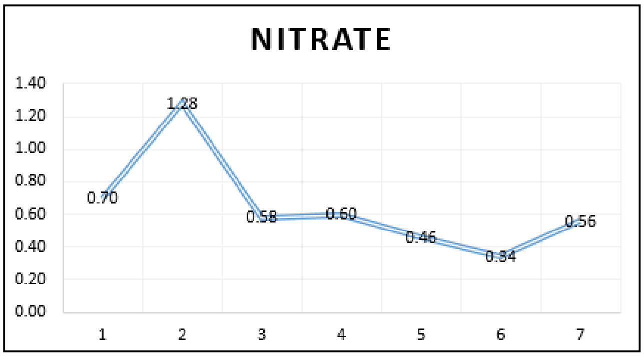

Fig.8: Variation of Nitrate (mg/L)

3.5 Total Phosphate

The Phosphate PO_4^{-3} ions can be found in three forms: orthophosphate, metaphosphate (or polyphosphate) and organically bound phosphate. These forms of phosphate occur in living and decaying plant and animal remains, as free ions or weakly chemically bounded in aqueous systems, chemically bonded to sediments and soils, or as mineralized compounds in soil, rocks, and sediments. Phosphate is a crucial element for plant life but when there is an excessive amount of phosphate in river water, it will speed up the eutrophication process [7]. The maximum amount of phosphate (0.46 mg/L) was found near the Kaduwela Expressway Entrance while the lowest amount (0.08 mg/L) was found near Pattivila area. Fig. 7 shows the variation of total phosphate.

3.6 Nitrate

Nitrogen is important for all living things as it is a component of protein. Bacteria convert various forms of nitrogen to nitrate (NO_3^-) in the soil. Excessive amounts of nitrate can cause an unpleasant condition for the survival of aquatic species and also it will help the algae to grow rapidly. This will create an environment which will speed up the eutrophication process [7]. As shown in Fig. 8, the highest amount of nitrate (1.28 mg/L) was found near the Water Board Pump Division while the lowest amount (0.34 mg/L) was found near the Lion Brew Factory.

3.7 Water Quality Index

Finally the WQI values were calculated for the seven sampling sites. The overall WQI ranges from 0 to 100 and the quality of water is graded into five classes [6].

- 100-90 Excellent
- 90-70 Good
- 70-50 Medium
- 50-25 Bad
- 25-0 Very bad

As shown in Fig. 9, five of the samples are in good quality range while two of them are in medium quality range.

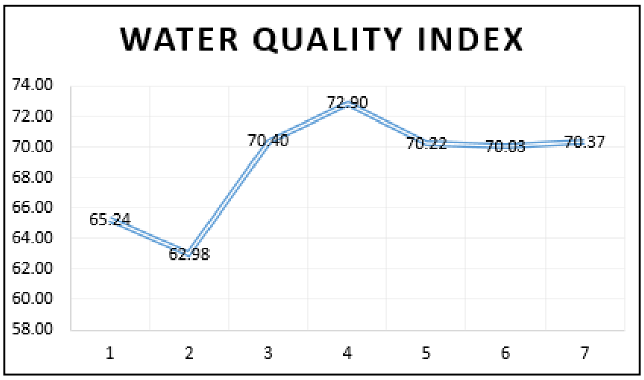

Fig.9: Variation of overall WQI

The highest WQI (72.90) was reported near the Water Treatment Plant in Biyagama, while the lowest WQI (62.98) was achieved near the Water Board Pump in Ambathale.

As the selected stretch of Kelani River has increased number of industries, hotels, restaurants, factories etc. along both sides of the watershed, here it is difficult to expect the quality of water to be in excellent condition. All of these places discharge their waste effluents into Kelani River [4].

$$WQI = 96.4790 + 0.3607(DO) - 2.5073(pH) - 1.1784(TEMP) + 1.7175(TP) + 1.9844(TN) + 1.0368(TURB)$$

Fig.10: Mathematical model for WQI

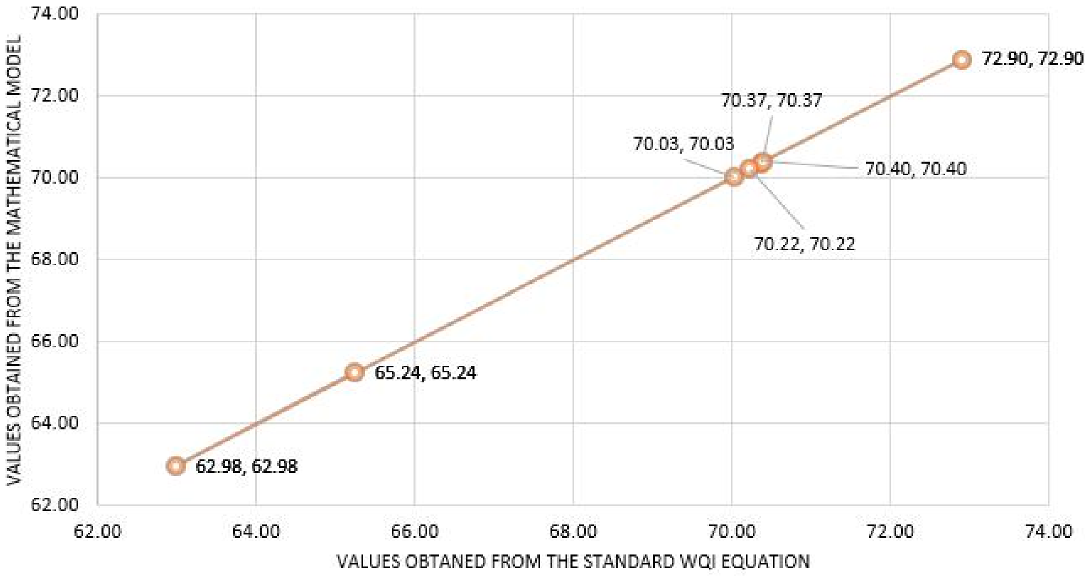

Fig.11: Comparison of values obtained from the mathematical model and the standard WQI equation

3.8 Mathematical model for Water Quality Index

The R Project for Statistical Computing was used in order to create a mathematical model for the collected water quality data. Table 2 shows the coefficients which were found for each and every parameter with the use of forward selection method. Intercept was found as 96.4790.

Table.3: Coefficients for the water quality parameters

Parameter	Coefficient
DO	0.3607
pH	-2.5073
Temperature	-1.1784
Total Phosphate	1.7175
Nitrate	1.9844
Turbidity	1.0368

Fig. 10 shows the ultimate mathematical model for the Water Quality Index. By comparing the values which were obtained using the newly created model and the standard Water Quality Index equation, it can be seen that both the values are same for each and every sampling site. This is illustrated in Fig. 11.

Using this model is much easier than the standard WQI equation, as additional values such as q values and weighting factors are not considered. The raw data which were collected as inputs for the standard WQI equation can be used with this model.

IV. CONCLUSION

With the rapid growth of industries along the sides of the Kelani River watershed, the amount of waste effluents that are released into the river has been increased. Understanding the water quality variation in an area like Kaduwela, which is highly populated, will be useful for the implement of pollution mitigation strategies in the future. The highest WQI (72.90) was reported near the Water Treatment Plant in Biyagama, while the lowest WQI (62.98) was achieved near the Water Board Pump in Ambathale. Water quality of five of the samples were in good quality range and the other two samples were in medium quality range.

The use of standard WQI equation can be stressful as there are some additional values to consider apart from the raw data but this newly created mathematical model will be easier to use, as the raw data can be directly entered. This mathematical model can be recommended for use, with the absence of q value graphs and weighting factors.

Acknowledgements

We take this opportunity to express our gratitude to the Department of Civil Engineering of General Sir John Kotelawala Defence University, Sri Lanka for providing all the laboratory facilities.

REFERENCES

[1] Chapman, D., 1996. *Water Quality Assessments- A Guide to Use of Biota, Sediments and Water in*

http://dx.doi.org/10.22161/ijeab/2.6.1 *ISSN: 2456-1878*

Environmental Monitoring. Cambridge: E&FN Spon, an imprint of Chapman & Hall.

[2] Goonetilleke, A., Thomas, E., Ginn, S. and Gilbert, D. (2005). *Understanding the role of land use in urban stormwater quality management. Journal of Environmental Management*, Vol. 74 (No. 1), pp. 31-42.

[3] Muiz, S. (2015, September 28). *Poisoning Kelani River, Slow and Steady.* Retrieved from Daily Mirror: http://www.dailymirror.lk/89167/poisoning-kelani-river-slow-and-steady

[4] Nizam, I. (2016, April 3). *Water Quality Of Kelani River Continues To Decline*. Retrieved from The Sunday Leader:http://www.thesundayleader.lk/2016/04/03/water-quality-of-kelani-river-continues-to-decline/

[5] Sharma, M.R. and Gupta, A.B., 2004. *Prevention and control of pollution in streams of outer Himalayas*

[6] Shweta Tyagi, B. S. (2013). Water Quality Assessment in Terms of Water Quality. *American Journal of Water Resources, 2013, Vol.*, 34-38.

[7] *The USGS Water Science School*. (2016, December 2). Retrieved October 16, 2017, from https://water.usgs.gov/edu/ph.html

[8] Wijesinghe, U. (2010, August 13). *Splendor of Sri Lanka*. Retrieved October 16, 2017, from https://splendorofsl.wordpress.com/2012/08/13/kelani-river/

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Comparative Economic Analysis of Rice Processing Methods in Benue State, Nigeria

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***Abstract*— *The study examined the comparative economic analysis of rice processing methods in Benue state, Nigeria. Random sampling technique was used to select 63 respondents made up of modern and traditional rice processing methods. The study also identified the major inputs used in rice processing, estimate the cost and returns in processing rice; identify the major factors militating against the modern rice processing methods .primary data were collected using structured questionnaire. The data were analyzed using descriptive statistics, gross margin and cobb-Douglas production model. The result of the analysis showed that the modern rice processors were dominated (74.6%) by male while the traditional were dominated (75.4%) by female. The gross margin for the modern rice processing methods was N16,770.00 per 100kg of rice higher than the traditional with N4,143.00 per 100kg of rice. The milling capacity of the modern was 200 kg/min. as against 50 kg/min. in the traditional methods. The study identified lack of awareness, low capital, poor infrastructure, and lack of skilled technical workers as factors militating on the adoption of the modern methods. The study recommended that the government should subsidized the cost of modern technology equipment's for the processors. The processors should form cooperative groups to help train their members to acquire technical skills and also to access inputs and other resources that will boost their business.*

Keywords*— *Comparative, Economic, Analysis, Rice, Processing, Methods.

I. INTRODUCTION

Rice supplies 7% 0f total per capital calorie consumption in Nigeria (1RR1,2015), and occupies about 1.88 million hectares of arable land, making it rank second most important cereal in the world after wheat in terms of processing (CBN,2014). The domestics consumption of rice rose from 5kg /person/week in 2012 to about 10kg/person/week in 2013 (Okafor and Chima, 2014).Currently, annual per capital consumption of milled rice is 25kg/person/month (Musa,2014).The relative ease of its preservation and cooking has influenced the processing trend in its consumption.

The quality of rice has become an important issue among Nigerian consumers who clearly show strong concern for imported rice, because of its quality in terms of cleanliness (WARDA,2015).This has brought about competition of imported rice and locally processed rice. The low quality of locally processed rice reflects low level of improved processing technology. This can reduced the efforts in achieving progress of raising output to meet the consumers demand.

The difficulty of processors in Nigeria to adopt and develop modern technology is due to inadequate resources. Poverty has become a significant factor in increasing processing of rice in Nigeria (Jerry ,2016).One of the major problem of rice processing in Nigeria is to develop appropriate technology. If the cost and returns of processing locally produced rice is known, it will be easy to address the problem of quality in locally processed rice.

Rice processing in Nigeria contributes to food security, employment, poverty reduction and national development. Rice processing is increasingly creating employment for new processors while the old processors have diversified into processing tree crops like cocoa and rubber in which their prices are unpredictable over years now. Income and employment generation in rice processing has been substantial (Msendoo,2016).

1.1 Objective

The specific objectives were to:

i. identify major inputs used in processing rice in the study area,
ii. determine the cost and returns in modern and traditional rice processing method,
iii. identify the factors militating against the adoption of the modern method of rice processing in the study area.

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II. METHODOLOGY

2.1 Study Area

Benue state is located between latitudes 6° 11´ and 11° 20´ N and longitudes 5° 25´ and 7° 15´ E of equator. It covers an area of 6,250 km². The mean rainfall ranges between 750 and 1000mm.The average annual number of rainy days ranges from 190 to 230days.The rains start from April and end in October with the highest point in July. The dry season is from November to March making it conducive for agro-processing. The minimum average temperature is about 27°c while the maximum average temperature is 37°c. The mean relative humidity ranges between 60% from January to February and 80% from June to September. The State falls within the guinea savannah vegetation zone. The vegetation supports the production of grains and root crops. The predominant crops are rice, sorghum, millet, yam, maize, groundnut and soya-beans. Benue Agricultural and Rural Development Authority (BNARDA, 2015).This also justified the selection of the study area.

2.2 Data collection

For the objective of the study to be achieved, data was collected through primary and secondary sources. This was done through structured questionnaires and internet. The data was collected based on the intensity of the rice processors in the study area.

2.3 Sampling techniques

Benue state is divided into three agro-processing zones (A, B and C) consisting of 7 (Ukum, Logo, Kwande, Katsina-Ala, Vande-ikya, Ushongu, Konshisha) Local Government Areas in zone A, and 6 (Makurdi, Gboko, Guma, Gwer, Gwer-west, Buruku) local Government Areas in zone B while zone C has 7(Otukpo, Ohimini, Adhoc, Okpokwo, Ogbadigbo, Oju, Obi) local Government Areas. In each of the zone, 3 local Government Areas were purposively selected based on their intensity in rice processing, making a total of 9 Local Government Areas,7(2 modern and 5 traditional) rice processors were randomly selected making a total of 63 rice processors in the study area.

2.4 Data Analysis

Data collected for this study were analyzed using simple descriptive statistics such as frequency tables, percentages and average. Cost and returns of the processors were also determined. Cobb-Douglas production function models was used to determine the efficient use of resources by the processors. The choice of the model was based on a similar study previously conducted by Aondofanan (2016). The ordinary least square (OLS) was used for estimating the parameters in line with different independent variables.

The model is specified as follow.

$$Y=a.X_1^{c1},\ X_2^{c2}{}_{,}\ X_3^{c3},\ X_4^{c4}{}_{,\ d}{}^{c5} \qquad \ldots(1)$$

Where: Y= output from capacity of processed rice

a=constant

X_1=cost of paddy rice/ 100kg of processing

X_2 =cost of firewood/100kg of processing

X_3= cost of labour /100kg of processing

X_4= cost of water used/100kg of processing

C = capacity (100 kg bag)

D = dummy

Gross margin (GM) analysis was used to determine the difference between the total revenue and total variable cost for the processors.

$$GM=TR-TVC \qquad \ldots (2)$$

Where: GM=Gross margin

TR= total revenue

TVC= Total variable cost

The Net income (NI) or profit is the difference between the gross margin and total fixed cost of the rice processors.

$$NI=GM-TFC \qquad \ldots (3)$$

Where: NI= Net income

TFC= Total fixed cost

$$\pi = TC-TR \qquad \ldots (4)$$

Where: π=profit

TC=Total cost

TR=Total revenue

III. RESULTS

3.1 The major inputs used in processing rice outside the processing equipments are, Paddy rice, slab for drying, firewood, water, labour, transportation, drums, rakes and sieves.

Table1, shows the estimates of regression in the modern rice processing methods, the result of Cobb-Douglas production function was fitted to find out the relationship between the output of paddy and the independent variables, as supported in a similar study previously conducted by Msendoo (2015). Firewood, labour and paddy rice were significant at 1% and 5% respectively. Jerry (2016) in his study confirmed that the cost of paddy rice dominated the processing cost with the processors spending more on paddy. The quantity and quality of rice may have effect on the cost and returns.

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The coefficient of the cost of firewood and labour showed that there was 1% and 5% increase in expenditure from its mean level to have a negative effect on output or revenue, while the cost of milling was insignificant. The increase in the cost of firewood and labour will have a negative effect on revenue. However, the cost of water may not affect revenue negatively; but the quantity and quality of water may affect the quality of rice which may in turn affect the revenue. The coefficient of the multiple determinations R^2 of the function was 0.568, which shows that 58.7% of the variation in output was explained in the independent variables included in the model.

Table.1: Estimates of Regression of Modern Rice Processors

Variables	Symbols	Regression Coefficient	Standard error	T-value
intercept	A	10.54	29.40	0.458
Qty of paddy kg	X_1	4·30	0·64	8·245
Cost of firewood	X_2	5·60*	0·354	-2·895*
Cost of water	X_3	6·70	0·425	-1·683
Cost of labour	X_4	9·404 **	0·498	2·905**
Cost of milling	X_5	7.50	0.456	-1.954

***Significant at 5%, **Significant at 1%**

Table 2 shows regression estimates for the traditional rice processing method. The firewood used in the traditional rice processing system was significant at 10%, while water, labour and milling were insignificant. The coefficient indicated increase in the cost of firewood by 1% indicating 25% decrease in revenue without equal increase in the quantity of paddy from its mean. Water, labour and milling cost were insignificant indicating that water, milling and labour had no significant influence on output or revenue in the study. The cost of water was negligible because most of the processors had their own sources of water supply. It was the quality of water used that affect the quality of rice which in turn affects the revenue.

The coefficient of multiple determination R^2 of the function was 0.600, indicating 60% of the variation in revenue or output as explained in the three independent variables included in the model.

Table.2: Regression Estimates of Traditional Rice Processing System

Variables	Symbols	Regression coefficient	Standard error	T-value
Intercept	A	20·89	40·08	0·534
Qty of paddy kg	X_1	0·0037	0·008	2·284
Cost of firewood	X_2	-0·350*	0·038	-1·834*
Cost of water	X_3	0.308	0·041	1·594
Cost of labour	X_4	0.218	0.018	1.684
Cost of milling	X_5	0.421	0.043	1.754

Significant at 10%

Gross margins can be used to evaluate various rice processing situations by comparing different processing methods, estimating profit and loss, calculating costs in processing rice and assist in making investment decisions (Jerry 2016). The gross margin from the modern rice processing method in the study area was ₦16,770 higher than the traditional rice processing method by ₦4,143 per 100kg. This may be as a result of adopting the modern technology in processing rice. Despite the fact that the modern rice processing activities add cost to processors, the products could be sold at a fixed price. The result shows that the modern rice processing method is more profitable than the traditional rice processing method.

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Table.3: Gross Margins per 100 Kg of Rice in Modern and Traditional Method

Imput	Modern rice processing method(₦)	Traditional rice processing method(₦)
Paddy rice	10,000	10,000
Fire wood	1,000	400
Labour	1,000	257
Water	500	100
Transportation	530	100
Packing	200	-
Total-variable cost(TVC)	13,230	10,857
Fix cost(FC)	500	150
Revenue	30,000	15,000
Quantity kg\min. milled	200kg	50kg
Output kg\min.	200kg	50kg
Selling price /100kg	30,000	15,000
Total revenue (TR)	30,000	15,000
Gross margin (GM)	16,770	4,143

Source: Field survey 2016

The result in table 4 shows that the modern rice processing method milled more (200 kg) quantity of rice at a time compare to the traditional method that milled only 50 kg at a time. This is in agreement with Usman (2015) and Yusufu (2014). The benefits of the modern rice processing method include higher (200 kg) quantity of paddy milled at a time with modern performance operations such as cleaning with water before soaking, parboiling, drying, milling, de-stoning, grading and packaging. These operations are not practiced in the traditional system thus resulting in broken grains thereby reducing its quality and value.

The modern method soaked paddy for only 6 hours because of its use of hot water while the traditional method takes 24 hours resulting to fermentation of grains giving it an odour after milling. The milling time is shorter (30mins.) in the modern method giving it a high turnover and improved quality as compared to the traditional method.

Table.4: Summary Data on Operations of the Modern and Traditional Rice Processing Method

Parameters	Modern system	Traditional system
Maximum capacity(kg)	200	50
Number of kg\day (6 working hrs=1 day)	1,200	300
Cleaning operation time (minutes)	50	-
Soaking time (hours)	6	24
Steaming time (minutes)	40	60
Drying time (hours)	4	6
Milling time (minutes)	30	35
De-stoning (minutes)	20	-
Grading (minutes)	5	-
Packaging (minutes)	5	-

Source: (NARPEN)

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Descriptive statistics such as frequency tables and percentages were used to analyze the socio-economic characteristics of rice processors in the study area.

Table 5 shows that the male constitute majority (76.6%) in the modern rice processing method while the female constituted 75.4% to form the majority in the traditional rice processing method. The reason could be that the technology involved in the modern rice processing method requires training to handle, maintain and operate them for effective performance. The men could accept the responsibility as an occupation but the female may not because of their position in the family as mothers and would not want to waste time in training before handling the equipment's.

The female had the highest (75.4%) over the male (24.6%) in the traditional rice processing method because the traditional rice processing method is not complex and does not require special training to operate. This gives the female the advantage to dominate it. Tondo and Iheanacho (2015) confirmed in their study that male were more than the female in the modern rice processing enterprise while the female were more than the male in the traditional rice processing enterprise.

Majority of the respondent (60%) falls within the age range of 21-40 years. In the modern rice processing method, while 45% were between 41 and above years constituting majority in the traditional rice processing method. This means that the modern enterprise is dominated by the younger generations who are more active and stronger. This can be attributed to the fact that rice processing in the study area is tedious and laborious. The success or failure of rice processing depends largely upon how labour and other associated resources are efficiently utilized. Akombo(2015) confirmed in his study that young people within the age bracket of 20-40 years dominated the modern groundnut processing enterprise.

The table also shows that 30% of the respondents in the traditional rice processing method had no formal education while 40% of the modern rice processing had tertiary education with others having various level of educational attainment. This implies that majority of the respondents in the modern rice processing method are literate.

The table revealed that 30% of the modern rice processing spends between 11-20 years processing rice while 30% of the traditional rice processors had between 21-30% years of experience in rice processing. This implies that rice processing seems to be a profitable business in the study area, since there is a general believe that nobody will spend several years in an unprofitable business.

The table also shows that 45% of the processors in the modern rice method had between 1-5 household size while 45% of processors in the traditional rice processing method had between 10 and above household size. This indicated that the traditional rice processors use members of their household to increase labour that is unpaid, to maximize profit.

Table.5: Distribution of Respondents According to Socio-economic Characteristics.

Modern rice processing method			**Traditional rice processing method**		
Variables	percentage	frequency	variables	percentage	frequency
Sex	(%)			(%)	
Male	74.6	75	male	24.6	16
Female	25.4	16	female	75.4	76
Total	100	90	total	100	92
Age (years)					
50-20	25	30		30	27
21-20	60	50		35	33
41 and above	15	15		45	34
Total	100	95		100	94
Education					
No formal education	5	12		30	35
Primary education	20	15		25	23
Secondary school	35	25		25	23
Tertiary education	40	40		20	11
Total	100	92		100	92
Experience (years)					
1-10	21	20		27	23

11-20	30	25	22	25
21-30	27	23	30	21
31 above	22	22	21	20
Total	100	90	100	89
House hold size				
1-5	45	40	15	27
6-10	40	30	40	30
10 above	15	27	45	40
Total	100	97	100	97

Source: Field survey 2016

Table 6 shows the factors militating against the adoption of modern rice processing method in the study area, indicating that 19.06% of the rice processors lack awareness of the modern method of processing rice. The same (19.06%) of processors suffered from low capital. Poor infrastructure and high cost of equipment were among the factors militating against the adoption of the modern method of processing rice constituting 17.04%. Processors that lack loan facilities to support their business constituted 16.08%, while 14.26% of processors lacked technical skills in handling the modern rice processing equipment's.

Table.6: Major Factors Militating the Adoption of Modern Rice Processing Methods.

Factors	Frequency	percentage
Lack of loan facilities	14	16.08
Poor infrastructure	15	17.11
Lack of awareness	16	19.06
High cost of equipment	15	17.04
Lack of technical skill	12	14.26
Low capital	16	19.06
Total	88	100

Source: Field survey 2016

IV. CONCLUSION

In conclusion, the study revealed that the modern and traditional rice processing methods were practiced in the study area. However, the traditional method was commonly practiced with few modern methods. The few modern rice processing methods had higher (200kg\min.) capacity of milling rice than the traditional (50kg\min.) method and is more efficient though underutilized due to inadequate supply of paddy rice.

The study also revealed that the cost of paddy rice dominated the processing cost which means that, the processors spend more on purchasing paddy rice. Although the cost of water did not negatively affect revenue but the poor quality and quantity of the water may affect the quality of rice which in turn affect the revenue.

There was increase in the cost of firewood by 1% indicating 20% decrease in revenue. The cost of water, labour and milling was insignificant indicating that water, labour and milling had no influence on output and revenue. However, the quality and quantity of water affected the quality and revenue of the rice.

The gross margin for the modern rice processing method was ₦16,770 per 100kg of rice higher than the traditional (₦4,143 per 100kg) method. This shows a clear result of adopting the modern rice processing method. The modern rice processing method had a higher (200kg\min.) capacity of milling rice than the traditional with the capacity of milling 50kg\min. of rice. The modern rice method soak paddy in a mechanically hot water tank for 6 hours and dry for 40 minutes before milling. While the traditional method manually soak paddy in cold water for 24 hours resulting in fermentation of the paddy giving it an odour after milling which lead to reduction in quality and revenue.

Majority (74.6%)of the modern rice processors were male while 75.4% of female were in the traditional method, indicating that male were more in the modern method of processing rice while the female were more in the traditional method. The modern rice processors constituting

60% were within the age range of 21-40 years while 45% of the traditional were 41 and above years. This shows that young rice processors were more in the modern rice processing method than the traditional method.

A higher (30%) of traditional rice processors had no formal education while 40% of the modern rice processors had tertiary and various levels of educational attainment. This is an indication that the modern rice processors were more literate and could plan better on how to utilize their human, materials and financial resources for better output and revenue.

The modern rice processors who had 11-20 years of experience in processing rice constituted 30% while 30% of the traditional had 21-30 years of experience in processing rice. This shows that rice processors with high years of experience were more in the traditional than the modern method, indicating that the traditional existed long before the modern.

The modern rice processors who constituted 45% had household size of 1-5 persons while 45% of the traditional rice processors had household size of 10 and above persons. This result is an indication that the traditional rice processors were using their household size to complement their labour force that is not paid in other to maximized profit.

The study also identify factors that are militating against the adoption of the modern rice processing method to include lack of awareness and low capital to constitute 19.06%, poor infrastructures and high cost of equipment to have 17.04% while the rice processors who lacked technical skills constituted 14.26%.

4.1 Recommendation

Rice processing is discovered to be a profitable business that can create employment to reduce the rate of unemployment in Nigeria. It also provide revenue through tax to the government, generate income for the owner, contribute in addressing the social needs of the society and above all, address the problem of food insecurity in Nigeria.

Based on the above, there is need for the government to encourage rice processing business, most especially the modern method in Nigeria. This could be achieved by providing counterpart funds to financial institutions to loan to rice processors who have seen the need to adopt the modern method of processing rice for higher capacity. This may contribute in addressing the shortage of locally processed rice in Nigeria.

The government should also provide basic infrastructures such as storage houses, good rural roads to help reduce cost on the side of the processors. Government should also help to subsidize the cost of modern equipment to enable processors replace their spoiled parts to put back their equipment's to work.

The processors should form cooperative groups to help train their members to acquire technical skills that will help them handle their equipment efficiently and effectively. The cooperative groups will also help their members to access inputs and other resources that will help to boost their business.

REFERENCES

[1] BNARDA. (2015). Annual Report on Climate Change in Benue State From 2010-2014. Benue Agricultural and Rural Development Authority. Makurdi.

[2] CBN (2014). Annual Report on Rice Production, Processing and Marketing. Central Bank of Nigeria. Makurdi

[3] IRRI. (2015). Rice Almanac. International Rice Research Institute Los Banos.

[4] Jerry, A.T (2016). *Profitability of Rice Processing Among Youths in Benue State, Nigeria.* A Paper Presented in Vande-Ikya Youths Rice Cooperative Society. 14th April, 2016.

[5] Msendoo,N.T. (2016). *Adoption and Impacts of Improved Rice Processing Technology:* A Case Study of the Cameroon Grains Development Project. Economics Working Paper (80-100).

[6] Musa, D.A (2014).*Comparative Economic Analysis of Improved Rice Processing Technology and the Traditional Technology in Selected Local Governments Areas of Taraba State, Nigeria*. A conference paper presented at Taraba State University, Jalingo .17th May, 2015.

[7] Okafor, C.A, Chima,K.J. (2014). *Economic Analysis of Local Rice Processing in Selected Local Government Areas in Kwara State, Nigeria.* A conference paper presented at Benue State University, Makuridi.19th July, 2015.

[8] WARDA. (2015). Essence of WARDA, The Reason Why Nigerians Are Hooked To Rice Importation. Insights from a Comprehensive Rice Sector Study. *The African Rice Center 2:1-4.*

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Establishment of Dipstick Development Technology for Detection of Cry1Ac in Transgenic Plants

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***Abstract*— *The insecticidal Bt Cry1Ac protein is, currently used for transgene expression in numerous crops or deliberating resistance against lepidopteron pests. Since the introduction of Bt cotton in Pakistan. It has been demonstrated that the technology has achieved the goal of providing an effective tool for lepidopteron control.*

In this study, single step, sensitive and specific dipstick strip test for the revealing of recombinant Cry1Ac protein in the transgenic plants was established. Anti-Bt-Cry1Ac antibodies and goat anti-rabbit IgG antibodies were used in test and control lines, respectively. The distance betweenthese lines were optimized as 0.5 cm. Polyclonal rabbit anti Bt-Cry1Ac antibody conjugated to nanocolloidal gold (20 nm of OD 15 and 40 nm; OD 1 in separate experiments) at pH 9.2 was used to serve as a probe for detecting Cry1Ac protein in transgenic Bt cotton samples. Both conjugate solutions were coated on separate polyester conjugate pads (0.7 cm × 0.5 cm). The total size of strip was 7.5 cm × 0.5 cm. For 20 nm gold conjugated strip, purple color test line and for 40 nm gold red color test line indicated the binding of gold labeled antibodies to antigen. The assay was corroborated with transgenic cotton samples with protein extraction buffer 1X PBS of pH 7.

This on-site test offers fast screening for any genetically modified crop devouring Cry1Ac transgenic protein.

Keywords— *Nitrocellulose membrane, Cry1Ac, Dipstick, Nano-colloidal gold particles.*

I. INTRODUCTION

Transgenic technology has provided a very powerful tool to develop crop varieties, which are tolerant to various biotic and abiotic stresses, and improved qualitative or quantitative traits. Many genetically modified (GM) crops have been developed with

different important traits by introducing various transgenes like insecticidal genes (*Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, Cry3A, Vip3*) [1], herbicide tolerant genes (*epsps, bar, pat, als*) [2], virus resistant (*cp, prsv-cp, rep, hel*), delayed ripening genes (*sam-k, acc, pg*) [3] genes for color modification (*dfr, hfl, bp40*) in a numeralcrops species like canola,cotton, corn, potato, tomato, brinjal, papaya, rice, tobacco, soybean, wheat, sunflower, alfalfa, etc. The two most important traits that have been successfully introduced in different commercially available GM crops are insect resistance and herbicide tolerance [4].

Transgenic plants expressing insecticidal genes that were initially derived from common soil bacterium, *Bacillus thuringiensis* (Bt), have been found to give an environmentally safe and efficient control of many insect pests Bt-cotton containing Cry1Ac gene provides protection against the lepidopteron insect pests commonly known as cotton bollworms. Bt cotton has been introduced in many other countries like Australia (1996), China (1997), Argentina (1997), South Africa (1998), Mexico (1998), Colombia (2002), India (2002) and Pakistan (2010). (http://www.agbioworld.org/biotech-info/articles/biotech-art/safety-bt-cotton.html).

Since GM crops have been entered the food chain, public and scientific domain discussions related to their safety and manipulability have been continued. Before the commercial release of any GM crop, their biosafety evaluation is required to assess the environmental influence and effect on health of the consumers. It was demonstrated that unauthorized and possibly unsafe GM products may sometimes be found in the market [3]. Unauthorized GM crops altogether present a significant socioeconomic risk through their possible undesired effects on human and animal health, and the environment. Therefore, several countries have implemented thresholds for unintended mixing of GM crops; fixed at 5% in Taiwan and Japan, 3% in Korea, 1% Australia, New Zealand, Brazil, and 0.9% in the European Union. Hence, for regulatory compliance of GM labeling, there is a dire need for easy and steadfast detection methods of such GM crops. Protein based GM crops detection is particularly useful for monitoring transgene expression both at qualitative and quantitative levels [5].

Previously methodologies are being used to detect the manifestation of GM materials in food stuff which emphasis on target either transgenic DNA or transgenic protein expression in GM crops[6]. Recently, Dong et al. have developed a database "GMO Detection method Database (GMDD)"which placid almost all the earlier developed and conveyed GMOs detection methods. The frequently used DNA based methods include polymerase chain reaction (PCR) and real-time PCR, while protein based methods include immuno PCR, near infrared (NIR) spectroscopy, micro fabricated devices, chromatography mass spectrophotometry and DNA chip technology which offer solutions to current technical issues in GM crop analysis [7], but these methods are costly, time taking and advantages along with the disadvantages[8]. Other protein based methods include enzyme linked immunosorbent assay (ELISA) and western blot. These are more accurate, cost effective but required trained and well equipped lab. Almost all of the above mentioned methods are not suitable for onsite testing of transgenic crops. Another protein based method named 'dipstick test' has been found quicker, simpler, less expensive, and suitable for onsite testing and does not require specific skills [9]. The current study is fixated on immunological based detection of transgenic plants expressing Bt (Cry1Ac) gene through developing a dipstick.

The scientific basis of the lateral flow immunoassay was consequent from the latex agglutination assay, which was established in 1956 by Plotz and Singer. During this period, plate-based immunoassays were being settled[10]. The basic principles of the lateral flow technology continual to be advanced through the early 1980s and were further recognized during the latter years with the filing of several major patents on this technology format by companies such as Becton Dickinson & Co. and Unilever and Carter Wallace. Since then, at least other 500 patents have been filed on various aspects of the technology [11]. The technology has been successfully applied for diagnostic purposes in the areas of agriculture, veterinary, environmental health,food and safety, industrial testing, as well as new areas such as molecular diagnostics and theranostics[12].

The dipstick (Lateral Flow Immunoassay) assay uses a membrane based detection system. Previous studies shown a number of names found for the strip based immunoassay tests, such as lateral flow devices (LFD), immunochromatographic (IC) tests, one-step tests,lateral flow technology (LFT) and dipstick tests[1]. The dipstick technology is a variant of enzyme-linked immunosorbent assay (ELISA), using nitrocellulose membrane strips rather than micro-titer wells and offers a qualitative or semi-quantitative test.

The objective of this study was to develop a quick, unpretentious, qualitative, and subtlenanocolloidal gold based sandwich IC strip assay for one step detection of transgenic Cry1Ac protein expressed in Bt cotton. The developed dipstick strip can successfully be used in diagnostic labs and by the cotton growers and farmers to perform the purity test of seed lots[13].

II. MATERIAL AND METHODS

2.1 Chemicals and reagents

The Material Starter Kit (Cat. No. 010) was purchased from Diagnostic Consulting Network UK, For nanocolloidal gold "Gold-in–a-Box™ kit (Cat. No. NGIB01-B044) was procured from BioAssay Works, LLc., USA Highly purified polyclonal antibodies, which were Rabbit IgG (Cat. No. 41-GR30) and Bt Cry1Ac antibody (Cat. No. 70r-BR005) were purchased from Fitzgerald International, country.

1X PBS (10 mM), 5% sucrose solution, BSA blocking buffer (3% BSA in 10 mM PBS) and PBS-Tween 20 (3%) with 5% BSA and 2 ml polyvinyle alcohol (blocking buffer) were prepared separately.

2.2 Preparation of Dipstick Strip by using Seven Different Types of NC Membranes

Dilutions of anti Cry1Ac Antibody (2 mg/ml in 1XPBS buffer) were prepared. The IEF point for Cry1Ac was estimated to be 8.8 (CLC bio workbench). Conjugated solution were prepared having pH 8.8 and 9.2 after optimized conditions.

Seven membranes of different pore sizes were short listed. Each membrane was cut into strip of 3 × 1.5 cm size. The each membrane was treated with same protocol. Two lines were assigned in the middle of the membrane; which were 1 cm apart from each other, upper line was named as control line the goat anti rabbit antibody (1 µl/line; 1 mg/ml) was dispensed on control line and lower was named as test line Anti-Bt Cry1Ac antibody (1µl/line; 1 mg/ml) mixed with 3% methanol was immobilized on test line. Each strip of NC membrane was placed in an incubator at 37ºC to dry for 1 hour. After drying of antibodies the unoccupied sites were blocked with blocking buffer (10 mM PBS containing 3% tween-20, 5% BSA and 2% polyvinyl alcohol). These membranes were incubated with blocking buffer for 4 hrs. at RT on orbital shaker. When blocking process was completed then these membranes were soaked in 5% sucrose solution (w/v) and dried for overnight at 37ºC

finally parts of strip were assembled into coordinating parts as a one unit assay on adhesive plastic backing. After assembly of dipstick strips, assay was performed using Bt positive (Cry1Ac) cotton seeds and commercially available Immuno Strip (Agdia) were also used as a control.

2.3 Blot test for the determination of antibody and antigen reaction

A blot test was performed for the confirmation of antibody and antigen reaction according to protein extraction buffers. For this purpose following four different experiments were conducted using Fermentas NC membrane.

1. Nanocolloidal gold conjugated Cry1Ac antibody (3µl) was dropped on NC membrane and labeled this spot number
2. Seeds of Bt cotton were crushed in 1X SEB4 buffer of pH 10.5 and used its 2 µl supernatant as antigen (Cry1Ac protein) and mixed it with 3 µlnanocolloidal gold conjugated Cry1Ac antibody. Total volume of 5 µl was dropped on NC membrane and labeled it as spot number 2.
3. A secondary antibody IgG (goat anti rabbit antibody) was dropped 1 µl on NC membrane and dried at 37ºC for 30 minutes. After 30 minutes Bt positive seeds were crushed in 1X SEB4 buffer of pH 10.5 and used its 2 µl supernatant as antigen (Cry1Ac protein)and mixed it with 3 µlnanocolloidal gold conjugated Cry1Ac antibody. Total volume of 5 µl was dropped on NC membrane exactly at the same place where IgG was dropped and labeled it as spot number 3.
4. 1 µl of Anti-Cry1Ac Antibody was dropped on NC membrane and dried it at 37ºC for 30 minutes. After 30 minutes Bt positive cotton seeds were crushed in 1X SEB4 buffer of pH 10.5 and used its 2 µl supernatant as antigen (Cry1Ac protein) and mixed it with 3 µlnanocolloidal gold conjugated Cry1Ac antibody. Total volume of 5 µl was dropped on NC membrane exactly at the same place where Anti-Cry1Ac Antibody was dropped and labeled it as spot number 4.

2.3Blot test for the determination of antibody and antigen reaction by using 1X PBS as protein extraction buffer

Again a blot test was performed for the determination of antibody and antigen reaction. For this purpose following four different experiments were conducted by using Fermentas Nitro Cellulose Membrane as an experimental base. Secondly, simple 1X PBS (10mM) buffer was used as protein extraction buffer.

1. Non-Bt cotton seeds were crushed in 1X PBS buffer of pH 7.4 and used its supernatant 2 µl and mixed it with 3 µl Cry1Ac antibody conjugated nanocolloidal gold. Total volume of 5 µl was dropped on NC membrane and labeled it as spot No. 1.
2. Bt cotton seeds were crushed in 1X PBS buffer of pH 7.4 and took 2 µl of its supernatant as antigen (Cry1Ac protein) and mixed with 3 µl Cry1Ac antibody conjugated nanocolloidal gold. Total volume of 5 µl was dropped on NC membrane and labeled it as spot No. 2.
3. A secondary antibody IgG (goat anti rabbit antibody) was dropped (1 µl) on NC membrane and dried at 37ºC for 30 minutes. After 30 minutes Bt cotton seeds were crushed in 1X PBS buffer of pH 7.4 and used its (2 µl) supernatant as an antigen (Cry1Ac protein) and mixed with 3 µlnanocolloidal gold conjugated Cry1Ac antibody. Total volume of 5 µl was dropped exactly at the same place on NC membrane where IgG was dropped and labeled it as spot No. 3.
4. Anti-Cry1Ac Antibody was dropped (1 µl) on NC membrane and dried at 37ºC for 30 minutes. After 30 minutes Bt cotton seeds were crushed in 1X PBS buffer (pH 7.4) and used its 2 µl supernatant as an antigen (Cry1Ac protein) and mixed with 3 µl of nanocolloidal gold conjugated Cry1Ac antibody. Total volume of 5 µl was dropped exactly at the same place on NC membrane where AntiCry1Ac Antibody was dropped and labeled it as spot No. 4.

NOTE. The gold antibody conjugate used in all these experiments for determination of antibody and antigen reactions was of pH 8.8.

2.4 Preparation of dipstick strips by using different NC membranes without any treatments

In this experiment three NC membranes of different pore sizes from different manufacturers were selected. These were Millipore HF135, Millipore HF120 and Fermentas NC membranes. Each membrane was cut into strip of 2.5 × 0 .5 cm size. Each membrane was treated with same protocol as mentioned above. Unoccupied sites on membranes were not blocked by any treatement.

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After assembly of 10 dipstick strips, assay was performed by using Bt positive cotton seeds and Bt negative cotton seeds. Commercially available (AgdiaImmunoStrip®) strip was also used for a control. For protein extraction 1XPBS (10mM) of pH 7.4 was used.

2.5 Prepared dipstick strips and test for different protein extraction buffers

Four dipstick strips were prepared by using MilliporeHF135 NC membrane. The purpose of this experiment was to observe the effect of different extraction buffers in attachment of antibody-antigen on blocked and unblocked NC membranes used in dipstick strips.

Four strips of HF135 NC membrane were prepared for construction of dipsticks with 2.5 × 0.5 cm size as reaction area. The each membrane was treated with same protocol as mentioned above. After drying of antibodies the unoccupied sites of 3 membranes were blocked with blocking buffer (1XPBS and 3% BSA). These membranes were incubated with blocking buffer for 4 hrs. at RT on orbital shaker. When blocking process was completed then these membranes were soaked in 5% sucrose solution (w/v) and dried for one hour at 37ºC. The 4th membrane was remained unblocked and untreated and labeled as 4. Conjugate pad was prepared and strip was assembled.

Bt positive Cotton seeds were taken in 3 pestle mortars and crushed them separately with different buffers.1XPBS, 0.5XPBS and protein extraction buffer (100 mM Sodium Phosphate buffer pH 7.0, β-mercaptoethanol, 5 mM EDTA, 0.1% Triton X-100, 2% PVP-40) and labeled the each pestle and mortar accordingly.200µl of each supernatant was taken in different eppendorf tube and dipped the strip (table 2.4).

Table.2.4: Different treatments of strips and different types of protein extraction buffers.

Strip number	Treatment of NC membrane	Protein Extraction buffer
1	Blocked unoccupied sites	1XPBS
2	Blocked unoccupied sites	Protein Extraction Buffer
3	Blocked unoccupied sites	0.5XPBS
4	Un-blocked	1XPBS

2.6 Preparation of unblocked NC membranes for strips with different antibody concentrations

The hypothesis behind this experiment was to observe that concentration of test antibody on NC membrane and its distance from conjugate releasing pad has any effect on color intensity of test line. For this purpose NC membrane (Millipore HF120) of high pore size and with a back protection sheet was used.

Two strips of MilliporeHF120 NC membrane (was in 2.5cm × 0.5 cm) were activated by 1XPBS. Anti-Bt Cry1Ac antibody (1mg/ml) mixed with 5% methanol was immobilize on 3 test lines; 0.5 mm apart, from bottom to top arrangement of lines were ; line 1(1µl/line; 1mg/ml) ,line 2(2µl/line; 1mg/ml) ,Line 3(3µl/line; 1mg/ml).

These strips were dried at 37ºC for 1 hr. One membrane was washed with 1XPBS buffer after drying process and other was not washed so labeled them accordingly. Washed membrane was dried for 30 minutes at 37ºC.

After assembly of 2 dipstick strips, assay was performed with Bt (Cry1Ac) positive cotton seeds along with AgdiaImmunoStrip®. For protein extraction 1XPBS (10mM) of pH 7.4 was used.

2.7 Preparation of unblocked NC membranes for strips with different antibody concentrations (washed, unwashed and reverse orientation of lines)

In this experiment strips were prepared according to above mentioned protocol but test lines were in reverse order.

2.8 Preparation of NC membranes with different blocking timings for development of strips

This experiment was designed to check the effect of different hybridization (blocking of unoccupied sides) timings of blocking buffer (1XPBS, 5% BSA) on NC membranes for the attachment of antibodies.

Five different NC membranes were prepared according to optimized parameters. the unocupied sites were blocked with blocking buffer (10 mM PBS containing 5% BSA) for different times.

Table.2.5: Blocking times for unoccupied sites on NC membranes with blocking buffer

No of strip	Blocking Duration
1	30 minutes
2	1 hour
3	2 hours
4	Unblocked (negative)
5	Unblocked (positive)

After assembly of 5 dipstick strips, assay was performed with Bt (Cry1Ac) positive cotton seeds and Bt negative cotton seeds by using protein extraction 1XPBS (10mM) of pH 7.4. For a control the commercially available (AgdiaImmunoStrip®) strip was also tested along with these strips.

2.9 Final preparation of dipstick strip with optimized conditions

The Gold conjugated antibodies were prepared according to optimized conditions.For preparation of conjugate pad The pH of gold-antibody solution which was used in this

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experiment was 9.2. About 10 µl of this prepared solution was finally dispensed on a fiber glass conjugate pad of 7mm × 5 mm. This conjugate pad was placed in an incubator at 37ºC for overnight incubation. NC membranes were prepared according to above optimized conditions and assembled them as dipstick strip and test was performed.

2.10 Final preparation of dipstick strip under optimized conditions by using 40 nm colloidal gold particles

In above experiments 20 nm colloidal gold was used of 15 OD. In this experiment 40 nm colloidal gold was used of OD 1 under optimized conditions.The Gold conjugated antibodies were prepared per supplier instruction given in the Gold in a Box kit. For preparation of conjugate pad instructions provided by supplier in kit was followed. Each 1 ml of gold-antibody conjugate solution was mixed with 0.1 ml of gold drying buffer. The pH of gold-antibody solution which was used in this experiment was 9.2. About 15 µl of this prepared solution (40nm of OD 1) was finally dispensed on a fiber glass conjugate pad of 7mm × 5 mm. This conjugate pad was placed in an incubator at 37ºC for overnight incubation. This incubation made gold-antibody conjugate dried on fiber glass pad and this was called as dried conjugate pad. Nc membranes were prepared according to above optimized conditions and assembled them as dipstick strip and test was performed.

III. RESULTS

3.1 Selection of Suitable NC membrane

Variation was found when different membranes were tested using Agdia SEB4 extraction buffer at pH ranging from 7 to 10.5 (Table 3.1; Fig 3.1)

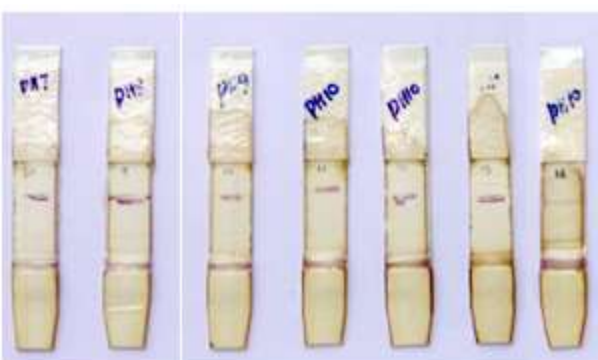

Fig.3.1: Variations in dipstick strip performance due to different NC membranes.1: Immunopore FP, 2: Millipore HF075, 3: Millipore HF090, 4:Millipore HF120, 5:Millipore HF135, 6:Millipore HF240, 7: WhatmanFusion5

Table.3.1: Results of dipstick strips for detection of Cry1Ac protein using different membranes at varying pH of extraction buffer.

Strip No	pH*	Membranes	Line appearar
1	7	Immunopore FP	Control line
2	8	Millipore HF075	Control line
3	9	Millipore HF090	Control line
4	10	Whatman Fusion5	No line
5	10.5	Millipore HF120	Control line
6	10.5	Millipore HF135	Control line
7	10.5	Millipore HF240	Control line

* pH of agdia SEB4 extraction buffer.

3.2 Blot Test for the Determination of Antibody and Antigen Reaction

1. First spot gave reddish purple colour such as antibody-gold conjugate colour was (Fig. 3.2).
2. Second spot gave a circular appearance of dual lining with light green colour on inner side and light reddish purple colour on external margins (Fig. 3.2).
3. On 3rd spot, where a secondary antibody goat-anti-rabbit antibody (IgG) was immobilized and a mixture of conjugate solution (pH 8.8) and Bt positive extract was applied. Spot of IgG repelled that mixture and gave two overlapping circles of dual margins. The external margin of one circle was overlapping with external and smaller with internal margin of second (Fig. 3.2).
4. The 4th spot gave two semi circles of dual margins; internal margin was smaller interacting with antibody spot (Fig. 3.2).

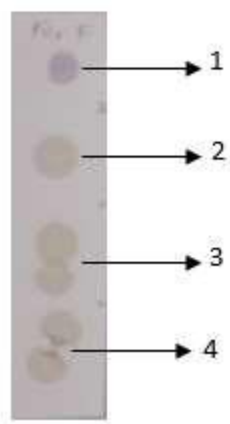

Fig.3.2: Determination of antibody–antigen interaction. 1: antibody gold conjugate solution, 2: conjugate solution and antigen mixture, 3: IgG, conjugate solution and antigen mixture, 4: Cry1AC antibody, conjugate solution and antigen mixture.

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3.3 Blot Test for the Determination of Antibody and Antigen Reaction by using 1X PBS as Extraction Buffer

The purpose behind this experiment was to check the role of protein extraction media in antibody–antigen interaction. So to determine antibody and antigen reaction, 1X PBS was used as protein extraction buffer (Fig 3.3) and results were the followings:

1. A light green colored spot appeared when Bt negative sample and antibody-gold sconjugate was used.
2. A light reddish circle of double margin appeared by using Bt positive sample and antibody-gold conjugate.
3. On 3rd spot goat-anti-rabbit antibody (IgG) was spotted, dried on 37º for half an hour. A mixture of antibody-gold conjugate and Bt positive extract in 1X PBS was dropped. The mixture interacted with the antibody spot and gave dark reddish purple colored circle.
4. On 4^{th} spot Anti-Bt Cry1Ac antibody was spotted, dried on 37º for half an hour. A mixture of antibody-gold conjugate and Bt positive extract in 1X PBS was dropped, the mixture interacted with the antibody spot and gave dark reddish purple colored circle like shape.

Both the spots of antibodies (Anti-Bt Cry1Ac antibody and goat-anti-rabbit antibody) gave almost similar results, which showed the antibody-antigen interaction.

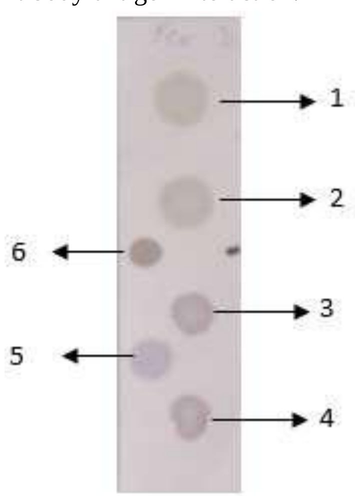

Fig.3.3: Blot test for the determination of antibody-antigen reaction by using 1X PBS as protein extraction buffer. 1: Bt negative sample and antibody-gold conjugate, 2: Bt positive sample and antibody-gold conjugate,3: Goat-anti-rabbit antibody (IgG), Bt positive sample and antibody-gold conjugate, 4: Cry1Ac-antibody, Bt positive sample and antibody-gold conjugate, 5: only antibody labeled gold, 6: nano-colloidal gold without antibodies.

3.4 Preparation of Dipstick Strips by using Different NC Membranes without blocking and washing treatments

This experiment was conducted to check that do the blocking and washing treatments have any effect on NC membranes performance. So NC membranes used in experiment were neither blocked nor washed with any buffer or any reagent. Second purpose of this experiment was that either sealing tape (lamination) used for the covering of strip has any effect on the membrane; therefore, two strips (Millipore HF135) were tested without lamination. Four strips with Millipore HF135 were dipped in extract from Bt positive sample, two strips of Millipore HF120 were dipped (one in Bt positive sample and second in Bt negative sample) and two strips with Fermentas NC membrane were also tested.

It was observed that, 4 strips of Millipore HF135 gave a sharp control line and a light line on test line. The 1 strip of Millipore HF120 with positive sample gave both control and test line and 2^{nd} gave only control line with negative sample. Strips with Fermentas NC membranes gave no signals i.e. any line with positive samples (fig 3.5 and Table 3.2).

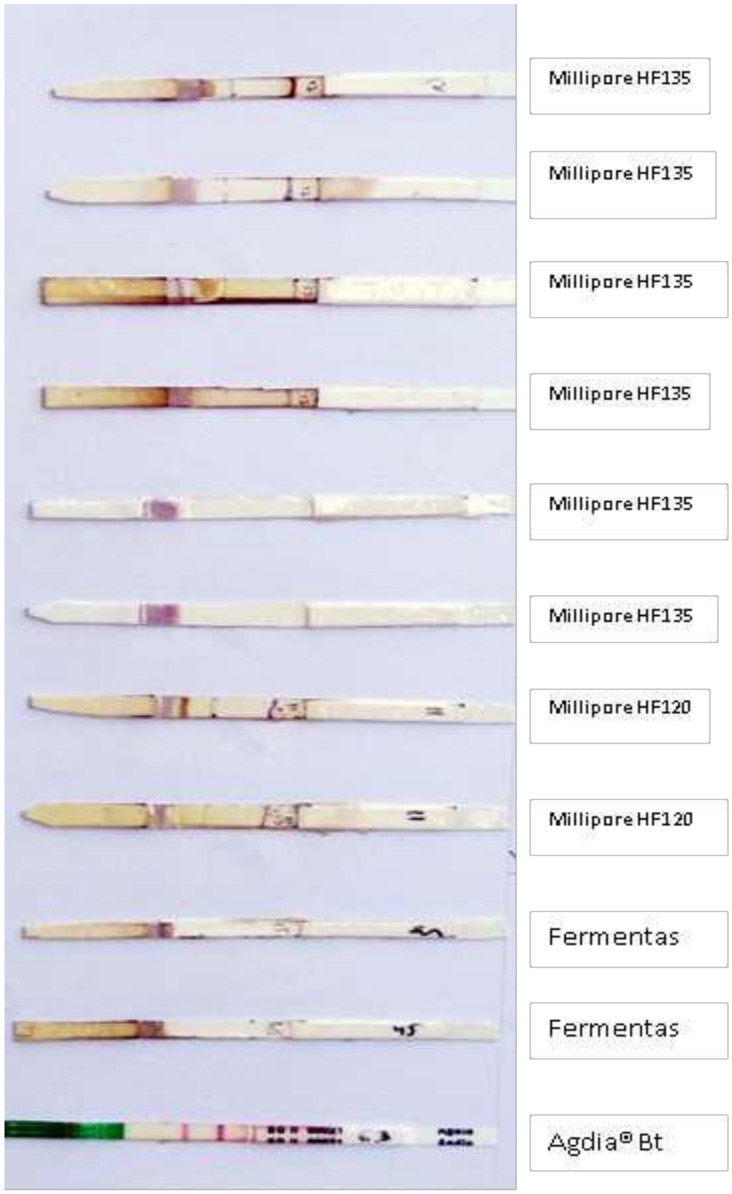

Fig.3.5: Dipstick strips prepared by using three types of membranes without any treatment. Agdia® Bt strip was also tested as a control.

Table.3.2: Results of dipstick strips prepared by using three types of membranes without any treatment. Agdia® Bt strip was also tested as a control.

Strip No.	NC membrane Used	Blocking	Gold particles size	pH of conjugate Solution	Extraction Buffer	Results
1	Millipore HF135	No	20 nm	8.8	1XPBS	**light test and control lines**
2	Millipore HF135	No	20 nm	8.8	1XPBS	**light test and control lines**
3	Millipore HF135	No	20 nm	8.8	1XPBS	**light test and control lines**

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4	Millipore HF135	No	20 nm	8.8	1XPBS	**light test and control lines**
5	Millipore HF135	No	20 nm	8.8	1XPBS	**Not tested**
6	Millipore HF135	No	20 nm	8.8	1XPBS	**Not tested**
7	Millipore HF120	No	20 nm	8.8	1XPBS	**visual test line**
8	Millipore HF120	No	20 nm	8.8	1XPBS	**visual test line**
9	Fermentas	No	20 nm	8.8	1XPBS	**Not any line**
10	Fermentas	No	20 nm	8.8	1XPBS	**Not any line**
11	**Agdia® Bt strip**				**1XPBS**	**Both test and control lines**

.

3.5 Preparation of Dipstick Strips and Test for Different Protein Extraction Buffers

Table.3.3: Dipstick strips tested using different extraction buffers

Strips	**Treatment of NC membrane**	**Protein extraction buffer**	**Line appearance**
1	Blocked unoccupied sites	1X PBS (pH 7.4)	**control Line**
2	Blocked unoccupied sites	Extraction Buffer in Lab (pH 7)	**control Line**
3	Blocked unoccupied sites	0.5X PBS (pH 7.4)	**Not any line**
4	**Un-blocked**	**1X PBS (pH 7.4)**	**Both test and control lines but light in colour**

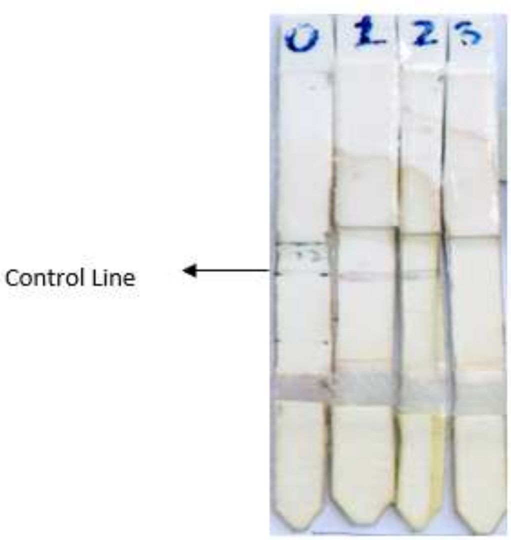

Fig.3.6: Dipstick strips tested using different extraction buffers. 1: membrane blocked and strip dipped in 1X PBS (pH 7.4), 2: membrane blocked and dipped in extraction buffer (pH 7), 3: membrane blocked and dipped in 0.5X PBS (pH 7.4), 4: membrane unblocked and dipped in 1X PBS (pH 7.4).

3.6 Preparation of Strips with unblocked NC Membranes and different Antibody Concentrations

In this experiment Millipore HF120 NC membrane was used and it was pre-soaked with 1X PBS buffer to check either it has any effect on antibody binding efficiency with NC membrane with the hypothesis that it might be possible that pre-soaking may help for proper application of antibodies on membrane. Anti-Bt Cry1Ac (1 mg/ml) antibody mixed with 5% methanol was applied on this membrane in different concentrations at different distances from the conjugate pad. Anti-Bt Cry1Ac antibody (1 mg/ml) was immobilized on 3 test lines; 0.5 mm apart each (Fig. 3.7). Results are tabulated in Table 3.4.

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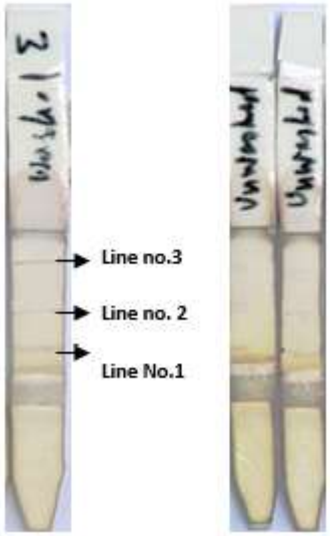

Fig.3.7: Unblocked NC membranes for strips with different volumes of antibody (1 mg/ml). 1: 1 µl/line, 2: 2µl/line, 3: 3µl/line.

Table.3.4: Strips with unblocked NC Membranes and different antibody concentrations as test lines on same membrane.

Line No. (bottom to top)	Antibody (1 mg/ml)	Washed membrane	Un-washed membrane
1	**1 µl/line**	**light purple colour line**	**sharp light purple colour line**
2	**2 µl/line**	**very light purple colour line**	**very light purple colour line**
3	**3 µl/line**	**invisible line**	**invisible line**

3.7 Preparation of strips with unblocked NC membranes and different antibody concentrations (washed, unwashed and reverse orientation of antibody lines)

This experiment was designed to evaluate that either colour the intensity of signal line (test line) depends upon antibody concentration in line at NC membrane or distance of antibody line from conjugate pad or any other factor. The major difference between this and previous experiments was that here antibody gold conjugated solution of pH 9.2 was used for the preparation of antibody gold conjugate pad. Anti-Bt Cry 1Ac antibody (1 mg/ml) mixed with 5% methanol was immobilized on 3 test lines; 0.5 mm apart each (Table 3.4; Fig, 3.8).

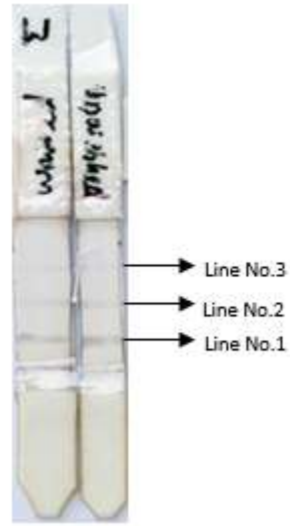

Fig.3.8: Results of unblocked NC membranes for strips with different antibody concentrations; washed: Line No.1; 3µl/line; 1mg/ml, Line No.2; 2µl/line; 1mg/ml, Line No.3; 1µl/line; 1mg/ml, unwashed; Line No.1; 3µl/line; 1mg/ml, Line No.2; 2µl/line; 1mg/ml, Line No.3; 1µl/line; 1mg/ml.

Table.3.5: Unblocked NC membranes for strips with different volumes of antibody (1 mg/ml) washed with 1X PBS, unwashed and reverse orientation of antibody lines.

Line No. (bottom to top)	Antibody (1 mg/ml)	Washed (treated) strip	Unwashed (un-treated) strip
1	3 µl/line	A sharp purple colour line	**A sharp purple colour line**
2	2 µl/line	Light sharp purple colour line	**Light purple colour line**
3	**1 µl/line**	**A light visible line (sharp)**	**light visible line**

3.8 Preparation of NC Membranes with different Blocking Timings for development of Strips

This experiment was designed to see the effects of different time durations for blocking the membranes with blocking buffer (1X PBS, 5% BSA) on stability of antibody binding with membranes. For this purpose, un-blocked membrane strips were also tested at same time with positive and negative sample. Results are given below in Table 3.5 and shown in Fig. 3.9.

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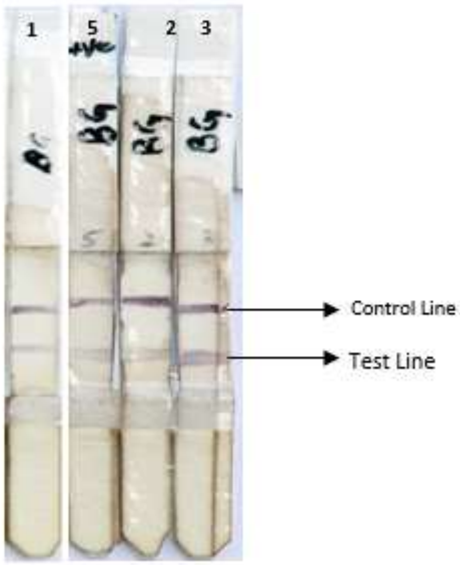

Fig.3.9: Dipstick strips with NC membranes blocked for different blocking duration. 1: 30 minutes, 2: 1 hour, 3: 2 hours, 5: unblocked membrane

Table.3.6: NC Membranes blocked for different blocking times.

Strip No.	Blocking duration	Line appearance
1	30 minutes	A sharp purple colour control and test line
2	1 hour	A sharp control line but smaller diffused test line
3	2 hours	A sharp control line but more diffused test line
4	Unblocked (negative)	A sharp control line
5	Unblocked (positive)	Both test and control lines very well

3.9 Final Preparation of Dipstick Strip with Optimized Conditions using 20 nm Colloidal Gold Solution of OD 15

Under optimized conditions Anti-Bt Cry1Ac-Antibody gold conjugate solution was prepared. It gave no precipitation or change in colour; that showed the labeling of antibody or correct concentration of antibody for gold particles for conjugation.

After the experimentation, finally strips were prepared under optimized conditions. 1x PBS buffer was used to extract protein from both Bt positive and Bt negative cotton seed samples. Three strips were tested with Bt positive extract 200 µl and one strip with Bt negative extract as negative control. Thesample solution through wicking migrated onto the strip by capillary action. As the sample flowed successively through the detection antibody (conjugate pad) and the capture antibody, the Cry1Ac proteins got captured on test line and a dark purple coloured band was visible with positive samples (Fig. 3.10). A second dark purple coloured line was also observed on the control line on the membrane, generated by excessive gold conjugates, indicating the proper test performance. The detection was completed in less than 10 minutes.

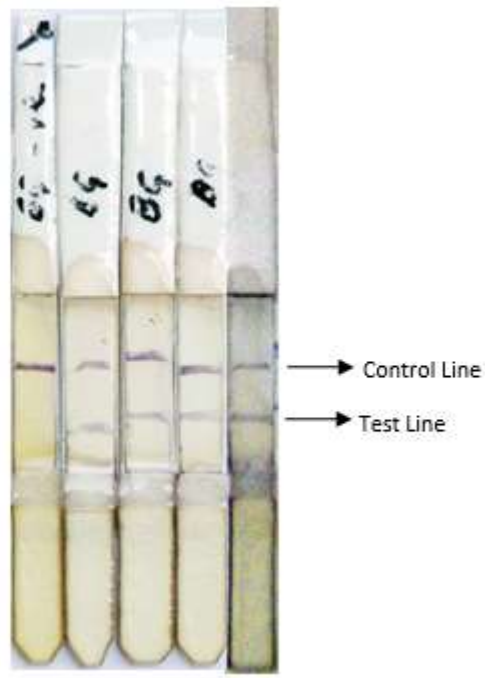

Fig.3.10: Dipstick strips prepared with optimized conditions and using 20 nm colloidal gold Solution of OD 15.

3.10 Final Preparation of Dipstick Strip with Optimized Conditions by using 40 nm Colloidal Gold Particles of OD 1

Antibody gold conjugate solution did not show any precipitation and change in colour; remained red. And strips were tested with positive and negative samples. Thesample solution by means of wicking traveled onto the strip by capillary action. As the sample flowed continually through the conjugate pad and the capture antibody, the Cry1Ac proteins got captured on test line and a red colored line appeared. A second red coloured line was also observed on the control line of the NC membrane, produced by excessive gold conjugates, indicating the appropriate test performance (Fig. 3.11).The detection was completed in less than 10 minutes.

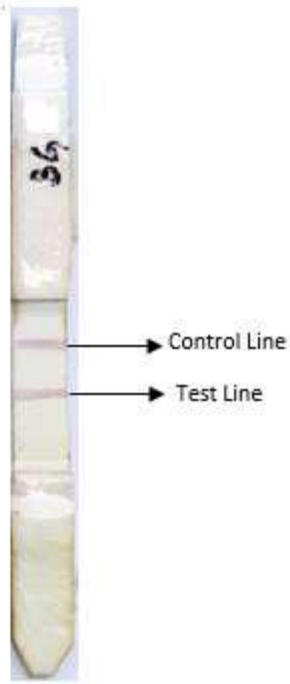

Fig.3.11: Dipstick strip prepared with optimized conditions and using 40 nm colloidal gold particles of OD 1, and tested with Bt positive cotton seed sample.

IV. DISCUSSION

During the past decade, a large number of genetically modified (GM) crops have been established. These GM crops shown inimitable agronomic traits such as insect resistance or herbicide tolerance, which offer noteworthy remunerations to the farmers. The development of GM crops is consummate by molecular biology methods, fundamentally by the incorporation of novel DNA sequences into the plant genome. The new DNA encodes in the novel protein expression in the targeted tissues, resulting in the distinctive agronomic trait. The DNA and novel protein are present in the plant, in harvested grain, and often in the food and feed prepared from them [14].

There are different detection methods of GM crops, which are based on phenotype or DNA and protein detections. The protein based detection of GM crops depend onsolely on the immunoassay technology applications[15]. Commercial immunoassays are available for GM crops and have been used in a variety for large-scale applications.

Immunoassays are based on the reaction of an antigen (Ag), e.g., transgenic protein, with a specific antibody (Ab) to give a product (Ag-Ab complex) that can be measured. Dipstick strip test is purely qualitative method for proteins detection in transgenic plants in which the results are interpreted by visual observation. The result provides yes or no answer for presence or absence of protein in the test samples. The appearance of two lines on dipstick strips after performing test shows the positive results and appearance of control line gives negative results as shown in fig 3.10.

The objective of the present study was to maturefor dipstick strip for the Cry1Ac protein detection expressed in Bt cotton. For this purpose the technology was optimized, which included the selection of best NC membranes for this particular assay, optimum pH, optimum extraction buffer, optimum antibody concentration and size of the gold nano particles suitable for detection.

In order to achieve quality results and stable protein-nano gold particles, numerous parameters should be deliberated. These include: the optimum pH of the reaction, the appropriate concentration of protein loaded onto the colloids, determining the isoelectric point (pI) of the protein, and the stability of the colloids. Generally, most proteins can be adsorbed onto the metal surface in an optimal manner using buffer systems close to their pI value, away from this pH the adsorption decreases [16]. Gold nano particles were being used in current study as color producing probes because these are stated to be non-toxic, inert and have long retention of their optical properties, which creates them a better choice as a signal generator. The gold nanoparticles accumulation produces a characteristic red color on the surface plasmon resonance [17]. Macromolecular ligands adsorb onto colloidal gold through a combination of electrostatic and hydrophobic interactions. Cry1Ac antibodies (macromolecules) were conjugated with gold particles after adjusting the pH of colloidal gold solution. gold with a suitable amount of adsorbed macromolecules are not subject to flocculation with high salt, making this an excellent tool for determining whether the colloid has been sufficiently ‘‘protected’’ [6]. After preparation of antibody gold conjugate solution and to check stability of conjugation reaction; 10 µl of coated gold solution was mixed with 10 µl 1M NaCl. The results under the optimized condition indicated color stability, which indicated that antibody-gold particles are stable enough to be utilized in the strip development assay.

In colloidal gold conjugation process, it is significant to control the pH of the colloidal and gold ligand. Both preparations should be attuned to a pH slightly above the isoelectric point (pI) of the ligand before conjugation. Below the pI of the ligand, ligand-induced flocculation may ensue, whereas, above the pI of the ligand, there is limited

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adsorption due to charge repulsion between the ligand and the colloid. In experiments, which were designed for optimization process of gold antibody conjugation, 20 nm gold was used with OD 15. Optimized pH values for conjugation process were 8.8 and 9.2, which were close and slightly above the calculated pI value of Cry1Ac antibody i.e. pH 8.8.

The conjugation of antibody with colloidal gold is controlled by pH of the solution and that concentration was used for full saturation of colloidal gold surface, which increased the chance of antigen-antibody interaction after collision with antigen and also increased the stability by protecting the surfaces of colloidal gold particles against coagulation[18]. The optimized concentration of Cry1Ac antibody for conjugation with colloidal gold was 14 µl of 2 mg/ml antibody solution in 1X PBS.

After addition of optimized concentration of antibody to the colloidal gold solution, BSA blocking stabilizer buffer was added with the concentration of 50µl/0.5ml. A major disadvantage of the affinity of colloids for proteins is that the colloidal particles can also adsorb to other components of the system, causing non-specific labeling. Hence, colloidal metal conjugates must also be steadied with an inert macromolecule such as gelatin, bovine serum albumin (BSA) or polyethylene glycol. This can usually be done through washing the conjugates with buffer containing the macromolecule after the absorption of the wanted protein onto the nanoparticles. In addition to the non-covalent adsorption, proteins can also be devoted to the nanoparticles through covalent bonds via functional groups such as amines and thiols[19].

Boththe antibody loading concentration and the pH of the reaction as well as the buffer conformation and incubation time, resolute the degree of adsorption of the protein onto the nanoparticle and the aggregation probability[20]. For the proper adsorption of antibody on the surface of gold particles, the gold conjugate solution should be left for 30 minutes of incubation after addition of antibody. It is necessary to give a proper time to the blocking buffer, so incubate the gold conjugate solution for overnight after addition of BSA blocking buffer. It is important to know that if antibody or nano particle is changed, then the optimal conjugation conditions will also differ. An optimised protocol for one system may not necessarily be readily applicable for another one, because once OD or size of colloidal gold changes the conditions for conjugation with antibody will also change [21].

In immune chromatographic assays, the primary function of a protein applied to a membrane is to act as a capture reagent for the target analyte in a sample [8]. In the present research work, Cry1Ac antibody was applied on membrane as a capture reagent and target reagent was Bt Cry1Ac protein extracted from Bt cotton seeds or leaves. Since the test result is entirely dependent on attaining a good binding of the capture reagent to the membrane, therefore, triumphing a high and consistent level of protein binding is imperative.

If one selects a buffer that too greatly reduces either hydrophobic or electrostatic interactions, the protein binding level could be vividly reduced. Similarly, it is extensively recognized that adequate drying of the membrane after protein application is an essential for ensuring the long-term constancy of the protein membrane bond[22]. In strip preparation antibody was dissolved in 1X PBS as 1 mg/ml and applied on NC membrane as 1 µl/line mixed with 5% methanol. The sufficient time was also given to the NC membrane for drying that is 30 minutes at 37ºC.

Blocking the unoccupied sites of the NC membrane after application of antibodies, with a proper blocking buffer is also an important factor in a successful dipstick strip development. Composition of blocking buffer and incubation times are key points in blocking process. As incubation time increases the binding of capture antibody on the NC membrane will be lose (Fig. 3.9).

The components of blocking buffer should be selected according to the properties of capture protein (antibody). Materials and chemicals can have an effect on the binding of antigens and antibodies to nitrocellulose membrane. These materials can interrupt protein binding can be divided into three types: nonspecific antigenic proteins, materials that can interfere with electrostatic interactions and materials that interact with hydrophobic interactions [23]. Generly used materials that ihibit protein attachment are those that compete for binding sites, such as the bulking proteins (BSA, animal sera), as well as those that can interfere with hydrogen bonding (formamide, urea) and those that can disturb hydrophobic bonding (Tween, Triton). synthetic polymers such as polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP) and polyethylene glycol (PEG) can also effect protein binding. Their mode of action may be a combination of effects that reduce one or more of the forces essential to protein membrane binding.

So, the optimized composition of blocking buffer was the 5% BSA in 1X PBS. All the detergents like triton X-100 and PVP were removed from the ingredients of the blocking buffer. The incubation time for blocking process was optimized as 30 minutes at room temperature (RT).

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Several efforts were made for appropriate protein extraction buffer and optimized to evade false positive and false negative results. Because protein capture reagents vary, therefore, maximizing the binding of a given protein may also require optimum buffer conditions. There are two important factors that need to be optimized through modifications to the application buffer. These are the solubility of the protein and the stability of the protein molecules.

To ensure that required protein is vacant in the dispensed capture line, it is essential that the capture protein be soluble in the appropriate application buffer. To allow the protein to be dissolved, it is mandatory to have some ions present in the application buffer. So, the ionic strength of the buffer can help to control the pH of the capture reagent. The pH level of an extraction buffer can also have a significant effect on immunoassay properties. The solubility of a typical protein is minimum at its isoelectric point. Since scientists are aiming to minimize the molecular stability of the capture protein in solution, the ideal pH of the extraction buffer should be around the pI of the capture protein being used. With these experiments it was found that 1X PBS at pH 7.5 should be used as protein extraction buffer in initial experiments and during optimization process [21].

The discovery sensitivity of the test was also reliant on the pore size of the NC membrane. The assay was accomplished separately with NC membranes of different pore size. We perceived that the detection sensitivity was reduced with growing pore size of NC membrane (Fig 3.1). It is due to the faster wicking rate in case of NC membranes with large pore size (10 and 15 µm) which, in turn, provides no sufficient time for antibody and protein interaction. The better sensitivity was obtained with NC membrane of 5 µm pore size (MilliporeHF120); it was, therefore, selected for final assay development (Fig. 3.10).

Table.4.1: Comparison of optimized method with other studies of strip development

Ref×	**Pre- Treatment of pads**	**Test and Control lines on NC membrane**	**Drying of antibodies on Test and Control lines**	**Blocking NC membrane**
1		I) Pab-Dog IgA solution of 650 µl was mixed with 20wt%sucrose solution diluted with 50mM KH2PO4 buffer (pH7.5)of 50µl and 50µl of 2-propanol. (IgA solution at 1mg ml−1in PBS) II) 40 µl of polyclonal anti-mouse IgG mixed with both 60µl of 2-propanol and 1100µl of 50mMKH2PO4 buffer (pH7.5).	drying for 1 h at room temperature	
2	Sample and the conjugate pads treated with PBS	DON–CBSA (1 mg /mL) conjugate and goat anti-mouse IgG antibody (1.5 mg/ mL) were sprayed onto the bottom and the top of NC membrane	vacuum-dried at 37 °C for 2 h.	By immersing in 50mM boric acid solution containing 0.5% casein (pH8.5) and incubating for 30min at RT
3		(NC membrane HF135MC100, Millipore) A solution of 1 mg/ml of recombinant Staphylococcus Protein-A (Sigma) and a solution of 1 mg/ml of mAb MM3, both in PBS	Dried overnight at 37°C.	
4	Sample pad was treated with 50 mMboratebuffer, pH 7.4, containing 1% BSA, 0.5% Tween-20, and 0.05%	Clenbuterol–BSA (0.038mg/mL) and goat anti-mouse antibody (1.123 mg/mL)	Dried at 35°C	

	sodium azide, and dried at 60°C			
5		3µl anti-HBsAg antibody (0.1 µg / ml) test line. 3µl of goat anti-rabbit IgG (0.1 µg / ml)	air dry for 45 minutes at 4°C	Incubating in mixture 3% BSA and 2% gelatin in TBS for 30 minutes.
6		Anti-O1 LPS mAb (2 mg/ml), anti-O139 LPS mAb (2 mg/ml) and goat anti-mouse Ab (1 mg/ml) 4 µl/cm	Dried overnight in a desiccator at room temperature	With 50 mM PBS (pH 7.4) containing 1% western blocking reagent and 0.05% Tween-20.
7		MAb 4D1 (2mg/ml) and goat anti-mouse IgG (2mg/ml) were dispensed at the test or the control line	drying for 2 h at 37 ◦C	Incubating with PBS (pH 7.4) containing 2% (w/v) nonfat driedmilk for 30min **Wash** three times with PBS containing 0.1% (v/v) Tween-20 for 3min each time
8*		Anti-Bt Cry 1Ac antibody (1µl/line; 1mg/ml) mixed with 3% methanol, Goat anti rabbit antibody(1µl/line; 1mg/ml)	Dried for 1 hr at 37ºC.	10 mM PBS with 3% BSA, 0.05 %,. Incubate NC membrane 30 minutes at RT. **Soaked with 5 % sucrose solution.**

1: [24] 2: [25] 3: [26] 4:[27] 5: [28] 6: [29] 7: [30] 8*: methode adopted in present study.

V. CONCLUSION

In conclusion, the developed technology for qualitative colloidal gold based dipstick strip using antibody sandwich immunoassay format can detect specific transgenic Cry1Ac protein. The results can be visualized by naked eyes without any complex instrumentation, which provides the convenience for assay on-site. In addition, the test is performed within 10 min without the need of using expensive equipment. It, therefore, could be used directly in the field for the rapid qualitative screening of GM samples. Additionally, the method is economic, simple, and easy-to-use.

Conflict of interest: All authors have no conflict of interest.

REFERENCES

[1] Brada, D., & Roth, J. (1984). "Golden blot"—Detection of polyclonal and monoclonal antibodies bound to antigens on nitrocellulose by protein A-gold complexes. *Analytical biochemistry, 142*(1), 79-83.

[2] Campbell, R. L., Wagner, D. B., & O'connell, J. P. (1987). Solid phase assay with visual readout: Google Patents.

[3] Chandler, J., Gurmin, T., & Robinson, N. (2000). The place of gold in rapid tests. *IVD technology, 6*(2), 37-49.

[4] Fren, G. (1973). Preparation of gold dispersions of varying particle size: Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature Physics, 241*, 20-22.

[5] Frens, G. (1973). Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature, 241*(105), 20-22.

[6] Grothaus, G. D., Bandla, M., Currier, T., Giroux, R., Jenkins, G. R., Lipp, M., . . . Pantella, V. (2006). Immunoassay as an analytical tool in agricultural biotechnology. *Journal of AOAC international, 89*(4), 913-928.

[7] Gupta, A. K., & Chandak, V. (2005). Agricultural biotechnology in India: ethics, business and politics. *International Journal of Biotechnology, 7*(1-3), 212-227.

[8] Hansen Jesse, L. C., & Obrycki, J. J. (2000). Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly. *Oecologia, 125*(2), 241-248.

[9] Henderson, K., & Stewart, J. (2002). Factors influencing the measurement of oestrone sulphate by

dipstick particle capture immunoassay. *Journal of immunological methods, 270*(1), 77-84.

[10] Herring, R. J. (2008). Opposition to transgenic technologies: ideology, interests and collective action frames. *Nature Reviews Genetics, 9*(6).

[11] Herring, R. J. (2008). Whose numbers count? Probing discrepant evidence on transgenic cotton in the Warangal district of India. *International Journal of Multiple Research Approaches, 2*(2), 145-159.

[12] Holst-Jensen, A. (2008). GMO testing—trade, labeling or safety first? *Nature biotechnology, 26*(8), 858-859.

[13] James, C. (2010). A global overview of biotech (GM) crops: adoption, impact and future prospects. *GM crops, 1*(1), 8-12.

[14] Jones, K. D. (1999). Troubleshooting protein binding in nitrocellulose membranes, Part 1: Principles. *IVD Technol, 5*(2), 32-41.

[15] Katrukha, A. G., Bereznikova, A. V., Esakova, T. V., Pettersson, K., Lövgren, T., Severina, M. E., . . . Gusev, N. B. (1997). Troponin I is released in bloodstream of patients with acute myocardial infarction not in free form but as complex. *Clinical chemistry, 43*(8), 1379-1385.

[16] Kumar, K. S., & Kang, S. H. (2007). Ultra-fast simultaneous analysis of genetically modified organisms in maize by microchip electrophoresis with LIF detector. *Electrophoresis, 28*(22), 4247-4254.

[17] Leimanis, S., Hernandez, M., Fernandez, S., Boyer, F., Burns, M., Bruderer, S., . . . Philipp, P. (2006). A microarray-based detection system for genetically modified (GM) food ingredients. *Plant Molecular Biology, 61*(1), 123-139.

[18] Li, Y., Hou, L., Ye, J., Liu, X., Dan, H., Jin, M., Cao, S. (2010). Development of a convenient immunochromatographic strip for the diagnosis of infection with Japanese encephalitis virus in swine. *Journal of virological methods, 168*(1), 51-56.

[19] Lin, T., Shao, J.-j., Du, J.-z., Cong, G.-z., Gao, S.-d., & Chang, H. (2011). Development of a serotype colloidal gold strip using monoclonal antibody for rapid detection type Asia1 foot-and-mouth disease. *Virology journal, 8*(1), 418.

[20] May, K., Prior, M. E., & Richards, I. (1997). Capillary immunoassay and device therefor comprising mobilizable particulate labelled reagents: Google Patents.

[21] McCabe, A. F., Eliasson, C., Prasath, R. A., Hernandez-Santana, A., Stevenson, L., Apple, I., . . . Corish, P. (2006). SERRS labelled beads for multiplex detection. *Faraday discussions, 132*, 303-308.

[22] McCabe, A. F., Eliasson, C., Prasath, R. A., Hernandez-Santana, A., Stevenson, L., Apple, I., . . . Corish, P. (2006). SERRS labelled beads for multiplex detection. *Faraday Discuss., 132*(0), 303-308.

[23] Ponti, J. S. (2009). Material platform for the assembly of lateral flow immunoassay test strips *Lateral Flow Immunoassay* (pp. 1-7): Springer.

[24] Samra, Z. Q., Aslam, M. S., Shaukat, H., Dar, N., & Athar, M. A. (2007). Development of diagnostic dip strip immunoassay using antibodies of PreS~ 2 region of hepatitis B surface antigen. *PAKISTAN JOURNAL OF ZOOLOGY, 39*(3), 185.

[25] Singer, J. M., & Plotz, C. M. (1956). The latex fixation test:: I. Application to the serologic diagnosis of rheumatoid arthritis. *The American journal of medicine, 21*(6), 888-892.

[26] Takahashi, A., Uchiyama, S., Kato, Y., Yuhi, T., Ushijima, H., Takezaki, M., . . . Miyahara, T. (2009). Immunochromatographic assay using gold nanoparticles for measuring salivary secretory IgA in dogs as a stress marker. *Science and technology of advanced materials, 10*(3), 034604.

[27] Thobhani, S., Attree, S., Boyd, R., Kumarswami, N., Noble, J., Szymanski, M., & Porter, R. A. (2010). Bioconjugation and characterisation of gold colloid-labelled proteins. *Journal of Immunological Methods, 356*(1-2), 60-69.

[28] Vermij, P. (2006). Liberty Link rice raises specter of tightened regulations: Nature Publishing Group.

[29] Xu, Y., Huang, Z.-B., He, Q.-H., Deng, S.-Z., Li, L.-S., & Li, Y.-P. (2010). Development of an immunochromatographic strip test for the rapid detection of deoxynivalenol in wheat and maize. *Food chemistry, 119*(2), 834-839.

[30] Yu, C. Y., Ang, G. Y., Chua, A. L., Tan, E. H., Lee, S. Y., Falero-Diaz, G., . . . Acosta, A. (2011). Dry-reagent gold nanoparticle-based lateral flow biosensor for the simultaneous detection of Vibrio cholerae serogroups O1 and O139. *Journal of microbiological methods, 86*(3), 277-282.

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Geospatial Technology Based Rainfall Precipitation Assessment with Landslides in Mettupalayam – Aravankadu Highway, Tamilnadu

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***Abstract*— *The present study reveals that the relation between rainfall Precipitation with landslides was carried out. The Precipitation data were collected from IWS (Institution of Water Studies) and analyzed for annual and season wise for the period from 2006 to 2015. The Precipitation data were interpret tolated through spatial distribution methods in GIS and correlated with existing landslide locations. The spatial output of rainfall contour shows that larger area of rainfall is covered with higher amount in Northeast Monsoon when compared to other seasons. However, an almost equal amount of rainfall was noticed in Southwest Monsoon. The above data were taken into a GIS. Using this data, spatial interpolation maps were prepared. It clearly reveals that, high amount of rainfall and existence of landslides occurs throughout the Coonoor region and Wellington and Moderate amount of rainfall and existence of landslides in Kothagiri and Ooty region. This paper highlights the application of GIS in spatially locating the relation between precipitation and landslides.*
Keywords*— *Geospatial Technology, Aravankadu Highway, IWS.

I. INTRODUCTION

A landslide is an event of nature that leads to sudden disruption of normal life of society, causing damage to property of nations, to such an extent those normal, social and economic mechanisms available are inadequate to restore normalcy. Landslides are defined as the mass movement of rocks, debris or earth along a sliding plane. They are characterised by almost permanent contact between the moving masses and sliding plane (Butler, 1976; Crozier, 1984; and Smith, 1996). Landslides cause substantial economic, human and environmental losses throughout the world. Examples of devastating landslides at a global scale include the 1972 Calabria landslide in Italy, the 1970 Hauscaran landslide in Peru (McCall, 1992), the 1966 Aberfan landslide in wales, and the 1985 Armero landslide in Colombia (Alexander, 1993). It is estimated that in 1998, 180,000 avalanches, landslides, and debris flow in different scales occurred in China, estimated at 3 billion dollars' worth of direct economic losses (Huabin *et al.,* 2005).

II. STUDY AREA

The study area is the Nilgiris district, which is located in Tamilnadu state. The Mettupalaym to Aravankadu ghat section of length 273.30 km2 has taken as the study area to identify the landslide prone areas. It lies in the toposheet Nos. 58 A/15 of survey of India and located in between 76° 48' 8.34'' and 76°54' 2.48'' E longitudes and 11° 17' 41.25'' and 11° 17' 47.48'' N latitudes with an area of (273.30 km^2). The study area is blessed with deltaic system with different active and inactive distributaries and shown in figure 1. The proposed study area is covered include villages like Mettupalayam, Odanthurai, Adatturai, Burliyar, Hulical Drug, Kallar, Killpilur, Marrapalam, wellington, Aravankadu, Lambs rock and Tiger hill.

III. METHODOLOGY

The base map is prepared from Survey of India (SOI) Toposheets 58A/11 &15 at a scale of 1: 50000. In the present study, the average monthly rainfall of a ten years period (2006 - 2015) have been collected from five rain gauge stations and variation diagrams are prepared. Rainfall contour map has been prepared of rainfall variation is found at all the rain gauge stations. The spatial variability of mean annual precipitation depends upon the topographic factors such as exposure of station to the prevailing wind, elevation, orientation and slope of the mountain (Basist A and Bell G.D., 1994).

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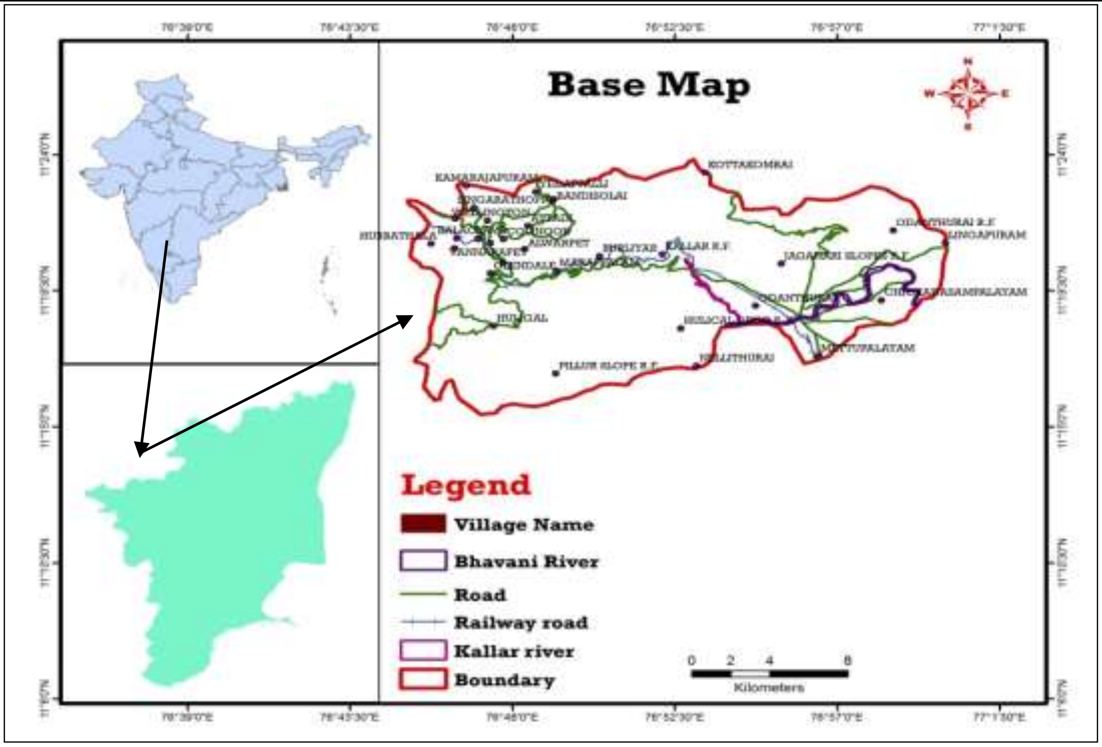

Fig.1: Study Area Base Map

Arithmetic mean is used for measurements of selected duration at all rain gauges are summed and the total divided by the number of gauges. Arithmetic method is the simplest objective methods of calculating the average rainfall over the area (Basavarajappa *et al.*, 2015a).

Thiessen polygon method provides the individual areas of influence around each set of points. Thiessen (1911), an American engineer adopted the polygon method for rainfall measurements at individual gauges as first weighted by the fractions of the catchment area represented by the gauges, and then summed. Thiessen polygons are the polygons whose boundaries are mathematically define the area (perpendicular bisectors) that is closest to each point relative to all other points (Basavarajappa *et al.*, 2015 a).

Iso-hyetal method is a line drawn on a map connecting points that receive equal amounts of rainfall. It is one of the convenient methods that views continuous spatial variation of rainfall areas. The main aim of the method, to draw lines of equal rainfall amount (isohyets) using observed amounts at stations (Reed W.G and Kincer J.B., 1917). In iso-hyetal map, the x-axis represents East Longitude, while y-axis represents North Latitude (Basavarajappa *et al.*, 2015 a).

IV. RESULTS AND DISCUSSION

The results of post-monsoon, pre-monsoon, southwest, northeast and average annual rainfall data for the period 2006- 2015 were used for the preparation of spatial distribution contour map using geospatial technology and the data's are given in figures 2 to 11 and in table.1.

Pre monsoon Season

During the pre-monsoon season, study area recorded an average rainfall of 453.83 mm. During this Season, the highest rainfall of 148.78 mm was recorded in Runneymedu station and the lowest rainfall of 43.79 mm was recorded in Gurrency station.

Post monsoon Season

During the post monsoon season, study area recorded an average rainfall of 1231.04 mm. During this Season, the highest rainfall of 347.22 mm was recorded in Coonoor station and the lowest rainfall of 162.43 mm was recorded in Gurrency station.

South-West Monsoon Season

During the South–West Monsoon season, study area recorded an average rainfall of 1435.73 mm. During this Season, the highest rainfall of 403.19 mm was recorded in Hilgrove station and the lowest rainfall of 236.31 mm was recorded in Aderly station.

North-East Monsoon Season

During the North-East Monsoon season, study area recorded an average rainfall of 2934.07 mm. During this Season, the highest rainfall of 953.93 mm was recorded in Coonoor station and the lowest rainfall of 351.38 mm was recorded in Mettupalayam station.

Table.1: Average occurrences of rainfall during various seasons

Stations	Post-monsoon	Pre-monsoon	SW monsoon	NE monsoon	Average Rainfall	Year
COONOOR	72	368	297.4	1430.6	542	2006
RUNNEYMEDU	56	392	346	1292	521.5	
HILGROVE	103	397.6	454	625	394.9	
GURRENCY	74	450.8	372	1094	497.7	
ADERLY	52	312	198	481	260.75	
METTUPALAYAM	7	277	107.9	571	240.725	
COONOOR	31.6	131.3	2038.1	662.8	715.95	2007
RUNNEYMEDU	85	143.8	773.8	350.9	338.375	
HILGROVE	166	348	1714.2	413.4	660.4	
GURRENCY	15.8	54	1470	519	514.7	
ADERLY	8	206.1	768.2	528.4	377.675	
METTUPALAYAM	33	96	173	314	154	
COONOOR	352.9	556.5	492.1	509	477.625	2008
RUNNEYMEDU	499	472	178.6	547.2	424.2	
HILGROVE	291.6	329.4	133.7	598	338.175	
GURRENCY	136.4	455.8	125	580.2	324.35	
ADERLY	236	316	172	422.6	286.65	
METTUPALAYAM	44	286	264	300	223.5	
COONOOR	10.2	190.9	371.4	1509.3	520.45	2009
RUNNEYMEDU	0	284	264	1473	505.25	
HILGROVE	0	257	251	541	262.25	
GURRENCY	0	200	142.7	106.4	112.275	
ADERLY	0	172	116.1	84.2	93.075	
METTUPALAYAM	0	249	153	378.7	195.175	
COONOOR	41.6	138.1	342.8	897.9	355.1	2010
RUNNEYMEDU	32	96	161.3	1551	460.075	
HILGROVE	7	117.5	134	709	241.875	
GURRENCY	4	14.8	104.8	438.6	140.55	
ADERLY	4.3	44.7	111.6	147.2	76.95	
METTUPALAYAM	16	159.2	209.20	603.90	247.08	
COONOOR	654.8	262.5	427.5	1239.3	646.025	2011
RUNNEYMEDU	682.8	161.4	273.5	1100.4	554.525	
HILGROVE	762.8	174.8	342	884	540.9	
GURRENCY	175.7	37	95.4	248.8	139.225	
ADERLY	27.2	24.3	24.2	393.1	117.2	

Stations	Post-monsoon	Pre-monsoon	SW monsoon	NE monsoon	Average Rainfall	Year
METTUPALAYAM	235	191.30	75.90	593.70	273.97	
COONOOR	14	214	206.6	925.10	339.93	2012
RUNNEYMEDU	0	264	194	698.6	289.15	
HILGROVE	0	196	161	650.2	251.80	
GURRENCY	0	128.9	309	625	265.73	
ADERLY	5	175.5	294.4	443.5	229.60	
METTUPALAYAM	18.40	123.70	126.50	359.60	157.05	
COONOOR	45.80	370.20	303.20	417.70	284.22	2013
RUNNEYMEDU	39.00	281.00	211.00	554.00	271.25	
HILGROVE	20.00	305.00	140.00	650.20	278.80	
GURRENCY	32.00	283.00	33.20	0.00	87.05	
ADERLY	36.00	255.50	222.00	501.00	253.63	
METTUPALAYAM	63.10	102.40	78.40	393.00	159.22	
COONOOR	108.20	283.40	277.60	913.20	395.60	2014
RUNNEYMEDU	94.00	299.00	486.40	1061.00	485.10	
HILGROVE	100.00	406.00	403.00	1080.00	497.25	
GURRENCY	0.00	0.00	0.00	0.00	0.00	
ADERLY	107.50	401.00	225.10	648.80	345.60	
METTUPALAYAM	46.40	185.30	0.00	0.00	57.92	
COONOOR	9.00	957.30	402.00	1034.40	600.68	2015
RUNNEYMEDU	0.00	800.50	374.00	774.00	487.13	
HILGROVE	0.00	500.00	299.00	818.30	404.33	
GURRENCY	0.00	0.00	0.00	0.00	0.00	
ADERLY	0.00	305.50	231.50	604.00	285.25	
METTUPALAYAM	0.00	0.00	0.00	0.00	0.00	

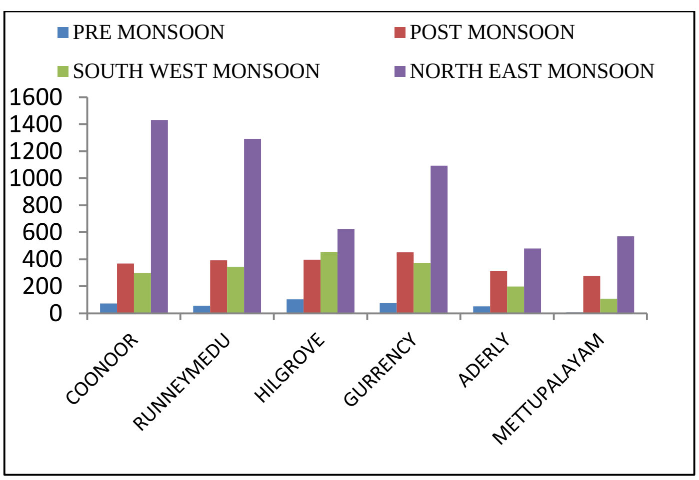

Fig.: 2 Annual Rainfall (in mm) 2006

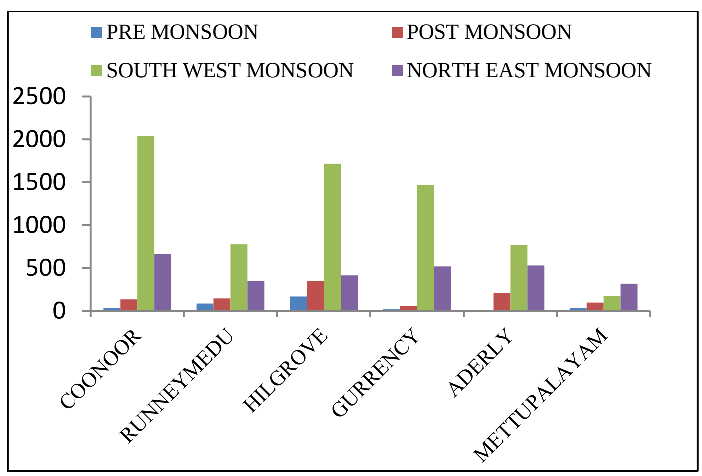

Fig.: 3 Annual Rainfall (in mm) 2007

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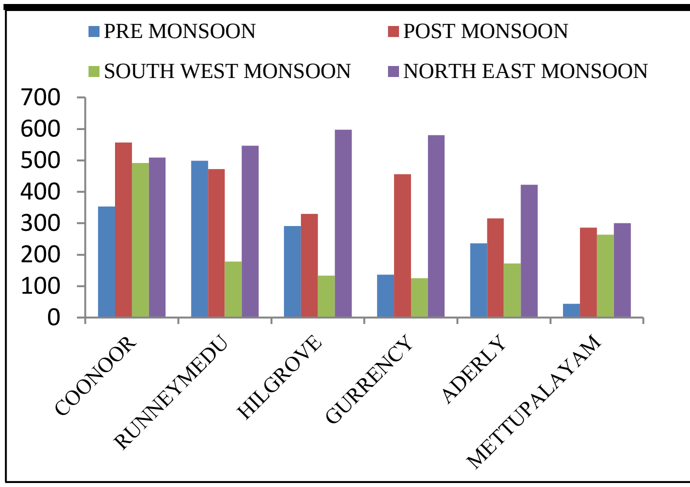

Fig.: 4 Annual Rainfall (in mm) 2008

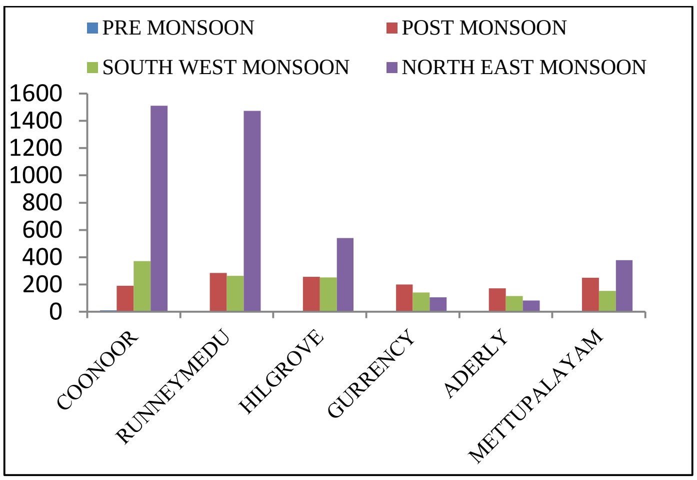

Fig.: 5 Annual Rainfall (in mm) 2009

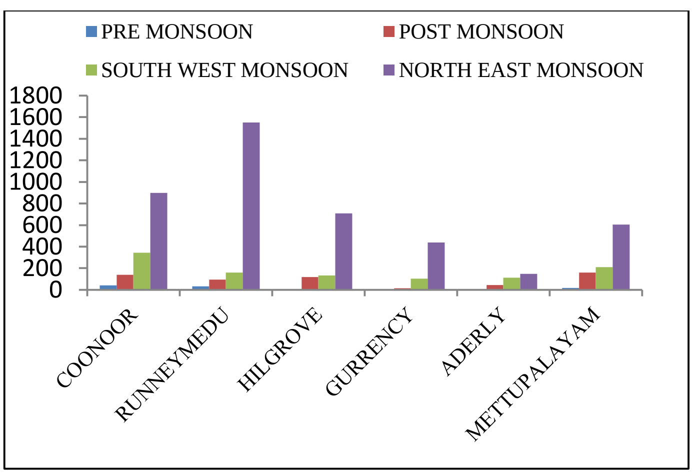

Fig.: 6 Annual Rainfall (in mm) 2010

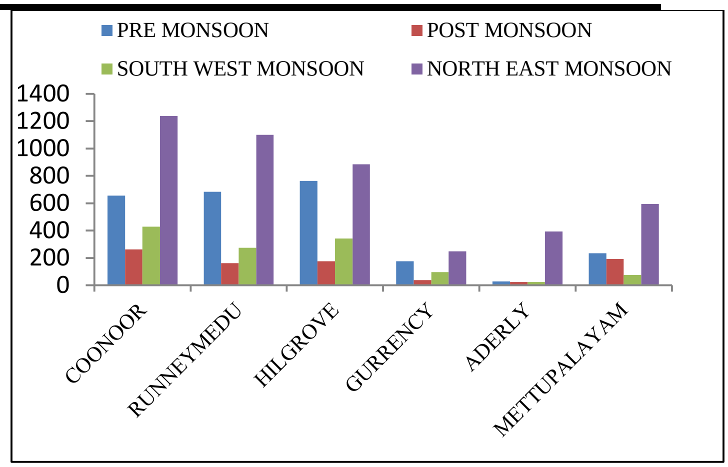

Fig.: 7 Annual Rainfall (in mm) 2011

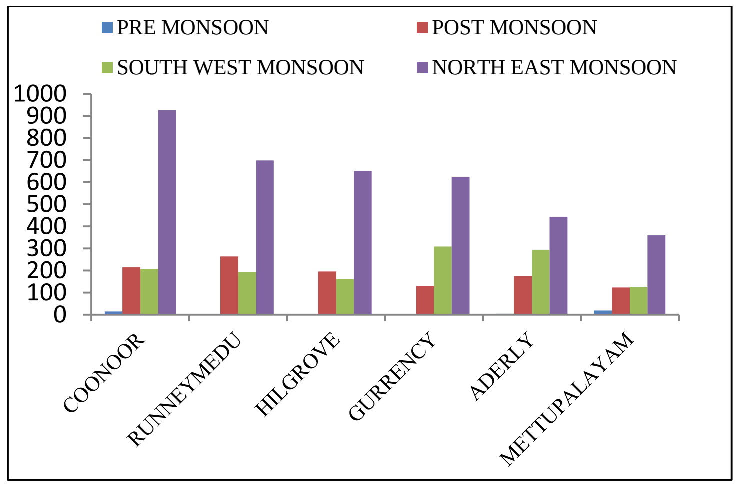

Fig.: 8 Annual Rainfall (in mm) 2012

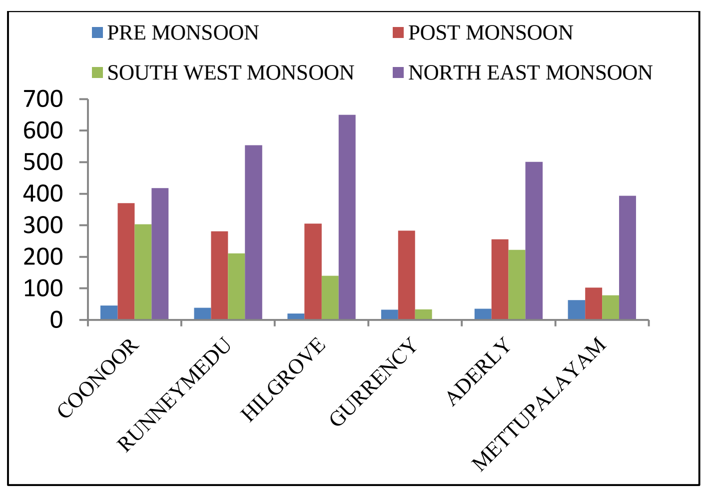

Fig.: 9 Annual Rainfall (in mm) 2013

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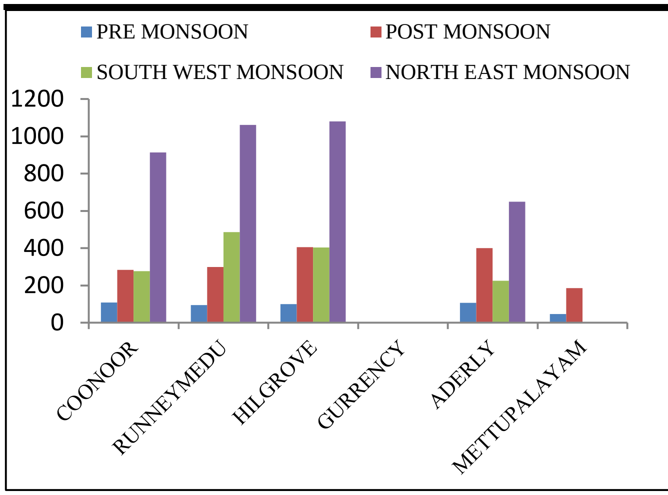

Fig.: 10 Annual Rainfall (in mm) 2014

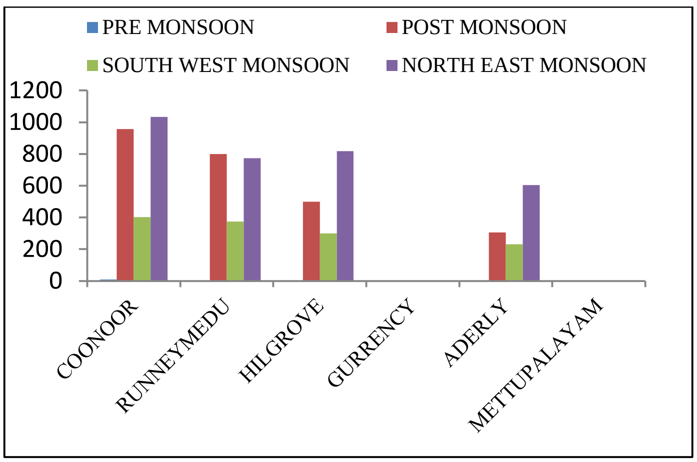

Fig.: 11 Annual Rainfall (in mm) 2015

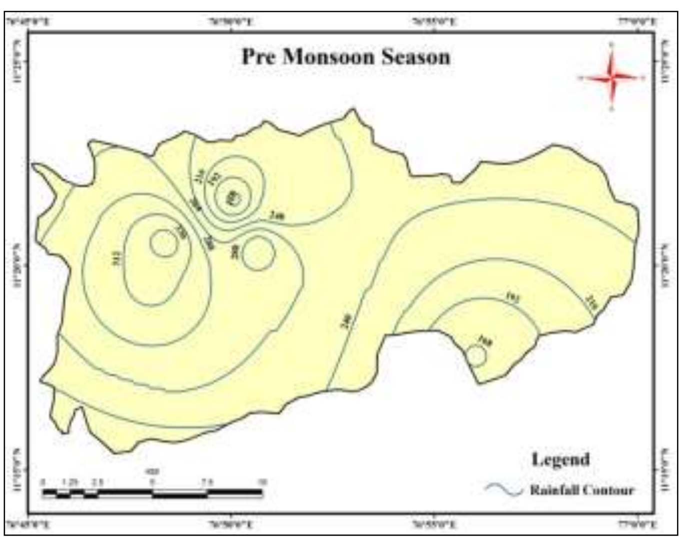

Fig.: 12 Rainfall contour Pre monsoon

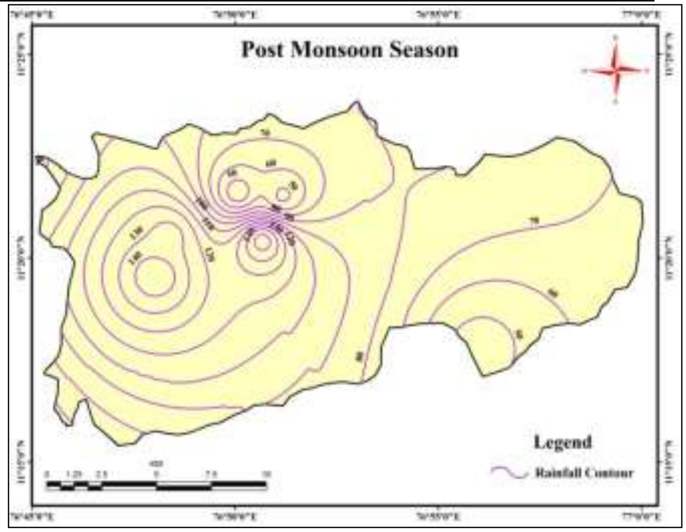

Fig.: 13 Rainfall contour Post monsoon

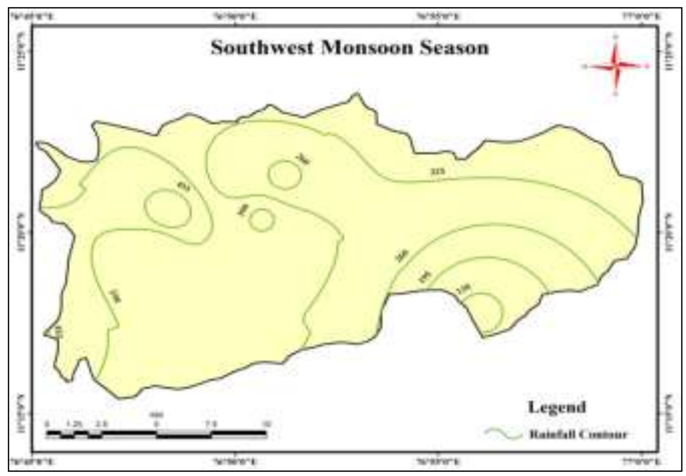

Fig.: 14 Rainfall contour south west monsoon

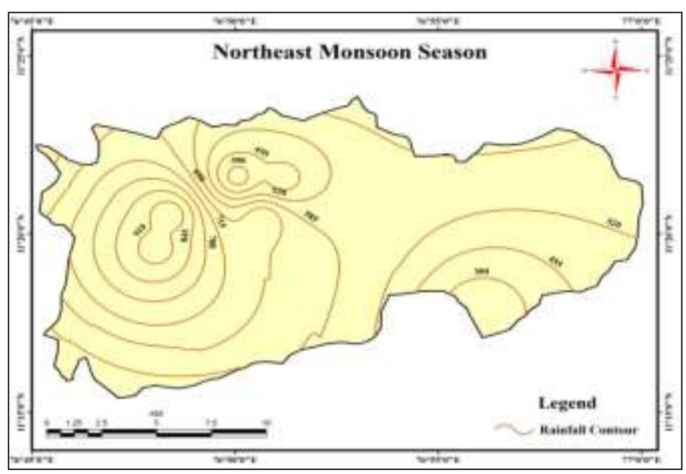

Fig.: 15 Rainfall contour north east monsoon

It is observed from the figures 12 to 15 that the isohyetal maps of pre-monsoon is lesser than post monsoon. In these maps, the increasing order of intensity is noticed towards the coonoor rain gauge station. However, in NE monsoon isohyetal map shows that scenario is quite high with compare to other monsoons. Though the contribution of pre monsoon rainfall is too low, the intensity increases towards NE in the basin.

The rainfall is considered as one of the prime triggering mechanism. Mostly the landslides that are happening in this region as triggered by rainfall so as in any landslides in Tamilnadu. The high hills with steep slopes are controlled by newer evolution of the plateaus probably tectonic plateaus, bounded by N S- N E and SW lineaments. These differently oriented lineaments make the slope of the plateaus very much vulnerable to landslides. Moreover the plateaus are highly dissected, may be as a result of cumulative effects of all the tectonic events in this region. Higher degree of deformation and recrystallization makes the rocks break easily and ready to slide.

V. CONCLUSION

Monthly rainfall analysis concludes that the highest intensity of rain showers is recorded during the month of October, while the lowest intensity is usually recorded during January at all the six rain gauge stations. Analysis of seasonal rainfall concludes that the percentage contributions of rainfall during various monsoon periods are in the following order: NE monsoon (52.34%) > SW monsoon (23.66%) > Post-monsoon (16.70%) > Pre-monsoon (7.30%). Spatial distribution pattern of rainfall indicates that the intensity of rainfall increases towards NE monsoon. Lesser intensity was found in pre-monsoon with compare post-monsoon season.

REFERENCES

[1] Alexander, D. (1993) Natural Disaster, London, University College Library Press.

[2] Basavarajappa H.T, Manjunatha M.C and Pushpavathi K.N (2015a). Mapping and Reclamation of wastelands through Geomatics technique in Precambrian terrain of Mysuru district, Karnataka, India., International Journal of Civil and Structural Engineering (IJCSE), Vol.5, No.4, Pp: 379-391.

[3] Basist A and Bell G.D., (1994), Statistical relationships between topography and precipitation patterns, Journal of Climate, 7, pp 1305-1315.

[4] Butler, R. and H. Kanamori (1976). Long-period ground motion in Los Angeles from a great earthquake on the San Andreas Fault, to be submitted to Bull. Seism. Soc. Am.

[5] Crozier M.J.: 1984, Field Assessment of Slope Instability in D Brunsden and D Prior (eds), Slope Instability, , New York, John Wiley and Sons.

[6] Huabin, W., Gangjun, L., Weiya, X., & Gunghui, W. 2005. GIS-based landslide hazard assessment: an overview. Progress in physical geography, 29(4):548-567. [Online] Available: ppg.sagepub.com [3 June 2011].

[7] McCall, G.J.H. (1992) Geohazards Natural and Man-Made, London. Chapman and Hall.

[8] Reed W.G and Kincer J.B (1917). The preparation of precipitation chart, Monthly Weather Rev., Vol.45, Pp: 233-235.

[9] Smith, K., 1996. Environmental Hazards: Assessing Risk and Reducing Disaster. Routledge, London, p. 478.

[10] Thiessen A.H., (1911), Precipitation for large areas, Monthly Weather Revision, 39, pp 1082-1084.

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Economics of Household Demand for African Breadfruit (*Treculia Africana*) in Owerri Agricultural Zone of IMO STATE, Nigeria

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Abstract— *The need to build up the knowledge of the current situation regarding the household demand for African breadfruit (Treculia africana) in Owerri Agricultural Zone of Imo State, Nigeria led to this study. The specific objectives of the study were to determine the household demand for African breadfruit and to estimate price, income and cross-price elasticities of demand for African breadfruit. One hypothesis was tested. Multi-stage random sampling technique was used in selecting sixty (60) households who consume breadfruit who were interviewed with a well structured questionnaire. Data collected were analysed using frequency distribution, percentages and elasticity of demand model. The findings of the study showed that the household demand for breadfruit is very high (63%) in the study area. The mean quantity demanded per month was 7kg (40 cups). Majority of the respondents consume 70% of their harvest. The result of multiple regression analysis showed that increase in household demand and price of substitute will lead to an increase in quantity demanded of African breadfruit. It was recommended that more farmers need to get involved in the production of breadfruit as it is highly demanded for its food and non-food uses.*

Keywords— Analysis, household demand, African breadfruit.

I. INTRODUCTION

The African Breadfruit (*Treculia africana)* is an unbuttressed large tree, a representative of Moraceae (Akubor & Obiegbuna, 2014). This non-timber forest product is a member of the mulberry family and one of the four members of the Genera, *Treculia* (Muojekwu, Ugwumba & Chidebelu, 2017). It produces matures fruits during the hungry season (March-July) following the planting of major staples such as yam, maize, rice, cocoyam etc in Imo and Anambra states in Nigeria (Nwufo and Mba, 1990). The fruits attain 40cm in diameter, weigh 8014kg and contain many orange seeds of about 1cm buried in spongy pulp. In Nigeria, African Breadfruit is very common in the Western and Eastern States. This forest product is given many names in various localities where it is found. The Ibos call it "*ukwa*", Yorubas "*afon*", Benin "*ize*", Efiks, Ibibios and Annangs "*ediang*". According to Okafor (2005), african breadfruit flour contains 4.05% protein, 76.70% carbohydrate and 331 calories.

The household demand for produced commodities is important to an economy because of its influence on the level of production. Households ultimately own in common all wealth, including resources that they make available to businesses or government. African breadfruit is a high employer of labour. It has several food and non-food potentials:- Confectionery: several products prepared from breadfruit flour include cookies, buns, cakes, soup thickening agent and weaning diet (Anazonwu-Bello in Okafor, 2005); Beverages: Ejiofor, Obiajulu and Okafor (2012) prepared a non-alcoholic beverage from breadfruit seeds and was found acceptable when taken without milk and sugar; Livestock feeds: the fruit head pulp (mesocarp) and the bran (pericarp) are used for livestock feed formulation (Okafor, 2005); Fibre: breadfruit wood is useful in pulp and paper making (Uju & Ugwuoke, 1996); Fuel: the wood is suitable for firewood and charcoal; and Medicinal uses: for curing malaria, cough, rheumatism, diabetes and leprosy (Nwabueze, 2006; Muojekwu et al., 2017). African breadfruit oil can be used for making soaps and hair shampoo (Ajiwe, Okeke & Agbo in Akintayo & Bayer, 2002).

Problem Statement

African breadfruit has restricted distribution, sparse density and is threatened with extinction, yet it has a high potential for selection and genetic improvement. It is currently included in the list of endangered species of Southern Nigeria and this is quite worrisome (Nuga & Ofodile, 2010). The output of this important crop is continuously declining to the extent that the household demand for it is no longer met.

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Limited studies have been made on African breadfruit with respect to its demand by households and this has created a gap in knowledge. The need to fill this gap led to this study. The study is therefore expected to provide answers to these questions: What is the household demand for African breadfruit? What is the nature of the price, income and cross-price elasticity of demand for breadfruit by households in the study area?

II. OBJECTIVES OF THE STUDY

The specific objectives of the study were to:

1. determine the household demand for African breadfruit and
2. estimate price, income and cross-price elasticities of demand for African breadfruit.

Hypothesis of the Study

One hypothesis was tested in the study:

Household demand for African breadfruit is price inelastic in the study area.

Theoretical Framework

The demand theory is applicable to this study. It is vital to distinguish three possible uses of demand theory. The first use is to study the aggregate behavior of all households. The second use is to make statements about an individual household's possible actions. The third is to make statements about what each household will certainly do (Lipsey …, 1990). The assumption of household irrational behavior applies primarily to the third use of demand theory.

III. METHOD

The study was conducted in Owerri Agricultural Zone of Imo State, Nigeria. Owerri Agricultural Zone is one of the three Agricultural Zones in Imo State.. The breadfruit producing areas of Imo State lie within longitude $6^{0}50^{1}$E and $7^{0}25^{1}$E and latitude $4^{0}45^{1}$N and $7^{0}15^{1}$N (Udo, 1993). The study adopted a descriptive survey design. Multi-stage random sampling technique was used for sample selection. The study was carried out in four Local Government Areas (LGAs) out of the eleven (11) LGAs in the study area. The LGAs are Ohaji/Egbema, Ngor Okpala, Ikeduru and Ezinihitte Mbaise.

Sixty (60) household heads formed the sample of the study. A 33-item researcher-developed questionnaire was validated by three experts and used for data collection. A pre-test was conducted to improve the reliability of the questionnaire used for the study. Data were analysed using frequency distribution, percentages and Ordinary Least Squares (OLS) multiple regression model.

Model Specification

Ordinary Least Squares (OLS) Multiple Regression Model

To estimate the price, income and cross-price elasticity of demand for African breadfruit, the variables were analysed and tested by first fitting the OLS multiple regression model to the demand function. The demand function is specified implicitly as follows:

Q_d= f (Pb, Yh, Ps, e)

Where: Q_d = Quantity of African breadfruit demanded (kg/month)

Pb = Price of breadfruit (₦)

Yh = Household income (₦)

Ps =Price of substitute (beans)

e = error term

It is expected *a priori* that the coefficients of Yh, Ps > 0; Pb < 0.

Four functional forms: linear, exponential, double-log and semi-log were fitted into the model. The functional form that best fits the regression line, according to economic, statistical and econometric criteria was selected as the lead equation.

The parameter estimates of each model gave the Marginal Physical Product (MPP) as the regression result. The elasticity of demand (Ed) was obtained by dividing the MPP by the Average Product (AP) as shown:

$$Ed = \frac{\text{MPPx}}{\text{APx}}$$

Elasticity of demand is derived from the parameter estimates as follows:

$$Ep = \frac{\text{Percentage change in quantity demanded}}{\text{Percentage change in price}}$$

$$Ei = \frac{\text{Percentage change in quantity demanded}}{\text{Percentage change in income}}$$

$$Exy = \frac{\text{Percentage change in quantity } x}{\text{Percentage change in price } y}$$

Where:

Ep = Price elasticity of demand

Ei = Income elasticity of demand

Exy = Cross-price elasticity of demand

If the elasticity of demand for African breadfruit is greater than 1 (Ed>1), it means that demand is elastic. If it is less than 1 (Ed<1), it means that demand is inelastic and if it is equal to 1 (Ed=1), it means that demand is constant.

IV. RESULTS AND DISCUSSION

The results of field survey were presented according to the objectives of the study as follows:

Objective 1: Determine the household demand for African breadfruit

The distributions of breadfruit consumers according to frequency of breadfruit consumption, rate of demand for breadfruit and quantity of breadfruit demanded per month are presented in Table 1

Table.1: Quantity of breadfruit demanded by households

Quantity demanded		Frequency	Percentage
Frequency of breadfruit consumption	Once a week	6	10
	Once in two weeks	21	35
	Twice a week	13	22
	Rarely (not often)	6	10
	Often (frequently)	14	23
Rate of demand for breadfruit	Very high	38	63
	High	16	27
	Average	6	10
	Low	0	0
	Very low	0	0
Quantity (kg) of breadfruit demanded by households Mean = 7.0kg (40 cups)	$\leq$ 4.9	34	57
	5.0-14.9	22	37
	15.0-24.9	2	3
	25.0-34.9	2	3

Table 1 shows that most (35%) of the breadfruit consumers eat breadfruit twice a week and 23% eat it often. Majority (63%) of the breadfruit consumers rate the demand for breadfruit as very high and none of them rated the demand for breadfruit as low or very low. Majority (57%) of the breadfruit consumers demand 4.9kg of breadfruit and below, 37% of them demand up to an average of 7kg while 3% demand up to 15-35kg of breadfruit per month. These findings reveal that the demand for breadfruit is high in the study area. Again, it may be possible that breadfruit is expensive in the study area thereby limiting the quantity purchased in the market.

The distributions of breadfruit consumers according to quantity of breadfruit harvest consumed and sold per month are presented in Figures 1 and 2.

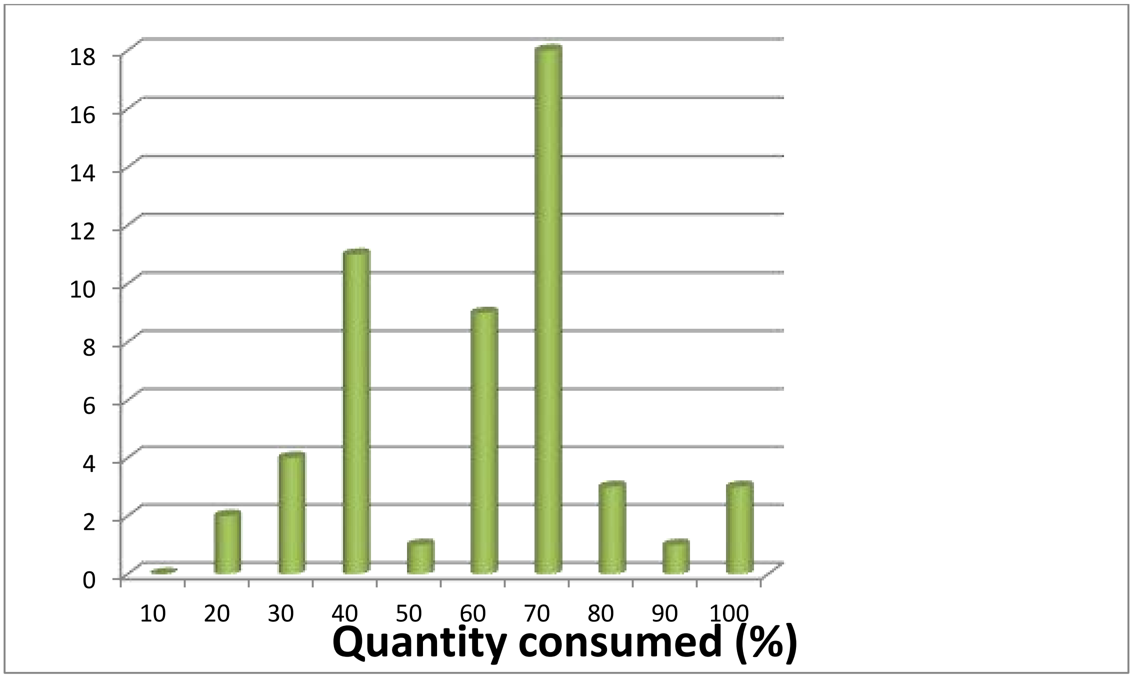

Fig.1: Bar chart showing quantity of breadfruit harvest consumed per month

Figure 1 shows that majority (18 persons) of the breadfruit consumers consume much (70%) of their harvest, 11 persons consume 40%, 9 consume 60%, 4 persons 30%, 3 persons 80%, 3 persons also consume 100% of their harvest, 2 persons 20% and 1 person consume 50% and 90% respectively. Six (6) persons do not own any breadfruit tree. The chart reveals that a greater number of breadfruit consumers prefer to consume it than to sell it. This implies that demand outweighs supply.

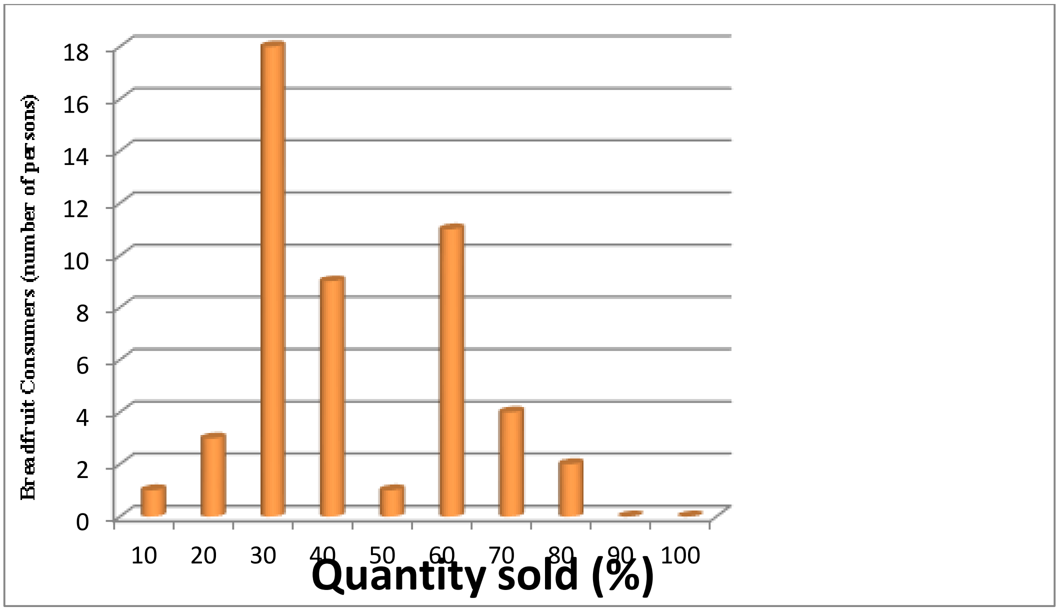

Fig.2: Bar chart showing quantity of breadfruit harvest sold per month

Figure 2 shows that majority (18 persons) of the breadfruit consumers sell (30%) of their harvest, 11 persons sell 60%, 9 persons 40%, 4 persons 70%, 3 persons 20%, 2 persons 80% and 1 person sell 10% and 50% respectively. 3 persons do not sell their harvest at all and 6 persons do not own any breadfruit tree. The chart reveals that a lesser number of breadfruit consumers prefer to consume it than to sell it. This implies that demand outweighs supply.

Objective 2: Estimation of price, income and cross-price elasticities of demand for African breadfruit.

The relationship between quantity of breadfruit demanded and demand factors is shown in table 1.

Table.1: Results of OLS multiple regression analysis on relationship between quantity of breadfruit demanded and demand factors

Explanatory Variables	Linear Function	Semi-log Function	Double-log Function	Exponential Function
Price of Breadfruit (P_b)	-9.8417 (1.1981)	-1.5266 (1.0969)	-0.6839 (3.3941)**	-0.0072 (2.6667)***
Household Income (Yh)	7.1904 (2.3152)*	2.5328 (1.0374)	0.8136 (3.6698)**	0.0066 (2.4444)*
Price of Substitute (Ps)	5.1186 (1.1148)	0.5912 (1.2073)	0.1942 (2.3887)*	0.0064 (1.0847)
Constant	176.0039	104.4127	91.4303	83.1014
R^2	0.4922	0.3928	0.8126	0.6421
Std Error	17.5213	16.5513	0.0318	0.0892
F-Value	18.0293**	12.1235**	82.0808**	33.4427**
Sample Size	60	60	60	60

Figures in parenthesis are the t-ratio
*** = t-ratio significant at 5%**
**** = t and f-ratios significant at 1%**

Table 1 shows the results obtained in four functional forms. The functional form that produced the highest value of coefficient of multiple determination (R^2), highest number of significant variables (at 1% and 5%), highest F-value and

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conformity to *a priori* expectations was selected as the lead equation. Based on these, the double-log functional form was selected as the lead equation for discussion.

The coefficient of multiple determination as shown in table 1 is 0.8126. This implies that 81% of the variation in quantity of breadfruit demanded can be accounted for by the joint action of the explanatory variables investigated in the study. The three demand factors included in the model were significant at at 1% and 5% levels of probability. The coefficients of household income (Yh) and price of substitute (Ps) were significant and positive while the coefficient of price of breadfruit (Pb) was significant but negative. This result implies that increase in Yh and Ps will lead to an increase in quantity of breadfruit demanded while increase in Pb will lead to a decrease in quantity demanded.

Hypothesis: Household demand for African breadfruit is price inelastic in the study area.

The results of computed price, income and cross-price elasticities of demand are shown in table 2. The table was derives from table 1 where the double-log function gave the lead equation.

Table.2: Computation of Elasticities of Demand

Demand Factor	Marginal Physical Product (MPP)	Elasticities of Demand	Classification
Price of breadfruit (Pb)	-0.684	-0.684	Inelastic
Household income (Yh)	0.814	0.814	Necessity
Price of substitute (Ps)	0.194	0.194	Substitute

In a double logarithmic function, the coefficients of regression (MPP) are also elasticities (Ohajianya & Onyenweaku, 2002). Table 2 shows that the price elasticity of demand for breadfruit is -0.684 which is inelastic demand and implies that if the price of African breadfruit increases, its demand would fall. The income elasticity of demand for breadfruit is 0.814. The value of income elasticity is positive and less than unity, hence breadfruit is classified as a necessity. The cross-price elasticity of demand for breadfruit represented by the price of substitute is 0.194. Since the cross-price elasticity value is positive, the commodity that had the price is a close substitute to breadfruit in the study area.

Therefore, the hypothesis is accepted.

V. CONCLUSION

The household demand for African breadfruit for diverse usage constitutes a useful ground upon which farmers can expand production. To sustain optimum demand for African breadfruit by households, more farmers need to get involved in the production of breadfruit as it is highly demanded for its food and non-food uses.

REFERENCES

[1] Akintayo, E.T. & Bayer, E. (2002). Characterisation and some possible uses of Plukenetia conophora and Adenopus breviflorus seeds and seed oils. *Bioresource technology, 85*(1), 95-97.

[2] Akubor, P.I. & Obiegbuna, J.E. (2014). Effect of Processing Methods on the Quality of Flour and Bread from African Breadfruit Kernel Flour. *Food Science and Quality Management, 24. 32-41.*

[3] Ejiofor, M.A.N., Obiajulu, O.R. & Okafor, J.C. (2012). Diversifying utilities of African breadfruit as food and feed. *International Tree Crops Journal, 5*(3), 125-134.

[4] Muojekwu, C.A., Ugwumba, C.O.A. & Chidebelu, S.A.N.D. (2017). Marketing of African breadfruit seeds (Treculia Africana) in Anambra state, Nigeria. *Scholars Journal of Agriculture and Veterinary Sciences*, 4(4), 167-174.

[5] Nuga, O.O. & Ofordile, E.A.U. (2010). Potential of *Treculia african* Decne: An endangered species of Southern Nigeria. *Journal of Agricultural and Social Research, 10*(2), 91-99.

[6] Nwabueze, T.U. (2006). Effect of hydration and screw speed on the nutrient and acceptability of extruded ready-to-eat African breadfruit (*Treculia africana*) snack. *Nigerian Food Journal, 24*(1), 107-112.

[7] Ohajianya, D.O. & Onyenweaku, C.S. (2002). Farm size and Relative Efficiency in Nigeria: Profit Function Analysis of Rice Farmers. Journal of Association for Advancement of modeling and simulation Techniques in Enterprises, France, *23*(1), 1-16.

[8] Okafor, J.C. (2005). Value addition and commercialization of plants and plant products developed through biotechnology approaches. pp. 4-13.

http://dx.doi.org/10.22161/ijeab/2.6.5 *ISSN: 2456-1878*

[9] Onyemauwa, C.S. (2012). Analysis of women participation in cassava production and processing in Imo State, Southeast Nigeria. *Journal of Economics and Sustainable Development*, *3*(5), 81-90.

[10] Ugwu, F.M. & Oranye, N.A. (2006). Effects of some processing methods on the toxic components of African breadfruit (Treculia Africana). *African Journal of Biotechnology, 5*(22), 2329-2333. Available online at https://www.ajol.info/index.php/ajb/article/view/55989/44445.

[11] Uju, G.C. & Ugwuoke, C.E. (1996). Studies on the dimensions and suitability of wood fibres of selected tree species of the family 'moraceae' in paper making. *Nigerian Journal of Botany*,*9*(10),7-13.

Vermicomposting of green Eucalyptus leaf litter by *Eisenia foetida* and *Eudrilus eugenia*

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***Abstract*— *Effective clearance of different types of waste has become significant to sustain healthy environment. Vermicomposting has become a suitable substitute for the safe, hygienic and cost effective disposal of organic solid wastes. Earthworms decompose organic waste leading to the production of compost which is high in nutrient content. The present work has been designed to reveal competitive and / or beneficial interactions by studying the inter-specific interactions in terms of growth, maturation, survival and vermicomposting efficiency of two earthworm species Eisenia foetida and Eudrilus eugenia exposed to green leaf litter of Eucalyptus and measured physical variables during entire process. The complete process was taken fourteen weeks. Work was done in plastic beans in four sets. 100 % cattle dung was also taken as a control. During the process following parameters viz. pH, temperature, biomass reduction and moisture content were analysed. pH of vermicomposting substrate was recorded low initially acidic but at last stage set in alkaline range. In case of temperature, it was changed 16-18°C ± 1°C from initial value. This was higher than control cattle dung (13°C± 1°C). Organic biomass was also depleted during process which was about 70-71 % ±1 % from initial level as compaired to cattle dung (46 %). Moisture content was lower initially then increased and set at high level.*

Keywords*— *Vermicompost, earthworm, Eucalyptus, leaf litter, physical parameters.

I. INTRODUCTION

Generation of the substantial quantity of leaf litter produced by trees growing in the forests, sideways the roads, rail lines, in the garden as well as in farm has always been problem. Improper management of these leaf waste such as burning, dumping in public place etc. has detoriate the health and environment. Sustainable remediation practices can only way to resolve this problem (Tilman *et al.*, 2002).

Vermicomposting is an important practice of sustainable remediation, which has been used in many countries and product that obtained by this process called vermicompost. It is rich in plant nutrients. Vermicompost is a microbiologically active organic material formed from the interactions between earthworms and different type of microorganisms (Domínguez, 2004).Through the vermicomposting process, environmental risk of leaf waste material reduces by transforming into a safer and more stable product suitable for application to soil (Lazcano *et al.*, 2008), and also reduces the transportation costs because of the significant reduction in the water content of the raw organic matter. Composted materials are therefore gaining acceptance as organic fertilizers in sustainable agriculture, and there has been a considerable increase in research dedicated to the study of the effects of compost-like materials on soil properties and plant growth. Vermicompost production have been ever reported from leaf of different plant such as saw dust, paddy straw and wheat straw (Indrajeet and Singh 2010),Sugarcane leaf (Alagesan and Dheeba,2010), Ashoka tree leaf litter (*Polyalthialongifolia*), Teak tree leaves litter (*Tectonagrandis*) and Neem tree leaf litter (*Azadirachtaindica*) (Jayanthi*et.al*., 2010),Tendu leaf litter (Mushan and Rao,2012), Mango and Guava leaf (Vasanthi*et.al*., 2013), Rubber leaf litter (Nath and Chaudhuri,2014;), Teak leaf litter (Nagalakshmi and Prakash 2016) etc.

We studied the vermicomposting of Eucalyptus leaf litter with two different species of earthworms i.e. *Eisenia foetida* and *Eudrilus eugenia. Eucalyptus globulus* was discovered on the island of Tasmania in 1792 by French explorers and was one of the first eucalypt species to be formally described. Eucalyptus belongs to the Myraceae family in the world, including more than 740 species. It is a long tree to a height of 35 to 40 meters, but it grows to a height from 80 to 100 meters in most of the country. Eucalyptus species is remarkable for their rapid growth. Nearly all Eucalyptus is evergreen but some tropical species lose their leaves at the end of the dry season (Kumar and Sahoo, 2011). Although leaf litters provided shelter and food to the terrestrial life and when it undergoes decomposition to produce nutrients that nourishes the soil. Eucalyptus litter has often been cited as of poor quality and slow breakdown rate (Boulton, 1991), characteristics which are linked to its high content of

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phenolic and tannins, and to its waxy cuticles (Bunn, 1988a; Campbell *et.al.*, 1992; Bärlocher*et.al*., 1995). However, this genus has hundreds of different species growing in a wide variety of climatic and edaphic conditions, and showing broad differences in litter texture and composition (Mitchell, 1988). These disparities also show effects on composting of organic biomass (Graciano *et al*. 2005). The first of these studies suggested that breakdown of low quality leaves such as eucalyptus was mainly increased by concentrations of dissolved nitrogen and phosphorus (Pozo, 1993).Briones & Ineson (1996) also observed that the mass loss of Eucalyptus *globulus* increases when it mixed with *Betula pendula*.

In present investigation leaf litter of Eucalyptus sp. Mixed with cattle dung and allowed to vermicomposting. During the process physical parameters were also analysed.

II. MATERIAL AND METHODS

2.1 Collection of plant leaves and cattle dung:

In present experiment cattle dung was collected from cattle houses in large-sized rectangular plastic pot containers and was brought to vermicomposting unit, Govt. Madhav Science College Ujjain. *Eudrilus eugeniae* and *Eisenia foetida* equally used in this study were obtained from vermiculture centre of Govt. Madhav Science College, Ujjain (M.P.) (Shouche *et.al.* 2011).

The leaf litter of Eucalyptus (*Eucalyptus globulus*) was used as a substrate was collected at random from the College campus of Govt. Madhav Science P.G. College, Ujjain (M.P).

2.2 Preparation for vermicomposting:

Collected leaf wastes were chopped into small pieces. The chopped waste was mixed with cattle dung in 50: 50 ratios. Control of both cattle dung (100 %) and leaf litter (100 %) was also taken for study.

Before chopping

After chopping

Fig.1: Green Eucalyptus leaf used in experiment.

2.3 Process of vermicomposting:

Vermicomposting process was done in plastic container. Mixture was prepared and kept in bins (plastic container) and were kept in vermicomposting room. The windrow compost method was used which composting materials was not covered and ventilation was not provided with pipes. Waste materials were agitated or turned on a regular basis for enhances passive aeration (NRAES, 1992). Bins were sprinkled with distilled water after turning it upside down to maintain high moisture content. The waste was pre-decomposed for fifteen days prior adding of earthworms. Twenty earthworms (*E. eugeniae and E .foetida*) were added in each composting bins (Singh *et.al.*2004).As the surface appear black granular indicated vermicomposting process almost completed. At this stage Watering was stopped before seven days of harvest. Prepared vermicompost was stacked so that the earthworms settle at bottom and the vermicompost was collected from the top without disturbing the bottom layers. The harvested vermicompost was filtered through fine sieve in order to get fine uniform vermicompost.

2.4 Measurement of physical parameters:

During vermicomposting of green Eucalyptus leaf litters some physical variables viz. pH, temperature, biomass reduction and moisture changes were measured. Determination of pH was done by a digital pH meter, electrical conductivity by a conductivity meter using 1:10 (w/v) vermicompost-water (double distilled) suspension. (Alidadi*et.al*., 2005; Munnoli and Bhosle,2009). Temperature was taken with the help of Mercury thermometer at the depth of 10 cm from two different sites and their mean value was taken in centigrade (Shouche*et.al.* 2011).Biomass reduction was measured with the help of scale in centimetre. For moisture measurement, 5 gm. sample was taken, and then kept it in incubator for 24 hrs. at 105°C. After drying, dry weight was taken and on that basis percentage of moisture was determined (Fairey, 2002)

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Fig.2: Different stages of vermicomposting of green Eucalyptus leaf.

III. RESULT AND DISCUSSION

Most of the organic wastes when subjected to the feeding by earthworm also involve microorganisms then it converted into blackish powdery form called vermicompost. In present investigation leaf litter of Eucalyptus was used as organic substrate separately or with cattle dung in 50:50 ratios. During vermicomposting physical variables were also measured.

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Table.1: Variation in pH, temperature, biomass reduction and moisture percentage during vermicomposting of green Eucalyptus leaf litter.

No. of Weeks	pH			Temperature in °C			Biomass depletion in Centimetre			Moisture percentage		
	100 % EL	50 % EL+ 50 % CD	100 % CD	100 % EL	50 % EL+ 50 % CD	100 % CD	100 % EL	50 % EL+ 50 % CD	100 % CD	100 % EL	50 % EL+ 50 % CD	100 % CD
1	5.5	5.6	8.5	33.7	36.3	38.5	28.9	26.42	14.84	67.62	64.33	60.35
2	6.2	6.4	8.4	32.6	34.4	35.4	25.39	23.45	12.78	69.36	64.45	61.37
3	6.5	6.7	8.3	30.6	32.5	33.8	23.86	21.23	11.11	70.28	65.45	62.95
4	6.7	6.9	8.2	28.7	29.8	32.5	20.42	19.84	10.68	72.53	66.69	63.83
5	6.9	7.2	8.2	27.5	28.8	29.9	18.77	17.38	10.34	74.29	67.79	64.69
6	7.2	7.3	8.1	26.7	26.9	27.5	16.57	15.83	9.72	75.58	68.83	64.89
7	7.3	7.4	8.1	24.9	25.7	25.5	14.42	14.89	8.74	75.43	70.52	65.65
8	7.5	7.5	8.0	21.9	24.5	24.7	13.83	13.95	7.95	77.75	71.19	66.28
9	7.6	7.7	----	21.6	22.9	----	11.59	12.25	----	78.92	72.78	----
10	7.7	7.8	----	20.8	21.7	----	10.9	10.52	----	79.35	73.32	----
11	7.8	7.9	----	20.3	21.6	----	9.37	9.58	----	80.65	74.35	----
12	7.9	8.0	----	19.6	20.6	----	9.22	9.12	----	80.89	75.49	----
13	8	8.1	----	18.8	19.7	----	8.65	8.1	----	81.35	75.39	----

Note: EL=Eucalyptus leaf, CD = cattle dung.

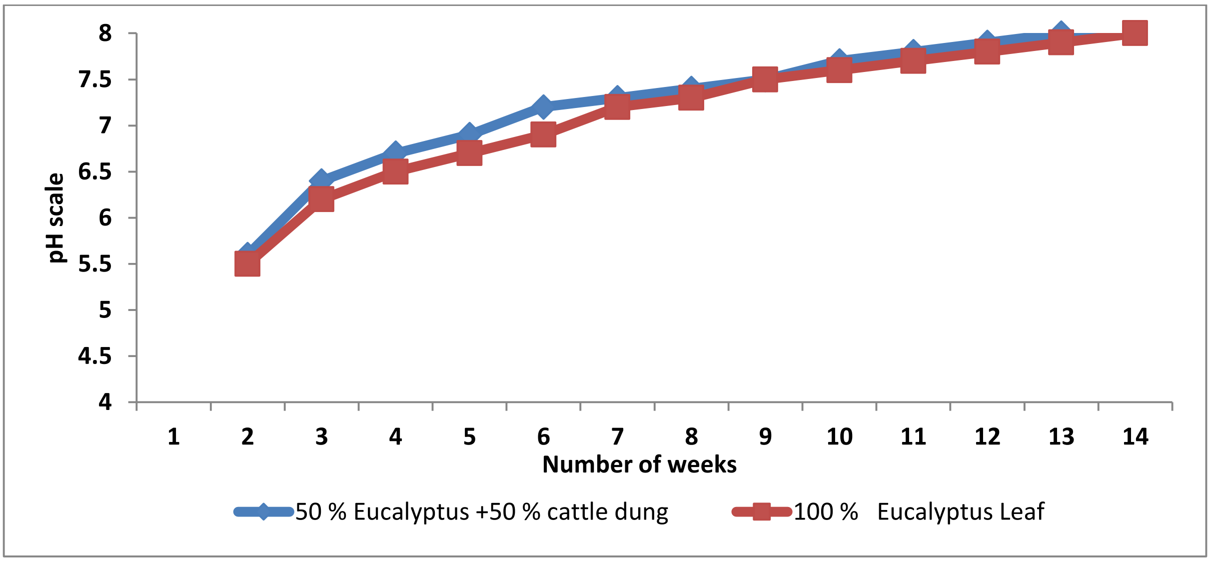

Fig. 3: Graph showing drift of pH during vermicomposting of green Eucalyptus leaf litter.

During measurement of pH it was found that initially pH in both 100% and 50 % leaf waste was in acidic (5.5 ±0.2) as compaired to alkaline in 100 % cattle dung (8.5±0.2).As process increased the pH was turn into alkaline and at the end of process set at alkaline range (8.0±0.2) while in control (100 % cattle dung) it was decreased and set around 8.0±0.2. The observed differences between the pH at the start and end of vermicomposting were +2.5, +2.5 and -0.5 in 100% leaf, 50 % leaf and 100 % cattle dung respectively. Our result concordance with Venkatesh and Eevera (2008) who have studied reduction and recovery of nutrients though vermicomposting by using *Eudrilus eugeniae* for a period of 60 days and stated that the final pH content of the vermicompost was towards the alkaline side. The initially lower pH was recorded due to production of organic acid and CO_2 by microorganisms due to presence of carbohydrate abundantly (Elvira *et.al.* 1998; Haimi and Huhta 1986). As carbohydrate source depleted, microbial metabolism shift into nitrogenous organic compound which leads to generate ammonia as a result pH turn increase (Ndegwa *et.al*. 2000).

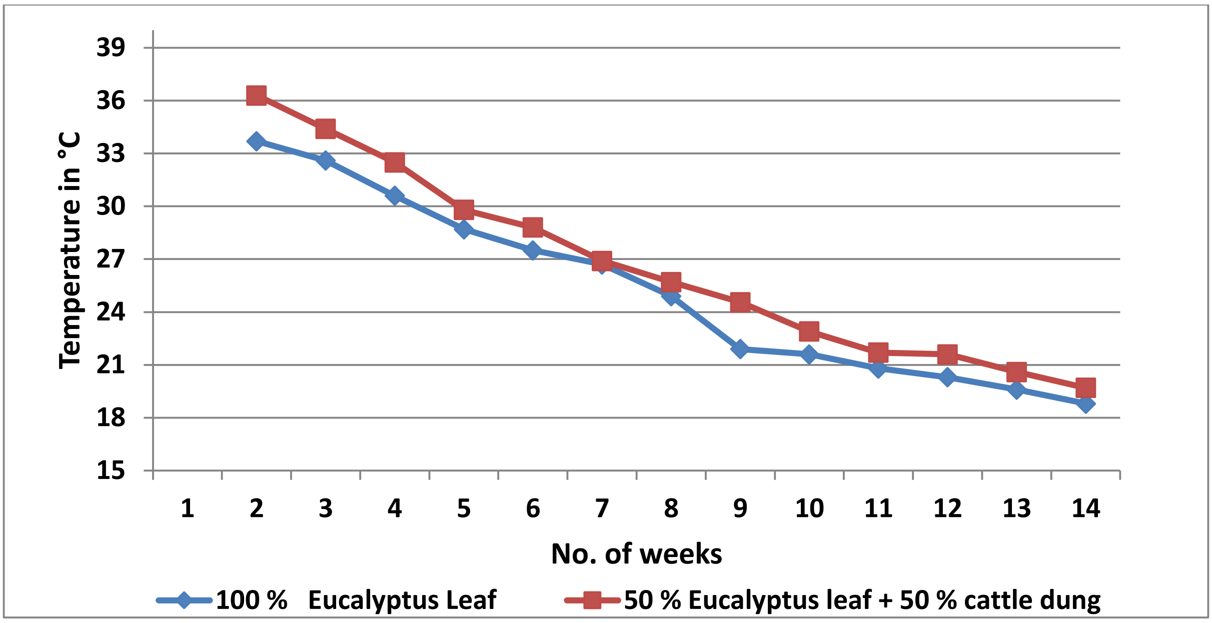

Fig. 4: Graph showing changes in temperature during vermicomposting of green Eucalyptus leaf litter.

During vermicomposting, temperature was also measured throughout the process which showing in graph no. 4. It was observed that at beginning stage temperature was high that gradually decreased and at the end of vermicomposting set at constant level. Initial temperature of 50 % leaf waste was higher (36.3°C) than 100 % leaf waste (33.7°C) as compaired to control that was 100 % cattle dung (38.5°C). After completion it was decreased up to around 19°C±1°C. The similar result also found with study of other researchers. Atchley and Clark (1979) carried out composting of different organic substances viz. card board, news paper, paper towel etc. and found that temperature was risen up to 60°C±2°C and fall down with in 24h.According to Mckinley and Vestal (1985), during composting of municipal sewage waste, temperature was raised in between 55-60°C initially then it was down gradually. Allan, (1979) concluded that the temperature of the composting pile was raised due to the biological oxidation of carbon.

The higher temperature in 50 % leaf was due to presence of more microbial community than 100 % leaf waste because the cattle dung serves the purpose of providing inoculum of microbes which carry out degradation of organic waste. During the process of composting, mesophilic flora predominates with their metabolic activity resulting in the increase in temperature of the organic waste. They are replaced by thermophilic organisms which survive at temperatures greater than 45°C to facilitate composting. When the temperature gets fall, mesophilic microorganism are become again active (Ansari, 2011).

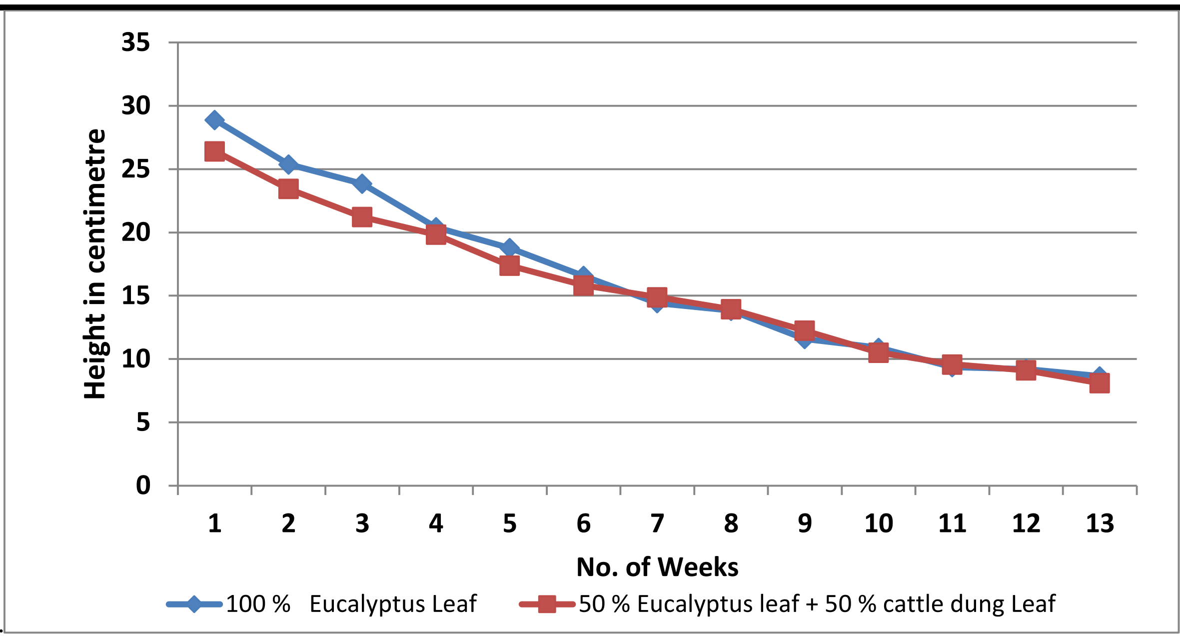

Fig. 5: Graph showing reduction in biomass during vermicomposting of green Eucalyptus leaf litter.

The result of biomass reduction is shown in table no.1 and graph no.5 revelled that height of biomass showed decrease than initial contents in both 100 % leaf litter and 50 % leaf litter with 50 % cattle dung after decomposing wastes through earthworms. Initially height of biomass were 28.9 cm. (100 % leaf litter), 26.42 cm. (50 % leaf litter) as compaired to cattle dung (14.84 cm) which depleted and set at 8.65 cm., 8.1 cm. and 7.95 cm. respectively. The maximum depletion was 71 % in 100 % leaf litter (70 % in 50 % leaf litter) as compaired to 46 % in control (100 % cattle dung) .The loss of carbon as CO_2 due to microbial respiration and assimilation of simple carbohydrates leads to biomass reductions from waste mixtures. Moreover, carbohydrates and other polysaccharides which are considered major source of carbon are digested rapidly by earthworms and some fraction of digested substances is then assimilated into worm biomass. The greater biomass reduction may be due to microbial richness of substrate materials. In such beddings the microbial respiration may leads to rapid C loss through CO_2 production. Also, earthworm fragments the waste feedstock into fine fractions which results in increased sites for microbial hydrolytic enzyme actions. The biological mutuality between earthworms and associated microbes is primarily responsible for C loss from the organic wastes. Kaviraj and Sharma (2003) reported 20–45% reduction in organic carbon contents of vermicomposted municipal solid waste mixed with CD. Similar observations have been reported by Prakash and Karmegam (2010) during vermicomposting of press-mud from sugar industry.

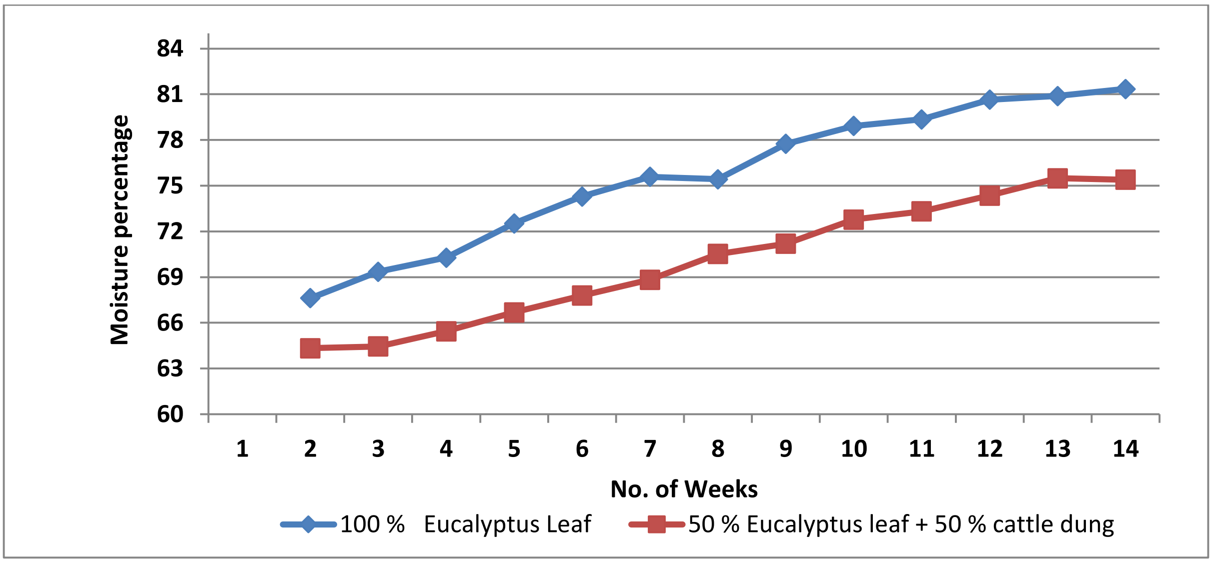

Fig. 6: Graph showing changes in moisture percentage during vermicomposting of green Eucalyptus leaf litter.

As shown in table 1 and figure 4, the level of moisture content in leaf litter was found to be maximum on 14th week of vermicomposting. Moisture is critical to the survival of earthworm species because it is the moisture within worm's body that gives it shape, enables it to move and aids in the worm's ability to absorb oxygen and it is also helpful for higher microbial activity due to which food matter is easy to feed upon. Moisture level was maintained at around 70% by addition of water every after 5^{th} day. It was notice that initially moisture level was lower and then increased day by day. During vermicomposting process due to microbial decomposition of organic matter, heat is generating that turn water into vapour and moisture content decrease initially. As organic matter consumed, no further heat generate and in that condition moisture level remain high.

IV. CONCLUSION

In the present investigation, Eucalyptus green leaf litter waste was decomposition by *E. foetida* and *E. eugeniae and* produced vermicompost that had an increased level of moisture level, pH and decreased level of biomass and temperature. This process was also taken one hundred days (14 weeks) for turn leaf into vermicompost. The present study clearly suggests that either leaf or cattle dung vermicomposted separately with earthworm but if leaf litter mixed with cattle dung then degradation rate of leaf litter increase comparatively. The study recommends that Eucalyptus leaf wastes is suitable mix for making vermicompost rich in nutrients and microorganisms which can be used as suitable organic soil amendment.

V. ACKNOWLEDGMENT

We are thankful to Principal, Govt. Madhav Science College for providing permission for conducting vermicomposting of leaf waste. The authors also wish to express their warm gratitude to the Department of Botany, Biotechnology & Microbiology for accompanying laboratory experiment.

REFERENCES

[1] P. Alagesan, and R. Dheeba (2010).Utilization of earthworms in organic waste management. *Proceedings of the 15th International Forestry and Environment Symposium*.26-27
[2] Alidadi H., Paravaresh A. R., Shahmansouri M. R. and Pourmoghadas P. (2005) Combined compost and vermicomposting process in the treatment and bioconversion of sludge. *IranJ. Environ. Health. Sci. Eng.* 2(4),251-254.
[3] Allan F.M.B.(1979).Resource recovery and recycling.John Wiley &Sons.USA.
[4] Ansari A. A.(2011.).Vermitech: an innovation in organic solid waste management.*Journal of Sustainable Development and Environmental Protection*.1(1), 107–113,
[5] Atchley K and Clark J. B.(1979). Variability of temperature, pH, and moisture in an aerobic composting process. *Applied and Environmental Microbiology*. 38(6),1040-1044.
[6] Bärlocher F., C. Canhoto& M. A. S. Graça.(1995). Fungal colonization of alder and eucalypt leaves in two streams in central Portugal. *Arch. Hydrobiol*. 133, 457–470.
[7] Boulton A. J.(1991). Eucalypt leaf decomposition in an intermittent stream in south-eastern Australia. *Hydrobiologia*.211,123–136.
[8] Briones M.J.I, Ineson P. (1996). Decomposition of eucalyptus leaves in litter mixtures. *SoilBiol. Biochem.* 28,1381–88.
[9] Bunn S. E., (1988a). Processing of leaf litter in a northern jarrah forest stream, western Australia: I. Seasonal differences. *Hydrobiologia*. 162, 201–210.
[10] Campbell I. C., James K. R.,Hart B.T.&DevereauxA. (1992). Allochtonous coarse particulate organic material in forest and pasture reaches of two south-eastern Australian streams. II. Litter processing. *Freshwat. Biol*. 27, 353–365.
[11] Domínguez J. (2004). *State of the art and new perspectives on vermicomposting research*. In: C.A. Edwards (Ed.). Earthworm Ecology (2nd edition). CRC Press LLC. 401-424.
[12] Elvira C.,Sampedro L, Benitz E, Walter I, &Calbo R (1998).Vermicomposting of sludge from paper mill and dairy industries with Eisenia Andrei: A pilot scale study. Bioresource Technology.63:205-211.
[13] Fairey N.A.(2002). An evaluation of five methods for the determination of moisture in grass seeds, *Can. J. Plant Sc.*, 82(2), 401-405.
[14] FaireyN.A.(2001) An evaluation of five methods for the determination of moisture in grass seeds. *CanadianJournal of Plant Science*.401-405.
[15] Graciano C, Guiamet, JJGoya. JF(2005). Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptusgrandis*seedlings. *For. Ecol. Manage*. 212, 40-49.
[16] Haimi J andHuhta V,(1986).Capacity f various organic residues to support adequate earthworm biomass for vermicomposting. *Biology and fertility of soils*.2,23-27.
[17] Indrajeet and Singh, J. (2010). Preparation of recipe for quality production of vermicompost. *Journal of recent advances in applied sciences* (JRAAS) 25,12-14.

[18] JayanthiB., Ambiga G. and Neelanarayanan P. (2010). Utilization of mixed leaves litter for converting into vermicompost by using an epigeic earthworm *Eudrilus eugeniae*.*Nature Environment and Pollution Technology*. 9(4),763-766.

[19] Kaviraj, Sharma, S.(2003)**.** Municipal solid waste management through vermicomposting employing exotic and local species of earthworms. *Bioresour. Technol*. 90 (2), 169–173.

[20] Kumar H.D. and Sahoo L. (2011).A review onPhytochemical and pharmacological of *Eucalyptus globulus*:A multipurpose tree.*International Journal of Research in Ayurveda & Pharmacy*.2 (5),1527-1530.

[21] Lazcano C., Gómez-Brandón, M. and Domínguez, J. (2008). Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere*, 72, 1013-1019.

[22] Mckinley, V. L. and Vestal, R.(1985) Physical and chemical correlates of microbial activity and biomass in composting municipal sewage sludge. *Applied and Environmental Microbiology*. 50(6), 1395-1403.

[23] Mitchell A.(1988). A field guide to the trees of Britain and Europe.2nd. ed., Collins & Sons Ltd.

[24] Munnoli P. M. and Bhosle S. (2009) Effect of soil and cow dung proportion on vermicomposting by deep burrower and surface feeder species, *J. Sc. Industr. Res.* 68(1), 57-60.

[25] Munnoli P.M. and BhosleS.(2009) Effect of soil and cow dung proportion on vermin-composting by deep burrower and surface feeder species. *Journal of Scientific and Industrial Research*.68,57-60.

[26] Mushan, L.C. and Rao, K. R.(2012). Physico-chemical analysis of tendu leaf litter vermicompost processed By E*udrilus eugeniae*. *DAV International Journal of Science* 1(2),100-102.

[27] Nagalakshmi P. K. and Prakash, M.(2016). A Microcosm Study of Cast and Gut of an epigeic earthworm *Perioynxceylanensis*reared on different substrates**.** *Int. J. Curr. Trend. Pharmacobiol. Med. Sci.*1(2),45-51.

[28] Nath, S. and Chaudhuri, P. S.(2014).Growth and reproduction of *Pontoscolexcorethrurus* (Muller) with different experimental diets. *Tropical Ecology* .55(3),305-312.

[29] Ndegwa PM,&Thompson SA, (2000). Effects of C-to-N ratio on vermicomposting in the treatment and bioconversion of biosolids. *Bioresource technology*.76,107-112.

[30] NRAES (1992). On-farm composting (Ed. Rynk, Robert). Natural Resource, Agriculture, and Engineering Service, Cooperative Extension, Ithaka, New York.

[31] Pozo, J.(1993). Leaf litter processing of alder and eucalyptus in the Agüera stream system (North Spain). I. Chemical changes. *Arch. Hydrobiol*. 127, 299–317.

[32] Prakash, M. &Karmegam, N.(2010). Vermi stabilization of press mud using *Perionyxceylanensis* Mich. *Bioresource Technology*.10 , 8464–8468.

[33] Shouche, S. Bhati, P. and Pandey, P. (2011). Study about the changes in physical parameters during vermicomposting of floral wastes. *Journal of Environmental Research and Development.*6(1),63-68.

[34] Singh N.B., Khare A.K., BhargavaD. and Bhattacharya, S.(2004).Effect of substrate depth on vermicomposting. *Journal-EN*. 85,16-21.

[35] Tilman D., Cassman K.G., Matson P.A., Naylor R.and Polasky S. (2002). Agricultural sustainability and intensive production practices. *Nature*, 418, 671-677.

[36] Vasanthi K., Chairman K., and Ranjit Singh A.J.A.(2013).Vermicomposting of leaf litter ensuing from the trees of Mango *(Mangiferaindica)* and Guava *(Psidiumguujuvu)* leaves.*International Journal of Advanced Research*. 1(3),33-38.

[37] Venkatesh R.M. and Eevera T. (2000).Mass reduction and recovery of nutrients through vermicomposting of fly ash. *Applied Ecol. Environ. Res.* 6(1),77-84.

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Role of stocking density of tilapia (*Oreochromis aureus*) on fish growth, water quality and tomato (*Solanum lycopersicum*) plant biomass in the aquaponic system

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Abstract— *The present study reports the results of the production of Nile tilapia (Oreochromis aureus) and tomato (Solanum lycopersicum) in the classical aquaponic system (one-loop) with different fish density. The experiment as the first scientific aquaponics study in Turkey was conducted at the Ankara University, Faculty of Agriculture, using in-door, small-scale classical aquaponic systems. Ninety six tilapia juveniles (O. aureus) were stocked at different ratio; 25 kg/m3 (Group I), 35 kg/m3 (Group II) and 50 kg/m3 (Group III) and fed with 45% raw protein feed at the level of 2% body weight for 126 days. Fish density affected the fish growth parameters and the most densiest group showed the best results in terms of fish growth and feed efficiency. Water quality parameters measured fluctated during the experiment even the exceed of the optimal ranges for the fish. However, tilapia tolerated the changes of water quality. Total plant biomass was low with the various limiting factors including insufficient lighting of in-door aquaponics system and low level of water potassium. The results of this study clearly illustrate the fish stocking rate has an impact on total biomass in the aquaponics and in one-loop aquaponics the water quality fluctation is the main challenging factor.*

Keywords— aquaponics, tilapia, tomato, fish growth.

I. INTRODUCTION

One of the main challenges of agriculture in 21th century to feed the growing population is finding more efficient and sustainable food production systems and adapting to climate change. There is also a gap in the availability of freshwater and land to increase the yield with minimal environmental effect [1]. To overcome the problems that the worls is facing with such as water scarcity, soil degradation, climate change and the population increase the aquaponics appear an alternative solution as the aquaponics are an environmental friendly and sustainable food production system [2,3].

Aquaponics, basically, the symbiotic growing of fish and vegetables in recirculating water systems is emerging as one of the most important areas of sustainable agriculture. Aquaponics is the systems that integrating aquaculture recirculating production systems with hydroponics. With aquaponics dual production of both fish and plants is possible by using the water from the fish tanks for plant growth. The essential elements of an aquaponic system consists of fish rearing tank, a suspended solid removal component, a biofilter, a hydrponic component and a sump [4]. In the aquaponic system, nutrients, which are excreted directly by the fish or generated by the microbial breakdown of organic wastes, are absorbed by plants cultured hydroponically. Through microbial decomposition, the insoluble fish metabolite and unconsumed feed are converted into soluble nutrients which then can be absorbed by plant [5] . Fish feed provides most of the nutrients required for plant growth [6]. Aquaponics work on the principle of nitrogen cycle, where in dissolved waste generated from the production system is effectively converted to plant nutrients by beneficial nitrifying bacteria. Plants can utilize these nutrients for their growth [6, 7, 8]. Plants in hydroponics and aquaponics grow more rapidly compared to their counterparts which grow in the soil because the root system is in direct contact with nutrients and nutrient uptake is more efficient in an aqueous phase [9]. Water, energy and fish feed are the three main physical inputs for aquaponic systems although the aquaponic operations vary in size and type of production system [10]. Palm et al. [11] highlighted that economic sustainability of aquaponics depends on a variety of factors including system and feed design, animal welfare and pathogen control. There is a need to establish the macro- and micronutrient proportion that fish can release in the water for a given feed in a given system; this depends on fish species, fish density, temperature, and type of plants [12]. It is clear that feed and stocking rate of fish are directly related and to maintain the balance between metabolic products the stocking rate is critical in the aquaponics as a reflection of feed. Therefore, the present study was carried out to assess the production of Nile tilapia (*Oreochromis aureus*) and tomato (*Solanum lycopersicum*) in the aquaponic system with different fish density.

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II. MATERIAL AND METHODS

This research was carried out in the small-scale aquaponic system with a grow bed form, producing tilapia (*O. aureus*) and tomato (*S. lycopersicum*) in Ankara University, Faculty of Agriculture, Department of Fisheries and Aquaculture. Aquaponic system was installed *in-door.*

The protocol for the experiment was approved by the ethics committee of the Ankara University with the reference number of 2014-2-9.

Experimental set up

Ninety six tilapia juveniles (*O. aureus*) were stocked at different ratio; 25 kg/m3 (Group I), 35 kg/m3 (Group II) and 50 kg/m3 (Group III). Individual fish weight was 5-7 g at the beginning of the experiment. Fish were fed with commercial rainbow trout feed with 45 % raw protein with 2% body weight for 126 days. Chemical composition of the feed is presented in Table 1. The aquaponics experimental system comprises of a nine fish tank (80x60x50 cm) and nine plastic tanks (65x40x35 cm) filled with hydraton for vegetable beds. Each vegetation tank planted with 4 plantlet (30-35 days old) of tomato (*S. lycopersicum*). Each fish tank was filled with 100 L of tap water and aerated continuously with air stone. Nitrifying bacteria; *Nitrosomonas europaea* and *Nitrobacter winogradskyi* were added to the system at the initial period. Experiments were run in three replicates. A lighting system made of eight Ostram HO 80w/865 lumilux cool daylight fluorescent lamps was placed above the units. Water loss due to sampling and evaporation was replenished with the addition of distilled water.

Analytic procedures

After 126 days of rearing the fish was harvested and their growth performance was measured with the parameters using the formulas as below.

i) Feed Conversion Ratios (FCR): FCR= food intake/ weight gain
ii) Protein efficiency ratio (PER): (PER) = (Wt-Wt0)/crude protein fed
iii) Feed efficiency (FE): FE= weight gain/feed fed
iv) Specific growth rate (SGR%): SGR%= (lnWt – lnWt0 x100) / t-t0

where, lnWt = the natural logarithm of the final weight, lnWt0 = the natural logarithm of the initial weight, t = time (days) between lnWt and lnWt0

v) Average daily gain (ADG): ADG% = 100[Wt-Wt0/Wt x (t-t0)]

where, Wt =Mean final fish weight, Wt0 =Mean initial fish weight and t-t0 = number of days on feed

vi) Daily growth index DGI (%): DGI%= (final weight1/3 - initial weight1/3) ×100/day

Table.1: Chemical composition of the feed

Component (%)			
Protein %	45,0	Digestible energy kcal/kg	4125
Lipid %	20,0	Metabolic energy kcal/kg	3742
Moisture %	8,5	Vitamin A IU/kg	5.000
Ash %	11,0	Vitamin D IU/kg	1.500
Cellulose %	3,0	Vitamin E IU/kg	100
Nitrogen free extract %	12,5	Vitamin K IU/kg	20
Phosphorus %	1,5	GE (Gross energy) kcal/kg	5124

At the end of the experiment, plant (*S. lycopersicum*) parts were weighted separately (as leaf, stem and root) for determination of fresh and dry weight. For measuring dry weight of the plant samples was dried in 65 ºC for 3 days.

Water Quality Measurements

Water quality parameters in fish tanks were routinly measured. During the experimental period the water temperature was kept at 23°C. Dissolved oxygen (DO), temperature (T) and pH were measured every week with portable equipments. Other water quality parameters; ammonia (NH3), Nitrat (NO3-), Nitrit (NO2-) and potassium (K) were measured every 15 days by using Standard Methods [13].

Statistical Analysis

This experiment were conducted as completely randomized design with three replicates. Data were analyzed by analysis of variance (ANOVA) with the SAS package. Duncan's multiple-range test was used to compare differences among individual means. Treatment effects were considered significant at $p<0.05$. Percentage and ratio data were transformed to arcsine values prior to analysis[14].

III. RESULTS

Growth and production of tilapia in the aquaponic system are given in Table 2. The mean group weight gain was 544.1±57.9 in Group I (stocking rate: 25 kg/m^3), 849.7±30.8 in the Group II (stocking rate: 35 kg/m^3) and 1003.3±49.8 for Group III (stocking rate: 50kg/m^3). The differences in mean group weight gain were statistically significant ($p < 0.05$) and the highest weight gain was in Group III with the highest fish density. Feed conversion ratio (FCR) differed among the groups ($p < 0.05$) however, the FCR was similar in Group II and III. The FCR was higher in Group I than that of Group II and III. Thus, feed efficiency (FE) was lower in Group I. Protein efficiency ratio (PER) showed significant differences among the groups. PER was the lowest in Group I and the highest in Group III. Specific growth rate was higher in Group III. Average daily growth was the highest in Group III with the value of 12.833±0.829 %. Daily growth index (DGI) differed among the groups ($p < 0.05$) and the minimum DGI percentage was in Group I. Survival rate showed significant differences among the groups ($p < 0.05$) and was the highest in Group II.

Table.2: The growth parameters of tilapia (O. aureus) in the aquaponics system by the stocking ratio

Growth Parameters	**Experimental groups**		
	Group I Stocking rate: 25 kg/m^3	Group II Stocking rate: 35 kg/m^3	Group III Stocking rate: 50 kg/m^3
Mean group initial body weight (g)	44.967±1.08b*	68.733±0.994a	70.067±3.18a
Mean group Final body weight (g)	589.0±58.4b	918.4±31.8a	1073.4±50.0a
Mean group weight gain (g)[1]	544.1±57.9c	849.7±30.8b	1003.3±49.8a
Food Consumed (g)[2]	621.87±23.0c	788.90±12.1b	913.83±2.39a
Feed Conversion Ratios (FCR)[1]	1.1600±0.0777a	0.9300±0.0231b	0.9133±0.0406b
Feed efficiency (FE)[1]	0.8710±0.0618b	1.0765±0.0257a	1.0977±0.0516a
Protein Efficiency Ratio (PER)[1]	11.828±1.26c	18.471±0.669b	21.812±1.08a
Specific Growth Rate (SGR %)	2.2891±0.0763b	2.3138±0.0176b	2.4366±0.0533a
Percentage average daily growth (ADG %)	10.788±1.02b	11.030±0.238b	12.833±0.829a
Daily growth index (DGI %)	4.2943±0.228c	5.0193±0.0815b	5.4583±0.145a
Survival (%)	80.952±9.52a	96.970±3.03b	85.714±10.9a

*Values with different superscripts in a row differ significantly ($p<0.05$)
1 Expressed as the percent of the initial body weight after 126 days.
2 Moisture-free basis.

The tomato (*S. lycopersicum*) plant biomass as fresh and dry weight of tomato plant leaf, stem and root branches were presented in Table 3. Significant differences were observed in the fresh weight and dry weight of tomato plant ($p < 0.05$). Final total weight values were the maximum in Group III. Fresh and dry weight of total plant correlated with fish density (R^2=0.92).

Table.3: Biomass of tomato (S. lycopersicum) plants grown in the aquaponic system by fish stocking density groups.

	Fresh Weight (g pot^{-1})				Dry Weight (g pot^{-1})			
Group	Leaf	Stem	Root	Total	Leaf	Stem	Root	Total
I	1252,5	621,6	131,2	2005,3a*	192,5	66,1	20,4	278,9a
II	1405,9	902,6	90,0	2398,5b	216,0	95,9	14,0	326,0b
III	1728,1c	1108,3	139,8	2976,2c	265,6	117,8	21,7	405,1c

*Different letters in a column indicate significant differences ($p < 0.05$) among the groups.

Water quality parameters measured in the experiment (DO, pH, ammonium, nitrite, nitrate, potassium) are presented in the Fig 1. Water quality parameters except water temperature showed significant differences by the time ($p<0.05$) and the experimental groups ($p<0.05$). During the experimental period the water temperature was kept around 24-25°C. The range of pH was between 5.83 and 7.31 in Group I, 5.60-7.22 in Group II and 5.50-7.12 in Group III. Dissolved oxygen level providing with artificial aeration ranged between 5.80 mg/L (min) and 7.13 mg/L (max). Ammonium levels during the experiment varied between 0.68 and 3.70 mg/L in Group I, 0.15 and 3.49 mg/L in Group II and 0.40 and 2.92 mg/L in Group III. Nitrite levels were between 0.05 and 0.80 mg/L in Group I, 0.16 and 0.90 in Group II and 0.10 and 0.53 mg /L in Group III. Nitrate levels ranged from 1.85

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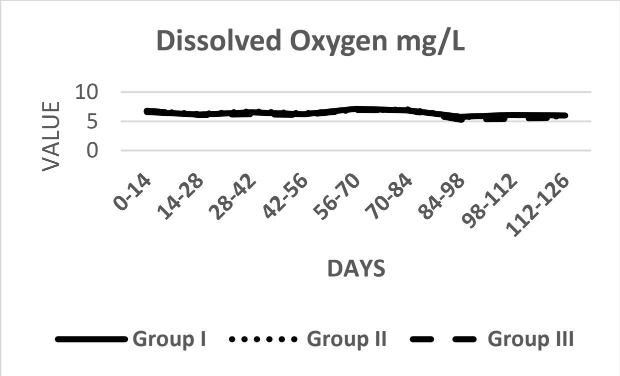
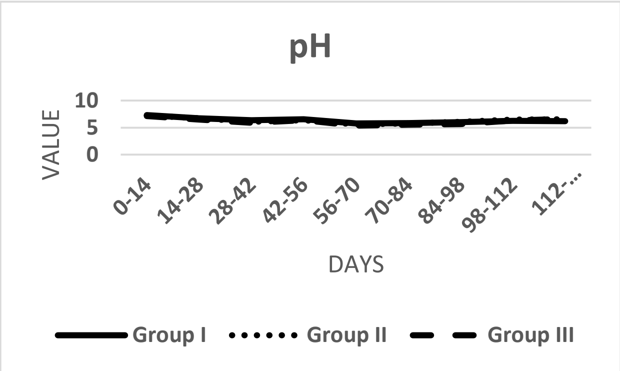
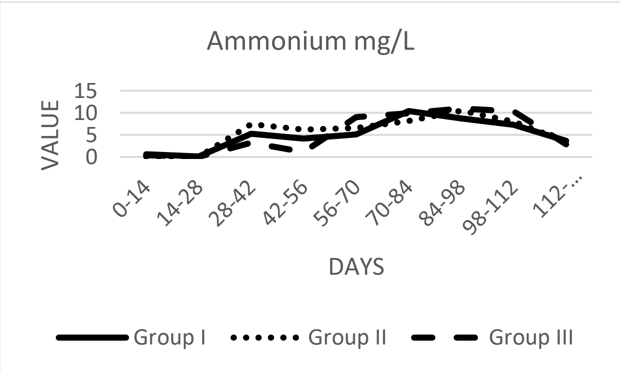
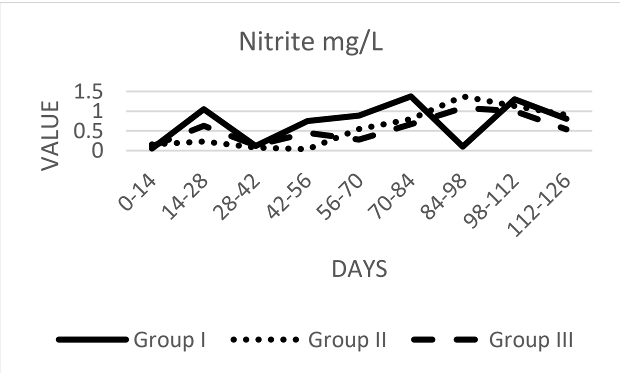
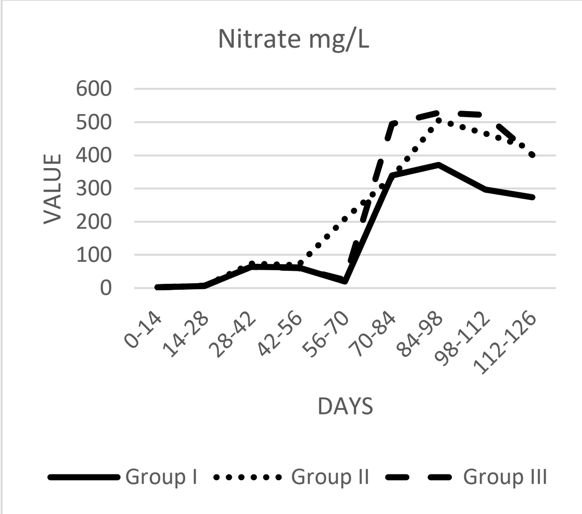
to 275 mg/L in Group I, from 2.33 to 419 mg/L Group II and from 2.38 to 400.93 mg/L in Group III. Potassium values in water ranged from 0.13 to 0.36 meq/L in Group I, from 0.10 to 0.37 meq/L in Group II and from 0.10 to 0.38 meq/L in Group III.











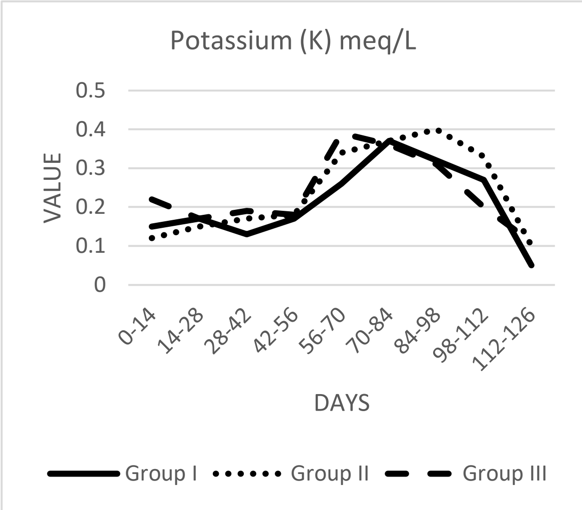


Fig.1: Water quality parameters in the aquaponic system with different tilapia density and tomato plant (Group I:Stocking rate: 25 kg/m³, Group II: Stocking rate:35 kg/m³, Group III: Stocking rate: 50 kg/m³)

IV. DISCUSSION

In this aquaponic system, three different stocking rate of tilapia were analysed for *i)* Feed Conversion Ratios (FCR), *ii)* Protein efficiency ratio (PER) *iii)*Feed efficiency (FE) i*v)*Specific growth rate (SGR %), *v)* Average daily gain (ADG), *vi)*Daily growth index DGI (%) and all fish were fed with the same feed containing 45% raw protein. We observed that the growth parameters were better in the group having the maximum fish density with 50 kg/m3. Total plant biomass values were also better in the group of 50 kg/m3 than the groups of 25 and 35 kg/m³. Nevertheless, tilapia in oxygenated water can be grown at the 120 kg/m3 by providing better nutrient supply [15]. FCR as one of the most import parameters in terms of economy of the aquaponic system should optimize in parallel to fish density and feding ratio. Thus, in our case, the minimum FCR was observed in the group of the highest stocking rate (50 kg/m3) with the feding ratio of 2% of total body weigt daily.

The average FCR as 1.2-1.3 in the couple system with 40 kg fish /m^3 in the study of Monsees et al [13] was considered as favouring for the commercial aquaculture. Endut et al [16] reported that feed conversion ratio (FCR) values were in the range of 1.23–1.39 for catfish (*Clarius gariepinus*) in the aquaponic system with stocking ratio of 25 kg/m3 at different flow rates, by stating that the FCR values were close to the ideal value for aquaculture. Thus, in our study FCR of the all groups (Group I 1.16; Group II 0.93 and Group III 0.91) are appropriate when compared to the economic FCR values in aquaculture. Here, SGR values were 2.28 (Group I), 2.31(Group II) and2.43 (Group III), presenting good growth performance. SGR values are higher than the values assessed by Al-Hafedh [17], Monsees [15] and Endut [16] for the aquaponic production.

pH values fluctuated in all groups during the present study. pH is one of the crucial factors in aquaponics and should be kept around 7 for the success in nitrification; converting ammonia and providing nitrate for the plants ([12, 15, 18]. Although the pH values were below the optimal value for the fish in this experiment tilapia tolerated the pH changes. On the other hand, pH values were suitable for the plant in the present study hence, most plants need a pH value between 6 and 6.5 in order to enhance the uptake of nutrients [12]. It is known that pH<6.5 disrupts the nitrification process with eventual risk of ammonia and nitrite toxicity. Here, ammonia and nitrite exhibited high values in parallel to low pH, the peak of ammonia and nitrite corresponds to the lowest pH values. However, in our case, the nitrate values reached higher values and this may be explained by the insuffcent nitrate uptake of the plant due to weak lighting. Thus, the interaction of the water quality parameters in the aquaponics with media based growing bed is more complicated and difficult to keep within optimal ranges. In terms of optimal production parameters decoupled systems are taken into consideration, as stated by Monsees et al. [15]. In this study, water potassium showed low levels. This was also reported by Graber and Junge [19] to explain a poor vegetable quality in aquaponics.

In the present study, total plant biomass was low when compared with the previous studies on tomato plant in the aquaponics [20, 21]. Total biomass of the plant showed differences depending on the fish stocking ratio and total plant biomass increased with decreasing the fish density. However, the proportion of root to total biomass decreased with fish density. Here, more leave portion was observed in one-loop system. This has been reported before by the fact that of suboptimal nutrient supply [22]. Leaves portion to total biomass increased in the one-loop system here. Thus, Bloom et al [23] reported that when exposed to low light, plants usually respond by increasing allocation of biomass to leaves, by actively creating a dynamic balance where all resources should be equally limiting to growth. Goddek et al [12] reported that every plant and fish species have different nutritional needs that are also dependent on the growth stage/life-cycle and external factors (including system design). Hence, the optimization of whole aquaponics system to dual production is highly complicated. Regarding the fish reaction to water quality fluctations, tilapia tolerated the sharp changes in water quality as reported by Rakocy [24]. Survival ratio is considered in normal ranges as found in RAS.

V. CONCLUSION

The effects of stocking rate were determined for the tilapia growth and plant biomass in one-loop system. The growth performance and feed conversion assessed in this study were better in the group with the maximum density (initial stocking rate, 50 kg/m3). Total plant biomass was found to be low with the various limiting factors including insufficient lighting of aquaponics system used. The most important factor was to control the water quality, particularly pH and nitrogenous substances. Thereby, the dynamic action of water quality in one-loop systems may not meet the expectations in terms of co-production performance. To optimize fish stocking density in the aquaponics the complexity of the water quality should be considered in one-loop system.

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REFERENCES

[1] FAO report 2009. Global agriculture towards 2050.

[2] Tyson, R.V., Treadwell, D.D. and Simonne, E.H. 2011. Opportunities and Challenges to Sustainability in Aquaponic Systems. Horttechnology, 21(1), 6-13.

[3] Salam M.A., Asadujjaman M, Rahman M.S., 2013. Aquaponics for Improving High Density Fish Pond Water Quality through Raft and Rack Vegetable Production. World J. Fish Mar. Sci., 5: 251-256.

[4] Rakocy J.E., Hargreaves J.A. and Bailey D.S., 1993. Nutrient accumulation in a recirculating aquaculture system integrated with vegetable hydroponics. In: Wang, J.K., ed., Techniques for Modern Aquaculture, ASAE Publ. 02-93, St. Joseph, MI, 1993, pp. 148–158.

[5] Rakocy J.E., Masser M.P. and Losordo T.M., 2006. Recirculating Aquaculture Tank Production Systems: Aquaponics-Integrating Fish and Plant Culture, SRAC Publication, pp: 454.

[6] Nelson, R. L. 2008. Aquaponic Food Production. Montello, WI: Nelson and Pade Inc Press.

[7] Quillere, I., L. Roux, D. Marie, Y. Roux, F. Gosse, and J.F. Morotgaudry, 1995. An artificial productive ecosystem based on a fish bacteria plant association. 2. Performance. Agriculture, Ecosystems and Environment 53:19–30.

[8] Ghaly, A. E., M. Kamal, and N. S. Mahmoud, 2005. Phytoremediation of aquaculture wastewater for water recycling and production of fish feed. Environment International 31:1–13.

[9] Azad, K.L., K. Ishikawa, J.C. Diaz-Perez, T.E. Eaton and N. Takeda, 2013. Growth and development of komatsuna (*Brassica rapa* L. Nothovar) in NFT (nutrient film technique) system, as influenced by natural mineral. Agric. Sci. J., 4: 1-7.

[10] Love, D.C., Fry, J.P., Ximin, L., Hill, E.S., Genello, L. Semmens, K., Tompson, R.E. 2015. Commercial aquaponics production and profitability: Findings from an international survey. Aquaculture 435: 67-74.

[11] Palm, Harry W., M Nievel, and Ulrich Knaus. 2015. "Significant Factors Affecting the Economic Sustainability of Closed Aquaponic Systems. Part III: Plant Units." AACL Bioflux 8 (1): 89–106.

[12] Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K.V., Jijakli, M.H.; Thorarinsdottir, R. 2015. Challenges of Sustainable and Commercial Aquaponics *Sustainability* **2015**, *7*, 4199-4224; doi:10.3390/su7044199

[13] American Public Health Association (APHA) (2005) Standard method for examination of water and wastewater, 21st edn. APHA, AWWA, WPCF, Washington.

[14] Zar J.H., 1984. Biostatistical Analysis, 2nd ed.Prentice Hall, Englewood Cliffs, NJ. 718 pp.

[15] Monsees H, Kloas W, Wuertz S (2017) Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes. PLoS ONE12(9): e0183056.

[16] Endut, A. Lananan, F., Abdul Hamid, S.H., Jusoh,A.& Wan Nik,W.N. 2016. Balancing of nutrient uptake by water spinach (Ipomoea aquatica) and mustard green (*Brassica juncea*) with nutrient production by African catfish (*Clarias gariepinus*) in scaling aquaponic recirculation system. Desalination and Water Treatment Vol. 57 , Iss. 60.

[17] Al-Hafedh, Y.S., Alam, A. and Beltagi, M.S. 2008. Food Production and Water Conservation in a Recirculating Aquaponic System in Saudi Arabia at Different Ratios Of Fish Feed to Plants. Journal of The World Aquaculture Society, 39(4), 510-520.

[18] Kloas, W., Roman, G., Daniela, B., Johannes, G., Monsees,H. Schmidt,U, Staaks, G., Suhl, J., Tschirner, M., Wittstock, B., Wuertz, S., Zikova, A.,Rennert, B. 2015. "A New Concept for Aquaponic Systems to Improve Sustainability, Increase Productivity, and Reduce Environmental Impacts." Aquaculture Environment Interactions 7 (2): 179–92.

[19] Graber, A. and Junge, R. 2009. Aquaponic Systems: Nutrient Recycling from Fish Wastewater by Vegetable Production. Desalination, 246(1-3), 147-156.

[20] Roosta, H.R. and Hamidpour, M. 2013. Mineral Nutrient Content of Tomato Plants In Aquaponic and Hydroponic Systems: Effect of Foliar Application of Some Macro- and Micro-Nutrients. Journal of Plant Nutrition, 36(13), 2070-2083.

[21] Khater, E.S.G., Bahnasawy, A.H., Shams, A.E.H.S, Hassaan, M.S., Hassan, Y.A. 2015. Utilization of effluent fish farms in tomato cultivation. Ecological engineering, 83(01): 199-207

[22] Hermans C, Hammond JP, White PJ, Verbruggen N. How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci. 2006;11(12):610–7. pmid:17092760

[23] Bloom, A. J., F. S. Chapin, and H. A. Mooney. 1985. Resource Limitation in Plants - an Economic Analogy. Annual Review of Ecology and Systematics 16:363-392

[24] Rakocy, J.E., 1999. Aquaculture engineering – the status of aquaponics, part 1. Aquacult. Magaz, 25(4): 83-88.

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Relationship between Media Counselling, Farmer's Attitudes and Adoption of Integrated Crop Management Technology of Chili

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Abstract— *Counseling of integrated crop management technology is done to reduce the negative impact on the environment through various ways either through formal or informal media, but in fact the utilization of technological innovation tends to decrease because of difficult farmer attitude accept technological innovation offered. The purpose of this research is to see the relationship of agricultural extension media, consumer behavior and adoption of integrated crop management technology of chili. The study was first conducted in Maros regency, conducted in April-August 2017. Data were collected randomly (simple random sampling) and interviews were conducted to 85 respondents in a structured manner using a pre-prepared questionnaire. Data analysis was done using two methods: (1) descriptive statistical analysis, and (2) quantitative analysis. Descriptive analysis to explain extension media, farmer attitude and farmer adoption, measured using index aid. Quantitative analysis uses Chie Square analysis. The results showed that there was no significant correlation between media of counseling with farmer attitude and there was significant correlation between media of counseling with application of integrated crop management technology of chili by farmer. Extension activities through extension media need to be improved and the choice of extension media should be in accordance with the needs and conditions of local farmers, so that counseling can be achieved. Farmers' attitude toward technology should be directed to arrive at a process of action to adopt technology.*

***Keywords—* Adoption, attitude, counseling, media, relationship.**

I. INTRODUCTION

One type of vegetable that has a broad development prospect is chili pepper, this is because the chili pepper has a wide market, both as a commodity consumed in the country and for export to overseas markets. In addition, chili pepper has a fairly high economic value, and at certain times the price of chili pepper will soar high enough. Chili pepper also has a high adaptability to be cultivated in various conditions (Kusmana et al., 2009).

In 2014, the area of chili pepper land in Indonesia reaches 128,734 ha, with production of 1,074,602 tons and productivity of 8.35 tons / ha. Nationally, chili pepper production in 2014 has increased 6.09 percent or about 61,723 tons while the contribution of chili pepper production to national vegetable production is 9.02 percent (Ministry of Agriculture, 2015). In 2014, the area of chili harvest in the South Sulawesi region reaches 3,920 ha, with production of 20,516 tons and productivity of 5.23 tons / ha. There was an increase in production from the previous year at 4,540 tons or 20.13% (South Sulawesi Statistics Agency, 2014). In addition, the area of harvest of chili pepper in Maros Regency reaches 583 ha with a production of 2.79 tons / ha (Statistic Center of Maros Regency, 2015). Although statistically the production of chili pepper increased, but nationally the production has not been able to meet national needs.

In an effort to solve the problem, the government has designed a technology assembly of chili pepper that can increase production, reduce the use of chemical pesticides and increase the use of organic fertilizer, called Integrated Crop Management Technology abbreviated PTT. But the perpetrator of chili pepper farming has not fully apply the recommended technology, so the quality and production level of chili pepper produced is still low. This is based on the results of research by Awaluddin et al (2014) that the level of utilization of technological innovations by farmers tend to slow down, the level of knowledge of farmers are still lacking, access to information sources far enough and the availability of information that is not in accordance with the needs of farmers.

According to Indraningsih (2011) research, the results of external and internal evaluation indicate that the speed and utilization rate of technological innovation produced by the Ministry of Agriculture through research institutions tends to decrease due to the attitude of farmers who still reject the technological innovation. In addition, agricultural counseling is currently only a program or it can be said that farmers only accept if there is an activity using the media counseling and has been determined without being able to choose what the preferred way farmers.

Problems of utilizing information media that have not touched stakeholders and the use of information that has not been widespread makes the position of farmers, fishermen, and breeders become increasingly weak (Andriaty & Endang, 2012). In addition, according to research by Sasongko et al (2014), the factors that significantly affect the behavior of communication is the credibility of the media where the higher the media credibility by farmers, the higher the communication behavior of farmers. Factors that significantly affect attitude of farmers are communication, motivation, and education behavior. Media consumption correlates significantly with the effectiveness of communication between counseling workers and farmers. The most dominant characteristic of farmers related to communication effectiveness is farmer education level and frequency of following counseling.

Research by Harmoko and Erik (2016) said that the factors that affect farmers in accessing agricultural information is the cosmopolitan level. The higher the farmers look for agricultural information then access to information will be higher. The information most needed by farmers is related to production technology, followed by marketing and post-harvest information. The need for such information remains unfulfilled. Farmers use meetings, print media, and electronic media to access information (Andriaty et al., 2011).

The attitude of farmers to the theme of counseling with the needs of farmers, is closely related to the technology that will be applied by farmers. Due to the fact on the field, there is still a gap between the technology recommended through the counseling media with the attitude of farmers and the adoption of integrated crop management technology of chili pepper. Therefore, the research aims to analyze the relationship between counseling media, farmer attitude and adoption of integrated crop management technology.

II. METHOD

1. Location and Research Design

This research was conducted in Maros Regency, Tanralili District, from April until August 2017. This research according to the explanation including correlation research type which aims to find whether there is relationship between two variables or more. Correlation research does not answer cause, but only explains the presence or absence of relationship between variables studied.

This study aims to explain the relationship between counseling media, attitude and adoption of integrated crop management technology of chili pepper. The sample in this research is 85 chili farmers, selected by simple random sampling method, the sample is homogeneous and comes from three villages of Todoopulia, Lekopancing and Borong in Maros Regency.

III. METHOD OF COLLECTING DATA

Data collection methods were conducted through interviews using semi-structured questionnaires. The data collected were farmer characteristics information, data on farmer attitude aspects; as well as the level of farmer adoption related to integrated management technology of chili. Then the secondary data that is in the form of statistical data from related institutions and previous research reports derived from the journal.

IV. DATA ANALYSIS

Variable measurement scale is done so that research variables can be measured through numbers so that it can be used in statistical tests. In measuring this research variable used ordinal scale.

Data analysis was done by using two methods: (1) descriptive statistical analysis, and (2) quantitative analysis. Descriptive analysis was conducted to find out the level of intensity of counseling media, farmer attitude toward counseling media and adoption rate of integrated crop management technology of chili measured by using an index. Quantitative analysis is used to see the correlation between extension media, farmer attitude and adoption of Integrated Crop Management Technology of Chili in Maros Regency. The analysis tool used is Chie Square test.

V. RESULT

1. Characteristics of Farmers Respondents

The average age of the farmers was 39 years, with variations ranging from 18 to 72 years. The results showed that 77.65 percent of farmers respondents, aged between 30 years to 54 years and 15.29 percent of farmers under the age of 30 years. Most of the respondent farmers have high school level of 41.67 percent, junior high school as much as 32.14 percent, elementary school as much as 17.86 percent and bachelor as much as 8.33 percent, thus providing a picture of sufficient capacity in access information technology. The average farmer of the respondent has a long experience of chili farming that is 5 to10 years as much as 74.12 percent, the experience of 11 to 15 years of farming as much as 20 percent and farmers with experience chili peppers above 15 years as much as 5.88 percent. The family dependent of the respondent farmers is 68.24 percent as many as 3 people, while the farmers who have a large number of dependents of the family with the number of dependents 4 to 7 people amounted to 31.76 percent.

Farming done by the farmers of respondents get a high profit and the farming is feasible to continue because the profit earned reached IDR 101.643.518, with the R / C ratio of 19.16, the value of R / C ratio is> 1 which means the farming is feasible to be cultivated and developed. Respondents belonging to farmer groups were 100 percent, with 67 percent of farmers joining farmer groups for 1-5 years.

2. Relationship Between Counseling Media, Farmers Attitudes and Adoption of Integrated Crop Management Technology of Chili

Based on Chi-Square analysis, relationship between counseling media, farmer attitude and Adoption of Integrated Crop Management Technology of Chili, obtained that X^2_{count} = 3,391. Chi-Square X^2_{table} with α = 0.05 is 9.49. Thus $X^2_{counts} < X^2_{tables}$, then H0 is accepted and H2 is rejected, where there is no significant relationship between counseling media and farmer attitude. Chi-Square analysis results, obtained that X^2_{count} = 27,376. Chi-Square X^2_{table} with a = 0.05 is 9.49. Thus $X^2_{count} > X^2_{table}$, then H0 is rejected and H1 accepted, where there is significant relationship between counseling media with farmer adoption rate.

VI. DISCUSSION

Most respondent farmers are of productive age where the average age of respondents is the ideal age for work and has the ability to increase work productivity, and has a great ability to absorb information and innovative technology in agriculture. Farmer education is quite well educated, because 41.67 percent are high school graduates. Education is one of the factors that determine the productivity of labor, in this case farmers. Farmers who have higher levels of education have a better ability to understand and apply Integrated Crop Management Technology of Chili so that productivity becomes higher. (Luluk et al., 2008).

Based on farming experience owned by farmers, farmers are expected to be able to overcome the problems faced in the struggle. Therefore, the experience of chili farming is very influential on the attitude and decision to adopt a technology. Research from Rukka et al (2006), explains that farmers' experience in farming has an effect on how to respond to an innovation. The longer the experience of farming, the level of response to a technology will be higher. Based on the number of dependents most (68.24%) of farmers belonging to the small families. Family members are one of the agricultural human resources owned by farmers, especially those who are productive and help in their farming activities. Family members can also be a burden for their families if they are not actively working to support farming activities (Syafrudin, 2003).

Farming conducted by the farmers of respondents is reasonable to be cultivated where R / C ratio is greater than 1. The ultimate goal of farming is to obtain income and profit as much as possible from the farming process undertaken. Farmers work their farms in order to maximize profits so as to compensate for the expenses incurred during the production process. In addition, the participation of farmer respondents in farmer groups is very large. Farmers recognize that the function of farmer groups is as a forum for learning, working together and as a unit of farm production. Group experience makes it easier for farmers to communicate with peasants, more open and easy to receive information about agriculture and help each other in the process of farming. One of the factors that can determine the success in farming is the length of time to become a member of the farmer group because with the length of membership of the group then more experience of farming more developed (Rizal & Rahayu, 2015).

From the result of analysis of relationship between counseling media with attitude of farmer to Integrated Crop Management Technology of Chili, hence as many as 53 people accept the technology, but the counseling media can not influence attitude or behavior of farmer. Counseling media offered by government agencies is not maximized. Formation of attitude requires a process that is influenced by internal factors (individual self) and external factors (environment). Counseling media is a supporting factor not a dominant factor. The higher the education, the farmers experience, the farmers are not easily influenced by the media, because of the many considerations and interaction of farmers with the environment so that the decision or attitude of farmers to a technology is different from the purpose of the counseling. Kokolakis (2015) research results say that the attitude and behavior of a person is a phenomenon that is paradox. There is a real inconsistency between the attitude and behavior of farmers to the counseling media.

Based on the results of research, the counseling media related in the process of technology adoption to farmers. Media of counseling through demonstration plots, printed media, and electronic media affect the farmers change in adopting agricultural innovation (Rushendi, et.al, 2016). The farmers receives information from counseling media, but the information they receives is not directly accepted and applied by the farmers, because the farmers will adapt it to the environment and customs that are usually done in the field. The choice of appropriate media in transferring information technology should be the focus of attention, the combination of the use of information media into

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alternative media counseling, so that farmers more easily absorb the information with the ability of the senses it has.

VII. CONCLUSION AND RECOMMENDATION

There is no significant correlation between media of counseling with farmer attitude. There is a significant relationship between the counseling media and the adoption of Integrated Crop Management Technology of Chili by farmers. Internal and external factors from farmers are the main reason for farmers to be receptive to information and adopt technology. It is advisable that evaluation of technological innovations that have been disseminated needs to be done, whether the technology is adopted or not by farmers. Counseling is the process of disseminating information and can not be separated from the media or tools used, preferably before doing counseling needs to be conducted survey of counseling media that is easily accepted and understood by farmers.

REFERENCES

[1] Andriaty, E., Bambang, S., & Endang, S. (2011). Study of Agricultural Technology Needs in Several Regencies In Java. *Journal of Agricultural Library*, 20 (2): 54-61.
[2] Andriaty, E., & Endang, S. (2012). Availability of Agricultural Technology Resources in Several Regencies in Java. *Journal of Agricultural Library*, 21 (1): 30-35.
[3] Awaluddin., Sukesi, K., & Sugiyanto. (2014). Study of Farmers Empowerment Model Using Three Media Communication in Bima District (Case In Nggembe Village, Bolo Sub District) .*Jurnal of Habitat*, 25 (1): 1-14.
[4] Statistics Centre of South Sulawesi. (2014). Regional Statistics of South Sulawesi Province 2014. Central Bureau of Statistics of South Sulawesi Province.
[5] Statistic Center of Maros Regency. (2015). Maros In Figures 2016. Maros Central Statistics Agency.
[6] Harmoko., & Erik, D. (2016). Access to Agricultural Information through Communication Media at Farmer Group in Sambas District and Singkawang City. *Journal of Communicators*, 8 (1): 1-10.
[7] Indraningsih, K.S. (2011). The Effect of Counseling on Farmers' Decisions in Adoption of Integrated Farming Technology Innovation. *Journal of Agro Economics*, 29 (1): 1-24.
[8] Kusmana, R., Kirana, I., Hidayat, H., & Kusandriani, Y. (2009). Adaptation Test of several Red Chili Drag in Garut Highlands and Lembang Plateau. *Journal of Horti*, 19 (4): 371-376.
[9] Ministry of Agriculture. (2015). Statistics of Holtikultura Production Year 2014. accessed October 16, 2017. Available from: http://hortikultura.pertanian.go.id.
[10] Rukka, H., Buhaerah & Sunaryo. (2006). Relationship of Farmer Characteristics with Farmers Response to the Use of Organic Fertilizer on Wetland Rice (Oryza sativa L.). *Journal of Agrisistem*, (2) 1: 12-18.
[11] Rizal, M., & Rahayu, P. (2015). Farmer participation level in wetland paddy farming group to support M-P3MI Program in Paser Regency, East Kalimantan. Proceedings of SemNas Masy Biodiv Indon 1 (2): 352-357.
[12] Rushendi., Sarwititi, S., & Retno, M. (2016). Influence of Interpersonal Communication Channel Against Decision of Agricultural Innovation Adoption of Bioindustry Integration of Seraiwangi-Ternak In West Java Province. *Journal of Agro Economics*, 34 (2): 135-144.
[13] Syafrudin. (2003). Influence Media Print Brochure In Process Adoption and Diffusion Innovation Breed Chicken Broiler in town of Kediri. Retrieved 24 July 2017. Available from: http://repository.usu.ac.id.
[14] Sasongko, W. A., Witjaksono R., & Harsoyo. (2014). The Influence of Communication Behavior Against Attitude and Adoption of Red Onion Cultivation Technology In Sandy Beach Area Sanden District Bantul Regency. *Journal of Agro Economics*, 24 (1): 35-43.
[15] Luluk, S., Rudi, C., Tarumingkeng., Bunasor, S., & Dadang. (2008). Knowledge, Attitudes and Action of Red Onion Farmers in the Use of Pesticides (Case Study in KabupatenNganjuk East Java Province). *Journal of Agroland*, 15 (01): 12-17.
[16] Kokolakis, S. (2015). Privacy attitudes and privacy behaviour: A review of current research on the privacy paradox phenomenon. Computers & Security. In Press, Corrected Proof, Available online 10 July.

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Productive and Reproductive Traits of Sheep Fed *Acacia saligna* Leaves-Based Diets

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Abstract— *Investigating effects of partial (50%) or total (100%) substitution of clover hay by tannins-rich plant (Acacia saligna) on productive and reproductive performance of ewe lambs was the main goal of this study. Two experiments were conducted: first focusing on digestibility and N balance using 9 Barky rams (live body weight, 43 ± 2.5 kg) where animals were randomly divided into 3 groups (n = 3); control (C), 50% Acacia ($AS_{50\%}$) and 100% Acacia ($AS_{100\%}$); second focusing on productive and reproductive performance of ewe-lambs (n=18) where animals were divided into three groups (n=6); C, $AS_{50\%}$ and $AS_{100\%}$. This experiment started 2 months before mating and continued till weaning. Dry matter intake decreased ($P<0.05$) linearly due with treatment. The digestion coefficients of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were lower ($P<0.05$) in treated than C. The nitrogen intake and urinary nitrogen were reduced ($P<0.05$) by treatment, while fecal nitrogen increased ($P<0.0\%$) with treatment. No change was found in conception rates among the three groups whereas fertility rates and lambing rates were higher in the treated compared to the control. $AS_{100\%}$ reduced ($P<0.05$) total protein and blood urea nitrogen (BUN) compared to other groups. No differences in progesterone concentration were found among groups. $AS_{50\%}$ resulted in higher ($P<0.05$) milk yield than other groups. Treatment decreased ($P<0.05$) milk fat percentage, whereas didn't change protein and lactose. Therefore, partial replacement of acacia leaves in sheep diets could be beneficial for productive and reproductive performance.*

Keywords— Feed additives, digestibility, rumen fermentation, blood metabolites, prolificacy.

I. INTRODUCTION

The major limitation to ruminant's production in many tropical regions is poor nutrition, which is characterized by low nitrogen and high fiber content in native grasses and crop residues. In Egypt, the primary constraints to livestock production are scarcity and fluctuating quantity and quality of the year-round feed supply. The gap between available and required amounts of animal feedstuffs in Egypt was estimated to be 3.5 million tons. There are two alternative strategies to overcome the problem of feeds shortage in Egypt. The first is to maximize utilization of agricultural and industrial byproducts and there are several studies focused on this point. The second strategy is seeking alternative nonconventional feed resources e.g. tannins-rich plants or shrub legumes, which have high protein contents and are potentially promising to overcome nutrient deficiencies.

The multipurpose trees and tannins-rich plants represent an important fodder reserve for ruminants in periods of feed scarcity and play vital role in bridging the wide gap between supply and demand of feeds **[1]**. Use of these forages in animal diets may participate in reducing the shortage of animal feed resources, enhance the fertility performance of animals and subsequently increase milk and meat production in tropic regions. The shrub foliages already play an important role in ruminant feeding systems in Mediterranean and many tropical environments around the world **[2- 5].**

To efficiently use alternative feed resources like tannins-rich plants, their effects on various aspects need to be considered. This needs more research to be done for fair judgment within different points of view. Therefore, the objectives of this study were to assess effects of the partial or total replacement of clover hay by acacia leaves as an alternative feed resource on the dry matter intake, nutrients utilization, ruminal fermentation profiles, reproductive and productive performance of Barky sheep.

II. MATERIAL AND METHODS

This study was carried out at the Nubaria Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Dokki, (Exp.1) and the Research Farm of the Department of Animal and Fish Production, Faculty of Agriculture, University of Alexandria (Exp. 2), Egypt. Two experiments were designed to investigate the potential replacement of clover (*Trifolium alexandrina*) hay by acacia (*Acacia saligna*, AS) leaves either partially (50%, $AS_{50\%}$) or totally (100%, $AS_{100\%}$). First experiment was designed to investigate digestibilty and nitrogen balance trial. However, the second experiment focused on the productive and reproductive performance of ewe-lambs.

Acacia samples collection

Acacia leaves were collected weekly during both experimental periods in order to offer as fresh materials for animals. Extra samples of acacia leaves were collected monthly during a year to analyze total phenols (TP), total tannins (TT) and condensed tannin (CT). The collection region was located at latitude of 30.91and longitude: 29.68. The region climate was semi-arid Mediterranean climate, which is characterized by a brief mild and rainy winter and long warm summer months with no rain. The chemical composition and tannins content of the commercial concentrate mixture, clover hay and acacia leaves for both experiments are presented in Table 1.

Table.1: Chemical composition and tannins content of the concentrate mixture, clover hay and Acacia leaves (Based on dry matter)

Item (%)	Concentrate	Clover hay	Acacia leaves
OM	88.5	89.3	89.6
CP	14.1	12.5	13.2
EE	5.8	1.0	4.8
NDF	38.3	64.2	64.9
ADF	10.6	55.8	60.6
Tannins content			
TP*	-	10.2	42.1
TT*	-	1.6	27.1
CT**	-	2.0	72.0

OM: Organic matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber. *TP: total phenols (eq-g tannic acid kg^{-1}DM), *TT: total tannins (eq-g tannic acid kg^{-1}DM), **CT: condensed tannins (eq-g leucocyanidin kg^{-1}DM).

Experiment 1

Animals and experimental design

Nine mature Barky rams (mean live body weight 43±2.5 kg) were randomly divided into three equal groups (n = 3) as control (C), $AS_{50\%}$ and $AS_{100\%}$ according to live body weight. Animals were housed in well-ventilated shade and adapted to the treatment feeds for 4 weeks before being subjected to the digestibility trial and had free access to fresh water. The animals were fed in groups on a commercial concentrate feed mixture and roughages according to NRC **[6].** The acacia leaves were offered fresh daily as partial or total replacement to the clover hay by 1.0 kg and 2.0 kg/h/d, respectively, while the clover hay was offered to the control and partial replacement group by 1.0 and 0.5kg/h/d, respectively. The commercial concentrate feed mixture was offered to all groups by 0.75 kg/h/d for all groups. After familiarization to the diets, the animals were kept in metabolic cages for 8 days of which 3 days for adaptation and 5 days for samples collection. Experimental animals were offered concentrate and roughages individually in buckets twice daily at 8:00 and 14:00 hr. During the collection period, animals were individually kept in metabolic cages and feed refusals, feces and urine were collected for the purpose of measuring nutrients digestibility and N balance.

To measure apparent total tract nutrients digestibility, two representative samples of feces 10 % of the total quantity) were collected daily from each animal; one of the two samples was sprayed with citric acid (10%) and stored under -20°C, the second was used for determination of the feces DM. Immediately, at the end of collection period stored feces samples during 5 days for each animal were pooled, mixed well and a sample was obtained for further analyses. Representative portions of feces were dried in a forced air-oven at 50°C for 48 h and then ground to pass through a 1 mm-screen and stored at -20°C thereafter until analysis.

The urine was collected daily throughout the collection period in plastic buckets containing 100 mL of H_2SO_4 (10%). Also, representative samples (10% of total volume) were collected daily from each animal and kept in dark bottles. At the end of collection period, urine samples from each animal were mixed well and obtained a sample, which kept under -20°C until analysis. Upon

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analysis, thawed urine samples were centrifuged at 2000 rpm for 20 min and sub-samples were analyzed for Kjeldahl N **[7]**. Ruminal fluid samples were obtained from each animal via a stomach tube before morning feeding for two consecutive days. The ruminal fluid samples were separated from the feed particles through four layers of cloth sheets and then stored under -20ºC for VFA and ammonia N analyses.

Experiment 2

Animals and experimental design

Eighteen ewe-lambs (live body weight of 35±2.5 kg and age of 10 months) were divided into three equal groups (n=6) according to the body weight; control (C), $AS_{50\%}$ and $AS_{100\%}$ to study the reproductive and productive performance. Ewe-lambs were fed on a commercial concentrate feed mixture, clover hay and acacia leaves according to NRC **[6].** The acacia leaves were collected and offered fresh daily. The Alexandria University guideline for the ethics and use of experimental animals was approved.

Reproductive performance of ewe-lambs

This study was started two months before mating and continued till lambing. The control ewe-lambs were fed on a commercial concentrates feed mixture and clover hay (1.0 kg/h/d). The $AS_{50\%}$ animals were fed a commercial concentrate feed mixture and clover hay (0.5 kg/h/d) and acacia leaves (1.0 kg/h/d). The $AS_{100\%}$ animals were fed a commercial concentrate feed mixture and acacia leaves (2.0 kg/h/d).

Ewe-lambs were fed the commercial concentrate mixture in groups, hay and acacia leaves in about two equal parts at 8:00 and 14:00 hr daily. The ewe-lambs had free access to fresh water through the experimental period. Blood samples were collected every month from the jugular vein, before access to feed and water in test tube without anti-coagulant. The samples were centrifuged at 3000 rpm for 20 min to get the serum for biochemical parameters assay. Total protein and albumin were assayed by colorimetric kits (Stanbio, Boerne, Texas, USA). Glucose was assayed using colorimetric kits (Futura System, Formello, Rome, Italy) and blood urea nitrogen (BUN), creatinine and cholesterol were assayed by colorimetric kits (BioSystems, Costa Brava, Barcelona, Spain). Globulin concentration was calculated as the difference between total protein and albumin.

After two months of the beginning of the experiment, all ewe-lambs were injected with prostaglandin $PGF_{2\alpha}$ (0.5 ml of estroPLAN with the concentration of 125 µg Cloprostenol; Parnell Technologies, Alexandria, New South Wales, Australia). The animals were submitted to estrus detection twice daily using mature teaser ram at 7.00 and 19.00 hr for 3 days, ewe-lambs which were mounted by teaser rams, were mated by fertile rams. The ewe-lambs which was insensitive to the effects of first $PGF_{2\alpha}$ received a second injection of $PGF_{2\alpha}$ (0.5 ml of estroPLAN with the concentration of 125 µg Cloprostenol) after 12 days from the first injection then submitted to estrus detection twice daily using mature teaser ram at 7.00 and 19.00 hr for 3 days. Ewe-lambs which were mounted by teaser rams were mated by fertile rams. A blood sample was taken from each ewe-lamb at the days of injection, estrus, 5, 10 and 20 after estrus for determination of BUN and progesterone.

Productive performance of ewes

Birth weight of the neonates were recorded at the day of birth and their weaning weights were recorded when lambs reached the age of 4 months. Daily weight gain of lambs was calculated using weekly body weight for each lamb.

Milk production was measured weekly postpartum and for seventeen weeks (119 day). Daily milk yield for each ewe was performed using weigh suckle-weigh technique **[8]**. Lambs were separated from their dams at 14:00 hr the day before milking and in the day after. The lambs were weighed at 6:00 hr and left to suckle their dams till satisfactions. They were weighed again and kept in closed pens till next milking at 14:00 hr. In the meantime their dams were striped to estimate the stripping milk. The same procedure was followed again at 14:00 hr in the same day. The daily milk yield was calculated by summing the weight of suckled milk (differences between lamb's weight before and after suckling) and the weight of striped milk in both morning and afternoon milking. Milk production was evaluated using a graduated cylinder (±5 mL). The amount of milk obtained was adjusted for 24 hr on weekly basis. Milk samples (100 mL) from individual ewes were taken for proximate analysis using Milk Analyzer (Milko Tester Instruments Inc., Bulgaria).

Milk urea nitrogen (MUN) was determined by adding 2 mL trichloroacetic acid (10%) to 5mL of milk sample for protein precipitation and left for 30 min then the mixture was centrifuged at 3000 rpm for 20 min in order to separate the supernatant which was used to determine milk urea nitrogen by colorimetric method (Stanbio, Boerne, Texas, USA) **[7].**

Blood samples were collected every month until the end of the experiment from the jugular vein, before access to feed and water in test tube without anti-coagulant to get coagulated blood samples. The samples were centrifuged at 3000 rpm for 20 min and sera were harvested for biochemical assays.

Samples analysis

The dry matter (DM) of feed and feces were analyzed by drying at 105°C for 24 h. The AOAC (2006) analytical procedures were used for the organic matter (OM) determination (No. 968.08) by ashing at 600 °C for 2 h, N estimation (No. 988.05) by a Kjeldahl technique and ether extract. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the procedure of Van Soest *et al.* **[9]** without sodium sulfite or α amylase and expressed with residual ash. Concentrations of NH_3-N and total volatile fatty acid (VFA) were determined according to Preston **[10]** and Warner **[11]**, respectively.

Total phenols (TP) were determined with the Folin-Ciocalteau reagent according to Makkar **[12]**. Total tannins (TT) were determined as the difference in TP before and after treatment with insoluble polyvinyl polypyrrolidone (PVPP) **[13].** Condensed tannins (CT) were measured by the HCl- butanol method according to Makkar (2003). TP and TT were expressed as tannic acid equivalents and condensed tannins were expressed as leucocyanidin equivalent **[12]**.

Statistical analysis

First experiment data were subjected to analysis of variance (ANOVA) using the general linear model procedure (GLM) of SAS software package **[14]**. The used model was: $Y_{ij}= \mu+ F_i + e_{ij}$ where: μ is the overall mean, F_i is the treatment effect, e_{ij} is the random error term. The significant differences between individual means were identified by using Tukey test.

Second experiment data were analyzed using GLM of SAS **[14]**. The model included effect of treatment, time, and their interaction on different variables that were tested in a repeated measurement design as follows: $Y_{ijk} = \mu + T_i + P_j + (TP)_{ij} + A_{kt} + e_{ijk}$; Where: μ is overall mean, Ti is a fixed effect of the treatment (i=1 to 2), Pj is a fixed effect of the time (j=1 to 8), TP_{ij} is an interaction between treatment and time, A_{kt} is random effect of the animal (within treatment) and e_{ijk} is random error assumed to be independent by and normally distributed with mean = 0 and variance = σ2. The reproductive traits results were compared among studied groups using Chi-square.

RESULTS

Experiment 1.

Data of the effect of partial (50%) and total (100%) replacements of clover hay by acacia leaves on dry matter intake (DMI) and apparent total tract nutrient digestibility of Barky sheep are shown in Table (2). There was a linear reduction (P<0.01) in DMI by increasing acacia leaves compared to the control. Percentages of reductions in DMI due to partial (50) and total (100%) replacement by acacia leaves were 9.2 and 28.5%, respectively compared to the control. The apparent total tract digestion coefficients of dry matter (DM), organic matter (OM), crude protein (CP), natural detergent fiber (NDF) and acid detergent fiber (ADF) were significantly lower (P<0.01) in treated than control animals.

Table.2: Effect of partial and total replacement of clover hay by acacia leaves on apparent total tract nutrients digestibility in Barky ewe lambs

Item	Control	Acacia		*P* Value
		$AS_{50\%}$	$AS_{100\%}$	
Intake (g/h/d)				
Clover hay	900.2±5.6	454.6±5.6	0	-
Acacia leaves	0	314.7±20.5	462.8±25.1	-
CFM	634.1±6.9	622.8±6.9	634.1±8.5	-
DMI g/d	1534.3±21.1^a	1392.2±21.1^b	1096. 9±25.9^c	<0.0001
Digestion coefficient (%)				
DM	72.2±1.3^a	67.0±1.3^b	57.3±1.6^c	<0.0001
OM	74.0±1.2^a	69.7±1.2^b	60.3±1.5^c	<0.0001
CP	68.9±1.6^a	55.7±1.6^b	38.0±2.0^c	<0.0001
NDF	73.0±1.5^a	65.8±1.5^b	52.6±1.9^c	<0.0001
ADF	68.8±2. 5^a	57.8±2. 5^b	35.5±3.0^c	<0.0001

Means in the same row with different superscripts significantly differ (*P*<0.05).
CFM : Concentrate feed mixture; DMI : Dry matter intake; DM : Dry matter; OM : Organic matter; CP :Crude protein; EE :Ether extract; CF :Crude fiber; NDF : Neutral detergent fiber; ADF : Acid detergent fiber.

Table (3) presents data of the effects of acacia leaves replacement on nitrogen utilization, rumen NH_3-N and VFA concentration of Barky sheep. There were linear (P<0.01) reductions in both nitrogen intake (NI) and urinary nitrogen due to replacement compared to control. In contrast, the fecal nitrogen increased (*P*<0.01) up to 48% with acacia replacement. Significant reductions (*P*<0.01) in nitrogen balance were found in animals given partial (26%) and total (79.9%) acacia leaves compared with control. There were no significant differences in the concentrations of VFA and ammonia due to acacia replacement.

Table.3: Effect of partial and total replacement of clover hay by acacia leaves on nitrogen utilization, rumen NH_3-N and VFA concentration in Barky sheep

Item, g/d	Control	Acacia		*P* Value
		$AS_{50\%}$	$AS_{100\%}$	
N intake	32.30 ± 0.26^{a}	29.83 ± 0.26^{b}	24.10 ± 0.26^{c}	<0.0001
Fecal N	10.01 ± 0.72^{b}	13.14 ± 0.72^{a}	14.85 ± 0.72^{a}	0.0086
Urinary N	9.90 ± 0.76^{a}	7.52 ± 0.76^{ab}	6.74 ± 0.76^{b}	0.0597
Nitrogen balance	12.39 ± 0.51^{a}	9.17 ± 0.51^{b}	2.51 ± 0.51^{c}	<0.0001
VFA (meq/dl)	8.68±0.75	8.52±0.75	7.13±0.75	0.3066
NH_3-N (mg/dl)	14.35±0.64	13.06±0.78	13.41±0.64	0.4131

Means in the same row with different superscripts significantly differ ($P<0.05$).
VFA: Volatile fatty acids

Experiment 2.

The chemical composition of the total phenols (TP), total tannins (TT) and condensed tannins (CT) of acacia leaves throughout the experimental period is presented in Figure 1.

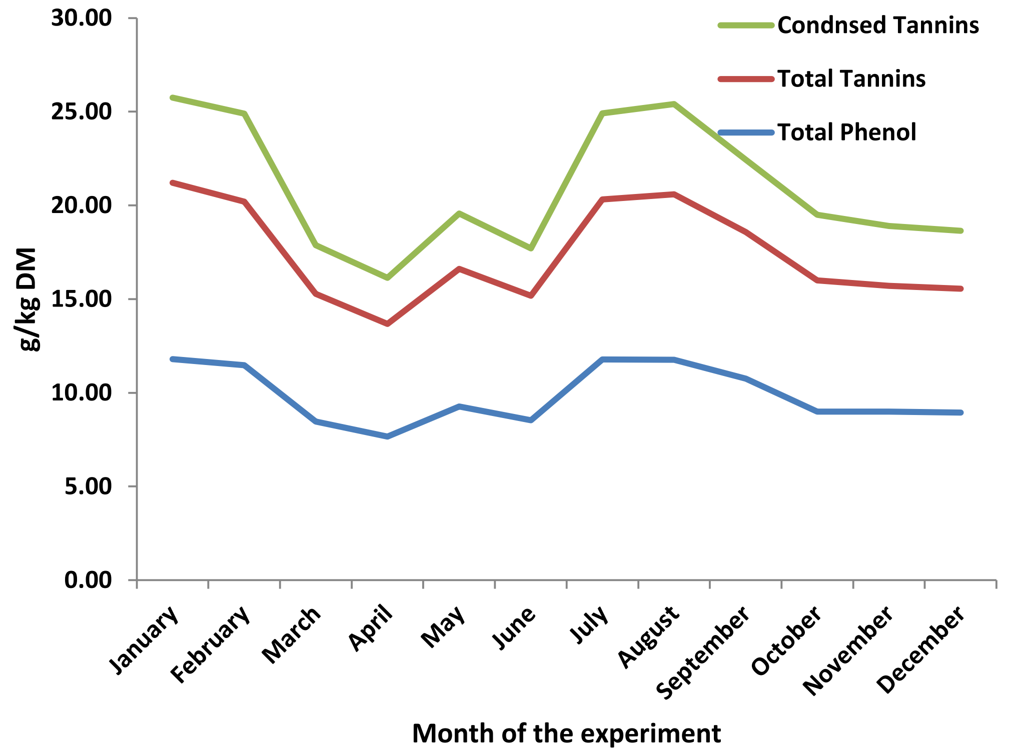

Fig.1: Total phenols, total tannins (eq-g tannic acid /kg DM) and condensed tannins (eq-g leucocyanidin /kg DM) throughout the experimental period

The phenolic compounds like TP, TT and CT concentrations exhibited similar trends throughout the entire experimental period. They tended to decrease gradually from February till June then increased slightly again to reach peaks in August and decreased thereafter till December. Mean values of TP, TT and CT in acacia leaves were 100.4 g tannic acid kg^{-1} DM, 76.8 g tannic acid kg^{-1}DM and 36.1 g leucocyanidin kg^{-1} DM, respectively.

Table 4 shows data of the effects of partial and total replacement of clover hay by acacia leaves on ewe's reproductive parameters. Results showed that number services per conception were similar (1.17) among groups. Likewise, conception rate have similar percentage among groups, while fertility rate and lambing rate were less in control than treated animals. The replacement of acacia leaves has no significant effect on litter size.

Table.4: Effect of partial and total replacement of clover hay by acacia leaves on some reproduction traits of Barky ewes

Item	Control	Acacia	
		$AS_{50\%}$	$AS_{100\%}$
Number services per conception	1.17	1.17	1.17
Conception rate, %	83.33 (5/6)	83.33(5/6)	83.33(5/6)
Fertility rate, %	66.66 (4/6)	83.33(5/6)	83.33(5/6)
lambing rate, %	66.66 (4/6)	83.33(5/6)	83.33(5/8)
litter size	1.0	1.0	1.0
Abortion rate, %	20	0	0

Effects of partial and total replacement of clover hay by acacia leaves on blood biochemical parameters in pregnant ewes are shown in Table (5). There were no significant changes in all serum biochemical parameters during premating period between control and treated animals. However, BUN declined ($P<0.05$) by 23% in the 100% acacia-treated ewes compared to control. While during pregnancy, serum total protein, globulin, glucose, and BUN concentrations significantly decreased ($P<0.05$) by the increase of acacia leaves compared to the control. Contrariwise, results revealed that partial and total replacements exhibited higher ($P<0.05$) concentration of serum albumin than the control. Serum cholesterol and creatinine were not affected during either stage by the inclusion of acacia leaves.

Data of the effects of partial and total acacia replacement on BUN concentration at $PGF_{2\alpha}$ injection and estrus and at 5, 10 and 15 days post estrus are presented in Table (6). The BUN was reduced ($P<0.05$) at day 10 after estrus, while there were no changes ($P>0.05$) in BUN concentrations at $PGF_{2\alpha}$ injection, estrus, 5 and 15 days after estrus due to partial and total replacement by acacia leaves.

Table.6: Effect of partial and total replacement of clover hay by acacia leaves on blood urea nitrogen (BUN) concentrations (mg/dl) at $PGF_{2\alpha}$ injection and estrus and at days 5, 10 and 20 post estrus in ewes

Item	Control	Acacia		
		$AS_{50\%}$	$AS_{100\%}$	*P* value
Day of $PGF_{2\alpha}$ injection	21.08±1.1	23.58±1.1	23.82±1.1	0.1895
Day of heat	19.25±1.68	18.80±1.68	15.56±1.84	0.3107
Day 5 after heat	20.80±1.52	20.75±1.39	17.03±1.39	0.1317
Day 10 after heat	20.20±1.59^{a}	22.45±1.45^{a}	15.48±1.59^{b}	0.0199
Day 20 after heat	21.22±1.79	22.48±1.79	22.00±1.79	0.8822

Means in the same row with different superscripts significantly differ ($P<0.05$).

Table 7 illustrates effects of partial and total replacement of clover hay by acacia leaves on progesterone concentration at $PGF_{2\alpha}$ injection, estrus, 5, 10 and 20 days after estrus. Results indicated that there were no significant differences ($P>0.05$) among groups. Typically, progesterone profile exhibited minimum values at the onset of heat and started to increase by time thereafter.

Table.7: Effect of partial and total replacement of clover hay by acacia leaves on progesterone concentration (ng/ml) in Barky ewes

Item	Control	Acacia		*P* value
		$AS_{50\%}$	$AS_{100\%}$	
Day of $PGF_{2\alpha}$ injection	4.90±0.68	3.90±0.68	4.60±0.68	0.5785
Day of heat	1.28±0.56	1.97±0.65	1.03±0.56	0.5598
Day 5 after heat	2.16±0.57	1.90±0.57	2.60±0.57	0.6888
Day 10 after heat	4.50±0.29	4.38±0.29	4.74±0.32	0.7094
Day 20 after heat	5.78±0.69	4.10±0.69	5.68±0.62	0.1959

Means in the same row with different superscripts significantly differ ($P<0.05$).
AS: *Acacia saligna*

Effects of partial and total replacement of clover hay by acacia leaves on milk yield, milk composition and milk urea nitrogen (MUN) in lactating ewes are presented in Table 8. The results revealed that there is a linear reduction in DMI with increasing proportions of acacia leaves in ewe's diets. The $AS_{50\%}$ group produced higher (P<0.05) milk yield than control and $AS_{100\%}$. The improvement due to partial substitution of acacia leaves was 11.8%, while the total substitution of acacia leaves decreased milk yield by 15.3% compared to the control. Both $AS_{50\%}$ and $AS_{100\%}$ decreased (P<0.05) milk fat percentage compared to control, while milk protein, lactose, SNF and MUN were not affected (P>0.10) by acacia replacement compared to control.

Table.8: Effect of partial and total replacement of clover hay by acacia leaves on Dry matter intake, milk yield, milk composition and milk urea N (MUN) in lactating Barky ewes

Item	Control	Acacia		*P* value		
		$AS_{50\%}$	$AS_{100\%}$	Treatment	Week	T×W
DMI g/d	1750	1685	1620	-	-	-
Milk yield g/d	509.9±22.0^{b}	570.1±19.7^{a}	431.7±22.0^{c}	<0.0001	<0.0001	0.9893
Fat %	5.47±0.22^{a}	4.77±0.19^{b}	4.46±0.22^{b}	0.0033	<0.0001	0.7655
Protein %	3.88±0.07	3.85±0.06	4.01±0.07	0.2269	0.2671	0.1262
SNF %	10.70±0.19	10.71±0.18	11.06±0.19	0.3673	0.2772	0.1397
Lactose %	5.83±0.10	5.81±0.10	6.02±0.10	0.2907	0.3160	0.1106
MUN mg/dl	20.19±0.95	18.50±0.82	19.07±0.93	0.3768	0.1999	0.8854

Means in the same row with different superscripts significantly differ (P<0.05).
DMI: Dry Matter Intake, SNF: Solid Not Fat, MUN: Milk Urea Nitrogen.

Data of the effects of partial and total replacement of clover hay by acacia leaves on blood biochemical parameters in the lactating Barky ewes are shown in Table 9. Serum total protein and globulins concentrations declined (P<0.05) due to partial substitution of acacia leaves ($AS_{50\%}$) but not in the $AS_{100\%}$ ewes compared to the control. There were no significant differences on serum albumin, glucose and creatinine concentrations due to the acacia leaves substitution at either level. Contrariwise, serum cholesterol concentration was higher (P<0.05) in the $AS_{100\%}$ than in C and $AS_{50\%}$ ewes. BUN concentrations were similar in C and $AS_{50\%}$ (23.87 *vs.* 22.59 mg/dl) being greater (P<0.05) than that in $AS_{100\%}$ (20.39 mg/dl) ewes.

Table.9: Effect of partial and total replacement of clover hay by acacia leaves on blood biochemical parameters in lactating Barky ewes

Item	Acacia			*P* value		
	Control	$AS_{50\%}$	$AS_{100\%}$	G	T	T x G
Total protein, (g/dl)	6.69±0.18^{a}	5.95±0.16^{b}	6.39±0.18^{ab}	0.013	0.031	0.938
Albumin (g/dl)	4.10±0.07	3.95±0.07	4.01±0.07	0.298	<0.0001	0.443
Globulin (g/dl)	2.59±0.17^{a}	1.99±0.16^{b}	2.38±0.17^{ab}	0.043	0.002	0.985
Glucose (mg/dl)	45.87±1.76	44.35±1.64	42.17±1.88	0.300	<0.0001	0.0100
Creatinine (mg/dl)	0.99±0.05	1.03±0.04	1.01±0.05	0.830	0.051	0.275
Cholesterol (mg/dl)	63.19±2.47^{ab}	56.67±2.30^{b}	66.16±2.47^{a}	0.029	0.011	0.006
BUN (mg/dl)	23.87±0.81^{a}	22.59±0.73^{a}	20.39±0.79^{b}	0.014	<0.0001	0.089

Means in the same row with different superscripts significantly differ (P<0.05).
AS: *Acacia saligna*; BUN: Blood Urea Nitrogen

Table 10 presents data of the effects of acacia replacement on birth weight, weaning weight and average daily gain of Barky lambs born of treated ewes. The highest birth weight was found in control-ewe's lambs (3.85 kg) which was comparable to those lambs born of $AS_{50\%}$ (3.09 kg) ewes. However, $AS_{100\%}$ ewe's lambs recorded the lowest (P<0.05) birth weight (2.97 kg). Similar trend was found with weaning weight as the lowest weaning weight was found on lambs born of $AS_{100\%}$ ewes (15.59 kg) representing 22.7% weight loss, however $AS_{50\%}$ - ewe's lambs had less weaning weight by 14.7% compared to C-ewe's lambs. Although the partial replacement of acacia leaves decreased the weaning weight by 14.7% but the difference was not statistically

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significant. Average daily gain of lambs decreased by 13.6 and 22.7% in $AS_{50\%}$ and $AS_{100\%}$, respectively, with non-significant difference between C and $AS_{50\%}$.

Table.10: Effect of partial and total replacement of clover hay by acacia leaves on birth weight, weaning weight and average daily gain (ADG) of Barky lambs

Item	Control	Acacia		
		$AS_{50\%}$	$AS_{100\%}$	P value
Birth weight, kg	3.85±0.26[a]	3.09±0.20[ab]	2.97±0.26[b]	0.043
Weaning weight, kg	20.17±1.21[a]	17.20±0.94[ab]	15.59±1.21[b]	0.033
ADG, g/d	145.77±9.19[a]	125.92±7.12[ab]	112.67±9.19[b]	0.042

Means in the same row with different superscripts significantly differ (P<0.05).
ADG: Average Daily Gain. Means in the same row with different superscripts significantly differ (P<0.05).

III. DISCUSSION

Feed intake and digestion

The reduction in DMI of sheep due to partial or total substitution of clover hay by acacia leaves hay might be due to astringency, decreased palatability, which possibly resulting in feeds avoidance and adverse effects on digestion as reported previously **[12, 15].** Moreover, Waghorn *et al.* **[16]** demonstrated that decreased ruminal turnover and rate of digestion were more important than palatability in reducing intake of sheep fed diets containing high levels of CT. Other researchers suggested that high concentrations of tannins could reduce the intake in the following ways: (1) physical distension of the rumen, resulting in a decrease in dry matter digestion; (2) hormonal response due to the binding of tannins to the gut wall; (3) reduction of the diet palatability caused by its astringency; and/or (4) binding of tannins to salivary and mucosal proteins **[17, 18].**

The depression in apparent total tract digestibility of CP associated with replacement of clover hay by acacia leaves might have been due to the formation of complexes between tannins and dietary proteins and carbohydrates **[19]**, as well as reducing rumen microbial proteolytic, ureolytic and cellulolytic enzyme activities, general fermentative activities and cell multiplication **[20]**. Tannins might also interfere with digestion by binding microbial enzymes and this might explain why acacia-given animals in this study decreased apparent total tract digestibility of cell walls digestion **[21]**. Animals given acacia in the current study tended to decrease N balance **[22]**. This is probably due to the presence of CT, high proportion of acid detergent insoluble nitrogen and high urinary N, which in turn was possibly attributed to an imbalance of high N relative to a low energy in the rumen. The interaction of tannins with protein alters the partitioning of N within the sheep guts, shifting the route of excretion away from urine toward feces **[3, 23]**. This reduction in urinary N reduces volatile N losses after land application with dairy manure, which in turn reduces environmental losses through nitrate leaching, NH_3 volatilization and nitrous oxide emissions **[24]**. Confirming these effects of CT-containing forages, Powell *et al.* reported that the ratio of N excreted in feces and urine was highest for low-tannin and high-tannin birds' foot trefoil treatment and lowest for the alfalfa treatment **[25]**. Current study data revealed that the higher fecal N excretion in the presence of active tannins was matched by lower urinary N excretion. This shift can be explained by the lower rumen degradability of nitrogenous compounds and is confirmed by effects of CT on rumen fluid ammonia and total N as previously stated **[26, 27]**. Volatile fatty acids production was not affected by acacia leaves replacement. The possible explanation for this might be due to lack of effect of supplemented tannins on rumen bacteria or to the adaptation of rumen microorganisms to tannins **[28]**.

Reproductive performance

Conception rate was similar among treatments; however fertility and lambing rates were higher by about 25% when acacia leaves were replaced in ewe's diets. These effects probably occur as a result of including tannins-rich plants in ewe's diets, which enhance the live body weight, body condition, and energy and protein intake and protein absorption from the small intestine **[29]**. Moreover, increased plasma of essential amino acids principally branched chain amino acids and plasma metabolic hormones especially insulin were found in tannin-fed animals **[30]**. Short periods of improved nutrient supply before and during mating and reproduction have been known to affect ovulation rate along with increased size and number of follicle **[31]**, reduce follicular atresia **[32]**, altered plasma gonadotrophin concentration **[33]** and affect ovarian sensitivity to gonadotrophins **[32]**. A large part of the dietary protein is hydrolyzed in the rumen to ammonia, some of which is re-incorporated into microbial protein. Excess ammonia is absorbed from the rumen and metabolized to urea in the liver, leading to increased plasma and uterine ammonia and urea concentrations **[29]** which may increase the number of early embryonic losses **[34]**. In agreement with the present finding, subsequent grazing experiments with sheep showed that CT in *L. corniculatus* increased both ovulation rate and lambing percentage by 20-27% **[29]**. The improvement of the conception rate in the current study might be ascribed to that BUN level was less than 20 mg/dL at the insemination day. The results of the BUN is confirmed by Butler who indicated that conception rate decreased when serum urea nitrogen concentrations exceeded 20 mg/dL on the day of insemination and suggested that degradation of excessive amounts of dietary protein in the rumen contributed to infertility **[35]**.

Milk production and composition

Effects of tannins-rich feeds on milk fat and protein composition varies markedly depending on the concentration of tannins present in the feeds. Condensed tannins in high concentrations (e.g. total replacement by acacia) generally have adverse effects on animal performance, while moderate concentrations (e.g. partial replacement by acacia) might have positive effects. The negative effects of tannin-diets on milk yield and fat content might be due the reduction of feed intake, decreased rate of digestion and development of conditioned aversion **[36, 37]**. Wang *et al.* reported that tannins from *Lotus corniculatus*, which contained moderate amounts of CT (44.5 g/kg DM), fed to lactating ewes increased milk yield, lactose and protein content and decreased milk fat as found in the present study**[38]**. One reason for these effects could be an increase in metabolizable protein supply from the protein binding action of CT because effects of tannins on ruminant productivity depend on the quality and quantity of dietary protein **[28]**. Protein protection by tannins from microbial degradation in the rumen resulted in an increase of milk production in dairy cows **[39]**, dairy goats **[40]** and sheep **[41]**. Contrariwise, reported no improvement in ewe's milk yield when acacia leaves were supplemented into their diets at 100 or 200g/day **[42]**.

Parallel to our finding, Maamouri *et al.* indicated that milk protein content hasn't been affected significantly in acacia-treated ewes **[42]**. On the contrary, these results disagree with those of Wang *et al.* for dairy sheep **[38]** and Woodward *et al.* (1999) for dairy cows **[39]** who found increased milk protein content when tannin was supplemented to the diet. The CT-containing plants can protect dietary protein against degradation in the rumen and increase N utilization, resulting in a reduction in MUN concentration and nitrogenous waste excretion and improved nutritional status of the animal. However, Ben Salem *et al.* found that supplementing lambs fed on spineless cactus pads with small amount (i.e. 100 g/day) of air-dried acacia and 200 g soybean meal significantly increased their growth rate compared to those receiving the same diet but without acacia leaves (102 g/day *versus* 75 g/day) **[44].** When the level of acacia was doubled, the beneficial effect of acacia disappeared since the average daily gain of lambs was 82 g/day. These findings raised the question as to whether the positive effect of 100 g acacia is because of the interaction between acacia tannins and soybean meal protein.

Blood metabolites

Plant secondary compounds may affect blood parameters by maintaining them **[45]**, while others may decrease **[46]** or increase **[47]** plasma glucose concentration, or alter serum insulin concentration **[48]**. In agreement with our finding, Waghorn *et al.* and Ben Salem *et al.* reported that BUN was lower when sheep and goats were fed legumes that contained tannins **[44, 49].** However, reduced proteolysis in ewes receiving *Acacia cyanophylla* with concentrate could have been caused by effects of acacia tannins on microbial proteolytic activity **[50]**. Additionally, others demonstrated that that BUN was also higher in the Bermuda grass hay-based ration compared with the *Lespedeza cuneata* forage (23.1 mg CT/mg soluble protein) in goats **[51, 52].**

Solaiman *et al.* reported that BUN, albumin, creatinine, triglycerides and glucose were unaffected in the diets of goats consuming different levels of the CT containing forage of sericea lespedeza (*Lespedeza cuneata*) **[53]**, however our data indicated elevation (P<0.05) of serum albumin due to partial substitution of clover hay by acacia leaves, while total substitution decreased (P<0.05) serum albumin. It is well known that serum albumin is a bio-indicator of the nutritional status of the animal and it decreases when animals are diseased. The glucose values ranged from 61.2 to 65.6 mg/dL in the current study, which were similar to previous studies **[51, 53].**

Silanikove *et al.* demonstrated that the blood metabolic profile was examined in non-lactating and non-pregnant goats consuming *Quercus calliprinos* (oak), *Pistacia lentiscus* (pistacia) and *Cerafonia siliqua* (carob) leaves **[54].** Overall view of the data of this study revealed that the controversy in the literature with the current study implying the ability of tannins to modulate the rumen fermentation, nutrients utilization and performance efficiency of ruminants is probably due to the great diversity in the structural features, ruminants species, and consequently in the reactivity of these plant secondary compounds. The dose-dependent effect of tannins and proportions of rumen degradable protein are another major issues because of the difficulty in selecting concentrations to positively affect a particular parameter without conferring a negative response on others (e.g., in overall diet utilization).

IV. CONCLUSION

Partial (up to 50% of the forage) replacement of clover hay by acacia leaves could be implemented in sheep feeding without detrimental effects on the utilization of nutrients, fermentation characteristics, reproductive and productive performance.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

[1] Makkar, H.P.S. (2005). Use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage: Achievements, result implications, and future research. Anim. Feed Sci. Technol. 122, 3-12.

[2] Wang, Y., Berg, B.P., Barbieri, L.R., Veira, D.M., McAllister, T.A. (2006). Comparison of alfalfa and mixed alfalfa-sainfoin pastures for grazing cattle: Effects on incidence of bloat, ruminal fermentation, and feed intake. Can. J. Anim. Sci. 86, 383-392.

[3] Waghorn, G.C. (2008). Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. Anim. Feed Sci. Technol. 147, 116-139.

[4] Kumara Mahipala, M.B.P., Krebs, G.L., McCafferty, P., Gunaratne, L.H.P. (2009). Chemical composition, biological effects of tannin and in vitro nutritive value of selected browse species grown in the West Australian Mediterranean environment. Anim. Feed Sci. Technol. 153, 203-215.

[5] Bunglavan, S.J., Dutta, N. (2013). Use of tannins as organic protectants of proteins in digestion of ruminants. J. Livest. Sci. 4, 67-77.

[6] NRC (2007). Nutrient Requirements of Domestic Animals: nutrient requirements of sheep. National Academy of Science, Washington, DC.

[7] AOAC (2006). Official Methods of Analysis, Arlington, VA, USA.

[8] Williams, J., Anderson, D.C., Kress, D.D. (1979). Milk production in Herford cattle. I. Effects of separation internal on weigh-suckle weigh milk production estimates. J. Anim. Sci. 49, 1438-1442.

[9] Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

[10] Preston, T.R. (1995). Biological and chemical analytical methods. In: T.R. Preston (Ed.) Tropical Animal Feeding: A Manual for Research Workers. FAO, Rome, pp:191-264 (Chap. 9).

[11] Warner, A.C.I. (1964). Production of volatile fatty acids in the rumen. Methods of measurements. Nutr. Abs. Rev. 34, 339.

[12] Makkar, H.P.S. (2003). Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rum. Res. 49, 241-256.

[13] Makkar, H.P.S., Blummel, M., Borowy, N.K., Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61, 161-165.

[14] SAS (2002). Statistical Analysis System, Version 9.1. SAS Institute, Cary, NC, USA.

[15] Hervás, G., Frutos, P., Giráldez, F.J., Mantecón, A.R., Álvarez del Pino, M.C. (2003). Effect of different doses of quebracho tannins extract on rumen fermentation in ewes. *Anim.* Feed Sci. Technol. 109, 65-78.

[16] Waghorn, G.C., Shelton, I.D., McNabb, W.C., McCutcheon, S.N. (1994a). Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. J. Agric. Sci. (Camb.) 123, 109-119.

[17] Kumar, R., Vaithiyanathas, S. (1990). Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30, 21-38.

[18] Mueller-Harvey, I. (2006). Unraveling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010-2037.

[19] Makkar, H.P.S., Singh, B., Dawra, R.K. (1988) Effect of tannin-rich oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. Brit. J. Nutr. 60, 287-296.

[20] Muhammed, S., Stewart, C.S., Acamovic, T. (1994) Effects of tannic acid on cellulose degradation, adhesion and enzymatic activity of rumen microorganisms. Proc. Soc. Nutr. Physiol. 3, 25-30.

[21] McSweeney, C.S., Palmer, B., McNeill, D.M., Krause, D.O. (2001). Microbial interactions with tannins nutritional consequences for ruminants. Anim. Feed Sci. Technol. 91, 83-93.

[22] Degen, A.A., Blanke, A., Becker, K., Kaman, M., Benjamin, R.W., Makkar, H.P.S. (1997). The nutritive value of *Acacia saligna* and *Acacia Salicinea* for goats and sheep. J. Anim. Sci. 64, 253-259.

[23] Beauchemin, K.A., Kreuzer, M., O'Mara, F., McAllister, T.A. (2008). Nutritional management for enteric methane abatement: a review. Aust. J. Exp. Agric. 48, 21-27.

[24] Misselbrook, T.H., Powell, J.M., Broderick, G.A., Grabber, J.H. (2005). Dietary manipulation in dairy cattle: Laboratory experiments to assess the influence on ammonia emissions. J. Dairy Sci. 88, 1765-1777.

[25] Powell, J.M., Broderick, G.A., Grabber, J.H. and Hymes-Fecht, U.C. (2009) Technical note: Effects of forage protein-binding polyphenols on chemistry of dairy excreta. J. Dairy Sci. 92, 1765-1769.

[26] Scharenberg, A., Arrigo, Y., Gutzwiller, A., Soliva, C.R., Wyss, U. Kreuzer, M., Dohme, F. (2007). Palatability in sheep and *in vitro* nutritional value of dried and ensiled sainfoin (*Onobrychis viciifolia*) birdsfoot trefoil (*Lotus corniculatus*), and chicory (*Cichorium intybus*). Arch. Anim. Nutr. 61, 481-496.

[27] Theodoridou, K., Aufrère, J., Andueza, D., Pourrat, J., Le Morvan, A., Stringano, E., Mueller-Harvey, I., Baumont, R. (2010). Effects of condensed tannins in fresh sainfoin (*Onobrychis viciifolia*) on *in vivo* and *in situ* digestion in sheep. Anim. Feed Sci. Technol. 160, 23-38.

[28] Patra, A.K., Saxena, J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. J. Sci. Food Agric. 91, 24-37.

[29] Min, B.R., Attwood, G.T., McNabb, W.C., Barry, T.N. (2001). Effect of condensed tannins on proteolytic bacterial populations in the rumen and on nitrogen flow to the abomasum of sheep. J. Anim. Sci. 79, 163 (Abstr.).

[30] Downing, J.A., Joss, J., Scaramuzzi, R.J. (1995). A mixture of the branched chain amino acids leucine, isoleucine and valine increases ovulation rate in ewes when infused during the late luteal phase of the estrus cycle: an effect that may be mediated by insulin. J. Endocrinol. 145, 315-323.

[31] Bellows, R.A., Pope, A.L., Meyer, R.K., Chapman, A.B., Casida, L.E. (1963). Physiological mechanisms in nutritionally induced differences in ovarian activity of mature ewes. J. Anim. Sci. 22, 93-108.

[32] Downing, J.A., Scaramuzzi, R.J. (1991). Nutrient effects on ovulation rate, ovarian function and the secretion of gonadotrophic and metabolic hormones. J. Reprod. Fert. 43, 209-227.

[33] Smith, J.F. (1988). Influence of nutrition on ovulation rate in the ewe. Aust. J. Biol. Sci. 41, 27-36.

[34] El-Zarkouny, S.Z., Ghobashy, H., Nour El-Din, A.N.M., Abdel-Gauad, E.I., Hassan, G.A. (2007). Lambing and embryonic mortality rates in Awassi and Barky ewes and their crosses throughout three different mating seasons in the west coastal region of Egypt. Alex. J. Agric. Res. 52, 15-24.

[35] Butler, W.R. (1998). Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci. 81, 2533-2539.

[36] Grainger, C., Clarke, T., Auldist, M.J., Beauchemin, K.A., McGinn, S.M., Waghorn, G.C. (2009). Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. J. Anim. Sci. (Camb.) 89, 241-251.

[37] Molle, G., Decandia, M., Giovanetti, V., Cabiddu, A., Fois, N., Sitzia, M. (2009). Responses to condensed tannins of flowering sulla (*Hedysarum coronarium L.*) grazed by dairy sheep. Part 1: Effects on feeding behavior, intake, diet digestibility and performance. Livest. Sci. 123, 138-146.

[38] Wang, Y., Douglas, G.B., Waghorn, G.C., Barry, T.N., Foote, A.G. (1996). The effect of condensed tannins in *Lotus corniculatus* upon lactation performance in ewes. J. Agric. Sci. (Camb.) 126, 353-362.

[39] Woodward, S.L., Auidist, M.J., Laboyrie, P.J., Jansen, E.B.L. (1999). Effect of *Lotus corniculatus* and condensed tannin on milk yield and milk composition of dairy cows. Proc. N.Z. Soc. Anim. Prod. 59, 152-155.

[40] Rouissi, H., Atti, N., Mahouachi, M., Rekik, B. (2006). Effect de la complementation azotée sur les performances zootechniques de la chèvre locale. Trop. Culture. 24, 111-114.

[41] Penning, P.D., Orr, R. J., Treacher, T.T. (1988). Responses of lactating ewes offered fresh herbage indoors and when grazing to supplements containing differing protein concentrations. Anim. Prod. 46, 403-415.

[42] Maamouri, O., Atti, N., Kraiem, K., Mahouachi, M. (2011). Effects of concentrate and *Acacia cyanophylla* foliage supplementation on nitrogen balance and milk production of grazing ewes. Livest. Sci. 139, 264-270.

[43] Ben Salem, H., Atti, N., Priolo, A., Efzaoui, A. N. (2002). Polyethylene glycol in concentrate or feed blocks to deactivate condensed tannins in *Acacia cyanophylla* Lindl. Foliage. 1-Effects on intake, digestion and growth by Barbarine lambs. J. Anim. Sci. 75, 127-135.

[44] Ben Salem, H., Makkar, H.P.S., Nefzaoui, A., Hassayoun, L., Abidi, S. (2005). Benefit from the

association of small amounts of tannin-rich shrub foliage (*Acacia cyanophylla* Lindl.) with soya bean meal given as supplements to Barbarine sheep fed on oaten hay. Anim. Feed Sci. Technol. 122, 173-186.

[45] Raghuvansi, S. K., Prasad, R., Mishra, A.S., Chaturvedi, O.H., Tripathi, M.K., Misra, A.K., Saraswat, B.L., Jakhmola, R.C. (2007). Effect of inclusion of tree leaves in feed on nutrient utilization and rumen fermentation in sheep. Bioresour. Technol. 98, 511-517.

[46] Joy, P.P., Thomas, J., Mathew, S., Skaria, B.P. (2001). Medicinal plants. In: Bose, T.K., Kabir, J., Das, P., Joy, P.P. (Eds.), Tropical Horticulture. Naya Prokash, Calcutta, India, pp. 449-632.

[47] Mohammed, N., Ajisaka, N., Lila, Z.A., Hara, K., Mikuni, K., Kanda, S. Itabashi, H. (2004). Effect of Japanese horseradish oil on methane production and ruminal fermentation *in vitro* and in steers. J. Anim. Sci. 82, 1839-1846.

[48] Devant, M., Anglada, A., Bach, A. (2007). Effects of plant extract supplementation on rumen fermentation and metabolism in young Holstein bulls consuming high levels of concentrate. Anim. Feed Sci. Technol. 137, 46-57.

[49] Waghorn, G.C., Shelton, I.D., McNabb, W.C. (1994b). Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 1. Non-nitrogenous aspects. J. Agric. Sci. 123, 99-107.

[50] Jones, G.A., Jakober, K.D., Bae, H.D., McAllister, T.A., Cheng, K.J. (1993). Some interactions between condensed tannins of forage legumes, bovine serum albumin and five strains of proteolytic rumen bacteria. Proceedings of the World Conference on Animal Production, Edmonton, Canada 36, 68-69.

[51] Turner, K.E., Wildeus, S., Collins, J.R. (2005). Intake, performance, and blood parameters in young goats offered high forage diets of lespedeza or alfalfa hay. Small Rum. Res. 59, 15-23.

[52] Moore, D.A., Terrill, T.H., Kouakou, B., Shaik, S.A., Mosjidis, J.A., Miller, J.E., Vanguru, M. Kannan, G., Burke, J.M. (2008). The effects of feeding *Sericea lespedeza* hay on growth rate of goats naturally infected with gastrointestinal nematodes. J. Anim. Sci. 86, 2328-2337.

[53] Solaiman, S., Thomas, J., Dupre, Y., Min, B.R., Gurung, N., Terrill, T.H., Haenlein, G.F.W. (2010). Effect of feeding sericea lespedeza (*Lespedeza cuneata*) on growth performance, blood metabolites, and carcass characteristics of Kiko crossbred male kids. Small Rum. Res. 93, 149-156.

[54] Silanikove, N., Gilboa, N., Perevolotsky, A., Nitsan, Z. (1996). Goats fed tannin-containing leaves do not exhibit toxic syndromes. Small Rum. Res. 21, 195-201.

Table.5: Effect of partial and total replacement of clover hay by acacia leaves on blood biochemical parameters in the premating and pregnant Barky ewe-lambs

Item	Premating period			Pregnancy period			*P* value		
	Control	$AS_{50\%}$	$AS_{100\%}$	Control	$AS_{50\%}$	$AS_{100\%}$	G	T	T xG
Total protein (g/dl)	8.56±0.32	8.29±0.34	8.23±0.32	7.70±0.16^{A}	7.13±0.16^{B}	6.97±0.18^{B}	0.000	0.004	0.509
Albumin (g/dl)	4.08±0.13	4.25±0.14	4.12±0.13	4.32±0.05^{B}	4.58±0.05^{A}	4.43±0.05^{A}	0.002	0.002	0.001
Globulin(g/dl)	4.50±0.34	4.05±0.35	4.12±0.34	3.38±0.17^{A}	2.54±0.17^{B}	2.56±0.19^{B}	0.0006	<0.001	0.524
Glucose(mg/dl)	55.59±4.15	61.96±4.35	61.38±4.15	68.91±2.86^{A}	57.01±2.86^{B}	62.45±3.32^{B}	0.020	0.001	0.089
Creatinine (mg/dl)	0.98±0.06	0.89±0.06	1.03±0.6	1.03±0.04	0.99±0.04	0.96±0.05	0.604	0.001	0.311
Cholesterol (mg/dl)	34.93±2.57	37.03±2.69	41.61±2.57	47.81±1.77	47.12±1.77	48.36±2.05	0.937	<0.001	0.032
BUN (mg/dl)	26.10±1.31^{A}	22.68±1.38^{AB}	19.99±1.31^{B}	20.69±0.65^{A}	20.13±0.65^{A}	13.13±0.75^{B}	<0.0001	<0.001	0.035

AS: *Acacia saligna;* BUN: Blood Urea Nitrogen.

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Treatment of Tannery Wastewater to Remove Hazardous Pollutants by Scoria (Volcanic ash) a Low cost Adsorbent

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Abstract — *In present study Scoria, a volcanic ash identified as potentially efficient low-cost and locally available filter media as an adsorbent for the treatment of tannery wastewater and investigated as a filter media for tannery wastewater treatment with selected physicochemical parameters. This study had been conducted in Addis Ababa University from May to August 2016.The volcanic rocks were collected from volcanic cones of refit valley area of Oromia region, Ethiopia and their chemical characteristics were determined using XRF analysis. Batch mode comparative experimental study design has been carried out. The filter media was crushed and graded and effective size was determined by using standard sieve. The composite sample of wastewater was collected from Dire tannery, Addis Ababa Ethiopia. The physicochemical analysis of wastewater samples has been done before and after 24, 48, and 72 hours treatment using standard methods. Mean and standard deviations were calculated for each parameter. R statistical software was run for data analysis. Based on this investigation, characterization of the untreated tannery wastewater revealed that the mean concentration of* BOD_5, *COD, TSS or orthophosphate, ammonium, nitrite, nitrate, sulfide, sulfate and chromium were 1081±159.55, 12913±6874.7, 2426±515.2, 168±74, 314±59.9, 1.7±0.29, 124±12.8, 417±130.7, 1307±224 and 35.7±8.6 mg/l respectively. Nitrate removal efficiency of scoria was 99% at RT= 72 hours. However phosphate removal was better by scoria on the first 24 and 48 hours but then again at RT=72hours scoria removes 63% only. The efficiency of scoria shows from 75-77%. In the first 24 and 48 hours retention time scoria achieved 71% chromium reduction. Considering all the selected tannery wastewater parameters for this study, the average treatment efficiency of scoriawas58.8% and 63.4% at RT=24 hours, and 67.5% at RT= 48 hours respectively and equivalent result (68.3%efficiency)was obtained after 72 hours. The results shows scoria substrates has a potential to treat tannery wastewater.*

Keywords— Tannery Wastewater, Scoria, Filter Media, Removal efficiency, Wastewater parameters.

INTRODUCTION

1.1 Introduction: Tanning is one of the oldest industries in the world Tannery effluent is among one of the most hazardous pollutants of industry. Major problems caused by tannery wastewater containing heavy metals, nutrients, toxic chemicals, chloride, lime with high dissolved and suspended salts, and other pollutants.

With the growth of population, the increasing requirement of leather and its products led to the establishment of large commercial tanneries. Tanneries are typically characterized as pollution intensive industrial complexes which generate widely varying, high-strength wastewaters. Nearly 30 m^3 of wastewater is generated during processing of one tone of raw skin/hide [1].

Tannery operation consists of converting of the raw hide or skin into leather which consume huge amount of water in several stages, generating an enormous amount of liquid effluents which are hazardous to the environment to which they are discharged, consequently, make it as a potentially pollution intensive industry. Tannery effluents again compromise the physical, chemical and biological properties of aquatic environment. Apart from the most toxic heavy metals like Chromium (Cr) chemical impurities of tannery effluents mostly includes the following dissolved substances such as inorganic salt cations (Fe, Zn, Cu, Ca, Na, etc.); anions such as SO_4^{2}, NO_3^- , PO_4^{3-} and parameters such as, BOD, COD, TSS, TDS etc.[2]. Therefore treating tannery wastewater using natural adsorbents is very important to protect the surrounding environment.

In Ethiopia Currently, there are more than 30 tannery industries in operation. Among them the majority found in Oromia region especially Mojo town and around six established in the capital city Addis Ababa. These tanneries have 153,650 sheep and goat skin soaking capacity and 9,725 cowhides soaking capacity per day. Together they also employ 4577 persons [3].

The total wastewater discharge estimation from tanneries is about 400 million m^3/year. About90% of world leather production uses chrome-tanning processes rather than vegetable tanning [4]. In Chrome tanning process tanneries utilize chromium in the form of basic chromium-sulphate for hide stabilization against microbial degradation and provision of flexibility of the leather. In chrome tanning process about 60% - 80% of chromium reacts with the hides and about 20%- 40% of the chromium amount remaining in the solid and liquid wastes.

Tanneries generate wastewater in the range of 30 – 35 liter per kilogram of skin or hide processed with variable pH and high concentrations of suspended solids, BOD and COD. Major problems are due to wastewater containing heavy metals, toxic chemicals, chloride, lime with high dissolved and suspended salts and other pollutants [5]. Hexavalent chromium from tannery wastewater is one of the major concerns of environmental pollution. This is due to discharge of tannery wastewater in large quantities without or with partial treatment [6].

Developing countries face numerous challenges related to preserving the environment from industrial wastewater pollution. Like many other developing countries, Ethiopia also grieve from environmental pollution problems of wastewater particularly Tannery industrial wastewater. This issue seems to be a subject which has not yet received adequate attention during the development of Tannery industries. Certainly very little and/or no investment has been made in the past to wastewater treatment facilities compared to drinking water supply. Therefore, there is a need to develop an efficient and low-cost wastewater treatment technologies for the removal of heavy metals and other pollutants. Among these technologies, adsorption is a user-friendly technique for this purpose.

Adsorption has been identified as one of the most promising mechanism for removal of dissolved heavy metal fractions and nutrients from wastewater. Although commercial adsorbents are available for use in adsorption, they are very expensive, resulting in various new low-cost adsorbents being studied by researchers. Babel and Kurniawan [7], reviewed the technical feasibility of various low-cost adsorbents for heavy metals removal from wastewater and concluded that the use of low-cost adsorbents may contribute to the sustainability of the surrounding environment and offer promising benefits for commercial purpose in the future.

Therefore identifying potentially efficient low-cost and locally available filter media as an adsorbent is critical for proper practice of environmental management by tanning industries. On the other hand ordinary sand for filter media is costly because of construction expansion in the country, not available readily and not efficient in removal of pollutants by adsorption hence there is a need to substitute scoria instead of sand.

1.2 Adsorbents and Adsorption in Wastewater Treatment Technology: Adsorption is recognized as one of the most effective purification and separation technique used in industry especially in water and wastewater treatment. Although the commercially available adsorbents are efficient in removal of heavy metals, they are costly and some cannot be regenerated and recycled. A number of approaches have been recently studied for the development of cheaper and more effective adsorbents for metal removal. Many non-conventional low cost adsorbents, including natural materials, bio-sorbents, and waste materials have been studied and proposed by several researchers [8].

Adsorption is a user-friendly technique especially for the removal of heavy metals. This process seems to be most versatile and effective method for removal of heavy metal [9]. The adsorption process is being widely used by various researchers for the removal of heavy metals [10] from waste streams and activated carbon has been frequently used as an adsorbent. Despite its extensive use in the water and wastewater treatment industries, activated carbon remains an expensive material. In recent years, the need for safe and economical methods for the elimination of heavy metals from contaminated waters has necessitated research interest towards the production of low cost alternatives to commercially available activated carbon.

Efficient methods of chromium removal from wastewater are important to attain environmental quality standards. Adsorption has been identified as one of the most promising method for removal of dissolved heavy metal from wastewater [11]. It has an advantage over other conventional methods due to its sludge free clean operation. Although commercial adsorbents are available for use in adsorption, they are very expensive, resulting in various new low cost adsorbents being studied by researchers. Babel and Kurniawan [7], reviewed the technical feasibility of various low-cost adsorbents for heavy metals removal from wastewater and concluded that the use of low-cost adsorbents may contribute to the sustainability of the surrounding environment and offer promising benefits for commercial purpose in the future.

1.3 Scoria as a low-cost Adsorbent: The volcanic ash scoria generally denser. Scoria is somewhat porous material with high surface area and strength with density larger than one. Scoria is an excellent media which holds water in its pores and allow air circulation to the root zone of the plant. Scoria is widely available in Rift valley area of Ethiopia.

Scoria is bomb-sized, generally vesicular pyroclastic rock with basaltic composition, which is reddish brown to black in color and is of low density. It has been used in several industrial applications, such as the manufacturing of a lightweight concrete mixture, a heating-insulating material, low-cost fillers in paints, and sorbents [12,13]. Scoria is abundant in many places worldwide including Central America, Southeast Asia (Vietnam, etc.), East Africa (Ethiopia, Kenya, etc.), and Europe (Greece, Italy, Spain, Turkey, etc.) [14,13].

Fig.1:Red Scoria: Photo by Mekonnen Birhanie March/2016, Ethiopia

Sorption of contaminants onto scoria mainly takes place at the outside surface at the initial stage. Changes of ionic composition during sorption experiments suggest that cation exchange is likely the dominant mechanism of heavy metals sorption onto scoria, while considerable As(III) removal by scoria is explained by specific sorption of the neutral As(III) species and electrical adsorption of negatively charged As(V) species via As oxidation onto hematite. The experimental investigation conducted demonstrates that the scoria is able to concurrently reduce concentrations of heavy metals and arsenic in aqueous solutions. Jang *et al*. [15], recommend that scoria can be used as an economic and efficient sorbent to treat contaminated water with heavy metals.

Taking into account the growth of industrialization in Ethiopia and the expected demand for industrial wastewater management, low-cost, appropriate and eco-friendly approaches will play a critical role in the development of future wastewater treatment technology in the country. In this practical approach, this work deal with the principles of adsorption and filtration for the removal of contaminants from tannery wastewater by replacing this volcanic ashes (scoria) as a filter media instead of conventional sand.

I. MATERIALS AND METHODS

2.1 Study Area and Period: This study has been conducted in Addis Ababa University by transporting sample wastewater from Dire tanning industry from May to August 2016.

2.2 Study Design: Batch mode comparative experimental study design has been carried out to determine the efficiency of scoria a volcanic ash as a filter media on the treatment of industrial wastewater, the case of tannery wastewater filtration.

2.3Experimental materials, Design and setup Establishments: The volcanic rocks were collected from volcanic cones of refit valley area of Oromia region, Ethiopia around Naziret, (Scoria: 8°30' N 39°19' E) approximately 100km East of Addis Ababa. The rocks are local volcanic rocks with various chemical and mineralogical structure and transported to Addis Ababa University. The chemical characteristics of filter media was determined by XRF analysis.(Table.1.)

Table.1: Physical and Chemical Characteristics of Scoria

Chemical Composition	Percent Weight of Scoria
SiO_2	52.46
Al_2O_3	18.14
Fe_2O_3	5.40
CaO	9.40
K_2O	0.20
Na_2O	3.28
MgO	7.44
MnO	0.12
P_2O_5	0.36
TiO_2	0.41
H_2O	1.12
LOI*	2.08
pH	7.81
**Physical properties, Particle size = 0.075–0.425mm.	
Porosity (%)	36
Particle density (gcm^{-3})	2.96
Specific surface area (BET) ($m^2\ g^{-1}$)	2.49
Cation exchange capacity (CEC), mequiv. 100 g^{-1}	0.09

**LOI= Loss on ignition*

This filter medium was crushed and graded. Effective size was determined by using standard sieve. Based on the analysis the effective size (ES) (d_{10}) of media was 1.5–

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4.5and the uniformity coefficient (UC) (d_{60}/d_{10}) is 3.5–4. After grading the filter material was washed by tap water and dray by sunlight for one week.

Filtration tank was made of metal sheet, with the following dimension, 60 cm height and 28 cm diameter and also was fitted with a half-inch an outlet tap(faucet)5cm above from bottom of tank. The filtration tank was installed at College of natural and computational science, Addis Ababa University. After installation the filter media was filled in the filtration tank 10cm depth with 10 – 25mm grain size drainage layer at the bottom, 30cm depth filter layer with a grain size of 1.5 – 4.5 mm at the middle and distribution layer (flat coarse gravel) was added 5cm depth at the top of the filter media to protect erosion of filter's top layer, then it is ready for sample tannery wastewater filtration.

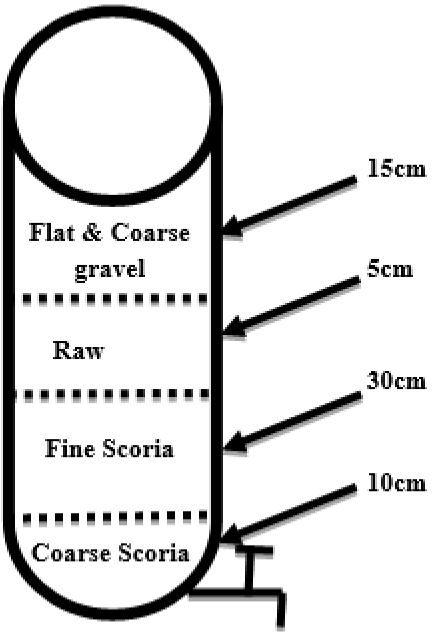

Fig. 2:Schematic layout of the Tanker Components of scoria filter

2.4 Wastewater Sample Collection and Filtration: The composite sample wastewater was collected from Dire tannery and transported to Addis Ababa University by using 40 liter plastic 'Jerican'. The onsite measurement of the physicochemical parameters were undertaken. The raw wastewater was added to the filtration tank at the time and a sample also transported to Addis Ababa EPA water and wastewater analysis laboratory and *Water Works Design and Supervision Enterprise* (WWDSE) for the raw wastewater characterization.

2.5 Filtrated Sample Collection and Laboratory Analysis: The physicochemical analysis of wastewater samples has been done before and after the treatment with the filter media, using standard methods. Optimum operating treatment time was determined for maximum removal of these impurities by running the experiment for 24, 48, and 72 hours, respectively. Filtrated Sample was taken by 2 liter plastic bottle after each fixed retention time that is after 24, 48, and 72 hours and transported to Addis Ababa EPA water and wastewater analysis laboratory and *Water Works Design and Supervision Enterprise* (WWDSE) after taking each sample.

The analytical parameters were pH, DO, BOD_5, COD, TSS, Ammonium N, Nitrite N, Nitrate N, Phosphate, Sulfide, sulfate and chromium. Onsite measurement of the wastewater like temperature, pH and DO were carried out at the site in the tannery environmental quality control laboratory using portable pH meter (Wagtech International N374, M128/03IM, USA) and DO meter (Hach P/N HQ30d, Loveland. CO, USA) for Dissolved oxygen and temperature.

COD, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphate, Sulfide and Sulfate were measured by using spectrophotometer (Hach model DR/3900 portable spectrophotometer, Germany) according to Hach instructions. $BOD_5$and total Cr were analyzed using BOD sensor and inductive stirring system AQUA LYTIC model type ET618-4 and Flame Atomic Absorption Spectrophotometer (AAS), (model AAS NOUA-400, Germany) respectively. Total suspended solids (TSS) were determined according to the Standard Methods for the Examination of Water and Wastewater gravimetric method [16].

The removal efficiency of the filter media for the selected parameters were calculated as:

$$\% \text{ removal} = Ci-Cf/Ci \text{X } 100$$

Where C_i is the parameter concentration in the untreated wastewater and C_f is the parameter concentration in the treated wastewater.

2.6 Statistical Data Analysis: Mean and standard deviations were calculated to estimate the concentration of each parameter of the samples. The hypothesis has been tested by student t-test using R statistical software: R version 3.2.2 (2015-08-14),Platform: x86_64-w64-mingw32/x64 (64-bit) to determine whether an observed difference between the means of the groups is statistically significant or not, based on the treatment efficiency of the filter materials.

2.7 Data Quality Management: To assure quality of the data by minimizing the errors the following measures had been undertaken: Apparatuses were calibrated; expiry date of reagents had been checked before starting the real analysis and standard control also prepared. Each test had been triplicates.

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II. RESULT AND DISCUSSION

3.1 Physicochemical **Characteristics of Dire Tannery Wastewater:** The raw wastewater was taken from Dire tannery around Asko area, Addis Ababa Ethiopia and transported to Addis Ababa EPA laboratory and water *works design and supervision Enterprise* (WWDSE) for physicochemical analysis. Based on this investigation the mean concentration of selected physicochemical parameters were presented at Table 2.

Table.2: Characteristics of Dire Tannery Wastewater June/2016

S.No.	Parameter	Concentration (mg/l) Except pH and T^o	Range
1	pH	9.1±3.1	6.5-12.5
2	T^o (^{o}C)	20.6±2.34	19-22
3	BOD_5	1081±159.55	924-1243
4	COD	12913±6874.7	8046-21025
5	TSS	2426±515.2	1849-2840
6	NH_4-N	314±59.9	259-378
7	NO_2-N	1.7±0.29	1.4-1.99
8	NO_3-N	124±12.8	110-135
9	PO_4-P	168±74	112-252
10	Sulfide	417±130.7	334-568
11	Sulfate	1307±224	1118-1555
12	Total chromium	35.7±8.6	28-45

This study revealed that the mean concentration of BOD_5, COD and TSS were 1081±159.55, 12913±6874.7 and 2426±515.2 mg/l respectively (Table 2.). This result is basically similar to different studies in Ethiopia with slight difference for different parameters for example a study done at Mojo tannery indicated that the mean concentration of COD was laid between 7950 to 15240 mg/l with the mean of 11123±563.9 mg/liter [17]. Another study also undertaken with same tannery wastewater showed that the mean concentration of BOD_5 was1054±448 mg/liter [18].But the concentration of total suspended solid was found from 1849 to 2840 (Table 2.) this is a bit greater than some studies for instance a study done in India indicated that 1244 mg/liter [19].

Nutrients like orthophosphate, ammonium, nitrite and nitrate concentration of Dire tannery were characterized in this study, the result revealed that 168±74, 314±59.9, 1.7±0.29, 124±12.8mg/l respectively. This result is comparable to a study done by Sivakumar *et al.*, [20] which indicates the concentration of nitrate in untreated tannery effluent was 116mg/l. the result of ammonium is in the range of the results done at Bahir Dar tannery wastewater characterization (96-420 mg/l) [21]. According to Arasappan and Kalyanaraman [22], the nitrite concentration of untreated tannery wastewater was 1.3 mg/l almost parallel to this study finding which accounts 1.7±0.29mg/l (Table 2.). Whereas the concentration of orthophosphate in this study was 168±74 mg/l, this result shows that the concentration of phosphate in Dire tannery wastewater is higher than other study results done previously to characterize another tannery wastewaters. The variation may be due to the utilization of phosphorus containing chemicals for different purposes and tanning activities in Dire tannery.

The total suspended solid in Dire tannery found to be 2426 mg/l this result is more or less similar with results of tannery wastewater analyzed by [23]. The concentration level of both sulfide and sulfate were 417±130.7 and 1307±224 mg/l respectively. In this case the amount of sulfide found in this study wastewater was more or less equivalent to study done by [24] that is 380±50 mg/liter [25], also characterize the tannery wastewater based on their result the concentration of sulfate was 1517mg/l which is almost parallel to this investigation. In terms of chromium concentration, Dire tannery comprised 35.7±8.6 mg/lit similar to other different results presented from various tannery wastewaters in Ethiopia for example a study done by [17] indicates 32.2±5.7 mg/l. On the other hand two more study results found to be in the chromium concentration ranges of this investigation result 28-45 mg/l (Table 5.1) [18,26].

Even though Wastewater of each tannery process consists of varying pH and temperature values, this study results (9.1±3.1and 20.6±2.34^{o}C) respectively were analogous to different studies. Likewise a large variation exists in values of physicochemical parameters in general like BOD_5, COD, TSS, phosphate, sulfide, sulfate, etc. in every tannery wastewater characteristics, this may be because of different tanning process, methods, technology and raw material utilization by various Tanning industries.

3.2 Tannery Wastewater Treatment by Filtration Technique Using Scoria as a Filter Media: Scoria is a volcanic rock found in the refit valley area of Ethiopia abundantly and different world. This study investigates the potential of this volcanic rock for the treatment of tannery wastewater using as filter media with three different retention time to filter out the sample wastewater. Based on this investigation scoria shows that promising result (Table 3).

Table.3: Tannery Wastewater Treatment Efficiency of Scoria as a Filter Media

Parameters	Mean Influent Concentration	Efficiency					
		RT= 24 hours		**RT= 48 hours**		**RT= 72 hours**	
		Mean Effluent Concentration	**% Removal**	**Mean Effluent Concentration**	**% Removal**	**Mean Effluent Concentration**	**% Removal**
BOD_5	1081±159.55	518±96	52	484±61.7	55	443±26	59
COD	12913±6874.7	6714±5735	48	6520±5398	50	5905±3621	54
TSS	2426±515.2	606±77.4	75	404±69.6	83	388±70.3	84
NH_4-N	314±59.9	213±5.9	32	230±11	27	296±23	6
NO_2-N	1.7±0.29	0.029±0.0035	98	0.039±0.0025	98	0.047±0.0035	97
NO_3-N	124±12.8	61.3±4.2	51	24.3±2.5	80	1.3±0.45	99
PO_4-P	168±74	73±54.9	57	66.7±53.4	60	63±3.5	63
Sulfide	417±130.7	111±12.5	73	106±22.9	75	96.7±6	77
Sulfate	1307±224	302±25.6	77	320±18.3	76	320±10	75
T. Chromium	35.7±8.6	10.4±1.29	71	10.2±1.26	71	11±1.22	69

According to this study, the maximum removal potential of scoria as a filter material for the reduction of BOD_5, COD and TSS from tannery wastewater was achieved at the retention time of 72 hours that accounts 59%, 54% and 84% respectively. In all of the three mentioned parameters the efficiency was directly proportional to retention time (Table 3).

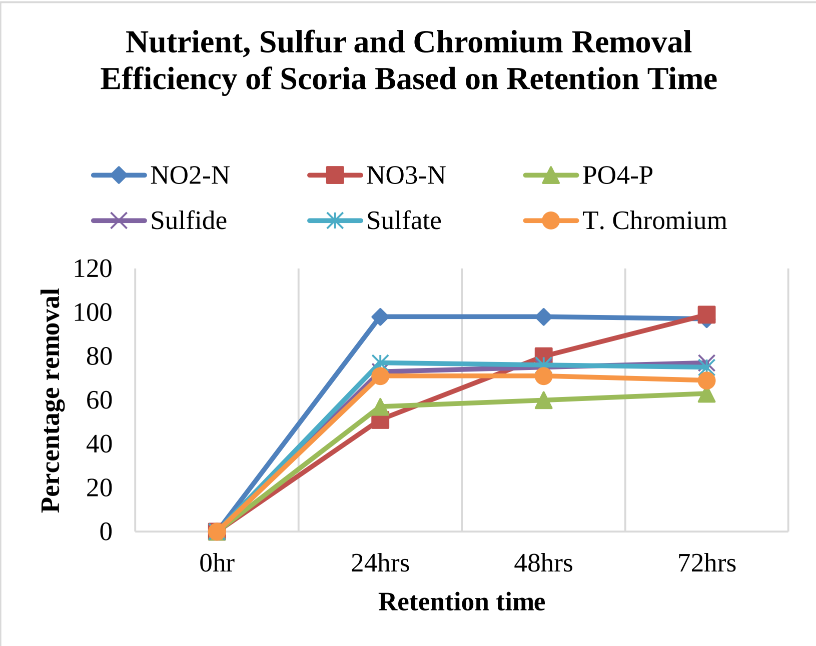

Fig. 3: Nutrient, Sulfur and Chromium Removal Efficiency of Scoria Based on Retention Time

The efficiency of scoria to reduce nutrients from tannery wastewater in general was high. The retention time and efficiency also directly related. In this study nitrite, nitrate and phosphate were reduce satisfactorily by 97%, 99% and 63% respectively after 72 hours (Table 3; Fig.3). This reduction may be obtained due to several mechanisms mainly adsorption, ion exchange, precipitation and finally filtration.

In this study significant reduction of nitrate was obtained which accounts 51%, 80% and 99% at RT= 24 hours, 48 hours and 72 hours respectively. One of the reason for this thought provoking results may be the retention time allowed is adequate for chemical equilibrium conditions to be reached between the filter material and nutrient in the wastewater in addition to the adsorption capacity of scoria. This study result shows that the same trend to a study done by [27] that indicates more than 95% nitrate removal efficiency from aqueous solution by adsorption mechanism. Another study result also analogous with this investigation that was done in 2004 by [28] to remove the nitrate from aqueous solution by using the original and activated red mud in batch adsorption technique. The similarity shown between the studies in terms of removal efficiency may be due to the similarity in the chemical composition of both scoria and red mud.

The result of phosphate reduction in this study was 57%, 60% and 63% at RT= 24 hours, 48hours and 72hours respectively. This result is almost in line with a study done in Ethiopia on the potential of scoria to reduce phosphate from aqueous solution that revealed 61% with 4mg/l initial concentration of the solution [29].

Wastewater from beam house of tanning industry contains high concentration of sulfide and sulfate ions. Since these effluents are toxic to aquatic environment, it is essential to reduce them and bring the discharge concentration levels of these species to below the toxic limit. In this study the better removal efficiency of both sulfide and sulfate by scoria filter were 77% at RT= 72 hours and 24 hours respectively (Table 3). In this study the adsorption technique is one of the efficient mode of physicochemical treatment for tannery wastewater likewise a study done in India on the treatment of tannery wastewater using adsorption technique and cactus powder as an adsorbent the result revealed that sulfate removal was found to be at the level of 90 % with initial concentration of 135 mg/l [30]. The reason for the result dissimilarity may be initial concentration and the adsorption potential of the adsorbents.

The concentration of chromium was reduce by 71%, 71% and 69% at RT=24 hour, 48 hours and 72 hours respectively. In the study of adsorption behavior of Cr(VI) onto macro and micro-vesicular volcanic rocks from water the maximum adsorption yield 77% for scoria was shown [31]. In another study it was observed that through increasing contact time, the removal efficiency of chromium in pre-determined optimum dose of scoria powder increased significantly from 73.28 to 86.63 % [32].

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REFERENCES

[1] Suthanthararajan R., Ravindranath E., Chits K., Umamaheswari B., Ramesh T., Rajamam S. (2004). Membrane application for recovery and reuse of water from treated tannery wastewater, *Desalination* 164, 151–156.

[2] Kawser Ahmed Md., Monika Das, Monirul Islam Md., Mosammat Salma Akter, Shahidul Islam and Muhammad Abdullah Al-Mansur (2011). Physicochemical Properties of Tannery and Textile Effluents and Surface Water of River Buriganga and Karnatoli, Bangladesh, *World Applied Sciences Journal* 12 (2): 152-159, ISSN 1818-4952.

[3] UNIDO (2012). United Nations Industrial Development Organization Vienna, Technical assistance project for the upgrading of the Ethiopian leather and leather products industry, Independent Evaluation Report Ethiopia, UNIDO project number: TE/ETH/08/008, available Online at *https://www.unido.org/fileadmin/user/Evaluation/Ethiopia_leather_valuation_FINAL_report_130208.pdf*.

[4] Rezic, I. and Zeiner, M. (2008). Determination of extractable chromium from leather, *Monatsheftefur Chemie -Chemical Monthly*, 140(3), pp.325-328.

[5] Durai G., Rajasimman M. and Rajamohan N. (2011). Kinetic studies on biodegradation of tannery wastewater in a sequential batch bioreactor, *Journal of Biotech Research*, 3:19-26.

[6] Lofrano, G., Aydn, E., Russo, F., Guida, M., Belgiorno, V., and Meric, S. (2008). Characterization fluxes and toxicity of leather tanning bath chemicals in a large tanning district area, *Water Air Soil Pollut.* 8, pp. 529-542.

[7] Babel S. and Kurniawan T. A. (2003). Low-cost adsorbents for heavy metal uptake from contaminated water: A review, *J. Hazard. Mat.* , 97, pp. 219-243.

[8] Kilonzo, F., Mutwiwa, U., Mutua, S., and Waweru, W. (2012). Evaluation of the use of constructed wetland in the treatment of tannery wastewater, *Kenya Science, Technology and Innovation Journal*, *ISSN 2079-5440*,PP. 16-22.

[9] Rao B.H., Dalinaidu A. and Singh D.N. (2007). Accelerated diffusion test on the intact rock mass, *Journal of Testing and Evaluation*, *ASTM*, vol. 35(2), pp. 111- 117.

[10] Ahmed R., Yamin T., Ansari M. S., and Hassany S. M. (2009). Sorption behavior of Lead (II) ions from aqueous solution onto Haro River sand, *The Nucleus*, vol. 24(6), pp. 475-486.

[11] Senthilkumar S., Bharati S., Nithynandhi D. and Subburam V., Bio sorption of toxic heavy metals from aqueous solution, Bioresour. Technol, 75,2000,163-165.

[12] Moufti, A.A. Sabtan, O.R. El-Mahdy, W.M. Shehata (2000). Assessment of the industrial utilization of scoria materials in Central Harrat Rahat, Saudi Arabia, *Eng. Geol*. 57: 155–162.

[13] Esayas Alemayehu, and Lennartz B. (2009). Virgin volcanic rocks: kinetics and equilibrium studies for the adsorption of cadmium from water, *J. Hazard. Mater*. 169: 395–401.

[14] Kwon, S.T. Yun, S.O. Kim, B. Mayer, I. Hutcheon (2005). Sorption of Zn(II) in aqueous solutions by scoria, *Chemosphere* 60: 1416–1426.

[15] Jang-Soon Kwona, b, Seong-Taek Yuna, Jong-HwaLeea, Soon-Oh Kimc, Ho Young Jo. (2010). Removal of divalent heavy metals (Cd, Cu, Pb, and Zn) and arsenic (III) from aqueous solution using scoria: Kinetics and equilibria of sorption, *Journal of Hazardous Materials*, 174: 307-313.

[16] American Public Health Association (APHA). (2005). Standard Methods for the Examination of Water and Wastewater.

[17] Seyoum Leta, Fassil Assefa and Gunnel D. (2003). Characterization of tannery wastewater and assessment of downstream pollution profiles along Modjo river in Ethiopia, *Ethiopian Journal of Biological Sciences*, 2 (2), 157 – 168.

[18] Tadesse Alemu Terfie and Seyoum Leta Asfaw, (2015). Evaluation of selected wetland plants for removal of chromium from tannery wastewater in constructed wetland, Ethiopia, *African Journal of Environmental science and technology* vol.9(5), pp. 420-427.

[19] Tamal Mandala, Dalia Dasguptab, Subhasis Mandala, Siddhartha Datta (2010). Treatment of leather industry wastewater by aerobic biological and Fenton oxidation process, *Journal of Hazardous Materials*, 180: 204–211, available at*www.elsevier.com/locate/jhazmat*.

[20] Sivakumar P., Kanagappan M. and Sam Manohar Das S. (2015). Physicochemical Characteristics of Untreated Effluent from Tannery Industries in Tamil Nadu: A Comparative Study, *Int. J Pharm Bio Sci.*, 6(1): (B) 446 – 451.

[21] Assefa Wosnie and Ayalew Wondi (2014). Bahir Dar tannery effluent characterization and its impact on the head of Blue Nile River, *Afr. J. Environ. Sci. Technol*., vol.8 (6), pp.312-318, available online at*www.academicjournals.org/AJEST*.

[22] Arasappan Sugasini and Kalyanaraman Rajagopal (2015). Characterization of Physicochemical Parameters and heavy metal Analysis of Tannery Effluent, *Int.J.Curr.Microbiol.App.Sci.* 4(9): 349-359, available at*http://www.ijcmas.com*.

[23] Saritha Banuraman and Meikandaan. T.P. (2013). Treatability Study of Tannery Effluent by Enhanced Primary Treatment, *International Journal of Modern Engineering Research (IJMER)*, Vol.3, Issue.1, pp-119-122, available online at*www.ijmer.com*.

[24] Islam B.I., Musa A.E., Ibrahim E.H., Salma A.A., and Babiker M. (2014). Evaluation and Characterization of Tannery Wastewater, *Journal of Forest Products & Industries*, 3(3): 141-150.

[25] Arasappan Sugasini and Kalyanaraman Rajagopal (2015). Characterization of Physicochemical Parameters and heavy metal Analysis of Tannery Effluent, *Int.J.Curr.Microbiol.App.Sci.* 4(9): 349-359, available at*http://www.ijcmas.com*.

[26] Asaye Ketema (2009). Evaluation of Selected Plant Species for the Treatment of Tannery Effluent in a Constructed Wetland System; *(Unpublished Thesis)*, Addis Ababa University, Available online at *en.wikipidia.org/winki/ITRC*.

[27] Nese and Ennil (2004). Nitrate removal from aqueous solution by adsorption onto various materials, *Journal of Hazardous Materials* B112: 155–162.

[28] Yunus Cengeloglu, Ali Tor, Mustafa Ersoz, Gulsin Arslan (2006). Removal of nitrate from aqueous solution by using red mud, *Separation and Purification Technology* 51: 374–378,available online at *www.sciencedirect.com*.

http://dx.doi.org/10.22161/ijeab/2.6.10 *ISSN: 2456-1878*

[29] Mekonnen Birhane, Alebel Abebe, Esayas Alemayehu & Embialle Mengistie (2014). Efficiency of locally available filter media on fluoride and phosphate removal for household water treatment system, *Chinese Journal of Population Resources and Environment*, 12:2, 110-115.

[30] Swathi M, Sathya Singh A, Aravind S, Ashi Sudhakar P.K, Gobinath R, Saranyadevi D. (2014). Experimental studies on tannery wastewater using cactus powder as an adsorbent, *Int. Journal of Applied Sciences and Engineering Research*, Vol. 3, Issue 2, available online at*www.ijaser.com*.

[31] Esayas Alemayehu, Soren Thiele-Bruhn, Bernd Lennartz (2011). Adsorption behavior of Cr(VI) onto macro and micro-vesicular volcanic rocks from water, *Separation and Purification Technology*, 78: 55–61, Available at *www.elsevier.com/locate/seppur*.

[32] Masoud Moradi, Lida Hemati, Meghdad Pirsaheb and Kiomars Sharafi (2015).Removal of Hexavalent Chromium from Aqueous Solution by Powdered Scoria-Equilibrium Isotherms and Kinetic Studies, *World Applied Sciences Journal* 33 (3): 393-400.

Management of Rust in Pearl millet caused by *Puccinia substriata* var. *penicillariae* using Plant Product, Bioagent and Fungicides

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***Abstract*—*Rust caused by Puccinia substriata var. penicillariae is one of the major disease affecting both forage and grain production in pearl millet. An attempt was made to manage pearl millet rust using plant product, bioagent and fungicides under screen house and field conditions. The experiment was conducted on susceptible hybrid HHB 197 both under screen house and field condition with eight treatments. Observation on rust severity recorded at grain filling stage. The experiment results indicated that all the treatments were effective in managing the disease but amongst them minimum disease severity (11.7%) and (21.7%) was contracted under screen house and field conditions respectively in treatment of Propiconazole 25% EC (0.1%) followed by Hexaconazole 5% EC (0.1%) and Copper oxychloride 50% WP (0.2%), Carbendazim 50% WP (0.2%), Mancozeb 75% WP (0.2%), Azadirachtin 0.15% (1500 ppm), Trichoderma viride (3%) treated pots and plot. Maximum grain yield (514.7 kg/acre), test weight (8.13 g) and Benefit: cost 3.98: 1 was observed in Propiconazole 25% EC (0.1%) sprayed plot followed by Hexaconazole 5% EC (0.1%) under field conditions.*

Keywords*—Plant product, Bioagent, Fungicides, Pearl millet, Puccinia substriata var. penicillariae.

I. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R.Br. Syn. *Pennisetum americanum* (L.) Leeke] is an important staple cereal in the arid and semi-arid region of the world, particularly in Asia and Africa. India is considered to be the secondary centre of pearl millet diversity (Rao and Wet, 1999). Being most tolerant to drought and salinity, the crop is by and large grown in different countries of the world. Due to its adaptability under very wide range of agro-climatic conditions this crop is mostly grown in the states of Andhra Pradesh, Gujarat, Haryana, Karnataka, Madhya Pradesh, Rajasthan, Tamil Nadu, parts of Delhi, Punjab and Uttar Pradesh. In India the total production of crop was 9.25 m ton with area of 7.89 m ha during 2013-2014 (Anonymous 2013-14). The yield of pearl millet has increased considerably with the introduction of hybrids, but these have become susceptible to fungal diseases. Among various diseases, rust is one of major concern in pearl millet growing areas of the world. *Puccinia substriata* var. *indica* Ramchar and Cumm (syn: *Puccinia substriata* Ell. and Barth. var. *penicillariae* Carvalho *et al.* 2006; *Puccinia penniseti Zimm*), causes rust disease in pearl millet. In present study attempts were made to find out cost effective spray schedule involving plant product, bioagent and fungicides.

II. MATERIALS AND METHODS

The studies were carried out at the experimental area of Plant Pathology, CCS HAU, Hisar during *Kharif* 2015. Plant product Azadirachtin 0.15 EC @1500 ppm, formulation of biocontrol agent *Trichoderma viride @ 3%* and five chemical compounds *viz.,* Carbendazim 50% WP @ 0.2%, Mancozeb 75% WP @ 0.2%, Copper oxychloride 50% WP @ 0.2%, Propiconazole 25% EC @ 0.1% and Hexaconazole 5% EC @ 0.1% were used as foliar sprays for management of pearl millet rust under screen house and field conditions.

III. SCREEN HOUSE EXPERIMENT

This experiment was conducted with eight treatments in completely randomized design (CRD) with five pots per treatment and each treatment had three replications. Five seeds of Hybrid HHB 197 were sown in each pot filled with sterilized soil-sand-FYM (farmyard manure) mix and placed in screen house. Inoculation was done by rubbing rust infected leaves to healthy leaves and infected leaves were taken from field after first appearance of rust. Rust severity (%) was recorded 15 days after inoculation. Different agents were sprayed one week after date of first appearance of disease. The fungicidal solutions of required concentrations were prepared by mixing them in the measured quantity of water and sprayed on the plants till run- off, with the help of Knapsack sprayer one week after appearance of rust. Observations on germination percentage (%), No. of tillers, date of first appearance of rust and per cent rust severity at maturity were recorded.

IV. FIELD EXPERIMENT

The experiment was conducted in randomized block design (RBD) with three replications of similar set with all the eight treatments in plot size of 5×3 m by maintaining row to row distance of 50 cm and plant to plant distance of 10 cm. Six lines were maintained in each treatment (Plot) and fifty seeds were sown in each row. Recommended agronomic practices were followed. Different agents were sprayed one week after first appearance of rust. The solutions of all treatment were prepared by mixing them in the measured quantity of water and sprayed on the plants till run- off, with the help of Knapsack sprayer. Spray operations were carried out in the evening to avoid wind drift when the wind was calm. The benefit: cost ratio was calculated based on total grain yield per acre. The increase in grain yield as compared to the control (unsprayed) was calculated by subtracting the yield of control (unsprayed) from the treated plot. For calculation of benefit cost ratio the grain yield was taken *vis-à-vis* expenditure incurred on disease management as per the formula given below.

$$\text{B: C} = \frac{\text{Additional income obtained by treatment}}{\text{Amount spent for disease treatment}}$$

Observations on germination percentage, total number of plants, date of first appearance of rust, rust severity at maturity, grain yield at maturity and test weight were recorded and then Benefit: cost ratio was calculated.

V. RESULTS AND DISCUSSION

Screen house and field experiment

The fungicides, plant product and bioagent used in this study were effective in managing the disease as compared to control under screen house (Table 1) and field conditions (Table 2). Among tested treatments minimum disease severity (11.7%) and (21.7%) was contracted under screen house and field conditions respectively in Propiconazole 25% EC (0.1%) followed by Hexaconazole 5% EC (0.1%) and Copper oxychloride 50% WP (0.2%), Carbendazim 50% WP (0.2%), Mancozeb 75% WP (0.2%), Azadirachtin 0.15 % EC (1500 ppm) treated plot. Baiswar *et al.* (2011) reported that Propiconazole 25 EC (0.1%) and Tebuconazole 250 EC (0.2%) were best in managing soybean rust followed by Triadimefon 25 WP (0.1%), Sulphur 80 WP (0.3%), Carbendazim 50 WP (0.2%), Copper oxychloride 50 WP (50%), Chlorothalonil 75 WP (0.2%), *Trichothecium roseum*, Mancozeb 75 WP (0.25%), Azadirachtin (0.03%), Cymbopogon leaf extract (3 ml/l), Hexaconazole 5 EC (0.1%), Tricyclazole75 WP (0.05%), *Trichoderma* sp. Highest Percent Disease Index was in case of control (65.4) and the yield was also lowest in this case (483.3 kg/ha). Cost benefit analysis also proved that Propiconazole (1.7) and Sulphur (1.6) are the best options for managing soyabean rust.

Among, five fungicides evaluated against pearl millet rust under field conditions, Propiconazole 25% EC @ 0.1% recorded the least rust severity (21.7%), highest seed yield (514.7 kg/acre) and 1000 seed weight (8.13 g) with highest benefit: cost ratio of 3.98 (Table 2). Nagarajan and Patil (2014) evaluated three fungicides *viz.*, Hexaconazole, Propiconazole and Triadimefon against pearl millet rust under field condition and reported that Hexaconazole @ 0.1% recorded the least rust severity (15.30%), highest seed yield (30.50 q/ha) and 1000 seed weight (10.51g) with highest benefit: cost ratio of 2.40.in present investigation grain yield in all the treatments of fungicides was significantly superior in comparison to control under field condition, maximum grain yield (514.7 kg/acre) and test weight (8.13 g) was observed in Propiconazole 25% EC (0.1%) sprayed plot followed by Hexaconazole 5% EC (0.1%) treated plot (480.0 kg/acre and 7.77 g) as compared to control (360.0 kg/acre and 6.37g). The Maximum B: C ratio of 3.98: 1 was observed in Propiconazole 25% EC (0.1%) followed by Hexaconazole 5% EC (0.1%) (3.38: 1), Copper oxychloride 50% WP @ 0.2% (2.59: 1), Carbendazim 50% WP @ 0.2% (2.43: 1), Mancozeb 75% WP @ 0.2% (2.27: 1) and Azadirchtin 0.15% EC (2.16: 1) (Table 2).

Biocontrol agents offer great potential in managing the disease. *Trichoderma* sp. are considered as one of the most important biocontrol fungi for improving plant growth and protecting crops from several fungal plant pathogens (Harman 2000). Rust of pearl millet managed to the extent 30% under screen house condition and 43.3% under field condition with *Trichoderma viride* (3%) spray and it was found significantly superior in comparison to control having rust severity of 56.7% under screen house condition and 91.7% under field condition. Bhushan *et al.* (2014) reported that *Trichoderma harzianum* an antagonistic isolate was able to cause significant reduction in seed-borne mycoflora

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in comparison with *Trichoderma viride* and *Trichoderma hamatum* in *Pennisetum americanum*.

The anti- fungal activity of Azadirachtin 0.15 % EC (1500 ppm) was studied against *Puccinia substriata* var. *penicillariae*. It was observed that in Azadirachtin 0.15 % EC (1500 ppm) treated plot disease was reduced 28.3% (under screen house conditions) and 41.7% (under field conditions) and yield increased significantly 440.0 kg/acre under field condition in comparison to control (360 kg/acre). Under field condition in Azadirachtin 0.15 % EC (1500 ppm) treated plot per cent increase in yield over control and Benefit: cost ratio was 22.22 and 2.16:1 respectively. Rao (2009) revealed that plant received the foliar spray with azadirachtin (0.03%) @ 3 mL/L on 30 and 45 DAS recorded lesser incidence of *Alternaria* leaf blight and powdery mildew. Zade *et al.* (2005) tested *in vitro*, the leaf extracts of neem (*Azadirachta indica*), mehandi (*Lawsonia inermis*), sadaphuli (*Catharanthus roseus*), bael (*Aegle marmelos*), custard apple (*Annona squamosa*) and marigold (*Tagetes patula*) at 50 and 100 per cent concentrations against *Puccinia arachidis*. Among the leaf extracts, the neem leaf extract at both concentrations was the best, followed by mehandi leaf extract at 100 per cent concentration. Kishore and Pande (2005) noted that aqueous leaf extracts of Datura metel and *L. inermis*, known for their high antifungal activity against *Phaeoisariopsis personata* completely inhibited the germination of uredospores of *Puccinia arachidis in vitro*.

Gopal *et al.* (2003) conducted an experiment to determine the efficacy of Difenoconazole (0.1 and 0.5%), Propiconazole (0.1 and 0.05%) and Chlorothalonil (0.25%), as well as different combinations of Tridemorph (0.1%), Carbendazim (0.05%) and Mancozeb (0.2%) in controlling late leaf spot and rust of groundnut. They reported that Difenoconazole (0.1%) treatment resulted in the highest mean yield (2150 kg/ha) followed by Carbendazim (0.05%) + Tridemorph (0.1%) treatment (2090 kg/ha). The incidence of late leaf spot and rust were lowest with the application of Carbendazim 0.05% and Tridemorph 0.1%, respectively. Rojasara *et al.* (2010) evaluated nine fungicides against rust (*Puccinia arachidis*) *in vivo* on groundnut variety GG - 2. Among them Difenoconazole (0.0125%) was the most effective fungicides in controlling the disease (62.43%) followed by Hexaconazole (59.22 %), Tridemorph (54.38%) and Propiconazole (50.03%). The highest pod (1307 kg/ha) and fodder (5173 kg/ha) yield was also recorded in the treatment Difenoconazole. Sunkad *et al.* (2005) tested the bioefficacy of six fungicides for the control of leaf spots and rust disease of groundnut in Karnataka. They recorded maximum disease control with higher dry pod and fodder yield in Hexaconazole, Difenoconozole and Propiconazole treatments. The Mancozeb gave better control of rust. Tirmali and Pawar (2000) examined the efficacy of different fungicides *viz.*, Mancozeb 0.2%, Carbendazim 0.2%, Neem (Neemata) at 5% and Cu chemicals (Copper & Boron at 1000 ppm, K at 30 and 60 kg/ha) against the rust disease of groundnut. All the fungicide and chemical treatments significantly reduced the disease incidence over the control. The Mancozeb was the most effective as it resulted in the highest per cent disease control (47.77%) and the lowest disease intensity (39.99%). This treatment also gave the highest values for pod yield (24.09 q/ha) and per cent increased in yield over control (70.61%).

These results indicate that Propiconazole is the best cost effective options for managing pearl millet rust. Since Propiconazole being a selective fungicide has more chances of development of resistance, so it should be used in conjunction with a non-selective fungicide which will minimize the chances of development of resistance.

Table.1: /Effect of plant product, bioagent and fungicides against pearl millet rust under screen house conditions

S. No.	Treatment	Germination (%)	Mean no. of tillers	Per cent rust severity at maturity
1.	Azadirachtin 0.15 % EC @ 1500 ppm	69.3 **(56.47)***	17.3	28.3 **(32.07)***
2.	Carbendazim 50% WP @ 0.2%	60.0 **(50.87)**	15.0	20.0 **(26.13)**
3.	Mancozeb 75% WP @ 0.2%	61.3 **(51.54)**	15.3	26.7 **(31.05)**
4.	Copper oxychloride 50% WP @ 0.2%	65.3 **(53.99)**	16.3	18.3 **(24.99)**
5.	Propiconazole 25% EC @ 0.1%	70.7 **(57.34)**	17.7	11.7 **(19.29)**

6.	Hexaconazole 5% EC @ 0.1%	62.7 **(52.40)**	15.7	15.0 **(22.28)**
7.	*Trichoderma viride* @ 3%	64.0 **(53.18)**	16.0	30.0 **(33.14)**
8.	Control (unsprayed)	64.0 **(53.12)**	16.0	56.7 **(48.87)**
	CD at 5%	NS	NS	10.75

* Figures in the parenthesis are angular transformed values, Date of first appearance of Rust **14/10/2015,** Date of observation **03/11/2015**

Table.2: Effect of plant product, bioagent and fungicides against pearl millet rust under field conditions

S. No.	**Treatment**	**Germination (%)**	**Mean no. of plants in each plot**	**Per cent rust severity at maturity**	**Grain yield at maturity (kg /acre)**	**Per cent increase in yield over control**	**B:C ratio**	**Test weight (g)**
1.	*Azadirachtin* 0.15 % EC @ 1500 ppm	69.4 **(56.42) ***	208.3	41.7 **(40.15)***	440.0	22.22	2.16:1	6.99
2.	Carbendazim 50% WP @ 0.2%	69.4 **(56.43)**	208.3	36.7 **(37.10)**	457.8	27.16	2.43:1	7.60
3.	Mancozeb 75% WP @ 0.2%	78.1 **(62.58)**	234.3	38.3 **(38.22)**	444.4	23.44	2.27;1	7.50
4.	Copper oxychloride 50% WP @ 0.2%	75.8 **(60.89)**	227.3	28.3 **(32.00)**	471.1	30.86	2.59:1	7.73
5.	Propiconazole 25% EC @ 0.1%	76.2 **(61.06)**	228.7	21.7 **(27.69)**	514.7	42.97	3.98:1	8.13
6.	Hexaconazole 5% EC @ 0.1%	78.8 **(62.62)**	236.3	26.7 **(30.98)**	480.0	33.33	3.38:1	7.77
7.	*Trichoderma viride* @ 3%	77.7 **(61.79)**	233.0	43.3 **(41.10)**	426.7	18.52	0.79:1	6.57
8.	Control (unsprayed)	74.9 **(59.95)**	224.7	91.7 **(76.24)**	360.0	_	_	6.37
	CD at 5%	NS	NS	10.78	29.93			0.389

* Figures in the parenthesis are angular transformed values, Date of first appearance of Rust **10/10/2015,** Date of observation **05/11/2015**

REFERENCES

[1] Anonymous (2013-14). Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India New Delhi. www.agricoop.nic.in

[2] Baiswar, P., Tiameren, Ao., Upadhyay, D.N. and Chandra, S. (2011). Management of soybean rust caused by *Phakopsora pachyrhizi* using fungicides, botanicals and biocontrol agent in mid-hills of Meghalaya. *Indian J. Hill Farmi.* 24(2): 33-37.

[3] Bhushan, G., Kumar S., and Singh, A.P. (2014). Antagonistic effects of *Trichoderma* against seed-borne fungi of *Pennisetum americanum. Ethiopian Int. J. Multidisciplinary Res.* 2: 13-19.

[4] Carvalho, A., Soares, D.J., Carmo, M.G.F., Costa, A.C.T. and Pimentel, C. (2006). Description of the life- cycle of the pearl millet rust fungus- *Puccinia substriata* var. *penicillariae* with a proposal of reducing var. *indica* to a synonym *Mycopathol.* 161: 331-336.

[5] Gopal, K., Jagadeswar, R. and Babu, G.P. (2003). Efficacy of new systemic fungicide in controlling late leaf spot and rust in groundnut. *Indian J. Pl. Prot.* 31(2): 76-79.

[6] Harman, G.E (2000). Myths and dogmas of Biocontrol: Changes in the perceptions derived from

research on *Trichoderma harzianum* T-22. *Pl. Dis*. 84: 377-393.

[7] Kishore, G.K. and Pande, S. (2005). Integrated application of aqueous leaf extract of Datura metel and chlorothalonil improved control of late leaf spot and rust of groundnut. *Astralasian Pl. Pathol.* 34(2): 261-264.

[8] Nagarajan, H. and Patil, P.V. (2014). Development of integrated spray schedule for the management of pearl millet rust in Northern zone of Karanatka. *Karnataka J. Agric. Sci.* 27 (3): 308-311.

[9] Rao, M. S. L., Kulkarni, L., Lingaraju, S. I. and Nadaf, H. L. (2009). Bio-priming of seeds: A potential tool in the integrated management of *Alternaria* blight of sunflower. *HELIA*, 32: 107-114.

[10] Rao, S., and Wet, J.M.J. (1999). Taxonomy and evolution. *In*: Pearl Millet Breeding. pp. 29-47 (Khairwal, I.S., Rai, K.N., Andrews, D.J. and Harinarayana G. eds.). Oxford & IBH Publishing Co., New Delhi, India.

[11] Rojasara, Y.M., Rajani, M.D. and Dhruj, I.U. (2010). Field evaluation of newer fungicides against groundnut rust (*Puccinia arachidis*) (Abst.). Anand and Indian Phytopathological Society, New Delhi. pp. 131.

[12] Sunkad, G., Mesta, R.K. and Reddy, M. (2005). Field efficacy of some fungicides for effective and economical control major foliar diseases of groundnut. *Karnataka J. Agric. Sci*. 18(4): 995-997.

[13] Tirmali, A.M. and Pawar, D.R. (2000). Efficacy of different fungicides against rust disease of groundnut. *J. Maharashtra agric. Univ*. 25(3): 248-249.

[14] Zade, S.R., Buldeo, A.N., Lanje, P.W. and Gulhane, V.G. (2005). Evaluation of plant extracts and culture filtrates of bioagents against *Puccinia arachidis* Speg. In groundnut. *J. Soils Crops*. 15(1): 150-154.

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In Vitro Selection of Calli for Salt Tolerance in Tomato (*Solanum lycopersicum* L.)

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***Abstract*— *Soil salinity is one of the most important abiotic stresses that limit crop production. Responses of six breeding lines (BD-7755, BD-7757, BD-9008, BD-9011, BD-10122, BD-10123 which were named as G1, G2, G3, G4, G5 and G6 respectively) of tomato (Solanum lycopersicum L) to NaCl stress were studied in callus induction. Hypocotyl and cotyledon segments were chosen as explants for callus induction in vitro. The six investigated tomato genotypes differed in their callus growth. Tomato seeds were cultured for callus formation and that callus were treated with 0 mM (control), 50 mM, 100 mM, 150 mM and 200 mM NaCl in nutrient solutions. The effect of the stress applied on the callus was evaluated in 10 DAT (Days After Treatment) and 17 DAT. Different concentrations of NaCl in the medium significantly affected the biomass callus size and callus weight of tomato. G2 and G6 showed excellent performance of tolerance up to 50 mM of NaCl. G6 showed better performance under high salt concentrations i.e., at 100 mM and 200 mM but not at low salt stress. It indicates the expression of functional gene occurs at high salt stress. However it is possible to select callus line tolerant to elevated levels of NaCl stress by sudden exposure to high of NaCl, accordingly a NaCl tolerant cell line was selected from hypocotyls and cotyledon derived callus of tomato which proved to be a true cell line variant. The interaction effect of variety and treatmentrevealed that genotype G6 and G1 were the highest and lowest performer respectively. These findings indicated some salt tolerant tomato genotypes which will be promising for regeneration and for future breeding program. It is quiet necessary to asses accumulation of proline and the anti-oxidant enzymes like Super Oxide Dismutase, Ascorbate Peroxidase and Catalase from the control and stressed callus as they are the indicator of salt tolerance. It is evident that tissue culture technique was able to evaluate several genotypes for salt tolerance into cell level under controlled environment with relatively little space and less time required comparing with such process studies at the whole plant level.*

***Keywords*— In vitro, Salt tolerance, Tomato.**

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important solanaceous vegetable crops in the world in terms of both production and harvested area. Though it is a self-crossing annual crop, nowadays, tomatoes are grown round the year. Due to increasing consumption of tomato products, the crop is becoming promising. In Bangladesh, the yield of tomato is not enough satisfactory in comparison to the other tomato growing countries of the World (Aditya *et al*., 1999). The low yield of tomato in Bangladesh however is not an indication of low yielding potentially of this crop but of the fact that the low yield may be attributed to a number of reasons, viz. unavailability of quality seeds of high yielding varieties, land for production based on light availability, fertilizer management, pest infestation as well as production in abiotic stress conditions especially high salinity.

Salinity is one of the major stress factors among the abiotic stresses. In the world, about 400 million hectares of land are affected by high salinity. In Bangladesh about 1 million hectares of land are affected by high salinity in the coastal regions and it is increasing day by day. Salinity affects almost every aspect of the morphology, physiology and biochemistry of plants and significantly reduces yield (Aazami *et al.*, 2010; Amini and Ehsanpour, 2006; Zhang *et al.*, 2004). As saline soils and saline waters are common around the world, great effort has been devoted to understanding physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. In spite of this great effort, only a small number of cultivars, partially tolerant to salinity, have been developed. Further effort is necessary if the exploitation of saline soils and saline waters that are not currently usable is to be achieved. Salinity affects yield and quality, so that yield characters must be taken into account when breeding for salinity tolerance. But not only yield-related characters are important. As salinity affects almost every aspect of the physiology and biochemistry of the plant, the enhancement of crop salt tolerance will require the combination of several too many physiological traits (Cuartero *et al*., 2006;

Cuartero and Ferna´ndez-Mun˜oz, 1999; Flowers and Yeo, 1995), not simply those directly influencing yield. As salinity in soils is variable and plant tolerance depends on the stage of plant development, plants should be phenotyped at several salinity concentrations and at the most sensitive plant stage(s).

As salinity is a major factor in limiting crop productivity in semi-arid areas of the world, the selection of salt tolerance lines continues to challenge plant scientists, especially those working in physiology and genetics. If it is possible to use cell and tissue culture techniques, together with conventional breeding and genetic engineering (Mohamed *et al*., 2011; Shatnawi, 2006; Cano *et al.*, 1998), for the development of plants with increased tolerance to salt stress, the major problem in selecting salt tolerant lines could be the difficulty of screening thousands of plants without a reliable selection for criterion. *In vitro* culture, besides its use as a tool for obtaining salt tolerant plants, may offer potential for quick evaluation of germplasm against salt stress. *In vitro* techniques make it possible to screen the required number of genotypes rapidly since *in vitro* plant exhibit their capacity to withstand the stress (Tewary *et al.,* 2000). In many species like tobacco, grape, rice citrus and carrot salt tolerant lines have been isolated using *in vitro* techniques (Vijayan *et al*., 2003; Tewary *et al*., 2000; Ben-Hayyim, 1987).

Tomato is sensitive to moderate levels of salt in the soil. Tomato genotypes' response to salinity is genetic and species dependant and there is too much interest in screening and breeding for higher salt tolerance (Mohamed *et al*., 2007; Amini and Ehsanpour, 2006). Tomato is also a favorable food crop for *in vitro* and genetic studies due to its low chromosome no i.e., 2n=2x=24 and due to comprehensive knowledge of tomato genetics. A considerable improvement has already been made by exploiting the natural variation through conventional breeding in tomato. Even though the success made in the last century, traditional breeding efforts alone cannot meet the increasing demand of tomato consumers in the 21st century. Therefore, plant cell and tissue culture techniques are being used for the genetic improvement and developing salt tolerant lines of tomato plant throughout the world. Several *in vitro* investigations have been conducted on tomato in different applications. In tomato, a positive correlation between growth of calluses and whole plants has been observed under saline condition (Tal *et al*., 1978; Perez-Alfocea *et al*., 1994). The genetics of physiological characters together with other tolerance components related to metabolic defenses against salinity have to be studied in order to advance the breeding of tomato genotypes tolerant to salinity.

This study was conducted to explore the bioassay so as to establish a reproducible protocol for selecting of different salt tolerant callus lines in tomato in different concentrations of NaCl. With conceiving the above scheme in mind, the present research work has been undertaken in order to fulfill the following objectives:

1. To study the *in vitro* biomass changing of callus under different concentration of NaCl.
2. To investigate the callus induction ability of six genotypes of tomato under different NaCl concentrations.
3. To identify and select the salt tolerant callus lines.
4. To find out the effect of NaCl at varied levels on biomass character of six tomato genotypes by using hypocotyls and cotyledons explants.
5. To establish an effective callus induction protocol for six genotypes of tomato under control and salt stress condition.
6. To develop of somaclonal variant from the salt tolerant calli.
7. To assay the magnitude of genetic divergence in tomato genotypes regarding callus induction.

II. MATERIALS AND METHODS

The study was conducted at the Genetics and Plant Breeding Laboratory of Sher-e Bangla Agricultural University, Dhaka, Bangladesh during the period from March, 2013 to June, 2014 to study the *in vitro* selection of salt tolerant callus lines in Tomato. The experiment was done in different steps:

1. *In vitro* seed germination of different genotypes.
2. *In vitro* callus initiation and sub-culturing of callus.
3. *In vitro* salt treatment of different concentration (0Mm, 50Mm, 100Mm, 150Mm and 200 Mm) in six genotypes.

The materials and methods of this experiment are presented in this chapter under the following headings –

Experimental site

The experiment was carried out at the Genetics and Plant Breeding Laboratory, Sher-e-Bangla Agricultural University, Dhaka during the period of March, 2013 to September, 2014. The place is geographically located at about $24^0$75' North latitude and $90^0$50' East longitude.

Experimental material

Plant materials

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A total of six genotypes of tomato originated from different places of Bangladesh were used in this experiment. The materials were collected from Plant Genetic Resource Centre (PGRC) at Bangladesh Agricultural Research Institute (BARI), Gazipur. The name and origin of these genotypes are presented in Table 1.

Table.1: List of the tomato genotypes used in the experiment

SL. No.	Genotypes No.	Name/Acc No. (BD)	Origin
01	G1	BD-7755	PGRC, BARI
02	G2	BD-7757	PGRC, BARI
03	G3	BD-9008	PGRC, BARI
04	G4	BD-9011	PGRC, BARI
05	G5	BD-10122	PGRC, BARI
06	G6	BD-10123	PGRC, BARI

PGRC=Plant Genetic Research Centre, BARI=Bangladesh Agricultural Research Institute

Laboratory materials

Laboratory preparation was started in early March 2013 by collecting chemical and instruments.

Chemicals

MS medium, Sterilizing chemicals (Sodium hypo chlorite NaOCl, 70% ethanol), Sucrose, Agar, NaOH (10 N, 1N), HCl, KCl (3M), NaCl (laboratory grade), Absolute Ethanol, Ethanol (70%), Methilated spirit, NAA (1-Naphthaleneacetic acid), BAP (6-Benzylaminopurine).

Instruments

Autoclave, Hotplate with magnetic stirrer, Automatic drying oven, Freezers, Furnaces, Incubators,Laminar Air Flow Chamber, Microwave oven, Pipettes, Plant Growth Chamber, Safety Cabinets, Shakers, Shaking Incubator, Water Purification System, pH meter, Course and fine electric balances, Scalpel, forceps, scissors etc., Culture vials (petridishes, test tubes, culture bottles).

Culture media

Success of any experiment depends on the culture media, hormone combination, tissue and employing cell. Murashige and Skoog (1962) medium were used with different hormone supplements as culture medium for callus induction. Different concentration of salt was added to the basal MS medium supplemented with auxin and cytokinine. Three types of culture media were used in this study, viz,

1. Hormone free basal MS medium for raising of seedling to get explants.
2. MS medium supplemented with 2 mg/l of BAP and 0.2 g/l of NAA for callus induction.
3. MS medium as 2. supplemented with different salt concentrations (0 mM, 50 mM, 100 mM, 150 mM and 200 mM) for salt stress treatment.

Murashige and Skoog (1962) medium were used with different NaCl concentration as culture medium for selection of salt tolerant callus. The composition of MS medium has been presented in Appendix 1. NaCl were added to MS media as per treatment of the experiment. For the preparation of media, stock solutions were prepared at the beginning and stored in the refrigerator at 4±1ºC. The respective media were prepared from stock solutions.

Preparation of the stock solutions

The first step in the preparation of the medium is the preparation of stock solutions of the various constituents of the MS medium. As different media constituents were required in different concentrations, separate stock solutions for the macronutrients, micronutrients, Fe-EDTA (Iron stock), vitamins and growth regulators were prepared separately for ready use.

Stock solution of macronutrients (stock 1)

Stock solution of macronutrients was prepared with 10 times of the final strength of the medium in one liter of distilled water (DW). Ten times the weight of the salts required for one liter of medium weighted accurately. All the macronutrient were dissolved one by one except $CaCl_2$. The stock solution of $CaCl_2$ should be prepared separately in order to avoid precipitation. And in this way, dissolved all the salts thoroughly in 750 ml of distilled water and final volume was made up to one liter by further addition of (distilled water) DW. The stock solution was poured into a clean sterilized glass container and stored in a refrigerator at 4°C for ready use.

Stock solution of micronutrients (stock 2)

A stock solution of all the micronutrients with 100x concentration is generally prepared. Since copper and cobalt are required in very small quantities, it was preferable to

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first make a separate stock solution of those two salts (100X) and then an appropriate volume were pipetted and put into the main micronutrient stock solution. This stock solution was also stored in refrigerator at 4°C.

Stock solutionof iron (Fe-EDTA) (stock 3)

Iron-EDTA should be added fresh and it was made 100 times the final strength of the medium in one liter DW. Here, two constituents, $FeSO_4.7H_2O$ and Na_2EDTA, were dissolved in 750 ml of DW in a conical flask by heating in a water bath until the salts dissolved completely and final volume was made up to one liter by further addition of DW. This stock should be stored in an amber color bottle or a bottle covered with an aluminum foil and stored in refrigerator at 4°C.

Stock solution of vitamins (stock 4)

The vitamins used in the present study for the preparation of MS medium were, Myo-inositol (Inositol), Nicotinic acid (Vitamin B_3), Pyridoxin HCl (Vitamin B_6), Thiamine HCl (Vitamin B_1) and Glycine. Each of the vitamins except myo-inositol were taken at 100 times of their final strength in measuring cylinder and dissolved in 400 ml of distilled water. The final volume was made up to 1000 ml by further addition of distilled water. This stock solution was also labeled and stored in a refrigerator at 4°C.

MS Media preparation

To prepare one liter of MS medium, the following steps were followed:

1. 500 ml double distilled water was taken into 1 liter beaker
2. 4.4gm MS mixture was added in this 500 ml double distilled water
3. 30g of sucrose was dissolved in this solution with the help of magnetic stirrer
4. 8 gm of agar was added and finally the whole mixture was then made up to 1 liter with further addition of double distilled water.

pH of the medium

pH of the medium was adjusted to 5.7±1 by pH meter with the addition of 1 N NaOH or 0.1 N HCl whichever was necessary.

Agar

The media was gelled with 8 g/l agar and the whole mixture was gently heated on microwave oven at 250 °C Temperature for 8-10 minutes.

Preparation of NAA (50X)

A stock solution of NAA with 50x concentration was generally prepared. 0.2 mg NAA was dissolved in few drops of 1N NaOH. In this way, this solution was dissolved in 750 ml of distilled water and final volume was made up to one liter by further addition of DW. The stock solution was poured into a clean sterilized glass container and stored in a refrigerator at 4°C for ready use.

Preparation of BAP (10X)

A stock solution of BAP with 10X concentration was generally prepared. 2 mg BAP was dissolved in few drops of 1N NaOH. This solution was dissolved in 750 ml of distilled water and final volume was made up to one liter by further addition of DW. This stock solution was also labeled and stored in a refrigerator at 4°C.

Preparation of 1N NaOH

40 g NaOH pellets were weighed and dissolved in 900 ml. of sterilized distilled water under stirring condition. The flask in a thermostat at 20^0C and maintain for 1 hour and volume with sterilized distilled water up to 1 L.

Preparation of 70% Ethanol

In a 100 ml measuring cylinder 70 ml 99.9% ethanol was poured. Double distilled water was poured up to the level of 100 ml. The solution was stored in a sterilized glass bottle. This solution was made fresh each time before use.

MS medium supplemented with 50 mM NaCl

The molecular weight of NaCl is 58.44 gm. For preparing MS medium supplemented with 1 M NaCl solution, we needed to add 58.44 g NaCl in 1000 ml of distilled water. So for preparing 50 mM concentration, 2.92g of NaCl was added to the MS medium.

MS medium supplemented with 100 mM NaCl

For 100 mM concentration, 5.84 g of NaCl was added to the 1 L of MS medium.

MS medium supplemented with 150 mM NaCl

For 150 mM concentration, 8.78 g of NaCl was added to the 1 L of MS medium.

MS medium supplemented with 200 mM NaCl

For 200mM concentration, 11.68 g of NaCl was added to the 1 L of MS medium.

Sterilization

Sterilization of culture media

Fixed volume of medium was dispensed into conical flasks. After dispensing the flasks were covered with aluminum foil paper and marked with different codes with the help of a permanent glass marker to indicate specific hormonal supplement. Then flasks were autoclaved at 15 psi pressure at 121°C for 20 minutes. The medium was then transferred into the culture room and cooled at 24°C temperature before used. Marking is also necessary. Fixed volume of medium was aliquoted into petridishes under laminar hood (Plate 1). After dispensing the petridishes were covered with thin polythene (Swaran wrap) and marked with different codes with the help of a permanent glass marker to indicate specific NaCl supplements. The petridishes containing media could be store at 4^0C until use. Marking was done for identification.

Sterilization of glassware and instruments

Glassware, culture vessels, beakers, petridishes, pipettes, slides, plastic caps, other instruments such as forceps, needles, scissor, spatula, surgical blades, brush, cotton, instrument stand and aluminum foil were sterilized in an autoclave at a temperature of 121°C for 20 minutes at 15psi pressure. Before this, all types of glassware instrument was washed properly by liquid detergent, cleaned with running tap water and finally washed with distilled water.

Sterilization of culture room and transfer area

At the beginning, the culture room was sprayed with formaldehyde and then the room was kept closed for one day. Then the room was cleaned through gently washing the floors, walls and rakes with a detergent. This was followed by careful wiping them with 70% ethanol. This process of sterilization of culture room was repeated at regular intervals. The transfer area was also cleaned with detergent and also sterilized twice in a month by 70% ethanol. Laminar air flow cabinet was usually sterilized by switching on the cabinet. The ultra violate ray kills the microbes inside the laminar airflow. It switches on 30 minutes before working in empty condition and for 20 minutes with all the instruments. The working surface was wiping with 70% ethanol, 30 minutes before starting the transfer work.

Raising of seedling *in vitro*

Seeds of six genotypes (Table 1) of *Solanum lycopersicum* L.were surface sterilized and germinated under *in vitro* conditions as per standard tissue culture procedure. Briefly, seeds were surface sterilized with 70% ethanol for 1 min followed by sodium hypochlorite, NaOCl (10%) for six minutes and then soaked with sterilized distilled water for 30 min. Seeds were inoculated in petridishes for germination in a hormone free MS (Murashige and Skoog, 1962) basal medium containing 30 g sucrose and 0.8% agar adjusted with pH- 5.8. Twelve seeds were inoculated per petridishes. The cultures were incubated in growth chamber under 16h/8h light/dark photoperiod with the illuminations of white fluorescence lights (50 µmol/ m^2/s^{-1}) at 25±2 ºC (Plate 2). Thereafter, three weeks-old seedlings were used for explants source. All of the sterilization and inoculation steps were performed under laminar hood.

Explants preparation and inoculation

Three weeks after germination, cotyledons and hypocotyls explants were excised by aseptic manipulations. Explants of hypocotyls and cotyledons (0.5 cm) were isolated and inoculated in test tubes containing MS (Murashige and skoog, 1962) medium supplemented with 30 g sucrose, 2 mg/l BAP and 0.2 mg/l NAA. The pH of the medium was adjusted to 5.8 and solidified by 8 g/l agar. The hypocotyls were cut into a lower, middle and upper segment. The explants were placed horizontally on the medium

surface, leaf discs explants with the adaxial surface in contact with medium. One explant was cultured in every test tube and test tubes were placed in slope condition. The cultures were incubated in normal growth room conditions (16/8 light/dark regime) having the same light intensity and temperature as above for four weeks.

Callus induction and subculture

After four weeks, the callus were induced and were the size was optimum to cut into 0.5 cm^2 pieces aseptically and sub-cultured in fresh MS medium with the same ingredients. The calli were sub-cultured as the nutrient media were exhausted and to amplify the calli for salt treatment in different concentrations. The incubation condition was same as mentioned earlier. Within four weeks of sub-culturing, the calli became organized for different salt treatment.

Salt tolerance assay

The salt tolerance assay was performed as Zeba (2009). Briefly, Four weeks old sub-cultured callus were cut into 0.5 cm^2 pieces under laminar hood and were inoculated in test tubescontaining MS medium mentioned in section 3.5 supplemented with 0 mM, 50 mM, 100 mM, 150 mM and 200mM of NaCl. One piece of callus of each genotype was inoculated per test tube. The culture plates were kept in the growth chamber in vertical position. The culture environment included, 25±2ºC, 60% relative humidity, and a 16-h photoperiod from white fluorescent lights (200 µmol photons/m^2/s^{-1}). The experiment was performed in three replications for each genotype and for each treatment.

Precautions to ensure aseptic conditions

All inoculation and aseptic manipulations were carried out under laminar air flow cabinet. The cabinet was usually switched on with ultra violet light half an hour before use and wiped with 70% ethanol to reduce the chances of contamination. The instruments like scalpels, forceps, needles, surgical blades, scissor, pipettes, slides, plastic caps, spatula, brush, cotton etc. were pre-sterilized by autoclaving and subsequent sterilization were done by dipping in 70% ethanol followed by flaming and cooling method inside the laminar flow cabinet. While not in use, the instruments were kept inside the laminar airflow cabinet into the instrument stand. Hands were also sterilized by 70% ethanol and wearing of hand gloves. It is also necessary to wear apron and mask to avoid contamination rate. Other required materials like distilled water, culture vessels, beakers**,** glass plates, petridishes etc. were sterilized in an autoclave following method of media sterilization. The neck of test tubes were flamed before open and also dipping with ethanol with the help of soaked cotton before closing it with the aluminum foil paper. Aseptic conditions were followed during each and every operation to avoid the contamination of cultures.

Data recorded and statistical analysis

Data were collected and evaluated in terms of the biomass callus weight with digital fine balance and size (diameter) using verneer caliper, after ten days and later after seventeen days of incubation. Tubes were arranged on the shelves of a controlled environment room according to a CRD (completely randomized design). Each tube had a single callus and was considered as an experimental unit. Callus response data were analyzed using MSTAT-C software. The means and the genotype environment interactions were analyzed and the test of significance was performed by DMRT (Duncan's Multiple Range Test).

III. RESULTS AND DISCUSSION

The experiment was carried out at the Genetics and Plant Breeding Laboratory, Sher-e-Bangla Agricultural University to study the performance of different concentrations of salton callus initiation and biomass changing of callus in terms of fresh weight and diameter of six genotypes. This study dealt with the *in vitro* selection of different salt tolerant callus lines in tomato. As salinity in soils is variable and plant tolerance depends on the stage of plant development, in this study, calli were phenotyped at several salinity concentrations and at the most sensitive stage (10 days old callus). The genotypes used in this study were, G1 (BD-7755), G2 (BD-7757), G3(BD-9008), G4(BD-9011), G5(BD-10122) and G6(BD-10123) and the salt concentrations were 0 mM (T1), 50 mM (T2), 100 mM (T3), 150 mM (T4) and 200 mM (T5).

Response of seedlings and callus induction

The seeds of six genotypes were surface sterilized and inoculated in hormone free basal MS medium. The seeds started to germinate within seven days of incubation. Within three weeks of seed inoculation the length of seedlings were in appropriate size to serve as explants source for hypocotyls

and cotyledon. The hypocotyls and cotyledon segments from six tomato varieties were used as explants and cultured on MS medium supplemented with NAA and BAP. The hypocotyls and cotyledon were cut into about 0.5 cm^2 size and inoculated in the test tubes. Within 3-4 days the explants became enlarge and start swelling. Within four weeks of culture the swelled explants gradually turned into green callus. For amplification and maintaining, the callus of each genotype cut into pieces aseptically and subculture in the fresh medium. The gradual change of cotyledon and hypocotyls to the callus is presented in (Plate 3). Auxin is NAA is an important media supplements for callus initiation in culture. The combined effect of different tomato varieties and hormone showed significant variation for days required to callus initiation.

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Plate 3. Gradual change of cotyledon and hypocotyls to the callus. A. Inoculation of seed. B. Germination of seedlings. C. Inoculation of hypocotyl. D. Enlargement and swelling of explants. E. Callus induction. F. Subculture.

Performance of different genotypes under control and salt stress condition

To investigate the salt tolerance in six genotypes, firstly callus were grown and subcultured and then treated with different salt concentration containing 0 mM, 50 mM, 100 mM, 150 mM and 200 mM NaCl. Callus size and weight is the indicator for salt tolerance. So, the callus diameter and weight were assayed twice after treating with different salt concentrations. Initial weight and diameter of each callus of every genotype were also measured to compare with those

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data which were taken after 10 days and 17 days after treating NaCl. The callus growth was the highest in control condition (0mM of NaCl) and gradually decreases as the salt stress increases that is 50 mM , 100mM, 150mM and 200mM. The callus diameter and fresh weight were measured and the results obtained from these studies have been presented and discussed separately under different headings. Each of the parameter as influenced by genotypes, treatments and their interactions were discussed below.

Biomass changing of callus under salt stress at 10 DAT

Size and weight of callus of six genotypes were recorded for 10 days after treatment in different NaCl concentration and significant differences were recorded (Plate 4 and Fig. 1). After 10 days of treatment in case of G6 the highest biomass size was recorded up to 50 mM salt concentration and the lowest was recorded in G5 up to 100 mM salt concentrations. In case of 100 mM the highest biomass size was found in G2 and it was static upto 200 mM salt concentration and the lowest size was found in G1 for both 150 mM and 200 mM salt concentrations. Genotypic variation for biomass size is evident in control (0 mM) and in stressed condition (50 mM, 100 mM, 150 mM and 200 mM) (Fig. 1A). Biomass size reduced gradually as the salt concentration increases. Variable callus size was obtained at 10 DAT in different genotypes.

So, the size of root reduction per treatment was evaluated (Fig. 1B). Callus size reduction is negative in G5 from 50 mM to100 mM, that is root was not reduced at 100 mM rather increased than that of 50 mM. G2 also showed better performance at 50 mM. The biomass size was almost similar to control. G4 size reduced at 50 mM but after that at 100 mM it was similar as in 50 mM (Fig. 1B). After 10 days of treatment in case of G6 the highest biomass weight was recorded upto 50 mM salt concentration whereas biomass size of G5was the lowest from 0 mM to 50 mM salt concentrations In case of 100 mM the highest biomass weight was found in G2 and it was static up to 200 mM salt concentration and thelowest weight was found in G6 for 100 mM and the lowest biomass weight was found in G6 for both 100 mM and 150 mM. In case of 200 mM the lowest weight was recorded for G3. Genotypic variation for biomass weight is evident in control (0 mM) and in stressed condition (50 mM, 100 mM, 150mM and 200 mM) (Fig. 2A). G2 did not loss much weight under 50 mM salt stress (Fig. 2B). Gradually it reducedweight but at severe stress (200 mM) it was showed tolerance to salt (Fig. 2B). Reduction in growth with increasing salinity in growth media may be attributed to water deficit or ion toxicity associated with excessive ion uptake particularly of [Na.sup.+] and [Cl.sup.-] (Satti and Lopez, 1994). Nutrientsimbalance as a result of depressed uptake, shoot transport and impaired internal distribution of minerals especially [K.sup.+] and [Ca.sup.+2] may also explained the reduction in plant growth (Munns, 2008).

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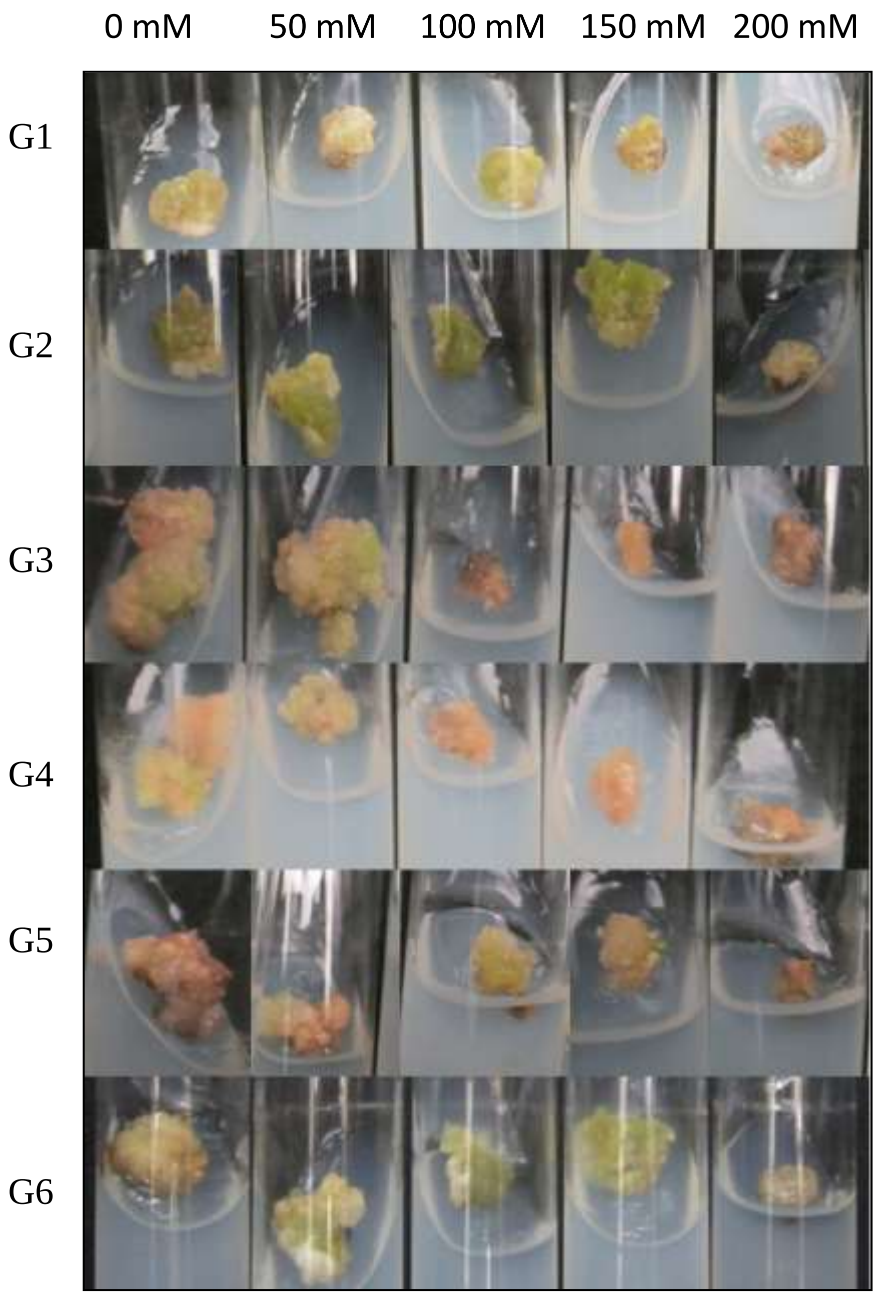

Plate.4: Biomass changing of callus under salt stress at 10 DAT.

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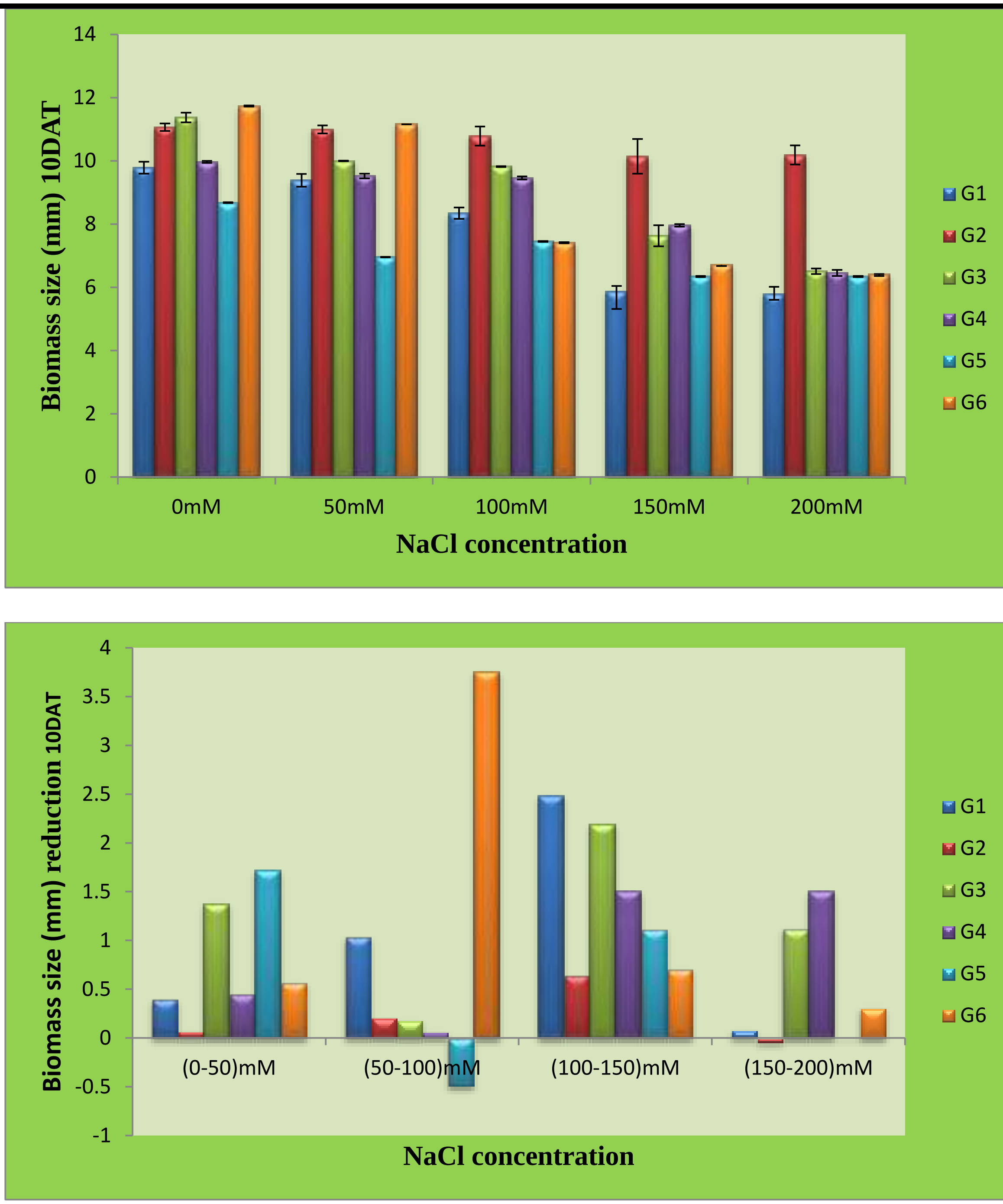


A

B

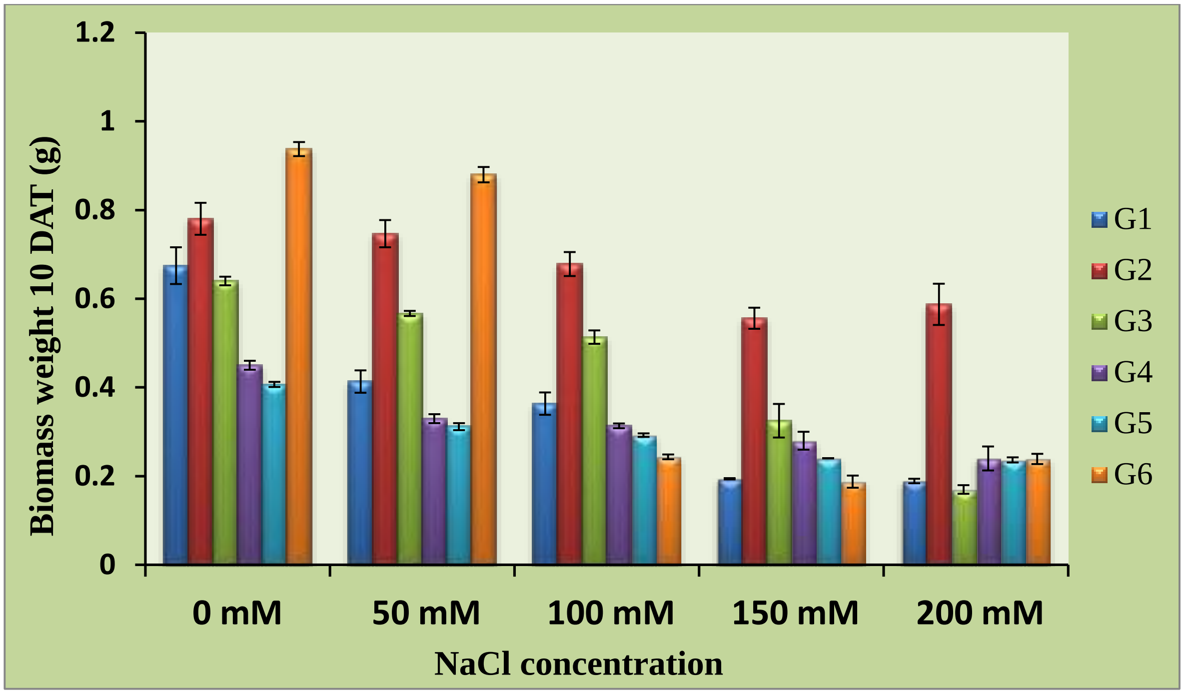
Fig.1: Biomass changing of callus size under salt stress at 10 DAT

A. Mean Biomass size. B. Mean Biomass size reduction 10DAT

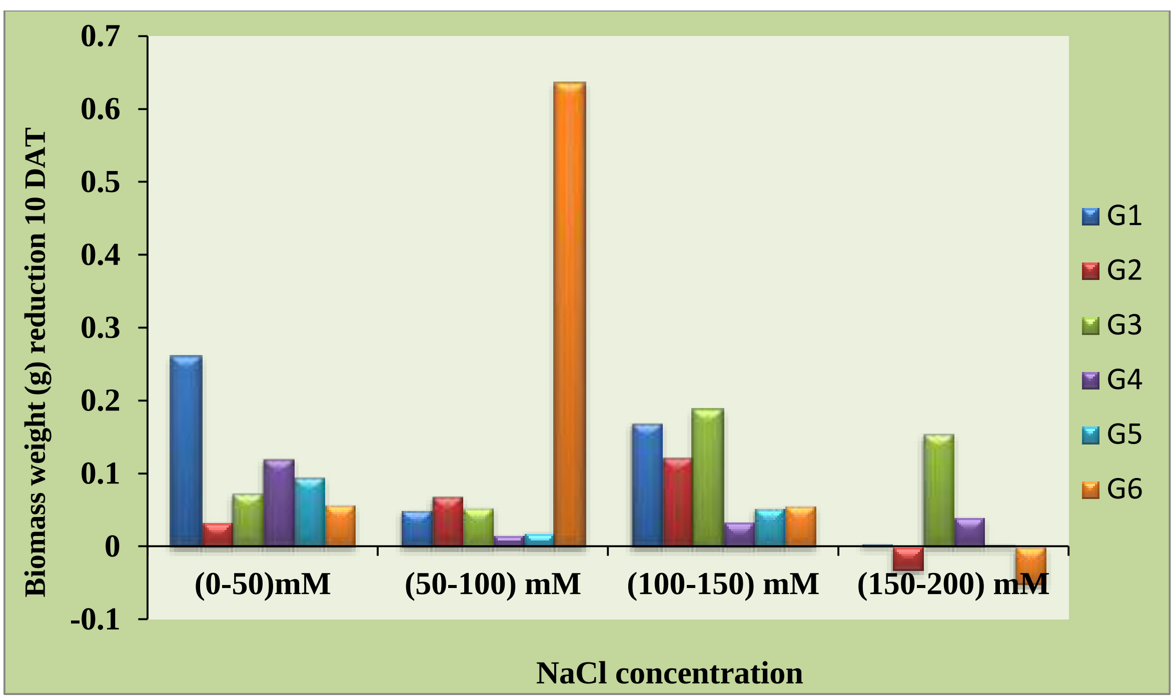
Biomass changing of callus under salt stress at 17 DAT

Size and weight of callus of six genotypes were recorded for 17 days after treatment in different NaCl concentration and significant differences were recorded (Plate 5 and Fig. 3). After 17 days biomass size was the highest in G4 at 0 mM and the lowest size was found in G3 at 0 mM to 50 mM. From 50 mM to 100 mM the highest size was recorded for G5.The highest size was recorded for G2 from 150 mM to 200 mM whereas the lowest size was recorded for G1 for both of these concentrations (Fig. 3A). The G2 was not significantly reduced in biomass size under 50 mM to 100 mM salt. G6 was significantly reduced under light salt stress (50 mM) but later became stable up to 200 mM salt concentrations. G2 and G3 callus also showed stable condition in size from lower to higher salt stress because size was not significantly reduced from control condition to stress condition (Fig 3B).

After 17 days, biomass weight was the highest in G2 from low stress (50 mM) up to the severe stress (200 mM) and size was almost same in control to severe stress condition indicating its tolerance. The lowest weight was found in G3 from 0 mM to 200 mM (Fig. 4A). There was no significant biomass weight reduction in G2 from control (0 mM) to severe stress (200 mM) condition. G6 has the lowest tolerance up to 50 mM regarding its weight but from 150 mM to 200 mM it recovered and showed slight tolerance (Fig. 4B). The addition of NaCl to the culture media decreased the osmotic potential of the media



A



B

Fig.2: Biomass changing of callus weight under salt stress at 10 DAT A. Mean Biomass weight. B. Mean Biomass weight reduction at 10DAT

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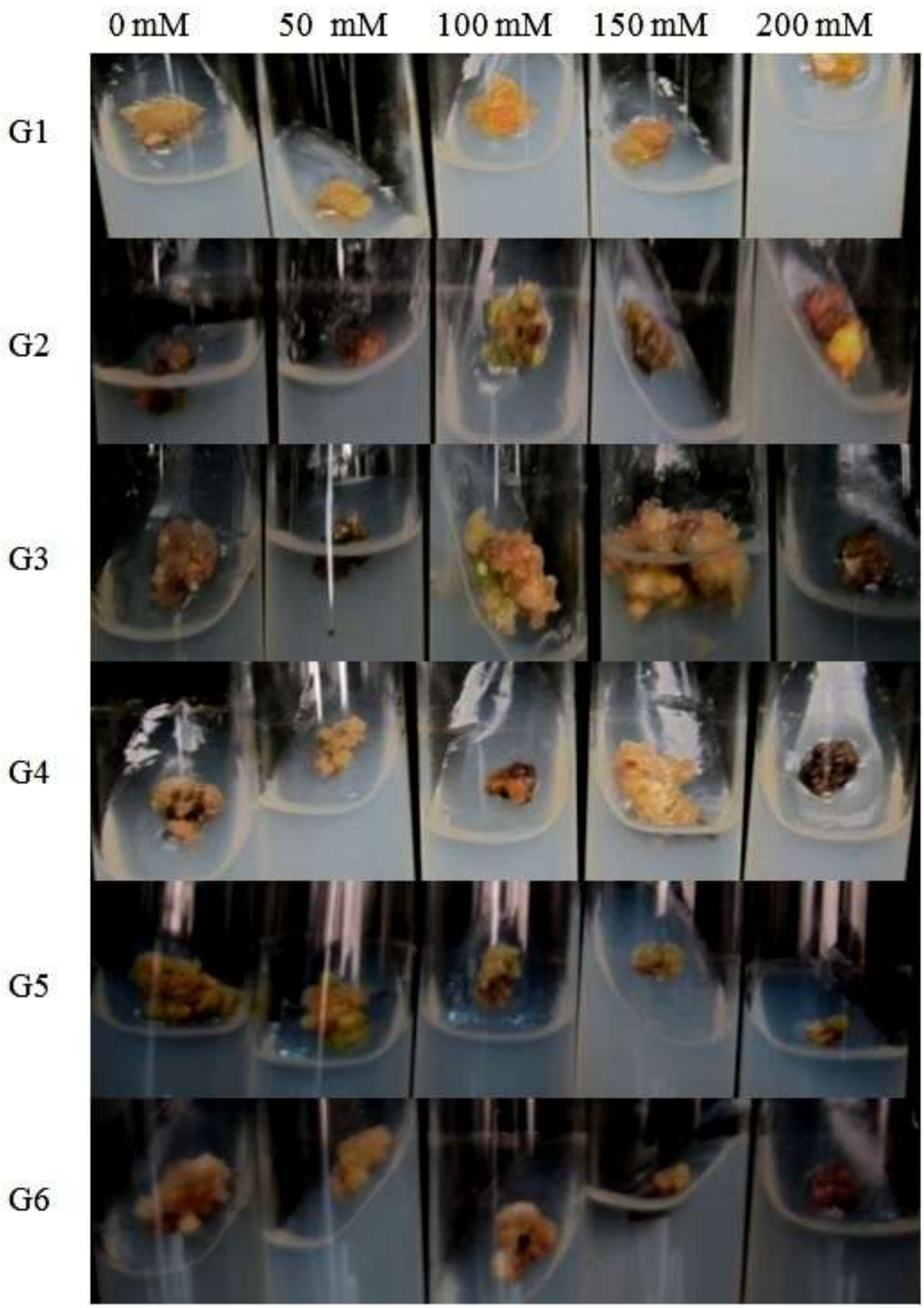

Plate.5: Biomass changing of callus under salt stress at 17 DAT.

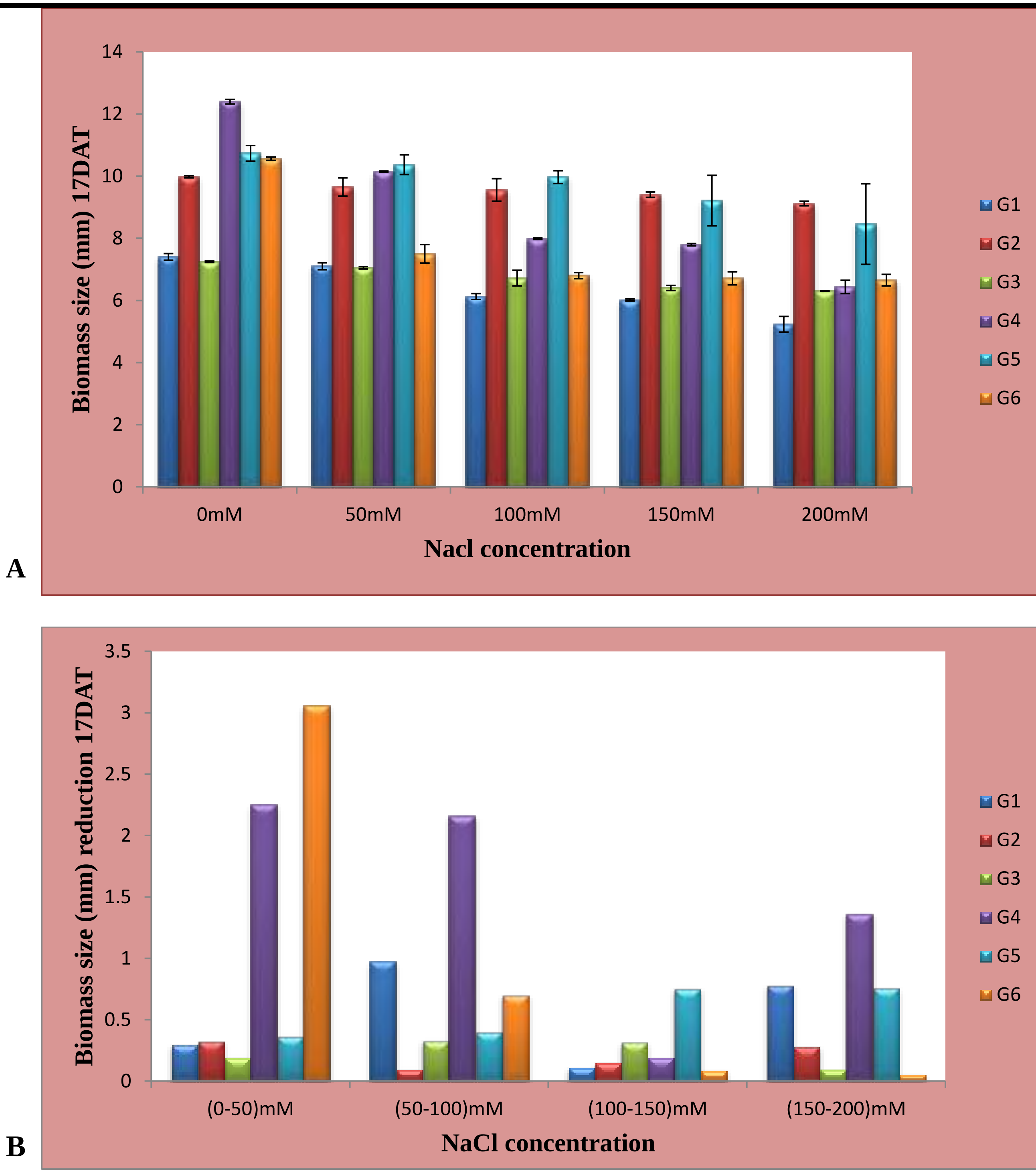

Fig .3: Biomass changing of callus size under salt stress at 17 DAT

A. Mean Biomass size. B. Mean Biomass size reduction 17DAT

inducing salinity stress that adversely affected the callus growth and in vitro regeneration capacity of tomato cultivars. In this study G2 showed moretolerance than the other genotypes might be due the osmotic adjustment. Yang *et al*. (1990) reported that osmotic adjustment in callus results from both Na + and Cl - accumulation. Similar results have been reported for calli of wheat genotypes (Farrukh, 2002). The tolerance of G2 might also be due to the accumulation of proline under stress condition. Proline protects hydration shell in the cell and helps protect from denaturation of protein. Aazami *et al*. (2010) was also in agreement with this concept. With increasing of NaCl, the

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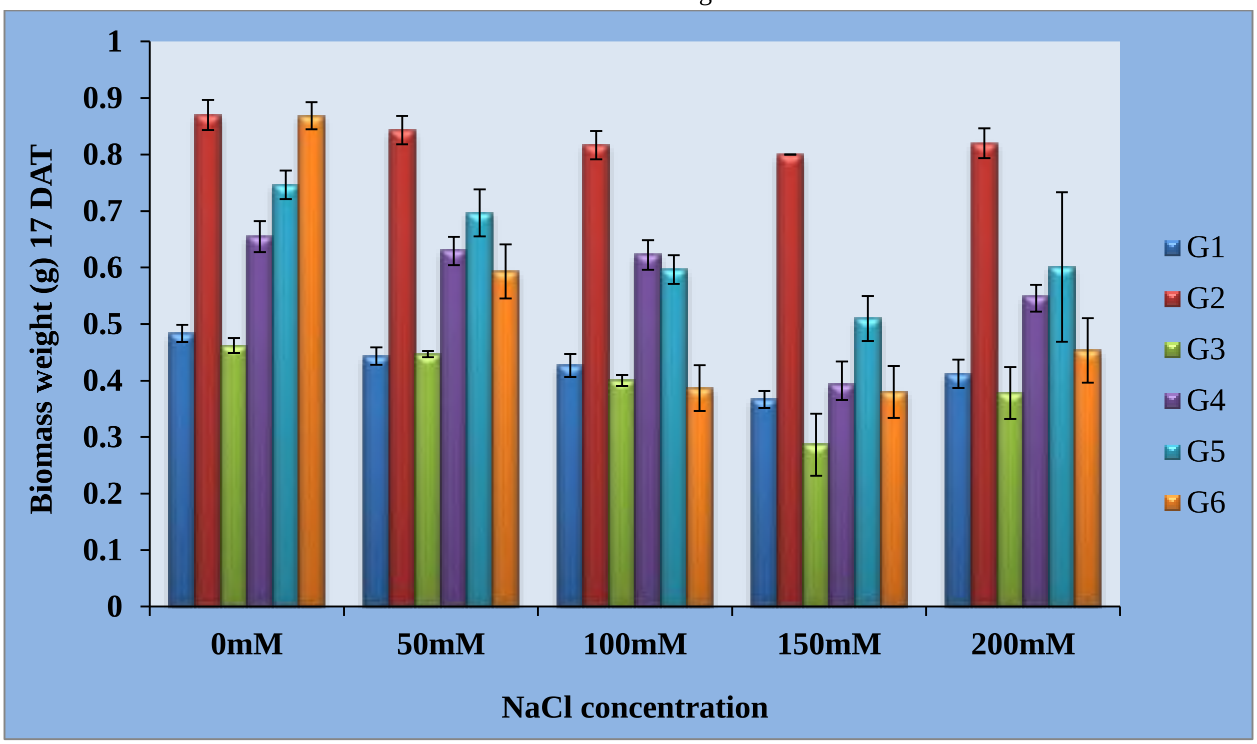
proline content of all cultivars significantly increased in the study of Aazami *et al*. (2010). The rates of accumulation were different depending on cultivars and NaCl levels. The results are in agreement with Emilio *et al*. (1998) for *L. esculentum* and *Lecopersicon pennellii*. Marthinez *et al*. (1996) reported a positive relationship between proline accumulation and NaCl tolerance in potato (Mohamed *et al.*, 2007). Salt tolerance also depends on the antioxidative defense. In spite of the large number of publications on the role of antioxidative defense under salt stress, the relative importance of this process to overall plant salt tolerance is still a matter of controversy. More study are needed for the generation and scavenging of reactive oxygen species (ROS) under normal and salt stress conditions in relation to the type of photosynthesis which are directly related to the stress tolerance because they scavenge toxic compounds in the cell.

Biomass changing in genotype, treatment and their interaction

Biomass changing of callus on genotypes

Salt treatment was given in every genotype and data were taken 10 DAT and 17 DAT. The highest size (diameter) of callus was found in the G2(10.63 mm) at 10 Days After Treatment (DAT) of callus (Table 2) but it was changed after 17 days and G2(9.539 mm)and G5 (9.749 mm) both of them werereached in the highest point. The lowest size of callus was found in G5 at 10 DAT (7.153 mm) and at 17 DAT G1 had the lowest size of callus (6.375). The highest weight was found in G2 for both 10DAT (0.6693 g) and 17DAT (0.8100 g). The lowest weight of callus was found in G4 (0.3227 g) and in G5 (0.2980 g) at 10 DAT (Table 2). At 17 DAT, G2 showed the highest callus weight (0.8100 g) and G3 showed the lowest callus weight (0.3593 g). Significant differences were also found

A



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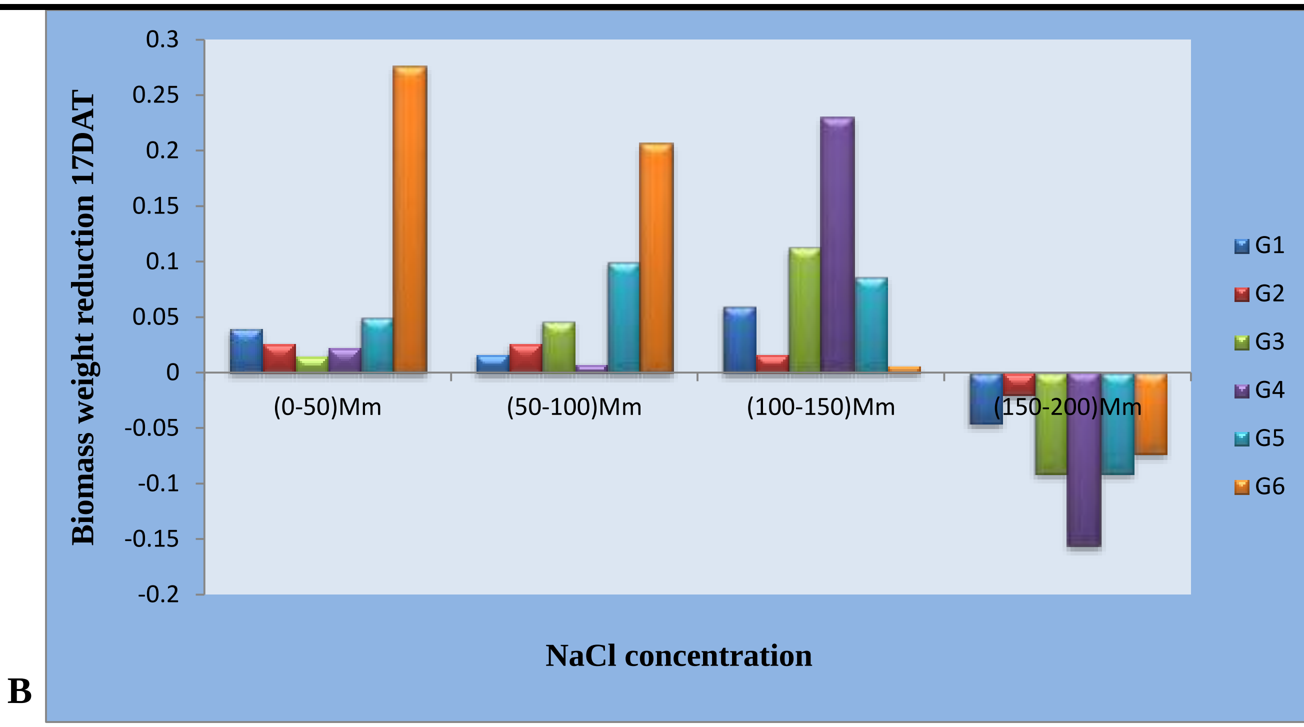

Fig 4. Biomass changing of callus weight under salt stress at 17 DAT

A. Mean Biomass weight. B. Mean Biomass weight reduction at 17 DAT

among cultivars regarding above traits by Aazami *et al*. (2010). Similar results were also found by other researchers (Vijayan *et al*., 2003; Tewary *et al*., 2000; Ben-Hayyim, 1987).

Biomass changing of callus on treatment

The main effect of different concentrations of salt showed significant variation on biomass changing of callus at different DAT. In all of the genotypes 0 mM salt treatment showed the best result whereas 200 mM showed the worst result (Table 3). Any increase in salinity levels in the media led to decrease of callus growth was also found by Aazami *et al*. (2010).

Biomass changing of callus on Genotype X Treatment interaction

The combined effect of genotypes and different concentrations (treatments) of salt showed significant variation on biomass changing of callus at different DAT. At 10 DAT the highest callus size was observed in G6T1 (11.73 mm) that is G6 treated with T1 and the lowest callus size was (5.783 mm) in G1T4 and G1T5. At 17 DAT, the highest biomass size was (12.40 mm) in G4T1 and the lowest callus size was in G1T5 (5.233 mm) (Table 4). As the salt concentration increases, the callus size decreases in G1 at 10 DAT as well as in 17 DAT. There was significant interaction effect between the genotypes and salt concentrations on callus weight also at 10 DAT and 17 DAT. In case of weight also all of the genotypes showed gradual decrease of weight from T1 to T5 at 10 DAT and at 17 DAT. In other genotypes also from G2 to G6, the biomass size of callus and weight were decreases gradually from 0 mM (T1) to 200 mM (T5) concentrations at 10 DAT as well as 17 DAT. The addition of NaCl to the culture media decreased the osmotic potential of the media inducing salinity stress that adversely affected the callus growth. Several authors reported the use of NaCl for *in vitro* salinity screening in different plants (Vijayan *et al*., 2003). In this study, the adaption capacity to different salt level varies with the genotype's degree of tolerance. The genotypic variation for salt tolerance in this study agreed with the result of Rus *et al.* (2001) and Perez-Alfocea *et al.* (1994) who detected different salt stress responses among several tomato cultivars, from an halophytic behavior. Callus growing in this study under the increasing NaCl concentrations reduced relative growth rate in all tomato cultivars as in the study of Ben-Hayyim, 1987, Zhang *et al*. 2004 and Amini and Ehsanpour, 2006. *In vitro* plant tissue culture is useful and quick tool to evaluate plant tolerance to salt stress. Many studies were carried out.

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Table.2: Biomass changing of callus on genotypes

Genotype	Biomass size after 10 days (mm)	Biomass size after 17 days (mm)	Biomass weight after 10days (g)	Biomass weight after 17days (g)
G_1	7.833 d	6.375 e	0.4633 b	0.4087 d
G_2	10.63 a	9.539 a	0.6693 a	0.8100 a
G_3	9.064 b	6.745 d	0.4433 b	0.3593 e
G_4	8.672 c	8.951 b	0.3227 c	0.5107 c
G_5	7.153 e	9.749 a	0.2980 c	0.5940 b
G_6	8.686 c	7.645 c	0.4973 b	0.5173 c
$s_{\bar{x}}$	**0.04397**	**0.08406**	**0.02828**	**0.01155**

G1= BD-7755, G2= BD-7757, G3= BD-9008, G4= BD-9011, G5= BD-10122, G6= BD-10123.

Table.3: Biomass changing of callus on Treatment

Treatment	Biomass size after 10 days (mm)	Biomass size after 17 days (mm)	Biomass weight after 10days (g)	Biomass weight after 17days (g)
T_1	10.43 a	9.719 a	0.6478 a	0.6811 a
T_2	9.670 b	8.636 b	0.5417 b	0.6094 b
T_3	8.879 c	7.859 c	0.4006 c	0.5417 c
T_4	7.441 d	7.589 d	0.3389 cd	0.4561 d
T_5	6.946 e	7.033 e	0.3161 d	0.3783 e
$s_{\bar{x}}$	**0.04014**	**0.07674**	**0.02582**	**0.01054**

T1= 0 mM, T2= 50 mM, T3= 100 mM, T4= 150 mM, T5= 200 mM.

Table.4: Interaction effect of genotype and treatment

Interaction	Biomass size after 10 days (mm)	Biomass size after 17 days (mm)	Biomass weight after 10days (g)	Biomass weight after 17days (g)
G_1T_1	9.783 fg	7.400 jk	0.6733 b-d	0.4833 gh
G_1T_2	9.383 h	7.100 kl	0.4133 f-i	0.4433 g-j
G_1T_3	8.347 j	6.127 no	0.3633 g-j	0.4267 h-k
G_1T_4	5.867 p	6.013 o	0.4433 e-i	0.3667 j-l
G_1T_5	5.783 p	5.233 p	0.4233 f-i	0.3233 l-n
G_2T_1	11.06 cd	9.97 d-f	0.7800 ab	0.8700 a
G_2T_2	10.99 cd	9.65 e-g	0.7467 a-c	0.8433 a
G_2T_3	10.78 d	9.55 e-g	0.6767 b-d	0.8167 ab
G_2T_4	10.14 e	9.400 fg	0.5567 c-g	0.8000 ab
G_2T_5	10.19 e	9.117 g	0.5867 b-f	0.7200 cd
G_3T_1	11.37 b	7.2 j-l	0.6400 b-e	0.4633 g-i
G_3T_2	9.997 ef	7.050 kl	0.5667 c-g	0.4467 g-j
G_3T_3	9.817 f	6.7 l-n	0.5133 d-h	0.4000 h-l
G_3T_4	7.630 l	6.4 m-o	0.3267 h-j	0.2867 mn
G_3T_5	6.510 no	6.303 m-o	0.1700 j	0.2000 o
G_4T_1	9.97 ef	12.40 a	0.4500 e-i	0.6533 d-f
G_4T_2	9.520 gh	10.1 c-e	0.3300 h-j	0.6333 ef

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G_4T_3	9.460 h	7.987 hi	0.3133 h-j	0.6233 ef
G_4T_4	7.957 k	7.793 ij	0.2800 ij	0.3933 i-l
G_4T_5	6.45 no	6.4 m-o	0.2400 ij	0.2500 no
G_5T_1	8.680 i	10.73 b	0.406 f-i	0.7467 bc
G_5T_2	6.960 m	10.37 b-d	0.313 h-j	0.6967 c-e
G_5T_3	7.450 l	9.970 c-f	0.293 ij	0.5967 f
G_5T_4	6.337 o	9.217 g	0.240 ij	0.5100 g
G_5T_5	6.33 o	8.457 h	0.236 ij	0.420 h-k
G_6T_1	11.73 a	10.56 bc	0.9367 a	0.8700 a
G_6T_2	11.17 bc	7.500 i-k	0.8800 a	0.5933 f
G_6T_3	7.41 l	6.800 lm	0.2433 ij	0.3867 i-l
G_6T_4	6.710 mn	6.710 l-n	0.186 j	0.3800 i-l
G_6T_5	6.403 o	6.653 l-n	0.24 ij	0.3567 k-m
$s_{\bar{x}}$	**0.09832**	**0.1880**	**0.06325**	**0.02582**
CV%	**1.98**	**3.99**	**24.21**	**7.33**

G1= BD-7755, G2= BD-7757, G3= BD-9008, G4= BD-9011, G5= BD-10122, G6= BD-10123, T1= 0 mM, T2= 50 mM, T3= 100 mM, T4= 150 mM, T5= 200 mM

Through using different tissue culture methods (Bhatia *et al*., 2008). The callus induction potential was decreased with increasing NaCl levels in this study. A similar observation was found by Yusuf *et al.* (1994), Cano *et al*. (1998) and Mercado *et al*. (2000) in tomato using tissue culture techniques for *in vitro* selection for salinity tolerance. Cultivated tomato is generally classified as being moderately salt-sensitive. Different genotypes of tomato displayed widely different degrees of salinity tolerance (Ghoshal and Bajaj, 1984). Marked differences in the behavior of both susceptible and tolerant tomato genotypes were evident (Cano *et al*., 1998; Rus *et al*., 2001). Yet, an understanding of the mechanisms that plants use to cope with high salinity is necessary to select and develop tomato plants that are more tolerant to salinity.

REFERENCES

[1] Aazami, M.A., Torabi, M. and Shekari, F. 2010. Response of some tomato cultivars to sodium chloride stress under *in vitro* culture condition. African J. Agril. Res. 5(18): 2589-2592.

[2] Aditya, T.L., Rahman, L., Shah-E-Alam, M. and Ghosh, A.K. 1999. Correlation and Path Coefficient Analysis in Tomato. *Bangladesh Agril. Sci*. 26(1):119-122.

[3] Amini, F., Ehsanpour, A.A. 2006. Response of tomato (*Lycopersicon esculentum* Mill.) cultivars to MS, water agar and salt stress in *in vitro* culture. Asian J. Plant Sci., 9(1): 170-175.

[4] Ben-Hayyim, G. 1987. Relationship between salt tolerance and resistance to polyethylene glycol-induced water stress in cultured citrus cells. Plant Physiol. 85: 430-433.

[5] Bhatia P., and Ashwath, N. 2008. Improving the quality of *in vitro* cultured shoots of tomato *(Lycopersicon esculentum* Mill. cv. Red Coat. Biotechnol. 7(2): 188-193.

[6] Cano, E.A., Perez, A., Moreno, V., Caro, M., and Bolarin, M. 1998. Evaluation of salt tolerance in cultivated and wild tomato species through in vitro shoot apex culture. Plant Cell Tiss. Organ Cul., 53 (1): 19-26.

[7] Cuartero, J., and Ferna´ndez-Mun˜oz, R. 1999. Tomato and salinity. Scientia Horticulturae, 78: 83–125.

[8] Cuartero, J., Boları´n, M.C., Ası´ns, M.J., and Moreno, V. 2006. Increasing salt tolerance in the tomato. J. Exp. Bot. 57(5): 1045–1058.

[9] Farrukh, J. 2002. *In vitro* salt tolerance in wheat. III. Water relations in callus. Int. J. Agri. Biol. 4(4): 665-667.

[10] Flowers, T.J., and Yeo, A.R. 1995. Breeding for salinity resistance in crop plants: where next? Aust. J. Plant Physiol., 22: 875–884.

[11] Ghosal, S.S., and Bajaj, Y.P.S. 1984. Isolation of sodium chloride resistant cell lines in some grain legumes. Indian J. Exp. Biol., 22: 209-214.

[12] Martinez, C.A., Maestri, M., and Lani, E.G. 1996. *In vitro* salt tolerance and proline accumulation in andean potato (Solanum spp.) differing in frost resistance. Plant Sci., 116: 177-184.

[13] Mercado, J.A., Sancho, Jimenez, B.S., Peran, U.R., Pliego, A.F., and Quesada, M.A. 2000. assessment of *in vitro* growth of apical stem sections and adventitious organogenesis to evaluate salinity tolerance in cultivated tomato. Plant Cell Tiss. Organ Cul., 62: 101-106.

[14] Mohamed, A.N., Rahman, M.H., Alsadon, A.A. and Islam, R. 2007. Accumulation of proline in NaCl-treated callus of six tomato (*Lycopersicon esculentum* Mill.) cultivars. Plant Tissue Cult. Biotech. 17(2): 217-220.

[15] Perez-Alfocea, F., Guerrier, G., Estañ, M.T. and Bolarin, M.C. 1994 Comparative salt responses at cell and whole-plant levels of cultivated and wild tomato species and their hybrid. J. Hort. Sci. 69: 639-644

[16] Rus, A.M., Estañ, M.T., Gisbert, C., Garcia-Sogo, B., Serrano, R., Caro, M., Moreno, V., and Bolarín, M.C. 2001. Expressing the yeast HAL1 gene in tomato increases fruit

[17] Shatnawi, M.A. 2006. Micropropagation and germplasm storage of Prunus amygdalus by the vitrification method. Jordan J. Agric. Sci.6(4): 446-450.

[18] Tal, M, Heikin H and Dehan K 1978 Salt tolerance in the wild relatives of the cultivated tomato: responses of callus tissues of Lycopersicon esculentumand Solanum pennellito high salinity. Z. Pflanzenphysiol. 86: 231-240.

[19] Tewary, P.K., Sharma, A., Raghunath, M. K. and Sarkar, A. 2000. *In vitro* response of promising mulberry (*Morus* sp.) genotypes for tolerance to salt and osmotic stresses. Plant Grow. Regul. 30: 17-21

[20] Vijayan, K., Chakraborti, S.P. and Ghosh, P.D. 2003. *In vitro* screening of mulberry (*Morus* spp.) for salinity tolerance.Plant Cell Rep. 22: 350-357.

[21] Yang, Y.W., Newton, R. J. and Miller, F. R. 1990. Salinity tolerance in sorghum. II. Cell culture response to sodium chloride in *S. bicolor* and *S. halepense.* Crop sci.30: 781-785.

[22] Zhang, F., Yang, Y.L., He, W.L., Zhao, X., Zhang, L.X. 2004. Effects of salinity on growth and compatible solutes of callus induced from *Populus euphratica*. *In vitro* Cell Dev. Biol., 40(5): 491-494.

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A comparative Quantitative study on Momordin in the fruit and leave extracts of two different cultivars of *Momordicacharantia* Linn

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Abstract— *Momordica charantia, is widely used as a medicinal plant. Studies have revealed that they contain an array of biologically active proteins like momordin which act as anti-tumor, anti-diabetic, and anti-rheumatic. Since momordin is an active compound, we have made a thorough study on the presence of momordin in the leave and fruit extracts of white and green varieties of the plant. Momordin eluted at 3.84-3.85 min under the standardized HPLC condition. It was found that the momordin was present only in the methanolic extracts of fruit and leave samples and not in the water extracts. The leave samples were found to be contained more quantity of momordin (2878.57 µg/mL) when compared with the fruit extract (72.72 µg/mL). It was also observed that green variety of bitter gourd contained more momordin than white varieties.*
Keywords— Momordin, Charatin, HPLC, methanolic extract, Momordica Charantia.

I. INTRODUCTION

Momordica charantia Linn., belonging to the family Cucurbitaceae, is a widely cultivated plant for medicinal and food uses. The fruits of the plant are used for culinary preparations all over the world. The cooked fruits are eaten as a remedy for catarrh, eye and cough. The juice from the green fruit is drunk as a remedy for chronic colitis and dysentery[1].The fruits of the plant are consumed regularly as food and medicine all over the world. The different phytochemical compounds present in the bitter gourd make it suitable for its medicinal use. It is widely used as a medicinal herb and the major bioactive compounds present in the plant are charatin, momordin, momordicineetc. The plants synthesize an array of metabolites characterized as 'phytoanticipins' or as general 'phytoprotectants' that are stored in specialized cellular compartments and released in response to specific environmental stimuli like damage due to herbivores, pathogens or nutrient depletion[2].Bitter gourd also produces an array of secondary metabolites which show medicinal properties. The present study was an attempt to compare the quantity of the momordin present in the leaves and fruits of two cultivars (white and green fruited varieties) of *Momordica charantia*.

II. MATERIALS AND METHODS

Standard: Momordin (1mg/mL). The standard stock was diluted to 100 and 200µg/mL in HPLC grade methanol and used for HPLC analysis.

Samples:Bitter guard leaf and fruit aqueous and methanolic extracts (green and white varieties)

Sample preparation: Samples (1mL) were diluted with 4mL respective solvent (water or methanol) and passed through 0.45µ membrane filter and analyzed by HPLC.

A gradient HPLC system with ODS C column 18 (250x 4.6mm) was used. The HPL Csystem was equipped with software. The mobile phase components were Acetonitrile (90%): 0.0001% phosphoric acid in HPLC grade water (10). They were filtered through 0.2 m membrane filter before use and were pumped out the solvent reservoir at a flow rate 1ml/min. The Hamilton syringe was used for injecting 10µL of samples andeluted isocratically. The column temperature was maintained at 27°C. The absorbance was measured at 215nm.

Momordin Quantification (µg/mL of extract): Momordin content in the sample quantified using the standard curve fit analysis of standard momordin response.

Linear regression equation obtained for momordin standard was used for quantification, $Y = 1.177x – 7.575$, Where

Y = instrument response at 215nm in mV

X = Concentration of momordin in µg/mL.

III. RESULTS

The HPLC chromatogram of standard momordin is presented in Fig. 1 and Fig. 2. Momordin eluted at 3.84-3.85 min under the standardized HPLC condition. The summary of quantification of momordin content in samples are summarized in Table. 1. It was found that the momordin was present only in the methanolic extracts of fruit and leave samples and not in the water extracts. The leave samples were found to be contained more quantity of momordin (2878.**57 µg/mL)** when compared with the fruit extract (72.72 **µg/mL)**. It was also observed that green variety of bitter gourd contained more momordin than white varieties.

Table.1: HPLC summary report.

Sl. No	Sample Code	Retention Time (min)	Peak Area	Momordin Content (µg/mL)	Chromatogram reference
1	Momordin (100 µg/mL)	3.840	97.574		Fig.1
2	Momordin (250 µg/mL)	3.850	291.903	-	Fig. 2
3	Bitter guard_Whitefruit_methanolic extract	3.8	9.54	72.71	Fig. 3
4	Bitter guard_Whitefruit_Aquous	-	-	-	Fig.4
5	Bitter guard_White Leaf_ methanolic extract	3.8	542.98	2338.81	Fig.5
6	Bitter guard_WhiteLeaf_Aquous	-	-	-	Fig. 6
7	Bitter guard_Green fruit_ methanolic extract	3.87	0.71	35.19	Fig. 7
8	Bitter guard_Green Leaf_ methanolic extract	3.87	670.04	2878.57	Fig. 8
9	Bitter guard_Greenfruit_Aquous	3.8	5.76	56.65	Fig. 9
10	Bitter guard_GreenLeaf_Aquous	-	-	-	Fig. 10

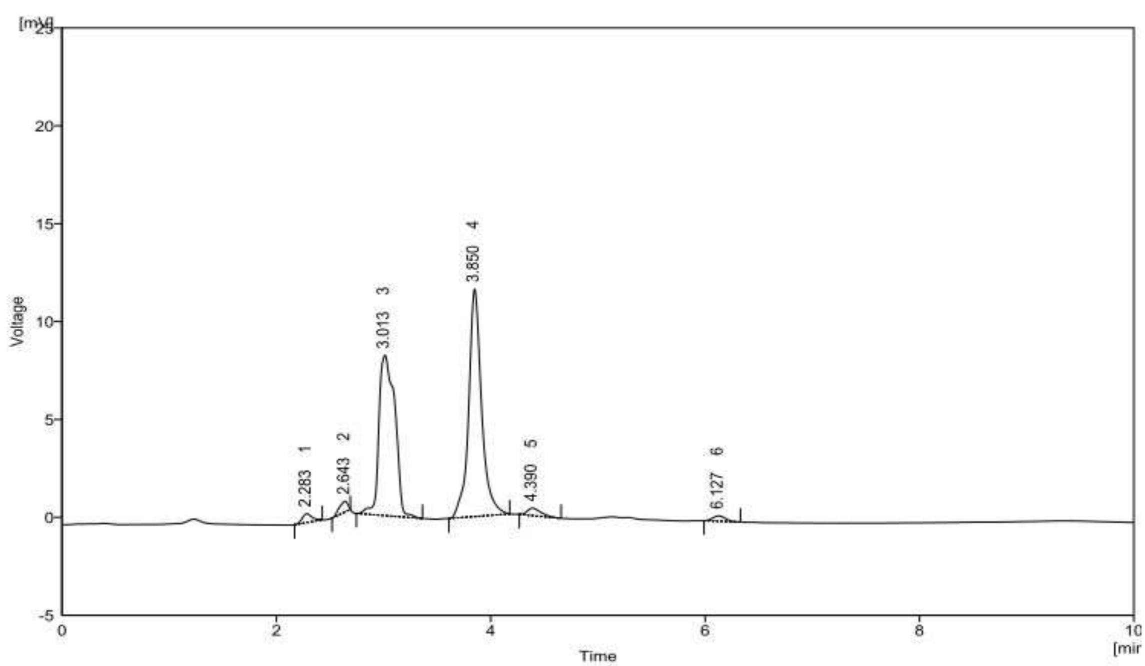

Fig.1: HPLC chromatogram of momordin (100 µg/mL)

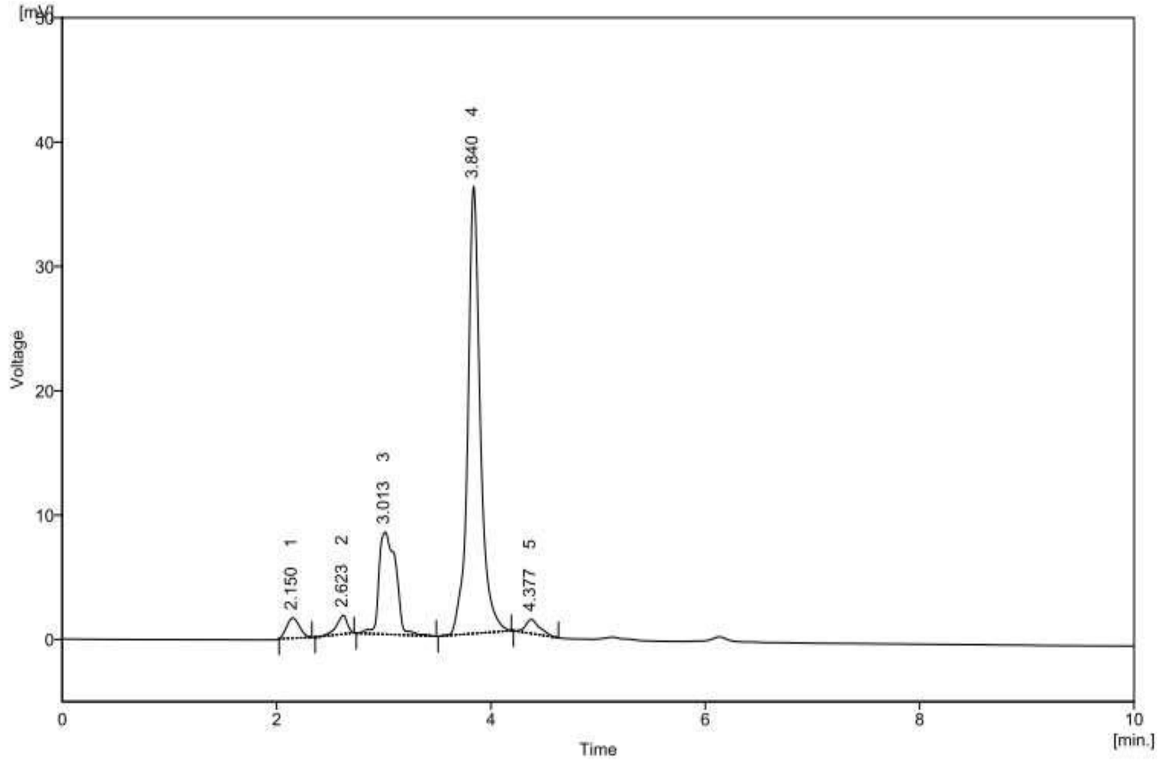

Fig.2: HPLC chromatogram of Momordin (250 µg/mL)

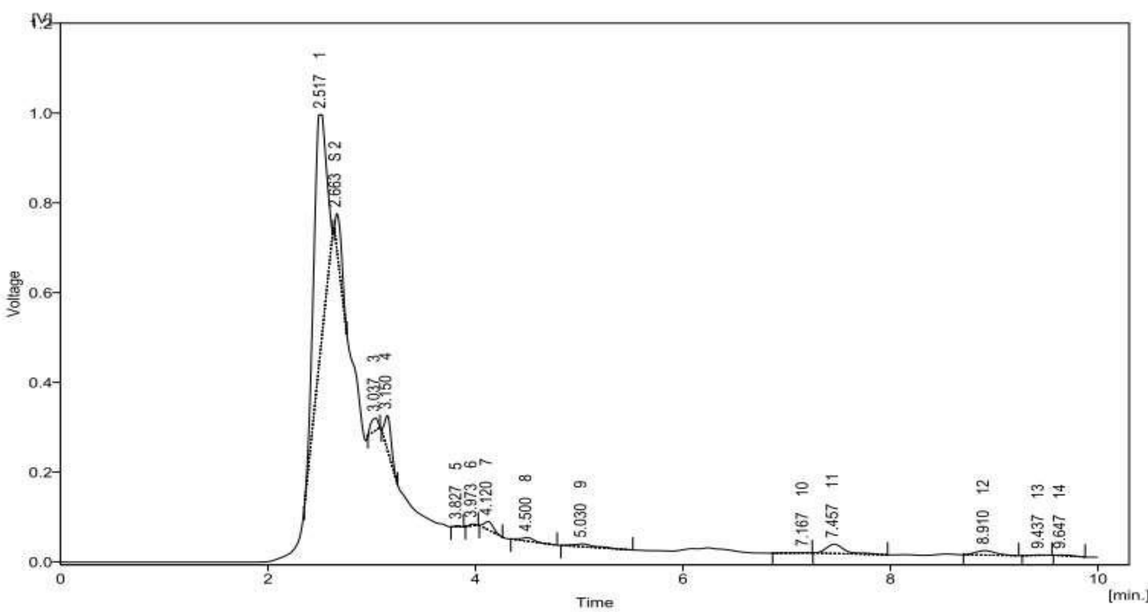

Fig.3: HPLC chromatogram of Bitter guard_White fruit_methanol (1:5 diln)

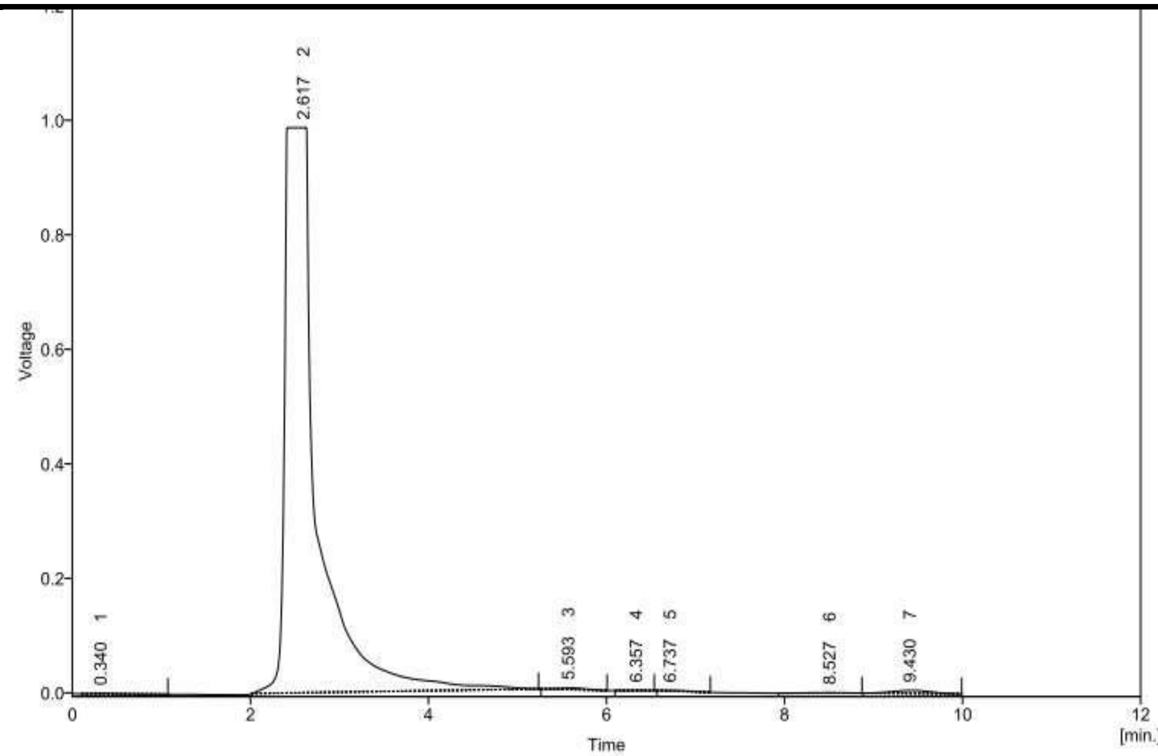

Fig.4: HPLC chromatogram of Bitter guard_Whitefruit_Aq (1:5 diln)

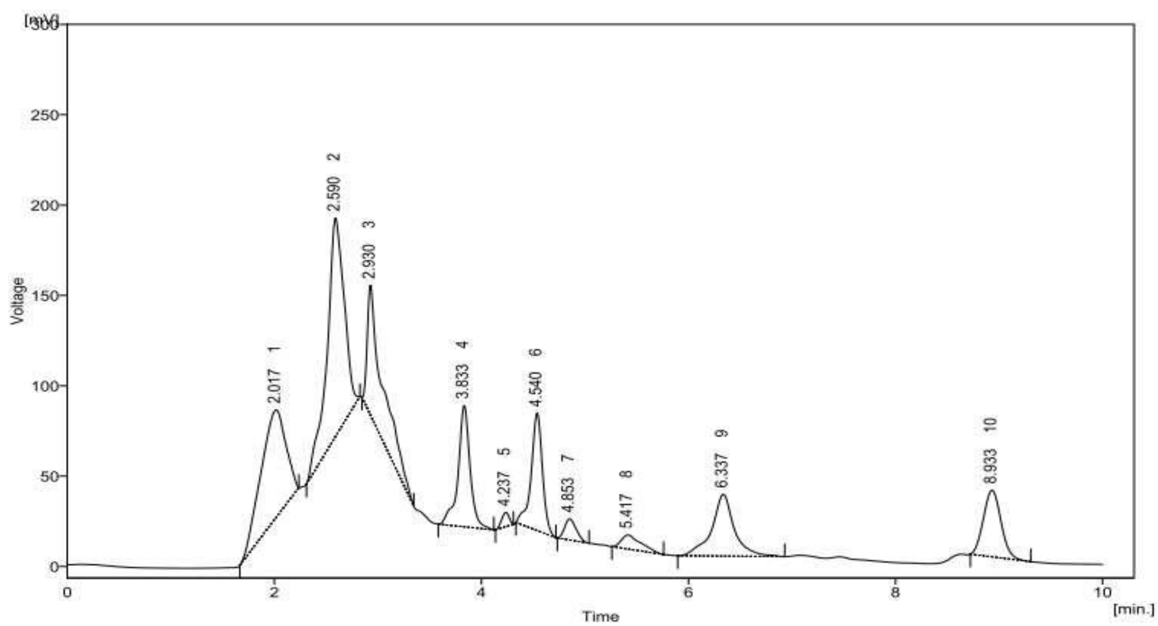

Fig.5: HPLC chromatogram of Bitter guard_White Leaf_methanol (1:5 diln)

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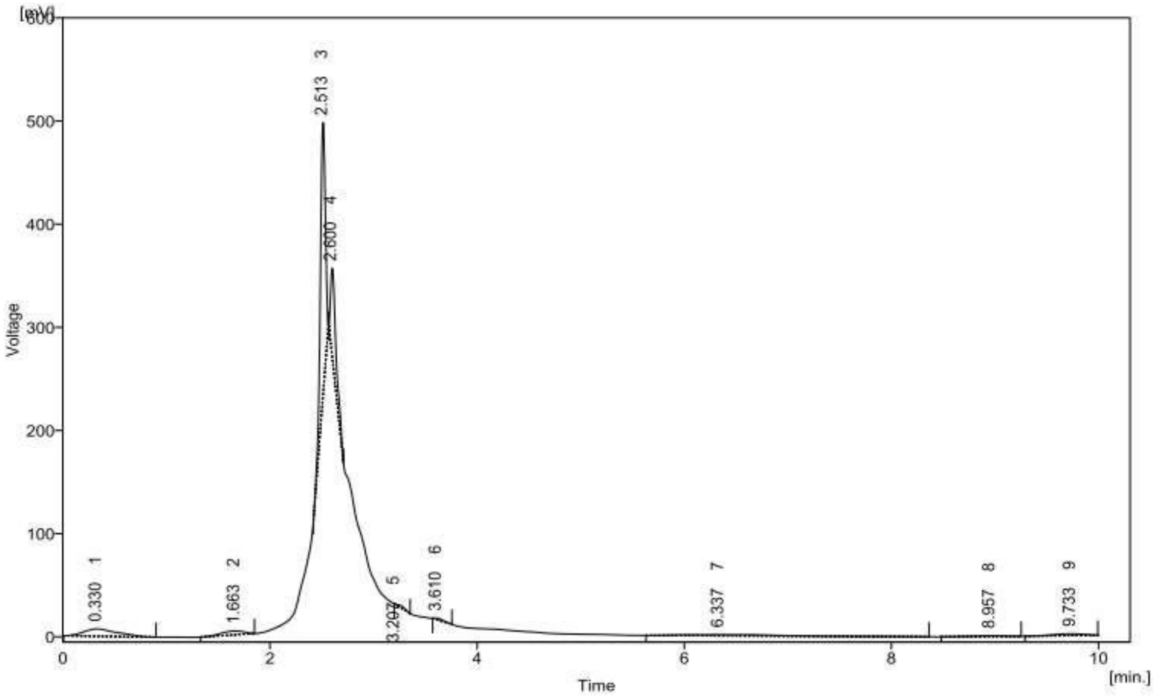

Fig.6: HPLC chromatogram of Bitter guard_WhiteLeaf_Aq (1:5 diln)

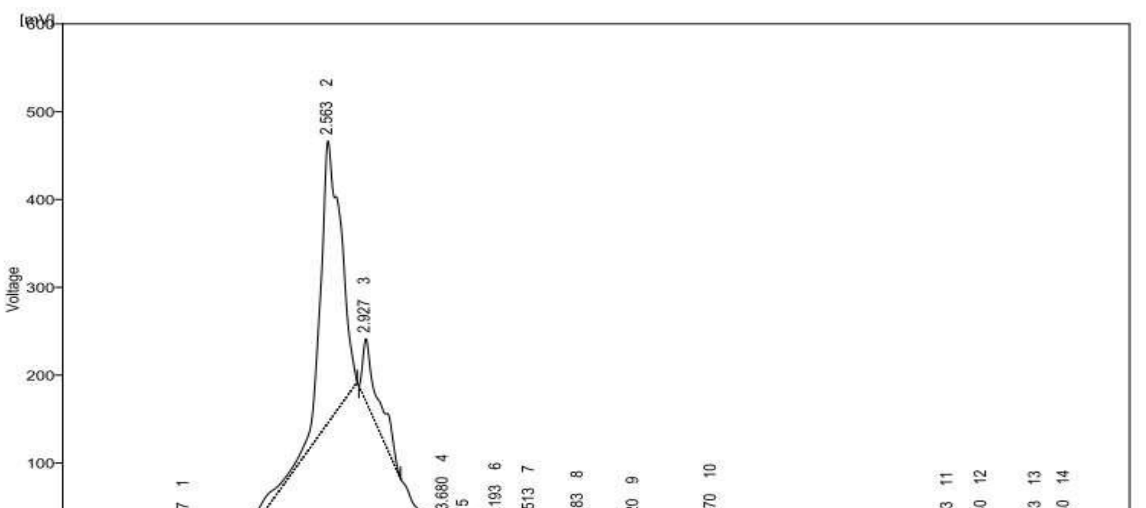

Fig.7: HPLC chromatogram of B Bitter guard_Green fruit_ methanol (1:5 diln)

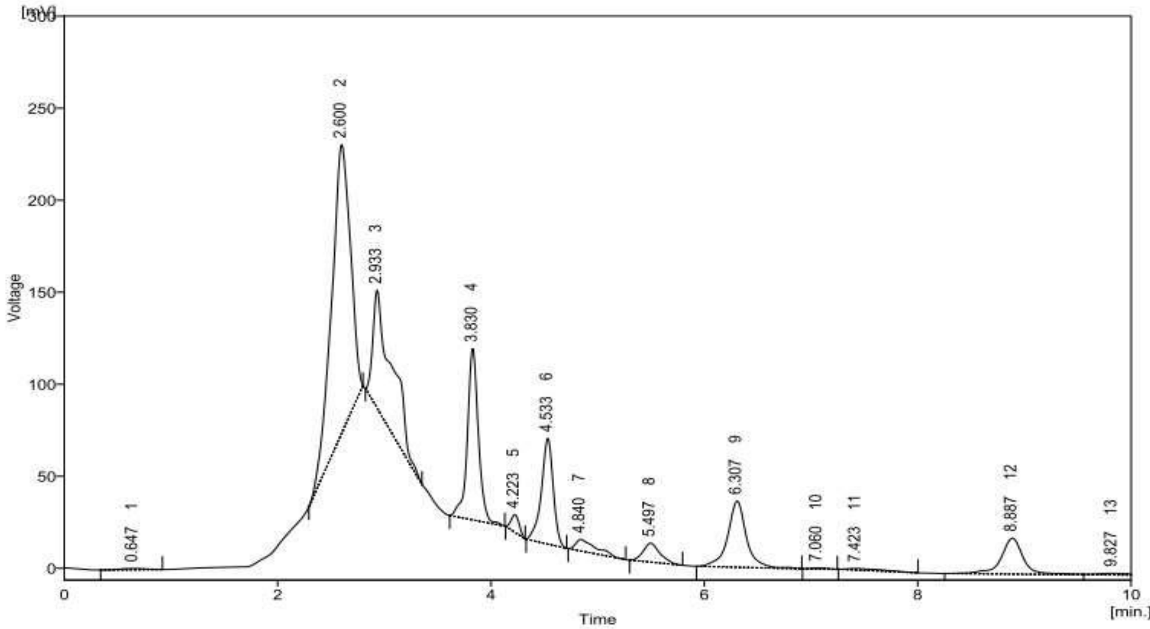

Fig.8: HPLC chromatogram of Bitter guard_GreenLeaf_methanol (1:5 diln)

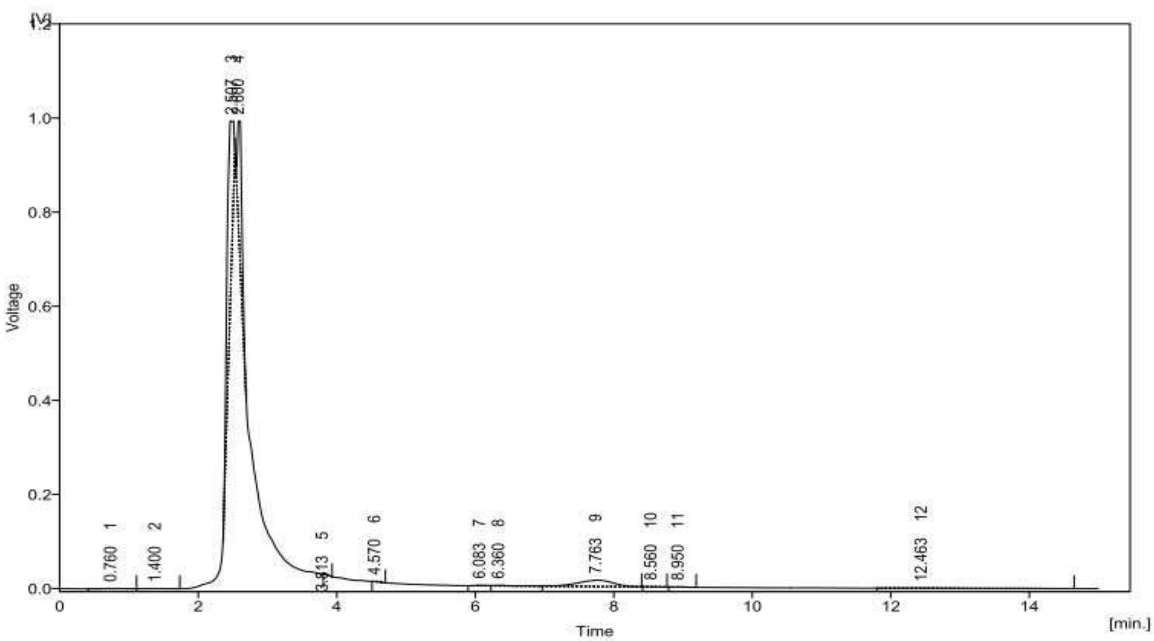

Fig.9: HPLC chromatogram of Bitter guard_Greenfruit_Aq (1:5 diln)

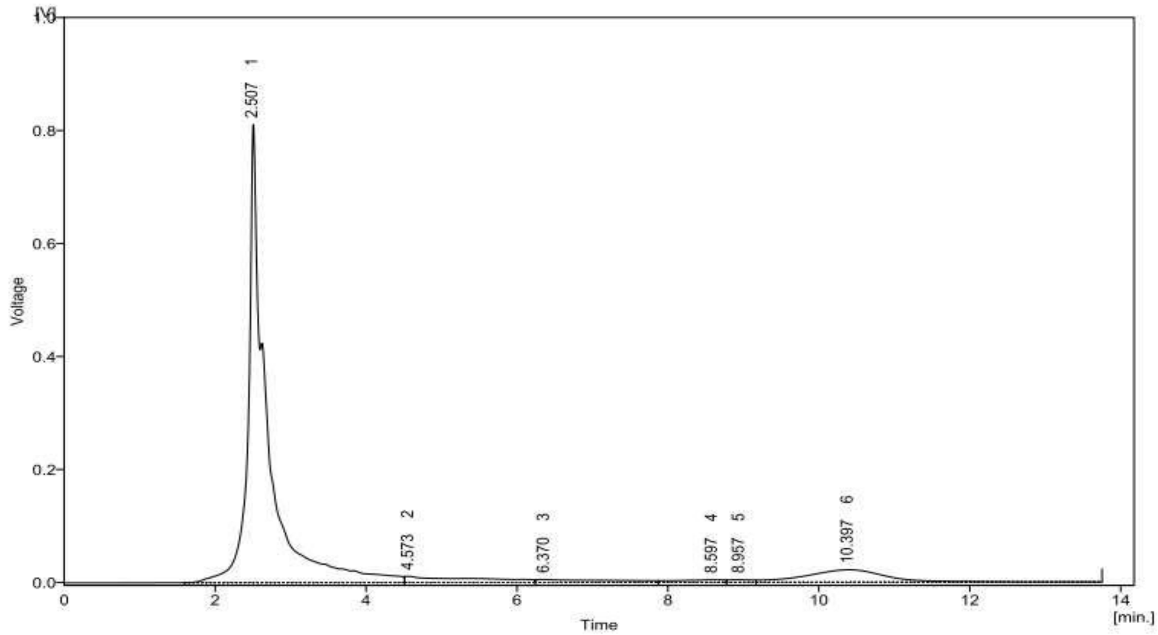

Fig.10: HPLC chromatogram of Bitter guard_GreenLeaf_Aq (1:5 diln)

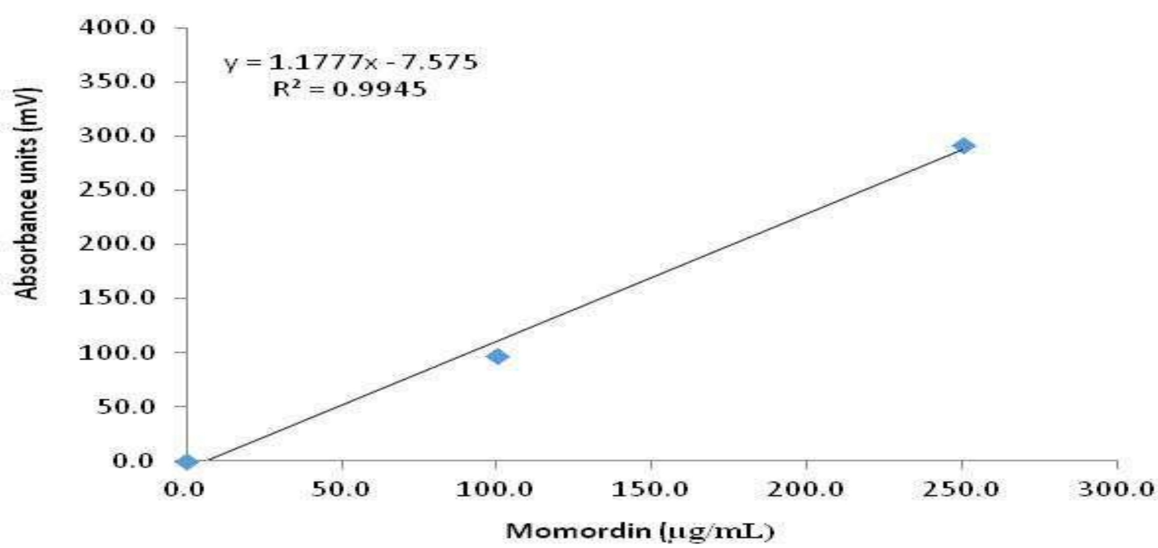

Fig.11: Standard graph of momordin

IV. DISCUSSION

Momordica charantia, widely used as a medicinal plant contains many phytochemical compounds. Many studies have proved that bioactive compounds present in the plant are responsible for the high medicinal value. Studies have revealed that they contain an array of biologically active proteins, namely, momordin, a- and b-momorcharin, cucurbitacin, and MAP30, that have shown to have highly effective anti-human immunodeficiency (HIV), anti-tumor anti-diabetic, and anti-rheumatic properties and to function as febrifuge medicine for jaundice, hepatitis, leprosy, hemorrhoids, psoriasis, snakebite, and vaginal discharge[3]. Since momordin is an active compound, we have made a thorough study on the presence of momordin in the leave and fruit extracts of white and green varieties of the plant. The present study revealed that momordin is high in the leave extracts compared to fruit extracts.

V. CONCLUSION

The study revealed the fact that the leave extracts of the plant *Momordica charantia* contains more quantity of momordin, one of the several saponins derived from oleanolic acid, a triterpenoid. Leaves can be more useful than fruit in terms of its medicinal value.

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REFERENCES

[1] Kuri E. Yuwai et al., “Chemical composition of *Momordica charantia* L. fruits”, J. Agric. Food Chem., 1991, 39 (10), pp 1762–1763.
[2] Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Advances in Nutrition 2011; 2: 32-50.
[3] Prarthna Daniel, UjjwalaSupe, M.G.Roymon “A review on Phytochemical analysis of Momordica charantia”,*International Journal of Advances In Pharmacy, Biology And Chemistry (IJAPBC),* Vol. 3(1), Jan - Mar, 2014.

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Suitability Evaluation of Soils of Ohimini Area of Benue State, Nigeria for Sustainable Rainfed Arable Crop Production

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***Abstract*— *Suitability evaluation of soils of ohimini area of Benue state, Nigeria for sustainable rainfed arable crop production was carried out.* *This study evaluates the suitability and limitations of soils of Ohimini area of Benue State for sustainable maize and Rice production. The research was carried out in Atlo,Ochobo, Atakpa, Ojano,Ijami and Anmoda areas within Ohimin area of Benue State, Nigeria. Soil sampling was carried out from July to August, 2014 at six different locations at 0 - 15 cm and 15 – 30. The bulk samples were air dried and gently crushed using mortar and pestle. The samples were then passed through 2 mm sieve for laboratory analysis. The suitability of the soils was assessed for Rice and Maize by matching their characteristics with the requirements of the crops and their critical limits. The suitability class of a soil is that indicated by its most limiting characteristics. Thus the classes S1, S2, S3 and N represent highly, moderately, marginally and not suitable respectively. The interpretation of critical limit of analytical parameter was done according to the procedure stated by Esu (1991). The soils were generally rated suitable for Rice and Maize production; however, CEC was identified as the most limiting factor. Based on the results, the N status of the soil should be increased by practicing O- minimum tillage and planting crops that are capable of fixing N. Organic carbon level should also be raised through appropriate organic matter maintenance strategies.*

Keywords*— *Suitability, Evaluation, Rainfed, Maize, Rice, arable Crops.

I. INTRODUCTION

Land evaluation is the process of estimating the potential of a land for alternate uses (FAO, 1976). Land evaluation tells the farmer the suitability of his/her land for specific uses and it limitations. This is achieved by matching land qualities/characteristics with the requirements of the envisaged land use (FAO, 1976).

Soil productivity is the capacity of a soil in its natural environment to produce a certain amount of crop per annum. Soil productivity is largely determined by its ability to provide water and nutrients to allow deep rooting of agricultural plants. To better understand the potential productivity of soil, it is important to examine key soil characteristics and indicators, such as soil texture, depth, pH, organic matter and fertility. Soil productivity evaluation remains the most valuable tool for assessing soil health, as a guide in to elucidating processes that could had to increased crop productivity. One of the most serious problems affecting agricultural productivity in tropical regions and developing countries like Nigeria is the ineffective and unplanned use of agricultural land. It is therefore necessary that every piece of land should be used according to its potential capacity (Fasina and Adeyanju, 2006).

The primary and most effective land conservation method is appropriate allocation of lands to uses for who they are most suitable. Land suitability assessment can aid a farmer on the suitability level of his land and its limitations.

Increasingly demand for food in Nigeria as a result of rapid population expansion necessitates a substantial expansion of cultivated areas. Some plants may grow under different soil and extreme agro-ecological conditions, yet not all can grow on the some soil and under the same environment (Dent and Young, 1981).

Rice and Maize are crops of economic importance; Rice is a staple food of over 50% of the total world population (Adesemuyi, 2014,). Rice is a unique crop grown both in upland and lowland. Yield under lowland could reach 2.3 t/ha and able to play dominant role in future production (IITA, 1989), Maize (*Zea mays)* is the most important cereal crop in Sub-Saharan Africa. Every part of Maize has an economic value; the grain, leaves, stalk, tassel and the cob can all be used to produce a large variety of food and non-food products. In industrialized countries, maize is largely used as livestock feed and as raw materials for industrial products, while in developing countries it is used mainly for human consumption, further more it is a source of carbohydrate, protein, iron, vitamin B and minerals.

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However, there is paucity of information in the study area on the extent to which the soils of Ohimini area can satisfy the agronomic requirements of Maize and Rice. This study therefore, evaluates the suitability and limitations of soils of Ohimini area of Benue State for sustainable maize and Rice production.

II. MATERIALS AND METHODS

Study Area:

The research was carried out in Atlo,Ochobo, Atakpa, Ojano,Ijami and Anmoda areas within Ohimin area of Benue State, Nigeria. The area experiences a hot tropical climate with distinct wet and dry seasons. The rainy season starts from April and last till November to March. The mean annual rainfall for this period ranges between 100 - 1600 mm. Crops like Maize, Rice, Soybeans and Cassava are the major crops grown in the area.(Idoga and Ogbu, 2012)

The study area (Ohimini) lies between 7 '30, and 8' 00E. The area has average annual temperature of 28º C. The average relative humidity is highest in September and lowest in December or January with an average of 80%. The major soil type is sandy.

Field Methods:

Soil sampling was carried out from July to August, 2014. Samples were collected from six different locations, in each location, soil samples were collected from an adjacent soil distances of about 50m away. In each location soil samples were obtained from three (3) different points at 0 - 15 cm and 15 - 30 cm using an auger making a total of six (6) soil samples in each location and 36 samples. The bulk samples were air dried and gently crushed using mortar and pestle. The samples were then passed through 2 mm sieve for laboratory analysis.

The Bouyoucos hydrometer method (1951) was used to determine the particle size distribution of the samples. The soil pH in water (1:1) and KCl (1:1) was determined by electromagnetic method as described by IITA (1979).The organic carbon content of the soil samples were determined using the Walkley - Black method described by IITA (1979). Cation Exchange Capacity (CEC) of the soil was obtained by the ammonium acetale (NHOAL) method (IITA 1979).Bray -1 method was used to determine the extractable Phosphorous (Bray and Kurts, 1945).Total Nitrogen was determined by the macro - Kjeldal digestion method (Jackson. 1965).Exchangeable acidity was determined by EDTA hydration method (Jackson, 1965).

Land Evaluation:

The suitability of the soils was assessed for Rice and Maize. Soil was placed in suitability classes by matching their characteristics with the requirements of the crops and their critical limits. The suitability class of a soil is that indicated by its most limiting characteristics. Thus the classes S1, S2, S3 and N represent highly, moderately, marginally and not suitable respectively.

Critical Limits for Interpreting Levels of Analytical Parameters

The interpretation of critical limit of analytical parameter was done according to the procedure stated by Esu (1991).

Table.1: Land and Soil Requirements for Maize, and Rice

Characteristics	Maize				Rice			
Climate	S1	S2	S3	N	S1	S2	S3	N
Rainfall	850-1250	750	600	<500	1800	1400	600	1250
Temperature	22-25	18-22	14-16	<14	22-25	18-22	14-16	<14
Length of dry Season	3-4	4-6	6-7	>7	4-5	5-7	>8	<3
Slope	0-2	4-8	8-16	>16	<4	<8	<16	<25
Drainage	CL.L	SL.LS	LSC	CS	L	LS	S	S
Soil depth	>100	50-75	20-25	<20	>90	>50	>20	>20
CECmolkg^{-1}	>24	16-24	<16	10	>16	>5	Any	Any
BS (%)	>50	35-50	<20	<10	>35	>15	>10	>5
OM (%)	>2	0.8-1.2	<0.8	<0.5	>1.5	>0.8	>0.8	<0.3

Source: **Sys (1985)**

Symbols: CL = Clay loam L = Loam LCS = Loamy Coarse Sand

SL = Sandy loam SC = Sandy clay CS = Clay sand LS = Loamy sand, S = Sandy

S1 = Highly suitable S2 = moderately suitable S3 = Marginally suitable N = Not suitable

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Table.2: Critical Limits for Interpreting Levels of Analytical Parameters

Parameter	Low	Medium	High
Ca^{2+} ($cmol_{(+)}$ kg^{-1})	< 2	2 - 5	>5
Mg^{2+} ($cmol_{(+)}$ kg^{-1})	<0.3	0.3 - 1	> 1
K^{+}($cmol_{(+)}$ kg^{-1})	<0.15	0.15-0.2	>0.3
Na^{2+} ($cmol_{(+)}$ kg^{-1})	<0.1	0.1-0.3	>0.3
CEC ($cmol_{(+)}$ kg^{-1})	<6	6-12	>12
Org. C (g kg^{-1})	<10	10-15	>15
Total N (g kg^{-1})	<0.1	0.1-0.2	>0.2
Avail. P (mg kg-1)	<10	10-20	>20
B.S (%)	<50	30-80	>80

Source: Esu (1991)

III. RESULTS AND DISCUSSION

Soil Properties of the Study Sites

The textural class of the surface and subsurface horizons was sandy loam. Generally, sand content was higher than clay and silt across the locations. The soils across the locations could generally, be described as sandy loam. The predominance of sand separates indicates that the water holding capacity of the soils is low; this could be due to high weathering in the tropics. The textural class of the soil indicates that the soils are likely to be well drained during the wet season and moderately hard during the dry season.

The soil reaction for the sites was strongly acidic in Ojano (4.8), moderately acidic in Atakpa and Atlo (pH 6.7) and alkaline in Ochobo and Ijami (pH 7.1). The low pH was associated with their silica rich parent material (Ojanuga 2006). Most of the pH values of the locations fell within the normal range of 5.5-7.0 reported to be optimum for the release of some plant nutrients (Brady and Weil 2010).The soils of Ojano, Atakpa and Atlo may require liming to raise their pH level to the normal range. The soils were characterized with decreasing pH down their profile. This trend may be due partly to Al^{3+} and H^{+} into the soil solution through isormophous substitution (Tisdale *et al.*, 1995) or maybe linked to the effect of nutrient bio-cycling.

The available P varied amongst the sites. P ranged from 0.28-0.52. The values for the surface horizon were 0.34-0.54 respectively and was rated low, the low P status in these soils maybe due to frequent bush burning or lack of applied phosphorus containing fertilizers by the farmers.

The organic carbon range from 0.40-2.57% and was slightly more in the subsurface, it was rated low for all sites irrespective of the depth. The low organic content of the soil is a characteristic of the Guinea savannah and mineralization of organic matter and to poor management sometimes by burning crop residues by farmers.

The N values across the locations range from 0.006 in Ijami to 0.0083 in Atlo and were rated low both at surface and subsurface. The increase in soil organic carbon and N in the soil profile depth is an indication of the young or immature nature of the profile due to seasonal deposition of materials. Generally, the C/N ratio may favour nitrogen mineralization in these soils (Brady and Weil 2010).

The Ca values ranged from 4.60 at Ojano having the highest value to 2.24 at Ijami and were rated high; Ca was the dominant cation in all sites probably because the alluvial materials from which these soils were formed were derived from sedimentary rocks. The dominance of Ca on the exchange sites may also be attributed to Ca being the least easily lost from the soil exchange complex. It has been said to be the most abundant cation in exchange complex of nearly all soils that are not so acidic as to have high aluminum saturation (Brady and Weil, 2010) in all the sites. Mg was high irrespective of soil depth and was more in the subsurface than the surface

K was high across all locations with values ranging from 0.38 at Ojano to 0.40 at Ochobo at the subsurface. Na was high in all sites except the subsurface region of Atakpa, Atlo, Anmoda And Ochobo, Na reduced with increasing depth and had values of 0.37 at the surface region of Atakpa to 0.21 at the subsurface region of Ijami. The CEC values ranged from 6.22-11.08 at the surface to 7.07-8.34 at the subsurface respectively for Atakpa, Atlo, Ijami, Ochobo and Ojano, CEC was rated medium to low, CEC values was probably as a result of fairly high clay content of the soils. The B.S% values ranged from 87.6-88.6 in Atakpa, 85.1-86.4 in Atlo, 86.6-87.8 in Anmoda, 83.7-84.9 in Ijami,87.00-88.6 in Ochobo and 88.7-89.2 in Ojano respectively. B.S (%) was high across all locations.

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Table.3: Soil Physical Properties of the Study Sites.

Location	Sand	Silt	Clay	Textural Class	Sand	Silt	Clay	Textural class
	0 – 15 cm				15 – 30 cm			
Atk1	78.36	6.0	15.64	SL	74.8	9.0	15.2	SL
Atk2	76.79	7.01	16.20	SL	75.8	9.64	14.0	SL
Atk3	76.8	8.36	16.24	SL	76.36	16.92	13.76	SL
Atl1	75.36	8.40	16.24	SL	76.64	10.0	13.76	SL
Atl2	78.08	7.28	14.64	SL	76.34	10.2	13.46	SL
Atl3	77.8	7.28	15.42	SL	75.72	11.0	13.28	SL
Anm1	77.08	9.02	13.92	SL	76.04	10.64	13.32	SL
Anm2	77.24	9.0	13.76	SL	76.08	9.9	14.02	SL
Anm3	76.82	9.0	14.13	SL	75.64	10.76	13.6	SL
Ija1	75.36	9.22	15.42	SL	75.6	10.40	14. 24	SL
Ija2	75.8	9.4	14.80	SL	75.2	11.0	13. 8	SL
Ija3	75.08	9.72	15.20	SL	75.64	10.7	13.66	SL
Och1	79.2	8.0	12.8	SL	74.08	10.9	15.02	SL
Och2	78.8	8.0	13.2	SL	75.02	9.9	15.08	SL
Och3	79.20	7.9	13.08	SL	74.8	9.96	15.24	SL
Oj1	76.36	10.0	13.64	SL	75.18	10.6	14.22	SL
Oj2	76.20	10.08	13.72	SL	75.34	10.06	14.06	SL
Oj3	76.08	9.9	14.02	SL	75.64	11.1	13.26	SL

SL = Sandy loam, Atk =Atakpa, Atl =Atlo, Anm =Anmoda, Ija =Ijami, Och = Ochobo , Oj=Ojano

Table.4a: Soil Chemical Properties of the Study Sites (0 – 15 cm)

Location	pH	O.C(%)	O.M(%)	P (mg/l)	N(%)	K	Na	Mg	Ca	E.A	TEB	CEC	BS(%)
Atk$_1$	6.02	1.40	2.41	0.40	0.084	0.38	0.34	3.6	3.8	1.04	8.12	9.16	88.6
Atk$_2$	5.88	1.64	2.83	0.34	0.081	0.35	0.31	3.3	3.5	1.00	7.46	8.46	88.2
Atk$_3$	5.96	1.76	3.04	0.48	0.084	0.42	0.36	3.4	3.6	1.10	7.78	8.88	87.6
Atl$_1$	5.04	0.64	1.10	0.32	0.077	0.28	0.23	2.28	3.10	1.12	6.41	7.53	85.1
Atl$_2$	5.78	0.80	1.38	0.34	0.079	0.31	0.26	2.6	2.92	0.96	6.09	7.05	86.4
Atl$_3$	6.27	0.80	1.38	0.32	0.077	0.30	0.26	2.7	2.84	0.98	6.10	7.08	86.2
Anm$_1$	6.30	1.60	2.76	0.44	0.070	0.34	0.30	3.20	3.42	1.12	7.26	8.38	86.6
Anm$_2$	6.74	1. 06	1.83	0.41	0.074	0.36	0.32	3.40	3.60	1.10	7.68	8.78	87.5
Anm$_3$	6.70	1.38	2.38	0.48	0.076	0.34	0.30	3.22	3.34	1.00	7.2	8.20	87.8
Ija$_1$	7.17	0.44	0.76	0.28	0.069	0.26	0.22	2.43	2.54	0.97	5.45	6.42	84.9
Ija$_2$	7.22	0.90	1.55	0.31	0.067	0.29	0.23	2.34	2.40	0.96	5.26	6.22	84.6
Ija$_3$	7.43	0.40	0.69	0.26	0.066	0.24	0.21	2.12	2.24	0.94	4.8	5.75	83.7
Och$_1$	7.42	2.59	4.48	0.56	0.071	0.40	0.37	3.52	3.70	1.20	7.99	9.19	87.00
Och$_2$	7.75	1.18	2.04	0.46	0.069	0.38	0.36	3.50	3.60	1.13	7.84	8.97	87.4
Och$_3$	7.06	2.33	4.04	0.52	0.072	0.43	0.39	3.38	3.90	1.10	8.52	9.62	88.6
Oja$_1$	6.32	2.60	4.48	0.55	0.077	0.46	0.42	4.40	4.60	1.20	9.88	11.08	89.2
Oja$_2$	4.81	2.55	4.42	0.54	0.076	0.44	0.40	4.0	4.40	1.12	9.24	10.36	89.2
Oja$_3$	4.37	2.63	4.55	0.54	0.074	0.46	0.41	4.10	4.42	1.20	9.39	10.59	88.7

Atk =Atakpa, Atl =Atlo, Anm =Anmoda , Ija =Ijami, Och = Ochobo, Oja=Ojano

Table.4b: Soil Chemical Properties of the Sites (15 – 30 cm)

Location	pH	O.C(%)	O.M(%)	P (mg/l)	N (%)	K	Na	Mg	Ca	E.A	TEB	CEC	BS (%)
Atk_1	5.66	1.36	2.35	0.38	0.077	0.40	0.37	3.20	3.50	1.20	7.47	8.67	86.2
Atk_2	5.95	1.38	2.38	0.40	0.077	0.30	0.29	2.70	3.20	1.10	6.69	7.59	85.5
Atk_3	6.00	1.62	2.80	0.44	0.075	0.36	0.31	2.80	3.00	1.00	6.47	7.47	86.6
Atl_1	5.67	2.39	4.14	0.52	0.083	0.39	0.33	2.70	3.20	1.00	6.62	7.62	86.9
Atl_2	6.18	2.63	4.55	0.56	0.084	0.42	0.38	3.40	3.80	1.22	8.02	9.22	87.0
Atl_3	6.22	2.23	3.86	0.43	0.081	0.38	0.34	3.00	3.30	1.20	7.02	8.22	85.4
Anm_1	6.63	2.25	3.90	0.44	0.070	0.36	0.31	2.50	2.90	1.00	6.07	7.07	85.9
Anm_2	6.93	2.57	4.45	0.53	0.069	0.35	0.31	2.60	3.00	1.10	6.36	7.36	86.4
Anm_3	7.16	0.74	1.28	0.32	0.073	0.36	0.32	2.80	3.10	1.10	6.58	7.68	85.7
Ija_1	7.31	2.31	4.01	0.46	0.074	0.39	0.33	2.81	3.20	1.21	6.73	7.94	84.8
Ija_2	7.30	1.56	2.69	0.41	0.073	0.36	0.32	3.00	3.30	1.20	6.98	8.18	85.3
Ija_3	7.46	0.98	1.69	0.37	0.074	0.35	0.29	2.60	2.80	0.97	6.14	7.11	86.4
Och_1	7.42	2.59	4.48	0.56	0.071	0.40	0.37	3.52	3.70	1.20	7.99	9.19	87.00
Och_2	7.75	1.18	2.04	0.46	0.069	0.38	0.36	3.50	3.60	1.13	7.84	8.97	87.4
Och_3	7.06	2.33	4.04	0.52	0.072	0.43	0.39	3.38	3.90	1.10	8.52	9.62	88.6
Oja_1	6.32	2.60	4.48	0.55	0.077	0.46	0.42	4.40	4.60	1.20	9.88	11.08	89.2
Oja_2	4.81	2.55	4.42	0.54	0.076	0.44	0.40	4.0	4.40	1.12	9.24	10.36	89.2
Oja_3	4.37	2.63	4.55	0.54	0.074	0.46	0.41	4.10	4.42	1.20	9.39	10.59	88.7

Atk =Atakpa, Atl =Atlo, Anm =Anmoda, Ija =Ijami, Och = Ochobo, Oj=Ojano

Fertility Status of Soils the Study Sites:

Critical nutrient status of the soils indicates that Ca^{2+} at both surface and subsurface was rated medium(M) for about 100% of the location, Mg^{2+} rated high both at surface and subsurface, K^{+} was rated high also both at surface and subsurface in about 98 (%) of the location, Na^{2+} was high (H) in about 60 (%) of the locations and medium for about 40 (%) of the same location except for Ijami at the subsurface, OC, N and P were rated low (L) for all locations both at surface and subsurface, B.S (%) was high (H) across the locations. The low levels of organic content, N and P of the soil are a characteristic of the Guinea savannah and mineralization of organic matter and to poor management sometimes by burning crop residues by farmers.

Table.5a: Analytical Status of the Soil Chemical Properties of Soils of the Study Sites

Sample Locations/ Depth(0-15cm)	Ca2+	Mg2+	K+	Na2+	CEC	O.C	N	P	BS(%)
ATAKPA 1	M	H	H	M	M	L	L	L	H
ATAKPA 2	M	H	M	M	M	L	L	L	H
ATAKPA 3	M	H	H	M	M	L	L	L	H
ATLO 1	M	H	H	M	M	L	L	L	H
ATLO 2	M	H	H	M	M	L	L	L	H
ATLO 3	M	H	H	M	M	L	L	L	H
ANMODA 1	M	H	H	M	M	L	L	L	H
ANMODA 2	M	H	H	M	M	L	L	L	H
ANMODA 3	M	H	H	M	M	L	L	L	H
IJAMI 1	M	H	H	M	M	L	L	L	H

IJAMI 2	M	H	H	M	M	L	L	L	H
IJAMI 3	M	H	H	M	M	L	L	L	H
OCHOBO 1	M	H	H	H	M	L	L	L	H
OCHOBO 2	M	H	H	M	M	L	L	L	H
OCHOBO 3	M	H	H	H	M	L	L	L	H
OJANO 1	M	H	H	H	M	L	L	L	H
OJANO 2	M	H	H	H	M	L	L	L	H
OJANO 3	M	H	H	H	M	L	L	L	H

KEY: **L** = LOW, **M** = MEDIUM, **H** = HIGH

Table.5b: Analytical Status of the Soil Chemical Properties of Soils of the Study Sites

Sample Locations/ depth(15-30cm)	Ca2+	Mg2+	K^+	Na^{2+}	CEC	O.C	N	P	BS(%)
ATAKPA 1	M	H	H	H	M	L	L	L	H
ATAKPA 2	M	H	M	M	M	L	L	L	H
ATAKPA 3	M	H	H	H	M	L	L	L	H
ATLO 1	M	H	H	H	M	L	L	L	H
ATLO 2	M	H	H	H	M	L	L	L	H
ATLO 3	M	H	H	H	M	L	L	L	H
ANMODA 1	M	H	H	H	M	L	L	L	H
ANMODA 2	M	H	H	H	M	L	L	L	H
ANMODA 3	M	H	H	H	M	L	L	L	H
IJAMI 1	M	H	H	H	M	L	L	L	H
IJAMI 2	M	H	H	H	M	L	L	L	H
IJAMI 3	M	H	H	M	M	L	L	L	H
OCHOBO 1	M	H	H	H	M	L	L	L	H
OCHOBO 2	M	H	H	H	M	L	L	L	H
OCHOBO 3	M	H	H	H	M	L	L	L	H
OJANO 1	M	H	H	H	M	L	L	L	H
OJANO 2	M	H	H	H	M	L	L	L	H
OJANO 3	M	H	H	H	M	L	L	L	H

KEY: **L** = LOW, **M** = MEDIUM, **H** = HIGH

Suitability Status of Soils of the Study Sites

Suitability status of soils of the study sites as it influenced the cultivation of Rice and Maize are presented in Tables 6 and 7. The annual rainfall for the study areas was highly suitable for rice and maize production. Mean Temperature for the locations was 28º and is suitable for both rice and maize production. Base Saturation was rated high across the locations; this indicates high fertility status in the areas. This could be as a result of the non acidic condition of the soils. Soils with high percentage base saturation have higher pH, therefore they are more buffered against acid conditions for plant roots, and they also contain greater amounts of the essential plant nutrient for use by plants. The CEC with respect to rice production was moderately suitable, while CEC with respect to maize production was not suitable. Organic matter content in the study sites was found to be suitable for rice and maize production. Drainage was moderately suitable for rice production and highly suitable for maize production.

Table.6: Suitability Status of Soils of the Study Sites for Rice Production

Location	Annual.R	M.Temp	Slope	Texture	CEC	B.S(%)	O.M	Drainage
ATK1	S1	S1	S2	S1	S2	S1	S1	S2
ATK2	S1	S1	S2	S1	S2	S1	S1	S2
ATK3	S1	S1	S2	S1	S2	S1	S1	S2
ATK3	S1	S1	S2	S1	S2	S1	S1	S2
ATL1	S1	S1	S2	S1	S2	S1	S2	S2
ATL2	S1	S1	S2	S1	S2	S1	S2	S2
ATL3	S1	S1	S2	S1	S2	S1	S2	S2
ANM1	S1	S1	S2	S1	S2	S1	S1	S2
ANM2	S1	S1	S2	S1	S2	S1	S1	S2
ANM3	S1	S1	S2	S1	S2	S1	S1	S2
IJA1	S1	S1	S2	S1	S2	S1	N	S2
IJA2	S1	S1	S2	S1	S2	S1	S1	S2
IJA3	S1	S1	S2	S1	S2	S1	S3	S2
OCH1	S1	S1	S2	S1	S2	S1	S1	S2
OCH2	S1	S1	S2	S1	S2	S1	S1	S2
OCH3	S1	S1	S2	S1	S2	S1	S1	S2
OJA1	S1	S1	S2	S1	S2	S1	S1	S2
OJA2	S1	S1	S2	S1	S2	S1	S1	S2
OJA3	S1	S1	S2	S1	S2	S1	S1	S2

KEY: S1, S2, S3 and N represent highly, moderately, marginally and not suitable respectively.

Table.7: Suitability Status of Soils of study sites for Maize Production:

Location	Annual.R	M.Temp	Slope	Texture	CEC	B.S(%)	O.M	Drainage
ATK1	S1	S1	S1	S1	N	S1	S1	S2
ATK2	S1	S1	S1	S1	N	S1	S1	S2
ATK3	S1	S1	S1	S1	N	S1	S1	S2
ATK3	S1	S1	S1	S1	N	S1	S1	S2
ATL1	S1	S1	S1	S1	N	S1	S2	S2
ATL2	S1	S1	S1	S1	N	S1	S2	S2
ATL3	S1	S1	S1	S1	N	S1	S2	S2
ANM1	S1	S1	S1	S1	N	S1	S1	S2
ANM2	S1	S1	S1	S1	N	S1	S1	S2
ANM3	S1	S1	S1	S1	N	S1	S1	S2
IJA1	S1	S1	S1	S1	N	S1	N	S2
IJA2	S1	S1	S1	S1	N	S1	S1	S2
IJA3	S1	S1	S1	S1	N	S1	S3	S2
OCH1	S1	S1	S1	S1	N	S1	S1	S2
OCH2	S1	S1	S1	S1	N	S1	S1	S2
OCH3	S1	S1	S1	S1	N	S1	S1	S2
OJA1	S1	S1	S1	S1	N	S1	S1	S2
OJA2	S1	S1	S1	S1	N	S1	S1	S2
OJA3	S1	S1	S1	S1	N	S1	S1	S2

KEY: S1, S2, S3 and N represent highly, moderately, marginally and not suitable respectively.

IV. CONCLUSION

The soils were generally rated suitable for Rice and Maize production; however, CEC was identified as the most limiting factor. Based on the results, the N status of the soil should be increased by practicing O- minimum tillage and planting crops that are capable of fixing N. Organic carbon level should also be raised through appropriate organic matter maintenance.

REFERENCES

[1] Adesemuyi EA (2014). Suitability Assessment of soils for Maize (Zea mays) production in a humid tropical area of South-western Nigeria. Int. J. Adv. Res. 1(2): 538-546.

[2] Brady,N.C and R Weil (2010).Element of the nature and properties of soils.3rd edition,Pearson Education,Inc.,Upper Saddle Rier,New Jersey07458.163pp.

[3] Bray,R.H and L.T. Kurtz(1945).Determination of total organic available forms of P in the soil, soil science 59:39-45.

[4] Bouyoucos, G.H. (1951) A Recalibration of the Hydrometer Method for making mechanical analysis of the soil. Argon J. 43: 434-438

[5] Dent D, Young A (1981). Soil Survey and Land Evaluation. George Alien and Unwin Ltd., London, UK.

[6] Esu,I.E(1991)Detailed Soil survey of NIHORT Farm at Bunkure, Kano state, Nigeria, institute for Agricultural Research. Ahmadu Bello University Zaria,Nigeria 72 pp.

[7] FAO (1976). A Frame work for Land Evaluation. FAO Soils Bull. 32: FAO, Rome, 87pp.

[8] Fasina A.S, and Adeyanju , S(2006). Suitability classification of some granitic soils of humid west Nigeria for rain fed maize, cassava and swap rice production. Niger. J. Soil Sci. 16:1-9.,

[9] Jackson, B (1965) USA monograph No 9 methods of soil analysis part || Kang, B.T

[10] IITA (1989). The cowpea biotechnology and natural pest control Research Briefs 9 . (2) 1-3 international institute of Tropical Agriculture, lbadan, Nigeria.

[11] Idoga, S and Ogbu, J. O (2012). Agricultural potentials oh the Anbilla Plateau of Benue state, Nigeria. Nig. Journal of Soil Sci. 22(2) : 224 – 238.

[12] IITA, (1979). Selected Methods for Soil and plants Analysis. Manual Series No. 1 IITA, Ibadan Nigeria.

[13] Ojanuga, A.G (2006).Management of fadama soils for Food Security and Poverty. Soil science Society of America (SSA 1975).Glossary of soil science terms. Medison, Wiscon sin.

[14] Sys C. (1985). Land Evaluation in State University of Ghent part I and II

[15] Tisdale,,S.L., Nelson and J.D.Beaton (1985).Soil Fertility and Fertilizer 4th Edn. MacMillian publishers, New York, pp 382-391.

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Effect of Tillage and Mulch on Growth and Performance of Maize in Makurdi, Benue State, Nigeria

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Abstract— *Field experiments were conducted during the 2015 and 2016 cropping season at the Teaching and Research Farm of the University of Agriculture Makurdi, Benue State, Nigeria to evaluate the effect of different tillage systems and mulch application on the growth and performance of maize. Four tillage systems (minimum tillage, flat bed, ridge tillage and no tillage) and mulch at two levels (mulched and unmulched) were used. Data was recorded on plant height (cm), leaf area (cm^2) of maize, dry cob length (cm), dry cob width (cm) 1000-grain weight (g) and grain yield (t ha^{-1}). Tillage methods significantly affected maize growth. The maximum plant height (178.8cm) leaf area (487.0cm) 1000-grain weight (0.2500g) and grain yield (1.4g) were observed in ridged tillage while no tillage as compared to minimum tillage and flat bed. Mulch significantly affected the growth of maize. The maximum values of plant height (144.5cm), leaf area (411.0cm) dry cob length (cm) (11.16cm), dry cob width (10. 52cm), 1000-grain weight (0.1717g) and grain yield (0.90 tons/ha) were obtained when mulch was applied compared to the unmulched plots. There was no significant difference between the interaction of tillage and mulch. Ridged tillage × mulch produced the best result on maize performance*

Key words— Tillage, mulch, maize, growth, and performance.

I. INTRODUCTION

Maize *(Zea mays L)* is one of highly consumed cereal crops ranked the first in terms of production and third in terms of consumption among the ten staples that feed the world and therefore, dominates agriculture in many regions of the world. In Nigeria, maize is an important food fodder and industrial crop grown both at commercial and subsistence levels, it is eaten fresh or made into flour and also as livestock feed. The increasing use of maize gives Agricultural production in Nigeria can be enhance through the use of various agronomic practices that ensure more efficient use of limited resources to improve the growth of crops and their yield. Management practices that leaves crop residue on soil surface have shown to enhance crop growth (Odofin, 2005).The use of inorganic fertilizer has proven to be more convenient and impactful, but the resulting rapid soil physical degradation, soil nutrient imbalance, increase soil acidity cast and security of fertilizer at the time required have drawn the attention of researcher to the use of other methods of improving productivity.

Mulch materials and tillage systems influence soil properties giving rise to significantly better root growth and yield of maize compared to no mulch treatment due to increase soil water content resulting from reduce evaporation and increase infiltration.

The aim of this work was to assess the effects of mulch application on maize growth and grain yield under four tillage systems in Makurdi, Benue State, Nigeria.

II. MATERIALS AND METHODS

Experimental Area/Site

This experiment was carried out at the Teaching and Research Farm, Federal University of Agriculture, Makurdi, Benue State. Makurdi lies between latitude 7^0 and 8^0N as well as longitude 8^0 and 9^0E. Makurdi has an average relief of 120 m above the sea level. The mean annual temperature range is between 22^0C and32^0C while the relative humidity ranged between 50% and 80% and is season dependent. The highest relative humidity occurs between June and September while the lowest is December and February (Adaikwu *et al,* 2012). The mean annual rainfall is 1250mm. Two peaks of rainfall are observable, June-July and September-October. Soil textural class is loamy sand. The land use of the study site includes arable crops (yam, cassava, soya bean, cowpea, and maize) while the trees include mango and citrus. The vegetable crops include: eggplant, amaranthus, ugu and okra.

Experimental Treatments and Design

The study was made up of two factors: Tillage at four levels: minimum tillage, flat bed, ridge tillage and no tillage and Mulch at two levels: mulch and unmulch. The treatment combinations was as follow: min-till x unmulched, min-till x mulched, ridge x unmulched, ridge

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x mulched, Flat bed x unmulched, Flat bed x mulched, No-till x unmulched and No-till x mulched

Field Layout

The treatments were laid out in a Randomized Complete Block Design (RCBD) and replicated three times. The field layout and its replication is presented in Figure 1.

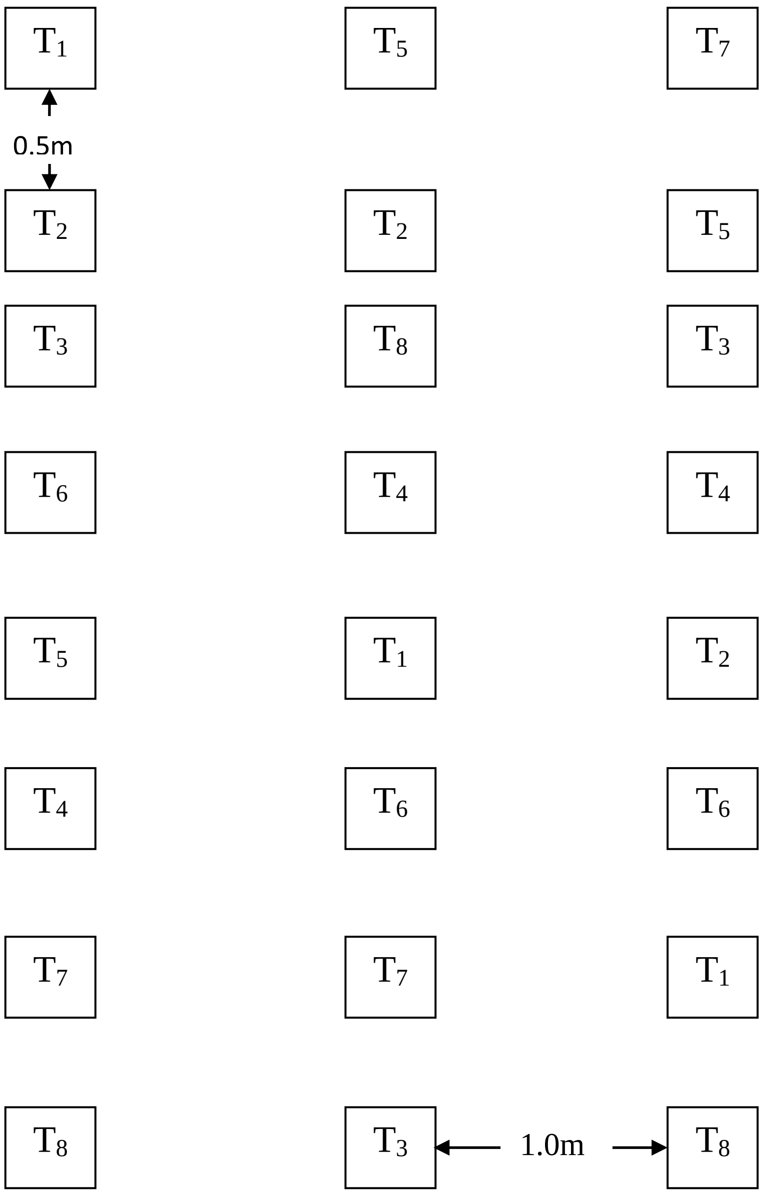

Fig.1: Experimental Layout

Land Preparation and Planting

The land was manually prepared using hoe and cutlass along the contour. Contour bond was constructed at the upper edge of the plots demarcation of the field into blocks and plots were carried out. Maize (*Zeamays* L.) seeds were gotten from the University of Agriculture Experimental Farm. Four maize seeds were planted using the standard plant spacing and latter thinned to one plant. Plant residues from the cleared plots were used to mulch the maize plots at the rate of 45 ton ha^{-1}. The plots measured 3mx5m ($15m^2$) with inter block spacing of 1 m and inter plot spacing of 0.5m were ensured and the harvest was done at maturity

Soil Sample Collection/Analysis

Initial soil sampling was carried out at a depth of 0-15cm from three locations using soil auger and core samplers. Soil samples were also collected at harvest using soil auger at 0-15cm at appropriate plots for analysis in order to assess the effect of management practices on soil. The collected soil samples were air-dried and ground to pass 2 mm sieve. Soil pH was determined in a 1:1 soil-water suspension by the glass electrode method, particle size analysis by the hydrometer method of Bouyoucos (1951) in which sodium hexametaphosphate (Calgon) was used as dispersing agent. Total organic carbon by the chromic acid oxidation procedure of Walkley and Black (1934),

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exchangeable bases by the neutral ammonium acetate saturation. Na and K in the extracts were determined by the flame photometer while Ca and Mg were determined with the Atomic Absorption Spectrophotometer (AAS), exchange acidity by the 1M KCl extraction and 0.01M NaOH titration. Nitrogen in the samples was determined by the Marco Kjeldahl method, Free Fe and Al oxides (Total oxides) were extracted by the citrate dithionate – bicarbonate method (Mebra and Jackson, 1960). Iron and Aluminum oxides in the extracts were determined with an Atomic Absorption Spectrophotometer (AAS) at 248.3 nm and 396.1nm wavelengths respectively.

Crop Data

Data was collected on plant height, Leaf length (cm) Leaf width, Leaf Area (cm^3)and Grain yield (kg)

The data generated were subjected to analysis of valiance (ANOVA). Means that showed statistically significant differences were separated using least significant difference (LSD) (Genstat, 2009)

III. RESULTS AND DISCUSSION

The information on rainfall, temperature and relative humidity is presented in Table 1. The highest temperatures are recorded at the end of dry season (November to April) when the average temperature is 35ºC. At the start of rain in April down to May, temperature decreased until August which showed 29ºC.The relative humidity was maximum in the month of August (81%) and then dropped until December (27%). However, mean annual rainfall recorded during the period of study was 80.1mm which is low, treatments that received mulch cover had the highest soil water content at the end of the cropping season. One of the major roles played by mulch cover during the cropping season was probably reducing soil water evaporation which contributed to the maintenance of soil fertility and biological activities. Based on long term experiment, Boomsma *et al,* (2010) observed that substantial crop residue cover and cool, moist early season soil conditions are common characteristics of continuous maize no-tillage systems which often delay seed germination, seedling emergence and early root and seed development. Residue removal had also a significant impact on the noon temperature and water content in the soil if soil drying can be delayed for few days as a result of surface mulch, both temperature and soil strength will be lower during the emergence of crop seedlings (Bristow 1988).

Table.1: Meteorological Data for Makurdi (2015)

Months	**Rainfall (mm)**	**Temperature**		**Relative Humidity (%)**
		Max (^{0}C)	**Min (^{0}C)**	
January	0.0	33	18	47
February	109.0	35	24	48
March	4.0	35	24	55
April	14.0	35	24	65
May	36.0	35	25	73
June	152.0	33	24	78
July	128.0	31	22	79
August	135.0	29	23	81
September	283.0	30	22	79
October	80.0	32	24	78
November	20	34	20	69
December	0.0	33	18	27
Mean	80.1	32.9	22.3	64.9

Source: Nigerian Meteorological Agency (NIMET)

Soil Properties of the study site

Table 2 shows the physical and chemical properties of the experimental site before planting. The soil of the experimental site prior to planting was characterized by low level of organic carbon (0.93), total Nitrogen(0.05%).The PH was slightly acidic(6.62) which is conducive for maize production. Exchangeable Calcium (Ca) and Magnesium were 3.01cmol/kg and 2.4cmol/kg respectively. The organic matter content of the soil was 1.60% which is low. The available Phosphorus and nitrogen was 0.31ppm. The exchangeable cations indicated low K^+ with 0.23cmol/kg and Na^+2.40cmol/kg. The percentage base saturation was 85.5%.the particle size distribution of sand, clay and silt is78.36%,8.02% and 13.62% indicating loamy sand using the textural classes. According to Metson (1961), textural class of the soil has high influence on the physical and chemical properties of the soil. The total nitrogen content

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(0.5gkg-1) is moderate. The organic matter (1.60 is very low, thus the maintenance of soil organic matter is paramount to sustaining other soil quality factors (Robertson et al.,1991)

Table.2: The physical and chemical properties of soil in the study site prior to 2015 cropping season

Soil Parameters	Values
Sand (%)	78.36
Silt (%)	8.02
Clay (%)	13.62
pH	6.62
Organic Carbon (C)	0.93
Total Nitrogen (%)	0.05
Available Phosphorus (ppm)	0.31
Potassium (K^+)(Cmol/kg)	0.23
Calcium (Ca^+) (Cmol/kg)	3.01
Sodium (Na^+) (Cmol/kg)	2.40
Acidity (Cmol/kg)	0.26
Basicity (Cmol/kg)	1.00
Organic matter (%)	1.60
Effective cation exchangeable capacity (ECEC) (Cmol/kg)	6.90
Textural Class	Loamy sand
Total Porosity (%)	48.68
Bulk density (gcm^{-3})	1.36
Base Saturation (%)	85.50

Effect of Tillage and Mulch on maize Performance

The Main Effect of Tillage and Mulch on Maize Height is Presented in Table 3. Analysis of data indicated that the effect of mulch practices was not significant. Plant height was highest under mulch treatment compared to the un-mulch this might be due to moisture retention in the soil and decomposition of organic matter in the soil. According to (Holland, 2004) soil biota increase under mulched soil environment thereby improving nutrient cycling and organic matter build up over a period of several years. Yonghe (1994) also reported that plastic mulch significantly raised the soil temperature keeping the soil water content stable, which resulted in faster growth with higher dry matter yield as compared to uncovered treatments. However, tillage systems showed significant differences in plant height except at 6 weeks after planting. The tallest plant was located in the ridge tillage treatment at 8 weeks after planting while shortest plant was found in the no tillage plots. This might be due to proper root penetration due to that of Kayode and Adenileuyi (2004) who observed the shortest maize plant in the no tillage plots in comparison with that in the tilled plots on a sandy clay loan Alfisols in south western Nigeria.Alkins and Afuaka (2010) also reported taller cowpea plants in the tilled plots compared to that of the No-tilled plots

The effect of interaction of tillage and mulch on maize height is presented in Table 4. There was no significant effect ($p \leq 0.05$) between the various interactions on plant. The tallest plant was found in the ridge-tillage and mulch interaction which penetration while mulch reduced recompaction of the soil, increased water and nutrient absorption. The combined positive effects led to increase in maize plant height growth and establishment

Table.3: The Main Effect of Tillage and Mulch Practices on Maize Height in Makurdi

	Treatment	Plant Height (cm)			
		2WAP	4WAP	6WAP	8WAP
1	Mulch	11.78	50.78	97.2	144.5
2	Unmulch	10.97	40.75	91.6	136.0
	LSD(0.05)	NS	NS	NS	NS
	CV (%)	8.1	6.2	14.8	13.4
	Tillage practices				
1	Flatbed	11.22	85.97	104.5	152.8

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2	No-till	10.20	33.04	76.7	85.8
3	Ridge-till	11.72	37.67	85.0	178.8
4	Min-till	12.34	67.37	111.9	143.5
	LSD (0.05)	1.146	3.846	NS	23.29
	CV(%)	8.1	6.2	14.8	13.4

Table.4: The Interactive Effect of Tillage and Mulch on Maize Height

Interaction		Plant Height (cm)			
Tillage	Treatment	2WAP	4WAP	6WAP	8WAP
Flatbed	Mulch	11.78	65.48	102.4	156.8
	Unmulch	10.07	66.45	106.0	148.8
No-tillage	Mulch	10.85	84.80	80.9	90.9
	Unmulch	9.55	31.28	72.4	80.8
Ridge-till	Mulch	12.04	38.61	87.4	180.0
	Unmulch	11.40	36.66	82.6	177.6
Min-till	Mulch	12.43	64.11	118.2	150.3
	Unmulch	12.26	60.62	105.6	136.8
LSD (0.005)		NS	NS	NS	NS
CV (%)		8.1	6.2	14.8	13.4

The Main Effect of Tillage and Mulch on Leaf Area is Presented in Table 5. Results show significant differences between the mulch treatments. Leaf area was highest at 2, 4, 6 and 8 weeks after planting in the mulch treatment compared to the un-mulch. This indicated y that the presence of mulch materials on the soil surface helped to retain moisture and improved the fertility status of the soil which lead to increase in crop establishment, growth and development.

Among the tillage systems, ridge tillage produced the largest leaf area at 8 weeks after planting compared to the other systems of tillage. Leaf area was lowest under No tillage system. Although No-tillage did not hinder the establishment and early growth of maize, yet later on may have affected root development as compared to the other tillage systems. The negative effect on root development may have led to shower flow of water and nutrients from soil to the plants. These results are similar to that of Karunatilake (2000) who also reported higher leaf area plant in conventional tillage compared to no-tillage in maize and thus was attributed to higher leaf area plant in conventional tillage abundant root growth compared to that of zero tillage.

The interaction effect of tillage and mulch on leaf area is shown in Table 6. There was no significant difference between the interactions. At 8 WAP the highest leaf area was observed in the ridge tillage and Mulch treatment while the least leaf area was observed in No-tillage and un-mulch interactions.

Table.5: Main Effect of Tillage and Mulch on Leaf Area of Maize in Makurdi

	Treatment	Plant Leaf Area (cm)			
		2WAP	4WAP	6WAP	8WAP
1	Mulch	32.22	191.2	364.0	411.0
2	Unmulch	27.43	163.7	297.0	334.0
	LSD(0.05)	3.973	22.23	53.8	71.7
	CV (%)	15.2	14.3	18.6	22.0
	Tillage practices				
1	Flatbed	30.93	241.0	401.0	428.0
2	No-till	24.03	123.3	218.0	256.0
3	Ridge-till	31.24	156.9	372.0	487.0
4	Min-till	33.11	188.7	331.0	319.0
	LSD (0.05)	5.618	31.44	76.0	101.3
	CV(%)	15.2	14.3	18.6	22.0

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Table.6: The interaction Effect of Tillage and Mulch on Leaf Area of Maize in Makurdi

Interaction Tillage	Treatment	Leaf Area (cm^2) 2WAP	4WAP	6WAP	8WAP
Flatbed	Mulch	34.55	262.9	450.0	481.0
	Unmulch	27.31	219.0	352.0	374.0
No-tillage	Mulch	24.58	124.3	221.0	266.0
	Unmulch	23.49	122.2	216.0	246.0
Ridge-till	Mulch	35.23	174.5	391.0	540.0
	Unmulch	27.26	139.3	346.0	435.0
Min-till	Mulch	34.55	203.1	388.0	356.0
	Unmulch	31.66	174.3	274.0	282.0
LSD (0.005)		NS	NS	NS	NS
CV (%)		15.2	14.3	18.6	22.0

Main effect of tillage and mulch on dry cob length (cm), wt 1000g, grain yield (crop yield) of maize is presented in table 7

Analysis of variance showed no significant differences between the mulch treatments. Mulch treatment had the highest dry cob length, dry cob width, weight of 1000grains and grains yield (t/ha). The increase in grain yield of corn under mulching conditions may be due to increased soil moisture storage and suppression of weed growth (Mastana, 1988)

Similarly, Tolk *et al.,* (1999) and Liv *et al.,* (2002) concluded that mulch increases soil moisture and nutrients availability to plant roots in turn, leading to grain yield. Mulch significantly increased grain yield.

Among the tillage treatments the highest dry cob length (cm) was obtained in flat bed while ridge-tillage plot Presented the highest dry cob width, weight of 1000grains and grain yield (t/ha) this might be due to proper soil loosening which led to deep rooting ability, water utilization and nutrient uptake for crop growth and yield. The lowest dry cob length (cm), dry cob width (cm) weight of 1000grams and yield (t/ha) were obtained in no-tillage systems. These results are in agreements with that of Videnovil *et al.,* (2011) who observed higher maize yield in conventional tillage plots in comparison with that of the no-tillage plots in comparison with that of the no-tillage plots in the chenozen soil type in Cemunpolje, Serbia. This is particularly due to the fact that no-tillage environments are more likely to exhibit no-uniform germination, emergence and early growth and development which cause great plant to variability for multiple morpho-physiological traits that are associated with yield reduction (Liv*et al.,* 2004; Tokattidis *et al.,* 2004)

The effect of interaction of tillage and mulch on maize crop yield is shown in Table 8 Significant differences were not observed in all the interactions. Flat bed and mulch produced highest fry cob length, ridge-tillage and mulch produced the highest dry cob width while the highest weight of 100grains was observed in the ridge tillage and mulch and ridge tillage and un-mulch tillage and mulch interaction.

Table.7: Effects of Tillage and Mulch on Maize Crop Yield (t/ha)

	Treatment Mulch	Maize yield DCL (cm)	DCW (cm)	WT 1000g	Grain yield (t/ha)
1	Mulch	11.16	10.52	0.1717	0.90
2	Unmulch	11.14	10.12	0.1540	0.66
	LSD(0.05)	NS	NS	NS	NS
	CV (%)	17.2	10.2	29.4	67
	Tillage practices				
1	Flatbed	13.82	12.07	0.200	0.68
2	No-till	5.79	5.71	0.0788	0.29
3	Ridge-till	13.70	12.85	0.2500	1.41
4	Min-till	11.29	10.64	0.1230	0.74
	LSD (0.05)	2.372	1.300	0.05935	0.653
	CV(%)	17.2	10.2	29.4	67.5

Table.8: The Interaction Effect of tillage and Mulch on Maize Grain Yield in Makurdi

Interaction		Maize yield			
Tillage	Treatment	DCL(cm)	DCW (cm)	Wt 1000g	Grain yield (t/ha)
Flatbed	Mulch	13.54	12.23	0.1967	0.77
	Unmulch	14.11	11.90	0.2083	0.59
No-tillage	Mulch	6.25	6.23	0.1000	0.36
	Unmulch	5.33	5.18	0.0567	0.22
Ridge-till	Mulch	14.00	13.73	0.2500	1.68
	Unmulch	13.40	11.97	0.2500	1.14
Min-till	Mulch	10.86	9.87	0.1400	0.80
	Unmulch	11.72	11.41	0.1060	0.68
LSD (0.005)		NS	NS	NS	NS
CV (%)		17.2	10.2	29.4	67.5

IV. CONCLUSION

This study examined the effect of tillage and Mulch practices on maize performance. Mulch treatment proved to be most effective in promoting maize growth, development and yield. Ridge-tillage showed to be most effective and no-tillage was least. Ridge-tillage and mulch was most beneficial while no-tillage and un-mulch were least beneficial in promoting maize growth, performance and yield in Makurdi. Much application and ridge tillage is therefore recommended for improved maize growth and yield.

REFERENCES

[1] Adaikwu, A. O., M. E. Obi and A. Ali (2012).Assessment of degradation Status of Soils in Selected areas of Benue State, Southern Guinea Savanna of Nigeria.*Nigerian Journal of soil science.* 22(1): 171-180..

[2] Boomsma C.R., Santini J.B., West T.D., Brewer J.C., McIntyre L.M., Vyn T.J. (2010): Maize grain yield responses to plant height variability resulting from crop rotation and tillage system in long-term experiment. Soil and Tillage Research, 106: 227–240

[3] Bouyoucos, G. H. (1951). A Recalibration of the Hydrometer Method for Making Mechanical Analysis of soil.*Agronomy Journal.* 43:434-438.

[4] Bristow, K. L (1988), the role of mulch and its architecture in modifying soil temperature. Australian Journal of soil research. 26, 269-280.

[5] Holland, J. M. (2004). The environmental consequences of adopting conservation tillage in Europe: Reviewing evidence. Agriculture Ecosystems and Environment, 103: 1-25.

[6] Karunatilake, U., H.M. van Es and R.R. Schindelbeck. 2000. Soil and maize response to plow and no-tillage after alfalfa-to-maize conversion on a clay loam soil in New York. Soil and Tillage Res., 55(1-2): 31-42.

[7] Kayode and Ademiluyi, B (2004). Effects of tillage methods on weed control and maize performance in southwestern Nigeria location, Journal of sustainable Agriculture 23(3).39-45

[8] Liv, J., S.A. Xu, Zhou, G. Y. and H.H. Lu, (2002).effects of transplanting multicropping spring maize with plastic film mulching on the ecological effect of, plant growth and grain yield, Agric. Coll., 2: 100 - 102

[9] Mastana, P S, (1988) "Effect of crop residue management practices on nitrogen balance in water eroded cultivated lend, M.Sc. thesis, Punjab agricultural university, Ludhiana, India.

[10] Mebra, O.P and M.I Jackson (1960). Iron oxide removal from soils and clays by a dithionate-citrate system buffered with sodium bi carbonate. Proceedings of 7th National Conference on clays and clay minerals pp 317-327.Peragon press, New York.

[11] Metson, A. J. (1961). Methods of Chemical Analysis for Soil Survey Samples.New Zealand DSIR Soil Bulletin 12. Government Printer, Wellington New Zealand. 84 pp.

[12] Odofin, A. J. (2005). Effects of no-tillage with mulch on soil moisture condition, penetration resistance and maize performance in Minna area of Nigeria's southern guinea savanna. Nigerian Journal of Soil Science, 15(2):1-8.

[13] Robertson, E. B., S. Sarig and M.K. Firestore 1991.Cover crop management of polysaccharide – mediated aggregation in an orchard.Soil Science Society of American Journal.55:734 – 739.

[14] Tokatlidis I.S., Koutroubas S.D. (2004): A review of maize hybrids' dependence on high plant

populations and its implications for crop yield stability. Field Crops Research, 88: 103–114.

[15] Tolk, J.A., T.A Howell and S.R. Evert, (1999), Effect of mulch, irrigation and soil type on water use and yield of maize soil Tillage, Pp; 50: 137-147

[16] Videnovic, Z., Simic, M., Srdic. J. and Dumanovic, Z. (2011). Long term effects of different soil tillage systems on maize (Zea mays L) yields. Plant soil and Environment. 57(4):186-192.

[17] Walkley, J. T. and C. A. Black (1934).An examination of the Degtjarefft method of Determining the Organic Matter and a proposed Modification of the chronic acid Titration Method.*Soil Science* 37: 29-88.

[18] Yonghe, Z. (1994), study on the impact of plastic mulch on selected crop agroecosystems in yunna province peoples; republic of china, philipinesuniv, los banos, college, laguna (philipines), university library, university of the philipines at los banos, benquet state university, la Trinidad, Bequet 2601, philipines.

Acidity, Vollatyl Fatty Acid and Digestibility In-Vitro of Corn Straw Silage as Energy Source

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***Abstract*— *The research aims to determine the best silage formulation using pollard and molasses to acidity (pH), vollatyl fatty acid, and in vitro digestibility. The experiment use completely randomized design (CRD) with four treatments and four times replication, so it has 16 units experiment. The four treatments are treatment A = 100% corn straw + 20% pollard + 0% molases; B = 100% corn straw + 10% pollard + 10% molases; C = 100% corn straw + 0% pollard + 20% molases; D = 100% corn straw + 10% pollard + 0% molases; E = 100% corn straw + 5% pollard + 5% molases; dan F = 100% corn straw + 10% pollard + 0% molases. Variables observe are pH, vollatyl fatty acid, and in vitro digestibility in rumen fluid including dry matter, and organic matter digestibility. Results of the experiment showed that nutrient content and acidity on treatment A is highest. Level of VFA total, dry matter digestibility, organic matter digestibility are significant different (P<0,05). Microbes need energy and protein for the growth and could be fullfill from corn straw silage that combination of pollard and molases. It could be concluded that combination of 10% pollard and 10% molases could use by microbes to fullfill the need of energy and protein*.

Keywords*— *corn straw silage, pH, in vitro digestibility, VFA.

I. INTRODUCTION

Ruminant need forage as main energy and protein source, beside consentrate as feed supplement. Feed that usually give to ruminant are grass, leguminose and tree such as *Kibatalia arborea* and *Hibiscus tiliaceus*. The availability of forage especially grass is fluctuated where it will be abundant during rainy season but the availability is limmited even deficiency during draught season.

Utilization of waste of agricultural crop resources as a feed source is an efficient step to overcome the lack of grass production. Agricultural waste including an in-situ forage source is available in abundance and easily available. Most of the agricultural waste can be used for cattle feed. The great potential of agricultural waste as a source of forage is corn straw (BPTP West Sumatra, 2011).

Corn is an agricultural commodity that is quite widely developed by farmers in Indonesia. According to Kushartono and Iriani (2003), if the high yield of corn crops can be developed in Indonesia, it is hoped that corn crops can contribute to the provision of forage beside grass, leguminous and rice straw. One of the factors causing underdevelopment of corn crop as livestock feed in Indonesia is the limited of farmer for its utilization. Therefore it is necessary to conduct an economical assessment and development of corn crops more intensive and more introducing how to grow crops and utilization of corn crops as a source of feed.

Plant sugars are fermented by anaerobic bacteria to organic acids which reduce the pH of the plant material. This process preserves the crop during long-term storage. High quality corn silage results when lactic acid is the predominant acid produced during fermentation. Lactic acid is the most efficient fermentation acid and will drop the pH of the silage the fastest (University of Wisconsin, 2017).

The principle of silage making is to maintain airtight conditions in silo to the maximum extent possible. Airtight conditions can be pursued by solidification of silage material as much as possible and the addition of fermentable carbohydrate sources (Hidayat and Indrasanti, 2011).

The advantage of silage making are stabile composition of the feed (silage) for a longer period (up to 5 years, plants can be harvested at optimal phase of development and are efficiently used by livestock, and reduction of nutrient loses (Food and Agriculture Organization of United Nation and USAID)

Retnani *et al.* (2009) study using field grass, corn leaves and corn cloves made biscuits found that 50% of field grass and 50% of corn leaves have the lowest water content and water absorption. Waste corn biscuits can be used as a substitute for the source of fiber for sheep because it has the same palatability as the field grass biscuits.

Bahri (2012) study using field grass and complete ration diets based on corn straw for 6 weeks showed that a complete silage ration treatment based on corn straw and field grass with 60%: 40% ratio showed a better response than other rations on consumption rations and weight gain of bali cattle.

The objective of this study was to obtain a formulation of corn silage using pollard and molasses on pH, vollatyl faty acid, in vitro digestibility.

II. MATERIALS AND METHODS

Materials

The materials used in making corn silage straw are corn straw, pollard, and molasses. Tools used include knives for cutting corn straw, board as cutting pad, plastic sheet to mix silage, plastic bag, and bucket as silo.

Methods

The research was conducted at Animal Nutrition Laboratory of Faculty Animal Husbandry Udayana University and Ciawi Bogor Livestock Research Laboratory for 3 months from preparation until end of research.

Corn straw is cut into 3-5 cm, and mix with pollard, and molasses as per treatment. Then put in a plastic bag, pressed and compressed until there is no air in the plastic bag to create anaerobic state. Then tied tightly and kept in a cool place and not exposed to the sun. Laboratory analysis was conducted after 21 days of fermentation.

The design used in this study was a completely randomized design with six treatments and four replications, so there were 24 experimental units. The six treatments were: A = 100% corn straw + 20% additive (20% pollard + 0% molases); B = 100% corn straw + 20% additive (10% pollard + 10% molasses); C = 100% corn straw + 20% additives (0% pollard + 20% molasses); D = 100% corn straw + 10% additive (10% pollard + 0% molases); E = 100% corn straw + 10% additive (5% pollard + 5% molasses); F = 100% corn straw + 10% additive (0% pollard + 10% molases).

Variables observed were pH (acidity), the digestibility of dry matter and organic matter in rumen fluid in-vitro, and Vollatyl Fatty Acid (VFA).

The data obtained in this study were analyzed by using variance analize, if the average value of treatment significantly affected the variables followed by Duncan test at 5% (Steel and Torrie, 1991).

III. RESULTS AND DISCUSSION

Acidity (pH value) silage of corn straw silage showed a tendency decreases with decreased pollard supplementation and increased molasses in 20% supplementation in treatments A, B, and C and 10% in treatment D, E, and F. This indicated that the type (pollard and molasses) and the amount of additives used will affect the quality of silage. The pH value of the research results ranged from 3.88-4.22 and according to the Agricultural Departement (1980) is very good criteria. The lower the pH value or the more acidic the silage, the better of silage quality. The use of molasses can decrease the pH due to molasses as a soluble carbohydrate source which will form a higher lactic acid compared with pollard. The pH value in the supplementary ingredients with different levels it is seen that the use of additives 10%, pH value is more alkaline compared to 20% because the amount of additive very decisive the formation of lactic acid from lactic acid bacteria. Microbial growth will be higher in silage containing more additives than with the use of lower additives.

pH value of corn straw silage in treatment A is higher compared with treatment B and C because the pH value is influenced by the content of VFA and NH3. Silage of corn straw in treatment A has the lowest total VFA content so that the pH value becomes high because the higher the VFA content, so the lower pH value or will be acid. The low VFA total content of corn straw silage in treatment A is caused by the utilization of VFA by microbes in the presence of NH-3 availability of the pollard protein, so that microbial proteins are formed and after death will be able to increase the silage protein content.

Tabel.1: Acidity (pH value) and Volattile Fatty Acid (VFA) of corn straw silage

Variables	Treatments[1)]						SEM[2)]
	A	B	C	D	E	F	
Acidity (pH value)	4,18[d 3)]	3,95[b]	3,90[a]	4,22[e]	4,01[c]	3,88[a]	0,01
VFA partial:							
Acetic acid (mM)	16,35 [b]	25,02 [c]	25,22 [c]	22,38 [c]	10,04 [a]	6,93 [a]	1,98
Propionic acid (mM)	0,74 [a]	0,45 [a]	0,21 [a]	0,24 [a]	0,08 [a]	0,05 [a]	0,17
Iso-butyric acid (mM)	0,29 [b]	0,21 [ab]	0,14 [ab]	0,15 [ab]	0,04 [a]	0,04 [a]	0,06
N-butyric acid (mM)	0,29 [ab]	0,55 [b]	0,18 [a]	0,05 [a]	0,06 [a]	0,13 [a]	0,09
Iso-valeric acid (mM)	0,28 [b]	0,08 [a]	0,01 [a]	0,11 [a]	0,06 [a]	0,07 [a]	0,06
N-valeric acid (mM)	0,06 [a]	0,08 [a]	0,06 [a]	0,03 [a]	0,04 [a]	0,04 [a]	0,02

VFA total (mM)	18,02 b	26,38 c	25,81 c	22,97 bc	10,31 a	7,25 a	1,96

Noted:
1) A = 100% corn straw with 20% pollard + 0% molasses supplementation; B = 100% corn straw with 10% pollard + 10% molasses supplementation; C = 100% corn straw 0% pollard + 20% molases; D = 100% corn straw with 10% pollard + 0% molases supplementation; E = 100% corn straw with 5% pollard + 5% molasses supplementation; F = 100% corn straw with 0% pollard + 10% molases supplementation
2) Standard Error of the Treatment Means
3) Different alphabet on the same row show significantly different (P<0.05)

The highest total VFA content in treatment B was 26,3750 mM and 31,70%, 60,92%, and 72,53% significantly (P<0,05) higher than treatment A, E, and F, respectively, Whereas with treatment C and D respectively 2,13% and 12,92% not significant (P>0.05) higher (Table 4). When viewed as a whole, total VFA content in treatment B, C, and D is significantly higher than treatment A, the high total VFA levels have no positive effect on the nutritional value of silage. Trisandewi *et al.* (2016) find that treatment A contained 16.19% crude proteinand and significantly (P<0.05) higher than other treatments and the crude fiber content of treatment A is 15.13% and significantly lower (P<0.05) than other treatments. High levels of VFA in silage B, C, and D are not useful because nitrogen is not available for microbial growth so that VFA is remaining or not utilized by microbes. This can be attributed to the high content of crude fiber in silage B, C, D, E, and F prove that fermentation does not work perfectly because of the low microbes in the silage treatment.

Dry matter and organic matter digestibility on treatment B supplemented with 10% pollard and 10% molases is the highest (P <0.05) among all treatments. This occurs because the silage of corn straw on treatment B contains pollard and molasses additives where pollard and molasses are microbes nutrients. Pollard as an energy and nitrogen source and molasses as energy sources. Energy and nitrogen content in pollard and molasses for microbial growth, so could produce enzymes for in-vitro fermentation. Increasing degradation by enzymes could increase dry matter digestibility. This also occurs in silage treatment E with 5% pollard and 5% molasses additive so that the yield ofdry matter digestibility in treatment E is slightly lower than treatment B but not significantly different (P>0.05).

Table.2: Dry matter and organic matter digestibility of corn straw silage

Variables	Treatments 1)						SEM 2)
	A	B	C	D	E	F	
Dry matter digestibility (%)	54,87 ab 3)	62,77 d	51,32 a	57,37 bc	61,35 cd	56,66 **ab**	1,49
Organic matter digestibility (%)	56,08 a	64,66 c	52,52 a	58,50 ab	62,33 bc	57,73 ab	1,87

Noted:
1) A = 100% corn straw with 20% pollard + 0% molasses supplementation; B = 100% corn straw with 10% pollard + 10% molasses supplementation; C = 100% corn straw 0% pollard + 20% molases; D = 100% corn straw with 10% pollard + 0% molases supplementation; E = 100% corn straw with 5% pollard + 5% molasses supplementation; F = 100% corn straw with 0% pollard + 10% molases supplementation
2) Standard Error of the Treatment Means
3) Different alphabet on the same row show significantly different (P<0.05)

Organic matter and dry matter digestibility have the same tendency where organic matter digestibility on additives with combination of pollard and molasses shows highest percentage both in 20% and 10% adiditive. Organic matter digestibility with 20% additive was obtained on treatment B while with additive 10% on treatment E. It showed that addition of singularly additive is not good enough because microbes require nitrogen from protein degradable and energy (VFA) from soluble carbohydrate fermentation, so that the addition of pollard and molasses will be able to meet the needs of microbes for protein and energy.

IV. CONCLUSSION

Based on results and discussion, it can be concluded that corn straw silage with combination of 10% pollard and 10% molasses supplementation or additives provides better results on pH, total VFA, and dry matter and organic matter digestibility because rumen microbes require energy and proteins that contained in pollard and molasses additives.

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REFERENCES

[1] Bahri, S. (2012). Respon Silase Ransum Komplit Berbasis Jerami Jagung Sebagai Pakan Penggemukan Sapi Bali. Laporan Hasil Penelitian Dasar Keilmuan. Jurusan Peternakan Fakultas Ilmu-Ilmu Pertanian Universitas Negeri Gorontalo.

[2] BPTP Sumatera Barat. (2011). Teknologi Pembuatan Silase Jagung untuk Pakan Sapi Potong. Badan Litbang Pertanian Kementerian Pertanian Republik Indonesia. Sumber: http//sumbar.litbang.pertanian.go.id. Diakses 15 Maret 2015.

[3] Food and Agriculture Organization of United Nation and USAID. (-). Silage Making For Small Scale Farmers Sorce: http://pdf.usaid.gov/pdf_docs/PNADQ897.pdf. Acces: May 1 2017.

[4] Hidayat, N dan Indrasanti, D. 2011. Kajian Metode *Modified* Atmosfir dalam Silo dan Penggunaan Berbagai Additif Pada Pembuatan Silase Rumput Gajah. Laporan Penelitian. Fakultas Peternakan. Unsoed. Purwokerto.

[5] Kushartono, B. and N. Iriani. (2003). Prospek Pengembangan Tanaman Jagung sebagai Sumber Hijauan Pakan Ternak. Prosiding Temu Teknis Fungsional Non Peneliti. halm 26-31.

[6] Retnani Y., L. Herawati, W. Widiarti, and E. Indahwati. (2009). Uji Sifat Fisik dan Palatabilitas Biskuit Limbah Tanaman Jagung sebagai Substitusi Sumber Serat Untuk Domba. Buletin Peternakan Vol. 33(3): 162-169.

[7] Steel, R. G. D., and J. H. Torrie. (1991). Prinsip dan Posedur Statistik. Suatu Pendekatan Biometrik. Edisi Kedua. Alih bahasa B. Sumantri. Jakarta. Gramedia.

[8] Trisnadewi, A. A. A. S., I G. L. O. Cakra., dan I W Suarna. (2017). Kandungan Nutrisi Silase Jerami Jagung Melalui Fermentasi Pollard Dan Molases. Majalah Ilmiah Peternakan Vol. 20(2): 55-59. Source: https://ojs.unud.ac.id/index.php/mip/article/view/32217.

[9] University of Wisconsin, Division of Cooperative Extension. (2017). Corn Agronomy Wisconsin. Source: http://corn.agronomy.wisc.edu/Silage/S005.aspx. Cited May 1st 2017

Comparative effects of Varying Rates of Moringa Leaf, Poultry Manure and NPK Fertilizer on the Growth, Yield and Quality of Okra (*Abelmoschusesculentus* L. Moench)

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Abstract— *The fertilizer management practices have not ensure the desired improvement in yield for okra (Abelmoschusesculentus L. Moench) due to differences in fertilizer types. The search continues for nutrient sources that would provide adequate nutrition for the crop on the season. A pot experiment was carried out at the Teaching and Research Farm, Ekiti State University, Ado Ekiti, Nigeria to evaluate the growth and fruit yield responses of okra (Abelmoschusesculentus L. Moench) to the application of air-dried milled moringa leaf (MML), poultry manure (PM) and NPK fertilizer. The MML was applied at 400, 800, and 1200 kg/ha; NPK 15-15-15 at 250 kg/ha and PM at 10 t/ha separately and in all possible combinations in completely randomized design in three replicates. The parameters measured were plant height, stem girth, number of leaves, leaf area, number of fruits and fruit weight. The single treatments differed significantly (P = 0.05) with the combinations of the treatments giving better performance. The 800 kg/ha MML + PM treatment gave the tallest plants (103.33 cm) and followed by single application of PM (102.33 cm). The application of 400 kg/ha MML + PM + NPK produced the highest number of fruits but 800 kg/ha MML + PM + NPK gave the highest fresh fruit (42.70 g) and dry fruit (20.50 g) weight. 800 kg/ha gave best growth performance among MML but 1200 kg/ha gave best yield. This suggests that MML can be used as source of nutrients to grow okra.*

Keywords— ***npk, milled moringa leaf, okra (Abelmoschusesculentus), poultry manure.***

I. INTRODUCTION

Okra (*Abelmoschusesculentus*L., Moench) is grown for fresh fruits in the tropical and subtropical regions and ranks first in terms of calorie for human consumption (Babatunde, 2007). The output of okra constituted about 4.6 percent of the total staple food production in Nigeria between 1970 and 2003 (CBN, 2004). Inadequate weed management, infertile soils, cultivation of low-yielding varieties and sub-optimal planting densities are some of the major constraints to high okra yield and production in Nigeria (Iyagba, *et al*., 2013) which necessitated the development of various agronomic practices the farmers can adopt to improveokra growth and fresh pod yield.

The soil in Nigeria are inherently poor in fertility on account of low available nutrients and organic matter contents such that the application of organic and inorganic fertilizers would be the rule for high crop production. Inorganic fertilizers have been promoted as the panacea tothis low fertility and nutrient losses, more so as the added fertilizer nutrients become immediately availablein the soils for uptake by crops. The fresh fruit yields of okra increased with NPK fertilizer application and the recommended optimum rates had differed among the varieties (Babatola, 2006).However, the long term dependence on high rates of inorganic fertilizer has demerits: soil acidification, nutrient imbalance and trace element deficiencies especially of manganese (Mn) and zinc (Zn) (Asadu*et al*., 2004). These have catalyzed the identification and use of organic materials as alternative nutrient sources. Besides, the scarcity of fertilizers and resultant high prices which are beyond the reach of resource-poor farmers mean that the recommended fertilizer rates are hardly met if any at all (Rahman, 2004). Organic manures are relatively resistant to microbial degradation but are essential for enhancing soil nutrient availability and maintaining optimum soil physical conditions.

Poultry manure have been reported to influence positively the growth and fruit yield of okra (Ashraf *et* al., 2016; Ali*et al*., 2013; Tiamiyu*et al*., 2012) which had led to the increase in the its use as nutrients sources by farmers. Poultry manure is a very cheap and effective source of nutrients, especially nitrogen (N) but ready availability remains an important issue since large amounts must be applied to give optimum yield. Also, plant residues: banana peels (Jonathan *et al*., 2012), *Senna siamea*, *Leucaenaleucocephala*and*Gliricidiasepium* (Akande*et al* 2010; Olujobi and Ayodele, 2013), sea weeds (Khan *et al*., 2009), *Moringaoleifera* (Fahey, 2005) are sources of nutrients needed to improve crop production. *Moringaoleifera* is a good sources of green manure as it compared very well with other green manure crops such as lablab beans (Fuglie, 2008). Fuglie (2008) reported the use of moringa seedlings as green manure for crop production. *Moringaoleifera* was one of the green manure used by Makinde*et al*. (2016) in the production of fluted pumpkin who concluded that plant materias can be used as an alternative to synthetic fertilizers. Moringa leaves are rich in zeatin, a naturally-occurring cytokinin and other compounds such as ascorbates, vitamin E, and phenolics which confer on the leaf extract the status of a natural plant growth enhancer (Nagar *et al*., 2006).

Harlin*et al.* (2004) advocated for the integrated use of organic manure and inorganic fertilizers to supply the nutrients required to sustain maximum crop productivity and profitability while minimizing the negative environmental impacts from nutrient use. Therefore, this study was carried out to evaluate the comparative effects of moringa leaf, poultry manure and NPK fertilizer singly and in combination on the growth and yield of okra (*Abelmoschusesculentus)* in Ado – Ekiti. Southwestern Nigeria.

II. MATERIALS AND METHOD

Experimental Site

The experiment was conducted at the Teaching and Research Farm,Ekiti State University, Ado-Ekiti, during the 2015 cropping season. The study site lies on latitude 5˚45′ N and longitude 8˚15′ E and experiences tropical climate characterized by a wet and dry seasons. The long wet season is from late March to November and divided into early and late seasons by little dry season in July to August.

Collection and analysis of soil, moringa and poultry manure samples

Top soil (0-15 cm) samples were randomly collected from cultivated farm, bulked to form a composite sample, air-dried and sieved using a 2mm mesh size. The routine analyses as described in Udo *et* al. (2009) for physical and chemical properties were carried out on the soil sample. 10 kg of the soil sample were measured into 10l plastic containers that were perforated at the base. Fresh *Moringaoleifera* leaves were air-dried and milled. Poultry manure was also obtained from the dump site of the Poultry House on the Farm, air-dried and finely crushed.

Experimental design

The treatments consisted of milled moringa leaf (MML) at 400, 800 and 1200 kg/ha, 10 t/ha poultry manure (PM) and 250 kg/ha NPK 15-15-15 fertilizer singly and in all possible combinations and control. The MML and PM were applied 2 weeks before planting while the NPK fertilizer was applied 2 weeks after planting (WAP). Two seeds of okra (NHAe 47-4 variety) were sowed to each pot and thinned to one seedling after emergence at 2 WAP. The experiment was laid out in a Completely Randomized Design (CRD) with three replicates. Adequate watering, weeding and pest control were carried out as required.

Data collection and statistical analysis

Data were collected on plant height, number of leaves and leaf area at intervals of two (2) weeks from 2 WAP. The leaf area was calculated as the product of leaf length and leaf breath and coefficient factor obtained with the graphical method (Pandey and Singh, 2011). Harvesting of fresh fruits begins at 9 WAP which was done in 4 days interval. The number of fruits per plant was counted while the fruit weight per plant (fresh and dry) were recorded.All data collected were subjected to analysis of variance (ANOVA) and the treatment means were separated by Fisher's Least Significant Difference (LSD) at 5% probability.

III. RESULTS

Moringa, poultry manure and soil samples

Table 1 shows the pre-cropping soil properties and some chemical properties of the PM and MML. The soil was a slightly acidic (pH=6.24) loamy sand, containing 0.09%N, 1.48% organic matter and16.59 mgkg^{-1} available P while exchangeable K, Na, Ca, and Mg were 0.25, 0.03,2.38,1.12 cmolkg^{-1} respectively. The MML was slightly acidic (pH=6.37) while PM was slightly alkaline (pH=8.25). MML was higher in exchangeable K (10.40 cmolkg^{-1}), total N (4.51%) and available P (7.16 mkg^{-1}) than PM with 0.09 cmolkg^{-1} exchangeable K, 3.76% total N and 3.00 mgkg^{-1} available P.

Plant height

Table 2 shows that okra plant height increased with theMML levels and the application of NPK 15-15-15 and

PM. Among the single application treatments, M_2 and M_3 gave the highest values which did not different significantly at 2 WAP. NPK gave the highest value at 4 WAP
which did not differ from M_2 and M_3 at 4 WAP. M_2 gave the highest value at 6 WAP while
PM gave the highest followed by M_2 which was similar to NPK fertilizer application at 8 WAP. Okra treated with M_2 gave the tallest plant (71.33 cm) among the MML rates which was significantly different from M_1 and M_3. M_3 + NPK produced the tallest plants at 2, 4 and 6
WAP and did not differ from M_2 + PM at 2 and 4 WAP which gave the best value at 8 WAP. M_2 + PM + NPK produced the tallest plants throughout the sampling period.

Table.1: Chemical and physical properties of soil, poultry manure and dried milled moringa leafsamples

Parameter	Soil	Poultry manure	Dried milled moringa leaf
pH	6.24	8.25	6.37
Organic C (%)	0.86	21.35	51.87
Organic matter (%)	1.48	36.80	89.40
N(%)	0.09	3.76	4.51
C:N ratio	9.56	5.68	11.5
Available. P ($mgkg^{-1}$)	16.59	3.00	7.16
Exchangeable K ($cmolkg^{-1}$)	0.25	0.09	10.40
Exchangeable Na ($cmolkg^{-1}$)	0.03	-	-
Exchangeable Ca ($cmolkg^{-1}$)	2.38	0.13	2.11
Exchangeable Mg ($cmolkg^{-1}$)	1.12	1.57	2.20
ECEC ($cmolkg^{-1}$)	4.19	-	-
Physical Characteristics		-	-
Sand (gkg^{-1})	840	-	-
Silt (gkg^{-1})	98	-	-
Clay (gkg^{-1})	62	-	-
Textural Class	Loamy Sand		

Table.2: The Comparative effects of Moringa Leaf, Poultry manure and NPK Fertilizer on the Plant Height (cm) of Okra (Abelmoschusesculentus)

Treatments	Week after planting			
	2	4	6	8
Control	8.50c	21.33f	38.17f	54.57e
M_1	9.23c	22.50f	38.67f	59.70e
M_2	21.50ab	36.00d	55.67d	71.33cd
M_3	20.13b	34.33de	47.00e	66.00de
NPK	15.13bc	38.00cd	51.67de	70.40d
PM	13.67c	28.33e	50.00de	76.67cd
M_1+NPK	21.50ab	36.50d	57.00d	78.60c
M_2+NPK	18.00bc	31.25e	50.00de	70.50d
M_3+NPK	25.83a	60.17a	85.67a	102.33a
M_1+PM	13.33c	41.83c	65.00c	83.00c
M_2+PM	19.27b	52.17b	81.67ab	103.33a
M_3+PM	16.10bc	47.67b	73.00b	94.33b
M_1+PM+NPK	17.00bc	42.33c	70.33bc	94.67b
M_2+PM+NPK	25.83a	60.33a	80.00ab	98.33a
M_3+PM+NPK	17.50bc	48.67b	75.07b	94.00b
LSD (5%)	5.26	4.62	7.91	7.69

M: Moringa, (M_1=400kg/ha, M_2=800kg/ha, M_3=1200kg/ha); NPK 15:15:15 Fertilizer, PM: Poultry manure. Mean with different letter in the same column are significantly different at 5% probability.

Number of leaves

Single and combined application of MML, PM and NPK 15-15-15 did not significantly affect the number of leaves in okra (Table 3). At 2 WAP, the single application of NPK and M_2 gave the highest number of leaves while PM produced most leaves at 4-8 WAP followed by M_1, M_2 and NPK at 4 and 6 WAP and M_3 at 8 WAP. M_2 + PM gave the highest number of leaves over the 2-8 WAP while M_2 + PM + NPK and M_1 + PM + NPK produced the highest number of leaves at 2-4 and 6-8 WAP respectively.

Leaf area

Sole application of M_2 gave the largest leaf area 2-4 WAP while the largest area was with PM at 6-8 WAP. M_3 + NPK gave largest leaf area at 2 WAP, M_2 + PM produced largest leaf area at 4 WAP while M_3 + NPK, M_1 + PM, M_2 + PM and M_3 + PM gave highest values which were not significantly different at 6 WAP. M_2 + PM + NPK gave the highest value at 2-8 WAP.

Fruit yield

Table 5 shows that PM and NPK produced the same average number of fruit per plant (4) followed by M_3 (3). NPK has the highest average fresh and dry fruit weights of 37 and 17.10 g respectively which were different significantly from PM with 22.80 and 10.20 g. M_3 gave the best performance in okra yield compared to M_1 and M_2. M_3 + NPK and M_2 + PM did not differ in their average number of fruit per plant but M_3 + NPK gave higher values of average fresh fruit weight that is significantly different to M_2 + PM. The treatment combination M_1+PM+NPKproduced the highest average number of fruits per plant (5)while M_3+PM+NPK had the highest average fresh fruit weight (42.72g) and dry fruit weight (20.50 g). The fresh and dry fruit weight yields recorded from the combination of MML, PM and NPK were significantly higher than other combinations.

Table.3: The comparative effects of milled moringaleaf, poultry manure and NPK fertilizer on the number of leaves of okra

Treatments*	**Week after planting**			
	2	**4**	**6**	**8**
Control	3.33	4.67	5.00	5.00
M_1	3.00	5.00	5.00	6.00
M_2	5.00	5.00	5.00	6.00
M_3	3.00	4.33	5.00	6.33
NPK	5.00	5.00	5.00	6.00
PM	4.67	5.67	6.00	6.67
M_1+NPK	3.67	4.33	5.00	5.67
M_2+NPK	4.00	4.50	5.50	6.00
M_3+NPK	3.50	4.50	5.00	6.00
M_1+PM	3.33	5.00	5.67	6.00
M_2+PM	5.00	5.33	6.00	6.33
M_3+PM	3.67	5.33	5.67	6.33
M_1+PM+NPK	3.33	5.00	6.00	6.67
M_2+PM+NPK	5.33	6.33	5.67	6.33
M_3+PM+NPK	4.00	5.00	5.67	6.67
LSD (5%)	1.64	1.60	1.00	1.00

M: Moringa, (M_1=400kg/ha, M_2=800kg/ha, M_3=1200kg/ha); NPK 15:15:15 Fertilizer, PM: Poultry manure, NS: Not significant.

Table.4: The Comparative effects of Moringa Leaf, Poultry manure and NPK Fertilizer on the Leaf Area (cm^2) of Okra (Abelmoschusesculentus)

Treatments	**Week after planting**			
	2	**4**	**6**	**8**
Control	11.43g	32.89i	63.83g	95.67i
M_1	11.85g	35.86h	83.57f	127.11h
M_2	51.87c	84.13e	131.74de	167.32g
M_3	29.18e	64.49f	94.56f	156.09g
NPK	27.87e	62.66fg	103.37e	190.07f

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PM	19.72f	47.87g	164.43cd	285.92e
M_1+NPK	33.95d	72.17f	180.54c	274.15e
M_2+NPK	21.21f	53.24h	61.04	136.17
M_3+NPK	83.42a	177.96c	227.93b	341.43bc
M_1+PM	28.33e	156.33d	224.98b	338.22c
M_2+PM	35.46d	202.49b	217.35b	333.67cd
M_3+PM	33.50d	185.73c	232.21b	334.75c
M_1+PM+NPK	33.53d	92.00f	210.00b	322.19d
M_2+PM+NPK	64.95b	292.89a	328.68a	418.59a
M_3+PM+NPK	33.11d	152.19e	236.53b	350.55b
LSD (5%)	3.79	10.29	36.48	12.10

M: Moringa, (M_1=400kg/ha, M_2=800kg/ha, M_3=1200kg/ha); NPK 15:15:15 Fertilizer, PM: Poultry manure. Mean with different letter in the same column are significantly different at 5% probability.

Table.5: The comparative effects of milled moringa leaf, poultry manure and NPK fertilizer on the number of fruit, fresh and dry fruit weight of okra.

Treatments	**Number of Fruit**	**Weight of Fruit (g)**	
		Fresh	**Dry**
Control	1.10e	2.10i	1.30i
M_1	2.20d	5.50h	3.40h
M_2	2.67c	10.50g	5.10g
M_3	3.00c	16.70f	6.80f
NPK	4.00b	37.80b	17.10b
PM	4.00b	22.80de	10.20e
M_1+NPK	3.00c	19.40e	7.50f
M_2+NPK	3.00c	7.90gh	3.00h
M_3+NPK	4.00b	38.90b	16.30bc
M_1+PM	3.00c	20.60e	9.60d
M_2+PM	4.00b	34.50c	17.30b
M_3+PM	3.00c	23.70d	15.30c
M_1+PM+NPK	5.00a	23.40d	12.00d
M_2+PM+NPK	3.00b	42.70a	20.50a
M_3+PM+NPK	4.00b	38.50b	19.60a
LSD (5%)	0.47	2.27	1.08

M: Moringa, (M_1=400kg/ha, M_2=800kg/ha, M_3=1200kg/ha); NPK 15:15:15 Fertilizer, PM: Poultry manure. Mean with different letter in the same column are significantly different at 5% probability.

IV. DISCUSSION

The pH value of the soil (pH=6.24) was within the pH range of 6 – 7 considered as suitable for optimum performance of vegetables (Purselglove 1992). The total N was very low compared to the critical level of 0.1% for N in the soils of Nigeria (FMANR, 1990) suggesting the need for its increased supply in the soil to improve the growth and yield of okra.This expectation was met with the application of NPK, PM and MML singly and in all combinations which increased the selected growth parameters at all sampling occasions.

Studies have shown that MML and PM are rich in nutrients (Patterson *et. al., 1998;* Fahey, 2005; Mark, 2010; Annette, 2012) and can thereby be used as soil amendments. Treatments with PM significantly influenced the height of okra plants. Moringa has been used as a growth enhancer (Fahey, 2005, Aluko, 2016) through foliar spray of the leaf extract but not as soil amendment. The response of okra to soil-applied MML was reflected in the growth parameters and fruit yield. Fuglie (2008) andMvumi*et al*. (2012 and 2013) had reported increased in the yields of crops with the application of moringa leaf extract. The increase in MML rates resulted in higher fruit yield which is similar to the observation of Aluko (2016) that the increase in concentration of moringa leaf extract as foliar spray improved pepper fruit yield. The significant increase in the growth parameters confirms the ability of plant residues to compete favorably with the inorganic fertilizers as sources of nutrients (Olujobi and Ayodele, 2013).MML and PM increased okra growth in the same magnitude as NPK 15-15-15 fertilizer and confirms that organic materials improved crop production by gradual release of nutrients (Akande*et al*.,2010). The use of organic and inorganic fertilizer mixture to improve okra crop production observed by Akande*et al (*2010) and Olujobi and Ayodele (2013) is similar to the response of MML + PM + NPK which would ensure steady release of nutrients, especially N that exerts the greater control on growth and yield potentialities of the soil in south western Nigeria (Olaniyi, 2006).

The integrated application of NPK 15-15-15, PM and MML gave higher yield compared to the sole application of these sources. Havlin*et al* (2004) had advocated the integrated approach to nutrient management in crop production for better performance. Akande*et al* (2010) noted that the combination of organic and inorganic fertilizer produced better yields of okra. The sole application of NPK 15-15-15 gave a better performance in terms of number of fruits and fresh fruit and dry fruit weights compared to the sole application of MML and PM. This is associated with ready availability of nutrients from NPK 15-15-15 produced in water-soluble form whereas PM would undergo microbial decomposition and mineralization through which nutrients are slowly released over a long period.

V. CONCLUSION

The results showed that the application of NPK 15-15-15, poultry manure and milled moringa leaf had significant effect on the performance of okra. The combination of NPK 15-15-15, poultry manure and milled moringa leaf gave better performance. Application of MML at different rates gave significant effect in growth characters except in number of leaves. Thus, milled moringa leaf can serve as source of nutrients for the production of okra.

REFERENCES

[1] **Akanbi, W. B. (2010).** Growth, Dry Matter and Fruit Yield Components of Okra under Organic and Inorganic Sources of Nutrients. American – Eurasian Journal of Sustainable Agriculture, 4(1): 1-13.

[2] **Akande M. O., Oluwatoyinbo F. I., Makinde, E. A., Adepoju A. S. and Adepoju I. S. (2010).** Response of Okra to organic and inorganic fertilization. *Nature and Science*. 8 (11): 261-266.

[3] **Akinyele, B. O. and Osekita, O. S. (2006).** Correlation and path coefficient analyses of seed yield attributes in okra *(Abelmoschusesculentus*(L.) Moench). Afri. J. Biotechnol., 5(14): 1330-1336.

[4] **Ali, M. B., Lakun, H. I., Sani, S. M., and Adamu, H. M. (2014).** Effect of organic manure and sowing date on the growth and yield of okra (AbelmoschusesculentusMoench) in Samaru Zaria, Nigeria. *International Journal of Agronomy and Agricultural Research (IJAAR)*, *5*(5), 111-117.

[5] **Aluko, M. (2016).** Moringa leaf extract on the growth and yield of pepper (*Capsicum annuum*). APRN Journal of Agricultural and Biological Science. 11 (3): 106-109.

[6] **Annette F. (2012).**Moringa: Tree helps in Niger's food crisis. Australia: The World Today/ABC News. Retrieved 13 August 2013.

[7] **Asadu, C. L. A., Ezeaku, P. I., and Nnaji, G. U. (2004).** The soils of Sub-Saharan Africa and management needs for sustainable farming. *Strategies and Indices of Sustainable Agriculture in the Tropics*, *2*: 1-27.

[8] **Ashraf, I., Ahmad, I., Nafees, M., Yousaf, M. M., and Ahmad, B. (2016).** A review on organic farming for sustainable agricultural production. Pure and Applied Biology. 277-286

[9] **Babalola, L. A. (2006).** Effect of NPK 15:15:15 on the performance and storage life of okra (*Abelmoschusesculentus*). *Proceedings of the Horticultural Society of Nigeria Conference*. 125-128

[10] **Babatunde, R.O., Omotesho, O.A and Sholotan, O.S. (2007).** Socio-economic characteristics and food security status of farming household in Kwara State, North – Central Nigeria. Pakistan Journal of Nutrition. 6 (1): 49-58.

[11] **Central Bank of Nigeria (CBN) (2004).** Annual Report and Statistical Bulletin. 6 (12) December 2004.

[12] **FMANR, (1990).** Literature Review on Soil Fertility Investigation in Nigeria (in Five Volumes). Federal Ministry of Agriculture and Natural Resources, Lagos. 32-45

[13] **Fahey, J. W. (2005).***Moringaoleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. *Trees for life Journal*, *1*(5).

[14] **Fuglie, L. J.(2008).** New Uses of Moringa Studied in Nicaragua: ECHO's Technical Network Site-networkingglobal hunger solutions. ECHO, Nicaragua: 1-7.

[15] **Havlin, J. L., Beaton, J. D., Tisdale, S. L. and Nelson, W. L. (2004).** Soil fertility and fertilizer: an introduction to nutrient management. Pearson Education. India, 2004: 106-153.

[16] **Iyagba, A. G., Onuegbu, B. A. and Ibe, A. E. (2013).** Growth and yield response of okra (*Abelmoschusesculentus*(L.) Moench) to NPK fertilizer rates and weed interference in South-

Eastern Nigeria. Int. Res. *J. Agric. Sci. Soil Sci. 3*(9):328-335.

[17] **Jonathan, W. C., Wong, D. J., Lee and Jaya Nair. (2012).** An evaluation of aerobic and anaerobic composing of banana peels treated with different inoculums for soil nutrient replenishment. In Advances in Biological Waste Treatment and Bioconversion Technologies. Eds: Jonathan, W. C., Wong, D. J., Lee and Jaya Nair. Bioresource Technology. 126: 375-382

[18] **Khan, W., Rayirath, U. P., Subramanian, S. *et al.*(2009).** Seaweed extracts as biostimulants of plant growth and development. Journal of Plant Growth Regulation. 28 (4): 386-399.

[19] **Makinde, A. I., Are, K. S., Oluwafemi, M. O., Ayanfeoluwa, O. E. and Jokanola, O. O. (2016).** Green Manure Source Affects Growth and Vegetative Yield of Fluted Pumpkin. *American Journal of Experimental Agriculture* 12 (4): 1-6.

[20] **Mark, O. (2010).**MoringaceaeMartinov. Drumstick Tree Family. Flora of North America, 1993. Flora of North America North of Mexico 7: 167 – 169.

[21] **Mvumi, C., Tagwira, F. and Chiteka, A. Z. (2012).** Effect of moringa extract on growth and yield of tomato. Greener Journal of Agricultural Sciences. 2 (5): 207-211.

[22] **Mvumi, C., Tagwira, F. and Chiteka, A. Z. (2013).** Effect of moringa extract on growth and yield of maize and commonbeans. Greener Journal of Agricultural Sciences. 3 (1): 055-062.

[23] **Nagar, P. K., Iyer, R. I. and Sircar, P. K.(2006).** Cytokinins in developing fruits of *Moringapterigosperma*Gaertn. Physiol Plant. 55: 45-50.

[24] **Olaniyi, J. O. (2006).** Influence of nitrogen and phosphosrus fertilizers on seed yield and equality of egusi melon *(Citrulluslanatus* (Thunb) Mansf) in Ogbomoso, South – Western Nigeria. Ph.D Thesis, University of Ibadan, Ibadan. 57 – 155.

[25] **Olujobi, O. J. and Ayodele, O.J. (2013).** Growth and yield of okra (*Abelmoschusesculentus*) in response to tree lrgume manure and urea fertilizer. IJAFS 4(12): 502-509

[26] **Pandey, S. K and Singh, H.(2011).** A simple, cost-effective method for leaf area estimation. Journal of Botany. 2011: 1-6.

[27] **Patterson, P. H., Lorenz, E. S. and Weaver, Jr. W. D. (1998).** Litter production and nutrients from commercial broiler chickens. Journal of applied Poultry Research, 7: 247-252.

[28] **Purseglove, J. W. (1992).** Tropical crops. Dicotyledon. Longman. 1:1-719.

[29] **Rahman, S. A. (2004).** The place of organic manure in sustaining agricultural development in Nigeria. Paper presented at Science Technology and Society National Workshop in Lafia, Nasarawa State, 11th July, 2004.

[30] **Tiamiyu, R. A., Ahmed, H. G., & Muhammad, A. S. (2012).** Effect of sources of organic manure on growth and yields of okra (Abelmoschusesculentus L.) in Sokoto, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, *20*(3), 213-216.

[31] **Udo, E.J., Ibia, T. O., Ogunwale, J. A., Ano, A. O. and Esu, I. E. (2009).** Manual of Soil, Plant and Water Analyses. Sibon Books Limited, Lagos, Nigeria, Pages: 183.

Contrast of a Quality Control Model for Sustainability in a Mexican Organization in Central Mexico

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Abstract— *Often, the total quality has been instrumented before being weighted. The strategies even precede a diagnosis in Mexican organizations, but in an opposite sense, the present work set out to establish the reliability and validity of an instrument to measure the perception of total quality based on three indicators related to management. , production and transfer of knowledge. A nonperimental study was carried out with a non-probabilistic selection of 124 administrative staff and employees from an organization in central Mexico. From a structural model* $\lceil X^2 = 123{,}24\ (23df)\ p = 0{,}010;\ GFI = 0{,}990;\ CFI = {,}991;\ IFI = 0{,}993;\ RMSEA = 0{,}007 \rfloor$*, it was found that management affects production (0,38) and this about the total perceived quality (0,35), although there are lines of research concerning empathy, commitment, entrepreneurship, satisfaction and happiness in relation to the implementation of continuous improvements to the quality of processes and products.*

Keywords—Client omission, Control strategy, Logistics mistake, Wrong delivery.

I. INTRODUCTION

No doubt, organizations have some mistakes in its organizational context, however, sometimes, mistakes are over-dimensioned because of clients' honest lack. It is when the organization need to have a severe control of it processes, even administrative, financial, sales, production or logistics ones. Organizations which promote the use, production or consumption of green energies, also are attached to negative factors occurrence over its processes. Present document, look forward to be a path on mistake occurrence, when it is considered the logistics' or deliveries' mistakes, in the framework of sustainability's context, due to the need of green organizations hold in the market to promote clean energy methods.

Concern about sustainability has been grown in people's mind. Debate since the release of the World Conservation Strategy in 1980, "Our Common Future" the report of the World Commission On Environment and Development in 1987 and Agenda 21 in 1992 has resulted in gradual acceptance that sustainability must integrate ecological integrity, economic efficiency and social equity (Côté& Cohen-Ronethal, 1998).

In Molina Ruiz (2013), it is mentioned that there exists an alarming situation, due to planets situation. In Mexico, it is possible to see the negative influence of population impact over environment (Molina-Ruiz, 2015). It is also possible to observe some social deterioration and economic problems. Cavagnaro & George (2017) propose a framework in which they are recognized the three main dimension of sustainability.

It is important to promote wellbeing inside the organizations. In the framework of sustainability, organizations which promote use of clean energies, sometimes are in a constant risk that threaten its stability.

It is natural for organizations to have some mistakes along its development and historical path, however, when client shows a lack of honesty and omit information sharing, the organization have a higher spend of resources to correct the mistake or repair the problem. Between organizations it is necessary to create a supporting environment in which the stakeholders share information with each other.

In order to survive on the market and achieve profitability, the companies need to meet customer requirements and perform their activities in an efficient way (Andrejić, Kilibarda&Popović, 2015). However, some clients abuse of the organizations good will, bringing extra cost in the organizational use of resources.

Sometimes, inside of the organizations, low compromised personnel have cheating attitudes that affect directly the organization performance. In Bohte& Meier (2000), it is defined organizational cheating as an attempt to manipulate performance criteria; it is also identified three major forms of organizational cheating:1) cutting corners (doing sloppy work); 2) lying (making up organizational results); and 3) biasing samples (reporting most conductive cases).In the organizational context it can be identified another way of organizational cheating, "client's snuggling", which means that a stakeholder inside of the organization overprotect the client, giving to it privileged information and covering bad client (or supplier) behavior that affects the organization.

Cialdini, Petrova& Goldstein (2004), proposed that organizational dishonesty can increased surveillance, (mis)matches between values of employee and organization and/or reputation degradation. It is also possible to state that organization dishonesty can make that enterprise run out of business (bankruptcy), loss of clients, loss of suppliers, loss of bank or credit-agents' support.

Enterprise in which it happened the case under study had certain particularities. It is an enterprise relatively new in the photovoltaics sector in Mexico, it was created in 2013. Due to its recent creation, there was a lack in the control and organization of different activities inside of the organization. That organization has the second place in sales in Mexican market, during 2015. During 2017, it has increased its market share to North America and Central America. In Mexico, the enterprise recovers the second position in importance by Mexican PV-market.

First detected particularity was, as here exist a cordial and close communication, delivery of final product would be required via a piece of paper written by sales manager and given to production manager.

Despite there exist four main steps to deliver a merchandize, sales manager, due to urgency of delivery, avoid the sequence of steps. The correct step by step in the enterprise would be as follows: (a) quotation price document, in which sale's agent sent the price and characteristics of the product to client, in case client accept the price and characteristics, it is generated (b) the request document, in which warehouse is notified that a product need to be packaged, it also is sent to the client so he/she can make the payment, to make (c) the invoice document, which is the official document and ensures that merchandize is now client's property, once invoice is created, it is made a (d) warehouse authorization, a list of the allowed merchandise's delivery to client, via Delivery-service outsourcing.

Sometimes it was authorized the delivery of merchandize, when the quotation price document was just generated, because of the request of sales manager.

There were some situations in which sales manager sent a "request document" to logistics department (warehouse), with missed information, and after, she resent mentioned document with extra information or with corrections in the information, or sales manager hold the (extra) information document (or the corrected one) for itself.

Warehouse do not have a complete folder for each delivery. Deliveries were just registered in a list with very little information, and the folder for each delivery (invoice) do not have all of the documents.

II. THEORY OF PERCEIVED QUALITY

In the anthropocentric paradigm in which companies circumscribed their total quality control to the demands of the market and the specific demand of their clients, the function of the leader was that of an intermediary who managed and managed the risks without considering the environment or capital nor the possibilities of human or intellectual capital in face of the imbalance that the situation implied (see Figure 1).

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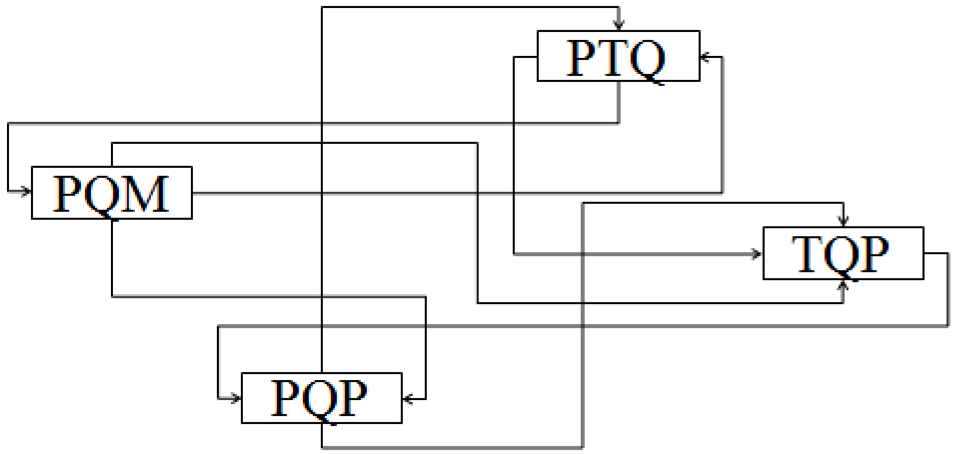

Fig.1: Theory of Perceived Quality

TQP = Total Quality Perceived, PQM = Perceived quality management, PQP = Perception of quality production, PTQ = Perceived Transfer of Quality
Source: Elaboration with study data

In the paradigm of sustainability, the total quality lies in the evaluation, certification and accreditation of processes based on the availability of resources, policies against climate change, the effects on environmental public health and the risks inherent in the Industrial production (Acar&Acar, 2014).

While in the old anthropocentric paradigm the responsibility was centered on the leader, the manager or administrator, in the new ecocentric paradigm the responsibility is shared (Hernandez & Valencia, 2016). This implies a unilateral communication versus a bilateral communication, a unidirectional motivation versus a bidirectional motivation. It is about the confrontation of two cultures, one authoritarian and the other democratic (Anicijevic, 2013).

Even the new environmental paradigm is distinguished from the previous dominant paradigm by the continuous improvement of processes (Mendoza, Ramirez &Atriano, 2016). This supposes the entrepreneurship and the innovation of the processes that in the previous paradigm was translated in a resistance to the change. That is to say that the responsibility of participation and initiative now concerns all those who integrate the organization (Carreón et al., 2014).

The achievement of a shared responsibility precedes a shared work commitment and a climate of emotional, affective and sentimental relationships regulated and oriented to coexistence, respect, solidarity and support among those who make up the organization (Cruz, Arroyo & Marmolejo, 2016).

Therefore, there to define quality standards and criteria for its continuous improvement, the organization involves leaders and managers, managers and employees in the objectives, tasks and goals according to the availability of resources, social responsibility and organizational capabilities (Escobar, 2014).

III. SPECIFICATION MODEL

Formulation

Will the relationships proposed in the theory of perceived quality be adjusted to empirical observations with leaders and employees of an organization in central Mexico?

Null hypothesis

The relationships between the variables specified in the theory of perceived quality will be adjusted to the data observed in an organization in central Mexico, since it is a universal asymmetric relationship between the demands of the environment and organizational capacities, which also mark differences between leaders and employees

Alternative hypothesis

Although the theory of perceived quality anticipates scenarios of differentiation between the requirements of the environment and the capabilities of the organization, among leaders and employees, the perceptions around the total quality process, as well as control management are different in each organization reason why the relationships established in the theory will not conform to the observations of a case study

Relations on the factors

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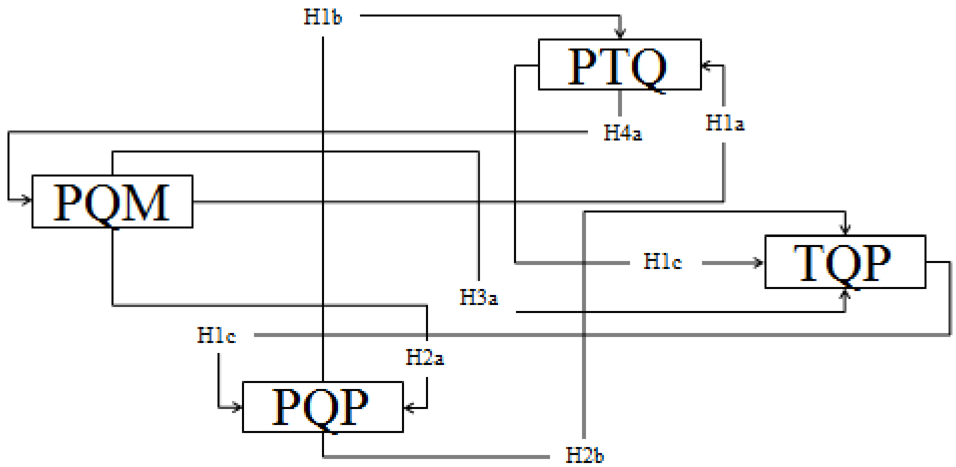

Fig.2: Specification model

TQP = Total Quality Perceived, PQM = Perceived quality management, PQP = Perception of quality production, PTQ = Perceived Transfer of Quality

Source: Elaboration with study data

In the following paragraphs, it is reported different events linked to wrong delivery made by the provider enterprise. Data have been changed or modified to protect confidential information of different enterprises and persons.

On Tuesday, July 5th, 2016 it was a wrong delivery of 8 panels of 260W and a 2.0 KW inverter, from the invoice X57X, whit tracking number AB00XX2970X, to our client, Renewable Energies Co. (SolarGroup). It because our last delivery to that client was to its address on the Southeast of Mexico.

In following figure, it is represented the invoice linked to mentioned delivery, that invoice was made by 12 PV-modules, 1 inverter of 2 KW and 1 WiFi stick for the inverter.

On Friday, July 15th, 2016, our sales manager communicates to us that the client complains because he was not received his product (delivery was sent to Southeast of Mexico). Sales department manager, request that logistics would sent 5 panels of 260W and a WiFi stick, to other address in the northwest of Mexico.

They were delivered on Saturday, July 16th, 2016. The 5 panels and 1 WiFi stick was sent to Delivery-service (center of Mexico's Office) by an outsourcing service by $ 500.00 plus taxes ($580.00), which take the merchandize from the factory to the Delivery-service's office.

It was stared the process to recover the merchandise on July 18th, 2016, with almost daily callings to Southeast's office of Delivery-service and occasional calling to Delivery-service's Call Center. Logistics department tried to establish a communication bridge. It is pointed out, that the communication with Southeast's office was very narrow and sometimes it is not possible communicate whit them.

There was also made some other calling to Southeast's Delivery-service Office. On Monday, July 18 it was made the phone call to Delivery-service's Call Center (XX XXX X10 8352), logistics department was attended by Attendant I so they were obtained the following phone numbers:

(XXX) XX3 0953

(XXX) XX3 0972

(XXX) XX3 0973

On Tuesday, July 19, 2016, it was contacted Delivery-service's Office in Southeast, so Attendant II ask to request the re-expedition of panels and inverter, by sending an e-mail to attx@deliveryserv.com.mx and attiii@deliveryserv.com.mx, to Attendant X and Attendant III

On Friday, July 22nd, 2016, it was made a call again to Delivery-service (Southeast's Office), Attendant II answered, and gave the extension number of Attendant X and Attendant III. Attendant II take the phone call to the extension of Attendant III. When Attendant III, answered said that she has already sent the quotation to send back panels and inverter to factory. It was set a price of $5634 pesos, so Logistics department request a quotation to Delivery-service Office (Center of Mexico's Office).

On Monday, July 25th, 2016, it was made another phone call to Southeast's Office, but there was no answer. On Tuesday 26th, 2016, it was called again to Southeast's

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Office, however in both lines the calling was stopped. It was made a phone call to Delivery-service's Call Center answering Attendant IV, she gave again the same phone numbers from Southeast's Office, and transferred the phone call to that office, that moment, answered Attendant II and logistics was hanged on the line, after a while, she asked to resend the request to Southeast's Office, so the request was resent.

On Thursday, July 28th, 2016, there was made another communication to Southeast's Office, and it was requested (Attendant X), to resend the last request, due to he has not received mail nor document.

On Friday, July 29th, 2016 and Monday, August 1st, 2016, logistics try to communicate to Southeast's Office but there was no answer. On Monday, August 8th, 2016, logistics try to communicate to Southeast's Office but there still was no answer. On Tuesday, August 16th, 2016, logistics try to communicate to Southeast's Office but again, there was no answer. On Friday, August 19th, 2016, logistics try to communicate to Southeast's Office but there was no answer. On Monday, August 22nd, 2016, logistics try to communicate to Southeast's Office but there still was no answer. On Wednesday, August 31st, 2016, logistics department try again to communicate with Southeast's Office but still no answer.

On Friday, September 2nd, 2016, logistics department make a phone call to Delivery-service's Call Center, answering Attendant V, she request the basic information of the delivery an she found out the that merchandise was already picked up by the client, the person who picked up the merchandise was named: Mauricio E. A., merchandise has been taken by that person on August 11th, due to a connection failure the calling was ended. However, logistics department call back again, attending Attendant VI, she communicate logistics with Attendant III (in Southeast's Office), and Attendant III said she was checking and she said she was calling logistics back, but Attendant III did not make any phone call.

On Monday, September the 5th, 2016, logistics try to communicate to Southeast's Office but again, there was no answer. On Tuesday, September 61h, 2016, there was made a phone call to Southeast's Office, that time answered Attendant III: she made the link with Attendant VII, and Attendant VII request to ask via mail for support to recover the information of the case. An e-mail was sent to request the support to recover the evidences that Southeast's Office, have in order to integrate a report or (if necessary) to start a legal motion.

On Tuesday, September 8th, 2016, there was made a phone to Southeast's Office, but there was no answer. On Friday, September 9th, 2016, there was made a phone call to Southeast's Office and answered Attendant III. It was requested to talk with Attendant VII, en the phone call was transferred to the Attendant VII's extension. Attendant II answered and she said, it was not possible to talk to Attendant VII, but Attendant II was told about the situation, so she inform that it wouldn't be possible to recover any picture or video due to Southeast's Office data base only cover 21 days of record. However, Attendant II, agree to look for the document linked to tracking number ABO0XX2970X, and send it via mail to Logistics Department, to check the person who had signed and toke the merchandise.

Some days after that communication it was received the e-mail in which a person of Renewable Energies Co.'s, required that merchandise would be given to Mauricio E. A.

On a general way, to avoid problems on merchandize delivery, it was adopted a very strong attitude over the sales manager informal requests, respecting the stablished procedure to deliver merchandize and it was stablished a delivery's binnacle in warehouse and security gate.

It has been mentioned that sales manager asked for deliveries with quotation price documents or with request document, so production and logistics department, avoid the informal authorizations to delivery products or material. The process was stablished as a four steps method: (a) quotation price document (b) request document (c) invoice document and, (d) warehouse authorization.

After the problems, it was integrated a complete folder for each delivery and added some documents to complete it. It has been mentioned that, previously, a delivery can be authorized with a quotation or request document, but with the new way of working, it was required the following documents to authorize a delivery: i) quotation, which have the price authorized to sale the merchandize; ii) request, which includes authorized price and correct data and address linked to merchandize sold; iii) payment, it is the ticket or voucher (scanned, picture taken, or PDF) in which it can be seen the linked payment for each bought material (in the case of check, it was necessary to wait three days, until the amount of money was contrasted in the enterprise's bank account); iv) invoice, generated invoice after payment check in; v) sent data ticket, which have the information to be delivered by the outsourcing delivery service; vi) warehouse binnacle, where they were registered each material (invoiced) delivered (and contains data like: date, quantity, model, client, invoice, client's Federal Taxpayer

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Registry, driver, license plate, sent mode); vii) tracking number, it is the obtained document linked to delivery service

IV. METHOD

Design.

A descriptive, exploratory and transversal study was carried out

Sample.

124 administrative and employees of a for-profit organization in the center of Mexico.34% men and 66% women. 75% under 29 years old (M = 24,13 SD = 0,18), 15% between 29 and 65 years old (M = 41,23 SD = 10,17) and 5% over 65 years old (M = 67,32 SD = 0,16). 22% with more than 7 working years (M = 7,12 SD = 0,12), 38% with less than 7 and more than 3 working years (M = 4,35 SD = 0,84), 28% with less than 3 working years (M = 2,43 SD = 0,93).

Instrument.

The Total Perceived Quality Scale of Carreón (2016) was used, which includes four dimensions related to the management, production and the perceived transference of the quality of processes. each reagent includes five answer options that go from 0 0 it does not look like anything to my organization up to 4 = it looks a lot like my organization.

Proceeding.

The Delphi technique was used for the processing of information and the elaboration of the reagents, comparing and integrating informative information to the total quality, as well as to the opinions of different administrative and employees in an organization for profit in the center of Mexico.

Subsequently, the surveys were applied in the human resources department as part of the staff recruitment and selection protocol, as well as part of the induction, training and training courses. The confidentiality and anonymity of the respondents was guaranteed in writing, as well as the warning that the results of the study did not affect their economic or work status.

The consistency of the instrument was estimated in terms of its questions from the answers, considering the Cronbach alpha parameter, as well as the Barttlet and KMO tests for adequacy and sphericity as preliminary tests to the validity, which was performed with a method of extraction of main axes with promax rotation. The comparison of the model with adjustment and residual parameters for the hypothesis test.

V. RESULTS

Table 1 shows the values of internal consistency of the instrument (alpha of 0.782 for the general scale and 0.780 to 0.795 for the subscales) which suggest that in other contexts and study samples the measurement of indicators and factors will be similar in up to 70% of cases.

Table.1: Descriptives of the instrument

Code	Item	M	SD	A	F1	F2	F3	F4
PQM1	Prevention against risks	3,21	0,19	0,701				0,439
PQM2	Disasterprevention	3,25	0,28	0,702				0,329
PQM3	Preventionagainstviolence	3,45	0,38	0,731				0,431
PQM4	Conflict prevention	3,25	0,43	0,721				0,403
PQM5	Accident prevention	3,46	0,54	0,742				0,325
PQM6	Prevention against epidemics	3,67	0,83	0,721				0,345
PQM7	Preventionagainstdiseases	3,93	0,48	0,742				0,392
PQP1	Production before demands	3,02	0,91	0,743			0,431	
PQP2	Competitiveness in the face of shortages	3,01	0,29	0,741			0,423	
PQP3	Entrepreneurship before needs	3,26	0,39	0,752			0,504	
PQP4	Continuous improvement in the face of backlog	3,46	0,40	0,704			0,593	
PQP5	Continuous improvement before absences	3,41	0,53	0,725			0,502	
PQP6	Continuous improvement against rotation	3,24	0,45	0,721			0,501	
PQP7	Continuous improvement against fraud	3,25	0,41	0,793			0,504	
PTQ1	Securities against corruption	3,44	0,24	0,783		0,305		
PTQ2	Empathy in the face of absenteeism	3,12	0,32	0,702		0,416		
PTQ3	Communication in disasters	3.11	0,22	0,771		0,406		
PTQ4	Conflict support	3,02	0,33	0,772		0,493		

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PTQ5	Disappearance rules	3,26	0,13	0,783		0,492
PQT6	Incentives for absenteeism	,345	0,21	0,711		0,501
PTQ7	Emergency response	3,46	0,34	0,705		0,403
TQP1	Attachment to the company	3,47	0,02	0,783	0,403	
TQP2	Thanks to the company	3,41	0,38	0,783	0,302	
TQP3	Recognition to the company	3,26	0,49	0,756	0,392	
TQP4	Delivery to the company	3,27	0,93	0,736	0,491	
TQP5	Put on the company shirt	3,38	0,12	0,747	0,302	
TQP6	Respect for company values	3,04	0,21	0,746	0,321	
TQP7	Execution of company protocols	3,36	0,32	0,726	0,301	

Method of extraction of the main axes, promax rotation. Adequacy and Sphericity ⌈X^2= 452,67 (56df) p = 0,000: KMO = 0,770⌋. M = Average, DE = Standard Deviation, A = Alpha, quitting the item value. F1 = Perceived Quality Management (alpha of the 0,780 and the 24% of the variance explained), F2 = Production Perceived Quality (alpha of the 0,785 and 21% of the variance explained), 3 = Perceived Quality Transfer (alpha of the 0,790 and the 16% of the variance explained), F4 = Perception of Total Quality (alpha of the 0,795 and the 11% of the variance explained). All the items are answered with five response options: 0 = it does not look like my organization, 1 = it seems very little to my organization, 2 = it seems little to my organization, 3 = it appears in something to my organization, 4 = it looks a lot like my organization

Source: Elaborate with study data

Figure 3 shows that the perceived management of quality determines the perceived production of quality (0,38), but this last factor is determinant of the total perceived quality (0,35).

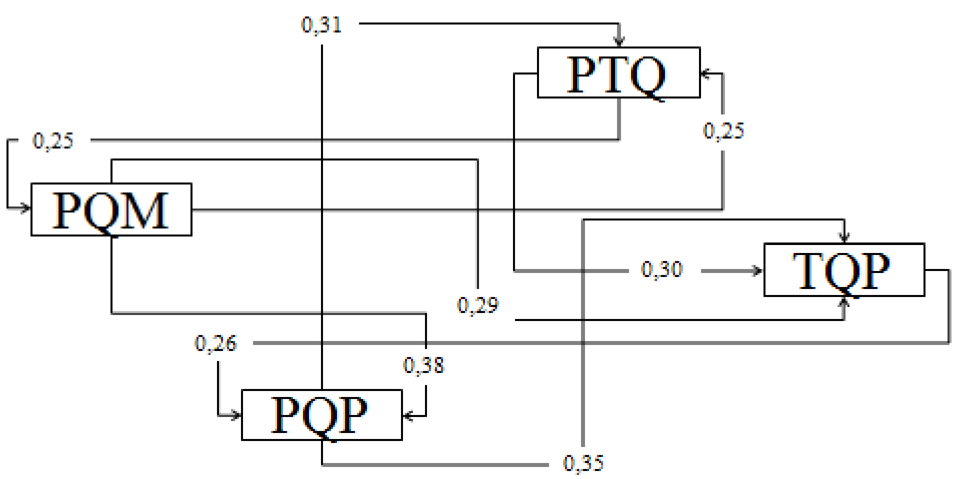

Fig.3: Structural model

Source: Elaborated with study data

The parameters of adjustment and residual ⌈X^2 = 123,24 (23df) p = 0,010; GFI = 0,990; CFI =,991; IFI = 0,993; RMSEA = 0,007⌋suggest the acceptance of the null hypothesis, relative to the relations of dependence between the factors used in the state of the question and demonstrated in the empirical test.

VI. DISCUSSION

The contribution of this study to the state of the question lies in the establishment of the reliability and validity of an instrument that measures the perception of management, production, transfer and the totality of the quality of the processes, but the type of non-experimental study , the type of non-probabilistic selection and the type of exploratory factor analysis limit the results of the study to the sample and the context of the investigation.

It is recommended to extend the study to other contexts and samples, using sophisticated analysis of factors such as the least squares technique in order to confirm the structure that underlies the perception of total quality, configured by three factors related to management, production and the transfer of knowledge.

It is so important for the organization to hold a substantial list of clients. It because, the client is the stakeholder that provides organization with the financial resource to going on with its labor and remain in its market share. However, it is more important to have a selected list of clients which can be recognized a loyal to the enterprise, and in which case can be a support for the organization.

With the strict control applied on the PV-modules enterprise, apparently mistakes where reduced. In the practice, there were some mistakes on deliveries, however, all of the was due to mistakes in the information provided by sale's agents, main mistakes detected still being in the address given by sale's agents and sale's manager.

With strict control strategy application, it was also possible to determine responsibilities. Due to wrong deliveries, responsibility for each mistake was charged to logistics department or production warehouse, however, when control strategy started it application, it was recognized that mistakes and/or omissions were mainly produced by data provided thorough sales department. Very little mistakes was due to Delivery-service omissions.

VII. CONCLUSION

In the economy, the total quality is a preponderant factor in the processes and the products, although the labor climate that supposes such company is centered in the analysis of positions, worker cycle and the motivation of the worker as determining factors of a system of management, production and transfer of knowledge oriented to the continuous improvement of the scientific, technological and industrial process.

REFERENCES

[1] Acar, Z. and Acar, P. (2014). Their organizational culture types and effects on organizational performance in Turkish hospitals *Emerging Markets Journal*, 3 (3) : 1-15 [DOI: 10.5195 / emaj.2014.47].

[2] Andrejić, M., Kilibarda, M. &Popović, V. (2015). Logistics failures in distribution process, 2nd Logistics International Conference, available at: [http://logic.sf.bg.ac.rs/wp-content/uploads/Papers/LOGIC2015/ID-41.pdf].

[3] Anicijevic, N. (2013). The mutual impact of organizational culture and structure. *EconomicAnnals, 58* (198), 35-60

[4] Bohte, J. & Meier, K. J. (2000). Goal displacement: Assessing the motivation for organizational cheating. Public Administration Review, 60 (2), 173-182, available at: [http://onlinelibrary.wiley.com/doi/10.1111/0033-3352.00075/full].

[5] Carreon, J. Hernandez, J., Garcia, C. Garcia, E., Rosas, F. Aguilar, J. (2014). Specifying a digital enterprise model for human development through intensive use of information and communication technologies. *Rural Perspectives*, 13 (25), 123-155

[6] Cavagnaro, E., & George, H. (2017). The three levels of sustainability. Routledge. ISBN-13: 978-1-906093-68-6, available at: [https://books.google.com.mx/books?hl=es&lr=&id=vqk0DwAAQBAJ&oi=fnd&pg=PT7&dq=herman+daly%27s+three+filter+economic+social+environment+1987&ots=ZWnL1VB-3E&sig=LVc-vxGU9RPT4ajTGlTbJvALQ4c&redir_esc=y#v=onepage&q&f=false].

[7] Cialdini, R.B., Petrova, P.K. & Goldstein, N.J. (2004). The hidden costs of organizational dishonesty: companies that engage in unethical practices face consequences far more harmful than is traditionally recognized. The resulting damage can easily outweigh the short-term gains, MIT Sloan Management Review. 45 (3), 67-73, available at: [http://go.galegroup.com/ps/i.do?id=GALE%7CA116484228&sid=googleScholar&v=2.1&it=r&linkaccess=fulltext&issn=15329194&p=AONE&sw=w&authCount=1&u=uaeh1&selfRedirect=true] & [http://mylearning.denverzoo.org/ets/companies/fbfdf7ad-f5a3-416e-8c31-62af837c7f0a/UserFiles/Article%20Archive/Culture%20Articles/The%20Hidden%20Cost%20of%20Organizational%20Dishonesty_Article.pdf].

[8] Côté, R.P. y Cohen-Ronethal, E. (1998). Designing eco-industrial parks: a synthesis of some experiences, Journal of cleaner production, 6 (3-4), 181-188, DOI: [https://doi.org/10.1016/S0959-6526(98)00029-8], available at: [http://www.sciencedirect.com/science/article/pii/S0959652698000298] & [https://ac.els-cdn.com/S0959652698000298/1-s2.0-S0959652698000298-main.pdf?_tid=6b56d41c-9fb0-11e7-a8e0-00000aacb35d&acdnat=1506096752_8c6e6bdac145b7fed82995e5a9dc42ff].

[9] Cruz, O., Arroyo, P. and Marmolejo, J. (2016). Technological innovations in logistics: inventory management, information systems and

http://dx.doi.org/10.22161/ijeab/2.6.18 *ISSN: 2456-1878*

outsourcing operations. In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 165-178). Mexico: Miguel Angel Porrua-UAEMEX.

[10] Escobar, R. (2014). Neural networks, cognitive processes and behavior analysis. *International Journal of behaviorism*, 2 (1), 23-43

[11] Hernandez, A. and Valencia, R. (2016). Innovation instruments: social networks in the internalization of micro, small and medium - sized Mexican companies. In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 47-66). Mexico: Miguel Angel Porrua-UAEMEX.

[12] Mendoza, E. Ramirez, L. and Atriano, R. (2016). Use of media and technology in creating an innovation system for the common good. In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 95-114). Mexico: Miguel AngelPorrua-UAEMEX.

[13] Molina Ruiz, H.D. (2013). Aproximación de cálculo de la huella de carbono en una institución de educación media superior y superior. Innovación y Desarrollo Tecnológico Revista Digital, 5 (3), ISSN: 2007-4786.

[14] Molina Ruiz, H.D. (2015). Three levels analysis of sustainability's environmental dimension in México. Innovación y Desarrollo Tecnológico Revista Digital, 7 (4), ISSN: 2007-4786.

[15] National Institute of Statistics, Geography and Informatics. . (2010) *XI National Population Census.* Mexico: INEGI

[16] Omotayo, and Adenike O., A. (2013). Impact of organizational culture on human resource practices: a study of selected Nigerian private universities. *Journal of Competitiveness,* 5 (4), 115-133 [DOI: 10.7441 / joc.2013.04.07]

[17] Quintero, M., Velázquez, E., Sales, and J. Padilla, S. (2016). A review of the state of the art on SMEs. What innovation studies? In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 31-43). Mexico: Miguel AngelPorrua-UAEMEX.

[18] Robles, C., Alviter, L., Ortega, A. and Martinez, E. (2016). Culture of quality and innovation in microenterprises. In M, Quintero., Sales, and J. Velázquez, E. (Coord.).*Innovation and technology challenges for practical application in companies.* (Pp. 11-30). Mexico: Miguel AngelPorrua-UAEMEX.

[19] Saansongu, E. and Ngutor, D. (2012). The influence of corporate culture of employee commitment to the organization. *International Journal of Business and Management*, 7 (22) : 1-8

[20] Sales, J., Quintero, M. Velázquez, E. (2016). Adaptation versus innovation: the formation of industrial districts from rural communities. Santa Cruz Atizapan and Chiconcuac.In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 181-199). Mexico: Miguel AngelPorrua-UAEMEX.

[21] Vazquez, C., Barrientos, B., Quintero, M. Velázquez, E. (2016). Government support for innovation, technology and training for small and medium enterprises in Mexico. In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies*. (Pp. 67-78). Mexico: Miguel AngelPorrua-UAEMEX.

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Impact of Selenium Nanoparticles on Growth, Biochemical Characteristics and Yield of Cluster Bean *Cyamopsis tetragonoloba*

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Abstract— *The present study deals with the impact of selenium nanoparticles on growth, biochemical characteristics and yield of Cluster bean Cyamopsis tetragonoloba grown for a period 60 days Sodium selenite and ascorbic acid was utilized for the synthesis of Selenium nanoparticles using precipitation method. Selenium nanoparticles were characterized by using SEM, EDAX, FTIR and XRD. Pot culture studies of cluster bean in different quantity of Selenium nanoparticles such as 0,100, 200, 300, 400 and 500mg for treatment T_0 (Control) T_1, T_2, T_3, T_4 and T_5 and growth biochemical and yield were estimated at the end of 60 days. SEM image of selenium nanoparticles was observed as spherical in shape. EDAX spectrum recorded on purity of selenium nanoparticles. The FTIR spectrum of selenium nanoparticles was analyzed in the range of 4000-400 cm^{-1} spectral bands were observed. The germination percentage in T_0, T_1, T_2, T3, T4 and T5 are 100,90,80,90,100 and 100 respectively. Among the treatments the shoot length is higher (21.8) in T_1 containing 100mg of selenium nanoparticles and lower in(12.01) T_5 containing 500mg of nanoparticles. Root length, fresh and dry weight and leaf area were higher in T_2. The vigor index is higher T_4.The chlorophyll a, b total Chlorophyll, carotenoids, anthocyanin, protein, L-proline, free amino acids and leaf nitrate were higher inT_4.Among the treatments yield of cluster bean is higher in T_4 and lower in T_0.*

Keywords— Impact, Selenium, Nanoparticles, Growth, Biochemical, Yield, Cluster bean.

I. INTRODUCTION

Nanotechnology is highly promising and rapidly progressing discipline in research and influencing every field of science and biology. Nanotechnology is creating many new materials and devices with a vast range of application such as medicine, biomaterials and energy production. Exploring comprehensive application profile nanoparticles may revolutionize research in crop science and transform agriculture in to industry (1). Application of nanotechnology in agriculture delivery to plant technology also holds the promise of controlled release of agro chemicals and its targeted delivery of various macromolecules needs for improved plant disease resistance, efficient nutrient utilization and enhanced plant growth. Recent research on nanoparticles in a number of crop like corn, wheat, soybean, tomato and cucumber have provided evidence of enhanced seedling growth, germination, nitrogen metabolism, photosynthetic activity and protein level indicating their potential use for crop improvement. Among nanoparticles, Selenium is proved to be an essential mineral required for proper health, immunity, and reproductive functions of animals. Plants are the main source of this element, it is important to increase its plant growth. A new approach to fertilization of plants is the use of selenium nanomaterials (2).The study related to the impact of selenium nanoparticles on growth, biochemical characteristics and yield of vegetable crop cluster bean is totally wanting. Hence the present study was carried out.

II. MATERIALS AND METHODS

2.1. Synthesis of Selenium Nanoparticles

Precipitation method is adopted for the synthesis of selenium nanoparticle. For the synthesis 0.7Mg of (700mg) sodium selenite were dissolved in 50ml of distilled water under stirring vigorously using magnetic stirrer for 20 minutes. After stirring, the precipitation was achieved by adding 50ml of ascorbic acid solution in drop wise under constant stirring. The initial pH was observed as 3 and it was increased to pH 14 .Then precipitating process was continued until the orange colour precipitate was obtained. Then this precipitate was centrifuged at 1500 rpm for 20 minutes. The centrifugal process was continued. The obtained precipitate was dried in room temperature. Finally selenium nanoparticles were obtained.

2.2. Characterization of selenium nanoparticles

The synthesized selenium (Se) nanoparticles were characterized by SEM, EDX,FTIR and XRD.

2.3. Collection of Red Soil for Pot Culture Studies

Garden soil (red soil) was collected from the Nursery, Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram. For the collection of red soil a trench of 25 cm depth was dug out and red soil was taken from the trench. The red soil was dried in the shade, powered using wooden mallet and sieved through a 2mm sieve before used for analysis. P^H

2.4 .Sources of Materials used in Pot Culture (Seeds and Cowdung)

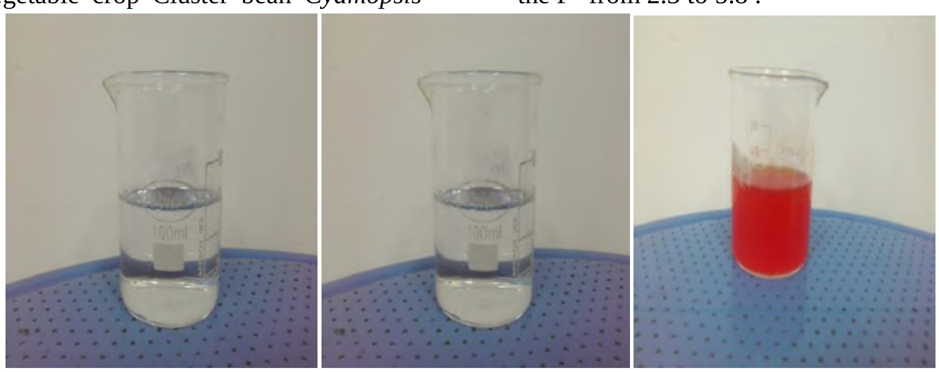
Seeds of Cluster bean were collected from Bavani store, Dindigul, Tamil Nadu, India, Cow dung was collected from School of Agriculture and Animal Science, Gandhigram Rural Institute- DeemedUniversity, Gandhigram, Tamil Nadu and India. Vegetable crop Cluster bean *Cyamopsis tetragonaloba* was selected for pot culture studies based on their easy availability, relative importance in daily diet of a common man, surviving capacity, growth capabilities and economic growth.

2.5. Pot Culture Studies:

For the pot culture studies, the seeds were soaked in ground water and kept as control. Both the control and experimental seeds were allowed to grow in plastic pots (25 cm diameter, 25 cm height) containing a mixture of red soil, cow dung manure in the ratio of 1:1 The experimental pots were supplied with different quantities of selenium nanoparticles such as 0,100,200,300,400 and 500 for treatment 1 (Control) 2, 3,4,5,6 respectively. Triplicates were maintained and grown in net house for a period of 60 days. Pots were irrigated with well water. After 60 days growth and biochemical characteristics were estimated.

III. RESULTS AND DISCUSSION

As $C_6H_8O_6$ was added to Na_2SeO_3, it is found to change colour from orange to red colour is shown in Fig.1 and this colour change indicates the synthesis of selenium nanoparticles (Se).Precipitation was observed by increasing the P^H from 2.3 to 5.8 .



a)SODIUM SELINITE ((Na_2SeO_3)   b) ASCORBIC ACID ($C_6H_8O_6$)   c) SELENIUM(Se)

Fig.1: Synthesis of Selenium Nanoparticles

The SEM image (Fig.2) showing the high density chemical by synthesized Se further confirmed the development of selenium nanostructures. Obtained nanoparticles showed spherical in nature. The microscopic image showed that the Se nanoparticles did not appear as discrete particles but form much larger dendritic flocks whose size could reached micron scale size range about 9.09mm (scale bar 5µm),9.07mm 27mm (scale bar 10µm),9.05mm (scale bar 20µm) for figure 1 a,b and c respectively. Scanning electron microscope (SEM) images were taken for the analysis of size and shape of SeNPs (Hitachi s-3400N) with resolution of 500 nm operated at 10 kV HV mode and detectors contain secondary electron; semiconductor BSE (Quad type)(3). The SEM images of selenium nanoparticles synthesized by different combinations were oval in shape with smooth surface. The particle size was found to be around 50–150 nm. Sonam Malhotra et al.,(2014)(4) suggested that properties forming a spherical shape nanoparticle having a size range of 20 to 30 nm as measured using particle size analyser, purity of the Nano selenium were further measured by the (SEM) Scanning Electron Microscope. Selenium nanoparticles were highly using

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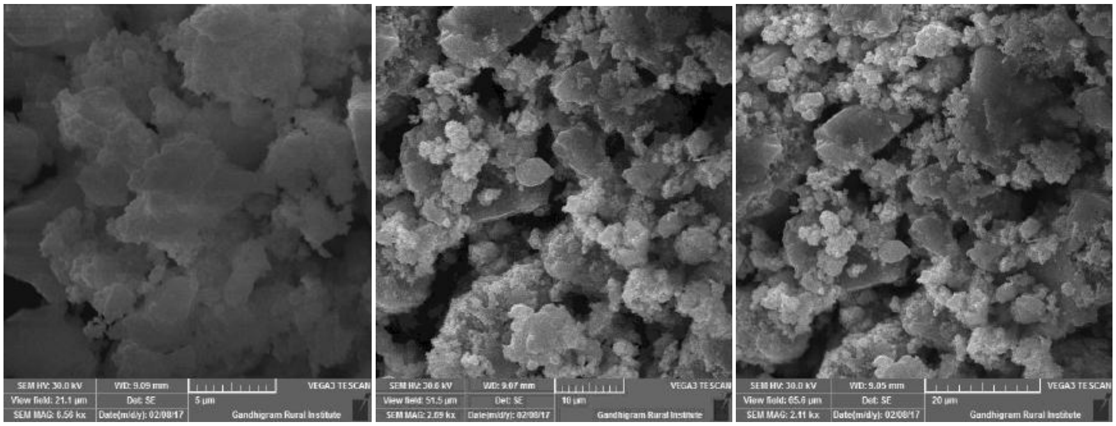
Dextrin obtained from Maize starch. Selenium nanoparticles coated varied from 5% - 20%.



a) 5µm of selenium nanoparticles b)10µm of selenium nanoparticles c) 20µm of selenium nanoparticles

Fig.2: Scanning Electron Microscopic (SEM) Image

EDAX spectrum recorded on the selenium nanoparticles is shown as two peaks located between 1.6Kev and 10.8Kev (Fig. 3),those maxima are directly related to the selenium characterized lines.The maximum peak located on the spectrum at 10.9Kev clearly coming from selenium. The second maximum peak located on the spectrum at 1.6Kev. JonneRodenburg et al.,(2014)(5) suggested that EDX profile shows a strong selenium signal along with weak sulfur group peaks. The result indicated that 92.76% (wt.) of the sample had the presence of selenium nanoparticles. The detection of the presence of sulfur 7.24% (wt.) in the EDX spectra, confirms the presence of sulfur containing protein/peptide molecules bound to the surface of the nanoparticles.

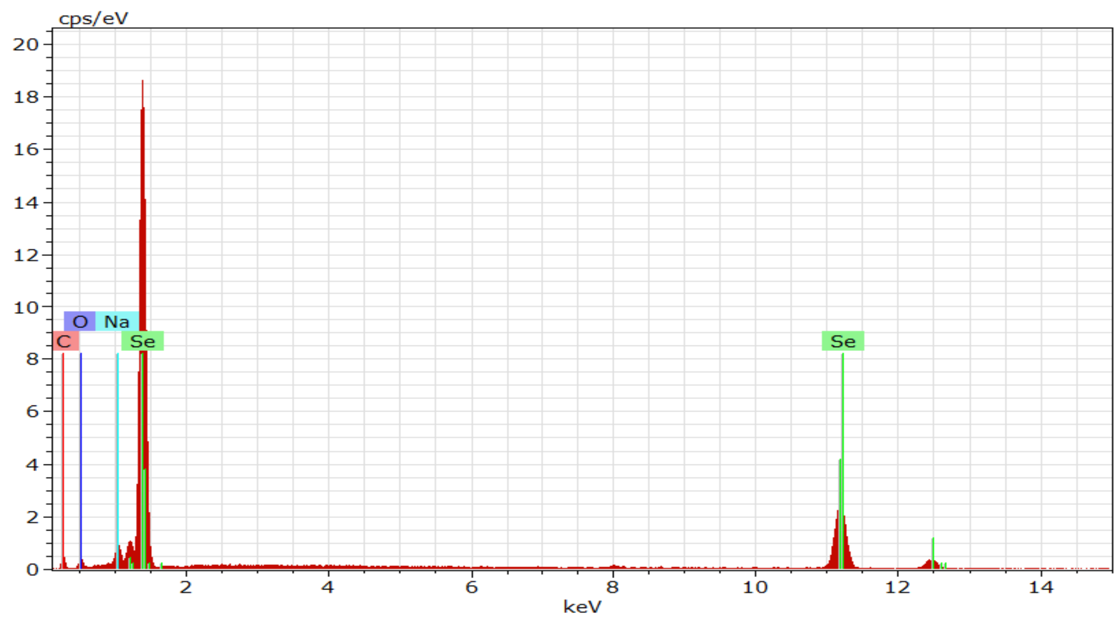


Fig.3: Energy Dispersive X-Ray Spectroscopy (EDX) Image

The XRD diffraction Peaks indexed as 22.01583,6.957353, 2.733253,0.9307381,0.3921799, 0.2205399(Fig. 4).All diffraction peaks indexed according to the hexagonal phase of selenium characteristic peaks of impurity phase except selenium are found which revealed that good crystalline in nature of the sample. The broading of the peaks in the above XRD pattern can be attributed to the small particles size of the synthesized selenium. This proves that pure selenium nanoparticles were synthesized.Similar X-ray diffraction (XRD) patterns of selenium nanoparticles was also reported(3).

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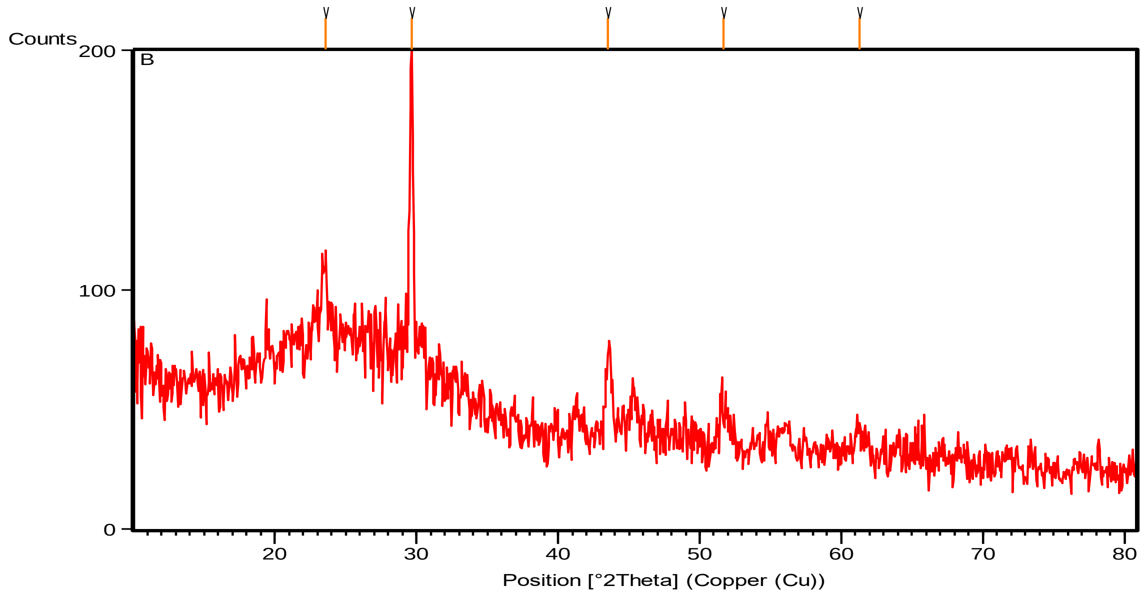

Fig.4: Analysis of Selenium Nanoparticles (XRD) Image

Fourier Transform Infrared Spectroscopy measurements were carried out to identify the possible functional group responsible for the reduction of the selenite in chemical synthesized selenium nanoparticle. The FTIR spectrum of the selenium was analyzed in the range 4000 – 400cm (Fig. 5) and bands observed at 3441, 2920, 2858, 1625, 1537, 1324,1025, 1032. Which are associated with O-H stretch, free hydroxyl-C-H stretch, H-C-H stretch=C-H asymmetric stretch=O bend, C-O stretch (Table 1). The peaks obtained were plotted as % transmit- trance in X axis and wave number (cm – 1) in Y axis. Salwa and Abbas.,(2012)(6)suggested that FTIR study was carried out to confirm the coating. In dextrin coated nanoparticles, shift in peak 1417 per cm in FTIR spectrum was observed indicating H-C-OH bond. As the concentration of Dextrin increases the shift in the peak from 1417 cm-1 to 1384 cm-1 was observed.) The FTIR analysis was performed to characterize the surface chemistry of selenium nanoparticles produced by BSA and analysis of FTIR indicated protein mediated synthesis of selenium nanoparticles, the strong absorption bands at 1649 and at 1551/ cm are characteristic of amide I and C-H vibrations of CH_2 groups of protein moiety respectively, with albumin as the stabilizing and capping agent surrounding the selenium nanoparticles.

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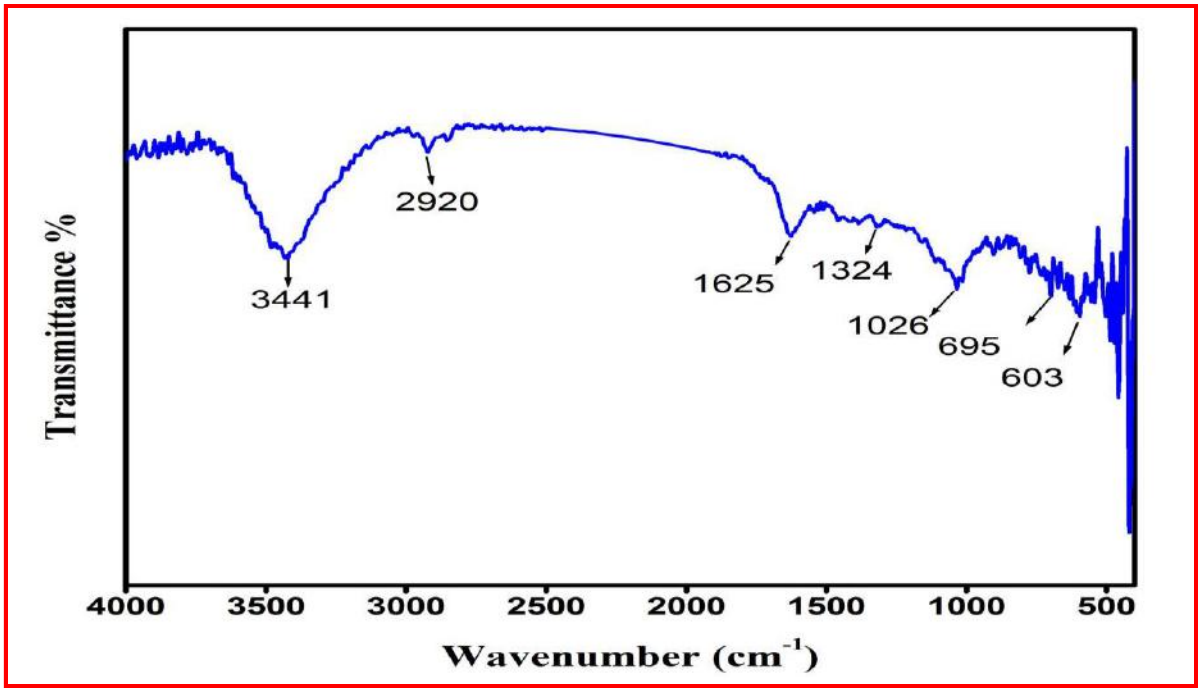

Fig.5: Fourier Transform Infrared Spectroscopy(FTIR) of Selenium Nanoparticles (XRD)Image

Table.1: FTIR Functional group representation

S.NO	WAVE NUMBER	BONDS	FUNCTIONAL GROUP
1	3441	O-H	Phenol and Alcohols
2	2920	H-C-H	Alkanes
3	2858	H-C-H	Alkanes
4	1625	C-C-=C Stretch	Aromatic rings
5	1537	N=O	Nitro group
6	1324	C-O	Esters
7	1025	C-O	Esters
8	1032	C-O	Esters

Effect of different quantities of selenium nanoparticles on growth characteristics of cluster bean is presented in table 2.The germination efficiency of cluster bean T_0,T_1, T_2T_3,T_4 and T_5 are 100,90,80,90,100 and 100 respectively. The nanoparticles and lower in T_5 (12.01) containing 500mg of nanoparticles. Similar result was also reported in Cluster bean treated with 100mg of Zinc(8). The root length of the cluster bean in control is 10.4cm. Among the treatments the root length is higher in T_2(10.6cm) and lower in T_3(7.3cm).Marisamy et al.,(2015)(9)suggested that shoot length is higher in control and lower in treatment 5(10mM).Similar root length was reported when pea nut is treated
with 100mg of Zno nanoparticles(10). The fresh weight of t he cluster bean in control is 3.18g. Sanghpriya Gautam et al germination percentage of peanut is 100% when treated with ZnO nanoparticles (7). The shoot length of cluster bean in control is 21.7cm. Among the treatments the shoot length is higher (21.8) in T_1 containing 100mg of selenium .,(2015) (11)reported that fresh and dry weight of *S. oleracea* increased at treatment T4 There was maximum increase in fresh weight (38.6 %) and dry weight (78.3 %) at T4. A reduction of fresh and dry weight was reported in *Chloroxylon* swietenia treated with sugar mill effluent for a period of 90 days(12). The leaf area is higher in$T_{3,}$ T_4 (16) and lower in T_1(10.6).Vijayarengan,(2013)(8) reported that total leaf area of cluster bean plants of 20th day were found to be 45.67, 58.72, 55.41, 37.40, 34.61 and 28.10 at control, 50, 100, 150, 200 and 250. But Gokila et al (2017)(13) reported that the leaf area decreased in the

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increasing quantity of zinc nanoparticles treated in Lady's finger.

Table.2: Effect of different quantities of selenium nanoparticles on growth characteristics of cluster bean.

S.No.	Parameters	Treatments					
		T_o	T_1	T_2	T_3	T_4	T_5
1.	Germination (%)	100	90	80	90	100	100
2.	Shoot Length (cm)	21.7±3.17	21.8±5.80	18.5± 0.58	14.85±4.53	13.67±2.90	12.01±2.95
3.	Root Length (cm)	10.4±0.85	10.03±1.36	10.6±0.7	7.10±1.59	9.70±1.15	8.70±0.32
4.	Fresh Weight (g)	3.18±2.43	2.08±1.64	4.3±2.09	3.30±0.80	2.50±1.75	2.03±1.75
5.	Dry Weight (g)	0.60±0.37	0.40±0.2	1.17±0.6	0.70±0.3	0.23±0.1	0.28±0.23
6.	Leaf Area (cm^2)	10.6±1.08	10.6±0.9	13.3±0.05	16.0 ±0.04	16.0 ±0.26	13.3±0.05
7.	Vigor Index	1656	1933	2448	3521	4373	2313

The chlorophyll a,b and total chlorophyll of cluster bean is presented in figure 6.The chlorophyll a is higher in T_4 and lower in T1. The chlorophyll b is higher in T_4 and lower in T_1. Total Chlorophyll is higher in T_4 and lower in T_1. Similar study was also reported when Lady's finger was treated with different quantities of zinc oxide nanoparticles (13). Se treatment at the lower concentration (16μM) recorded the highest values of chlorophyll a concentration (2.68 and 1.99 mg/ g FW). Vijayarengan(2013) (8)reported that chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content of cluster bean leaves increased at lower concentration. An increase in chlorophyll a and chlorophyll b contents of wheat seedlings may be attributed to selenium effect on protection of chloroplast enzymes and thus increasing the biosynthesis of photosynthetic pigments(6). High concentration of selenium induced reduction in photosynthetic pigments content. Marisamy et al.,(2015)(9)suggested that the chlotophyll a, and b are higher in control and lower in treatment 5(10mM) . Sanghpriya Gautam et al.,(2015)(11) reported that pigment contents (chlorophyll a, b) were increased maximum up to T3, whereas decreased at T4.

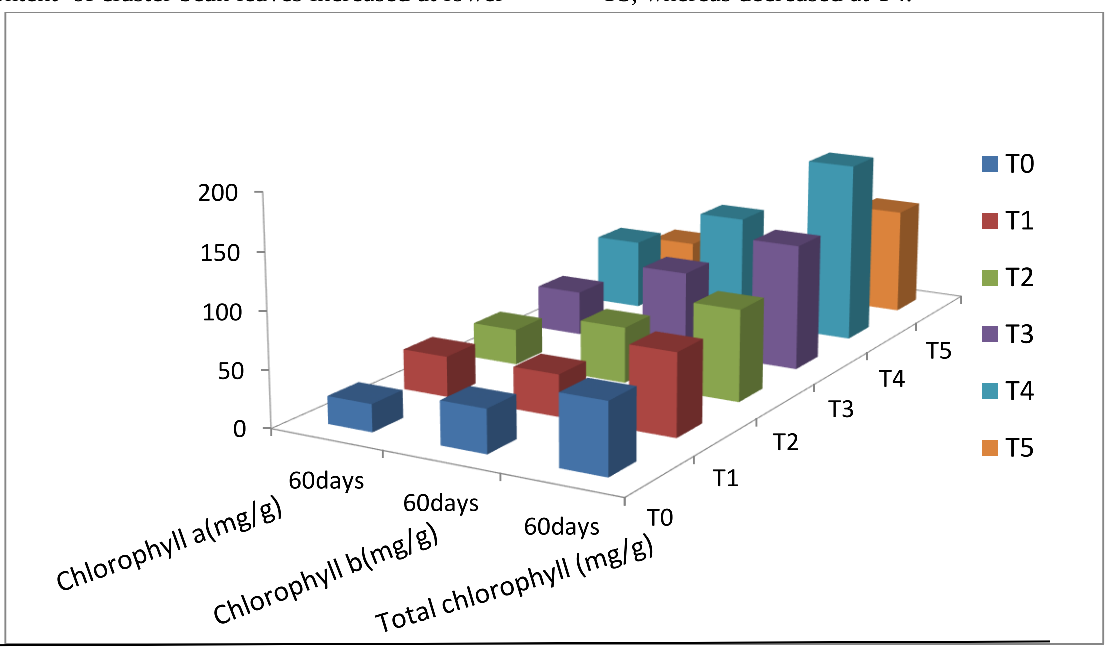

Fig.6: Chlorophyll a,b and Total Chlorophyll of Cluster bean

The carotenoids and anthocyanin of Cluster bean is presented in Figure 7. The carotenoids are higher in T_4 and lower in T_2..Salwa and Abbas.,(2012)(6) suggested that the selenium increased the contents of carotenoids and chlorophyll *a*,and in turn change in the photosynthetic pigments level is likely to have been connected with different effects of the selenium ions on the oxidation-reduction status of leaves. The carotenoids content of *Amaranthus caudatus* is higher in control and lower in treatment 5(10mM) when treated with barium(9). The

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anthocyanin is higher in T_4 and lower in T_2. Anthocyanin is a pigment to protect chlorophylls from photo oxidation, compared with the other components and high concentration of sodium selenate (12 mg per litter) reduced their contents(6). The results demonstrated that Selenium supply could increase anthocyanin content of seedlings. Marisamy et al.,(2015)(9)reported that anthocyanin is higher in control and lower in treatment 5(10mM) when Amaranthus caudatus was treated with nanoparticles.

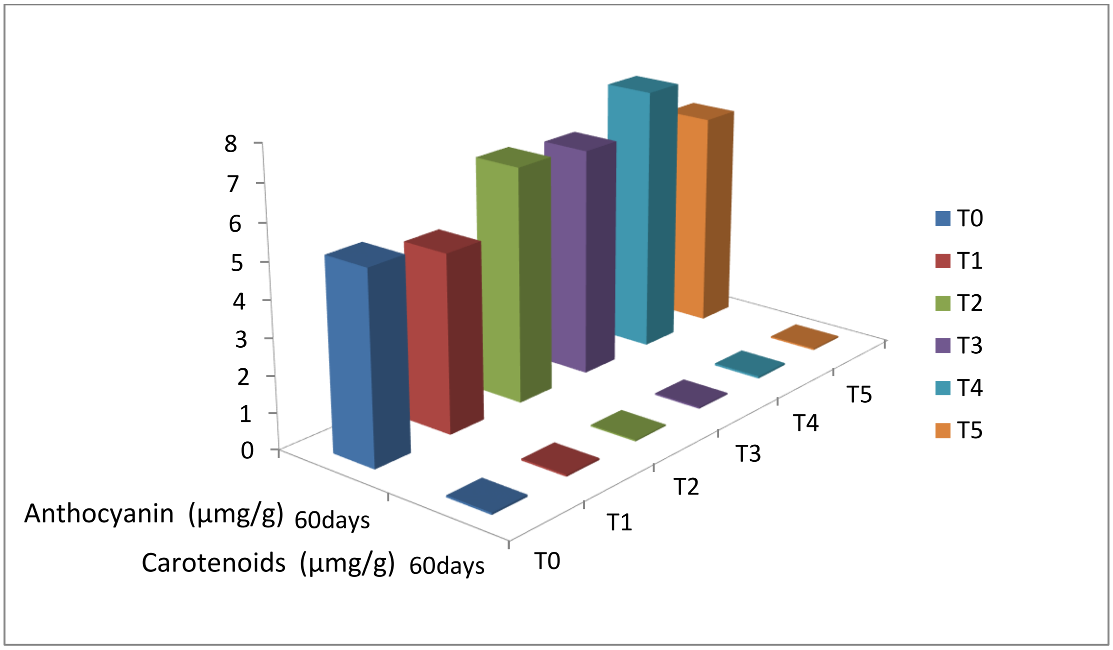

Fig.7: Anthocyanin and Carotenoids of Cluster bean

The protein and L-proline of the cluster bean is presented in Figure 8.The protein content is higher in T_4(5.5mg/g) lower in T_5(3.4mg/g). Vijayarengan (2013)(8) reported that the protein content of leaves was maximum at 50 mg of zinc treated with cluster bean. Suresh kumar and Total soluble protein content was found to be 35 % in 90 days at 50% treatment when compared to control in sugarcane effluent(12). The L-proline is higher in T_4(5.2mg/g) and lower in T_1 (4.1mg/g). Proline concentration was significantly increased in Lady's finger treated with Zinc oxide nanoparticles when compared to untreated ones(14). Also reported that L-proline accumulates in the leaves of many tree species when subjected to stress(12).

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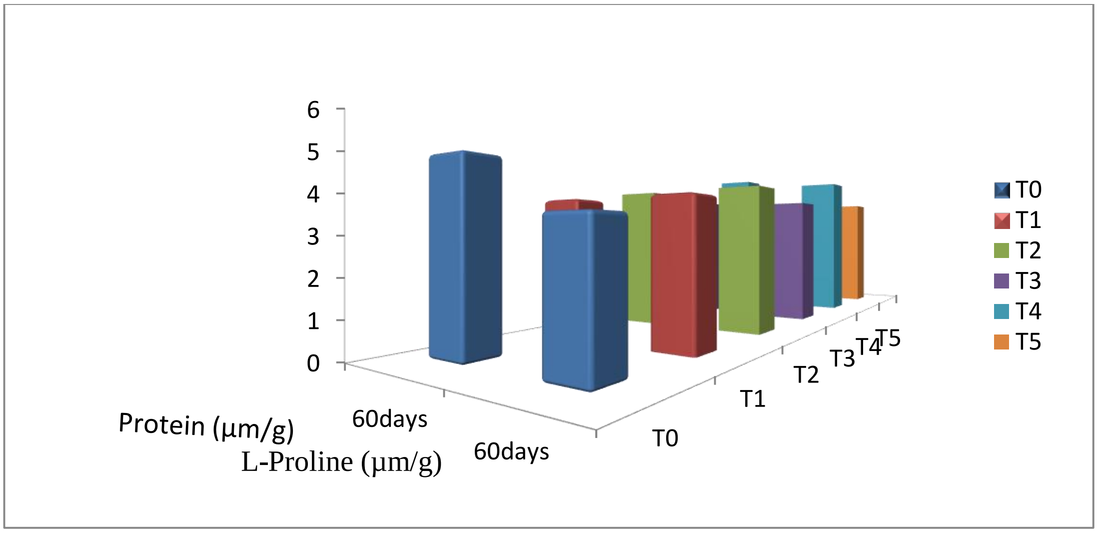

Fig.8: Protein and L-proline of Cluster bean

The free amino acid and leaf nitrate of the cluster bean is presented in Figure 9.The free amino acids are higher in T4 (6.8mg/g) lower in T_2(5.3mg/g).). The free amino acids content in *Helianthus annuus* significantly increased with increasing concentration of barium(9). Suresh Kumar and Mariappan.,(2013)(12) reported a reduction in soluble protein level eventually leads to increase in free amino acids content. The leaf nitrate is higher in T4(5.9mg/g) and lower in T_1(4.12mg/g). Marisamy et al.,(2015)(9)suggested that leaf nitrate content increased in control when compared to barium treatment. Suresh Kumar and Mariappan (2013) (12) reported that leaf nitrate accumulated in all the effluent treated tree species.

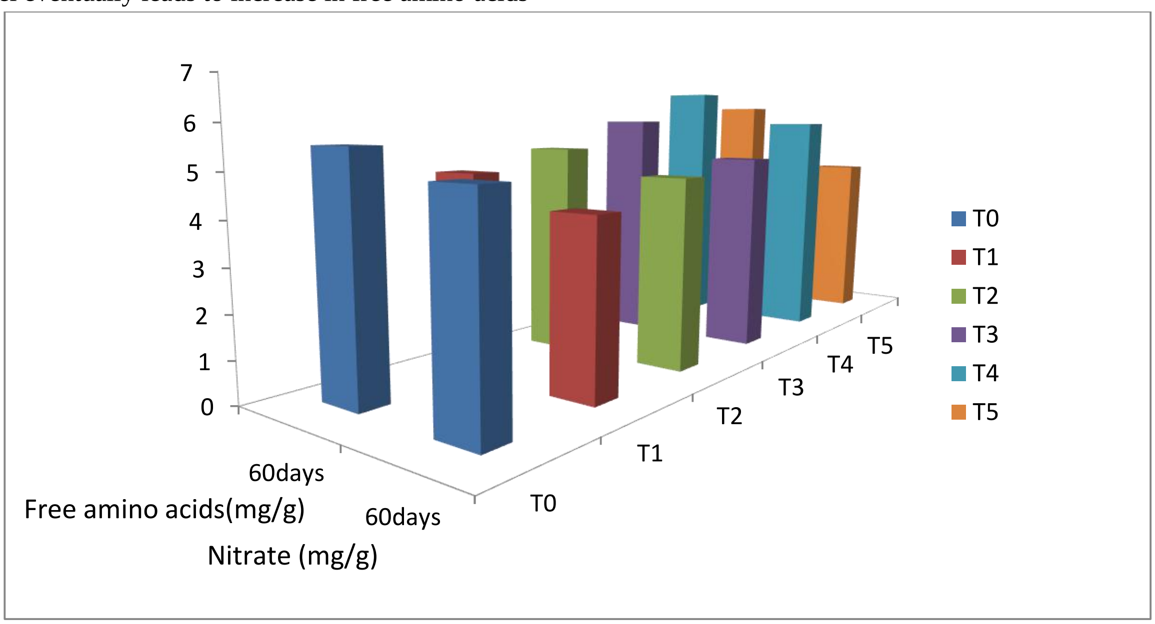

Fig.9: Free Amino acids and Nitrate of Cluster bean

Yield performance of cluster bean is presented in figure Table 3.Yield performance of cluster bean is higher in T_4(1.41g) and lower in T_2(0.69g). Sharma and Kansal(1984)(14) suggested that the yield parameters such as length, weight and number of the cluster bean showed increase over control up to 800mg and decrease gradually in the further higher concentrations. Manal et al.,(2014)(15) reported that imposition of drought stress reduced plant height, number of tillers, 1000-grain weight and grain yield of both rice cultivars. The treatment with either Se has a favorable effect on1000-grain weight which up to 9.5% and 5.2%.

Table.3: Yield performance of cluster bean

Treatment	Length(cm)	Weight(g)	Number
T0	13.11±5.39	5.4±1.03	8.0±1.00
T1	32.1±10.95	1.9±1.09	6.0±0.00
T2	29.1±5.58	7.3±0.65	10.0±1.00
T3	21.95±5.48	8.04±0.69	8±1.00
T4	28.37±6.34	2.45±1.41	15.0±1.0
T5	20.71±2.34	0.25±2.60	5.0±1.0

IV. CONCLUSION

The present study concluded that 400 mg of selenium nanoparticles influences the growth, biochemical characteristics and yield of Cluster bean.

V. ACKNOWLEDGEMENTS

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REFERENCES

[1] Maeda, H., Wu, J and Sawa, T.Tumor 2001.vascular permeability and the EPR effect in macromolecular therapeutics- A Review. J. Control Release, 65: 271-284.

[2] Bunglavan, S.J., Garg, A.K., Dass, R.S and Sameer Shrivastava, 2014.Effect of supplementation of different levels of selenium as nanoparticles/sodium selenite on blood biochemical profile and humoral immunity in male Wistar rats. Research Articles,7(12) : 1075 – 1081.

[3] Ramamurthy, C. H., Sampath, K.S., Arun Kumar., Suresh Kumar, M., Sujatha, V., Prem Kumar,K., Thirunavukkarasu.C, 2012.Green synthesis and characterization of selenium nanoparticles and its Augmented cytotoxicity with doxorubicin on cancer cells. Journals of Bioprocess and Biosynthesis Engineering, 36 : 1131–1139.

[4] Sonam Malhotra, Neetu Jha and Krutika Desai, 2014.A superficial synthesis of selenium nanospheresusing wet chemical approach. International Journal of Nanotechnologyand Application, 3(4) : 7 - 14.

[5] JonneRodenburg., Kazuki Saito., RunyamboIrakiza., Derek, W., Makokha, Enos, A., Onyuka and Kalimuthu Senthilkumar, 2014.Labor-Saving Weed Technologies for Lowland Rice Farmers in sub-Saharanm Africa. Journal of America Weed Society Science, 29 (4) : 751 - 757.

[6] Salwa, M and Abbas, (2012)Effects of low temperature and selenium application on growth and the physiological changes in sorghum seedlings. Journal of Stress Physiology and Biochemistry, 8 (1) : 268-286.

[7] Prasad, B., H. Feizi and Sharmila, P, 2012 Effect of nonoscale zinc particles on the germination, growth and yield of peanut, Journal of Plant Nutrition,39:905-927.

[8] Vijayarengan, P (2013) Changes in growth, biochemical constituents and antioxidant potentials in cluster bean *Cyamopsis tetragonoloba*L. Taub under zinc stress. International Journal of Current Science, 5 : 37- 49.

[9] Marisamy Kalingan., Duraipandian Muthaiah., Sevugaperumal Rajagopal and Ramasubramanian Venkatachalam (2015) Estimation of Barium Toxicity Mitigating Efficacy of Amaranthus caudatusL., Universal Journal of Environmental Research and Technology, 5 : 295 - 305.

[10] Liu, X.M., Y.Shi and Salama, H, 2016.Effect of nano ferric oxide on growth and nutrients absorption of Peanut. Plant Nutr. and Fert. Sci., 11:14-18.

[11] Sanghpriya Gautam,P., Kannaujiya and Srivastava.M.N (2015)Growth and biochemical responses of spinach (*Spinacea oleracea* L.) grown in Zn contaminated soils.International Journal of Recent Biotechnology, 3(1) : 7-12.

http://dx.doi.org/10.22161/ijeab/2.6.19 *ISSN: 2456-1878*

[12] Suresh Kumar,K and Mariappan,V (2013)Evaluation of sugarcane mill effluent and its impact on the growth, biochemical and DNA profile of C*hloroxylon swietenia*, dc. International Journal of Research in Environmental Science and Technology, 3 (3) :92 - 99.

[13] Gokila, B., V.Keerthika and M.R.Rajan, 2017.Impact of Zinc oxide Nanoparticles on Growth, Biochemical characteristics and Yield of Lady's finger. Indian J. Of Appl. Res.,,7(8):53- 56,

[14] Sharma, V.K. and Kansal, B 1984.Effect of Nitrogen, farm yard manure, town refuse and sewage water on the yield and Nitrogen content of Maize, J. Ecol.,11: 77-81.

[15] Manal,M., Emam, Hemmat E., Khattab, Nesma M. Helal, Abdelsalam E. Deraz (2014) Effect of selenium and silicon on yield quality of rice plant grown under drought stress. Australian Journal of Crop Science, 8 (4) : 596 - 653.

Identifying QTLs Associated and Marker-Assisted Selection for Salinity Tolerance at the Seedling, Vegetative and Reproductive Stages in Rice (*Oryza Sativa* L.)

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Abstract— *Salinity affects rice growth in all growth stages, with the seedling and reproductive stages being the most sensitive. Genetically improving salt tolerance of rice is an important objective of rice breeding programs. Hence, mapping quantitative trait loci (QTL) will be useful for marker-assisted selection in rice breeding programs. An advanced backcross population (BC_2F_2) was developed with the parents included OM5629 as a donor of salt tolerance and OM7347 as a recurrent parent with good quality traits and drought tolerance. Molecular markers associated with both qualitative and quantitative trait loci (QTL) salt tolerance were identified by using 416 polymorphic SSR markers. QTLs, associated with stress tolerance at EC = 15 dS/m at seedling stage, detected from the BC_2F_2 population of OM7347/OM5629, were located on chromosomes 1 and 3. Three QTLs were identified at the intervals of RM3252-S1-1 - RM10694, RM3740-RM5336 and RM11125-RM9 with genetic distance of 4.4, 4.5 and 18 cM on chromosome 1, respectively. Two QTLs at the intervals of RM3867-RM6959 and RM6876-RM4425 with genetic distance of 4.5 and 18.0 cM on chromosome 3, respectively. One QTL on chromosome 5 was detected at the interval of RM874 - RM10359, it was associated with salt stress tolerance under EC = 8dS/m at vegetative stage. Three QTLs at the regions of RM1324-RM2412, RM1185-RM24, and RM1282-RM2560 on chromosome 1, and one QTL of RM453-RM511 on chromosome 12, were related to salt tolerance under EC = 8dS/m at reproductive stage. Two tightly linked markers as RM3252-S1-1 and RM3867, were exhibited their effectiveness in identification of salt tolerance genotypes in BC_3F_6 population of OMCS2000/ Pokkali. The identification of new QTLs associated with salt tolerance will provide important information for the functional analysis of rice salinity stress.*

Keywords— ***Seedling, reproductive stage, salinity, SSR, QTL, vegetative stage.***

I. INTRODUCTION

Salinity is the second type of stress and is the most important loss rice yield production after drought [1]**.** Salty soil is one of the most common stress has a negative effect on crop production. Salty soil is the main limiting factor in the production of rice, a type of salt-sensitive plants, productivity is affected greatly by the ion toxicity [2]**.** The difference between plant species and on the tolerance to salinity [3]. Rice crops are relatively resistant to stress during germination, active and mature branches lying but very sensitive at the beginning stage of seedlings [4, 5]**.** Two stages of the rice plant sensitivity independent of each other and are controlled by genes, meaning, in the reproductive period the tree no resistant varieties in the stage of seedlings and vice versa. Moreover, salt tolerance at the reproductive stage is very important because the process of fertilization and seed formation occurs in this period and reproductive stages directly related to yield [6]. In rice, important traits such as salt-tolerance, yield and quality are controlled by polygenes that are described as quantitative trait loci (QTLs) [7]. QTL mapping related to environmental stresses, yield and quality are very important for the application of map-based cloning and marker-assisted selection (MAS) in rice breeding programs [8]. In rice, QTL analysis of salt tolerance has been conducted by several researchers [9, 10]. Lang *et al*, [11] reported that salt tolerant genes tagging based on SSR markers with advanced backcross populations (BC_2F_2) of IR64/ChengHui 448, IR64/OM1706 and IR64/ FR13A derived alleles nearly located at on chromosome 1 while in the population of IR68552-55-3-2/OM1706, the alleles are linked with RM223 on chromosome 8. The major gene for salinity tolerance (*Saltol*) was mapped on chromosome 1 and chromosome 8 [12, 11]. RM223 linked to salt tolerance gene at the distance of 6.3 cM on chromosome 8 at vegetative stage under EC = 10 dS.m^{-1} from F_3 population of IR28/Doc Phung [12]. Tiwari *et al,* [13]**.** reported a method for rapid identification of QTLs for reproductive stage salt tolerance in rice using bulked segregant analysis (BSA) of bi-parental recombinant inbred lines (RIL). The method was applied to CSR11/MI48 RILs segregating for reproductive stage salt tolerance. Genotyping of the parents and RIL bulks, made on the bas is of salt sensitivity index for grain yield, revealed 6,068 polymorphic SNPs and 21 QTL regions showing homogeneity of contrasting alleles in the two bulks. BSA with 50K SNP chip revealed 5,021 polymorphic loci and 34 QTL regions.

In this study, we established QTL maps for the agronomical traits related to salinity tolerance at different stages to aim at selecting suitable genotypes adapted to both seedling and reproductive stages under Mekong Delta of Vietnam.

II. MATERIALS AND METHODS

1. Plant Materials

Two different populations were used to identify QTL analysis and marker-assisted selection in this study. Two hundred fifty-three elite lines were divided from the BC_2F_2 population between OM7347, a good quality variety and drought tolerance and OM5629 which was considered as a donor of salt tolerance. Of 769 SSRs, 416 polymorphic markers related to salinity tolerance were applied to set up the QTL map. A second mapping population of 230 lines from the BC_3F_6 population of OMCS2000 / Pokkali was developed [14]. These plants were genotyped the Genetics and Plant Breeding Division of High Agricultural Technology Research Institute for Mekong Delta Vietnam and the Molecular Biology Lab of Biotechnology PCR Company, Cantho, Vietnam.

2. Phenotypic evaluation

This study carried out in two environments (EC of 15 dS/m and 8 dS/m) at seedling and reproductive stages, respectively. Pokkali is a tolerant variety and IR29 is a susceptible variety**.** The experiment consisted of in three replications, randomized complete design (RCD). Germinated seeds were put into the floating Styrofoam under sterilized water within 3 days. Then, the Yoshida solution was increased the EC up to 4dS/m and 8 dS/m, at pH = 5.0 – 5.5. After 21 days, the result was evaluated base on the survival day (SD) of the tolerant and susceptible genotypes based on the methods of Gregorio, [15] and IRRI [16] (Table 1). The susceptible variety was almost dead completely.

Table.1: Modified IRRI standard evaluation scoring (SES) system based on the visual symptoms of salt stress injury of rice.

SES	Description	Tolerance
1	Normal growth, only the old leaves show white tips while no symptoms on young leaves	Highly tolerant
3	Near normal growth, but only leaf tips burn, few older leaves become whitish partially	Tolerant
5	Growth severely retarded; most old leaves severely injured, few young leaves elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dried; only few young leaves still green	Susceptible
9	Almost all plants dead or dying	Highly susceptible

3. SSR markers genotyping

DNA was extracted using CTAB method which modified by Lang, [17]**.**. Genomic DNA samples were loaded in 0.9% agarose gel in TAE 1X to check for its quality .

PCR reactions were done in eight microliters of each reaction were run on the polyacrylamide gel with 769 SSR markers. Reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 ^{0}C, 1 min at 55 ^{0}C, and 2 min at 72 ^{0}C, with a final extension at 75 ^{0}C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 ^{0}C for 2 min. Amplified genes from PCR were assessed on agarose gel 3% in TBE 1X.

4. Map construction and QTL analysis

The program MAPMAKER/EXP v.3.0 [18, 19] was employed to establish a genetic linkage map using the Kosambi mapping function [20]. Link-age groups were inferred based on the existing RFLP and micro-satellite maps of rice [21, 22, 23]**.** MAPMAKER/QTL version 1.1 was used to identify loci affecting quantitative traits on the basis of interval analysis [24, 19]. A LOD score of 3.0 was selected as the threshold for the presence of a QTL based on the total map distance, and the average distance between markers [25]. With such a threshold, a false positive QTL would be detected anywhere in the genome with a probability of approximately 0.05 [24]. The independence test was performed where the initial scan suggested two or more QTLs located on the same chromosome as described by Paterson et al, [24]. and Lander and Bostein, [25]. The total phenotypic variation explained by all QTLs were estimated by fitting a multiple regression model in the MAPMAKER/QTL program. The interaction between all possible loci on the map was performed using QTLMapper version 1.0 [26]. (Wang *et al.*, 1999).

Single-marker QTL analysis using linear regression was employed [27]. The marker alleles linked to salt stress were coded 1; and in contrast, a code 0 for conducting regression analysis. To analyze markers-QTLs association for each trait, single-point (single marker) analysis of QGene version. 4.0.2 [28] was performed. This analysis served as the primary method of detecting the association between markers and the target traits.

To determine the precise location of the putative QTLs, interval mapping of the MAPMAKER/EXP version 3.0 [24, 19] was conducted. Interval mapping analysis was also used to confirm the results of the single marker analysis.

LOD ≥ 3,0 [with *P*-value $R^2 \geq 10\%$] was used as the threshold for detecting QTLs location. LOD peaks for significant QTLs were used to position the QTL on the linkage map and to identify significant markers linked to target genes.

GRAMENE database was used to widen putative gene regions and identify new markers for genetic diversity and shortening of target gene regions.

Combined data based on QGene, MapMarker and GGT (graphic genotyping) was used to analyze QTL maps. After that, only the most two specific traits were selected to build QTL maps. QTL maps were analyzed by using the direction of phenotypic effect (DPE), additive value and probability with small errors [29].

III. RESULTS AND DISCUSSION

1. QTL map related to salinity tolerance in BC_2F_2 population of OM7347/ OM5629 at seedling stage

A total 253 individuals of the BC_2F_2 population of OM7347/OM5629 were used to set a QTL map on twelve chromosomes to identify target genes (Fig 1). Out of 769 SSRs, 416 SSRs were found polymorphism between drought tolerant gene of the recurrent parent (OM7347) and the donor (OM5629) in previous studies [30, 31]. PCR products of population OM7347/OM5629 based on 416 polymorphism markers showed in Table 1. Twelve linked groups on twelve chromosomes constructed were 4,447.5 cM in total length, with average distance at 10.69 cM among contiguous regions.

2. Analysis of QTL map

To elucidate the genetic basis and physiology of traits related to salt tolerance in rice, the overlapped QTLs, and beneficial genes were identified. Through CIM analysis, there were many significant QTLs related to salinity tolerance from BC_2F_2 with the average distance of 10.69 cM. The QTLs scattered on chromosome 1 *viz.* RM3532S-RM10694; RM3740-RM5336 and RM11125-RM9RM explained 13.33%, 30.48% and 37.14% of phenotypic variance, respectively. QTLs located on chromosome 1 are major loci, which explained more than 50% of the phenotypic variance. The QTLs related to salinity tolerance under EC=18 dS/m at the seedling stage are located on chromosomes 1, 6, 8 and 9 [32, 30, 31]. Gong *et al*, [33], Bonilla *et al*, [34] and Lin *et al*, [35]. detected QTLs for salt tolerance on chromosome 1.

Table.1: Polymorphism of SSR markers on twelve rice chromosomes.

No.	Chromoeome	No. of linked markers	Length (cM)	Mean (cM)
1	1	72	872.3	12.11
2	2	48	518.4	10.80
3	3	53	549.7	10.37
4	4	31	254.6	8.21
5	5	33	414.5	12.56
6	6	27	411.0	15.22
7	7	21	213.3	10.14
8	8	21	153.3	7.30
9	9	45	385.7	8.57
10	10	19	198.2	10.43
11	11	23	232.0	14.04
12	12	23	244.7	10.63
Total		**416**	**4447.5**	**10.69**

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In addition, Graphical representation of QTLs was located on linkage on map is showed in Fig 2 using CIM analysis on chromosom1 and chromosome 3. Their LOD values, additive effects, and phenotypic variance are presented in Table 2. QTLs related to salinity tolerance at seedling stage on chromosome 1 (RM3532-S-RM10694; RM3740-RM5336 and RM11125-RM9) with genetic distance of 4.4 cM, 4.5 cM and 18 cM, respectively, and two QTLs revealed salinity tolerance at seedling stage with genetic distance of 4.5 and 18.0 cM on chromosome 3 (RM3867-RM6959 and RM6876-RM4425) in the BC_2F_2 population of OM7347/OM5629 under EC = 15 dS/m. [7]. reported that salt tolerance QTLs located on chromosomes 1 and chromosome 3 were QST01 and QST-3, respectively. Recently, Thomson *et al,* [37] reported that four QTLs related to salt tolerance are on chromosome 1 (1 QTL), chromosome 2 (1 QTL), chromosome 3 (1 QTL) and chromosome 12 (1 QTL). Markers linked the QTLs in MAS breeding permits to exactly identify the major and minor QTL regions. Results from QTL map analysis showed that marker RM3532S was tightly linked to *Saltol* locus (4.6 cM in genetic distance) on chromosome 1. QTLs for major traits detected on chromosome 1. The results are useful for MAS breeding as well as pyramiding breeding in the future.

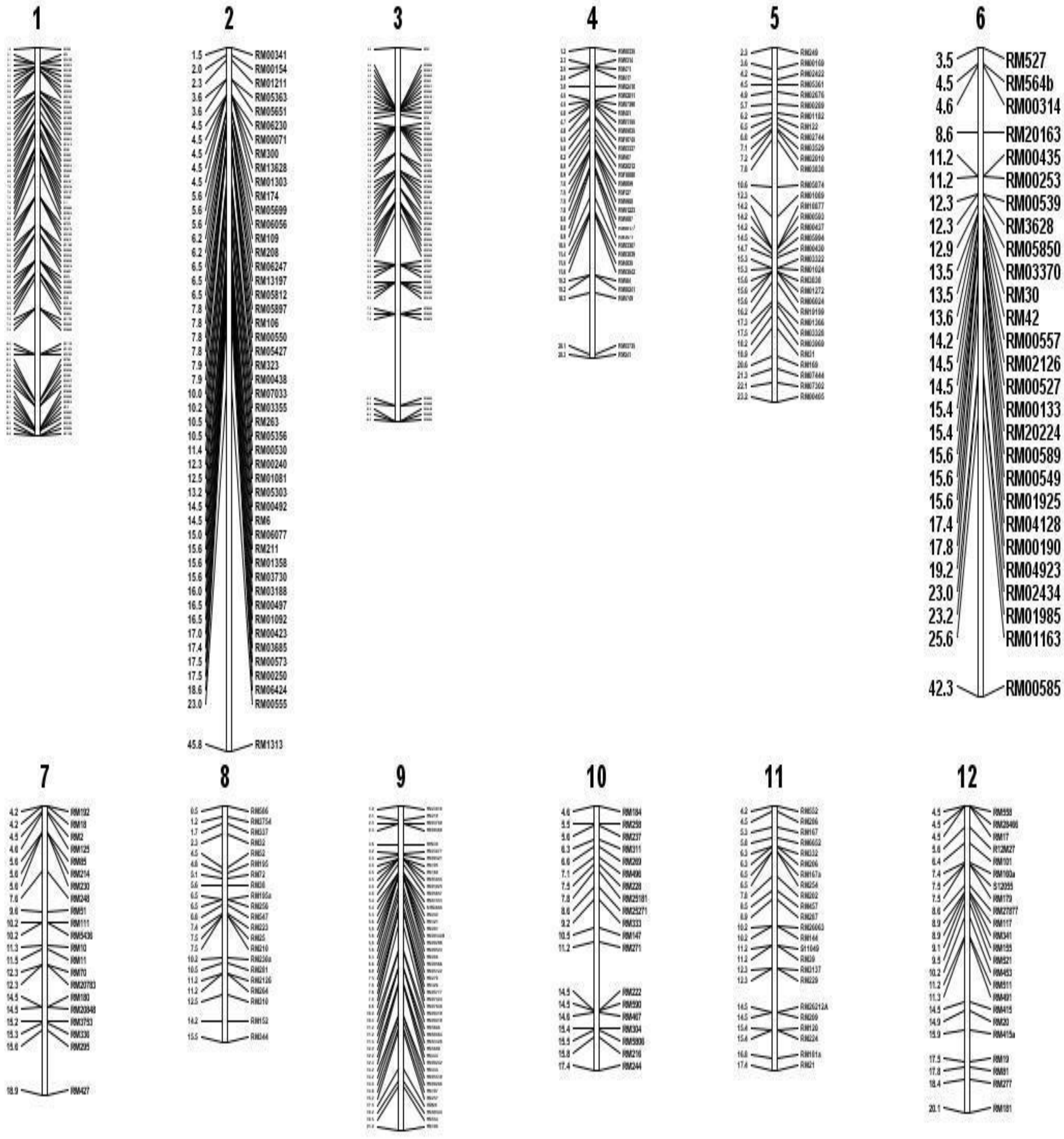

Fig. 1: QTL map for the traits related to salinity tolerance on twelve chromosomes in the BC_2F_2 population of OM7347/OM5629.

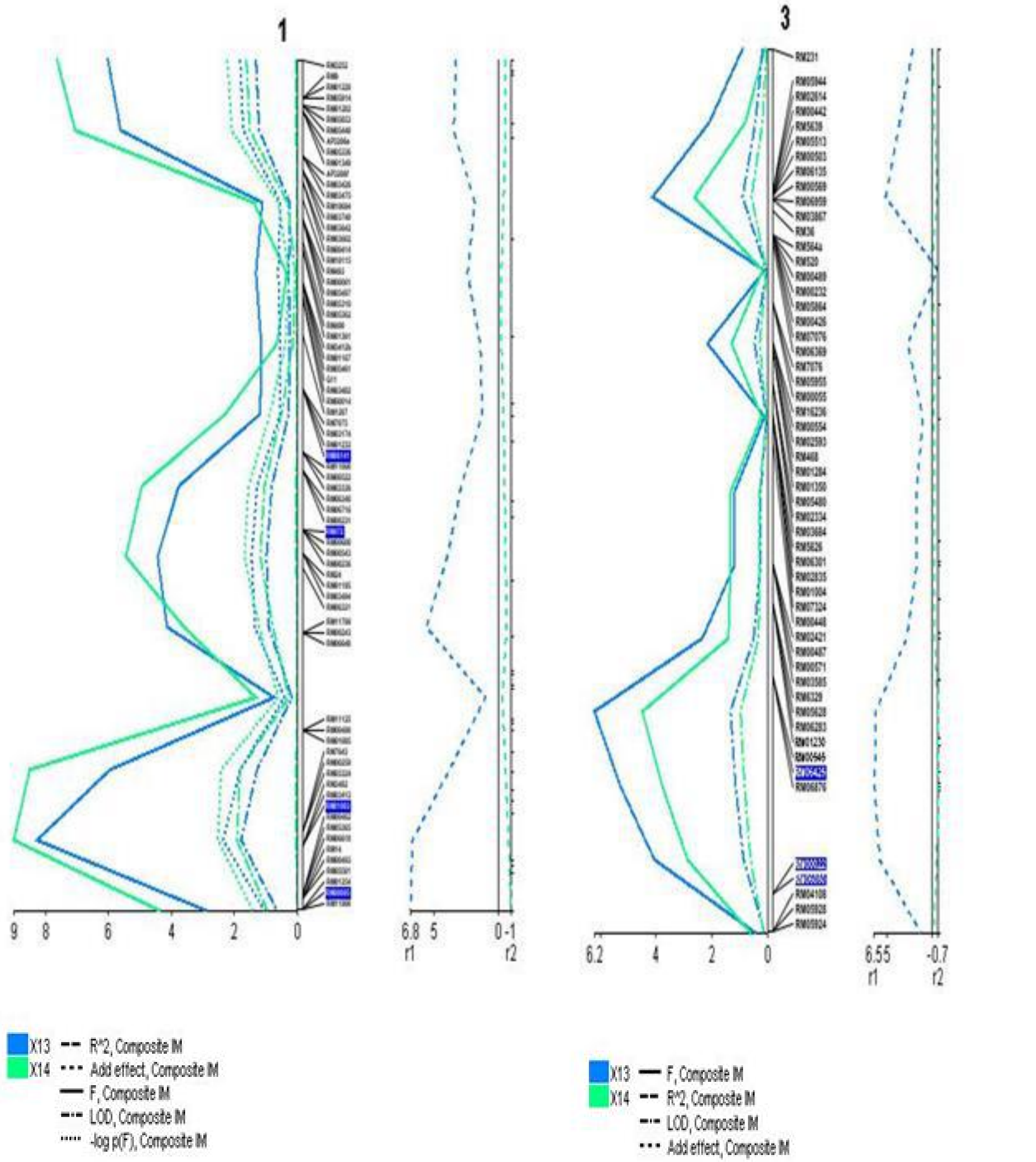

Fig. 2: LOD peaks for QTLs related to salinity tolerance at seedling stage on chromosome and chromosome in the BC_2F_2 population of OM7347/OM5629 under EC = 15 dS/m.

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Table.2: QTLs related to salinity tolerance in the BC_2F_2 population of OM7347/ OM5629 at seedling stage.

Chr.	QTL	Location (cM)	CIM (Interval cM)	LOD	A	D	R^2
1	SD QTL-1	4.4	RM3252-S1-1 -RM10694	4.3	0.29	15.18	13.33
1	SD QTL-1	4.5	RM3740-RM5336	2.8	13.15	23.67	30.48
1	SEIQTL-1	18.0	RM11125-RM9	3.0	11.43	81.08	37.14
3	SD QTL-3	4.5	RM3867-RM6959	4.6	12.56	23.67	11.41
3	SEIQTL-3	18.0	RM6876-RM4425	17.1	4.85	24.50	17.40

$P< 0.05$; A: Additive, D: Dominant, R^2: phenotypic variance explained, Chr: chromosome; SD: survival day; CIM: composite interval mapping

At vegetative stage

Similarly, the BC_2F_2 population of OM7347/OM5629 was assessed some key phenotypical traits at tillering stage. One QTL was detected at the region of RM874-RM10359 on chromosome 5 at LOD ≥ 3 (Fig 3).

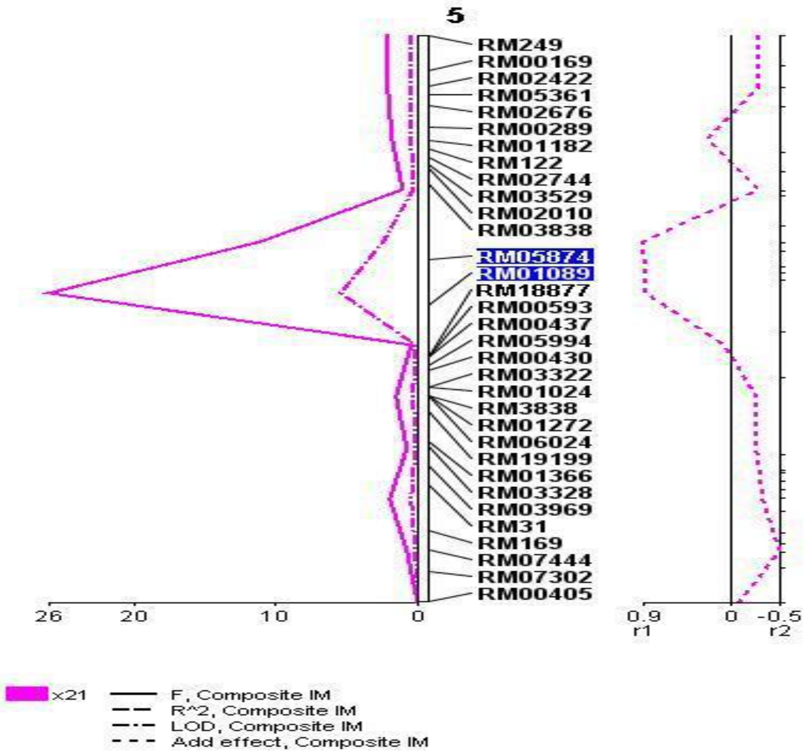

Fig. 3: LOD peaks for QTLs related to salinity tolerance at vegetative stage on chromosomes 5 in the BC_2F_2 population of OM7347 / OM5629 under EC = 8dS/m.

3. At reproductive stage

At reproductive stage, two hundred fifty-three individuals of BC_2F_2 population from OM7347/OM5629 were screened in Yoshida solution plus NaCl under salt stress of EC = 8dS/m. Yield components and grain yield were evaluated. The result showed that *Saltol* QTLs were mainly located on chromosomes 1 and 12. Three QTLs located at the intervals of RM1324-RM2412, RM1185-RM24 and RM1282-RM2560 on chromosome 1 were corresponded to grain yield. One QTL at the region of RM453-RM511 on chromosome 12 corresponded to survival day (SD) and salinity tolerance score under EC = 8dS/m at reproductive stage (Fig 4, Table 3).

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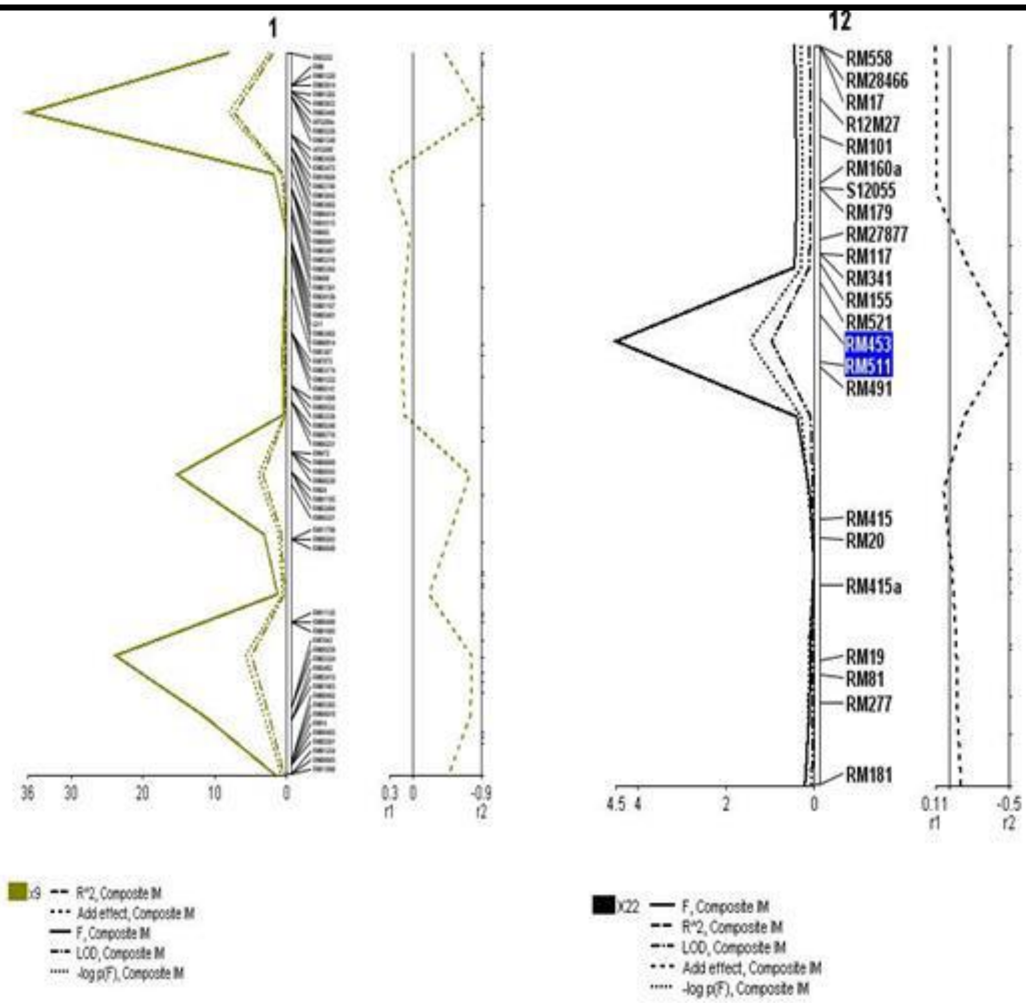

Fig. 4: LOD peaks for QTLs related to salinity tolerance at reproductive stage on chromosome 1 and chromosome 12 in the BC_2F_2 population of OM7347 / OM5629 under EC = 8dS/m.

Table.3: QTLs related to salt tolerance in the BC_2F_2 population of OM7347/OM5629 at reproductive stage.

Chr	QTL	Location (cM)	CIM (Interval cM)	LOD	A	D	R^2
1	SD QTL-1	6.8	RM1324- RM3412	7.1	1.61	7.19	12.15
1	SD QTL-1	11.6	RM1185-RM24	7.0	1.92	3.90	15.14
1	SD QTL-1	6.5	RM1282-RM2560	7.0	7.50	4.10	14.23
12	SD QTL-12	4.6	RM453-RM511	4.5	25.17	32.56	25.17

P< 0.05; A: Additive, D: Dominant, R^2: phenotypic variance explained, Chr: chromosome, SD.

4. Marker-assisted selection in BC_3F_6 population of OMCS2000/Pokkali

Markers tightly linked to *Saltol* genes were RM3252-S1-1 and RM3867 on chromosome 1 and 3, respectively. They were used in marker-assisted selection in BC_3F_6 population.

One line namely BC_3F_6-7 was exhibited at same band with Pokkali via RM3252-S1-1. Two lines as BC_3F_6-17 and BC_3F_6-22 expressed their heteromorphic bands. This meant that RM3252-S1-1 was significantly used to select the elite rice lines, which tolerate salinity on chromosome 1 (Fig. 5).

In term of marker RM3867 on chromosome 3, PRC products of 24 selected lines were highly polymorphic. Two elite lines as BC_3F_6-7 and BC_3F_6-13 were exhibited at the same band of Pokkali on 3% agarose gel. No heteromorphism was recorded in case of RM3867 (Fig 5) accordingly.

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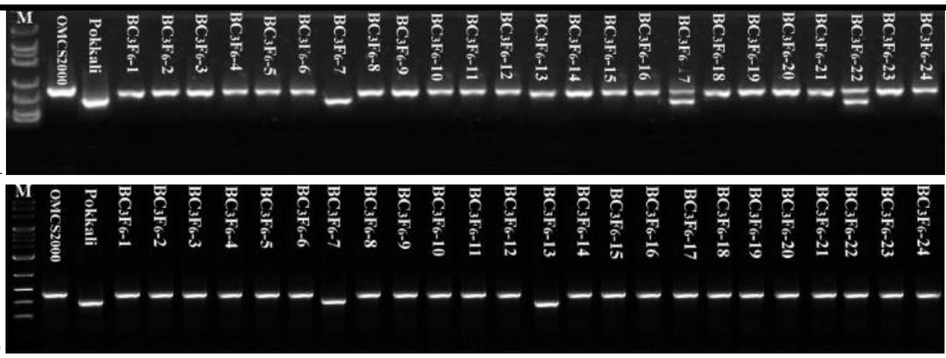

Fig. 5: PCR products of 24 lines of BC_3F_6 from OMCS2000/Pokkali on 3% agarose gel. (A): PCR products at locus RM3252-S1-1 on chr.1; (B): PCR products at locus RM3867 on chr. 3. M: Lambda leader (174/Hae III).

IV. CONCLUSION

In the backcross populations, parents were genotypically assessed on 12 chromosomes using SSR markers to identify the regions of rice genome where the contrary alleles (tolerant-susceptible alleles) showed "homogeneity" character of the polymorphic SSR markers. This method was applied at seedling stage. Success in QTLs mapping using polymorphic SSR markers in the BC population of OM7347/ OM5629 permits to identify new QTLs (located on chromosomes 1 and 3) beside previous QTLs reported. The candidate regions on chromosomes 1 and 3 from this study were then tested using the BC_3F_6 population of OMCS2000/Pokkali. The polymorphism in PCR products proved that two useful markers *viz.* RM3252-S1-1 and RM3867 were efficient.

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REFERENCES

[1] G.B. Gregorio, "Tagging salinity tolerance genes in rice using amplified fragment length polymorphism AFLP". Univ Philippines, Los Baños. Laguna, Philippines, 1997a.

[2] A.M. Ismail, S. Heuer, M.J. Thomson, and M. Wissuwa, "Genetic and genomic approaches to develop rice germplasm for problem soils", Plant Mol. Biol. 65(4), 2007, 547–570.

[3] S. Turan, K. Cornish, and S. Kumar, "Salinity tolerance in plants: Breeding and genetic engineering", Aust. J. Crop Sci. 6(9), 2012, 1337–1348.

[4] R.K. Singh, G. Gregorio, and A. Ismail, "Breeding rice varieties with tolerance to salt stress", JISCAR. 26, 2008, 16–21.

[5] R. Singh, and T. Flowers, "Physiology and molecular biology of the effects of salinity on rice", Handbook of Plant and Crop Stress, 2010, pp 899–939. doi:10.1201/ b10329-44.

[6] M.C. Shannon, "Adaptation of plants to salinity", AdvAgron. 60, 1998, 75–120

[7] S.Y. Lee, J.H. Ahn, Y.S. Cha, D.W. Yun, M.C. Lee, J.C. Ko, K.S Lee, and M.Y. Eun, "Mapping of quantitative trait loci for salt tolerance at the seedling stage in rice". Mol. Cells. 21(2), 2006, 192–196.

[8] K.M. Kim, Y.S. Kwon, J.J. Lee, M.Y. Eun, and J.K. Sohn, "QTL mapping and molecular marker analysis for the resistance of rice to ozone". Mol. Cells. 17, 2004, 151−155.

[9] M.L. Koyama, A. Levesley, R.M. Koebner, T.J. Flowers, and A.R. Yeo, "Quantitative trait loci for component physiological traits determining salt tolerance in rice". Plant physiol.125, 2001, 406−422.

[10] G.Y. Zhang, Y. Guo, S.L. Chen, and S.Y. Chen, "RFLP tagging of a salt tolerance gene in rice". Plant. Sci. 110, 1995, 227−234.

[11] N.T. Lang, Z. Li, and B.C. Buu, "Microsatellite markers linked to salt tolerance in rice". OMon Rice. 9, 2001a, 9−21.

[12] N.T. Lang, "QTL mapping for salt tolerance in rice. Final report". Japan fellowship, 1999.

[13] S. Tiwari, S.L. Krihnamurthy, V. Kumar, B. Singh, A.R. Rao, S.V. Amitha Mithra, V. Rai, A.K. Singh, and N.K. Singh, "Mapping QTLs for salt tolerance in rice (*Oryza sativa* L.) by Bullked segregant analysis of recombination inbred lines using 50K SNP chip". PloS. One., 11(4), 2016, e0153610.

[14] N.T. Lang, L.Q. Loc, P.T.T. Ha, B.P. Tam, and B.C. Buu, "Screening rice for salinity tolerance by phenotypic in population

OM1490/Pokkali//OM1490 cross at seedling stage". Int. J. Cur. Inn. Res. 3 (7), 2017, 700−706.

[15] G.B. Gregorio, "Screening rice for salinity tolerance". IRRI Discussion Paper Series No.22. International Rice Research Institute, Los Baños. Laguna, Philippines, 1997b, 1−30.

[16] IRRI, "Standard Evaluation System". Rice Note Book, The Philippines, 1996.

[17] N.T. Lang, "Manual for biotechnology Lab". Vietnam Agriculture Publisher, Ho Chi Minh City, Vietnam, 2002.

[18] E.S. Lander, and D. Botstein, "Mapping Mendelian Factors Underlying Quantitative Traits Using RFLP Linkage Map". Genetics. 121, 1987, 185−199.

[19] S. Lincoln, M. Daly, and E. Lander, "Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1". Whitehead Institute Techn Rep 2nd edn, Whitehead Institute, Cambridge, Massachusetts, 1992.

[20] D.D. Kosambi, "The estimation of map distance from recom-bination values". Ann. Eugen. 12, 1994, 172–175.

[21] M.A. Causse, T.M. Fulton, Y.G. Cho, S.N. Ahn, J. Chunwongse, K. Xu, J. Xian, Z. Yu, P.C. Ronald, S.E Harrington, G. Second, S.R. McCouch, and S.D. Tanksley, "Saturated molecular map of the rice genome based on an interspecific backcross population". Genetics. 138, 1994,1251–1274.

[22] Y. Harushima, M. Yano, A. Shormura, M. Sato, T. Shimano, Y. Kuboi, T. Yamamoto, S.Y. Lin, B.A. Antinio, A. Parco, H. Kajiya, N. Huang, K. Yamamoto, N. Nagamura, N. Kurata, G.S. Khush, and T. Sasaki, "A high-density rice genetic linkage map with 2,275 markers using a single F2 population". Genetics. 148, 1998, 479–494.

[23] S. Temnykh, G. DeClark, A. Lukashova, L. Lipovich, S. Cartinhour, and S.R. McCouch, "Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon association, and genetic marker potential". Genome. Res. 11, 2001,144–1452.

[24] A.H. Paterson, E.S. Lander, J.D. Hewitt, S. Paterson, S. Lincoln, and S.D. Tankley, "Resolution of quantitative traits into mendelian factors by using a complete linkage map of restriction fragment length polymorphisms". Nature. 335, 1988,721–726.

[25] E.S. Lander, and D. Bostein, "Mapping Mendelian factors underly-ing quantitative traits using RFLP linkage maps", Genetics. 121, 1989, 185–199.

[26] D.L. Wang, J. Zhu, Z.K. Li, and A.H. Paterson, 1999. "Mapping QTLs with epistatic effects and QTL envirment interactions by mixed linear model approaches". Theor. Appl. Genet. 99, 1999,1255–1264.

[27] S.D. Tanksley, "Mapping polygenes". Annu. Rev. Genet. 27,1993, 205–233.

[28] J.C. Nelson, "QGene: Software for marker-based genomic analysis and breeding". Mol. Breed. 3, 1997, 239–245.

[29] S.D. Tanksley, N.D. Young, A.H. Patterson, and M.W. Bonierbale,1989. "RFLP mapping in plant breeding: New tools for an old science", Bio. Technology.7, 1989, 257.

[30] N.T. Lang, S. Yanagihara, and B.C. Buu, "QTL analysis of salt tolerance in rice (*Oryza sativa* L.). SABRAO. 33(1), 2001b, 11–20.

[31] N.T. Lang, S. Yanagihara, and B.C. Buu, "A microsatellite marker for a gene conferring salt tolerance on rice at the vegetative and reproductive stages", SABRAO., 33(1), 2001c, 1-10.

[32] N.T. Lang, S. Yanagihara, and B.C. Buu, "Quantitative trait loci for salt tolerance in rice (Oryza sativa L.) via molecular markers". OMon Rice. 8, 2000, 37–48.

[33] J.M. Gong, P. He, Q. Qian, L.S. Shen, L.H. Zhu, and S. Chen, "Identified of salt tolerance QTLs in rice (*Oryza sativa* L.)". China Sci. Bull, 44, 1999, 68–71.

[34] P. Bonilla, J. Dvorak, D. Mackill, K. Deal, and G. Gregorio, "RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines", Philipp. J. Agric. Sci. 85, 2002, 68–76.

[35] H.X. Lin, S. Yanagihara, J.Y. Zhang, T. Senboku, K.L. Zheng, and S. Yashima, 1998. "Identification of QTLs for salt tolerance in rice via molecular markers". Chinese J Rice Sci. 12(2), 1998, 72–78.

[36] S.Y. Lee, J.H. Ahn, and Y.S. Cha, 2007. "Mapping QTLS related to salinity tolerance of rice at the young seedling stage". Plant breed. 126, 2007, 43–46.

[37] M.J. Thomson, M.D. Ocampo, and J.L. Egdane, "Characterizing the saltol quantitative trait locus for salinity tolerance in rice". Rice. 3, 2010, 148–160.

Analysis of Profitability and Constraints of Table Egg Production Enterprises in Benue State, Nigeria

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***Abstract*— *This study was carried out in Benue State, Nigeria. It was aimed at determining the profitability of table egg production enterprises and the constraints limiting their operations. The population of the study comprised all registered table egg production enterprises in Benue State, Nigeria. A sample size of 65 table egg production enterprises were selected using multi-stage random sampling technique. Cross sectional data were obtained from the 65 table egg producers with the aid of structured questionnaire. Data collected from the enterprises were analysed using descriptive statistics, farm budgeting technique and factor analysis methods. The results revealed that the table egg production enterprises in the state were of medium scale, with mean flock size of 1040 birds. An average producer in the enterprise was 44 years old and had a household size of 7 persons. All the producers had obtained one for of formal education or the other, with tertiary education being the majority (69.2%). Table egg production was found to be a profitable venture in the study area with Net Farm Income (NFI) of ₦ 1248.03 per bird. However, the enterprises are faced with a number of constraints militating against their efficient operation. These including financial/marketing, inputs and environmental constraints. Based on the findings of this study, it was concluded that table egg production in the State is profitable. The enterprise remains a viable investment option for investors, as it possesses high growth potentials which are achievable through increased investment and proper management of identified constraints. Hence, subsidization of poultry production inputs by the government, making loans/grants easily accessible to egg farmers at single digit interest rates, formation of cooperative societies by the producers in the enterprise, setting up of more feed mills in the state and support for researches geared toward developing Non-feed-food competing feed materials were recommended as means of increasing profitability of table egg enterprises in the Study area.*

Keywords*— *Table Egg, Profitability, Production, Constraints, Benue State.

I. INTRODUCTION

The Poultry industry is an important branch of the livestock sub-sector of Nigeria's agricultural sector. The importance of the poultry industry lies chiefly in the provision of meat and egg, the provision of employment either directly or indirectly and the generation of revenue; Gross Domestic Product (GDP) of the country (Adebayo and Adeola, 2005).Although poultry production began as a rural and subsistence backyard farming practice, today, it has become a fully commercialized industry. It is the most commercialized (capitalized) of all the sub-sectors of the Nigerian agriculture (Adene and Oguntade, 2006).

According to Okoli *et al.*(2004), poultry keeping is the commonest livestock production practice in rural areas of Nigeria. They added that it is an attractive business because birds adapt easily to new and changing environmental factors, relative to other livestock. They have high economic value, rapid generation time and a high rate of productivity that can result in the production of meat within eight weeks and eggs within eighteen weeks from the time the birds are day old. In the first year of its laying life, a fowl is capable of producing about 300 eggs but under tropical conditions this has averaged about 180 - 200 eggs, even though higher levels have been reported (Oluyemi and Robert, 1978; Kumar and Pandey, 1999).

Following the drive towards the attainment of food security in Nigeria and efforts to diversify the economy from oil to non-oil sectors, following the volatility of oil prices in recent years, the agricultural sector has received and continues to so much attention by the government and private sectors. The increased in activities in the sector have led to rising rate of investment in the various sub-sectors, including the poultry industry. Tijjani *et al.* (2014) noted that despite the growth in the country's egg production industry since the year 2000, domestic egg production has is yet to meet demand. Preliminary survey showed that despite the abundant production of some livestock feed materials like soya beans and maize, Benue State still depend on neighbouring states for her egg needs.

Literatures provide evidence of Studies on poultry egg production in different parts of Nigerian; Aihonsu (1999) assessed the optimal laying period for profitable and sustainable egg production., Tijjani *et al.* (2006) studied the profit efficiency among Nigerian poultry egg farmers in Ojo Area of Lagos State., Nigerai, Mukhtar (2007) analysed the economics of poultry-egg production in Bauchi Local Government Area, Bauchi State, Nigeria.,Yusuf and Malomo (2007) assessed the technical efficiency of poultry egg Production in Ogun, Nigeria., Jatto (2012) studied the economics and social characteristics of registered poultry egg Producers in Ilorin, Kwara State, Nigeria., Aji and Tanko (2011) compared the economic efficiency of broiler and egg production enterprises in Niger State, Nigeria., while Tanko *et al*. (2014) analyses the profit efficiency of small scale layer producers in some selected Local Government Areas in Sokoto State, Nigeria. This and many other related studies have been conducted in Nigeria, however, published information on the profitability or constraints of table egg production in Benue State is lacking. It is in this light that this study sought to determine the profitability and constraints of table egg production enterprises in Benue State, Nigeria. Specifically, the objectives of the study were to:

(i) Describe the socio-economic characteristics of table egg producers;
(ii) Determine the profitability level of table egg producers and
(iii) Identify the constraints to table egg production in the study area.

II. METHODOLOGY

This study was carried out in Benue Sate, Nigeria. Benue State is in the middle belt zone of Nigeria, between latitudes 8^0 - 10^0N and longitudes 6^0 - 8^0E, with the land mass of about 33,955Km2 and 23 local government areas three agricultural zones and total population of 4.2 million people (National Population Commission (NPC) (2006) and Hula (2010)). It shares boundaries with five other states, namely; Nasarawa to the North, Taraba to the East, Cross River to the South-East, Enugu to the South-West and Kogi to the West. The Republic of Cameroon shares boundary with the state on the South-East. It is bordered on the North by 280 km of River Benue and transversed by 202Km of River Katsina-Ala inland. Majority (75%) of the population; mostly farmers reside in the rural area (Benue State Agricultural and Rural Development Authority (BNARDA), 1999). Following the administrative demarcation of the state, it is divided into three agricultural zones namely; North-east, North-west and Southern zones.

The two main ethnic groups in Benue State are the Tiv and Idoma who represent 72% and 21% respectively of the entire population. The Igede ethnic groups makes up 6 percent, while the remaining one percent is composed of the Hausa, Fulani, Jukun, Abakwa, Nyifon, Etulo and the Igbos (National Population Commission, 2006).The State is a major producer of food and cash crops like Yam, Cassava, Rice, Sesame, maize, etc. Some of the livestock produced in the state include goats, pigs, cattle and poultry. Among the livestock produced in Benue State, Poultry ranks the highest (48.25%) in terms of population followed by goat, pig and sheep with percentage populations of 35.09%, 11.40% and 5.26% respectively (BNARDA, 1999).

Multi-stage sampling technique was employed in the selection of respondents for this study. First, one local government area (LGA) was purposively selected from each of the three agricultural zones, based on existing information on broiler production. These are namely Katsina-Ala from Northern East zone (Zone A), Makurdi from North West zone (Zone B) and Otukpo the Southern zone (Zone C) due to the preponderance of broiler production enterprises; this was followed by the simple random selection of 80% of the broiler enterprises in each of the three (3) LGAs to give a sample size of 65 table egg production enterprises. Data were collected with the aid of structured questionnaire which was administered to the producers (managers) of the enterprises. The data obtained were analyzed using descriptive statistics, farm budget technique and factor analysis. Descriptive statistics such as mean, percentage, and frequency were used to ascertain the distribution based on their socio-economic characteristics. Farm budget techniques such as Gross Margin and Net farm income (NFI) were used to estimate the costs and returns among broiler farmers. Gross Margin (GM) which is the difference between the total revenue and the total variable cost of production is expressed as:

GM= TR – TVC ……………………. (1).

GM= Gross Margin

TR=Total Revenue

TVC=Total Variable Cost.

On the other hand, Net Farm Income (NFI) which is the difference between the total revenue and total cost of production is expressed as:

NFI= GM –TFC……………………. (2)

Where;

NFI= Net Farm Income.

GM=Gross Margin

TFC=Total Fixed Cost.

Factor Analysis Model

$F1 = \alpha11X1 + \alpha12\ X2 + * * * + \alpha1nXn$ - - - (3)

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$$F_2 = \alpha_{21}X_1 + \alpha_{22} X_2 + * * * + \alpha_{2n}X_n \quad (4)$$

$$* \qquad\qquad *$$
$$* \qquad\qquad *$$
$$* \qquad\qquad *$$

$$F_n = \alpha_{n1}X_1 + \alpha_{n2} X_2 + * * * + \alpha_{nn}X_n \quad (5)$$

Where;

$F_1, F_2, * * * F_n$ = Unobserved underlying factors constraining broiler production.

$\alpha_1 * * * \alpha_n$ = Factor loadings or correlation coefficients.

$X_1, X_2, * * * X_n$ = Observed variables/Constraints of table egg production

$F_n < X_n$.

III. RESULTS AND DISCUSSION

Table 1 shows the socio-economic characteristics of table egg producers. The result on age showed that the mean age of the producers is 43 years with higher percentage of the farmers being relatively young and in their active age of production. The implication is that younger farmers are likely to adopt innovations faster than the older ones. The finding is in agreement with the findings of Jatto (2012) and Sani et al.(2007) who found that high percentages of poultry farmers were in their active ages.

The result on farming experience revealed a mean experience of 9.8 years, with majority (67.7%) having 10 and below years of experience. The implication is that most of the producers have garnered production experience and are likely to be profitable in their production. This result supports the finding of Onyebinama (2004), that previous experience in farm business management enable farmers to set realistic time and cost targets, allocate, combine and utilize resources efficiently and identify production risks. The result also supports that of Olasunkanmi *et al.* (2013) who found that poultry farmers in Peri-urban Lagos Nigeria had farming experience spanning between 1 and 15 years.

The mean flock size of chicken table egg farmers in the study area was found to be 1040 birds. This shows that a typical table egg enterprise in the study area is of the medium scale class. The implication is that the farmer are likely not able to meet the egg needs of the inhabitants of the state. This could partly explain the reason for importation of eggs from other states to complement that produced in the state. The classification here followed that of Olorunsanya (2004) and Akinkumi *et al.*(1979) who concluded that a farmer who maintains at least 5000 birds is classified a large scale producer and farmers with between 500 and 4999 birds are medium scale producers, while those with less than 500 birds are said to small scale producers.

Analyses of household size of the producers showed a mean household size of 7 persons with a modal household size of range of between 6 and 10 persons. The high mean household size means that there is high availability of family labour to the enterprises in the study area. This result is in line with that of Igbalajobi *et al.* (2016).

The result on gender showed that majority (78.5%) of the sampled producers were married. This shows that the producers are responsible according to the societal standard and therefore are likely to have some experience of life challenges which instils some level of discipline in making decision. This finding supports the result of Oluwatayo *et al.*, (2008) that married farmers tend to have large family to compliment family labour to enhance production and reduce the cost of hired labour.

On educational status of the producers, the result shows that majority (69.2%) of the producers had tertiary education indicating that they are highly educated. The implication is that the level of education of the producers will contribute significantly to their decision making. According to Obinne, (1991); Alabi and Aruna (2006) and Ndahitsa, (2008) education determines the quality of skills of farmers, their allocative abilities and how well informed they are to the innovations and technologies around them.

The result on occupational status showed that most of the respondents (53.8%) in the enterprise were engaged in the eggs production venture as their secondary occupation. This implies that they had alternative means of income. This also explains the reason for the relatively high average off farm income obtained by producers in the enterprise.

Analysis of the off farm income of the producers showed that the mean off farm income for broiler and pullet-egg enterprises were ₦351,590.91 and ₦113,034.17 per annum respectively. This implies that the producers in both enterprises are likely to cushion some of the effects of their financial constraints using their off farm income. This result agrees with Amaza (2000) who noted that it is common for some farm household to engage in other non-farming activities to complement their earnings from farming occupation for their livelihood.However, engaging in off farm production activities could reduce supervision and production efficiency of the farm.

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Table.1: Socio-economic Characteristics of Table Egg production Enterprise Owners

Variables	Frequency	Percentage
Age (Years)		
≤20	2	3.1
21 – 40	25	38.5
41 – 60	33	50.8
61 and above	5	7.7
Total	65	100.0
Mean	**43**	
Experience (years)		
≤10	44	67.7
11 – 20	14	21.5
21 – 30	7	10.8
31 and above	-	-
Total	65	100.0
Mean	**10**	
Flock Size (No of Birds)		
≤1000	44	67.7
1001 and above	21	32.3
Total	65	100.0
Mean	**1040**	
Household Size (No of Persons)		
≤5	23	35.4
6 – 10	38	58.5
11 and above	4	6.2
Total	65	100
Mean	**7**	
Gender		
Male	38	58.5
Female	27	41.5
Total	65	100
Marital Status		
Single	14	21.5
Married	51	78.5
Divorced/Separated	-	-
Widow/widower	-	-
Total	65	100
Education		
Primary	3	4.6
Secondary	17	26.2
Tertiary	45	69.2
Total	65	100
Occupation		
Poultry production as primary occupation	30	46.2
Poultry production as secondary occupation	35	
Total	65	53.8
		100
Off farm income (₦)		
≤200,000	57	87.7
200,001 – 600,000	4	6.2
600,001 – 1,000,000	4	6.2
1,000,001 and above	-	-
Total	65	100
Mean	**113,034.17**	

Source: Field Survey, 2016

3.1 Profitability of Table egg Enterprises

Analysis of the cost and returns components of the table egg production enterprises as shown on Table 2 indicated that a table egg production enterprise spent an average of ₦10342.31as total variable cost per bird and ₦ 116.28 as total fixed cost per bird. An average table egg production enterprise in the study area realized a total gross income of ₦11,590.33 per bird raised and kept in laying per annum in the study area. The average net profit realized per layer by the enterprise is ₦1248.02. Analysis of the variable cost item showed that feed constituted about 89.95% of the total cost of production. Second and third highest contributing variable cost items were cost of labour 6.26% and cost of obtaining day old chicks 1.84% respectively. While returns from sales of eggs contributed the most 92.21% to the total revenue. Returns from sales of spent of culled birds and sales of droppings made up the remaining 7.5% and 0.20% respectively. This is in consonance with the findings of Tanko *et al.* (2014), Emam and Hassan (2010), Samarend (2003) and Narahari (2002) who found that sale of eggs constituted the highest proportion of the total revenue. The average gross returns per bird of ₦11,590.33 and the average net income per bird of ₦1,248.02 suggest that table egg production is a profitable venture in the study area. Although these amounts are lower than those reported by Jatto (2012) and Tanko *et al.* (2014). This is in line with Tanko *et al.* (2014), Anang (2013), Adepoju (2008), Yusuf and Malomo (2007) and Samarendu (2003) who also found egg production to profitable and pointed out that price of egg, among other factors, influence the profitability of a poultry farm.

The result hasalso revealed that in table egg production, feed is an important cost component of poultry production. This is in agreement with Tanko *et al.* (2014), Anang (2013), Adepoju (2008) and Yusuf and Malomo (2007) who also found that, of the cost items in poultry production, cost of feed was the highest.

Table.2: Cost and Returns of Broiler and Table egg Enterprises

Cost/Returns Items	Amount(₦/Bird)	%
Variable Costs	**(₦/Bird)**	**(%)**
i) Feed and Supplements	9222.84	89.18
ii) Foundation stock (DOC & POL)	219.68	2.12
iii) Transportation	11.78	0.11
iv) Labour	678.34	6.56
v) Repairs and Maintenance	38.50	0.37
vi) Drugs and Vet.	37.99	0.37
vii) Debeaking	16.89	0.18
Total Variable Cost (TVC)	10226.02	98.88
Fixed Cost (Depreciation on fixed assets such as feeders cages, etc.)	116.28	1.12
Total Cost (TC)	10342.31	100
Revenue		
i) Egg	10716.78	92.46
iii)Culled/Spent layers	842.24	7.27
iv) By-Products e.g. Droppings	31.31	0.27
Total gross income	11,590.33	100
Gross Margin(GM)	1364.32	-
Net Farm Income (NFI)	1248.02	-

Source: Field Survey, 2016
DOC: Day old Chicks
POL: Point of Lay

3.2 Constraints to Table egg production

Table 3 shows the result for the rotated component matrix showing the extracted factors based on the responses of table egg producers on constraints to egg production. Factors 1, 2 and 3 were; financial/marketing constraint, input constraint and environmental constraint respectively. The specific constraint variables contributing to financial/marketing constraint are inadequate finance (0.921), Theft (0.909) and Seasonal egg glut (0.916).

Inadequate finance may be due to the low equity base of table egg producers and their inability to access credit. Hence, table egg production in the study area is still operating on small and medium scale levels. This result conforms with those of Ovwigho *et al.* (2009) and Mukhtar (2007) who found inadequate finance as one of the major constraints to poultry production.

Seasonal egg glut with a factor loading of (0.916) was identified as one of the financial/marketing constraint. This confirms the findings of Adebayo *et al.* (2012) who found that seasonal egg glut was a constraint to poultry egg production. Government policies, low per capita income and consequently, low purchasing power, lack of adequate education, availability and affordability of close substitutes and complementary food have been identified as some of the causes of seasonal egg glut.

Theft was also identified as an institutional factor militating against egg production in the study area. The failure on the part of government to provide effective security through its security institutions, allows crime, including theft of eggs and birds to be perpetrated. This result agrees with Salman *et al.* (n.d.) who found that theft by workers and non-workers were among the risks faced by poultry farmers in Ibadan, Nigeria.

The variables contributing to input constraints (factor2) were high input cost (0.910), inadequate feed formulation ingredients (0.868), poor quality chicks (0.784) and inadequate storage facilities (0.760).

Increasing cost of inputs, including feed, labour, day old chicks, etc. is a major problem faced by egg producers. This reduces the producers' profit and discourages investment in the enterprise. This confirms the findings of Mukhtar (2007), Esiobu *et al.* (2014) and Adebayo *et al.* (2015) who identified high cost of inputs, especially cost of feed as an important constraint faced by poultry egg producers. Inadequate/poor feed formulation ingredient is likely to result in low productivity and in consequently, low returns.

Poor quality day old chicks puts the egg production enterprise at the risk of being less profitable and unattractive to investors. This confirms the findings of Mukhtar (2007) who found that poor quality day old chicks is among the constraints to egg production. The result also supports the claim of Esiobu *et al.* (2014) that poor breeds of layers is a constraint to poultry egg production.

Good quality storage facilities are necessary in egg production due to the short storage period of egg in an uncontrolled environment. Inadequate and poor quality storage facilities will amount to losses of eggs to theft, predators and spoilage. This result agrees with Esiobu *et al.* (2014) and Mukhtar (2007).

The specific variables that loaded sufficiently on environmental constraint are adverse weather condition (0.880) and disease outbreak (0.904).The environment which is consist of everything around man possess high implications for poultry production; some positive and others negative.

Adverse conditions of weather does have negative implications for egg production. For instance, extremely warm weather have been associated with drop in egg production, high rate of egg deterioration and outbreak of infectious diseases. This result agrees with Mirski (2012).

Supporting the chief variable contributing to environmental constraint is outbreak of disease. Certain diseases tend to predominant in some climatic conditions than in others. Mirski (2012) established that there is an association between the outbreak of infectious diseases and climate change and variation in climatic factors like temperature, rainfall, humidity, etc.

Table.3: Constraints to Table egg Production

	Components		
Variables	Factor 1	Factor 2	Factor 3
Inadequate finance	0.921*	-0.166	0.305
Adverse weather Condition	0.306	-0.179	0.880*
Inadequate feed formulation ingredients	-0.117	0.868*	-0.046
Poor quality chick	-0.158	0.784*	-0.355
High input cost	-0.169	0.910*	-0.125
Inadequate extension	0.753*	0.011	0.505*
Theft	0.909*	-0.235	0.076
Seasonal egg glut	0.916*	-0.067	0.286
Inadequate Storage facilities	-0.005	0.760*	0.046
Diseases outbreak	0.315	-0.091	0.904*

Source: Analysis of field data, 2016.

* = Significant based on Kaiser Normalization

Method: Principal Component Method

Factor 1= Financial/marketing; Factor 2= Input constraints; Factor 3= Environmental constraints

IV. CONCLUSION

The result of this study has shown that table egg production Benue State, Nigeria is a profitable enterprise and does have the potentials for growth and better profitability,though, the adoption of innovations, increase in the scale of operation and more efficient management. The enterprise is however faced with a number of constraints that have continued to hinder it from realising these potentials. The constraints include financial/marketing constraints, input constraints and environmental constraints.

The following recommendations were made based on the findings of this study:

i. The government should subsidize production inputs used by table egg production enterprises especially feeds.
ii. Funds in the forms of loans and grants should be made available and accessible to egg producers in the study area by government, commercial banks and other financial bodies. Where loans are provided, they should be at a single digit interest rate.
iii. Egg producers in the state should form cooperative societies which will enable them procure production inputs at relatively cheaper rates than they are able to do as individuals.
iv. All stake holders in the enterprise should do their bit at ensuring security in and around the farms. The farmer could hire trained guards to provide security for individual farms or groups of farms within the same location and
v. Modern poultry feed mills should be established in the study area by stakeholders in the sector. Also, the government and corporate bodies should provide support for researches towards the discovery of efficient and less expensive feed materials.

REFERENCES

[1] Adebayo, O.O, Adeola, R.G. (2005). Socio-Economic Factors Affecting Poultry Farmers in Ejigbo Local Government Area of Osun State. *Journal of Human Ecology*, 18(1):39 – 41.

[2] Adene, D.F. and Oguntade, A.E. (2006). The structure and importance of the commercial and village-based poultry industry in Nigeria. FAO (Rome) Study, October 2006. 66 pp.

[3] Adepoju, A. A. (2008). Technical efficiency of Egg Production in Osun state, *International journal of Agricultural Economics and Rural Development*, (1): 7-14.

[4] Aihonsu, J.O.Y. (1999). Optimal laying period for profitable and sustainable egg production in Bamiro, O. M. and Shittu A. M. (2009). Vertical Integration and Cost Behaviour in poultry Industry in Ogun and Oyo States of Nigeria.

[5] Akinwumi, J. A., Adegeye, A. J., Ikpi, A. E. and Olayide, S. O. (1979). Economic Analysis of Nigerian Poultry Industry (A study commissioned by Federal Livestock Department) Lagos.

[6] Amaza, P.S. (2000). Resource Use Efficiency in Food Crop Production in Gombe State: A PhD Thesis (unpublished) cited in Abdulsa- lam R.Y. (2010). Economics of Smallholder Irrigated Farming System in the Hadejia Nguru Wetlands of North Eastern Nigeria. M.sc dissertation (unpublished).Department of Agricultural Economics and Extension,Faculty of Agriculture, Usmanu Danfodiyo University Sokoto.

[7] Anang, B. T., Yeboah, C. and Agbolosu, A. A. (2013). Profitability of Broiler and Layer Production in the Brong Ahafo Region of Ghana. *ARPN Journal of Agricultural and Biological Science*, 8(5):423-430.

[8] Benue State Agricultural and Rural Development Authority (BNARDA) (1999). *Highlights of BNARDA'S Programmes, Makurdi*. pp 1-15.

[9] Emam, A.A. and A.M. Hassan (2010). Econom- ics of egg poultry production in Khartoum State with emphasis on the open-system- Sudan; *African Journal of Agricultural Research* 5(18), 2491-2496.

[10] Esiobu, N.S; Onubuogu, G.C. and Okoli, V.B.N. (2014). Determinants of Income from Poultry Egg Production in Imo State, Nigeria: An Econometric Model Approach. *Global Advanced Research Journal of Agricultural Science*, 3(7): 186-199.

[11] Hula, M. A. (2010). Population Dynamics and Vegetation Change in Benue State, Nigeria. *Journal of Environmental Issues and Agriculture in Developing Countries*, 2(1): pp. 53-69.

[12] Igbalajobi, O., Fatuase, A. I. and Ajibefun I. (2012). Determinant of Poverty Incidence Among Rural Farmers in Ondo State, Nigeria. *American Journal of Rural Development* 1(5): 131-137.

[13] Jatto, N. A. (2005). Economics and Social Characteristics of Registered Poultry Egg Producers in Ilorin, Kwara State. *Russian Journal of Agricultural and Socio-economic Sciences*, 11(11): 18-23.

[14] Mirski, T. Bartoszeze, M. and Bielawska-Drózd (2012). Impact of Climate Change on Infectious Diseases. *Polish Journal of Environmental Studies*. 21(3): 525-532.

[15] Mukhtar, U. (2012). Economic Analysis of Poultry-Egg Production in Bauchi Local Government Area, Bauchi State, Nigeria. M.Sc. Thesis. Department of Agricultural Economics and Rural Sociology,

Faculty of Agriculture. Ahmed Bello University, Zaria 83pp.

[16] Narahari, D. (2002). All Layer Farms is More Economical than Chick, Grower Cum Layer Farm, Department of poultry science, Ma- dras veterinary college, India. Cited in Rajendran, K. and M. Samarendu (2003). Comparative Economic Analysis and Constraints in Egg Production under Cage vs. Deep Litter Systems of Rearing in India, *International Journal of Poultry Science* 2 (2): 153-158.

[17] National Population Commission. (2006). Population Census Statistics: Population Distribution by Age and Sex. Retrieved 10th February, 2016 from http://www.population.gov.ng/index.php/publications/141-population-distribution-by-age-and-sex-2006-census-priority-tables-vol-4

[18] Ndahitsa, M.A. (2008). Impact of Small Scale Irrigation Technologies on Crop Production by Fadama users in Niger state, 10th nation- al Annual Conference of National Associa- tion of Agricultural Economics (NAAEC).Held at university of Abuja main campus p 195.

[19] Obinne, C.P.O. (1991)."Adoption of Improved Cassava Production Technologies by Small Farmers in Bendel State" *African journal of Biotechnology* 7 (9): 1227-1286.

[20] Okoli, I.C., Anyaegbunam, C.N., Etuk, E.B., Uchegbu M. C. and Udedibie, A.B.I. (2004). Socio-Economic Characteristics of Poultry Business Entrepreneurs in Imo State, Nigeria. *Journal of Agriculture and Social Research*. 4(2), 100 – 111.

[21] Olasunkanmi, M. B., Otunaiya, A. O. and Adejumo, I. (2013). Profit Efficiency in Poultry Production in Peri-Urban Lagos, Nigeria. *International Journal of Applied Agricultural and Apicultural Research. 9* (1&2): 120-130.

[22] Olorunsanya E. O. (2004). Egg Farming Business in Kwara State, How profitable? *Agrosearch* 6(1), 9 – 14.

[23] Oluwatayo, I.B., Sekumade A.B. and Adesoji S.A. (2008). Resource use efficiency of maize farmers in rural Nigeria. Evidence from Ekiti state, *World Journal of Agricultural Science* 4(1): 91-99.

[24] Onyebinama, U.A.U., (2004). Farm Business Management for Smallholder Farm Firms in Nigeria, Owerri Alphabet Nigeria Publish-ers, Nigeria.

[25] Salman, K.K., Ashagidigbi, W.M. and Jabar, K.T.(n.d.). Correlates of Risk-Aversion among Poultry Egg Farmers in Ibadan, Nigeria. *Journal of Rural Economics and Development*,19 (1): 46 – 60.

[26] Sani, R.M., S.A., Musa, M.I. Daneji, M.T. Yaka- sai and O. Ayodele (2007). Cost and Returns Analysis in Poultry Production in Bauchi and Gombe metropolis area. *Continental Journal of Agricultural Economics* 1:14-19.

[27] Tanko, L. and Aji, D. A. (2011). Comparative Analysis of Economic Efficiency of Broiler and Egg Production Enterprises in Niger State, Nigeria. Retrieved 21st December, 2015 from

[28] http://www.academia.edu/5926880/corrected_comparative_analysis_of_economic_efficiency_of_broiler_and_egg_production_enterprises_niger_state_nigeria

[29] Tanko, L., Nabil, I. A. and Maikasuwa, M. A. (2014). Profit Efficiency of Small Scale Layer Producers in some selected Local Government Areas in Sokoto State, Nigeria. *International Journal of Modern Research and Review*, 2(1): 52-61.

[30] Tijjani, A. A., Alimi, T. and Adesiyan, A. T. (2006). "Profit Efficiency among Nigerian Poultry Egg Farmers: A Case Study of Aiyedoto Farm Settlement, *Nigeria. Research Journal of Agricultural Biological Sciences*, 2(6):256-261

[31] Yusuf S. A. and Malomo O. (2007). Technical Efficiency of Poultry Egg Production in Ogun State, *International Journal of Poultry Science*, 6(9): 622-629.

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Effect of Ethephon Stimulation on Downward Tapping in Latex Production Metabolism on Upward Tapping in PB 217 Clone of Hevea Brasiliensis

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Abstract**—*In Hevea brasiliensis, Ethephon is used as an exogenous stimulant of latex production. In downward tapping, it has been shown that its misuse has negative consequences on the metabolism of the latex cells of the tree. However, little is known about the impact of this downward tapping practice on latex-producing metabolism in upward tapping. The aim of this study was to determine the effect of stimulation in downward tapping on the latex-producing metabolism in clones PB 217, when the trees are subsequently tapped up. Study was carried out by monitoring the evolution of biochemical parameters of latex and rubber production. From this study, it was found during the last two years of downward tapping that in PB 217 clone, the optimal metabolism of latex production was obtained with 13 Ethephon stimulations per year. When subsequent tapping is done upward, the stimulation frequencies of the trees greater than 4 times per year in downward tapping have a negative impact on the latex-producing metabolism in PB 217 clone of Hevea brasiliensis. However, in cumulative production over the 10 years of downward tapping added to the 2 years of upward tapping, it is the frequency of 13 stimulations per year in downward tapping which is the highest.*

Keyword— Ethephon stimulation frequency, downward tapping, upward tapping, production potential, PB 217 clone of Hevea brasiliensis.

I. INTRODUCTION

In Hevea brasiliensis at maturity, latex production is usually done in three phases. The first phase consists of tapping the tree on the pristine bark of the trunk at a height of 1.20 m from the ground, according to the direction from top to bottom. This phase is called downward tapping. In the second phase, the tapping begins above the first opening and is conducted in the direction from the bottom to the top of the trunk. It is called upward tapping. Finally, the third phase is conducted like the first but on the newly formed bark resulting from the first phase. For this reason, it is called tapped on regenerated bark[1]. In each of these three phases, the Ethephon, an ethylene-generating compound, is used as an exogenous stimulant for latex production. This method, widely adopted by rubber planters, prolongs the flow of latex and stimulates the metabolism of latex cells[2,3,4,5]. However, it has been shown that, in downward tapping, its abusive use has negative consequences on the tree latex cells metabolism[6,7]. In the short or medium term, depending on the intensity of the stimulation or the clone, the production capacity of the trees, also called production potential, is strongly affected, in downward tapping. This is reflected in the external sign, which is the increase tapping panel dryness (TPD) rate[8,9].

However, the impact of this stimulant product used in downward tapping on the production potential of the tree, in upward tapping, has been little studied.

Some work[10,11] has shown that in the PB 260 clone which has a low tolerance to stimulation and the GT1 clone which has an average tolerance, stimulations beyond of 4 and 6 times per year respectively, in downward tapping, reduces the production potential of the trees, in upward tapping. For the clone PB 217 which has a better tolerance to Ethephon stimulation in downward tapping[12,13], the impact of the stimulation in downward tapping on the production potential, in upward tapping, is not well known. The aim of this study is to determine in PB 217 clone, the effect of downward tapping stimulation on latex-producing metabolism when trees are harvested following upward tapping.

The study was carried out by monitoring the evolution of the latex biochemical parameters such as dry rubber content (DRC), sugar (Suc), thiol (R-sh) and inorganic phosphorus (Pi) concentration, tapping panel dryness (TPD) rate and tree production. This follow-up was linked with different stimulation frequency in downward tapping.

II. MATERIAL AND METHODS

Plant Material

The Plant material used in this study was the clone PB 217 from Hevea brasiliensis planted at the CNRA Anguédédou experimental station in southeastern Côte d'Ivoire. This clone is characterized by a high tolerance to Ethephon stimulation in downward tapping. The first tapping of the trees was done at 6 years and 10 months.

Methods

Experimental device

The experiment was carried out using a totally randomized single tree plot design with 9 treatments in downward tapping which are stimulation frequencies and 33 trees per treatment for a total of 297 trees during the experimentation. The empty slots of the edge of the trees and the neighboring trees on the line were eliminated from the test to minimize edge effects.

The stimulation of production was made with a mixture (stimulating paste) of Ethrel and palm oil (adjuvant). Ethrel is a commercial product with 2-chloroethylphosphonic acid or Ethephon (ET) as active material (m.a.).

The production system used in downward tapping is the notched half-spiral every 4 days except sunday, stimulated with 1 g of paste with 2.5% of active material, applied to the regenerating panel above the notch (S/2 d4 6d/7 ET 2.5% Pa 1 (1)).

In upward tapping, a single stimulation frequency was applied for all treatments. That is 13 frequency stimulations per year (13/y). The production system used is the quarter spiral tapped every 4 days except sunday, stimulated with 1 g of paste with 5% active material applied to the regenerating panel below the groove notch (S/4 U d4 6d/7 ET 5% Pa 1 (1)).

The various treatments which are stimulation frequencies in downward tapping and in upward tapping are presented in Table 1.

Collected Data

The collected data are 4 biochemical parameters of the latex which are Dry Rubber Contents (DRC), Sucrose (SUC), inorganic Phosphorus (Pi) and Thiol groups (R-SH)) and Production in grams per tree per tapping (g/t/t), in grams per tree per millimeter of notch (g/t/mmN) and gram per tree cumulated (g/t.cum), 9, 10, 11 and 12 years old.

Measurement of latex biochemical parameters

The biochemical parameters were measured on fresh latex harvested in the field at the level of the trees of the various treatments and brought back to the laboratory.

The measured parameters are DRC, SUC content, Pi content and R-SH content of the latex[14].

Tapping panel dryness percentage determination

The tapping panel dryness (TPD) percentage at each treatment was estimated from a dry notch length measure (DNLM).

The DNLM is a visual assessment of the exudation of the latex at the indented area, called notch. During the DNLM, trees are considered " healthy " these are trees which exude the latex over the entire length of the notch after the tapping. They are scored zero (0). Others who do not exude any latex or a portion of the notch after tapping are considered diseased trees and are rated from 1 to 6 along the length of non-production of latex notch (Table 2).

The percentage of TPD is obtained by the following formula:

$$TPD=\frac{0.1*n1+0.3*n2+0.5*n3+0.7*n4+0.9*n5+1*n6}{N}*100$$

Where N represents the number of trees per treatment. Coefficients 0.1; 0.3; 0.5; 0.7; 0.9 and 1 are the mean percentages of classes of non-production notch length latex. Numbers n1; n2; n3; n4; n5 and n6 represent the number of trees observed by length class percentage of non-producing latex notch.

As part of this study, a DNLM was made in November each year on all living trees of the experimentation and TPD

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percentage of each treatment was calculated year by year.

Production estimation

The production of each treatment was estimated tree-by-tree by weighing the coagulated latex in the polybag sachets after tapping. A conversion coefficient of the dry rubber with fresh rubber was determined for each treatment by the following formula:

$$C=\frac{DW}{FW}$$

Where FW is the fresh weight of a coagulum sample, and DW is the weight of the same sample after spinning and drying.

This coefficient, which is calculated for each treatment, is multiplied by the total weight of fresh rubber of the 6 tapping to obtain the monthly dry rubber production of the treatment. For the production in grams per tree per millimeter of notch (g/t/mmN), notch lengths (NL) were calculated from the average circumferences (Cir) of the trees of each treatment measured at 1m 70 from the ground, after each tapping campaign, using the following formulas:

- in downward tapping:

$$NL = \frac{Cir}{2\cos 33}$$

- in upward tapping:

$$NL = \frac{Cir}{4\cos 45}$$

The values obtained were compared with the average annual production of treatments to determine the yield per tree per millimeter of notch.

Statistical methods

Analyzes of the variances for all the studied parameters were carried out using the Student-Newman-Keuls statistical test at the risk $\alpha = 5\%$. The statistical software used is Statistical Package for Social Sciences (SPSS).

TPDs, which are proportions, have been transformed into the Arc Sinus TPD Square Root (ASINTPD) to render the distribution normal and to stabilize the variances in order to make statistical analysis possible[15].

III. RESULTS

The data analyzed cover 4 years of observation at the level of the biochemical parameters and the rate of length of dry notch. These are the last two years of downward tapping (years 9 and 10) and the first two years of upward tapping (years 11 and 12). In addition to the data for these four years, the cumulated values of production up to the 12th year have also been analyzed.

Evolution of latex biochemical parameters in downward tapping as a function of Ethephon stimulation frequency

Biochemical parameters of latex in the penultimate year of downward tapping (year 9)

Depending on the Ethephon stimulation frequency, the biochemical parameters of latex in the penultimate year of tapping (year 9 of tapping) are as shown in Table 3. The dry rubber contents (DRC) and inorganic phosphorus of the latex did not vary, irrespective to the stimulation frequency in the 9th year of downward tapping. Conversely, the levels of sucrose and thiol groups show variation as a function of the Ethephon stimulation frequency. Latex sucrose content decrease significantly from the unstimulated treatment to reach these lower values in the treatments stimulated 38 and 78 times per year. As for latex thiol content, it remains statistically identical from the non-stimulated treatment to the stimulated treatment 8 times a year, then decreases significantly to reach its lowest value in treatment stimulated 78 times a year.

The levels of the biochemical parameters allow the 8 treatments to be grouped into 6 classes according to the resemblance of the biochemical profiles numbered D1 to D6. Class D1 groups the non-stimulated treatment. At this level, all biochemical parameters are at their maximum values. Class D2 groups stimulated treatments 2, 4 and 8 times per year. This class differs from the former by the significant decrease in the latex sugar content. At the level of class D3 which is constituted by the treatment stimulated 13 times a year, in addition to the significant reduction of latex sugar content, there is a significant decrease in the thiol content compared with D1 and D2 classes. Class D4 is constituted by treatment stimulated 26 times a year. At the level of this class, the sugar content is significantly lower compared to that of class D3. Class D5 is constituted by treatment stimulated 39 times a year. It has the lowest sugar content with thiol levels significantly lower than that of class D4. Treatment stimulated 78 times per year is class D6. This class, in addition to having low sugar content, has the lowest thiol value.

Biochemical parameters of latex in the last year of downward tapping (year 10)

In the last year of tapping, evolution of latex biochemical parameters is as indicated in Table 4. The dry rubber contents remain statistically equivalent from the non-stimulated treatment to the stimulated treatment 78 times per year. At the level of latex inorganic phosphorus (Pi) content, there was a significant increase in the non-stimulated treatment at the stimulated treatment 4 times a year when it reached its

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maximum value. From 4 to 39 stimulations per year, latex Pi content is statistically equivalent and then decreases significantly in the treatment stimulated 78 times per year. Regarding sugar levels, they remain statistically and significantly higher equivalent to non-stimulated treatment with stimulated treatment 4 times a year. The sugar content then decreases with increasing stimulation frequency to reach its lowest value significantly at the stimulated treatments 39 and 78 times per year. The sugar content at these last two frequencies is statistically equivalent. As for thiols, their concentrations do not statistically vary from non-stimulated treatment to treatment stimulated 4 times a year and show values that are significantly higher. Above 4 stimulations per year, the thiols in the latex decreased significantly to reach their lowest levels of treatment stimulated 78 times per year. The evolution of the thiol content in the latex as a function of the stimulation frequency in the last year of tapping is similar to that of sugars.

The combined analysis of the four parameters allowed the constitution of six classes according to the similarity of the biochemical profiles. Thus, class D'1, represented by the treatment that has not been stimulated has the lowest Pi content with the three other parameters at their statistically highest levels. The class D'2 is constituted by the stimulated treatments 2 and 4 times a year. At the level of this class, the four biochemical parameters have the highest values. The class D’3 regroups the stimulated treatments 8 and 13 times per year. The sugars and thiols are significantly lower compared to the D'1 and D'2 classes. In the case of class D’4 which is represented by the stimulated treatment 26 times a year, it is characterized by a concentration sugar and thiols significantly lower than those of class D’3. Concerning the class D'5 which was stimulated 39 times a year, the sugars and thiols in the latex still fell significantly with reference to D’4. The last class D'6 is constituted by the treatment stimulated 78 times a year. This treatment at a concentration of Pi in its latex is statistically similar to that of the non-stimulated treatment and is significantly lower. Added to this, are the concentrations of sugars and thiols of the latex which are also the lowest.

Evolution of the tapping panel dryness (TPD) rate as a function of the rate of stimulation in downward tapping

During the last two years of downward tapping (year 9 and 10), TPD rate in clone PB 217 (Table 5) does not change linearly. However, the lowest rates are generally observed in the treatment that is not stimulated or stimulated only slightly (2 to 4 stimulations per year). Conversely, the highest rates are those treatments that are most stimulated (39 and 78 times per year).

Evolution of rubber production as a function of the rate of stimulation in downward tapping

Rubber production in the penultimate year of downward tapping (year 9)

Rubber production values in the penultimate year of downdraft are shown in Table 6. Analysis of production in grams per tree per tapping (g/t/t) shows that there is a significant increase of unstimulated treatment at the lowest value to treatments stimulated 8 and 13 times per year. These two treatments have productions that are statistically identical and significantly higher. Above 13 stimulations per year, g/t/t declines significantly until treatment stimulated 78 times per year.

When production is expressed in gram/tree/millimeter of tapping notch (g/t/mmTN), to account for any differences in tree size, it still shows that the growth is significantly higher in non-stimulated low value to treatment stimulated 8 times a year. However, unlike production expressed in g/t/t, it remained maximal from treatment stimulated 8 times per year to treatment stimulated 39 times per year before decreasing significantly in the treatment stimulated 78 times per year. Within this range of stimulation frequency from 8 times to 39 times per year, the production expressed in g/t/mmTN is statistically identical.

For cumulative production over 9 years of downward tapping, there was a significant increase in unstimulated treatment with stimulated treatment 13 times a year, then remained statistically identical until treatment stimulated 39 times a year and decreased significantly at the level of treatment stimulated 78 times a year. Statistically, the value was lower than that of unstimulated treatment.

Rubber production in the last year of downward tapping (year 10)

Production data as a function of the stimulation frequency in the last year of downward tapping are presented in Table 7. They show that the highest productions in g/t/t are those of the stimulated treatments 8 times and 13 times per year. The productions of these two treatments are statistically identical. Below 8 stimulations per year, g/t/t increases significantly as a function of the stimulation frequency. Above 13 stimulations per year, g/t/t decreases significantly as the stimulation frequency increases. The significantly lowest g/t/t is that of non-stimulated and stimulated 78 times per year treatments. These two treatments gave statistically identical g/t/t. Values of g/t/t in the last year of downward tapping are lower than those of the previous year.

When the production estimate is made in g/t/mmTN, it is found that the latter increased significantly from the

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treatment which was not stimulated to reach its maximum values in the stimulated treatments 13 times and 26 times a year. Above 26 stimulations per year, production in g/t/mmTN decreases significantly with increasing stimulation frequency. The production in g/t/mmTN was significantly the lowest in non-stimulated treatment. As in g/t/t, the g/t/mmTNs in the last year of downward tapping are lower than those of the penultimate year.

Yield in grams per tree cumulative (g/t.cum) over the 10 years of downward tapping increased significantly from unstimulated treatment to treatment stimulated 13 times per year. It remained statistically equivalent until treatment stimulated 39 times per year, and then decreased significantly in treatment stimulated 78 times per year. The statistically lowest cumulative yield was that of the treatment that was not stimulated.

Evolution of the biochemical parameters of the latex in upward tapping as a function of the previous one in stimulation with Ethephon in downward tapping

Latex Biochemical parameters in the first year of upward tapping (year 11 of tapping)

Table 8 shows the values of the biochemical parameters in the first year of the upward tapping as a function of the previous one in stimulation in downward tapping. At DRC level, the values are more or less statistically equivalent and significantly lower than the treatment that was not stimulated to the treatment that received 4 stimulations per year in downward tapping. It subsequently increased to its significantly highest value in treatment receiving 26 stimulations per year and then significantly decreased in treatments that were stimulated 39 and 78 times per year in downward tapping. Pi increased in the latex of the treatment that was not stimulated to reach its significantly highest value in the treatment that was stimulated 4 times per year in downward tapping. Then, inversely proportional to the frequency of stimulation received, in downward tapping, it decreases significantly to reach its lowest value in the treatment that was most stimulated in downward tapping (78 times per year). Latex sugar (SUC) content, on the other hand does not evolve according to the stimulation gradient in downward tapping. The maximum value of SUC content is that of the treatment that has not been stimulated and the minimum is that of the treatment which has been stimulated 4 times per year in downward tapping. As for the thiols (R-SH) groups, concentrations in the latex remain statistically equivalent and significantly higher from the previous treatment in stimulation in downward tapping from 0 times to 4 times per year. Beyond this precedent in stimulation in downward tapping, the R-SH content of latex in upward tapping decreases significantly to reach its lowest value in the previous treatment in stimulation in downward tapping 78 times per year.

The combined analysis of the four biochemical parameters makes it possible to group the 8 treatments into 4 classes according to the resemblance of the biochemical profiles. The U1 class includes non-stimulated treatment and stimulated treatments 2 and 4 times a year. In this class, the treatments have the lowest values of DRC and the values of Pi, SUC and R-SH are significantly the highest. Class U2 is constituted by stimulated treatments 8 and 13 times a year. At the level of this class, latex DRC are similar and significantly higher than those of the U1 class. In contrast, the contents of Pi, SUC and R-SH in the latex are significantly lower. At the level of class U3 which is constituted by stimulated treatments 26 and 39 times a year, the Pi and R-SH values are statistically identical and significantly lower compared to those of the U2 group. As for the U4 class, It is represented by treatment stimulated 78 times a year. It is characterized by its Pi and latex R-SH which are the weakest of all treatments.

Biochemical parameters of latex in the second year of upward tapping (year 12)

Latex biochemical parameters in the second year of upward tapping are given in Table 9. Among the 4 biochemical parameters, the DRC did not statistically vary in the second year of upward tapping compare to the precedent in stimulation. PI is statistically equivalent to treatment that was not stimulated compare to treatment stimulated 4 times per year in downward tapping. These values are significantly higher. Beyond the previous one in stimulation, in downward tapping, 4 times a year, the latex Pi content decreases significantly to reach its lowest value at the treatment level which received 78 stimulations per year in downward tapping. Sugars (SUC) in the second year of upward tapping significantly increased treatment that was not stimulated in downward tapping to reach its significantly highest values with treatments stimulated 2 to 13 times per year in downward tapping. Latex SUC content in the second year of upward tapping decreases to its lowest treatment value which has been stimulated 78 times per year in downward tapping. Regarding the latex thiols (R-SH) concentration, it significantly increased from untreated treatment in downward tapping to reach its significantly highest value in the previous treatment in stimulation in downward tapping 4 times a year. Beyond this precedent in stimulation in downward tapping, latex R-SH concentration decreases significantly and in a way inversely proportional to the

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frequency of stimulation received in downward tapping. The lowest value of R-SH in the second year of upward bleeding is that of the treatment which was stimulated 78 times per year in downward tapping. The level of R-SH in latex in the second year of upward tapping is generally low compar to that observed in the first year.

When the 4 biochemical parameters are considered simultaneously depending on the resemblance of the biochemical profiles, the treatments can be grouped into 7 classes. Unstimulated treatment in downward tapping constitutes class U'1. In this class, DRC and Pi are at their maximum values in contrast to SUC and R-SH which have mean values. The class U'2 is represented by the treatment stimulated twice a year in downward tapping. The concentrations of SUC and R-SH in the latex of the trees of this treatment are superior to those of treatment U'1. The treatment stimulated 4 times a year in downward tapping constitutes class U'3. All parameters are at their maximum values at that level. Treatment stimulated 8 times a year in downward tapping with Pi and R-SH contents lower than those of class U'3, constitutes class U'4. At the level of class U'5, constituted by the treatment which was stimulated 13 times per year in downward tapping, the values of Pi, SUC and R-SH are low compare to those of class U'4. The class U'6 groups the treatments stimulated 26 times and 39 times per year in downward tapping. The concentrations of Pi, SUC and R-SH are lower than those of the first five classes. The treatment which received 78 stimulations per year in downward tapping constitutes class U'7. The Pi, SUC and R-SH contents of this class are the lowest of all treatments.

Evolution of TPD rate in upward tapping as a function of the rate of stimulation in downward tapping

TPD rates in the first and second year of upward tapping are presented in Table 10.In the first year of upward tapping the rate of TPD remains statistically equivalent to the treatment stimulated twice a year with treatment stimulated 78 times per year in downward tapping. Only the treatment that was not stimulated in downward tapping has a significantly lower TPD value.

In the second year of upward tapping, the significantly higher rates of TPD were those of treatments that were stimulated 2, 4, 8 and 39 times per year in downward tapping. They have statistically identical TPD rates. The lowest TPD rates are those treated with 13 and 78 stimulations per year in downward tapping. There is no TPD rate gradient linked to the previous one in stimulation in downward tapping.

Evolution of rubber production in upward tapping as a function of the rate of stimulation in downward tapping

Rubber production in the first year of upward tapping

Production values of the different treatments during the first year of upward tapping are shown in Table 11. In g/t/t, the treatment which was not stimulated in downward tapping and the stimulated treatments 2 and 4 times per year in downward tapping have statistically similar and significantly higher productions. Beyond the previous one in stimulation in downward tapping 4 times a year, the g/t/t decreases as the frequency of stimulation applied in downward tapping is high. The statistically lowest g/t/t is that of treatment which was stimulated 78 times per year in downward tapping.

The same evolution is observed when the production is expressed in gram/tree/millimeter of tapping notch (g/t/mmTN). The highest values were those of treatments that were stimulated 0 to 4 times per year in downward tapping. The g/t/mmTN decreased further to its lowest value in the treatment that received 78 stimulations per year in downward tapping.

Cumulative production values over the 11 years of total tapping show that treatments that had a precedent in stimulation in downward tapping 8 to 39 times a year are statistically of the same order and significantly higher than those of the other treatments. The treatment that was not stimulated in downward tapping has cumulative value of production significantly the lowest in spite of the high production observed in upward tapping.

Rubber production in the second year of upward tapping

Table 12 shows the productions in the second year of upward tapping (year 12) at the level of the various treatments.

Expressed in g/t/t, production in the second year of upward tapping increases from non-stimulated treatment in downward tapping to its significantly highest value in treatment that has been stimulated twice a year in downward tapping. It then decreased significantly in inverse proportion to the previous one in stimulation to reach its lowest value in the treatment which was stimulated 78 times a year in downward tapping.

When the production is related to the length of the tapping notch (g/t/mmTN), the previous treatments in stimulation in downward tapping 0 and 2 times per year have statistically similar and significantly higher values. Beyond the previous one of 2 stimulations per year in downward tapping, the g/t/mmTN in upward tapping is the lower of the higher stimulation frequency, although, this decrease is not significant between the previous ones in stimulation of 13 to 39 times a year. The lowest value of g/t/mmTN in the second year of upward tapping was that of the treatment that was stimulated 78 times per year in downward tapping.

Concerning cumulative production, it is the treatment having received 13 stimulations per year in downward tapping which has the statistically highest value over the 12 years of total tapping in downward tapping.

IV. DISCUSSION

The results of the present study show that in PB 217 clone, during the last two years of downward tapping, biochemical parameter DRC does not vary from non-stimulated treatment to stimulated treatment 78 times a year. In contrast, the latex contents in Pi, SUC and R-SH undergo fluctuations according to the frequency of stimulation of the trees. DRC is thus not a good indicator of the effect of stimulation on the metabolism of tree in this clone in downward tapping. On the other hand, the fluctuations of latex Pi, SUC and R-SH contents can be assessed for the metabolic status of the laticiferous cells in relation to the stimulation frequency in clone PB 217. Analysis of the level of its three biochemical parameters coupled with that of production reveals that the eight treatments can be reorganized into six groups. These six groups represent six different metabolic statuses of laticiferous cells[16]. These metabolic statuses are more or less similar to the penultimate year and the last year of downward tapping. The group 1 formed by the unstimulated treatment with the lowest g/t/t with very high SUC and R-SH levels coupled with a low Pi concentration especially in the last year of downward tapping is in a non-activated metabolic status. The 2 and 4 times per year stimulated treatments that make up group 2 are in partially activated metabolic status. At the level of these treatments, the production in g/t/t and the Pi concentration of the latex increased significantly compared to the non-stimulated treatment, while those of the R-SH and the SUC remain always maximal. The optimum of the metabolic is located at the level of group 3 formed by the stimulated treatments 8 and 13 times a year. Indeed, at the level of this group, g/t/t reached its maximum value. The three biochemical parameters significantly decreased without reaching the critical thresholds[7] and TPD rate is not in the ascending phase. From 26 stimulations per year, g/t/t decreases inversely proportional to the stimulation frequency. The R-SH falls below the minimum threshold of 0.5 mM[17] and TPD rate increases linearly. This evolution of production and biochemical parameters shows a gradual decline in the metabolic status from group 4 (treatment stimulated 26 times a year) to reach its final stage in group 6 (treatment stimulated 78 times a year). PB 217 clone therefore needs to be stimulated 13 times per year in the 9th and 10th year of downward tapping to reach its optimum metabolic. Similar results have been reported by Gohet[18]. PB 217 clone has a high tolerance to Ethephon stimulation[19]. Evolution of the metabolic status of the latex cells as a function of the stimulation frequency of PB 217 clone is different from those of PB 260 and GT 1 clones[10,11]. In these two clones, during the last two years of downward tapping, the metabolism of the latex cells is already at its optimum at zero stimulation per year. Stimulant intake during the last two years has a depressive effect on the metabolism of latex cells in these two clones. In PB 271 clone, when the production is expressed in g/t/mmTN, it is observed that it remains statistical maximum up to the stimulated treatment 39 times a year. This confirms the observations of Lacote[12] which show that PB 217 clone can withstand 39 stimulations per year up to 7 years of downward tapping. However, the present study shows that from 13 to 39 stimulations per year, the ceiling of the production expressed in g/t/mmTN shows a depressive effect of the stimulation on the trees growth, as mentioned by Gohet[20]. Analysis of TPD levels indicates that beyond 13 stimulations per year, this rate increases linearly, showing that stimulation becomes harmful to latex production of the trees. It thus appears that the physiological optimum of production of PB 217 clone is reached at 13 stimulations per year in downward tapping.

As a result of downward tapping with different stimulation frequencies, when the trees are tapped upwards with a frequency of stimulation standardized to 13 times per year, in the first year, analysis of biochemical parameters and production makes it possible to group the treatments into four metabolic statuses of the latex cells. Group 1 consists of the previous treatments in stimulation, in descending bleeding, from 0 to 4 times per year. The biochemical parameters in this group are at an optimal level, with respect to the thresholds[21]. The productions in g/t/t and g/t/mmTN of these three treatments are statistically identical and significantly the highest. The metabolic status of the laticiferous cells of the trees of these treatments can be described as good. Group 2 consisting of stimulated treatments in downward tapping 8 and 13 times a year, shows latex cells in a poorer metabolic state with productions in g/t/t and g/t/mmTN, which are significantly down from those in Group 1. However, this metabolic status can be described as fairly good because the level of biochemical parameters is still above the thresholds[21]. Group 3 is formed by treatments that have been stimulated in downward tapping 26 and 39 times a year. Given the level of biochemical parameters close to the threshold values and the production values in g/t/t and g/t/mmTN which are significantly lower compared to group 2, the metabolic status of the latex cells of this group 3 can be characterized as passable. The last group consisting of the previous treatment in stimulation in downward tapping of 78 times per year, has latex Pi and R-SH contents below the threshold[21]. The productions in g/t/t and g/t/mmTN are the lowest. The metabolic status of the latex cells can therefore be described as mediocre. It can be seen that when going from group 1 to group 4, the metabolic status of the latex cells decreases from good to poor. Thus, in the first year of upward tapping, the production potential of PB 217 clone decreased beyond the previous one in stimulation in downward tapping 4 times a year. In the second year of upward tapping, 7 classes of metabolic statuses were established, in connection with the stimulation frequency in downward tapping. Analysis of biochemical parameters and production at these classes shows a decreasing gradient of the latex-producing metabolism from treatment receiving 4 stimulations per year to the treatment which was stimulated 78 times per year in downward tapping. As a result, the tree potential of production in the second year of upward tapping decreases from the treatment that has been stimulated 4 times a year to reach its lowest level in treatment receiving 78 stimulations per year in downward tapping.

These results show that in the BP 217 clone, the stimulation frequencies greater than 4 times per year in downward tapping have a negative impact on the trees production potential in upward tapping. The present study also showed that in cumulative production over 12 years, 10 of which were in downward tapping and 2 years in upward tapping, the treatment stimulated 13 times a year was significantly higher. From an agronomic point of view, the frequency of 13 stimulations per year in downward tapping is therefore the one that best values the PB 2017 clone.

V. CONCLUSION

It is clear from this study that in the BP 217 clone, the frequency of stimulation of the trees in downward tapping has an impact on their production potential in upward tapping. Beyond 4 stimulations per year in downward tapping, the production potential of the trees in upward tapping decreases gradually to reach its lowest level with 78 stimulations per year.

The study also confirmed that in PB 217 clone, optimal production potential in downward tapping is reached when trees are stimulated 13 times per year. It is also this stimulation frequency of 13 times per year that gives the significantly highest cumulative production over the 10 years in downward tapping added to the 2 years in upward tapping. It therefore offers the best compromise between downward and upward tapping, economically.

REFERENCES

[1] DUSOTOIT-COUCAUD (2009) Caractérisations physiologique et moléculaire des transporteurs de sucres et de polyols des cellules laticifères chez *Hevea brasiliensis*, en relation avec la production de latex. Thèse de Doctorat ; Université Blaise Pascal - Clermont-Ferrand II. p 9 ; PP 155.

[2] JACOB J.L., PREVOT J.C., D'AUZAC J. (1983) Augmentation de la production de l'hévéa *Hevea brasiliensis* par l'éthylène = Enhancing the yield of hevea trees through ethylene. Revue Générale des Caoutchoucs et Plastiques 60 (631) : 87-89.

[3] ESCHBACH J.M., LACROTTE R., SERRES E. (1989) Condition which favor the onset of brown bast. In: D'Auzac J, Jacob JL, Chrestin H, Ed. Physiology of rubber tree latex. CRC Press, Inc., ISBN 0-8493-4893-5. Boca Raton Florida, USA: pp 443-454.

[4] GOHET E. (1996) La production de latex par *Hevea brasiliensis*. Relations avec la croissance. Influence de différents facteurs: origine clonale, stimulation hormonale, réserves hydrocarbonées. Thèse de Doctorat; Université de Montpellier-Science et Technique du Languedoc, France, pp 343.

[5] DUAN C. (2011) Etude de l'interaction entre l'éthylène et le jasmonate, hormones impliquées dans la production de caoutchouc naturel chez *Hevea brasiliensis.* Thèse de doctorat: Biologie intégrative des plantes: SupAgro Montpellier, France, pp 166.

[6] CHRESTIN H. (1985) La stimulation à l'Ethrel de l'hévéa : jusqu'où ne pas aller trop loin. Revue Générale Caoutchouc et Plastique 62: 75-78.

[7] JACOB, J.L., SERRES E., PREVOT J.C., LACROTTE R., VIDAL A., ESCHBACH J.M., D'AUZAC J. (1988) Development of Hevea latex diagnosis. Agritrop 12, 97–115.

[8] OKOMA K.M., DIAN K., ALLOU D., SANGARE A. (2009). Etude de la sensibilité des clones d'*Hevea brasiliensis* (Muell. Arg.) à l'encoche sèche. Sciences & Nature 6(1): 17-26.

[9] OKOMA K.M., DIAN K., SOUMAHIN E.F., ELABO A.A., DOUMBIA S., OBOUAYEBA S. AND KELI Z.J. (2016) Agricultural practices in Côte d'Ivoire and apparition and development of tapping panel dryness in *Hevea brasiliensis* muell. Arg. International Journal of Current Agricultural Sciences (IJCAS), Vol. 6, Issue, 7, pp. 74-80, July.

[10] DIAN K., OKOMA M.K., KOFFI E.K., POKOU D.N., KOUASSI M.K., KOUADJO G.Z., KOUASSI N.K., SANGARE A. AND OBOUAYEBA S. (2016a) Effect of Ethephon stimulation frequency in downward tapping on the production potential in upward tapping among PB 260 clone of Hevea brasiliensis in

Ivory Coast. International Journal of Agronomy and Agricultural Research (IJAAR). Vol. 8, No. 2, p. 51-63.

[11] DIAN K., OKOMA M.K., GNAGNE M.Y., GABLA R.O. AND OBOUAYEBA S. (2016) Impact of intensive stimulation with Ethephon in downward tapping on the potential of production in upward tapping in GT 1 clone of Hevea brasiliensis. International Journal of Current Sciences, 19(3): E 35-40.

[12] LACOTE R., GABLA O., OBOUAYEBA S., ESCHBACH J.M., RIVANO F., DIAN K., GOHET E. (2010) Long-term effect of ethylene stimulation on the yield of rubber trees is linked to latex cell biochemistry. Field Crops Research 115: 94-98.

[13] TRAORE M.S., DIARRASSOUBA M., OKOMA K.M., DICK K.E., SOUMAHIN E.F., COULIBALY L.F., OBOUAYEBA S. (2011) Long-term effect of different annual frequencies of ethylene stimulation on rubber productivity of clone GT1 of Hevea brasiliensis (Muell. Arg.) in South-East of Cote d'Ivoire. Agriculture and Biology Journal of North America 2: 1251-1260.

[14] INTERNATIONAL RUBBER RESEARCH AND DEVELOPMENT BOARD, (1995) Manuel of Biochemical and Physiological Tests. Ref. 1995/3.

[15] DAGNELIE P. (1994) Théorie et Méthodes Statistiques. Applications agronomiques Vol. 2.. Ed. Les presses agronomiques de Gembloux, Gembloux, Belgique A.S.B.L. Pp 463.

[16] JACOB J.L., PREVOT J.C., ROUSSEL D., LACROTTE R., SERRES E., D'AUZAC J., ESCHBACH J.M., OMONT H. (1989) Yield limiting factors, latex physiological parameters, latex diagnosis and clonal typology. In: D'Auzac J, Jacob JL, Chrestin H, Ed. Physiology of rubber tree latex. Boca Raton Florida, CRC Press, Inc., 345-382.

[17] DIAN K., SANGARE A., OBOUAYEBA S., BOA D. (1999) Exploitation intensive de quelques clones d'Hevea brasiliensis Müll. ARG. en Côte d'Ivoire. Agronomie Africaine 11(1) : 7-17.

[18] GOHET, E., CHANTUMA, P., LACOTE, R., OBOUAYEBA, S., DIAN, K., CLEMENT-DEMANGE, A., KURNIA, D., ESCHBACH, J.M. (2003) Latex clonal typology of Hevea brasiliensis: physiological modelling of yield potential and clonal response to Ethephon stimulation. In: Vijayakumar, K.R., Thomas, K.U., Rajagopal, R., Karunaichamy, K. (Eds.), Proceedings International Rubber Workshop on Exploitation Physiology, Rubber Research Institute of India, Kottayam, India, December 15–18, 2003, pp. 199–217.

[19] ELABO A.A.E., KOFFI K.E., OKOMA K.M., LIDAH Y.J., N'GUETTA A.S.P,, DIAN K., SANGARÉ A. (2012) Detection of *Hevea brasilensis* clones yield potential and susceptibility to tapping panel dryness in Côte d'Ivoire using the 32 and 35 KDa lutoidic proteins. African Journal of Biotechnology 11(44):pp. 10200-10206.

[20] GOHET, E., PRÉVÔT, J.C., ESCHBACH, J.M., CLÉMENT, A., JACOB, J.L. (1996b) Clone, growth and stimulation: latex production factors. Plant. Rech. Dév. 3, 30–38.

[21] JACOB J.L., PREVÔT J.C., LACROTTE R., ESCHBACH J.M. (1995) Le diagnostic latex. Plant. Rech. Dev. 2: 34- 37.

THE TABLES

Table.1: Treatments (Ethephon stimulation frequency) applied in downward tapping and upward tapping in PB 217 clone of Hevea brasiliensis.

Treatments	Modalities	
	Downward tapping (years 1-10) S/2 d4 6d/7 ET 2.5%	**Upward tapping (year 11-12)** S/4 U d4 6d/7 ET 5 %
A	Not tapped Trees	Not tapped Trees
B	0/Y	13/Y
C	2/Y	13/Y
D	4/Y	13/Y
E	8/Y	13/Y
F	13/Y	13/Y
G	18/Y	13/Y
H	26/Y	13/Y
J	39/Y	13/Y
K	78/Y	13/Y

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S/2 d4 6d/7 ET 2.5%: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon,
S/4 U d4 6d/7 ET 5 % 13/y: quarter spiral upward tapping every 4 day except Sunday stimulated with 5% Ethephon 13 times a year,
0 to 78/Y: number of stimulation per year,

Table.2: Rating of trees with dry notch in Hevea brasiliensis

Non-latex-producing notch length (%)	**Notation**
01 to 20	1
21 to 40	2
41 to 60	3
61 to 80	4
81 to 99	5
100	6

Table.3: Evolution of latex biochemical parameters and metabolic status of latex cells depending on the stimulation frequency at the 9th year downward tapping in PB 217 clone Hevea brasiliensis

Treatments	Latex biochemical parameters				Classification of latex cells Metabolisms
	DRC (%)	Pi (mM)	SAC (mM)	R-SH (mM)	
S/2 d4 6d/7 ET 2.5 % **0/y**	41,3 a	10,5 a	36,2 a	0,65 a	**Group D 1**
S/2 d4 6d/7 ET 2.5 % **2/y**	40,1 a	11,9 a	27,9 b	0,69 a	**Group D 2**
S/2 d4 6d/7 ET 2.5 % **4/y**	39,4 a	12,8 a	25,6 b	0,70 a	
S/2 d4 6d/7 ET 2.5 % **8/y**	40,9 a	13,0 a	25,0 b	0,69 a	
S/2 d4 6d/7 ET 2.5 % **13/y**	42,5 a	12,3 a	22,8 b	0,59 b	**Group D 3**
S/2 d4 6d/7 ET 2.5 % **26/y**	41,9 a	12,6 a	15,3 c	0,59 b	**Group D 4**
S/2 d4 6d/7 ET 2.5 % **39/y**	42,2 a	12,3 a	11,5 d	0,51 c	**Group D 5**
S/2 d4 6d/7 ET 2.5 % **78/y**	40,2 a	11,4 a	13,1 cd	0,41 d	**Group D 6**

S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,
TSC: Total Solid Content, Pi: inorganic Phosphorus, SUC: Sucrose, R-SH: Thiols Groups, mM: millimolar,
a, b, c, d: homogenous group according to the test of Newman and Keuil alfa risk of 5%, y: year,
a > b > c > d,

Table.4: Evolution of latex biochemical parameters and metabolic status of latex cells depending to the stimulation frequency at the last year downward tapping (year 10) in PB 217 clone of Hevea brasiliensis

Treatments	Latex biochemical parameters				Classification of latex cells Metabolisms
	DRC (%)	Pi (mM)	SAC (mM)	R-SH (mM)	
S/2 d4 6d/7 ET 2.5 % **0/y**	43,5 a	12,3 b	22,1 a	0,67 a	**Group D'1**
S/2 d4 6d/7 ET 2.5 % **2/y**	42,3 a	13,4 ab	18,3 a	0,68 a	**Group D'2**
S/2 d4 6d/7 ET 2.5 % **4/y**	42,5 a	15,1 a	19,1 a	0,65 a	
S/2 d4 6d/7 ET 2.5 % **8/y**	42,2 a	14,6 a	14,6 b	0,62 ab	**Group D'3**
S/2 d4 6d/7 ET 2.5 % **13/y**	42,6 a	14,8 a	13,6 b	0,61ab	
S/2 d4 6d/7 ET 2.5 % **26/y**	41,6 a	15,1 a	11,0 bc	0,53 b	**Group D'4**
S/2 d4 6d/7 ET 2.5 % **39/y**	42,5 a	14,0 a	8,5 c	0,45 c	**Group D'5**
S/2 d4 6d/7 ET 2.5 % **78/y**	42,1 a	12,6 b	9,3 c	0,36 d	**Group D'6**

S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78

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times a year,

TSC: Total Solid Content, Pi: inorganic Phosphorus, SUC: Sucrose, R-SH Thiols Groups, mM: millimolar,

a, b, c, d: homogenous group according to the test of Newman and Keuil alfa risk of 5%,

a > b > c > d,

Table.5: Evolution of tapping panel dryness (TPD) percentage in the tow last years downward tapping (years 9 and 10) depending of stimulation frequency in PB 217 clone of Hevea brasiliensis

Treatments	After last year downward tapping (Year 9)		Last year downward tapping (Year 10)	
	TPD (%)	ASNSR(TPD)	TPD (%)	ASNSR(TPD)
S/2 d4 6d/7 ET 2.5 % **0/y**	4,0	0,201 d	4,0	0,201 e
S/2 d4 6d/7 ET 2.5 % **2/y**	3,4	0,185 d	3,4	0,185 e
S/2 d4 6d/7 ET 2.5 % **4/y**	3,3	0,183 d	8,3	0,292 d
S/2 d4 6d/7 ET 2.5 % **8/y**	9,6	0,315 bc	12,4	0,360 c
S/2 d4 6d/7 ET 2.5 % **13/y**	1,6	0,127 e	5,3	0,232 de
S/2 d4 6d/7 ET 2.5 % **26/y**	7,8	0,283 c	14,2	0,386 c
S/2 d4 6d/7 ET 2.5 % **39/y**	12,6	0,363 b	25,0	0,524 b
S/2 d4 6d/7 ET 2.5 % **78/y**	18,9	0,450 a	42,5	0,710 a

S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,

TPD: tapping panel dryness,

ASNSR (TPD): arc sinus square root (TPD),

a, b, c, d, e: homogenous group according to the test of Newman and Keuil alfa risk of 5%,

a > b > c > d > e,

Table.6: Evolution of latex production based on frequency stimulation on after last year downward tapping (Year 9) in PB 217 clone of Hevea brasiliensis

Treatments	Production at after last year downward tapping (Year 9)		
	g/t/t	g/t/mmTN	g/t. cum
S/2 d4 6d/7 ET 2.5 % **0/y**	78,03 c	19,44 c	31712 c
S/2 d4 6d/7 ET 2.5 % **2/y**	90,92 bc	22,58 bc	40115 b
S/2 d4 6d/7 ET 2.5 % **4/y**	100,81 b	26,12 b	42988 b
S/2 d4 6d/7 ET 2.5 % **8/y**	112,14 a	29,15 a	48188 ab
S/2 d4 6d/7 ET 2.5 % **13/y**	117,13 a	32,05 a	52558 a
S/2 d4 6d/7 ET 2.5 % **26/y**	103,84 b	30,44 a	52150 a
S/2 d4 6d/7 ET 2.5 % **39/y**	108,15 ab	30,66 a	55069 a
S/2 d4 6d/7 ET 2.5 % **78/y**	86,63 bc	25,11 b	49719 ab

S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,

g/t/t: gram/tree/tapping,

g/t/mmTN: gram/tree/millimeter of tapping notch

g/t.cum.: gram/tree cumulated year 1 to year 8,

a, b, c: homogenous group according to the test of Newman and Keuil alfa risk of 5%,

a > b > c,

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Table.7: Evolution of latex production based on frequency stimulation in the last year of downward tapping (year 10 of tapping) on PB 217 clone of Hevea brasiliensis

Treatments	Production at last year downward tapping (Year 10)		
	g/t/t	g/t/mmTN	g/t. cum
S/2 d4 6d/7 ET 2.5 % **0/y**	65,32 c	15,72 c	36806 c
S/2 d4 6d/7 ET 2.5 % **2/y**	75,53 b	18,75 bc	46004 bc
S/2 d4 6d/7 ET 2.5 % **4/y**	84,14 ab	21,08 b	49548 b
S/2 d4 6d/7 ET 2.5 % **8/y**	88,31 a	22,65 ab	55075 ab
S/2 d4 6d/7 ET 2.5 % **13/y**	90,62 a	24,25 a	59624 a
S/2 d4 6d/7 ET 2.5 % **26/y**	83,43 ab	23,91 a	58655 a
S/2 d4 6d/7 ET 2.5 % **39/y**	70,62 b	19,6 b	60576 a
S/2 d4 6d/7 ET 2.5 % **78/y**	66,4 c	18,9 bc	54898 ab

S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,
g/t/t: gram/tree/tapping,
g/t/mmTN: gram/tree/millimeter of tapping notch
g/t.cum.: gram/tree cumulated,
a, b, c: homogenous group according to the test of Newman and Keuil alfa risk of 5%,
a > b > c,

Table.8: Evolution of latex biochemical parameters and metabolic status of latex cells in 1st upward tapping year (year 11 of total tapping) depending on the downward tapping frequency stimulation in PB 217 clone of Hevea brasiliensis

Treatments		**Latex biochemical parameters**				**Classification of latex cells Metabolisms**
1st year Upward tapping	**Previous Downward tapping**	**DRC (%)**	**Pi (mM)**	**SAC (mM)**	**R-SH (mM)**	
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **0/y**	45,4 bc	12,9 ab	27,0 a	0,74 a	**Group U1**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **2/y**	43,2 c	11,4 abc	24,6 abc	0,70 a	
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **4/y**	43,8 c	13,3 a	25,0 ab	0,68 a	
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **8/y**	46,4 abc	9,5 cde	20,1 c	0,57 b	**Group U2**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **13/y**	47,9 abc	10,7 bcd	23,4 abc	0,58 b	
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **26/y**	50,4 a	10,3 cd	21,7 bc	0,51 c	**Group U3**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **39/y**	47,7 abc	9,2 cd	23,4 abc	0,51 c	
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **78/y**	48,7 ab	7,5 d	21,6 bc	0,44 d	**Group U4**

S/4 U d4 6d/7 ET 5 % 13/y: quarter spiral upward tapping every 4 day except Sunday stimulated with 5% Ethephon 13 times a year,
S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,
TSC: Total Solid Content, Pi: inorganic Phosphorus, SUC: Sucrose, R-SH: Thiols Groups, mM: millimolar.
a, b, c, d, e: homogenous group according to the test of Newman and Keuil alfa risk of 5%,
a>b>c>d > e,

Table.9: Evolution of latex biochemical parameters and metabolic status of latex cells in 2nd upward tapping year (year 12 of total tapping) depending on the downward tapping frequency stimulation in PB 217 clone of Hevea brasiliensis

Treatments		Latex biochemical parameters				Classification of latex cells Metabolisms
2nd year Upward tapping	**Previous Downward tapping**	**DRC (%)**	**Pi (mM)**	**SAC (mM)**	**R-SH (mM)**	
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **0/y**	44,4 a	15,6 a	21,4 bc	0,41 b	**Group U'1**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **2/y**	44,0 a	14,8 ab	25,7 a	0,44 ab	**Group U'2**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **4/y**	43,2 a	15,8 a	25,7 a	0,46 a	**Group U'3**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **8/y**	42,1 a	13,0 bc	25,7 a	0,41 b	**Group U'4**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **13/y**	43,9 a	12,6 bcd	23,6 ab	0,39 bc	**Group U'5**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **26/y**	45,4 a	11,2 cd	22,7 b	0,36 c	**Group U'6**
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **39/y**	45,1 a	11,2 cd	21,4 bc	0,36 c	
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **78/y**	44,9 a	10,4 d	18,7 c	0,31 d	**Group U'7**

S/4 U d4 6d/7 ET 5 % 13/y: quarter spiral upward tapping every 4 day except Sunday, stimulated with 5% Ethephon 13 times a year,
S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,
TSC: Total Solid Content, Pi: inorganic Phosphorus, SUC: Sucrose, R-SH: Thiols Groups, mM: millimolar,
a, b, c, d: homogenous group according to the test of Newman and Keuil alfa risk of 5%,
a>b>c>d,

Table.10: Evolution of tapping panel dryness (TPD) percentage in the tow first years upward tapping (year 11 and 12 of total tapping) depending on the downward tapping stimulation frequency in PB 217 clone of Hevea brasiliensis

Treatments		1st year Upward tapping		2nd year Upward tapping	
Upward tapping	Previous Downward tapping	TPD (%)	ASNSR(TPD)	TPD (%)	ASNSR(TPD)
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **0/y**	1,0	0,100 b	2,0	0,142 b
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **2/y**	3,3	0,183 ab	7,0	0,268 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **4/y**	6,3	0,254 a	10,4	0,328 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **8/y**	4,0	0,201 a	8,7	0,299 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **13/y**	2,8	0,168 ab	1,0	0,100 c
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **26/y**	2,6	0,162 ab	3,7	0,194 b
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **39/y**	4,9	0,223 a	8,7	0,299 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **78/y**	5,4	0,235 a	1,1	0,105 c

S/4 U d4 6d/7 ET 5 % 13/y: quarter spiral upward tapping every 4 day except Sunday, stimulated with 5% Ethephon 13 times a year,
S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,
TPD: tapping panel dryness,
ASNSR (TPD): arc sinus square root (TPD),
a, b, c: homogenous group according to the test of Newman and Keuil alfa risk of 5%,
a > b > c,

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Table.11: Evolution of latex production in the 1st upward tapping year (year 11 of total tapping) based on downward tapping frequency stimulation in PB 217 clone of Hevea brasiliensis

Treatments		Productions		
1st year Upward tapping	Previous Downward tapping	g/t/t	g/t/mmTN	g/t. cum year 1 to 11
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **0/y**	121,20 a	30.92 a	46938,5 c
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **2/y**	116,93 a	30.88 a	56468,6 b
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **4/y**	117,21 a	31.06 a	60870,5 ab
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **8/y**	105,78 ab	28.21 b	65160,6 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **13/y**	098,65 b	28.18 b	67943,3 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **26/y**	074,18 c	22.49 c	65820,8 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **39/y**	068,08 c	20.00 cd	66958,7 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **78/y**	060,40 d	18.31 d	63523,4 ab

S/4 U d4 6d/7 ET 5 % 13/y: quarter spiral upward tapping every 4 day except Sunday, stimulated with 5% Ethephon 13 times a year,
S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,
g/t/t: gram/tree/tapping,
g/t/mmTN: gram/tree/millimeter of tapping notch,
g/t.cum.: gram/tree cumulated,
a, b, c, d: homogenous group according to the test of Newman and Keuil alfa risk of 5%,
a > b > c > d,

Table.12: Evolution of latex production in the 2nd upward tapping year (year 12 of total tapping) based on downward tapping frequency stimulation in PB 217 clone of Hevea brasiliensis

Treatments		Productions		
2nd year Upward tapping	Previous Downward tapping	g/t/t	g/t/mmTN	g/t. cum year 1 to 12
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **0/y**	136.57 ab	35.16 a	57534.3 c
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **2/y**	141.99 a	36.89 a	68555.2 b
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **4/y**	131.37 b	33.90 ab	72190.3 ab
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **8/y**	120.50 c	33.43 b	75620.8 ab
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **13/y**	111.27 d	29.48 c	78043.6 a
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **26/y**	096.07 e	28.58 c	73455.7 ab
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **39/y**	096.13 e	27.88 c	75082.5 ab
S/4 U d4 6d/7 ET 5 % **13/y**	S/2 d4 6d/7 ET 2.5 % **78/y**	083.88 f	23.94 d	70528.2 b

S/4 U d4 6d/7 ET 5 % 13/y: quarter spiral upward tapping every 4 day except Sunday, stimulated with 5% Ethephon 13 times a year,
S/2 d4 6d/7 ET 2.5% 0-78/y: half spiral downward tapping every 4 days except Sunday stimulated with 2.5% Ethephon 0 to 78 times a year,
g/t/t: gram/tree/tapping,
g/t/mmTN: gram/tree/millimeter of tapping notch,
g/t.cum.: gram/tree cumulated,
a, b, c, d, e, f: homogenous group according to the test of Newman and Keuil alfa risk of 5%,
a > b > c > d > e > f,

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Competence of Biopesticide and Neem in Agriculture

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Abstract— *Neem plant is considered as the most useful traditional plant in India. The various properties of different parts of neem tree are used as insecticide, antifeedant, hormonal, antifungal, antiallergic, antidermatic, anti-inflammatory, antiscabic larvicidal and spermaticidal activity etc. In recent era the major challenge is to increase the food production without harming the environment and can control the pest. Since, last decades pesticides have become an integral component in sustainable agriculture and the modern day cultivation practices uses of chemical pesticides and fertilizers are in eliminable. Enormous use of different chemical pesticides in agricultural fields is resistant to most of the pests. So that the natural pesticides from A. indica are considered to be less harmful, biodegradability, least persistence, lest toxic to non-target organism, economic and used to combat insects, pests are not ruled out. Neem derived products play an essential role in the pest management, in agricultural fields such as crop and stored grains. Fruitful results of application of formulated neem based products in agriculture field will provide a cost effective technology to the farmers.*

Keywords— Bio fertilizer, Biopesticides, Neem, Soil fertility.

I. INTRODUCTION

Agriculture is a significant sector for food, fibres, bio-fuels & other products for the wellbeing of human life. In the context of the growing Indian population, apart from fulfilling the demands of food, agriculture plays vital role in improving the socio economic status of country. In the other hand, with the increase in human population along with the scarcity of land, people are using chemical fertilizers and pesticides for enhancing food productivity and crop yielding capacity which are the primary causes for the reduction in soil fertility and degradation [Liu *et al.*, 2004].Neem plant namely *Azadirachta indica* commonly found in Indian subcontinents and belongs to family *Meliceae*. This tree grows rapidly and can reach a height of 15-20 meters in tropic and sub tropic climate, and also survive in dry and arid conditions. The barks of neem plant contain 14% of tannin; it is also strong in course fiber which is commonly used in ropes. In India 80% of neem oil is used in manufacturing of soaps. One of the most popular dishes namely *veppampoo charu* obtained from the shoots and flowers of neem are commonly used in Tamilnadu. The neem plant is used in medical treatment, and its oil is used in insecticides, lubricants, pesticides, and in variety of diseases such as diabetes and tuberculosis. The environment is adversely affected by the use of chemical fertilizer and pesticide like water hardness, development of insect resistance, genetic variation in plant, increase in the toxic level in food chain and other human health issues like skin cancer. These also cause water pollution due to the accumulation of nitrates by the breakdown of nitrogen fertilizer. Applying high level of chemical fertilizers & pesticides on agriculture can gradually reduce the growth of beneficial microorganism in soil. To make an eco-friendly plant protection field through an integrated pest management (IPM), it is necessary to introduce the substitutes of chemical fertilizers and pesticides called bio fertilizer and bio pesticide which are the inoculants of soil microorganism, which enrich the soil nutrient level & establish the symbiotic relationship with plants by producing nitrogen & phosphorous nutrient in the soil and harnessing the foresaid challenges [Dutta, 2015]. Bio-fertilizers are the substances which contain bio-organisms which colonize and supports to promote the growth of plants by supplying the primary nutrient. They have the ability to synthesize important nutritional element from non-usable to usable form. The study says that the application of bio-fertilizer to the seed or soil improves the yield by 10-20 % without harming the soil & environment [Roychowdhury *et al*., 2014].

II. PESTICIDES

Pesticides are toxic chemical compounds are used in agriculture, horticulture, olericulture, floriculture, to kill the pests, improve the quality of plant and increase the crop yield. Pesticides are used in public health to kill the vector of diseases such as mosquito and in agriculture to kill pests which cause crop damage. Ideal pesticides must be lethal to targeted pest but nontoxic to non-target species including man. There are two outcomes from the use of pesticide on agriculture field both beneficiaries as well as hazardous impact. Benefits of pesticide include improvement of productivity, protection of crop losses,

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and vector disease control improved quality of food [Aktar *et al.*, 2009]. Pesticides can be up two types based on the presence of active ingredient, such as Synthetic pesticides and Bio pesticides:

2.1. Synthetic Pesticides:

The synthetic are placed in different groups like Organ chlorines, Organophosphates, Phenoxies, Triazines and Thiocarbamates etc. According to different survey in European nation, some pesticides have hazardous impact on food stock, soil, water level, environment and finally human. It has been reported that pesticides Acephate, chlorpyriphos, methylated chloropyriphos, Methamidophos, Iprodione, Procymidone, Chlorothalonil, Benomyl and Moneb group have dangerous effect on fruits like apple, strawberries and grapes. Every sample contained some amount of residue of these applied pesticides [Aktar *et al.,* 2009].These pesticides are unavoidable imputes in our ecosystem and olericulture. India used maximum amount of synthetic pesticides in comparison to world. In India, about 76% insecticides are used whereas about 44% of insecticides are used in worldwide [Mathur *et al*., 1999].

2.2 Biopesticide: An excellent alternative of chemical pesticide

As we know pesticides are the toxic substances that kill the weeds, insects, fungus, and rodents. It is also used to manage the pest problem around the world [Lewis *et al.*, 1997]. Pesticides show a extensive range of human health risks from temporary to permanent impact. Among these conditions, acute conditions include nerve dysfunction, skin and eye irritation and long term chronic effects lead to cancer. In comparison to pesticides, bio pesticide are an eco-friendly nontoxic natural type of pesticides, derived from natural substances like plants, bacteria & minerals which has the capacity to control pests without affecting the environment. It was reported in 2014 that about 430 bio pesticides and 1320 active products has been registered for use in agricultural land [Roychowdhury *et al.,* 2014]. Bio pesticides have the properties like target pest specific and their close relatives, harmless to human beings and other beneficial organisms, and never cause pollution [Rabindra, 2005; Thakore, 2006]. So it is a major component of IPM (Integrated pest management) programme. According to the presence of different key components, bio pesticides are broadly classified as microbial bio pesticide (which include bacterial, fungal, viral), biochemical bio pesticide which include insect pheromone, different components of plant, growth regulatory hormone and the others are the formulated product of bio pesticide [Vinod *et al.,* 2015]. The challenges for bio pesticide industries are to develop a fruitful, safe and multifacted eco-friendly products [Mukhopadhay *et al.,* 1992; Kalra and Khanuja, 2007; Prabhat *et al.,* 2014].

2.2.1 Microbial bio pesticide

The active components in microbial bio pesticide are naturally evolved bacteria or genetically modified bacteria, fungi, viruses and protozoans. About 74% bacterial bio-pesticides, 10% of fungal pesticides, 5% of viral bio-pesticides and others include about 3% for all type of crops [Thakore, 2006]. Till now about 72 components of micro-organisms have been registered by USEPA (United States Environmental Protection Agency) which include 35 bacterial products, 15 fungal, 8 plants incorporated protectants (PIPs), 6 nonviable microbial bio-pesticides, 1 protozoan, 1yeast and 6 viruses.

2.2.2 Biochemical pesticide

Biochemical pesticides are different from the conventional pesticide. The key components evolve naturally, a mixture comprising essential oil, insect pheromone etc. *[Gelernter, 2006]*. The hormone released from insects to communicate other members of same species is called as insect pheromones (semiochemicals). These chemicals are the type of decoder produced by the living organism or synthetic analogues which cause behavioural responses in the same or other species. The pheromones are used in pest management without killing the target pest but by attracting the pest towards the lethal pesticide or by initiating mating disruption. As it monitors the larger group of pest, it is the part of IPM system [Thakore, 2006]. The mixture of chemical component derived from plants and used as bio-pesticide are more diverse with respect to target pest, mode of action and their composition [Kovach *et al.,* 2012]. Different plant products have different modes of action on target organism: the fragrance of some plant extracts attract the toxic insects but other used as deterrent. Lemongrass oil, cause the degradation of some weeds. Some products cause suffocation while some other products increase the immune system of crops naturally. The important components for the proper growth of plants are plant hormones & growth regulators that accelerate or retard the growth of plant naturally. Along with bacterial bio-pesticide, fungal bio-pesticide, insect pheromone and some growth regulators of insect also alter the growth & development of insects, along with this some insecticides having juvenile hormone are also used [Vinod *et al.,* 2015]. Some chitin synthesis inhibitors are used as insecticide which decreases the ability of insect for further synthesis of new exoskeleton [Karen *et al.,* 2009].

2.2.3 Formulated Bio pesticides

The key components of formulated bio-pesticides are all the microbes and plant products. In case of bio-pesticide processing, some problems are related to presence of other inert ingredients along with the active ingredients.

Different survey have been carried out to study the particular bio-pesticidal effects on pests in various agricultural crops [Rahman & Motoyama, 2000] such as repellant as well as insecticidal effect from entire garlic clove, its rubbed product and buoyant extract of garlic on two stored products, maize weevil (*Sitophilus zeamais)* and red flour beetle (*Tribolium casteneum).* Alliin highly reactive and unstable volatile compound present in garlic is converted to allicin, pyruvate and ammonia by the activity of an enzyme called allinase when garlic is chopped [Salas, 2001]. These allicin have the aromatic property which repels the target pest, but the mechanism is unsure. From some studies it has been observed that some plant material like leaves of neem, pongum, citrus, nocli, tulsi, fly ash, castor oil, red earth are strongly effective on the survival of *C. Maculates* flourishing the green gram [Dhakshinamoorthy and Selvanarayana., 2002]. Different doses of neem (*Azadirachta indica),* Bergera *(Bergera koenigii),* akk *(Calotropis gigentea),* ipoma *(Ipoma reptans),* chilli *(Capsicum amanda),* and mustard *(Brassica campestris)* have the strong effects against *sitophilis oryzae* [Petel *et al.,* 2004]. Repulsive activities of some essential oil of aromatic plants were also analyzed by GC-MS (gas chromatography – mass spectrometry) against *sitophilus zeamais (Coleoptera: curculinonidae)* [Nerio *et al.,* 2009].

2.2.4 Neem in Agriculture

Neem is considered as renewable resource with the potential in solving agricultural, environmental and public health problems. The common properties of neem are its non-toxicity; so they are beneficial in plant conservation and management. Neem derived products such as neem fruit, neem oil, neem seed cake and leaf extract used as bio-pesticide, fungicide and organic manure has been applied because of their different mode of action to control the insects, plant pathogen, pest which have the resistance against chemical pesticides. Studies say that neem can regulate around 300 species of insects. The most common form of neem used as pesticide in organic agriculture are the emulsifiable concentrate (EC) due to its biodegradable nature as well as it is easily mixes with synthetic pesticides and enhances their action. Now a day's dried neem extract is mixed with the stored grain, to prevent further proliferation of storage pest and even the insects stop feeding on them due to its anti-feeding property like, the most popular bio-pesticide Neem seed kernel extract (NSKE 5%) which is composed of well dried form of neem seed kernel, distilled water, detergent and neem oil (multineem) are used against chick pea pod borer *Helicoverpa armigera* [Bhatt and Patel, 2001]. Neem products intercede at various phases of the insect's life. The main function is to paralyze the activity of pests instead of wiping out the pests. Due to the powerful insect growth regulatory (IGR) activity of different parts of neem, they are now recognised as more covetable product than a rapid clash synthetic pesticide in integrated pest management programmes. Not only Neem obeys all precedences among environmental objectives, this particular plant is also perhaps the utmost symbolic example of how nature can blend divergent activities *i.e.,* the action of de-oiled Neem cake as a pesticide cum fertilizer.

2.2.5 Neem as an Insecticide

Neem trees are invincible produce various defensive chemicals during pest attack which act as repellent, antifeedant, ovipositional deterrent, growth inhibitor, toxicant [Saxena *et al.,* 1989].There are several application of different parts of neem tree in the field of ayurvedic medicine, as effective dentifrice *[Larson, 1989]*, neem cake (remained part after oil extraction) as an effective fertilizer and animal feed. Azadirachtin (tetranortritterpenoid) one of the major active components which is the most potent natural insect antifeedant [Isman *et al.,* 1990]. It is a mixture of seven isomers named as Azadirachtin A-G. Among all, isomer E is most effective against insect growth regulator. Azadirachtin is highly unstable and stored at -40ºC are not applied directly to the field. Oil based extracts stabilize these biologically active compounds. So the commercial azadirachtin preparation is more advantageous over pure azadirachtin [Isman *et al.,* 1990a].

Neem extract has been used in over 195 insect species and some rebellious insects are also controlled by the neem extract application [Salma et al., 2011]. Formulated neem product has significant effect against eggs of peach fruit fly (*Bactrocera zonata)* but has no effect on good insects like pollinator insects, bees and other useful organism [Salma et al., 2011].The insecticidal effect of Botanical toxin (*Bacillus thuringiensis)* and agricultural insecticide (*Azadirachta indica* and *Vitex negundo)* on the lactate dehydrogenase enzymatic activity in rice leaf folder called as Guenee (*Cnaphalocrocis medinalis)* was studied [Nathen *et al.*, 2006]. The combined effect of both bacteria and insecticides even in low concentration decreases the LDH activity strongly. This indicates that combinational product is an efficient enzyme inhibitor [Kumar, 2015].

Some of the studies are done by using aqueous form of neem seed powder on the survival, improvement and growth of Aphid (*Aphis gussypii)* Glover which is the most perinicious pest on cotton causing direct or indirect loss [Chinaberry and Jehu, 2001].This aphid normally live superficially on leaves, feed the phloem part and causes around 50 types of diseases in plant. Biological insecticides are used against these aphids instead of using aphid resistant chemical pesticide [Weathersbee, 1997; Kabissa *et al.,* 1996; Mann *et al.,* 2001]. The negative effects of different concentration of azadirachtin on different parameters like development & survival of nymph, number of molts of aphids *A. gossypii* has been checked [Stark and Rangus, 1994]. It was found that the fertility of various aphids like *A.pisum* on faba bean [Stark and Rangus, 1994; Stark and Wennergren, 1995] and *Myzus persicae* on sweet pepper were reduced at the highest concentration of neem extract. Neem extracts show high mortality rate, decreasing fertility, growth inhibitory activity on more than 400 insect species from different orders like Diptera, Hymenoptera, Coleoptera, Lepidoptera, orthoptera and Hemiptera etc [Ragsdale *et al.,* 2004; Liu *et al.,* 2004]. As neem extract is efficient to control several aphids in the field, so these are the most significant product to be used as biological pesticide.

The neem product Azadirachtin affect the activity of enzyme ecdysone by suppressing the moulting or ecdysis process of insects by entering into insect's larva through feeding and restrict the passage of larva to pupal stage, thus leading to death. The different parameter like survivorship, development, fecundity of soybean aphid (*Aphis glycine)* and its predator named *Harmonia axyridis* are also are affected by the action of neem seed oil and azadirachtin. Azadirachtin& neem seed oil are two key components which have been proven most fruitful agent, against the aphids like Brown citrus aphid, *Toxoptera citricida* (Kirk lady) by increasing in nymph as well as adult mortality and decreasing adult fecundity in all trial concentration [Tang *et al.,* 2002]. Along with this, neem seed oil has some muffling effect on pea aphid, *Acyrthosiphon pisum* (*Harris*) nymphal mortality *[Stark and Walter, 1995]*. Based on the aphid mortality neem oil and its processed product are more efficient than azadiractin [Adan *et al.,* 1998].

2.2.6 Neem as an ovipositional deterrent

Neem (*Azadiracht indica*, A.Juss, Meliaceae) as a plant based pesticides [Elisinary and Rizk, 2002] has an ovipositional deterrent activity on some pest like *Bactrocera carambolae* (fruit fly) which decreases economic value of fruits and vegetables. It is also used against the cabbage moth, *Mamestra brassicae* [Joger *et al.,* 2009], peach fruit-fly (*B. Zonata)* [Mohamoud and Shoeib, 2008] and potato tuber moth, *phthorimaea opercullela* [Elsinary and Rizk, 2002]. It has been found that neem leaves contain more polar components and the most dominant component found in n-hexane extract of neem is nonacosane, which is made up of a group of saturated fatty acid having the negative impact on egg as well as egg producing capacity and durability of larvae of *Stephanities pyriodes* [*Wang et al.,* 1999]. Nonacosane attracts different parasitoid like *Trichogramma chilonis* [Ananthakrishnan *et al.,* 1991], *T. Briasiliensis* and T. exiguum [Kumar *et al.,* 2011] and *Cotesia plutellae* [Kumar *et al.,* 2012; *Seenivasagan* and Paul, 2011].

2.2.7 Neem as Biofungicide

Neem oil is used as fungicide, to prevent the germination of fungal spores. The neem based products are used as natural biofungicide in the branch and collar canker disease caused by tea plant pathogen. The extracts of neem have a suppressive effect on the fungal pathogen like *Poriamonticolad* infecting wood [Dhyani *et al.,* 2004], *Pyricularia oryzae* infecting rice plant [Amadioha, 2000]. Components like Azadirachtin, nimbin , nimbidin and also some commercially available neem product like Achook, Bioneem, Nimbecidine, Neemark have the antifungal property against some pathogen like *Fusarium oxysporium*, *Altanaria solani*, *Curvulata lunata*, *Helminthosporium* sp. and *Sclerotium rolfsii* [Bonder et al., 1999]. Azadirachtin has significant inhibitory effect on the plant pathogenic fungi and acts as a most advance fungicide than Bavistin and mancozeb, the synthetic fungicide [Dubey and Kumar, 2009]. Beside this, neem oil also has the fungicidal property on some fungi like *Botryiscinera*.and *Glomerella cingulata* [Hirose et al., 2001]. Among the commercially available neem products, Nimbecidine is the oil based pesticide where the active ingredients are azadirachtin and other components like meliantrol, salnin, nimbin are used against the worms, weevils and wire worms of tomatoes [Schumutterer, 1990]. Another component of neem is Trilogy which is a clarified hydrophobic extract of neem oil, has multiple properties like insecticide, acaricide and fungicidal effect against the mildews on cucurbits [Meister, 1999]. It also acts as a growth regulatory agent and represses the growth of pathogen like *Podosphaera xanthii* on cucumber leaves [Aboellil, 2007]. Trilogy is more effective in bacteria like *Pseudomonas xanthii* at very low concentration as reported by [Polioakidou, 2005]. The growth inhibitory effect of nimbecidine is more than that of Trilogy in their higher concentration. Nimbecidine has an adverse effect on the growth parameter of *P. theae* and can be used by the farmers in the management of pest [Okigbo and Nmeka, 2005].

III. CONCLUSION

Now there is a need of cost effective, biodegradable, eco-friendly and potential, soft pesticide in agriculture field. As neem act as the most reliable source of pro-pesticide having no ill effect on human and animals, on agricultural product. So neem is used as organic fertilizer and pesticide for sustainable crop production. Neem based products play a crucial role in pest management, which acts across different pests of both crop field as well as stored grains like rice, wheat, corn, legumes, potato, tomato *etc*.

REFERENCES

[1] Aboellil, A.H. (2007). Trilogy, a product of Neem (Azadirachta indica), induces resistance in cucumber against (Podosphaeraxanthii). Reserve Journal of Microbiology, 2(5): 402-414.

[2] Adan, A., Soria, J., DelEstal, P., Sanchez-Brunete Cand Vinuela, E. (1998). Acci ´on deferencial de dos formulaciones de azadiractina sobrelos estados de desarrollo de Ceratitis capitata (Wiedemann) (Dipetera: Tephritidae). Boletín de sanidad vegetal. Plagas, 24:1009-1018.

[3] Ahmed, S. and Grainge, M. (1986). Potential of the neem tree (Azadirachta indica) for pest control and rural development. Economy Botany, 40: 201-209.

[4] Amadioha A.C. (2000). Controlling rice blast in vitro and in vivo with extracts of Azadirachta indica, Crop Protection, 19: 287-290.

[5] Ananthakrishnan, T.N., Senrayan, R., Murugesan, S. and Annadurai, R.S. (1991).Kairomones of Heliothisarmigeraand Corcyra cephalonicaand their influence on the parasitic potential of Trichogrammachilonis (Trichogrammatidae: Hymenoptera). Journal of CommunicationsIssue, 1: 23-24.

[6] Bhatt, N.J. and Patel, R.K. (2001). Screening of chickpea cultivars for their resistance to gram pod borer, Helicoverpaarmigera. Indian Journal of Entomology, 63(3): 277 – 280.

[7] Bhonde, S.B., Deshpande, S.G. and Sharma, R.N. (1999). In vitro evaluation on inhibitory nature of some neem formulations against plant pathogenic fungi. Hindustan Antibiotic Bulletin 4:22-24.

[8] Dhakshinamoorthy, G. and Selvanarayana, V. (2002). Evaluation of certain natural products against pulse beetle, Callosobruchus maculates F. infesting stored green gram. Insect Environment, 8(1): 29-30.

[9] Dhyani, S., Tripathi, S. and Inder, D. (2004). Preliminary screening of neem (Azadirachta indica) leaf extractives against Poriamonticolad-a wood destroying fungus. Journal of Indian Academics Wood Science, 1-2: 103-112.

[10] Dubey, N.K. and Kumar, A. (2009). Exploitation of natural products in eco-friendly management of plant pests. Business media B.V., 181-198.

[11] Dutta, S. (2015). Biopesticides: An Ecofriendly approach for pest control. World journal of pharmacy and pharmaceutical sciences, 4(6): 250-265.

[12] El-Sinary, N.H. and Rizk, S.A. (2002). Oviposition deterrence and other biological influences of aqueous leaves extracts of neem, colocasia and their mixtures alone or combined with gamma radiation to reduce the risk of the potato tuber moth, Phthorimaeaoperculella (Zeller). Pakistan Journal of Biological Science5 (9): 911-914.

[13] Gelernter, W. (2006). Biopesticides: visions vs. reality. From Presentation: 2006 (American Phytopathological Society Meeting). Quebec City, Quebec, Canada.

[14] Heidi, K. and Eileen, M.C. (2008). Insect growth regulator effect of azadirachtin and neem oil on survivorship, development and fecundity of Aphis glycines (Hemiptera: Aphididae) and its predator Harmoniaaxyridis (coleoptera: Coccinellidae). Pest Management science, society of chemical industry. Archived at http://orgprints.org/24349, pp. 660-668.

[15] Hirose, E., Neves, P.M.O., Zequl, J.A.C., Martins, L.H., Peralta, C.H. and Moino, A.J. (2000). Effects of biofertilizers and neem oil on the pathogenic fungi Beauvariabassiana (bals) vuill and Metarhiziumanisopliae (metsch.) sorok, Brazilian Archives of Biology and Technology, 44: 419-423.

[16] Isman, M.B., Koul, O., Luczynski, A. and Kaminski, J. (1990a). Insecticidal and antifeedant bioactivities of neem oil and their relationship to azadirachtin content. Journal of Agriculture and Food chemistry, 38: 1406-1411.

[17] Jogar, K., Metspalu, L., Hiiesaar, K., Loorits, L., Ploomi, A., Kiosk, A. and Luik, A. (2009). Influence of neem Azalea-T/S on Mamestra brassicae L. SodininkysteIr Darzininkyste., 28 (3): 85-92.

[18] Jonah, K.B. and Maingi, J. (2015). Invitro response of Phomopsistheae to the products of Azadirachta indica and extracts of Warburglaugandensis, unpublished.

[19] Kabissa, J.C.B. Kayumbo, H.Y. and Yapro, J.G. (1996). Seasonal abundance of chrysopids (Neuroptera: Chrysopidae) preying Helicoverpaarmigera (Hubner) (Lepidoptera: Noctuidae) and Aphis gossypii (Glover) (Homoptera: Aphididae) on cotton in eastern Tanzania. Crop Protection, 15: 5-8.

[20] Karen Peabody, O‟Brien, K.P., Franjevic, S. and Jones, J. (2009). Green chemistry and sustainable agriculture the role of bio-pesticide, pp 50.

[21] Kovach, J., Petzoldt, C., Degni, J. and Tette, J. (1992). A method to measure the environmental impact of pesticides. Newyork's food and lifesciences bulletin, Cornell University, Ithaca, 139: 0362-0069.

[22] Krischik, V. and Davidson, J. (2007). Integrated pest management of northwest Landscapes. Minneapolis

(MN): Regents of the University of Minnesota, 1(23-24): 315.

[23] Kumar, A., Paul, A.V.N., Zayeem, A. and Singh, A.K. (2011). The effect of leaf extracts of rice varieties on foraging behavior of Trichogrammabras iliensis and Trichogramma exiguum. Iranian Journal of Entomology,1: 1-7.

[24] Kumar, A., Zayeem, A. and Kanameni, S. (2012). Synomonal effect of cole crops on individual and associative learning behaviour of Cotesiaplutellae. International Journal of Biology, Pharmacy and Allied Sciences 1: 285-298.

[25] Larson, R.O. (1989). The commercialization of neem, In M. Jacobson [ed.], Focus on phytochemical pesticides, The neem tree. CRC press, Boca Raton, FL., Vol.1. pp. 155-168.

[26] Lavender, D.P., Parish, R. Johnson, C.M. Montgomery, G. Vyse, A. Willis, R.A. and Winston, D. [eds.], (1990). Regenerating British Columbia's Forests. University of British Columbia Press, Vancouver, B.C.

[27] Lewis, W., van Lenteren, C., Phatak, S. and Tumlinsen, J. (1997). A total system approach to sustainable pest management. Proceedings of the National Academy of Science USA, 94: 12243-12248.

[28] Liu, J., Wu, K., Hopper, K. and Zhao K. (2004). Population dynamics of Aphis glycines (Homoptera: Aphididae) and its natural enemies in soybean in Northern China. Annals of the Entomological Society of America, 97: 235-239.

[29] Mahmoud, M.F. and Shoeib, M.A. (2008). Sterilant and ovipositor deterrent activity of neem formulation on peach fruit fly Bactrocerazonata (Saunders) (Diptera: Tephritidae). Journal of Biopesticides, 1: 177-181.

[30] Mann, G.S., Dhaliwal, G.S. and Dhawan, A.K. (2001). Field efficacy of neem based insecticides against whitefly and their impact on insect pest complex of cotton. Pesticide Research Journal, 13: 79-81.

[31] Marian, J. and Sawchuk. (1994). An Environmentally friendly insecticide for conifer seed orchards. Simon Fraser University, unpublished.

[32] Mathur SC. (1999). Future of Indian pesticides industry in next millennium. Pesticide Information. 24(4):9-23.

[33] Md. Wasim Aktar, Dwaipayan sengupta and Ashim chowdhury (2009). Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary toxicology, 2(1):1-12.

[34] Mordue, A.J. and Nisbet, A.J. (2000). Azadirachtin from the neem tree Azadirachta indica: its actions against insects. Anais da Sociedade Entomológica do Brazil, 29: 615-632.

[35] Mukhopadhyay, A.N., Shrestha, A.M. and Mukherjee, P.K. (1992). Biological seed treatment for the control of soil-borne plant pathogens. FAO Plant Protection Bulletin, 40: 21-30.

[36] Nathan, S.S., Kalaivani, K. and Murugan, K. (2006). Effect of biopesticides on the lactate dehydrogenase (LDH) of the rice leaf folder, Cnaphalocrocismedinalis (Guenée) (Insecta: Lepidoptera: Pyralidae). Ecotoxicology and Environmental Safety, 65(1): 102-107.

[37] Nerio, L.S., Olivero, J.V. and Stashenko, E.S. (2009). Repellent activity of essential oils from seven aromatic plants grown in Colombia against Sitophiluszeamais Motschulsky (Coleoptera). Journal of Stored Product Research, 45: 212-214.

[38] Okigbo, R.N. and Nmeka, I.A. (2005). Control of Yam tuber rot with leaf extracts of Xylopiaaethiopica and Zingiber officinale. African Journal of Biotechnology, 14: 804-807.

[39] Petel, T., Jakhmola, S.S. and Bhadauria, N.S. (2004). Effect of plant materials on rice weevil (SitophilusoryzaeL.) in wheat. Indian Journal of Entomology, 66(2): 99-101.

[40] Poiloakidou, E. (2005). Biological control of powdery mildew, Pedoshaeraxanthii, on cucumber using plant extracts neem and Alicin, Honours thesis, University of Aberdeen Phytopathology100: 9913-9921.

[41] Rabindra, R.J. (2005). Current status of production and use of microbial pesticides in India and the way forward. In Rabindra, R.J., Hussaini, SS. and Ramanujam, B. (ed.), Microbial Biopesticde Formulations and Applications. Project Directorate of Biological Control, Technical Document No. 55: 1-12.

[42] Ragsdale, D.W., McCornack, B.P., Venette, R.C., Potter, B.D., Macrae, I.V., Hodgson, E.W., et al., (2007). Economic threshold for soybean aphid (Hemiptera: Aphididae). Journal of Economic Entomology, 100: 1258-1267.

[43] Ragsdale, D.W., Voegtlin, D.J. and O'Neil, R.J., (2004). Soybean aphid biology in North America. Annals of the Entomological Society of America, 97: 204-208.

[44] Rahaman, G.M. and Motoyama, N. (2000). Repellent effect of garlic against stored product pest. Journal of Pesticide Science,25(3): 247-252.

[45] Roychowdhury, D., Paul, M. and Banerjee, S.M. (2014). A review on the effects of Biofertilizers & Biopesticides on Rice and Tea cultivation and productivity. International Journal of Science,

Engineering and Technology. V2, Issue 8.2348-4098.

[46] Salas, J. (2001). Efficacy of a garlic best repellent on the reduction of whitefly (Bemisiatabaci) populations. Agron. Trop. Marc., 51 (2): 163-174.

[47] Salma, M., Kalita, J., Rajkhowa and Ratul. (2011). A review on the use of biopesticides in insect pest management. International Journal of Science and Advanced Technology (ISSN 2221-8386), V1 (7).

[48] Saxena, R.C., Jilani, G. and Abdul Kareem, A. (1989). Effects of neem on stored grain insects, In Jacobson, M. [Ed.]. Focus on phytochemical pesticides. The neem tree. CRC press, Boca Raton, FL., 1: 97-111.

[49] Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Annual Review of Entomology, 35: 271-297.

[50] Stark, J.D. and Walter, J.F. (1995). Neem oil and neem oil components affect efficacy of commercial neem insecticides. Journal of Agriculture and Food Chemistry, 43: 507-512.

[51] Stark, J.D. and Rangus, T.M. (1994). Lethal and sublethal effects of the neem insecticide formulation, 'Margosan-O', on the pea aphid. Pesticide Science, 41: 155-160.

[52] Stark, J.D. and Wennergren, U. (1995). Can population effects of pesticides be predicted from demographic toxicological studies? Journal of Economic Entomology, 88: 1089-1096.

[53] Sun, B., Liangm S.B. and Zhao, W.X. (2000). Outbreaks of soybean aphid in Suihua district in 1998 and its control strategies. Soybean Bulletin, 8:5.

[54] Tang, Y.Q., Weathersbee, A.A. and Mayer, R.T. (2002). Effect of neem seed extract on the brown citrus aphid (Homoptera: Aphididae) and its parasitoid Lysiphlebus testaceipes (Hymenoptera: Aphidiidae). Environmental Entomology, 31:172-176.

[55] Thakore, Y. (2006). The biopesticides market for global agricultural use. Industrial. Biotechnology, 2(3):192- 208.

[56] Vinod, K. (2015). A review on efficacy of biopesticides to control the agricultural insect's pest. International Journal of Agricultural Science Research, 4(9): 168-179.

[57] Wang, Y., Braman, S.K., Robacker, C.D., Latimer, J.G. and Espelie, K.E. (1999). Composition and variability of epicuticular lipids of azaleas and their relationship to azalea lace bug resistance. Journal of American Society of Horticulture Science,124: 239-244.

[58] Weathersbee, A.A., Hardee, D.D., Meredith- Junior, W.R. (1995). Differences in yield response to cotton aphids (Homoptera: Aphididae) between smooth leaf and hairy-leaf isogenic cotton lines. Journal of Economic Entomolgy, 88:749-754.

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Chemical Composition and Energy Nutritional Value of the Meat of Guinea Fowls (*Numidameleagris*), Fattened to different Ages

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Abstract*—*The aim of the study was to investigate the chemical composition and energy content of the meat of young guinea-fowls, with different duration of the fattening period, raised in a free-range, semi-intensive production system.

The authors establish the following data: dry matter content- from 27.08 to 28.82% in breast muscle and from 23.83 to 26.56% in thigh muscle; crude protein in dry matter –from 86.19to 93.54% in breast and from 82.02 to 87.84% in thigh muscle; crude fat in dry matter - from 5.64 to 7.58% in breast and from 9.02 to 11.05% in thigh muscles.

The average energy content in 100 g dry matter varies from 23.7 (breast muscle, 16 weeks of age) to 25.07 kJ (thigh muscle, 24 weeks of age).

Keywords*—*chemical composition, energy content, guinea fowl, meat.

I. INTRODUCTION

Over the last 30 years, much of Europe estimatedguinea-fowl as a source of dietary meatof a wild game flavour, attractive for the luxury food markets. Nevertheless, in Europe itself, there is a large gap in the production and marketing of that type of meat (Baeza et al., 2001; Sharma and Singh, 2006; Santiago et al., 2007).

The carcass of that type of fowlis characterized by a lower fat and cholesterol content and higher content of protein, essential amino acids and mineralsubstances, compared to broilers and pullets (Surdjiyska et al., 2006; Aisha Elfaki M. et al., 2012; Ayorinde, 2004; Cappa and Casati, 1978; Singh and Raheja, 1990). Consequently, guinea-fowl meat may be a more attractive alternative to meat of most other domestic animals and birds (Santiago et al., 2007).

Guinea-fowlmeat is an excellent and healthy alternative for the consumer, but studies in that area, except for France, are still scarce (Aisha Elfaki et al., 2012).

Considering the fact that,despite the rich composition and dietary characteristics, there are no traditions of consumption of meat of the species in our country, we set the aim of the study to investigate the chemical composition and energy content of the meat of young guinea-fowls, with different duration of the fattening period, raised in a free-range, semi-intensive production system.

II. MATERIAL AND METHODS

The experimental work was carried out at the poultry farm of the Training and Experimental Base of the Agricultural University – Plovdiv with three experimental groups in two replicates.

Studieswere conducted with a local for South Bulgaria guinea-fowlpopulation with a pearl-gray color of the plumage, distributed by the analogue method in three groups of 24 birdseach in the first and 20 birds each in the second year of study (an equal number of both genders). Birds were fedon complete compound forage, prepared according to recipes by Marinov et al. (2015).

Phase feedingwas applied according to the following scheme: First group (16 weeks fattening period): starter (0-5 week), grower (6-12 week), finisher (13-16 week); Second group (20 weeks fattening period): starter (0-5 week), grower (6-16 week), finisher (17-20 week). Third group (24 weeks fattening period: starter (0-5 week), grower (6-20 week), finisher (21-24 week).

The compound feed contained maize, wheat, soybean meal 46, sunflower meal 37, fish meal 72, L-lysine, DL-methionine, chalk, dicalcium phosphate, salt, sodium bicarbonate, vitamin mineral premix and sunflower oil.

During the three fattening periods (starter, grower and finisher), the basic substanceswere supplemented with different percentages of the major substances in 1 kg of native forage:

Starter:ME–12.1 MJ;crudeprotein (CP)– 26.27%; lysine – 1.63%; methionine+cysteine(M+C) – 0.98%; threonine – 1.09%;tryptophan – 0.27%;calcium (Ca)– 1.27%; available phosphorus (Avail.P)– 0.5%; Natrium (Na) – 0.18%.

Grower: ME– 12.41 MJ;CP– 21.82%;lysine – 1.3%;M+C – 0.87%;threonine – 0.95%; thryptophan – 0.21%Ca – 1%;Avail. P – 0.42%; Na – 0.18%.

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Finisher: ME– 12.7MJ;CP– 17.71%;lysine – 0.95%; M+C – 0.72%; threonine – 0.78%;tryptophan – 0.2%, Ca – 0.95%;Avail.P – 0.42%; Na – 0.18%.

Drinking water was given to the birds *ad libitum* throughout the fattening period.

Chemical and biochemical analysis of the meat was carried out in the Laboratory Test Complex of the Agricultural University – Plovdiv.

The analyses were carried out on standard parts of the carcass, skin-free, according to AOAC (2007) and included the following physical,chemical and biochemical analyses (Todorov et al., 2010): moisture content (according to BDS – ISO 6496); crude protein(%) by Kjeldahl (BDS – ISO 5983), crude fat (%) by Soxhlet (BDS – ISO 6492), crude ash (according to BDS – ISO 5984), gross energy – directly by microprocessor calorimeter KL 11 Mikado.

III. RESULTS

Table 1 shows the chemical composition and energy content of breast muscle depending on gender andeconomic yearand also the average data.

The dry matter content did not show statistically significant differences depending on bird gender and the duration of the fattening period and it was in the range of 27.08% and 28.82% in total for both replicates. This result was due to the insignificant accumulation of body fat with aging (Marinov, 2004). On the other hand, the result shows indirectly that in thatfowl species, the delicacy of the meat remains tender to an older age, even when raised in a free-rangefarming system. The lower rate of maturity (38 weeks) in thatspecies supports the above conclusion (Angelov,2017).

Variations in crude protein content were also not large. The reported values ranged from 86.19% (24-week old female birds in the experiment in 2016) to 93.54% (24-week old female birds in the experiment in 2015) in the dry matter. The statistical significance of the difference between the birds of 20and 24weeks of agewas mainly observed in the first year, while in the second, statistical significance was established only in the contentin the native muscle and only in females of the same age. The crude protein values of theseparate replicates showed significant differences, more on the basis ofthe native muscle, whereas when compared on the dry matter basis, the differences were smaller and in most cases insignificant.The data obtained, however, did not show any important trends to linear changes proportional to the age of fattening. The same trend was observed in terms of gender and between different replicates.

More significant differences, both by gender and age, were observed in the crude fat content, especially recalculated on the basis of the dry matter content. In the first year (2015), the highest content was reported in 20-week old birds – male (7.58%) and female (7.01%). In the second experimental year, the highest fat content was found in both sexes at 24 weeks of age (5.64 and 7.37%, respectively). In 24-week old birds, there was a marked tendency to higher fat deposition in the females, especially in fat recalculation based on the dry matter content. This can be explained by the deposition of body reserves in connection with the forthcoming egg-laying.

*Table.1: Chemical composition of guinea fowl's breast muscles**

		Indexes, %	16 weeksof age				20 weeksof age				24 weeksof age			
			Drymatter		Native		Drymatter		Native		Drymatter		Native	
			X	Sx	X	Sx	X	Sx	X	Sx	X	Sx	X	Sx
2015	Male	DM[1]	26,11	0,23	–	–	26,72	0,32	–	–	26,69	0,34	–	–
		CF[1]	5,45a	0,01	1,42a1	0,01	7,58ab	0,01	2,03a1b1	0,02	4,07a	0,33	1,09a1	0,09
		CP[1]	89,45	0,17	23,35a1	0,25	89,37ab	0,01	23,88b1	0,29	90,80ab	0,01	24,24a1	0,3
		Ash	4,70ab	0,01	1,22a1b1	0,01	3,25	0,25	0,87a1	0,07	3,93a	0,22	1,05a1	0,06
		Energy (MJ*kg-1)	23,64a	0,04	6,17	0,06	24,40b	0.01	6,52a1b1	0,08	23,46ab	0,12	6,26a1	0,09
	Female	DM	25,83	0,51	–	–	27,08	0,29	–	–	26,55	0,2	–	–
		CF	5,49a	0,16	1,42a1	0,07	7,01ab	0,19	1,90a1b1	0,04	4,23a	0,09	1,12a1	0,02
		CP	89,17a	0,12	23,04a1	0,49	92,59ab	0,01	25,08a1b1	0,27	93,54ab	0,01	24,84	0,18
		Ash	4,32b	0,01	1,11b1	0,0	3,48	0,52	0,94	0,14	4,17	0,16	1,13	0,05

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		Energy (MJ*kg-1)	23,59a	0,08	6,09	0,14	24,97ab	0,07	6,76a1b1	0,06	24,19ab	0,03	6,42a1	0,05
	Average	DM	25,95a	0,29	–	–	26,9	0,21	–	–	26,62a	0,19	–	–
		CF	5,48ab	0,09	1,42a1b1	0,04	7,30ab	0,13	1,96a1b1	0,03	4,15ab	0,17	1,11a1b1	0,05
		CP	89,29a	0,11	23,17a1	0,28	90,98a	0,49	24,48b1	0,26	92,17	0,41	24,54a1	0,19
		Ash	4,48a	0,08	1,16a1b1	0,02	3,37a	0,28	0,90a1b1	0,07	4,05a	0,13	1,09a1	0,04
		Energy (MJ*kg-1)	23,61ab	0,05	6,13a1	0,08	24,69b	0,09	6,64a1b1	0,06	23,82ab	0,12	6,34a1	0,05
2016	Male	DM	26,5	0,11	–	–	26,4	0,14	–	–	25,82	0,27	–	–
		CF	6,95ab	0,01	1,84a1b1	0,01	5,28a	0,25	1,39a1	0,06	5,64a	0,22	1,46	0,07
		CP	88,34	0,2	23,41	0,15	89,16	0,51	23,54	0,23	88,14	0,42	22,76	0,27
		Ash	3,79b	0,01	1,01b1	0,01	4,15	0,18	1,1	0,04	4,44	0,24	1,15	0,05
		Energy (MJ*kg-1)	23,92a	0,05	6,34a1	0,04	23,51	0,1	6,21a1	0,04	23,39a	0,04	6,04	0,07
	Female	DM	26,59	0,39	–	–	26,32a	0,12	–	–	26,78a	0,32	–	–
		CF	5,97b	0,01	1,59b1	0,02	5,57	0,21	1,47	0,05	7,37	0,79	1,98	0,24
		CP	88,91	0,32	23,64	0,32	89,31	0,45	23,51a1	0,22	86,19	1,11	23,07a1	0,09
		Ash	4,15b	0,06	1,09b1	0,01	4,33	0,33	1,14	0,08	4,26	0,67	1,14	0,19
		Energy (MJ*kg-1)	23,7	0,08	6,3	0,09	23,65	0,1	6,22	0,05	23,55	0,06	6,31	0,09
	Average	DM	26,55a	0,21	–	–	26,36ab	0,09	–	–	26,3	0,29	–	–
		CF	6,39b	0,2	1,70a1b1	0,05	5,43b	0,16	1,43a1b1	0,04	6,50b	0,53	1,72b1	0,16
		CP	88,66	0,22	23,54	0,18	89,24a	0,32	23,52a1b1	0,15	87,16a	0,69	22,92a1	0,14
		Ash	3,99	0,08	1,05b1	0,02	4,24	0,18	1,12b1	0,04	4,35	0,32	1,14	0,09
		Energy (MJ*kg-1)	23,80ab	0,06	6,32a1	0,05	23,58b	0,07	6,22b1	0,03	23,47ab	0,05	6,17a1	0,08

* The differences in the chemical composition are significant ($p<0,05$): a-a – in the dry matter under the different ages; a1-a1 – in the native muscles under the different ages; b-b - inthedrymatterbetween the sexes in the same age; b1-b1 - inthenative muscles between the sexes in the same age

[1]DM = dry matter; CF= crude fats; CP= crude protein

Ash content varied considerably depending on age and gender in each replicate. However, there were nostatistically proven differences by gender and duration of the fattening period between the separate replicates. Mineral content ranged from 3.25 in 20-week oldmales, (experimentin 2015) to 4.70% in 16-week oldmales (experimentin 2015) indrymatter.

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Table.2: Chemical composition of Guinea fowl's thigh muscles

		Indexes, %	16 weeksof age				20 weeksof age				24 weeksof age			
			Drymatter		Nativ		Drymatter		Nativ		Drymatter		Nativ	
			X1	Sx	X	Sx	X	Sx	X	Sx	X	Sx	X	Sx
2015	Male	DM[1]	23,83ab	0,08	–	–	24,73	0,23	–	–	25,62a	0,4	–	–
		CF[1]	10,77	0,33	2,56	0,07	11,05b	0,01	2,73b1	0,02	11,03b	0,44	2,83	0,14
		CP[1]	83,36ab	0,18	19,86a1 b1	0,11	87,84ab	0,01	21,73a1	0,2	87,12ab	0,01	22,32	0,35
		Ash	4,49b	0,01	1,07b1	0,01	3,19	0,56	0,79	0,14	4,1	0,45	1,01	0,11
		Energy (MJ*kg-1)	24,11a	0,08	5,75a1	0,01	25,30a	0,01	6,26a1	0,06	25,12	0,16	6,44	0,12
	Female	DM	24,57b	0,08	–	–	25,32	0,53	–	–	25,25	0,38	–	–
		CF	10,20a	0,01	2,51a1	0,01	13,36ab	0,06	3,38b1	0,07	13,29b	0,25	3,35a1	0,06
		CP	86,13b	0,88	21,16b1	0,17	87,37ab	0,01	22,12a1	0,46	87,26ab	0,01	22,03a 1	0,33
		Ash	5,01ab	0,01	1,23b1	0,01	4,36a	0,14	1,1	0,04	4,55	0,12	1,15	0,01
		Energy (MJ*kg-1)	24,58a	0,21	6,04a1	0,04	26,03	0,02	6,59	0,14	25,98a	0,09	6,56	0,09
	Average	DM	24,25a	0,16	–	–	25,03	0,29	–	–	25,44a	0,27	–	–
		CF	10,45a	0,17	2,53a1	0,03	12,20a	0,35	3,06	0,1	12,16	0,42	3,09a1	0,11
		CP	84,94a	0,73	20,60a1	0,28	87,61ab	0,07	21,92b1	0,25	87,19ab	0,02	22,18a 1	0,23
		Ash	4,79ab	0,1	1,16a1b 1	0,03	3,78ab	0,33	0,95a1b1	0,08	4,33	0,23	1,08	0,05
		Energy (MJ*kg-1)	24,38a	0,15	5,91a1	0,06	25,67ab	0,11	6,42b1	0,09	25,55b	0,16	6,50a1	0,08
2016	Male	DM	24,52	0,17	–	–	24,84	0,1	–	–	26,31	0,87	–	–
		CF	9,02ab	0,01	2,21a1	0,02	10,2	0,54	2,53	0,14	11,14a	0,09	2,93a1	0,12
		CP	85,23	0,01	20,9	0,14	83,75	1,12	20,8	0,22	82,47	0,69	21,69	0,53
		Ash	4,29a	0,01	1,05a1	0,01	4,31	0,29	1,07	0,07	4,62a	0,02	1,22a1	0,04
		Energy (MJ*kg-1)	23,93	0,01	5,87	0,04	24	0,25	5,96	0,06	24,04	0,14	6,32	0,17
	Female	DM	24,37a	0,24	–	–	25,39	0,48	–	–	26,56a	0,28	–	–
		CF	10,95b	0,75	2,67	0,19	11,91	1,67	3,04	0,48	11,84	0,66	3,15	0,21
		CP	84,29	0,93	20,54	0,34	82,27	1,55	20,86a1	0,14	82,02	1,28	21,78a 1	0,3
		Ash	4,41	0,2	1,08a1	0,05	4,86a	0,1	1,23a1	0,03	4,29a	0,13	1,14	0,04
		Energy (MJ*kg-1)	24,41	0,31	5,95a1	0,11	24,27	0,28	6,16	0,17	24,18	0,16	6,42a1	0,09
	Average	DM	24,44a	0,15	–	–	25,11a	0,25	–	–	26,43a	0,41	–	–
		CF	10,12	0,56	2,47a1	0,14	11,05	0,87	2,79	0,25	11,49	0,34	3,04a1	0,12
		CP	84,69a	0,53	20,7	0,2	83,01b	0,93	20,83a1b 1	0,12	82,25ab	0,66	21,73a 1	0,27
		Ash	4,35b	0,11	1,07a1b 1	0,03	4,58b	0,18	1,15b1	0,05	4,46	0,09	1,18a1	0,03
		Energy (MJ*kg-1)	24,2	0,19	5,91a1	0,06	24,13b	0,18	6,06b1	0,09	24,11b	0,1	6,37a1	0,09

* The differences in the chemical composition are significant ($p<0,05$): a-a – in the dry matter under the different ages; a1-a1 – in the native muscles under the different ages; b-b - inthedrymatterbetween the sexes in the same age; b1-b1 - inthenative muscles between the sexes in the same age

[1] DM = dry matter; CF= crude fats; CP= crude protein

The energy content of breast muscle ranged from 23.39 (males – 24 weeks of age, experiment in 2016) to 24.97 KJ (females – 20 weeks of age, experiment in 2015) in 100 grams of dry matter and 6.04 (males – 24 weeks of age, experiment in 2016) to 6.76 KJ (females – 20 weeks of age, experiment in 2015) in 100 grams of native muscle.

Statistically significant gender differences were reported between 20- and 24-week old birds in the first replicate, whereas the differences according to the age of fattening were significant in most cases between 16- and 24-week old birds. However, we assume that the energy content is relatively constant, both according to gender and the duration of the fattening period.

The chemical composition and the energy content of thigh muscle are presented in Table 2. The percentage of dry matter in thigh muscle was the lowest in 16-week old male birds in 2015 (23.83%) and the highest – in 24-week old females, experiment in 2016 (26.56%), which was due to the accumulation of age-related body reserves and it was more pronounced in the female birds in relation to egg-laying. Statistically significant differences were found between 16 weeks of age (23.83%) and 20 weeks of age (25.62%) in male guinea-fowls in the first year of the experiment and in female guinea-fowls in the same two periods of fattening in the second year – 24,37% (16 weeks) vs 26.56% (20 weeks), respectively. We also found out statistically significant differences in the dry matter content in male birds (23.83%) and in females (24.57%) in 2015 in the shortest period of fattening.

The average fat content in thigh muscle of guinea-fowls of both genders showed variations within 10.12% to 12.20% in the dry matter and 2.47% to 3.06% in native muscle. Significant differences in the average fat content between the two years of the study were not established. The differences between 16 weeks (9.02%), 20 weeks (11.05%) and 24 weeks (11.03%) of age of the males of these condreplicate were statistically significant. The same trend was observed according to the duration of fattening period in female birds of the same replicate – 10.95, 13.36 and 13.29% at 16, 20 and 24 weeks of age, respectively. In the first experimental year, differences in fat content in females from the shortest fat tening period (10.20%) and 20 weeks of age (13.36%) were also significant.

The established variations of the crude protein content in thigh muscle ranged from 82.02% (24-week old female fowls from the experiment in 2016) to 87.84% (20 week sold male guinea-fowls in the experiment in 2015) in the dry matter and from 19.86 to 22.32% in native muscle. Statistically significant differences were observed in the first replicate, both in all the three fattening periods and between the males and females. In the experiment in 2016, proven differences were established only in female guinea-fowls of 20 weeks of age (20.86%) and 24 weeks (21.78%) in native muscle.

The ash content in thigh muscle was the lowest in the males from the first replicate at 20 weeks of age (3.19%) and the highest (5.01% in the dry matter) in the females from the same replicate at 16 weeks of age, the differences being statistically significant in both the dry matter and native muscle (1.07% vs. 1.23%) in favor of the females. Significant differences were also observed in the female birds at 16 weeks of age (5.01%) and 20 weeks (4.36%) in the experiment in 2015. In the second replicate, statistical significance was established between the differences in the two longer fattening periods – 20 weeks (4.86%) and 24 weeks (4.29%).

Table 3 shows the averaged results of the two genders in both replicates. Although many authors think that the chemical differences between breast and thigh muscle of muscle also determined the significantly higher gross energy content in it. However, differences in the chemical composition were not as high as in broiler chickens.

The energy content of thigh muscle was with in the range of 23.93 (males – 16 weeks of age, experiment in 2016) to 26.03 KJ (females – 20 weeks of age, experiment in 2015), basedon 100 grams of dry matter, and, from 5.75 (malesat 16 weeks of age, experiment in 2015) to 6.59 KJ (females, 20 weeks of age, experiment in 2015), based on 100 grams of native muscle.

IV. DISCUSSION

The value of poultry meat is determined by its nutrient content. The chemical composition of guinea-fowl meat is considered delicacy because of its lower fat content and higher protein content compared to broiler chicke nmeat. In this direction, sufficient information has been compiled, mainly in foreign literature.

Table.3: Average results of the two genders in both replicates

Indexes, %		Breast muscles			
		Dry matter		Nativ	
		X	Sx	X	Sx
16 weeksof age	Dry matter	26,25	0,19	–	–
	Crude fat	5,93a	0,16	1,56	0,05
	Crude protein	88,98a	0,15	23,36	0,17

	Ash content	4,24	0,09	1,11	0,02
	Energy (MJ*kg-1)	23,70a	0,05	6,22	0,05
20 weeks of age	Dry matter	26,68	0,14	–	–
	Crude fat	6,55A	0,23	1,75	0,06
	Crude protein	90,28A	0,37	24,1	0,2
	Ash content	3,72	0,2	0,99	0,05
	Energy (MJ*kg-1)	24,24A	0,14	6,47	0,06
24 weeks of age	Dry matter	26,52	0,16	–	–
	Crude fat	4,94b	0,33	1,31	0,09
	Crude protein	90,50b	0,67	24	0,23
	Ash content	4,15	0,14	1,11	0,04
	Energy (MJ*kg-1)	23,71b	0,09	6,29	0,05
		Thigh muscles			
16 weeks of age	Dry matter	24,34	0,11	–	–
	Crude fat	10,28a	0,28	2,5	0,07
	Crude protein	84,82a	0,44	20,65	0,17
	Ash content	4,57	0,09	1,11	0,02
	Energy (MJ*kg-1)	24,29a	0,12	5,91	0,04
20 weeks of age	Dry matter	25,06	0,19	–	–
	Crude fat	11,74A	0,41	2,95	0,12
	Crude protein	85,77A	0,63	21,49	0,2
	Ash content	4,1	0,22	1,03	0,06
	Energy (MJ*kg-1)	25,05A	0,2	6,28	0,07
24 weeks of age	Dry matter	25,77	0,25	–	–
	Crude fat	11,94b	0,3	3,07	0,08
	Crude protein	85,54b	0,6	22,03	0,18
	Ash content	4,37	0,15	1,11	0,04
	Energy (MJ*kg-1)	25,07B	0,2	6,46	0,06

*The differences in the chemical composition of the DM of breast and thigh muscles are significant (p<0,05): a-a – by 16; A-A – by 20;b-b – by 24 week's fattening period

The dry matter in breast muscle in the present experiment with two replicates ranged from 27.08% to 28.82% and from 23.83% to 26.56% for thigh muscle, respectively. Kudryashov et al. (2015) found the same content of dry substance in both thigh and breast muscle in pearl gray, white and blue guinea-fowls. Those results were close to the results obtained in the present study, i.e. 25.32 – 26.93%. Moreki et al. (2012) also mentioned that the dry matter in guinea-fowls, fed on compound forages containing sorghum, ranged between 25.77% and 29.39% at the age of 6-12 weeks. In intensive and semi-intensive rearing of guinea-fowls, Saina (2005) established similar to our results: 22.9 – 26.1%.

The average content of crude fat in native muscle (male and female guinea-fowls) was comparable to that of Moreki et al. (2012). They announced the fat content of 2.18% (in 6- week old birds) to 2.99% (in 12-week old birds). According to the same author, fat accumulation was not influenced by the age of the birds. Similar to his finding, we also found that the age of guinea-fowls was not essential for the percentage of fats in the muscle.

In the first replicate, the fat percentage in breast (native) muscle in 16-week old males was 1.42%; in 20-week old – 2.03%; in 24-week old – 1.09%, and, in the females the results were as follows: 16-week old birds – 1.42%; 20-week old – 1.90%; 24-week old – 1.12%, respectively. Elhashmi et al. (2012) reported a fat content of 3% in breast muscle of 22-week old guinea-fowls, which differed significantly from our results. Tejerina et al. (2009), however, established that the fat content in thigh muscle of that bird species was within the range of 2.43 to 4%, which is close to our results.Tejerina et al. (2009), however, established that the fat content in thigh muscle of that bird species was within the range of 2.43 to 4%, which is close to our results.

The established results of the crude protein content in 2015-2016 (male + female guinea-fowls) were as follows: 22.92% to 24.54% in average in breast (native) muscle and 22.18% – 20.60% in thigh muscle. Moreki et al. (2012) announced results close to those established in our study: for 6-week birds – 22.90% and higher; for 12-week – 31.55%. Similar to our results were also published by Tlhong (2008) – 22.7% of crude protein reported in guinea-fowl muscle.

Unlike Moreki et al. (2012), which established an increase of the ash content with bird aging (from 2.56% to 5.54%), we did not find such a tendency in thigh muscle. In breast muscle (male + female, experiment in 2016), the increase of the ash content was insignificant (3.99% to 4.35%). According to Mareko et al. (2008) the method of rearing the birds had an impact on the chemical composition of the meat.

V. CONCLUSIONS

The dry matter content in guinea-fowlmuscle fattened to different ages, did not show significant differences in both gender and age and ranged from 27.08 to 28.82%in breast muscleand from 23.83% and 26.56% in thigh muscle, respectively.

The crude protein content in breast muscle(dry matter) ranged from 86.19% (24-weekold female birds) to 93.54% (24-weekold female birds), and from 82.02% (24-weekold female birds) to 87.84% (20-weekold male guinea-fowls)in thigh muscle. An apparent influence of the fattening age on thatcharacteristicwas not observed.

The crude fat content of breast muscle(dry matter) varied from 5.64 to 7.58% and a tendency to higher values was reported in females fattened to a higher age. The crude fatcontentinthigh muscle(dry matter) ranged from 9.02 to 11.05%. The differences in the contents in the male birds between 16 and 20/24 weeks of age were larger.

The leanermeat of the male birds is explained by the accumulation of body reserves in females prior to egg-laying.

The average energy content in 100 g of dry matter varied from 23.7 (breast muscle, 16 weeks of age) to 25.07 kJ (thigh muscle, 24 weeks of age). The energy content in thigh muscle(dry matter) was significantly higher than in breast musclefor the three fattening periods.

REFERENCES

[1] Angelov, A.(2017) Investigation on the egg productivity of local population of Guinea fowls (Numidameleagris), BG Journal of animal sciences (in print)

[2] AOAC international(2007) Official methods of analysis of AOAC, 18 Ed., Rev.2, Gaithersburg, MD, USA

[3] Kudrjavtsev, L., S., W. A. Zabijakin, T. B. Zabijakina (2015)Mjasn’ekachestva I himicheskijsostavtushekzessarokpasnogogenetiches kogoproishojdenja, Suchasneptahivniztvo, Nauko – virobnichijjurnal №7–8(152–153)

[4] Marinov, B.(2015) Feeding of Guinea fowls, In: Todorov, N., B. Marinov, A. Ilchev, D. Penkov, V. Georgieva, G. Ganchev, S. Chobanova, 2015, Applied feeding of domestic animals, BG ISBN 9789542944126

[5] Marinov, B.(2004) Feeding of layers,Textbook Sofia, 210pp

[6] Todorov, N., A. Atanasov, A. Ilchev, G. Ganchev, G. Mihajlova, D. Girginov, D. Penkov, Z. Shindarska, J. Najdenova, K. Nedjalkov, S. Chobanova (2010) Handbook of feeding of domestic animals, BG ISBN 9789543217335

[7] Surdjiyska, S., D. Stoyanov, V. Sredkova, S. Grigorova(2006) Bulgarian fodder additives, stimulating bird’s productivity, Ptizevadstvo (BG), 3: 18-20

[8] Aisha Elfaki, M., Z.Magzoub, M. Elhag, A. S. Mohamed(2012) Guinea fowl (Numidameleagris) as a meat bird, International Journal of Sudan Research Vol. 2 No. 1

[9] Ayorinde, K.L. (2004)The spice of life (the seventy first inaugural lecture), Library and Publications Committee. University of Ilorin, Nigeria

[10] Baeza, E., H. Juin , G. Rebours , P. Constantin , G. Marche, C. Leterrier(2001) Effect of genotype, sex and rearing temperature in carcassand meat quality of guinea fowl, Br. Poult. Sci., 42, 470-476

[11] Cappa, V., M. Casati(1978) Experiments of growing guinea fowl, amino acid composition of the carcass,Avicoultura, Vol. 47, 3, 21-29

[12] Elhashmi Y.H., E.L. Amin, F.A. Omer (2012) Growth and Development of Muscle, Bone and Fat of Guinea fowl (NumidameleagrisGaleata). Online J. Anim. Feed Res., 2(1): 6-12

[13] Moreki, J. C., Patrick,G., Nthoiwa, T. Kagiso, J. Podi, B. Machete(2012) Chemical Analysis and Sensory Evaluation of Guinea Fowl Meat Fed Diets Containing Three Cereal Grains as Energy Sources up to 12 Weeks of Age International Journal of Science and Advanced Technology (ISSN 2221-8386) Volume 2, No 10

[14] Saina, H.(2005) Guinea fowl (Numidameleagris) production under smallholder farmer management in Guruve district, Zimbabwe. Department of Animal Science, Faculty of Agriculture, University of Zimbabwe. http://www.life.ku.dk/upload/poultry/master_theses/poultry_master/happyson_saina_master_thesis_2005.pdf

[15] Santiago, H.L., V. Díaz, A.A. Rodríguez (2007) Processing yields, meat quality attributes and

http://dx.doi.org/10.22161/ijeab/2.6.24 ISSN: 2456-1878

nutrient composition of diverging genotypes of guinea fowl (Numidameleagris) broilers reared on various planes of nutrition in a tropical environment, Animal Science, Vol. 13, 236-238

[16] Singh, H., K. L. Raheja(1990) Genetic estimates of cholesterol and high density lipid components in indigenous guinea fowl serum, Proceedings of XI I Annual Conference and Symposium of Indian Poultry Science Association. Bombay Veterinary College, Bombay, India

[17] Sharma, D., H. Singh (2006) Future research priorities in guinea fowl breeding and Genetics, Poultry Research Priorities to 2020, 22-30.

[18] Tejerina D., M.M. López-Parra, S.García-Torres (2000) Potential used of near infrared reflectance spectroscopy to predict meat physico-chemical composition of guinea fowl (Numidameleagris) reared under different production systems. Food Chem., 113: 1290-1296

[19] Tlhong, T.M. (2008) Meat quality of raw and processed guinea fowl, Stellenbosch University. Retrieved on 18/08/12 from http://hdl.handle.net/10019.1/1898

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Growth Performance and Nutrient Digestibility of Growing Pigs Fed Cassava Peel Meal Based Diets Treated with Exogenous Enzyme

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Abstract— *A feeding trial was conducted to evaluate the growth performance and nutrient digestibility of grower-finisher pigs fed diets containing 0 %, 50 %, 75 % and 100 % levels of cassava peel meal treated with 0.035gNatuzyme®/100g CPM. Sixteen (16) pure bred male Landrace grower-finisher pigs, averaging 31.80kg were allotted to four dietary treatments in a completely randomized design such that each pig was housed and fed individually as a replicate. Four experimental diets T_1, T_2, T_3 and T_4 were formulated and 0 %, 50 %, 75% and 100% maize was replaced with Natuzyme® treated cassava peel meal and fed until the pigs reached 60kg ±2kg live weight. At the end of the feeding trial which lasted for 7, 7,8 and 9 weeks for treatment T_1, T_2, T_3 and T_4, respectively, two pigs from each dietary treatment were randomly selected and starved for 24hours;faecal samples were collected for seven days, oven dried, weighed and sampled for digestibility analysis. Feed intake and weight gain of pigs fed test diets decreased ($p>0.05$) while, feed conversion ratio increased ($p>0.05$) compared with the control. There was significant reduction ($p<0.05$) in the cost of feed per kilogram live weight gain as supplemented CPM increased in diets of finisher pigs. Nutrient digestibility of dry matter, crude fiber, crude protein, ash and nitrogen free extract decreased significantly ($p<0.05$) while ether extract digestibility decreased ($p>0.05$). 100% maize replacement with CPM treated with 0.035g of Natuzyme® in 100g of feed for grower finisher pigs proved cheaper though with a slower growth rate.*

Keywords— ***cassava peel meal, Natuzyme®, growth performance, nutrient digestibility, pigs.***
Abbreviations—CPM- cassava peel meal

I. INTRODUCTION

The pig has been noted to compete with human beings for available cereal and grains (Adesehinwa *et al*., 1998).In view of this development animal researchers have shifted their attention to materials that are available but underutilized as feed ingredients for livestock. One of such materials is the cassava peel, which is underutilized in Nigeria because it is often burnt or left to rot away on farms and homesteads after harvesting and processing of the tubers (Akinfala and Tewe, 2001). Cassava peel meal contains up to 5% crude protein, 20% crude fiber depending on the variety (Aro *et al*., 2010). The fibrous content of cassava peel meal has limited its use in monogastric nutrition. Hydrocyanic acid, an anti-nutritional factor is also present in cassava peel. However, sundrying appreciably reduces its level in the material (Aletor *et al*.,1997).Dietary addition of exogenous enzyme like Natuzyme® has been reported to enhance the breaking down of fibre encapsulating the more soluble constituents so that digestion can be effective. Effects on performance of grower-finisher pigs fed varying levels of cassava peel meal without exogenous enzyme have been investigated (Ikurior *et.al.*, 1996).This study was conducted to investigate the effects of varying levels of cassava peel meal diets supplemented with Natuzyme® on growth performance and nutrient digestibility of grower-finisher pigs.

II. MATERIALS AND METHODS

The experiment was carried out at the Pig production unit on the Livestock Teaching and Research Farm, University of Agriculture, Makurdi, Benue State of Nigeria. Cassava peels were obtained from garri processing agro-allied small-scale industries in Makurdi metropolis. The peels were washed and sun dried for seven (7) days to reduce the moisture content to about 10%. The peels were then crushed using a hammer mill to obtain cassava peel meal (CPM), sampled for analysis and stored in bags until included in the diets.

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Experimental diets

Four isonitrogenous experimental diets T1, T2, T3, and T4 were formulated as presented in Table 1. T1 contained 0% cassava peel meal (CPM) without Natuzyme® and diets T2, T3 and T4 contained CPM treated with 0.035g Natuzyme®/100g at 50%, 75% and 100%, respectively as replacement for dietary maize.

Table.1: Ingredient Composition of Grower-Finisher Pig Diets (g/100g)

	Dietary Treatments			
	T1	T2	T3	T4
	Levels of Cassava Peel Meal Replacement			
Ingredients	**0 %**	**50 %**	**75 %**	**100 %**
Maize	52.00	26.00	13.00	0.00
Cassava peel meal	0.00	26.00	39.00	52.00
Full fat soya bean	25.00	26.00	28.00	30.00
Maize offal	19.75	18.75	16.75	14.75
Bone meal	2.50	2.50	2.50	2.50
Common salt	0.50	0.50	0.50	0.50
Vitamins/Minerals premix[a]	0.25	0.25	0.25	0.25
Natuzyme®	-	+	+	+
Zinc oxide[b]	++	++	++	++
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Metabolizable energy (Kcal/Kg)	3,169.64	2,817.54	2,656.11	2,495.70
Crude protein (%)	16.81	16.04	16.06	16.09
Dietary cost (₦/kg)	77.68	62.43	55.54	48.65

[a]Biomix premix supplied the following per kg of diet: Vitamin A 12,000,000I.U, Vitamin D33,000,000 I.U , Vitamin B6 3,500 mg, Biotin 80 mg, Antioxidant 125,000 mg, Cobalt 250 mg, Selenium 250 mg, Iron 40,000 mg, Manganese 70,000 mg, Copper 8,000 mg, Zinc 80,000 mg, Choline chloride 200,000 mg , Calpan 10,000 mg, Vitamin B2 5,000 mg, Vitamin B1 2,000 mg, Iodine 1, 200 mg, Niacin 40,000 mg. Vitamin E 30,000 mg, Vitamin K3 2,500 mg, Folic acid 1,000 mg ,

[b] zinc oxide 0.0125 g/100 g, added to supply 100 ppm Zn, ++ = zinc oxide

- = Natuzyme® not added, + = 0.035/g Natuzyme®/100g diet.

Experimental design and management

Sixteen (16) male grower pigs were randomly allotted to four dietary treatments each of which had four replicates. Each pig was served drinking water *ad libitum.* Daily routine management activities were cleaning of pens, provision of experimental diets and drinking water, observation of each animal to know their health status.Each experimental animal was housed in a 183x75x106cm welded iron pipe, wire mesh, individual concrete floored pens while each pen housed four individual crates provided with concrete feeding and watering troughs measuring 52x29x21cm and 47x37x26cm, respectively. The experiment was a completely randomized design.

Data collection

The mean weekly body weights and feed intake were recorded throughout the experimental period of 63days. Feed conversion ratio was calculated from feed intake and body weight gain. Feed cost/kg gain and feed cost/kg diet were calculated from prevailing local market price of feed materials.

Nutrient digestibility was determined by the use of two (2) pigs from each dietary treatment which were randomly selected and starved for 24 hours.A weighed amount of feed was offered daily and fecal samples collected for seven days, oven dried, milled and analyzed for dry matter, crude fibre, crude protein, ether extract, ash and nitrogen free extract using standard methods (AOAC, 1995) The

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proximate analysis of the experimental diets was also carried out using the same standard methods.

All data collected were subjected to analysis of variance using the procedure of Steel and Torrie (1980) and where significant differences were observed treatment means were separated using Duncan multiple range test (Duncan, 1955)

III. RESULTS AND DISCUSSION

The experimental diets contained between 16-17 % crude protein (Table 1) in order to meet the protein requirement of grower pigs recommended by NRC (1997).Similarly, the metabolizable energy of the diets (2,495.70 - 3,169-64 kcal/kg) though reducing as the level of supplemented CPM increased in diet, were also within the energy requirement of growing pigs.

Table.2: Effect of Diets containing CPM treated Natuzyme® on Performance of Grower-Finisher Pigs

	Dietary Treatments					
	T1	**T 2**	**T3**	**T4**		
	Levels of cassava peel meal replacement					
Performance indices	**0 %**	**50 %**	**75 %**	**100 %**	**SEM**	LOS
Number of pigs	4	4	4	4		
Average initial live weight (kg)	31.45	31.75	31.88	32.50	0.89	NS
Average final weight (kg)	61.38	60.50	61.25	60.75	0.94	NS
Average daily weight gain (kg)	0.65	0.62	0.52	0.45	0.06	NS
Average daily feed intake (kg)	1.63	1.77	1.53	1.35	0.16	NS
Feed conversion ratio	2.55	2.85	2.93	3.03	0.15	NS
Feed cost/ kg live weight gain(₦)	198.84^{a}	177.93^{b}	162.73^{bc}	147.41^{c}	9.51	*
Average number of days fed	49.00	49.25	56.00	63.00	8.29	NS

^{a,b,c} Means within same row with different superscripts differed significantly ($p<0.05$)

LOS= Level of significance.; NS = Not significant ($p>0.05$) ; * = Significant SEM= Standard error of mean

The effect of the experimental diets on the growth response of grower- finisher pigs is presented in Table 2. It was observed that the diets had no significant effect ($p>0.05$) on the live body weight, weight gain, feed intake and feed conversion ratio. Significant effect ($p<0.05$) was observed for the feed cost/ kg live weight gain. These performance indices decreased as percent dietary maize replaced by CPM increased. This probably was due to CPM effect which increased the bulk of the feed thereby lowering the energy density of the diets and causing decrease in weight gain, feed intake and feed conversion ratio. This observed performance can be attributed to a synergy between the digestive system of the pig and the enhanced utilization of Natuzyme® treated CPM diets by the grower finisher pigs. This is in agreement with findings of Ikurior *et.al.*(1996) who reported that as animals grow older they tend to handle fibre more efficiently and Chesson (2001) that enzymes are a rich source of high quality protein (amino acids) making up for the short fall in the low quality of crude protein in CPM. It has been reported by Beachemin *et al*. (2003) that high producing/ growing animals require high level of available energy to meet the demand for lactation or meat production over and above maintenance needs, thus enzymes fed to low energy/ high fibre diets would help bridge the gap between potential and actual performance of the animal by assisting to release more digestible energy from fibrous fractions for use by the animal. The significant difference ($p<0.05$) in feed cost/kg live weight gain showed that it decreased at higher levels of CPM in the diets. Therefore, it was cheaper to feed pigs on Natuzyme® treated CPM diets than the control diet. This agrees with the finding of Adesehinwa *et.al.* (2008) who reported significant reduction in feed cost per kilogram live weight gain as a result of replacing maize in control diet with cassava peel supplemented with exogenous enzyme.

Table 3 presents the digestibility coefficient of grower-finisher pigs. Significant decrease ($p<0.05$) in nutrient digestibility occurred as CPM replacement of maize increased in diets of finisher pigs. However, digestibility of all nutrients: CP, CF, EE, NFE and overall dry matter digestibility was high,ranging from 60-90 percent despite the fibrous nature of CPM. This is traceable to the interactive effect between the pig's digestive system and Natuzyme® multi- activity. This was capable of breaking down the fibre encapsulating the more soluble constituent for easy digestion by the pigs, thereby enhancing the digestibility of other nutrients as reported by Irekhore *et al*. (2006).

Table.3: Effect of Diets Containing CPM treated with Natuzyme® on Apparent Nutrient Digestibility Coefficients of Grower-Finisher Pigs.

	Dietary Treatments					
	T1	**T2**	**T3**	**T4**		
	Levels of Cassava peel meal replacement					
Nutrients	**0 %**	**50 %**	**75 %**	**100 %**	**SEM**	**LOS**
Dry matter (%)	91.67^{a}	88.92^{ab}	84.76^{b}	77.69^{c}	2.06	*
Crude fibre (%)	87.13^{a}	80.92^{ab}	72.33^{b}	63.32^{c}	3.49	*
Crude protein (%)	94.75^{a}	92.77^{a}	90.49^{ab}	87.40^{b}	1.14	*
Ash (%)	76.85^{a}	74.47^{ab}	70.71^{ab}	61.57^{b}	2.60	*
Ether extract (%)	87.40	89.17	84.02	86.77	1.22	NS
NFE (%)	93.48^{a}	89.87^{a}	88.16^{a}	80.73^{b}	1.87	*

^{a,b,c}Means on the same row with different superscripts are statistically different ($p<0.05$),
NS=not significant ($p>0.05$) ; * = significant ($p<0.05$) ; LOS= level of significance
SEM= standard error of mean

Conclusion and Recommendation

In conclusion, the study revealed that the nutritive value of cassava peel meal as a feedstuff can be improved by treating it with Natuzyme® an exogenous enzyme at the rate of 0.035g Natuzyme® per 100g diet. The test diets compared favorably with the control diets in growth parameters. Cassava peel meal with 0.035g Natuzyme®/100g of feed at 100% CPM replacement of maize for grower-finisher pigs gave the least cost of feed per kilogram live weight gain though with a slower growth rate and is hereby recommended for finisher pigs.

REFERENCES

[1] A.O.A.C (1995). Association of Official Analytical Chemists. 15th edition. William Tryd Press. Richmond, Virginia. U.S.A.

[2] Adesehinwa A. O.K.,Dairo, F.A.S. and Olagbegi, B.S. (2008). Response of growing Pigs to Cassava Peel Based Diets Supplemented with Avizyme 1300: Growth, Serum and Haematological Indices. *Bulgarian Journal of Agricultural Science. 14(5):491-499*.

[3] Akinfala, O. and Tewe, O.O. (2001). Utilization of whole cassava plant in diets of growing pigs in the tropics. Livestock Research for Rural Development 13 (5): 13-21.

[4] Aletor, V. A. and Fasuyi, A. O. (1997). Nutrient Composition and Processing Effect on Cassava Leaf (*Manihotesculenta*, Crantz) Anti nutrients. In: Proc. 2nd Conference Livestock Production. 15-17 September 1996, Lagos, Nigeria, Pp 231 – 242.

[5] Aro, S.O., Aletor, V.A., Tewe, O.O. and Agbede. J.O. (2010). Nutritional Potentials of Cassava Tuber Wastes: A Case Study of a Cassava Processing Factory in South-Western Nigeria. *Livestock Research for Rural Development 22(11).*

[6] Beauchemin, K.A., Colonbalo, D., Morgari, D. P. and Yang, W.Z. (2003) Use of Exogenous fibrolytic enzyme to improve feed utilization by ruminants. *Journal of Animal Science. 81 (E. Supp1.2) E. 37-E47.*

[7] Chesson, A. (2001). Non- starch polysaccharide Degrading Enzymes in Poultry Diets: Influence of Ingredients on the Selection of Activities. *World Poultry Science Journal. 5:251-263.*

[8] Duncans, D.B. (1955). Multiple range and multiple F-test. Biometrics, 11:1-42

[9] Ikurior S.A., Onuh, S.O and Tegbe, T.S.B. (1996). Assessment of practical potential of cassava peels meal for growing and growing-finishing pigs in sub-humid tropics. *Bulletin of Animal Health Production* 44:209-124.

[10] Irekhore, O.T., Bamgbose, A.M. and Olubadewa, G.A. (2006). Utilization of Cassava Peel Meal as Energy

source for growing pigs. *Journal of Animal Veterinary Advances 5(10):849-851.*

[11] National Research Council(1997). Nutrient Requirements of Swine. 10th Revised Edition. National Academy Press, Washington D.C. Pp 23-114.

[12] Steel, R.G.D and Torrie, J.A. (1980). Principles and Procedure of Statistics. A biometrical approach. 2nd edition, McGraw Hill Book Co. New York, USA.

Response of Some Sunflower Hybrids (*Helianthus annuus* l.) to Different Nitrogen Fertilizer Rates and Plant Densities

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Abstract— *In order to improve sunflower productivity, this investigation aimed to study the performance of some genotypes to different doses of nitrogen and plant population density on seed yield and yield components. Two field Experiments carried out on the Experimental Farm of the Sakha Agriculture Research Station during 2015 and 2016 seasons. The objective of this study aimed to investigate the performance of some sunflower hybrids to different nitrogen fertilizer rates and plant population densities to growth, yield components, seed yield and its quality.The results indicated that tallest plants, highest leaves number/plant, number of achenes/head and highest values of head diameter were obtained from MS.sirena F1 genotype. Biest Brima genotype recorded the highest values of leaf area. The highest weight of 1000 seed and seed yield/ha were recorded from Nsovak genotype.The increases in nitrogen fertilizer rates to 168 Kg N/ha produced tallest plants,thick stems (cm), the highest leaves number/plant, leaf area (cm^2), number of achenes/head, head diameter (cm), 1000 achene weight (gm) and achene yield (Kg/ha) in both seasons. It could observed that increasing nitrogen fertilizer from 72 to 168 Kg N/ha significantly increased seed yield by 12.0 and 11.6 % in the first and second seasons, respectively. Increasing hill spacing from 15, 20 and 25 cm produced thickness stem, highest number of leaves/plant, highest values of leaf area, maximum number of achenes/head, head diameter and weight of 1000 achene. The tallest plants and highest and achene yield were produced from 20cm hill spacing. It could concluded that increasing nitrogen fertilizer from 72 to 168 Kg N/ha and sown Nsovak genotype at dense hill spacing of 15 cm between plants maximized seed yield per unite area.*

Keywords— Sunflower cultivars, nitrogen fertilizer rates, Plant population density.

I. INTRODUCTION

Sunflower (Helianthus annuus L.) plants the greatest significant oilseeds and occupies the fourth next to the soybean, palm oil and canola as anoil source.The cultivated area in Egypt is limited to old soils of Nile Valley and the Delta because its cultivation in it competes with other important summercrops like rice.Therefore, it could be cultivated on newlyreclaimed soils in the desert area, for minimizing this existing gap between production and import of edible oil; we should grow sunflower crops preferably. For increasing sunflower productivity, oil content and tothe oilyield to this area are the major target ofresearch programs.The hybrid S-278 erected out supreme for head diameters,seed numbers/head, weight of 1000-seed, seed yield. Maximum seed and oil percentageobtained from S-278 hybrid**(Ali et al., 2012).** The hybrid S-278 gave a higher seed yield/ha than Hysun-33 hybrid**(Ali et al., 2014)**.The great seed yield (2.06 t/ha) produced from sown sunflower hybrid PSH 569, excelling SH 332' by 3.5% and PSFH 118 by 13.8 % **(Parvender et al., 2014).**The highest seed yield/ha, thousand seed weight and head diameters obtained from Isera cultivar; the highoil content, oil yield/ha and plant height observed from C-70165, Isera and Teknosol cultivars **(Gul and Kara, 2015).**The highest percentages of seed oil recorded in Hysun-33, SMH-0917 and SMH-0907. Overall Hysun-33, SMH-0917 and SMH-0907 hybrids achieved improved for plant height, head diameters, seed numbers/head and seed yield/ha **(Iqrasanet al., 2017)**. Sunflower Hysun38 hybrids excelled the other two hybrids in seed yield/ha and oil percentage **(Nasim et al., 2017).**

Nitrogen fertilizerconsidered as one of the realelements that supplement the metabolic developmentsfounded on protein, reproducedupsurge on vegetative, generative growth stage and seed production. Different varieties need different plant densities and nitrogen requirements forproducing high seed and oil yields/ha.In this respect,

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nitrogen fertilizer rates muchexaggerated on seed numbers/head, yield to seed/head, weight of 1000–seeds, seed and oil yields/ha. Maximum seed and oil yields/ha produced from about 60 kg N/ha fertilization **(Killi, 2004)**.Fertilization of nitrogen decidedlyincreased the growth and seed yields, but occasionedreduced seed oil content. Application of 200 kg nitrogen/ha recorded the highest seed yield/ha, becauseapplication of 150 kg nitrogen/ha produced the higher oil yield/ha**(Al-Thabet, 2006).**Fertilization of nitrogen at levels of 150 kg/ha produced the highestyield/ha of achenewhich followed with nitrogen fertilization at a level of 125 kg N/ha while the lowest seed yield/haobtainedfrom fertilization of nitrogen at the rate of 100 kg N/ha **(Jahangir et al., 2006).** The greatest seed yield/ha was gottenfrom nitrogen fertilization ata rate of 50 kg N/ha for DW-2 cultivar and nitrogen fertilization at a rate of 80 kg N/ha for DW-2 and Trakya-80 cultivars. **(Süzer, 2010).**Seed yield and its attributes significantly increased, but decreased oil percentage with increasing nitrogen fertilizer rates**(Naseem et al. 2011).** Theincreases in rates of nitrogen had a significant effect and recorded the tallest plants, the highest biological and seed yields/haas well as seed oil percentage.Increasing nitrogen fertilizer rates of 225 kg/ha nitrogen were recorded the most of study traits **(Mollashahi et al., 2013).** Each nitrogen increase improves seed yield and its attributes, science oil percentage were enviably affected **(Awais et al., 2015).**

Plant density is among the factors affecting sunflower yield and seed oil percentages.Maximum yields to achenes obtained from an increasing sunflower population ofa certain level. Optimum population depends on cultural, environmental and field management factors **(Weiss, 2000).**Sunflower hybrids, plant height reduced retortedimproved to high plant populations than the standard and tall hybrids did **(Johnson, 2002).**The widerof hill spacing improvesto stem and head diameters, and weight of the seed/head,butthe narrow hill spacing significantly increased plant height, seed and oil yields/fed**(Allam et al., 2003)**.With the increase in plant populations per unit area significantly decreased head diameters, number and seed weight/head and a higher plant density of a boundaryinsignificantly effect of yield of achene/ha **(Majiri and Arzani, 2003).**The less plant population density produced the more head diameter, the higher seed number/head and seed yield/head as well as the heaviest of 1000–seed weight while, the more plant population densities produced the higher oil percentage, seed and oil yields/ha**(Killi, 2004).** Hill spacing influenced moststudiedtraits, except oil content. Sown at 25 cm hills spacing was the suitable hill spacing, butthe higher or the lower hill spacing had anundesirableresult of seed and oil yields/ha. Application of nitrogen fertilizerevidentlyimproved both growth and seed yield, neverthelesscaused decreases in the percentage of oil **(Al-Thabet, 2006).** Maximum yield toachenesyield and itsattributes as well as oil percentageproduced from the optimum with 60 x 20 cm. The lowestseed and oil yields/ha logged with planting decoration of 45 x 10cm **(Asghar et al., 2007).**Seed oil yieldsreduced with increasing plant population densities, while higher plant populations **(Ishfaq et al., 2009)** did not exaggerate the percentage of oil in the seed**.** Increasing plant population densities significantly reduced weight of 1000-seed and head diameters.The greatest seed yield in both studied hybrids produced from hill spacing of 15 × 70 cm **(Süzer, 2010).**Sow sunflower plants at hill spacing of 22.5cm recorded themost suitable planting population density, which recode the greatest of achene yield/ha, whilethe lossof hill spacing (17.5cm and 20cm) caused reducesof seed yield/ha(**Ali et al., 2011)**. Plant population density significantly differedfrom plant height, the diameters of the stem and head, the weight of achenes,weight of 100-seed and seed yield ha. The highest of seed yield/ha obtained by plant density of 48000 plants/ha in studying seasons (**Radwan et al., 2013)**.

In order to get the greatest seed and oil yield/ha, it can conclude that confection (Inegöl) and oilseed, sunflower should sow in high populations with nitrogen fertilization with60 kg N/ha **(Killi, 2004).**The hill spacing of 25 cm and nitrogen fertilization at a rate of 150-200 kg N/harecorded highest seed and oil yields/ha**(Al-Thabet, 2006).**It could noticed that hill spacing at 10 cm and fertilization of nitrogen at a rate of 60 kg N/fed maximized seed and oil yields/fed **(Osman and Awed, 2010).**Under studied hybrids, increasing plant population densities significantly reduced weight of 1000-seed and diameter of the heads.Nitrogen and plant populationinfluenced seed yield and itsattributes of different hybrids **(Süzer, 2010).**In view of the superiority, over 22.5cm hills spacing for both Hysun-38 hybrid recorded the high productivity. FH-331 hybrid recorded the lowest in seed yield compared to with hysun-38 hybrid**(Ali et al., 2011)**.Higher seed yield/ha recorded from sownat 20 cm hill spacing and 125 kg N/ha nitrogen fertilizer application **(Ali et al., 2012)**. Maximum seed yield produced with increases of plant population and the highest rate of nitrogen fertilization **(Ali et al., 2013).**Highest plant density increased seed oilspercentagewith sunflower. Similar results recorded with increasing nitrogen application phonological duration and achene yield/ha were increased, but the seed oil percentage reduced**(Ali et al., 2014)**.Nitrogen fertilization at a rate of 150 kg N/ha in 83,333 plants/ha plant population was the better treatment to extreme seed yield **(Awais et al., 2015). Nasim et al. (2017)**

summarized that Hysun-38 hybrid maximized seed yield/ha by fertilization of nitrogen at a rate of 180 kg N/ha. Therefore, the purposes of this study evaluating the outcome of plant population density of some sunflower hybrids at different application of nitrogen fertilizer rates on growth, seed yield, yield components, its seed quality, and the interaction effects among population densitiesof some hybrids at different nitrogen fertilization rates on seed yield and seed quality.

II. MATERIALS AND METHODS

2.1. Research time and location:

Two field Experiments carried out on the Experimental Farm of the Sakha Agriculture ResearchStation, ARC Egypt during the two consecutive summer seasons (2015 and 2016). A split-split plot of RCBD with four replication used.The main plots assigned to the three sunflower cultivars (Nsovak, MS.Sirena F1, BiestBrima), while the three nitrogen fertilizer rates (72, 120 and 168 Kg N/ha) which arranged in the sub-plot and the three hills spacing (15, 20 and 25 cm apart) were assigned to the sub-sub plot.Each experimental unit contained of five ridges with 3 m in length and 60 cm between hills. The size of each sub-plot was $12m^2$. The middle two rows used for determining seed yield and its components.Seeds of the studied sunflower cultivars obtained from the Field Crops Research Institute, A.R.C. Giza Egypt. The preceding crop was wheat.Analyses of chemical and physical properties of the experimental soil (0 to 30 cm depth) carried out according to the methods reported by Page et al. (1982). The soil was loamy clay in texture, the pH was 7.6, 7.8, organic matter was 4.9, 5.1%, 7.9, 7.6, E.C. dS/m^{-1} available nitrogen was 15.9 and 16.8 ppm and available phosphorus was 40.6 and 39.8 ppm of both seasons, respectively. After plotting and before the planting, sulfate of potassium (48 % K_2O) at a rate of 120 kg/ha and 240 kg/ha as calcium super-phosphate (15.5 % P_2O_5) supplied to experimental plots. Nitrogen at above rates of the form of ammonium nitrate (33.5 % N) added in two equal portions before the first and third irrigations.

2.2. Studied Characters:

Ten guarded plants occupied at harvest time from the 2^{nd} and 3^{th} ridges in each sub-plot to estimate plant height (cm),stem diameters (cm), number of leaves/plant, leaf area per plant (cm2), achene diameter (cm), achene weight (g), weight of 1000 seed (g), seed yields/head and per hectare. Leaf area per plant (cm2)It was determined according to the equation of **Schneiter (1978):** LA=[(LxW) x 0.6683] – 2.45

Where L = Maximum length of the leaf, W = Maximum width of the leaf.

2.3. Experimental analysis:

The analysis of data collected done, statistically by the analysis of variance technique using the MSTAT–C statistical package programmed as described by a procedure of Gomez and Gomez (1991).Lest significant differences test (LSD) at 5 and 1 % level of probability used to compare between treatment means according toSnedecor and Cochran (1980).

III. RESULTS AND DISCUSSIONS

3.1. Performance of cultivars:

Average of plant height (cm), leaves number/plant and leaf area (cm^2), number of achenes/head, head diameter (cm), 1000 achene weight (gm) and achene yield (Kg/ha) significantly differed in studied sunflower cultivars in both seasons as presented in Tables 1 and 2. However, steam diameter (cm) insignificantly affected in both seasons.

It could state that tallest plants (136.6, 138.4 cm), highest leaves number/plant (21.7, 21.7), number of achenes /head (869.7, 881.0 achenes /head) and highest values of head diameter (17.1, 16.86 cm) were obtained from MS.sirena F1 genotype in the first and second seasons, respectively. Meanwhile, Biest Brima genotype recorded the highest values of leaf area (178.8, 177.8 cm^2)in the first and second seasons, respectively. However, Nsovak genotype recorded the lowest values of plant height (cm), leaves number/plant and leaf area (cm^2), number of achenes/head and head diameter in both seasons. Whereas, the highest weight of 1000 seed (56.31, 56.40 g) and highest seed yield per hectare (3397.8, 3402.4 kg/ha) were recorded from Nsovak genotype in the first and second seasons, respectively. The results clearly indicated that Biest Brima genotyperecorded the lowest seed yield/ha and MS.sirena F1 genotype the lowest of 1000 achene weight in both seasons. The differences between studying genotypes in seed yields may attributed to the genotype genetics.The highest percentages of seed oil recorded in Hysun-33, SMH-0917 and SMH-0907. Overall Hysun-33, SMH-0917 and SMH-0907 hybrids achieved improved for plant height, head diameters, achene numbers/head and achenes yield/ha **(Iqrasan et al., 2017)**. The variances in sunflower hybrids seed yield and its components may be due to the genetic factors.These results in linewith those reported by**Ergen and Saglam (2005) and Smiderle et al. (2007).**Many investigators such as **Ali et al. (2012), Parvender et al. (2014), Gul and Kara (2015) and Nasim et al. (2017),** reported similar observations.

3.2. Effect of nitrogen fertilizer rates:

Average of plant height (cm), stem diameter (cm), leaves number/plant, leaf area (cm^2), number of achenes/head, head diameter (cm), 1000 achene weight (gm) and achene yield (Kg/ha) as affected by nitrogen fertilizer rates significantly affected as presented in Tables 1 and 2. Increasing nitrogen fertilizer rates from 72, 120 and 168 Kg N/ha significantly increased plant height (cm), stem diameter (cm), leaves number/plant, leaf area (cm^2), number of achenes/head, head diameter (cm), 1000 achene weight (gm) and achene yield (Kg/ha) in both seasons. Increasing nitrogen fertilizer rates up to 168 Kg N/ha produced the tallest plants (136.8, 137.5 cm), thickness stem (1.83, 1.98 cm), highest number of leaves/plant (22.2, 22.5), highest values of leaf area (191.7, 186.1 cm^2), maximum number of achenes/head (845.5, 860.4), head diameter (17.68, 17.97 cm), weight of 1000 achene (55.04, 55.93 g) and achene yield (3537.9, 3530.7 Kg/ha) Increasing nitrogen fertilizer rates up to 168 Kg N/ha in the first and second seasons, respectively. It could observed that increasing nitrogen fertilizer from 72 to 168 Kg N/ha significantly increased seed yield by 12.0 and 11.6 % in both seasons, respectively. Studied trials increased due to increases of nitrogen fertilization levels, this increase could be the effect of nitrogen encouraging the growth, seed yield and its attributes. Nitrogen fertilization significantly affected growth, physiological and metabolic procedures of sunflower **(Massignam et al., 2009)**. Growth trails, seed yield and its attributes increased with increases in nitrogen fertilizer rates due to the role of nitrogen in motivating development. Nitrogen fertilizer is an essential of the proteins and nucleotides that are vital to the metabolic purpose of sunflower **(Salisbury and Ross, 1994).** Nevertheless, increasing nitrogen fertilizer rates was reduced the oil percentage **(Nasim et al., 2012).** A reduction in growth and less translocation of photo-assimilates from source to sink organs of the sunflower crop due to deficiency of nitrogen fertilizer **(Nasim et al., 2017).** Dry matter, seed yield and its attributes improved due to increases in nitrogen fertilizer levels, but oil percentage was **decreased (Hussain et al., 2011 and Naseem et al., 2011).** A significant effect on plant height, biological and seed yields/ha and seed oil percentage with increasing nitrogen fertilizer rates. Increasing nitrogen fertilizer rates of 225 kg/ha nitrogen recorded the most of study traits **(Mollashahi et al., 2013).** Others **Osman and Awed (2010), Süzer (2010),** reported similar results. **Ali et al. (2011), (2012), (2013) and (2014) as well as Awais et al. (2015).**

Table.1: Average of plant height (cm), steam diameter (cm), leaves number/plant and leaf area (cm^2) as affected by sunflower cultivars, nitrogen fertilizer rates and hill spaces in both seasons.

Characters / Treatments	Plant height (cm)		Stem diameter (cm)		Leaves number/plant		Leaf area (cm^2)	
	2014	2015	2014	2015	2014	2015	2014	2015
A-Sunflower Cultivars:								
Nsovak	129.4	129.1	1.77	1.69	19.6	20.1	160.4	160.0
MS.sirena F1	136.6	138.4	1.75	1.81	21.7	21.7	169.4	165.4
Biest Brima	133.5	135.2	1.81	1.89	21.4	21.7	178.8	177.8
F. test	*	*	N.S	N.S	*	*	*	*
LSD at 5%	0.5	0.7	--	--	0.7	0.6	3.0	3.1
B-Nitrogen fertilizer rates:								
72 kg N/ha	127.9	129.0	1.53	1.66	19.4	19.8	146.5	149.1
120 kg N/ha	134.9	135.8	1.80	1.81	21.2	21.3	171.2	168.1
168 kg N/ha	136.8	137.5	1.83	1.98	22.2	22.5	191.7	186.1
F. test	*	*	*	*	*	*	*	*
LSD at 5%	0.5	0.7	0.07	0.05	0.7	0.6	3.0	3.1
C-Hill spacing:								
15 cm apart	141.5	142.2	1.40	1.54	20.8	20.9	149.4	150.2
20 cm apart	133.9	125.3	1.77	1.83	20.9	21.2	167.2	165.7
25 cm apart	124.2	124.8	2.00	2.07	21.0	21.6	191.9	187.3
F. test	*	*	*	*	*	N.S	*	*
LSD at 5%	0.8	0.7	0.07	0.06	0.6	--	4.3	2.7
D-Interaction F-Test:								
A x B	*	*	N.S	N.S	N.S	N.S	*	*
A x C	*	*	N.S	N.S	*	*	*	*
B x C	*	*	N.S	N.S	N.S	N.S	*	*
A x B x C	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

3.3. Effect of hill spacing:

Means of plant height (cm), stem diameter (cm), leaf area (cm^2), number of achenes/head, head diameter (cm), 1000 achene weight (gm) and achene yield (Kg/ha) as affected by nitrogen fertilizer rates significantly affected as presented in Tables 1 and 2 in both seasons except leaves number/plant was significantly affected only in the first season. Increasing hill spacing from 15, 20 and 25 cm significantly increased stem diameter (cm), leaves number/plant, leaf area (cm^2), number of achenes/head, head diameter (cm) and 1000 achene weight (gm), but, decreased plant height and achene yield (Kg/ha) in both seasons. Increasing hill spacing from 15, 20 and 25 cm produced thickness stem (2.00, 2.07 cm), highest number of leaves/plant (21.0, 21.6), highest values of leaf area (191.9, 187.3 cm^2), maximum number of achenes/head (894.1, 907.8), head diameter 18.30, 18.31 cm) and weight of 1000 achene (57.69, 57.88 g). However the lowest stem diameter (cm), leaves number/plant, leaf area (cm^2), number of achenes/head, head diameter (cm) and 1000 achene weight (gm) were produced from less hill space (20 cm). The tallest plants (141.5, 142.2 cm) and highest and achene yield (3629.6, 3649.6 Kg/ha) were produced from 20cm hill spacing.

Table.2: Average of number of achenes/head, head diameter (cm), 1000 achene weight(gm) and achene yield (Kg/ha) as affected by sunflower cultivars nitrogen fertilizer rates and hill spaces in both seasons.

Characters / Treatments	Number of achenes/head		Head diameter (cm)		1000 achene weight		Achene yield/ kg/ha	
	2014	2015	2014	2015	2014	2015	2014	2015
A-Sunflower Cultivars:								
Nsovak	751.3	774.5	15.33	15.46	56.31	56.40	3397.8	3402.4
MS.sirena F1	869.7	881.0	17.10	16.86	48.89	49.21	3359.5	3288.9
Biest Brima	806.9	812.7	17.00	17.26	52.31	53.40	3293.4	3297.9
F. test	*	*	*	*	*	*	*	*
LSD at 5%	15.0	12.9	0.25	0.07	0.65	0.95	28.3	25.90
B-Nitrogen fertilizer rates:								
72 kg N/ha	768.4	792.4	15.31	15.39	48.91	48.86	3113.2	3122.1
120 kg N/ha	814.1	815.4	16.43	16.24	53.58	54.22	3399.6	3336.5
168 kg N/ha	845.5	860.4	17.68	17.96	55.04	55.93	3537.9	3530.7
F. test	*	*	*	*	*	*	*	*
LSD at 5%	15.0	13.0	0.25	0.07	0.65	0.95	28.3	25.9
C-Hill spacing:								
15 cm apart	732.3	741.1	14.64	14.64	47.07	47.80	3629.6	3649.6
20 cm apart	801.5	819.3	16.64	16.65	52.74	53.33	3330.2	3330.5
25 cm apart	894.1	907.8	18.30	18.31	57.69	57.88	3029.5	3070.7
F. test	*	*	*	*	*	*	*	*
LSD at 5%	15.7	13.2	0.32	0.11	1.01	1.02	34.2	23.5
D-Interaction F-Test								
A x B	N.S	N.S	*	*	N.S	N.S	*	*
A x C	*	*	*	*	*	*	*	*
B x C	*	*	*	*	N.S	N.S	*	*
A x B x C	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

The shortest plants and lowest achene yield kg/ha was obtained from wide space (25 cm). The increases of these characters may be due to the sufficiency of environmental conditions reflected less competition between plants in wide spacing due to increases in light penetration within sunflower canopies that improved assimilation rate and oil creation. Plant population density significantly differed from plant height, the diameters of the stem and head, the weight of achenes, weight of 100-seed and seed yield ha. The highest of seed yield/ha obtained by plant density of 48000 plants/ha in studying seasons (**Radwan et al., 2013)**. The wider plant spacing of 25 cm seems to a good compromise between the highest seed yield/fad and good acid composition of the oil (**Abd El-Satar et al., 2017).** Similarly, others reported accordance results such as **Osman and Awed (2010), Süzer (2010), Ali et al. (2011), (2012), (2013), (2014) and Nasim et al. (2017).**

3.4. Interaction Effects:

3.4.1. Interaction between cultivars and nitrogen fertilizer rates:

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Cultivars and nitrogen fertilizer rates interaction significantly affected plant height (cm), leaf area (cm^2), head diameter (cm) and achene yield (kg/ha), however, insignificantly affectedsteam diameter (cm), leaves number/plant, number of achenes/head and 1000 achene weight (gm) in both seasons as presented in Tables 1 and 2. The results graphically illustrated in Figs. 1 the effect of the interaction between genotype sand nitrogen fertilization rates on plant height, the results clearly showed that sown MS.sirena F1 genotype and addition of nitrogen fertilizer at168 kg N/ha recorded the tallest plants (140.2, 141.5 cm). The highest values of leaf area (217.62, 209.88cm^2) and the thick heads (18.53, 18.98 cm) were obtained from sown Biest Brima genotype when fertilized with 168 kg N/ha in both seasons, respectively as graphically demonstrated in Figs 2 and 3. The highest achene yield (3588.8, 3608.5 kg/ha)was obtained from sown Nsovak genotype at higher nitrogen rates 168 N/ha as graphically illustrated in Fig 4 in both seasons, respectively.While, the lowermost values from plant height (cm), leaf area (cm^2), head diameter (cm) and achene yield (kg/ha) were recorded from sown Nsovak genotype when fertilized with the lowest nitrogen fertilizer (72 kg/ha) in both seasons. Highest seed yield/ha produced from sown Hysun-38 by nitrogen fertilizer supply at the rate of 180 kg N/ha **(Nasim et al., 2017)**

3.4.2. Interaction between cultivars and hill spacing:

The interaction between cultivars and hill spacing significantly affected plant height (cm), leaf area (cm^2), achene numbers/head, diameter of head (cm), 1000-achene weight (g) and achene yield (Kg/ha), except, stem diameter (cm) and leaves number/plant, in both seasons as presented in Tables 1 and 2. The results graphically illustrated in Fig. 5 indicated that the effect of the interaction cultivars and hill spacing on plant height, the tallest plants was produce from sown MS.sirena F1 genotype at hill spacing of 15 cm (145.1, 147.0 cm) in both seasons. The shortest plant was obtained from sownNsovak genotype at wider hill spacing (25.0 cm).

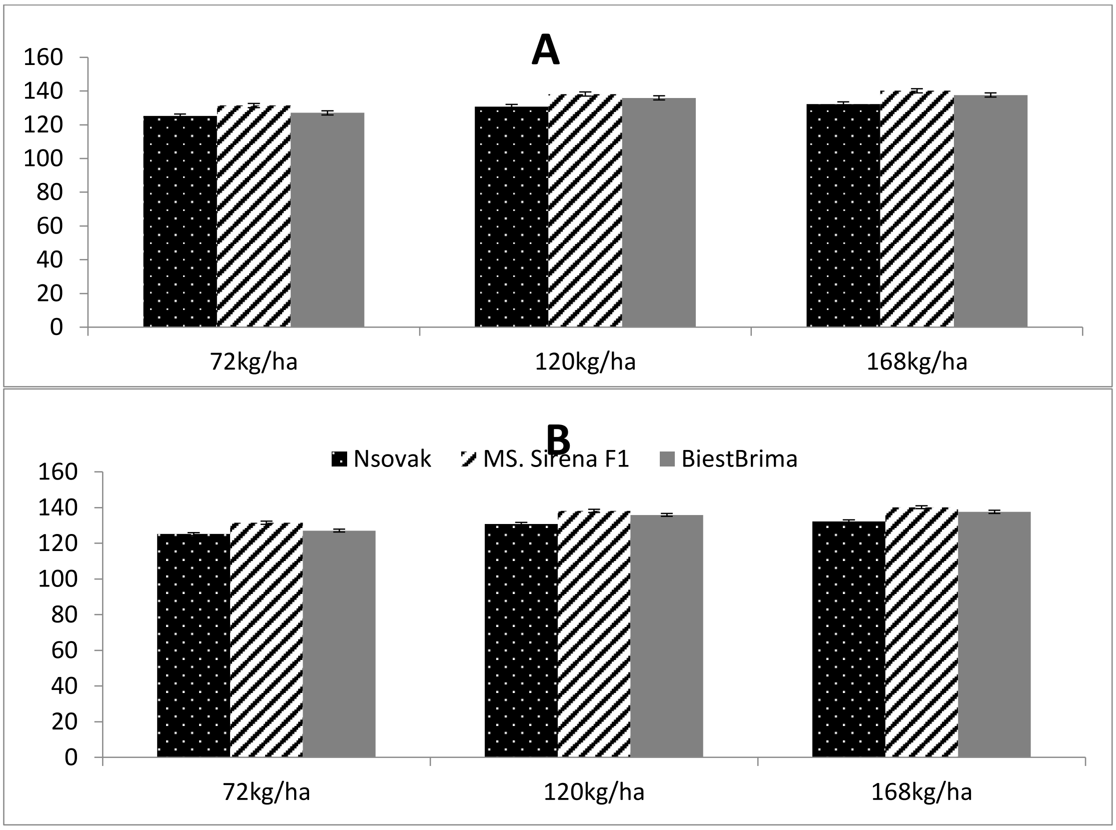

Fig. 1: Average of plant height (cm) as affected by interaction between sunflower cultivars and nitrogen fertilization during two season (A) 2014 and (B) 2015.

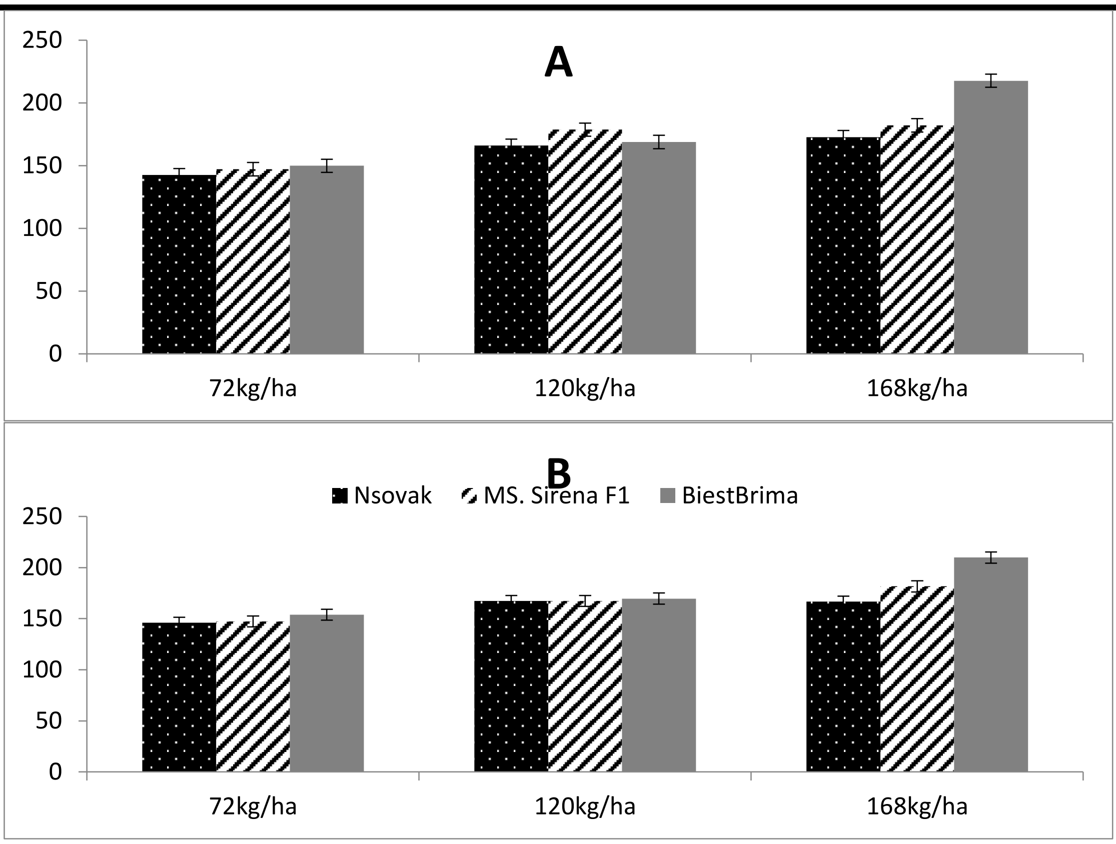

Fig.2: Average of leaf area (cm^2) as affected by interaction between sunflower cultivars and nitrogen fertilization during two season (A) 2014 and (B) 2015.

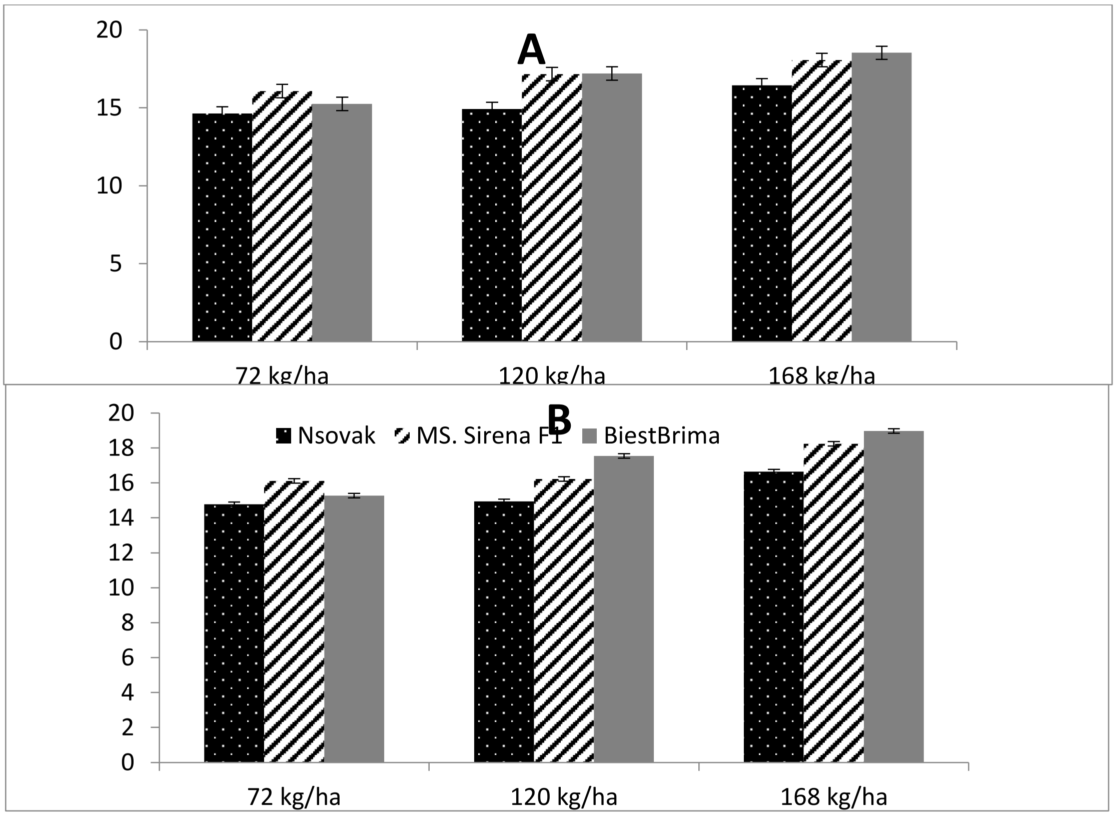

Fig. 3: Average of head diameter (cm) as affected by interaction between sunflower cultivars and nitrogen fertilization during two season (A) 2014 and (B) 2015.

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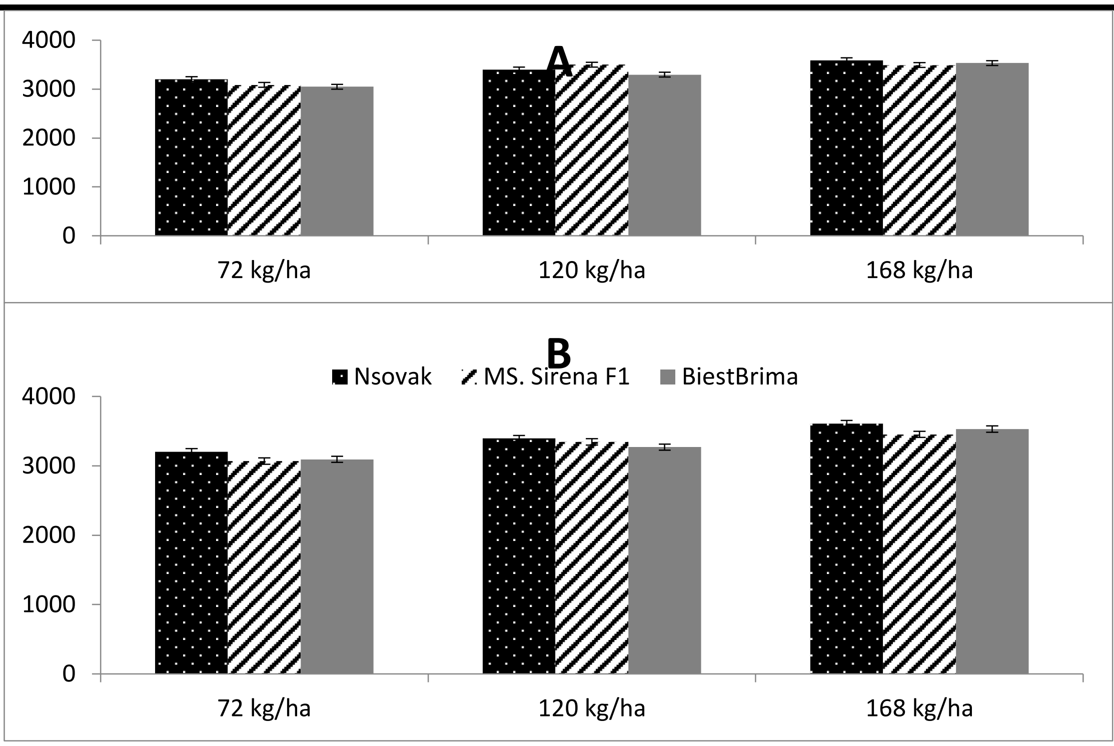

Fig. 4: Average of Achene yield/ ha (kg) as affected by interaction between sunflower cultivars genotypes and nitrogen fertilization during two season (A) 2014 and (B) 2015.

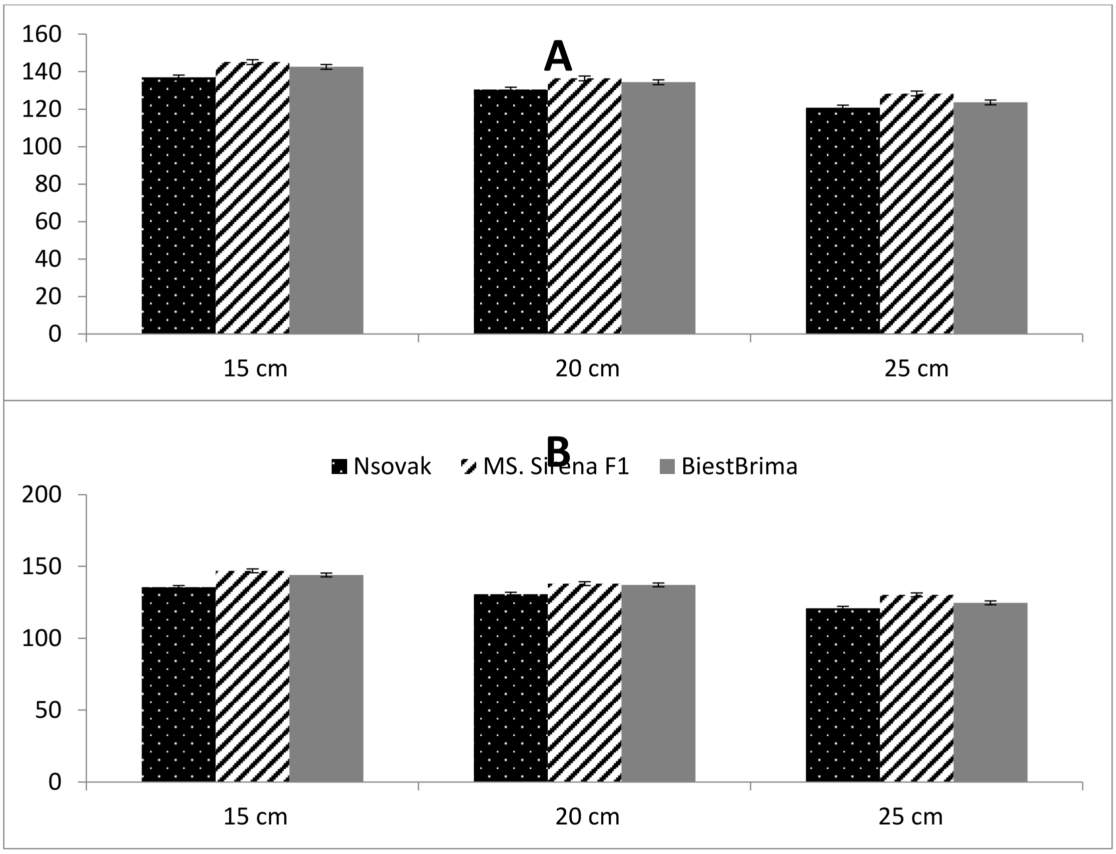

Fig. 5: Average of plant height (cm) as affected by interaction between sunflower cultivars genotypes and hill spacing during two season (A) 2014 and (B) 2015.

Data presented in Figs. 6, 7 and 8 showed the interaction between cultivars and hill spacing effects on leaf area and number of achenes/head. In both seasons. The results indicated that the highest values of leaf area (209.31,

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196.61 cm^2),number of achenes/head (1042.7, 1054.8), head diameter (19.33, 18.96 cm) were recorded from sown MS.sirena F1 at wider hill spacing (25 cm) in both seasons, respectively.In addition, the highest weight of 1000 achenes (60.7, 61.2 g)from sown Nsovak genotype graphically illustrated in Fig. 9. However, the lowest values of plant height (cm), leaf area (cm^2), achene numbers/head, diameter of head (cm), 1000-achene weight (g) were produced from sown Nsovak genotype at wider hills (25 cm). Results demonstrated in Fig. 10 clearly indicated that maximum achene yield (1558.7, 1520.9 kg/ha) produced from sown Nsovak genotype at dense hills (15 cm) in both seasons, respectively. The less yield obtained from sown Biest Brima genotypeat wider hills (25 cm). In view of the superiority, over 22.5 cm hills spacing for both Hysun-38 hybrid recorded the high productivity. FH-331 hybrid recorded the lowest in seed yield compared to with hysun-38 hybrid **(Ali et al., 2011)**.

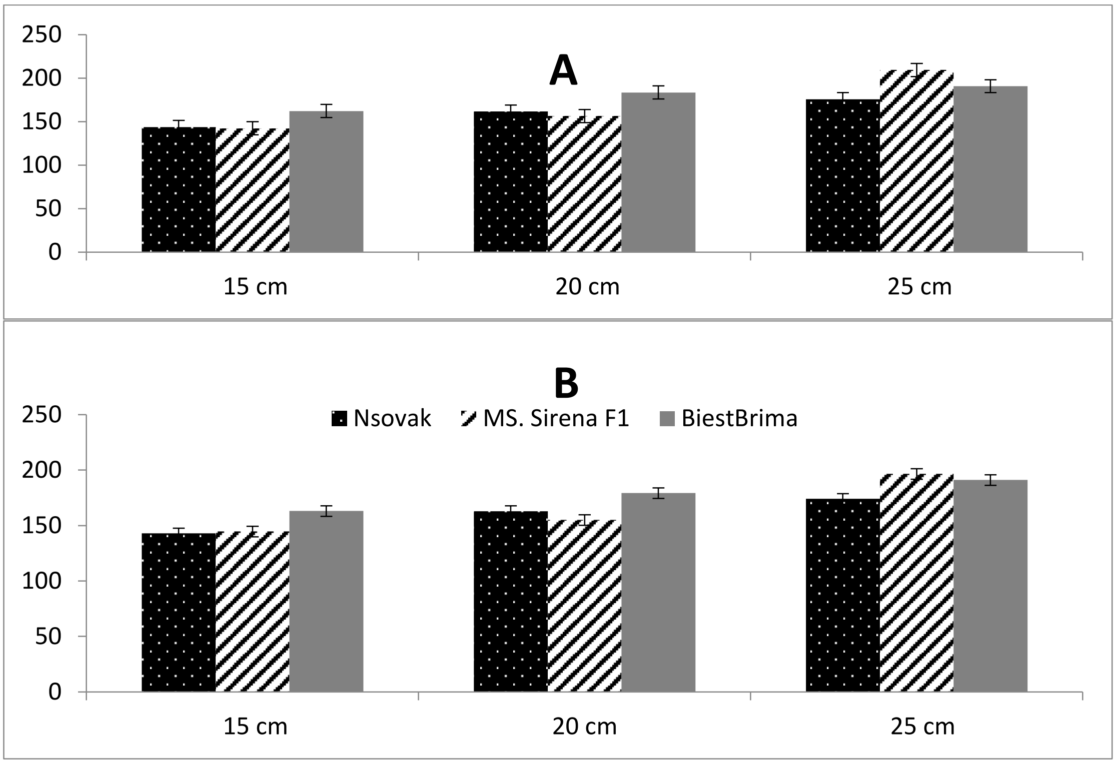

Fig. 6: Average of leaf area (cm^2) as affected by interaction between sunflower cultivars genotype and hill spacing during two season (A) 2014 and (B) 2015.

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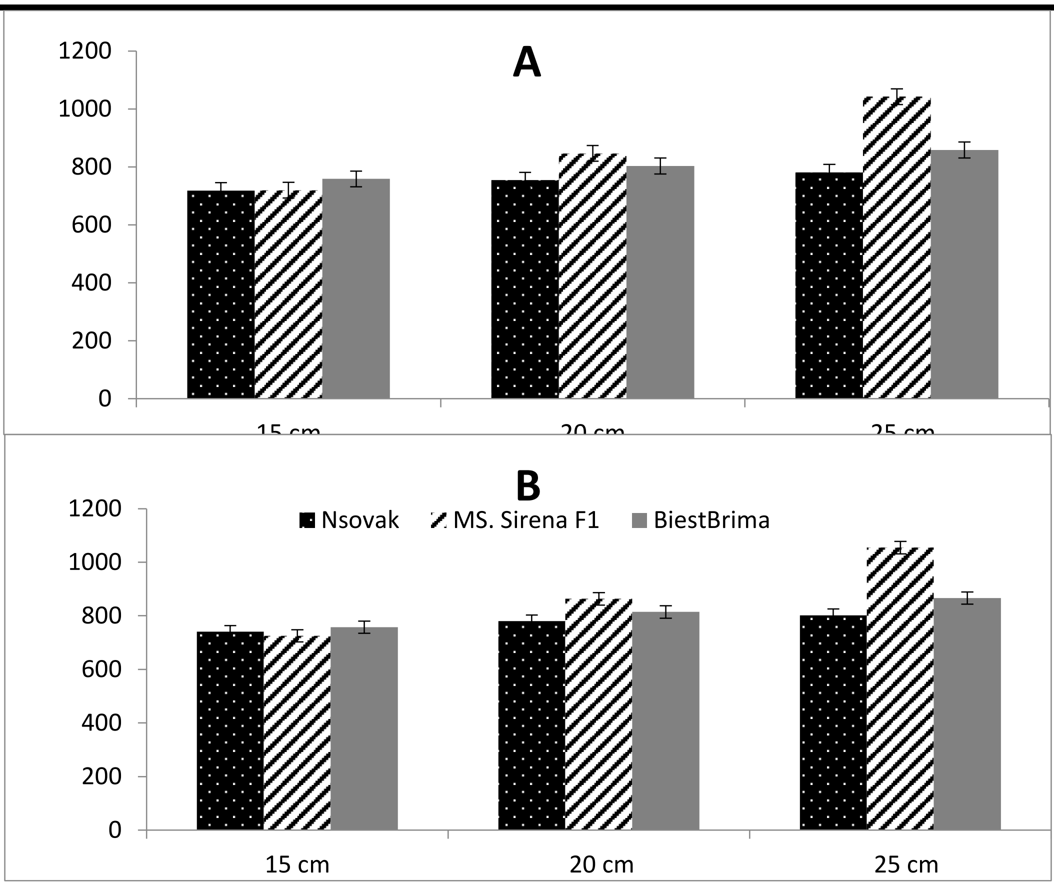

Fig. 7: Average of number of achenes /head as affected by interaction between sunflower cultivars and hill spacing during two season (A) 2014 and (B) 2015.

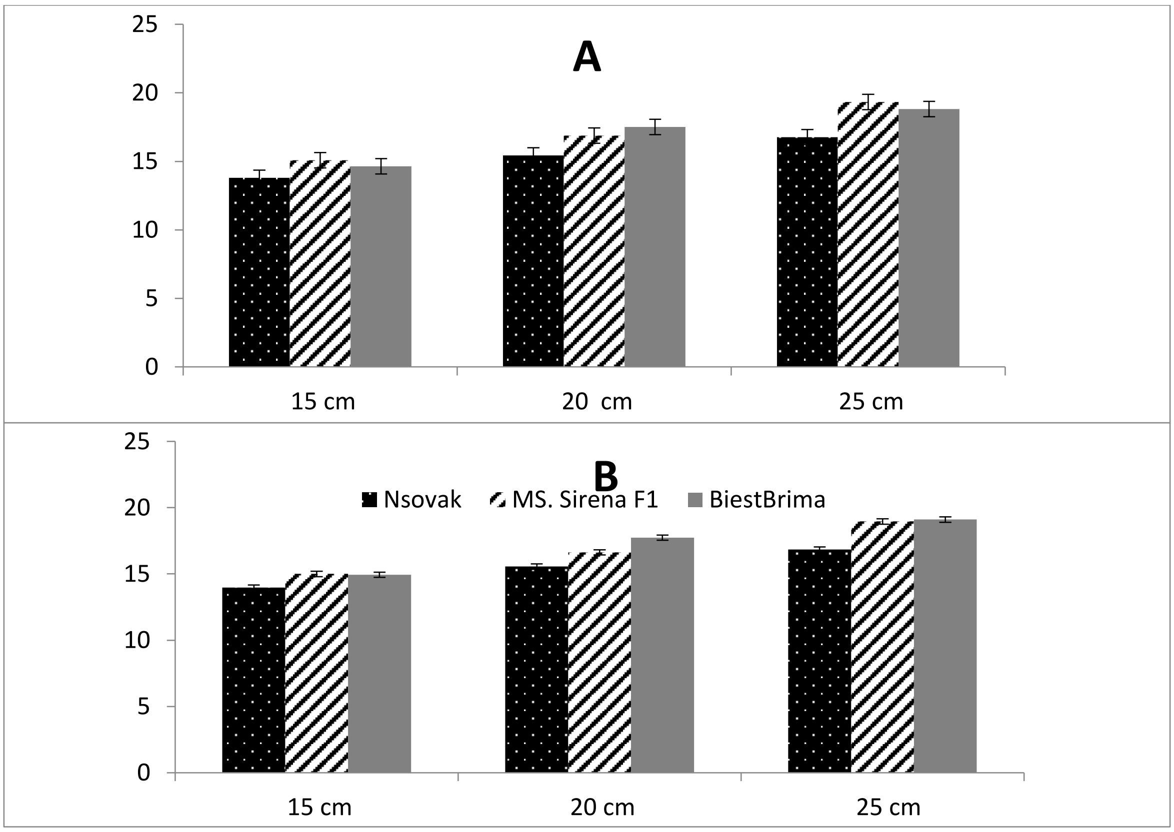

Fig. 8: Average of head diameter (cm) as affected by interaction between sunflower cultivars and hill spacing during two season (A) 2014 and (B) 2015.

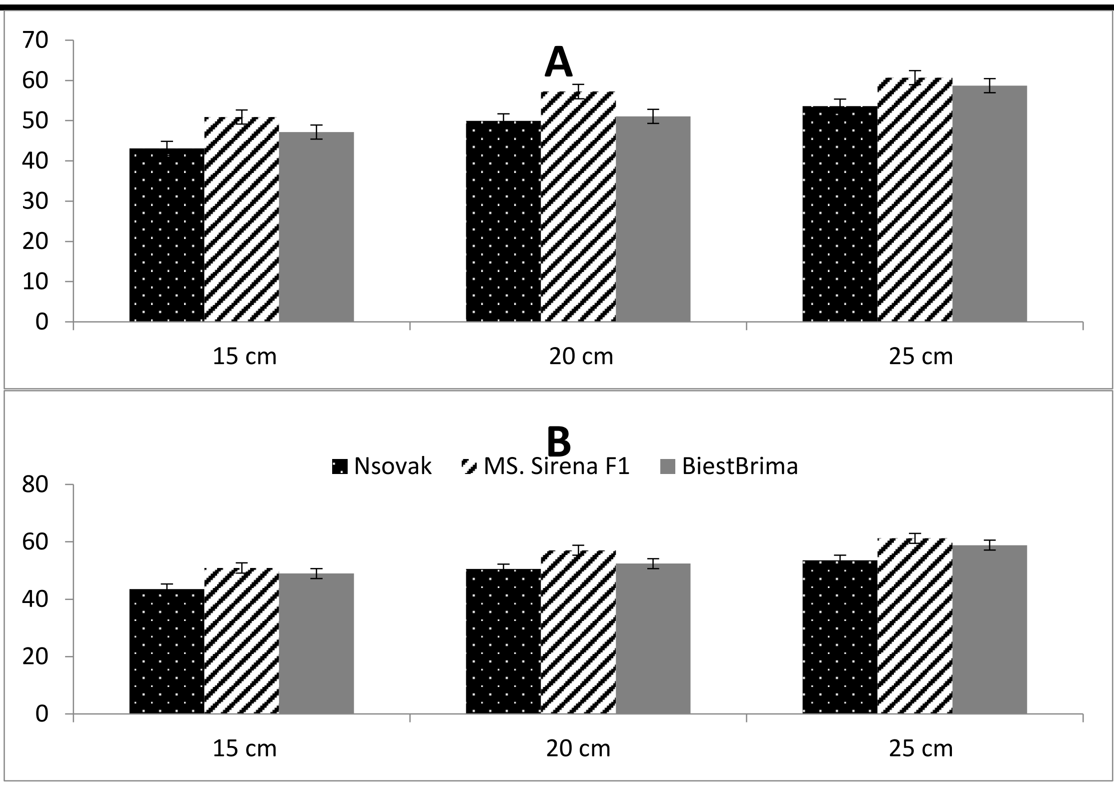

Fig. 9: Average of thousand achenes weight (g) as affected by interaction between sunflower cultivars and hill spacing during two season (A) 2014 and (B) 2015.

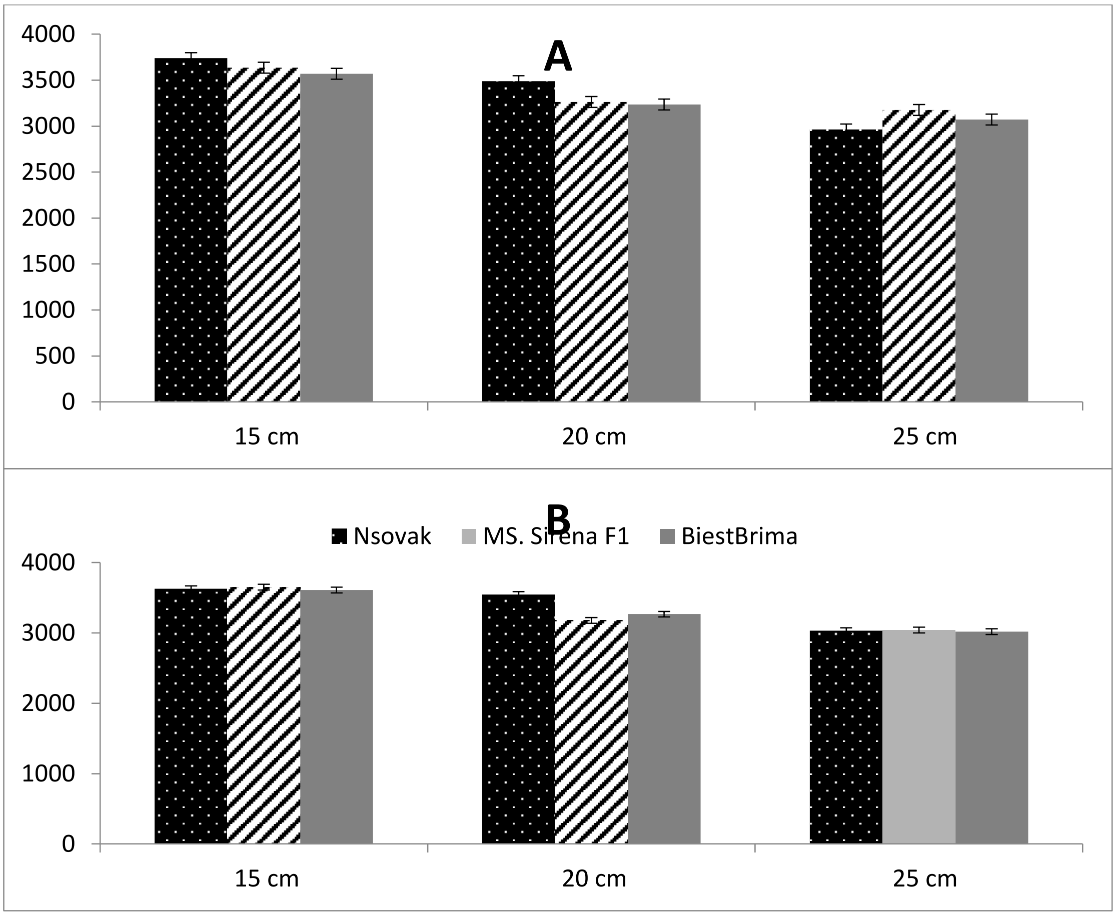

Fig. 10: Average of achene yield/ ha (kg) as affected by interaction between sunflower cultivars and hill spacing during two season (A) 2014 and (B 2015

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3.4.3. Interaction between nitrogen fertilizer rates and hill spacing:

The interaction between nitrogen fertilizer rates and hill spacing significantly affected plant height (cm),leaf area (cm^2), achene numbers/head, head diameter (cm), and achene yield (kg/ha), except, and stem diameter (cm), leaves number/plant and 1000 achene weight (g) in both seasons as presented in Tables 1 and 2. The results graphically illustrated in Fig. 11 indicated the interaction between nitrogen fertilizer rates and hill spacing effects on plant height, the tallest plants was produce from sown increasing nitrogen fertilizer up to168 kg N/haat dense hill spacing of 15 cm (144.7, 145.9 cm) in the first and second seasons, respectively. The results graphically demonstrated in Fig 12, 13 and14 showed that thehighest values of leaf area/plant (217.0, 209.5 cm^2), number of achenes/head (936.8, 952.5) head diameter and (19.2, 19.6 cm)were obtained from nitrogen fertilizer up to 168 kg N/ha and increasing hill spacing to 25 cm in both seasons, respectively. However, the lowest values of leaf area/plant (cm^2), number of achenes/head and head diameter were produced from reducing fertilizer to 72 kg N/ha at dense hill spacing of 15 cm in both seasons. In addition, the results in Fig. 15 showed that the highest achene yield (3903.4,3914.4 kg/ha) produced from increasing nitrogen fertilizer up to 168 kg N/ha and decreasing hill spacing to 15 cm between plants in both seasons, respectively. Sown sunflower plants at25 cm hill spacing and fertilization of nitrogen at a rate of 150-200 kg N/ha had maximized seed and oil yields/ha **(Al-Thabet, 2006).** Seed yield in addition, its attributes significantly affected by nitrogen fertilizer and plant population densities on of different-height sunflower hybrids **(Süzer, 2010).** Hill spacing at 10 cm and fertilization of nitrogen at a rate of 60 kg N/fed maximized seed and oil yields/fed **(Osman and Awed, 2010).** Higher seed yield/ha recorded from sown at 20 cm hill spacing and nitrogen fertilizer at a rate of 125 kg N/ha addition **(Ali et al., 2012)**. Seed yield and photosynthesis active increase radiation observation when nitrogen fertilization rates and plant density increased. Maximum seed yield recorded from increasing plant population densitywith highest nitrogen fertilizer levels **(Ali et al., 2013).**Plant spacing, nitrogen fertilization levels and genotypes as well as interactions in both seasons and their combined analysis significantly influenced yield and quality traits **(Abd El-Satar et al., 2017).**

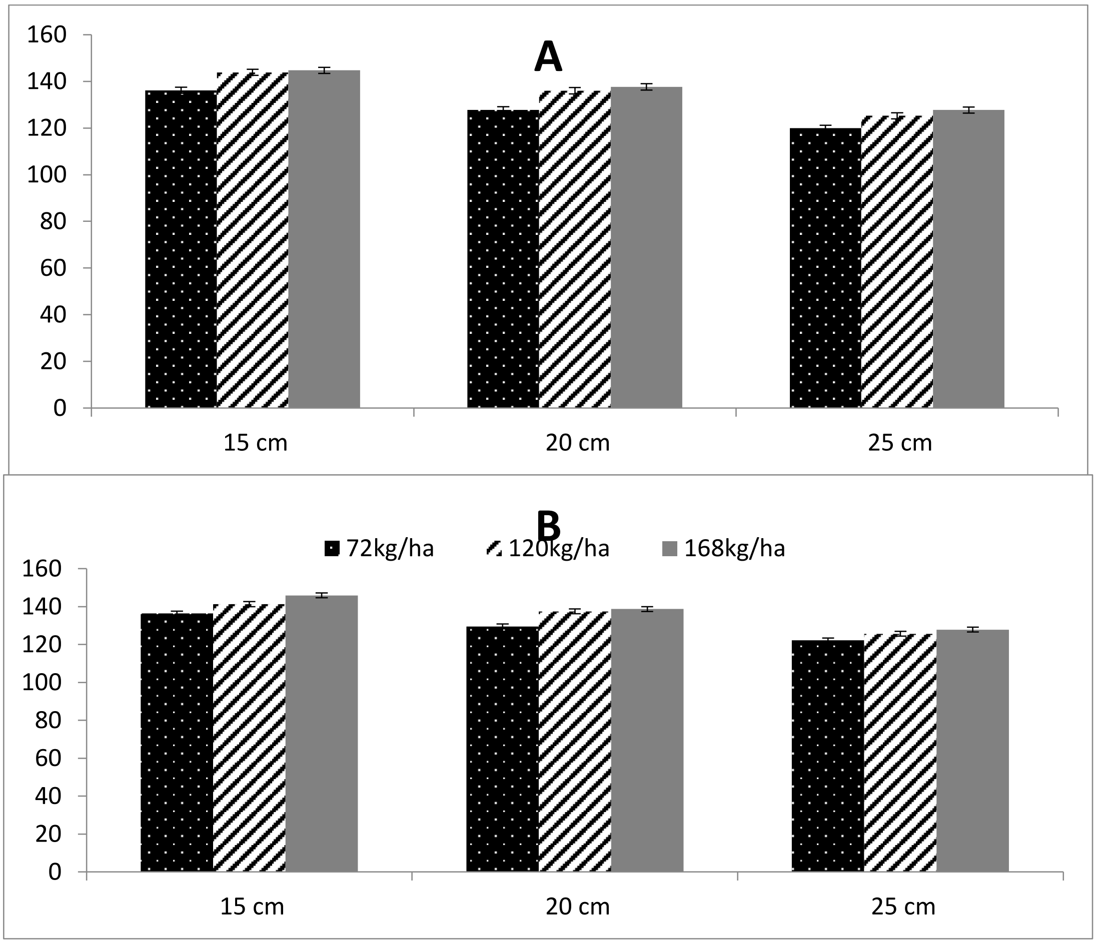

Fig. 11: Average of plant height (cm) as affected by interaction between nitrogen fertilizer rates and hill spacing during two season (A) 2014 and (B) 2015.

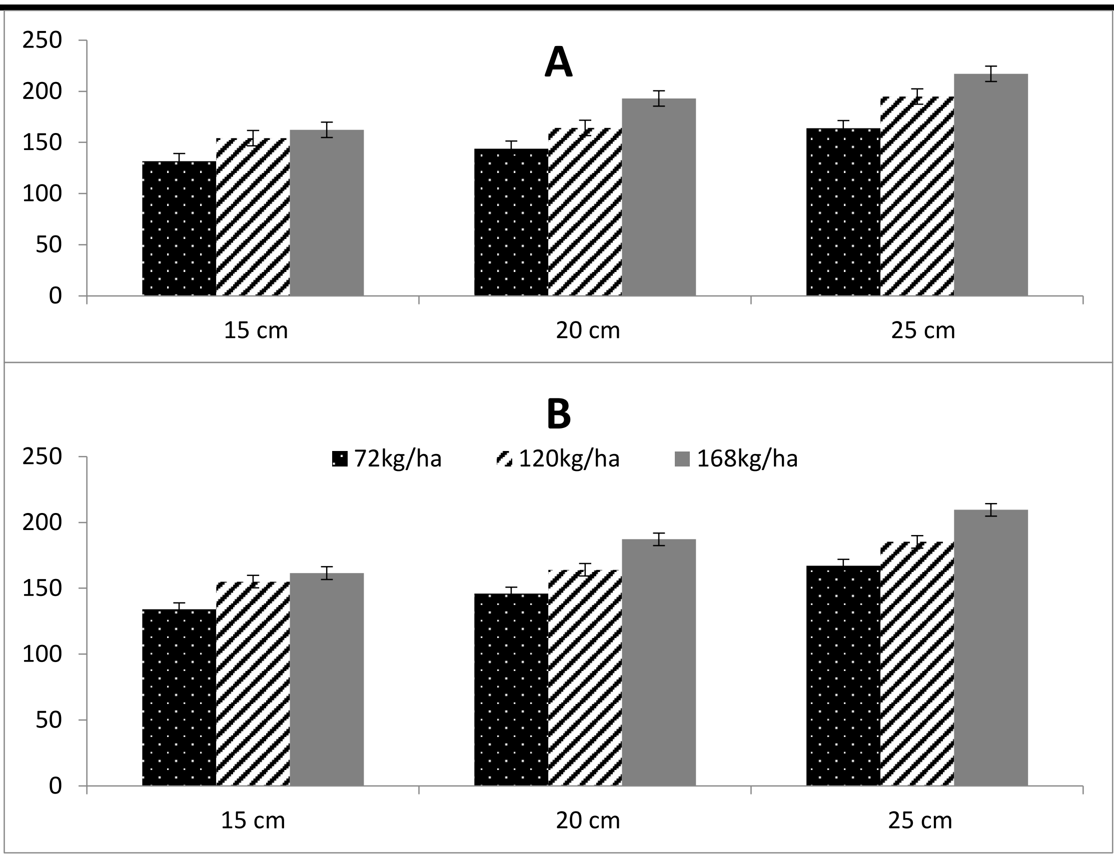

Fig. 12: Average of Leaf area (cm^2) as affected by interaction between nitrogen fertilization and plant density during two season (A) 2014 and (B) 2015.

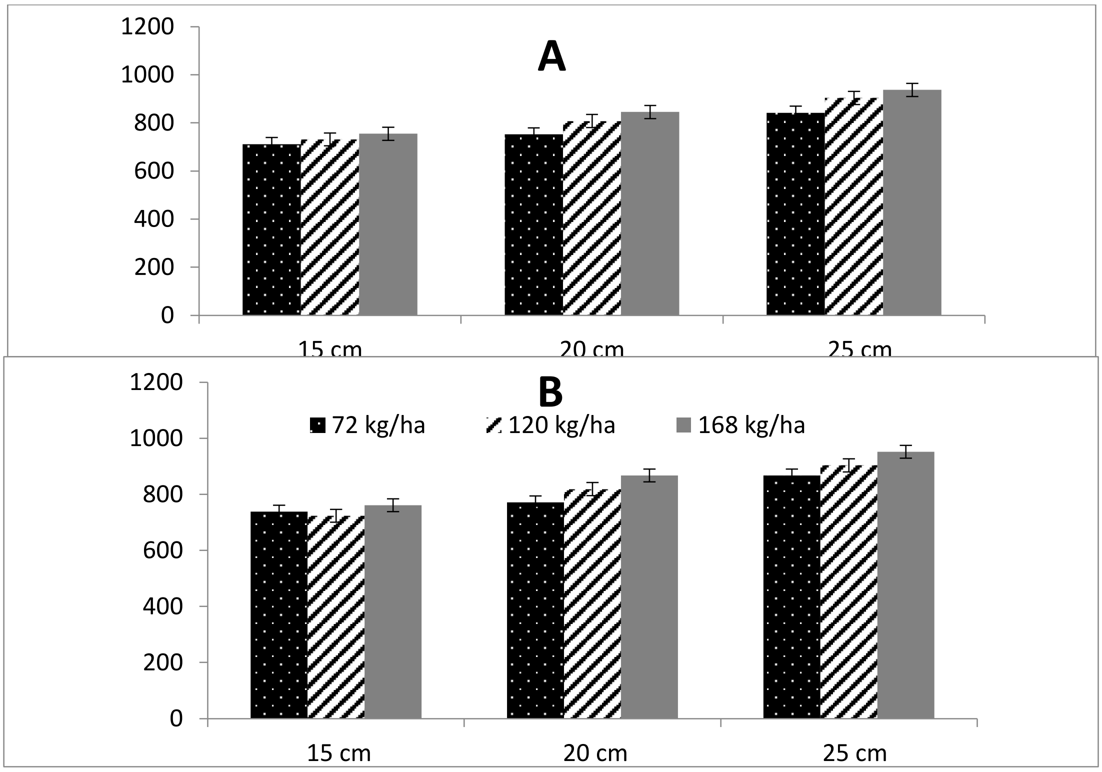

Fig.13: Average of number of achenes/head as affected by interaction between nitrogen fertilization and hill spacing during two season (A) 2014 and (B) 2015.

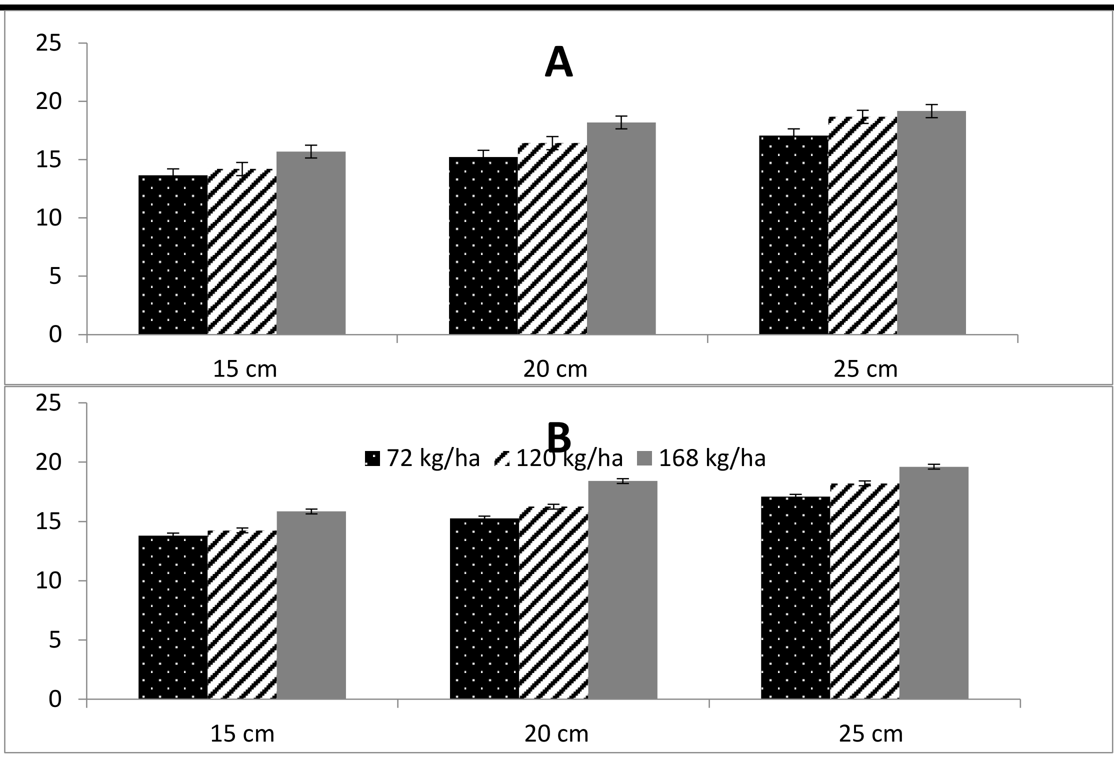

Fig.14: Average of head diameter (cm) as affected by interaction between nitrogen fertilization and plant density during two season (A) 2014 and (B) 2015.

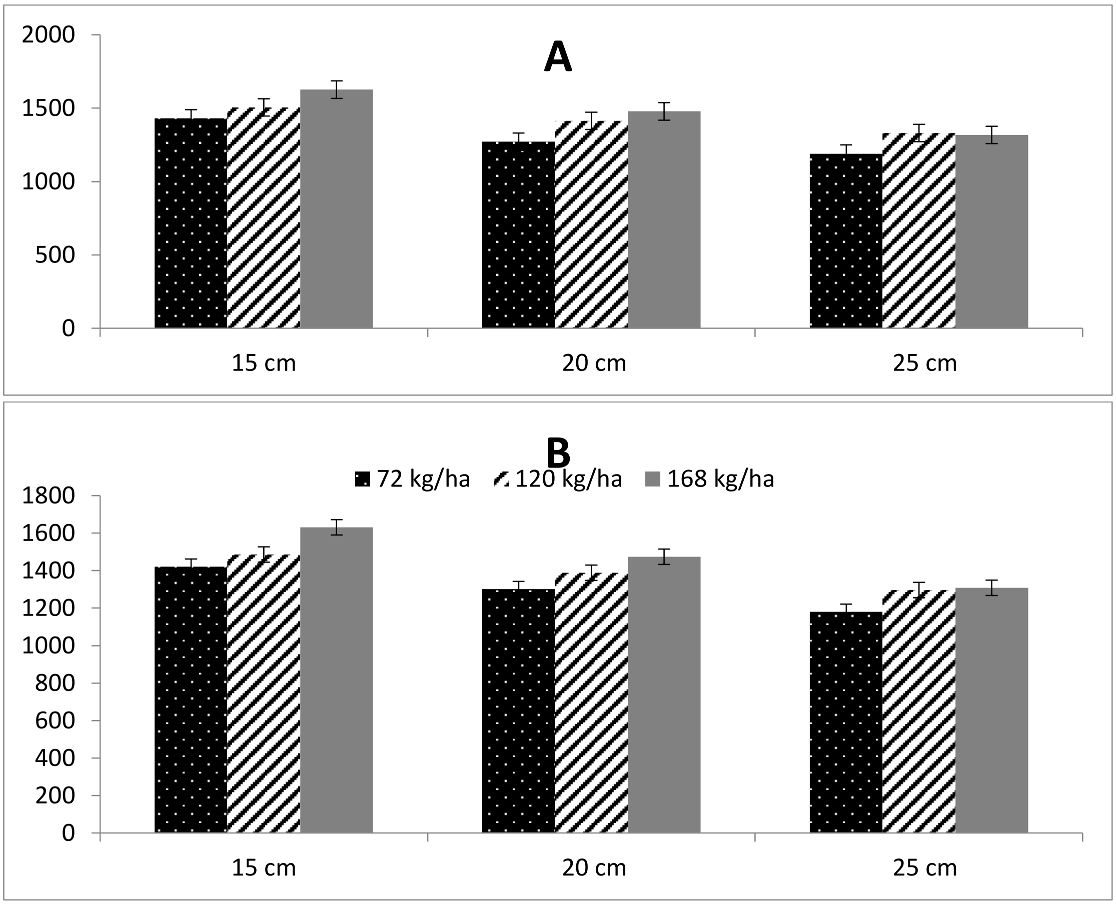

Fig.15: Average of Achene yield/ fed (kg) as affected by interaction between nitrogen fertilization and hill spacing during two season (A) 2014 and (B)2015.

3.4.4. Interaction between cultivars, nitrogen fertilizer rates, and hill spacing:

The interaction between cultivars, nitrogen fertilizer rates, and hill spacing plant height (cm), stem diameter (cm), leaves number/plant, leaf area (cm^2), number of achenes/head, head diameter (cm), 1000 achene weight (gm) and achene yield (Kg/ha) insignificantly affected as presented in Tables 1 and 2 in both seasons.

IV. CONCLUSION

It could concluded that the increases in nitrogen fertilizer rates from 72 to 168 Kg N/ha and sown Nsovak genotype at dense hill spacing of 15 cm between plants maximized seed yield per unite area under the environmental conditions of Egypt.

REFERENCES

[1] 95and fatty acid compositions for some sunflower genotypes to plant spacing and nitrogen fertilization. Information Processing in Agriculture. https://doi.org/10.1016/j.inpa.2017.05.003

[2] Ali, A., M. Afza, I. Rasool, S. Hussain and M. Ahmad 2011. Sunflower (Helianthus annuus L.) hybrids performance at different plant spacing under agro-ecological conditions of Sargodha, Pakistan. International Conference on Food Engineering and Biotechnology IPCBEE, IACSIT Press, Singapore 9: 317-322.http://www.ipcbee.com/vol9/61-B30012.pdf

[3] Ali, A., A. Ahmad, T. Khaliq, M. Afzal and Z. Iqbal 2012. Achene Yield and Quality Response of Sunflower Hybrids to Nitrogen at Varying Planting Densities. International Conference on Agriculture, Chemical and Environmental Sciences (ICACES'2012) Oct. 6-7, 2012 Dubai (UAE) pp. 73-77.http://psrcentre.org/images/extraimages/48%2010 12069.pdf

[4] Ali, A., A. Ahmed, T. Khaliq, A. Ali and M. Ahmed 2013. Nitrogen Nutrition and Planting Density Effects on Sunflower Growth and Yield: A Review. Pakistan Journal of Nutrition, 12(12):1024-1035.http://scialert.net/abstract/?doi=pjn.2013.1024. 1035

[5] Ali, A., A. Ahmad, T. Khaliq, M. Afzal, Z. Iqbal and R. Qamar 2014. Plant Population and Nitrogen Effects on Achene Yield and Quality of Sunflower (Helianthus Annuus L.) Hybrids. International Conference on Agricultural, Environmental and Biological Sciences (AEBS-2014) April 24-25, 2014, Phuket (Thailand).http://iicbe.org/upload/4834C414001.pdf

[6] Allam, A.Y., G.R. El-Nagar and A.H. Galal 2003. Response of two sunflower hybrids to planting dates and densities. Acta Agronomica Hungarica, 51 (1): 25-35.http://akademiai.com/doi/abs/10.1556/AAgr.51.2 003.1.4?journalCode=014

[7] A.O.A.C., 2007. Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Inc., Gaithersburg, MD, Method 04. http://www.eoma.aoac.org/

[8] Asghar, A., A. Tanveer, M.A. Nadeem, M. Tahir and M. Hussain 2007. Effect of varying planting pattern on growth, achene yield and oil contents of sunflower (Helianthus annuus L.). Pak. J. Agri. Sci., 44(3): 449-452.http://www.pakjas.com.pk/papers/290.pdf

[9] Awais, M., A. Wajid, A. Ahmad, M. F. Saleem, M. U. Bashir, U. Saeed, J. Hussain, M. and Habib-ur-Rahman 2015. Nitrogen Fertilization and Narrow Plant Spacing Stimulates Sunflower Productivity. Turk J. Field Crops, 20 (1): 99-108.http://dergipark.gov.tr/tjfc/issue/17157/179356

[10] Ergen, Y. and C. Saglam 2005. Yield and yield characters of different confectionery sunflower varieties in conditions of Tekirdag. Journal of Tekirdag Agricultural Faculty, 2(3): 221-227.https://doaj.org/article/2f16cf463bbe4dcba2753 ad802739c9a

[11] Gomez, K.A. and A.A. Gomez 1991. Statistical Procedures in Agricultural Research. John Wiley and Sons, New York, USA. http://pdf.usaid.gov/pdf_docs/PNAAR208.pdf

[12] Gul, V. and K. Kara 2015. Effects of Different Nitrogen Doses on Yield and Quality Traits of Common Sunflower (Helianthus Annuus L.). Turk J. Field Crops, 20(2):159-165.http://dergipark.gov.tr/tjfc/issue/17158/179368

[13] Hussain, S.S., F.A. Misger, A. Kumar and M.H. Baba 2011. Response of nitrogen and sulphur on biological and economic yield of sunflower (Helianthus annuus L.). Res. J. Agri. Sci. 2: 308-310.http://dergipark.gov.tr/download/article-file/158983

[14] Iqrasan, Q. A., S.U Khan,S.A. Khan, A. Mehmood, Y. Bibi,A. Sher, H. Khanand M.A. Jenks2017. Sunflower (Helianthus annuus) Hybrids Evaluation for oil Quality and Yield Attributes under Spring Planting Conditions of Haripur, Pakistan. Planta Daninha, 35: e017161596. Doi: 10.1590/S0100-83582017350100003

[15] Jahangir, A.A., R.K. Mondal, K. Nada, R. S. Afroze and M. A. Hakim 2006. Response of nitrogen and phosphorus fertilizer and plantspacing on growth and yield contributing character of sunflower. Bang. J. Sci. Ind. Res., 41(1-2), 33-40.

http://www.banglajol.info/index.php/BJSIR/article/view/258
[16] Johnson, B.L. 2002. Dwarf sunflower response to row spacing, stand reduction, and
[17] defoliation at different growth stages. Canadian Journal of Plant Science 83: 319-326.http://www.nrcresearchpress.com/doi/pdf/10.4141/P02-031
[18] Killi, F. 2004. Influence of Different Nitrogen Levels on Productivity of Oilseed and Confection Sunflowers (Helianthus annuus L.) Under Varying Plant populations. International Journal of Agriculture & Biology, 6(4): 594-598.https://www.fspublishers.org/published_papers/61972_..pdf
[19] Ishfaq, M., A. Ali, A. Khaliq and M., Yaseen 2009. Algometry, agronomic traits and yield of autumn planted sunflower hybrids under varying row spacing. Pak. J. Agric. Sci. 46: 248-257.
[20] http://old.parc.gov.pk/1SubDivisions/NARCCSI/CSI/sunflower.html
[21] Osman, E.B.A. and M. M. M. Awed 2010. Response of Sunflower (Helianthus annuus l.) to Phosphorus and Nitrogen Fertilization under Different Plant Spacing at New Valley. Ass. Univ. Bull. Environ. Res. 13 (1):11-19. http://www.aun.edu.eg/arabic/society/aubfer/res2_mar_2010.pdf
[22] Massignam, A. M, S. C. Chapman, G. L. Hammer, and S. Fukai 2009. Physiological determinants of maize and sunflower (Helianthus annuus L.) grain yield as affected by nitrogen supply. Field Crops Research 113: 256–267.https://www.cabdirect.org/cabdirect/abstract/20093248881
[23] Mojiri, A and A. A. Arzani 2003. Effects of nitrogen rate and plant density on yield and yield components of sunflower J. Sci. and technology of Agric. And Natural Resources, 7 (2): 115-125.
[24] http://jstnar.iut.ac.ir/browse.php?a_id=468&sid=1&slc_lang=en
[25] Mollashahi, M., H. Ganjali and H. Fanaei 2013. Effect of different levels of nitrogen and potassium on yield, yield components and oil content of sunflower. International Journal of Farming and Allied Sciences.2(S): 1237-1240.http://ijfas.com/wp-content/uploads/2013/12/1237-1240.pdf
[26] Nasim, W., A. Ahmad, A. Wajid, J. Akhtar and D. Muhammad 2011. Nitrogen effects on growth and development of sunflower hybrids under agro-climatic conditions of Multan, Pakistan J. Bot., 43(4): 2083-2092. http://www.pakbs.org/pjbot/PDFs/43(4)/PJB43(4)2083.pdf
[27] Nasim, W., A. Ahmad, A. Bano, R. Olatinwo, M. Usman, T. Khaliq, A. Wajid, H. M. Hammad, M. Mubeen, andM. Hussain. 2012. Effect of nitrogen on yield and oil quality of sunflower (Helianthus annuus L.) hybrids under subhumid conditions of Pakistan. American Journal of Plant Science 3: 243–251.https://www.scirp.org/journal/PaperInformation.aspx?PaperID=17565
[28] Nasim, W., A. Ahmad, S. Ahmad, M. Nadeem, N. Masood, M. Shahid, M. Mubeen, G. Hoogenboom, and S. Fahad 2017. Response of sunflower hybrids to nitrogen application grown under different agro-environments. Journal of Plant Nutrition, 40(1): 82–92.
[29] http://www.tandfonline.com/doi/full/10.1080/01904167.2016.1201492?src=recsys
[30] Radwan, F.I., M.A. Gomaa, E.E. Kandil and Marim M. Homany 2013. Effect of plant density and bio fertilization on sunflower "Helianthus annuus, L" cv. Sakha53 productivity Research Journal of Agriculture and Biological Sciences, 9(6): 287-295.http://www.aensiweb.net/AENSIWEB/rjabs/rjabs/2013/287-295.pdf
[31] Page, A.L., Miller, R.H. & Keeney, D.R. (eds) 1982. Methods of Soil Analysis: Part 2, Chemical and Microbiological Properties. Agronomy Series No 9, American Society of Agronomy, Madison, WI
[32] Parvender, S., S. Virender and S. Raj 2014. Evaluating productivity potential of spring planted sunflower (Helianthus annuus) hybrids in response to sowing time under changing climate scenario. Indian Journal of Agronomy, 59(1):124-132.http://www.iospress.nl/journal/indian-journal-of-agronomy/
[33] Salisbury, F.B. and C.W. Ross 1994. Plant Physiology. Belmont, California: Wadsworth Publishing Company, California Agric. Exp. Station, 1994.https://www.abebooks.com/Plant-Physiology-F.B-Salisbury-C.W-Ross/8129079659/bd
[34] Schneiter, A.A. 1978. Non-destructive leaf area estimation in sunflower, Agronomy Journal, 70:141-142. doi:10.2134/agronj1978.00021962007000010034x
[35] Smiderle, O.J., S.R.G. Silva and D.R. Schwengber 2007. Productivity of sunflower cultivars in cerrado of roraima. produtividade de cultivares de girassol em cerrado de roraima. Documentos-Embrapa Soja 292: 67-70.http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0044-59672005000300004

[36] Snedecor G.W. and W.G. Cochran 1980. Statistical Methods, 7th Ed. The Iowa State Univ. Press, Iowa, USA. https://www.amazon.com/Statistical-Methods-Seventh-isbn-0813815606/dp/B0012S4NIE
[37] Süzer, S. 2010. Effects of nitrogen and plant density on dwarf sunflower hybrids. Helia, 33(53): 207-214. http://www.doiserbia.nb.rs/img/doi/1018-1806/2010/1018-18061053207S.pdf
[38] Weiss, E.A. 2000. oil seed crops Blackwell Sci. Led. London. 364P. ISBN: 978-0-632-05259-2. http://eu.wiley.com/WileyCDA/WileyTitle/productCd-0632052597.html

Germination and Seedling Characters as Influenced by Sunflower Hybrids, Nitrogen Fertilizer Rates and Hill Spacing

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Abstract— *In order to study the performance of some sunflower genotypes to nitrogen fertilizer rates and plant population density to germination and seedling parameters. A laboratory experiment conducted in the Agronomy Department seed lab, Faculty of Agriculture, Mansoura University, Egypt during March and April 2017.The goals of this study aimed to study the effect of sunflower hybrids, nitrogen fertilizer rates and hill spacing on germination and seedling characters.The results indicated that Nsovak genotype recorded the highest values of abnormal seedling percentage. Highest mean germination time was obtained from MS.sirena F1 genotype. The highest coefficient of velocityand value of the vigour was produced from Biest Brima genotype without significant differences with sown Nsovak genotype.The results indicated that increasing nitrogen fertilizer rates from 72, 120 and 168 kg N/ha significantly increased mean germination timeandvalue of the vigour. The highest coefficient of velocity percentage produced from addition nitrogen fertilizer at 120 Kg/ha.The results designated that increasing hill spacing from 15, 20 and 25 cm reduced germination percentage and mean germination time, however, significantly increased coefficient of velocity anddead seed percentages and value of the vigour.It could be concluded that sown Nsovak genotype at hill spacing of 15 cm and fertilizing with 120 kg N/ha enhanced seed germination and viability.*

Keywords—* *Sunflower cultivars, nitrogen fertilizer rates, Germination and seedling parameters.

I. INTRODUCTION

Sunflower (Helianthus annus L.) has higher seed and oil yieldsfrom the essential oilseed crop that gives a main share in edible oil production. It cultivated widely in the arid and semi-arid regions. In order to obtainseeds good in quality,so essential to getabetter plant stand. Sunflower Hybrids, nitrogen fertilizer levels and plant population density may affect germination parameters and seedling characters. In this respect, Sakha 53 exceeded Giza 102 in seed yield, its attributes and percentage of oil **(Aml-El-Saiedy et al., 2011).**The higher germination index, germination rate, number of days to 50% germination, germination index, root and shoot length and their dry weight produced from Urfloar cultivar as compared with Blazar cultivar**(Moghanibashiet al., 2012).** Line S 102 surpassed other studied cultivars in final germination percentage, germination rate and germination index, energy of germination and seedling vigor index**(Kandil et al.,2016a).**Sunflower differed from germination and seedling parameters, theLine S 102 outdone Sakha 53, Giza 102 and Line S 1 in the length of shoots, length of roots,weight of fresh and dry roots and weight of fresh and dry shoots**(Kandil et al., 2016b).**

Germination percentage and seed vigor did not affect by increasing applications of nitrogen fertilizer rates of0, 66, 132 to 600 kg/ha **(Osechas and Torres 2002).** Nitrogen fertilization in wheat enhanced of seed vigor and percentages of final germination and decreasedmean germination time and the time that 50% of the seeds germinated**(Warraich, 2002).** Seed viability, seedling vigour and cool germination test performance of cottonseed were all found to increase due to the addition of nitrogen fertilizer rate of 142.8 kg N/ha **(Zakaria, 2007).** Environmental conditions affect seed quality of seed formation and affect seedling establishment of the next growing season **(Zakaria, 2009).** Fertilization of biological or artificial source as organic or inorganic material of nitrogen supplies nutrients essential to the soil and enhanced growth of plants **(SSSA, 2011)**. The late in germination time was obtained from of Okapi cultivar by increasing nitrogen fertilizer levels. Planting cultivar of Zarfam and fertilizing with 100 kg/h enhanced the amount of coefficient of speed of germination whichnitrogen fertilizer was applied of topdressing, however, the lowest amount was produced from sown

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Okapi cultivar without top dress of nitrogen fertilizer**(Oskouie and Divsalar, 2011).** Increasing nitrogen fertilizer increased seed yield for producing vigorous seeds **(Wambugu et al, 2012).**Nitrogen fertilization at a rate of 165 kg/ha produced the highest percentages of seed germination and seedling vigor index. Maximum electrical conductivity related to seeds that received nitrogen fertilizer at a rate of 165 kg/ha. They concluded that uses of 165 kg/ha nitrogen fertilizerson mother plants will produce seeds with higher vigor, germination characteristics and seedling establishment **(Farhidi et al., 2014).**Increasing nitrogen fertilizer rates in the mother plant field, genotypes and interaction of genotypes among nitrogen fertilizer influenced seed viability, germination, vigor of aromatic rice seeds **(Hossain, 2014).** Urea application produced the highest germination rate and followed by organic manure. Inorganic nitrogen fertilizer at Urea depicted maximum rate of shoot and root length. They suggested that urea is best fertilizer for germination of seedlings of sunflower and second one is FYM fertilizer (**Shahzaman et al., 2017)**. Therefore, the purpose of this investigation aimed to study the effect of nitrogen fertilizer rates application for mother plants of sunflower cultivars and hill spacing on germination and vigor characters of resulted seed.

II. MATERIALS AND METHODS

2.1. Research time and location:

An experiment was conducted in the laboratory of seed science, Agronomy Department, Faculty of Agriculture Mansoura University Egypt during March and April 2017. The goals of this study was aimed to investigate the response of some sunflower cultivars (Giza102, Sakha 53,) under three nitrogen rates (72, 120 and 168 Kg N/ha) and three hills spacing (15, 20 and 25 cm apart) to germination and seedling parameters.Afactorial experiment in Randomized Complete Block Design in four replication was used. The experiment includes three factors, the first sunflower genotypes (Nsovak, MS.Sirena F1, BiestBrima).The second factor included three nitrogen rates (72, 120 and 168 Kg N/ha) and the third cultivar includesthree hills spacing (15, 20 and 25 cm apart).Fifty seeds of uniform size in each treatment for each cultivar allowgerminating on Petri dishes in 9 cm diameter Petri dishes. Seeds were germinate in a germination chamber in 20 – 25ºC **(ISTA Rules, 2016).**The experiment included 108 Petri dishes that moistened with a distilled water.

2.2. Studied Characters:

Sunflower seed of both seasons subjected for determination of the following characters:1-Average of final germination percentage (FGP): Seed germinated count taken after 14 days from sowing date and stated as a percentage affording to the following formula as defined by **(Ellis and Roberts, 1981& Ruan *et al*. 2002).**

$$\mathbf{FGP} = \frac{\text{Number of germinated seeds}}{\text{Total Number of seed tested}} \text{ X } 100$$

2-Percentage of abnormal seedling = Number of abnormal seedling/total number of seeds

3-The mean germination time (MGT): It was determined according to the equation of **Ellis and Roberts (1981)**:

MGT= ? dn/ ? n

4-Average of coefficient of velocity (CV) was calculated using the following formula as described **by Scott et al., 1984:**

C V= 100[? Ni / ? Ni Ti]

The N mean the number of germinated seeds on day i and T is mean that the number of days from sowing.

5-Percentage of dead seed = Number of dead seed /total number of seeds

6-The value of the vigour (V) was calculated as following formula according to **Bradbeer 1988:**

$$V= (a/1 + b/2 + c/3 + d/4 + \ldots + x/n) \text{ x } 100 / S$$

Where a, b, c . . . respectively represent the number of seeds which germinated after 1, 2, 3 . . . days of imbibition, x is the number of seed for day n and S the total number of seeds sown. The range of the V values is from 0 to 100 (maximum rate). The rates were categorized as five numbers categories: 0= Null; 1= Slow; 2=Medium; 3= Fast; and 4= Very fast was divided according to **Mayer and Poljakoff, 1989.**

2.3. Experimental analysis:

The analysis of data collected done, statistically by the analysis of variance technique using the MSTAT–C statistical package programmed as described by a procedure of Gomez and Gomez (1991). For comparisons between treatment means, least significant differences test (LSD) at 0.05level of probability was used according to Snedecor and Cochran (1980).

III. RESULTS AND DISCUSSIONS

3.1. Performance of sunflower cultivar on seed germination and seedling characters:

Averages of abnormal seedling percentages, mean germination time, coefficient of velocity,dead seed percentages and value of the vigour significantly affected by sunflower cultivars as shown in Tables 1 and 2, except germination and dead seed percentages insignificantly differed. The results indicated that Nsovak genotype recorded the highest values of abnormal seedling percentage (6.67 %). Highest mean germination time (2.53) was obtained from MS.sirena F1 genotype.The highest coefficient of velocity (41.29 %) and value of the vigour (42.19) were produced from Biest Brima genotype without significant differences with sown Nsovak genotype.The differences in final percentage of germination of seed may due to the amount of saved matters in endosperm and different seed size of genotypes. The processes that inhibit germination probably involve cell division and no transfer of nutrients **(Bittman, 1989).**Zarfam and Licord cultivars recorded the highest seed vigor (98%) of both cultivars and Talaieh cultivar produced the lowest was(92%)**(Oskouie and Divsalar, 2011).**

3.2. Effect of nitrogen fertilizer rates on seed germination and seedling characters:

Averages of mean germination time,coefficient of velocity andvalue of the vigoursignificantly affected by different nitrogen fertilizer rates, however insignificantly influencedgermination and abnormal seedling anddead seed percentages as shown in Tables 1 and 2. The results indicated that increasing nitrogen fertilizer rates from 72, 120 and 168 kg N/ha significantly increased mean germination time (2.55) andvalue of the vigour (42.24). The highest coefficient of velocity percentage (41.56 %) produced from addition nitrogen fertilizer at 120 Kg/ha. Seed lots with high vigor have lower mean germination time (**Ellis and Robert, 1981**). The lowest germination and vigour of seeds produced under conditions of low-soil fertility **(Songa et al., 1994).** A fast and uniform field emergence is essential to do high yield with good quality and quantity of annual crops **(Yari et al., 2010).**The highest coefficient of speed of germination produced from nitrogen fertilization at a rate of 100 kg N/ha, for Zarfam cultivar and without nitrogen fertilization for Okapi cultivar produced the lowermost coefficient of speed germination **(Oskouie and Divsalar, 2011).**

3.3. Effect of hill spacing on seed germination and seedling characters:

Means of germination and abnormal seedling percentages and average of germination time, percentages of coefficient of velocity anddead seed as well as value of the vigour significantly affected by plant hill spacing as shown in Tables 1 and 2. The results designated that increasing hill spacing from 15, 20 and 25 cm reduced germination percentage (96.53 %) and mean germination time (2.41), however, significantly increased coefficient of velocity (41.54 %) anddead seed (3.55 %) percentages and value of the vigour (42.40).

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Table.1: Average of germination and abnormal seedling percentages and mean germination time as affected by sunflower cultivars, nitrogen fertilization and hill spacing.

Characters / Treatment	Germination %	Abnormal Seedling %	Mean germination time
A-Sunflower cultivars:			
Nsovak	96.96	6.67	2.43
MS.sirena F1	97.63	5.33	2.53
Biest Brima	97.18	5.04	2.40
F. test	NS	*	*
LSD at 5%	--	1.03	0.03
B-Nitrogen fertilization			
72 kg N/ha	97.85	5033	2.43
120 kg N/ha	97.33	5.48	2.41
168 kg N/ha	96.59	6.22	2.55
F. test	NS	NS	*
LSD at 5%	--	--	0.03
C-Hill spacing:			
15 cm apart	98.14	3.70	2.55
20 cm apart	97.11	6.07	2.43
25 cm apart	96.52	7.26	2.41
F. test	*	*	*
LSD at 5%	1.13	1.18	0.05
D-Interaction F- test:			
A x B	NS	*	*
A x C	*	*	*
B x C	*	*	NS
A x B x C	NS	NS	NS

3.4. Interaction Effects:

3.4.1. Interaction between cultivars and nitrogen fertilizer rates:

Averages of abnormal seedling percentage, mean germination time, coefficient of velocitypercentage and value of the vigour significantly influenced by the interaction between sunflower cultivars and nitrogen fertilization, however, germination and dead seed percentages insignificantly affected as shown in Tables 1 and 2. The results graphically illustrated in Figs. 1 and 2 clearly showed that the effect of the interaction between sunflower cultivars and nitrogen fertilization on abnormal seedling percentage and mean germination time. The highest percentages of abnormal seedling (7.11) and mean germination time (2.49, 2.70) were obtained from sown Nsovak orMS.sirena F1 genotypes when increasing nitrogen fertilizer up to 168 kg N/ha, respectively. The highest coefficient of velocitypercentage (42.41 %) produced from the interaction of planting Biest Brima genotype that fertilized with 72 kg N/ha as graphically demonstrated in Figs 3. The highest values of value of the vigour(42.86) was recorded from the interaction of planting MS.sirena F1 genotype that fertilized with 120 kg N/ha as graphically demonstrated in Figs 4.Supply of different fertilizer rates in the mother plant field, cultivar and the interaction of cultivar and fertilizer much affected seed germination of vigor of aromatic rice seeds**(Hossain, 2014).**

Table.2:.Average of coefficient of velocityanddead seedpercentages andvalue of the vigour as affected by sunflower cultivars, nitrogen fertilization and hill spacing.

Characters / Treatments	Coefficient of velocity %	Dead seed %	Value of the vigour
A-Sunflower cultivars:			
Nsovak	41.16	3.26	41.53
MS.sirena F1	39.67	2.37	40.82
Biest Brima	41.29	2.82	42.19
F. test	*	NS	*
LSD at 5%	0.42	--	0.62
B-Nitrogen fertilization			
72 kg N/ha	41.15	2.37	41.97
120 kg N/ha	41.56	2.67	40.33
168 kg N/ha	39.42	3.41	42.24
F. test	*	NS	*
LSD at 5%	0.42	--	0.62
C-Hill spacing:			
15 cm apart	39.32	2.07	40.78
20 cm apart	41.26	2.81	41.35
25 cm apart	41.54	3.55	42.40
F. test	*	*	*
LSD at 5%	0.70	1.13	0.51
D-Interaction F- test:			
A x B	*	NS	*
A x C	*	NS	*
B x C	NS	*	*
A x B x C	NS	NS	NS

3.4.2. Interaction between cultivars and hill spacing:

Averages of germination and abnormal seedling percentage, mean germination time, coefficient of velocitypercentage and value of the vigour significantly influenced by the interaction amonggenotypes and hill spacing, however, dead seed percentages insignificantly affected as shown in Tables 1 and 2.The results graphically illustrated in Figs. 5, 8 and 9 clearly showed that the highest germination percentage of (98.67 %),coefficient of velocitypercentage (42.90 %) and value of the vigour (42.33) were obtained from sown Biest Brima genotype when sown at dense hill spacing of 25 cm, respectively.The highest abnormal seedling percentage (9.48 %) was produced from the interaction of planting MS.sirena F1 genotype when sown at wider hill spacing of 20 cm as graphically demonstrated in Figs 6.The highest mean germination time (2.72) was produced from the interaction of planting MS.sirena F1 genotype when sown at dense hill spacing of 15 cm as graphically demonstrated in Figs 7.

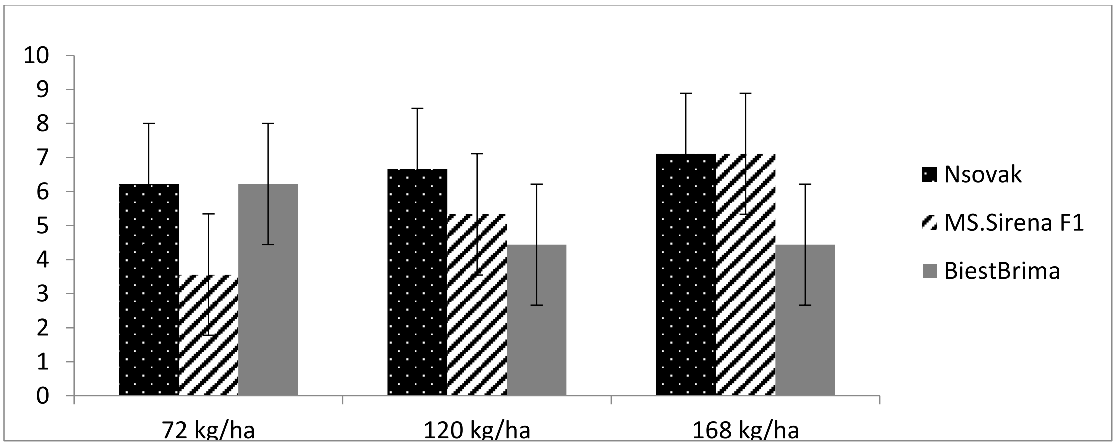

Fig.1: Average of abnormal seedling % as affected by interaction between sunflower cultivars and nitrogen fertilization.

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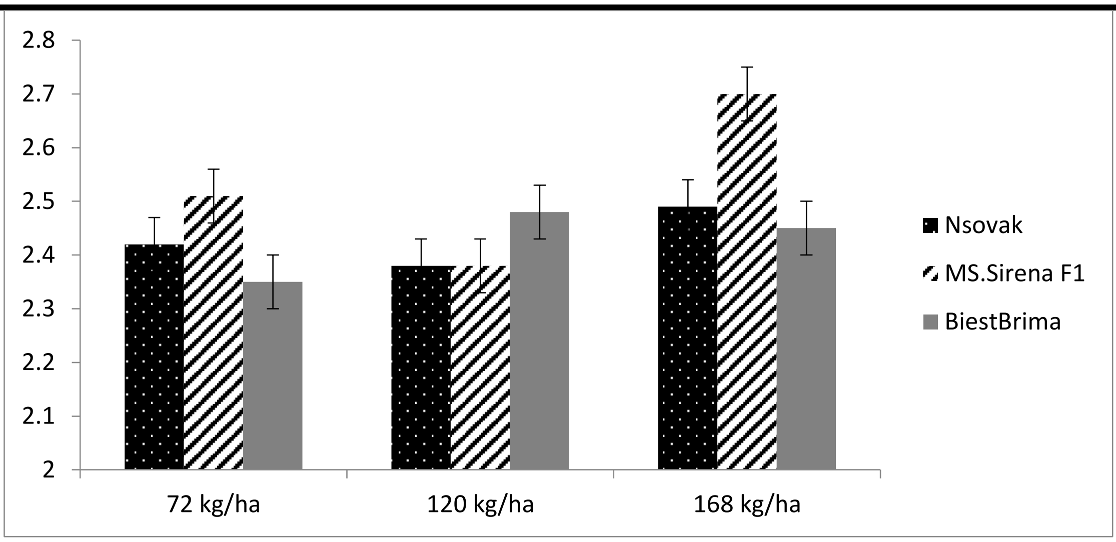

Fig.2: Average of mean germination time as affected by interaction between sunflower cultivars and nitrogen fertilization rates.

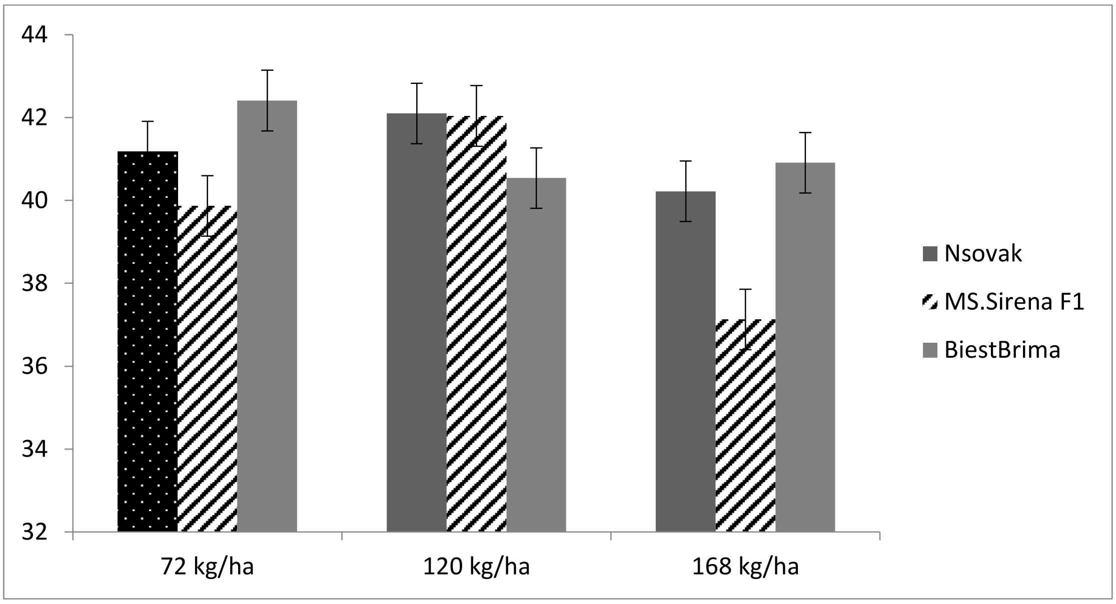

Fig.3: Average of coefficient of velocitypercentage as affected by interaction between sunflower cultivars and nitrogen fertilization rates.

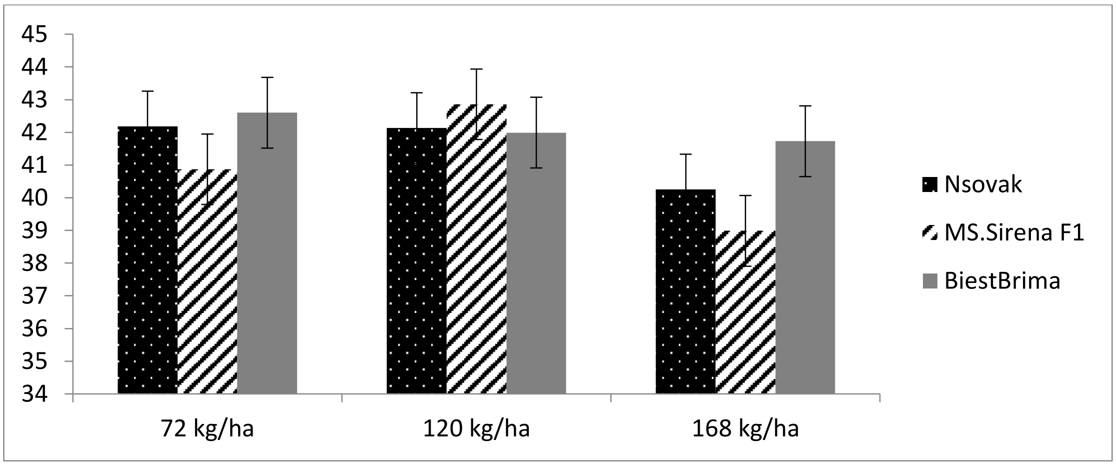

Fig.4: Average of value of the vigour as affected by interaction between sunflower cultivars and nitrogen fertilization rates.

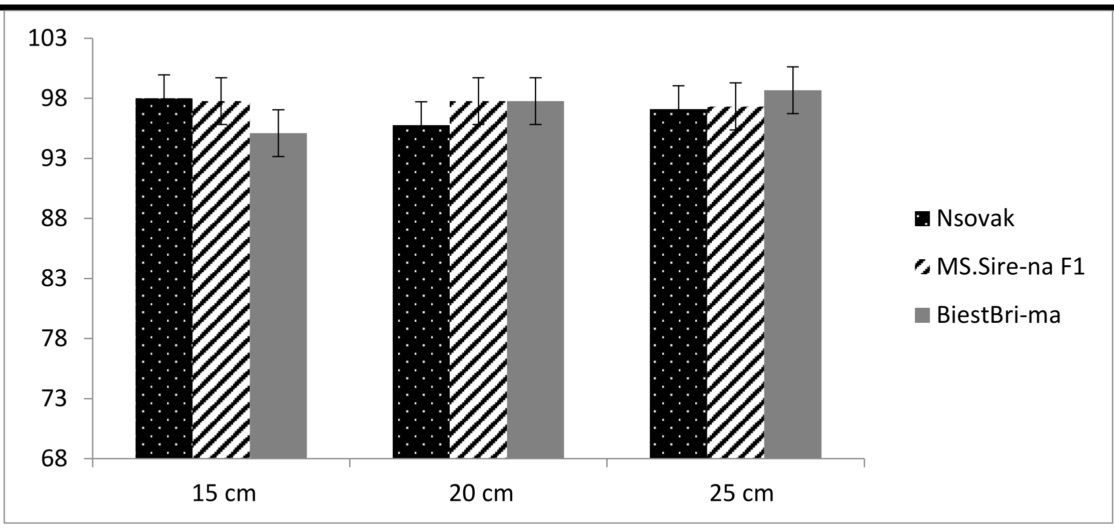

Fig.5: Average of germination percentage as affected by interaction between sunflower cultivars and plant density.

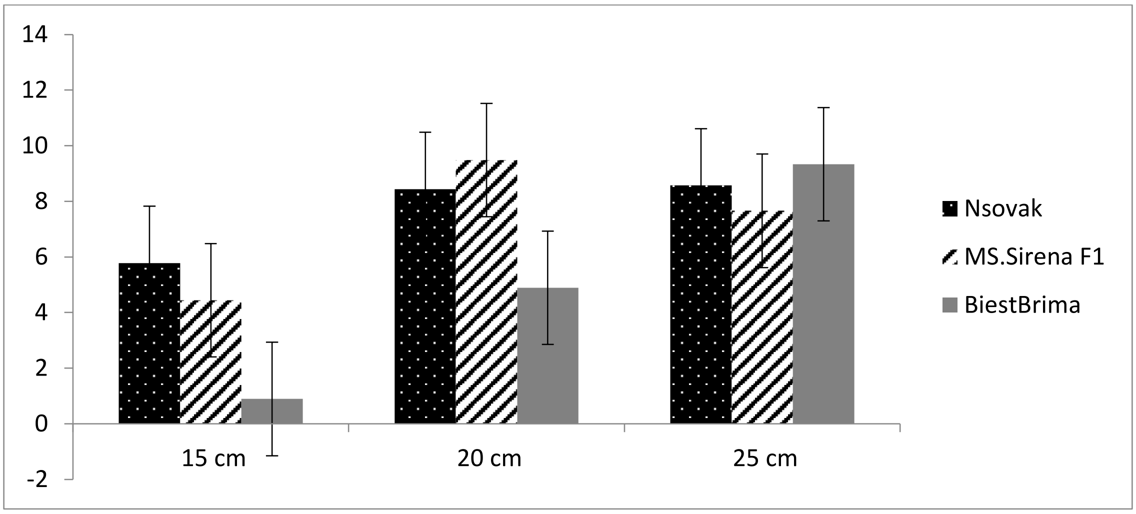

Fig.6: Average of abnormal seedling % as affected by interaction between sunflower cultivars and hill spacing.

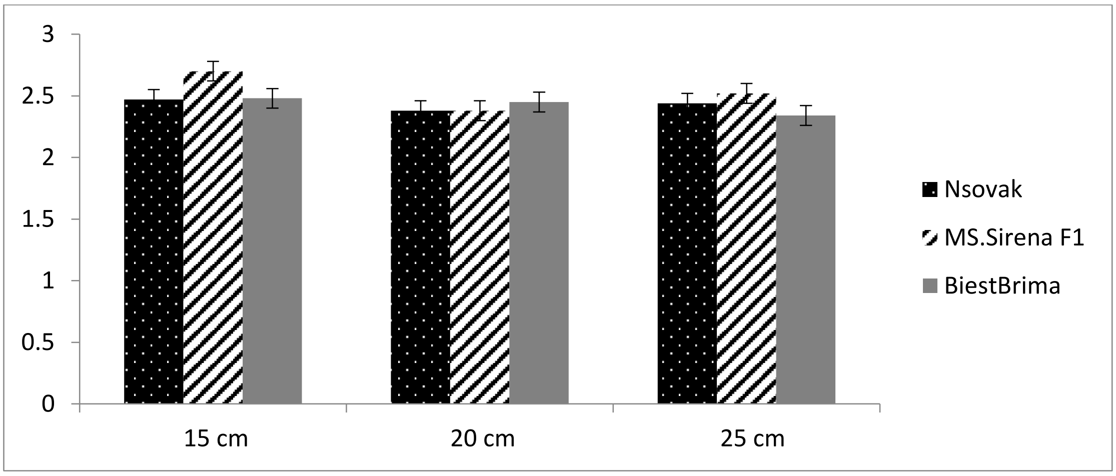

Fig.7: Average of mean germination time as affected by interaction between sunflower cultivars and hill spacing.

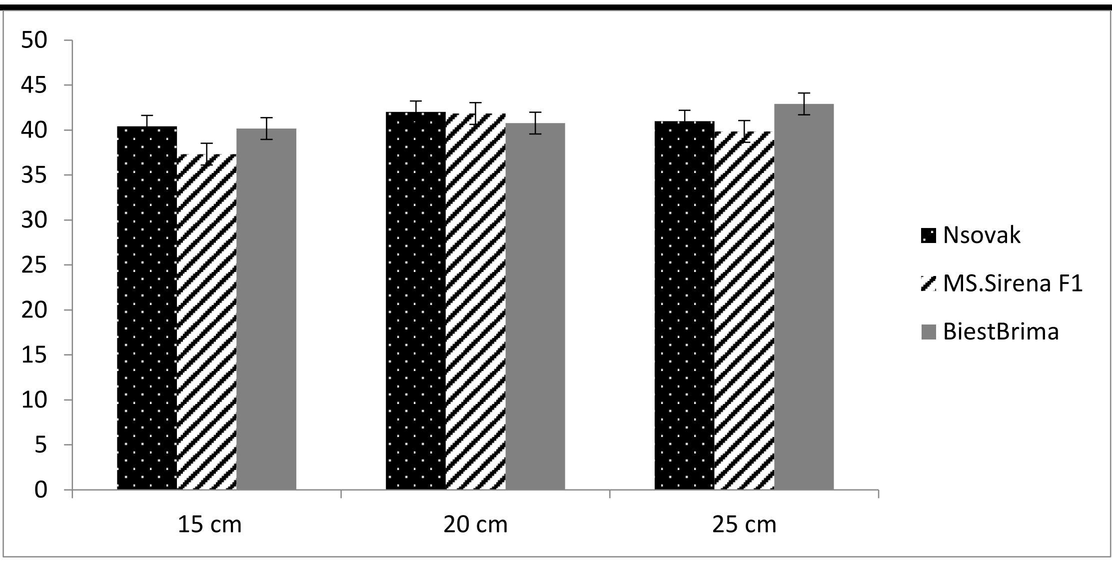

Fig. 8: Average of C.V % as affected by interaction between sunflower cultivars and hill spacing.

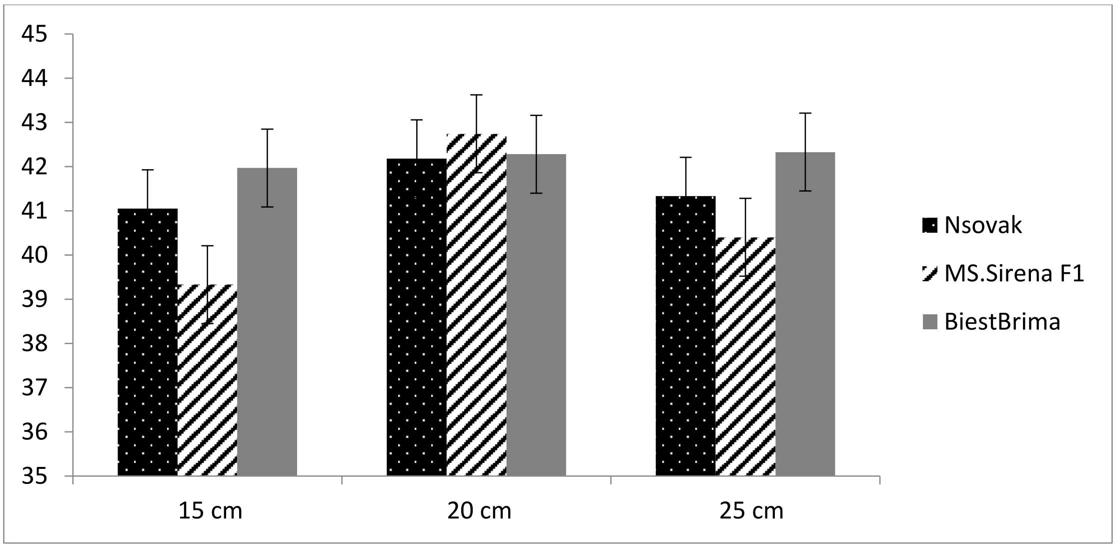

Fig.9: Average of value of the vigour as affected by interaction between sunflower cultivars and hill spacing.

3.4.3. Interaction between nitrogen fertilizer rates and hill spacing:

Averages of germination, abnormal seedling, dead seed percentages and value of the vigour significantly affected by the interaction between nitrogen fertilizer rates and hill spacing, however, mean germination time and coefficient of velocity percentage insignificantly affected as shown in Tables 1 and 2.The results graphically illustrated in Figs. 10 clearly showed that the effect of the interaction between nitrogen fertilizer rates and hill spacing on germination percentage, the results showed that the highest germination percentage (100 %) was obtained from sown sunflower fertilized with 120 kg N/ha and sown at hill spaces of 15 cm. The highest abnormal seedling percentage (9.78 %) was recorded from fertilization with 120 kg N/ha and sown at wider hill spacing of 25 cm as graphically demonstrated in Figs 11. The highest dead seed percentages percentage (4.89 %) w+as produced from fertilization with nitrogen at rate of 120 kg N/ha when sown at wider hill spacing of 25 cm as graphically demonstrated in Figs 12.The highest values of value of the vigour(43.26) was produced from fertilization with nitrogen at rate of 120 kg N/ha when sown at hill spacing of 20 cm as graphically demonstrated in Figs 13.

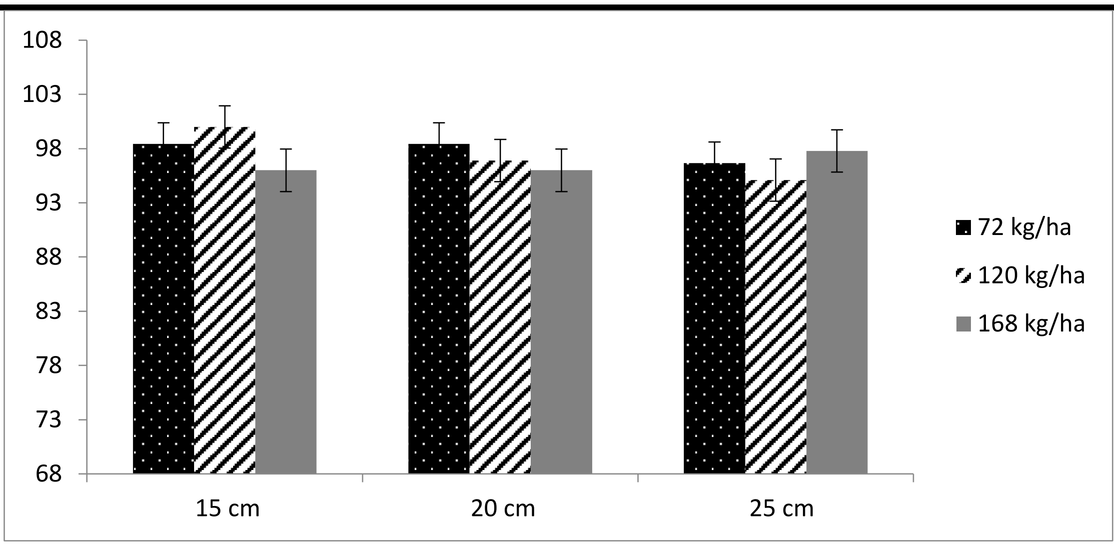

Fig.10: Average of germination percentage as affected by interaction between nitrogen fertilization and plant density

velocityand dead seed percentages and value of the vigour insignificantly affected by the interaction between sunflower cultivars x nitrogen fertilizer rates x hill spacing as shown in Tables 1 and 2.

3.4.4. Interaction between cultivars, nitrogen fertilizer rates, and hill spacing:

Averages of germination and abnormal seedling percentage, mean germination time, coefficient of

IV. CONCLUSION

Accordingly, it could be summarized that sown Nsovak genotype at hill spacing of 15 cm and fertilizing with 120 kg N/ha enhanced seed germination and viability.

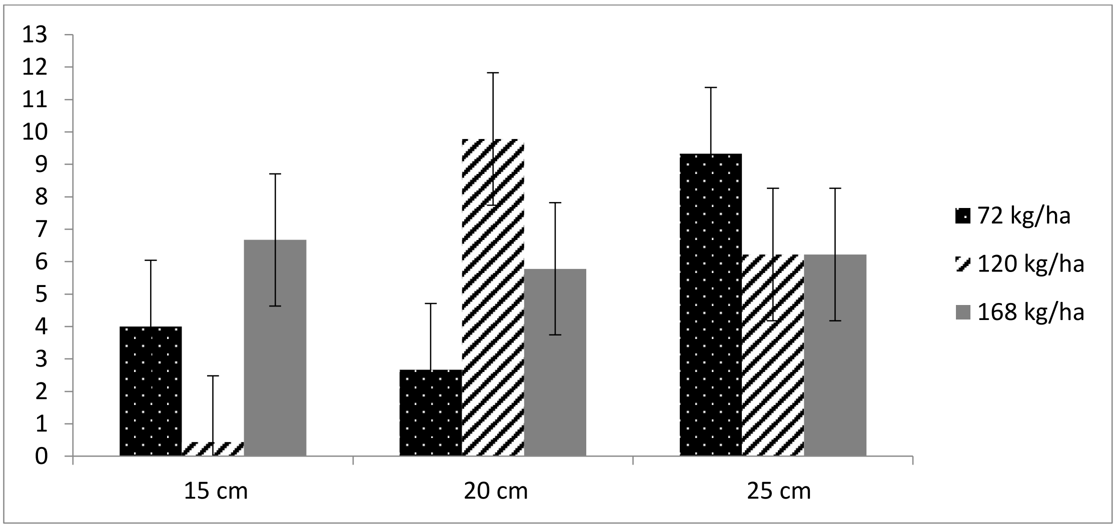

Fig.11: Average of abnormal seedling % as affected by interaction between nitrogen fertilization and hill spacing.

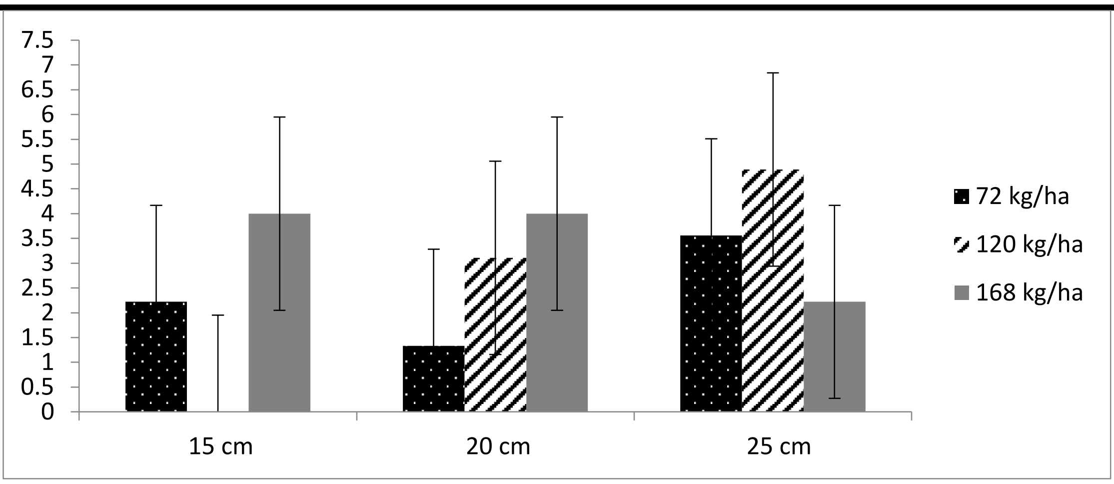

Fig.12: Average of dead seed% as affected by interaction between nitrogen fertilization and plant density.

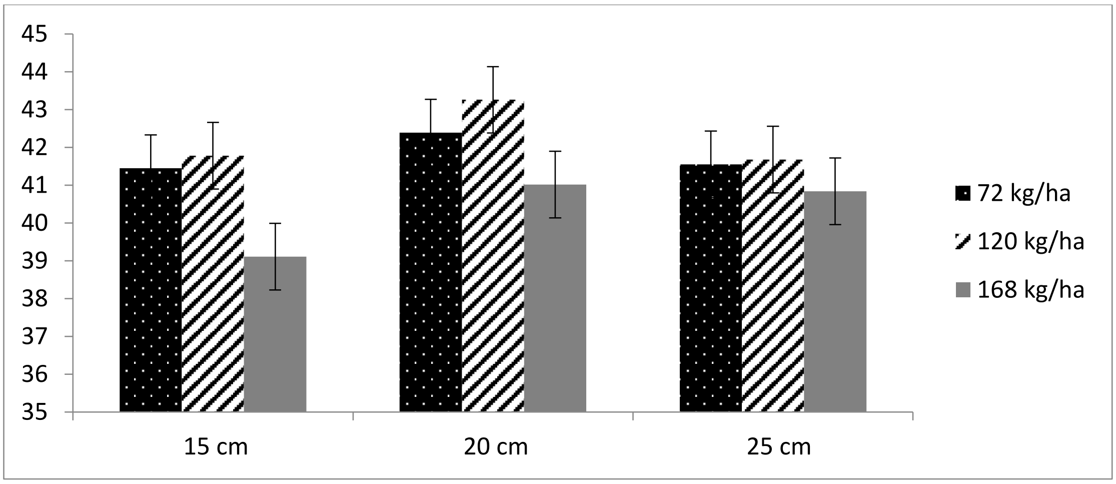

Fig.13: Average of value of the vigour as affected by interaction between nitrogen fertilization and plant den nitrogen fertilization and plant density during two season (A) 2014 and (B)2015.

REFERENCES

[1] Abdel-Baki, A. A. and J. D. Anderson 1973.Viability and leaching of sugars from germinating barley. Crops Sci., 10: 31 – 34.https://dl.sciencesocieties.org/publications/cs/abstracts/10/1/CS0100010031

[2] Aml, E.A. El-Saidy, S. Farouk and H.M. Abd El-Ghany 2011. Evaluation of Different Seed Priming on Seedling Growth, Yield and Quality Components in Two Sunflower (Helianthus annuus L.) Cultivars. Trends in Applied Sciences Research, 6: 977-991.http://scialert.net/fulltext/?doi=tasr.2011.977.991&org=10

[3] Bittman, S. and G.M. Simpsan 1989. Drought effect on water relation of tree cultivated grasses. Crop Sci., 29: 992-999. Carter, J.E. and Paterson, R.P. 1985. Use of relative water content as a seclusion tool for drought tolerance. In: Agron Abs ASA.

[4] Bradbeer J. W., 1988. Seed Dormancy and Germination.- Chapman & Hall, New York.

[5] Ellis, R.A. and E.H. Roberts 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol., 9: 373-409.http://agris.fao.org/agris-search/search.do?recordID=XE8182678

[6] Farhadi, E., J Daneshyan, A Hamidi, AH Shirani Rad and H.R. Valadabadi 2014. Effects of parent plant nutrition with different amounts of nitrogen and irrigation on seed vigor and some characteristics associated with hybrid 704 in Kermanshah region. Journal of Novel Applied Sciences, 3(5): 551-556.http://jnasci.org/wp-content/uploads/2014/05/551-556.pdf

[7] Gomez, K.A. and A.A. Gomez 1991.Statistical Procedures in Agricultural Research, John Wiley and Sons, New York. http://pdf.usaid.gov/pdf_docs/PNAAR208.pdf

[8] Hossain, M. F. 2014. Impact of Fertilizers on the Seed Quality of Aromatic Rice. Journal of Agricultural Science, 6 (6): 35-40.http://www.ccsenet.org/journal/index.php/jas/article/viewFile/31803/20680
[9] Islam, M.M. and M. A. Karim 2010. Evaluation of Rice Oryza sativa L. genotypes at germination and early seedling stage for their tolerance to salinity. The Agric., 8 (2): 57 – 65. http://www.banglajol.info/index.php/AGRIC/article/view/7578
[10] ISTA Rules 2016.International seed testing association. ISTA Germination Sec. Chapter 19: pp. 19 – 41. https://www.seedtest.org/upload/cms/user/OGM15-05-Proposed-Changes-to-the-ISTA-Rules-for-2016.pdf
[11] Karim, M.A., N. Utsunomiya and S. Shigenaga 1992. Effect of sodium chloride on germination and growth of hexaploid triticale at early seedling stage. Japanese Journal of Crop Science,61: 279 – 284. https://www.jstage.jst.go.jp/article/jcs1927/61/2/61_2_279/_article
[12] Kandil, A. A. and A. E. Sharief and Amira A. Mamoon 2016a. Germination characters as affected by seed priming of some sunflower cultivars under salinity stress. International Journal of Agronomy and Agricultural Research (IJAAR)., 9(2):100-116.http://www.innspub.net/wp-content/uploads/2016/08/IJAAR-V9No2-p100-116.pdf
[13] Kandil, A. A. and A. E. Sharief and Amira A. Mamoon 2016b. Seedling parameters of some sunflower cultivars as affected by seed priming and salinity stress. International Journal of Agronomy and Agricultural Research (IJAAR)., 9(2):117-136.http://www.innspub.net/ijaar/seedling-parameters-of-some-sunflower-cultivars-as-affected-by-seed-priming-and-salinity-stress/
[14] Karim MA, N. Utsunomiya and S. Shigenaga 1992. Effect of sodium chloride on germination and growth of hexaploid triticale at early seedling stage. Japanese J. of Crop Sci.,61: 279 – 284. https://www.jstage.jst.go.jp/article/jcs1927/61/2/61_2_279/_article
[15] Mayer, A.M. and M. A. Poljakoff 1989. The germination of seeds4th ed . Pergamon Press, Oxford.
[16] Moghanibashi, M., H. Karimmojeni, P. Nikneshan and D. Behrozi 2012. Effect of hydro priming on seed germination indices of sunflower (Helianthus annuus L.) under salt and drought conditions. Plant Knowledge Journal, 1(1): 10-15.http://www.sciencej.com/karimojani_1_1_2012_10_15.pdf
[17] Osechas D, Torres A and Becerra L. 2002. Effect of nitrogen fertilization on the production and quality of seed of signal grass (Urochloadecumbens, Stapf). Zootecnica Tropical, 20(1): 135-143.http://www.bioline.org.br/request?zt02010
[18] Oskouie, B. and M. Divsalar 2011. The effect of Mother Plant Nitrogen on Seed Vigor and Germination in Rapeseed. ARPN Journal of Agricultural and Biological Science, 5(5): 49-56.http://www.arpnjournals.com/jabs/research_papers/rp_2011/jabs_0511_275.pdf
[19] Ruan, S., Q, Xue and K. Tylkowska 2002. Effects of seed priming on germination and health of rice Oryza sativa L. seeds. Seed Science and Technology, 30: 451-458. http://www.uaiasi.ro/CERCET_AGROMOLD/CA3-15-05.pdf
[20] Russell, D.F. 1986.MSTAT-C computer based data analysis software Crop andSoil Science Department, Michigan State University USA. https://msu.edu/~freed/mstatc.htm
[21] Shahzaman, M., M. Ishtiaq and A. Azam 2017. Effect of different fertilizers on seed germination and seedling growth of sunflower (Helianthus annuus L.) from district Bhimber of Azad Jammu and Kashmir, Pakistan. International Journal of Botany Studies. 2(2): 10-15. http://www.botanyjournals.com/archives/2017/vol2/issue2/2-1-28
[22] Snedecor GW and WG. Cochran 1980. Statistical Methods. 7Th Ed. Iowa State University Press, Iowa, USA, ISBN-10: 0-81381560-6, Pp: 507. https://www.amazon.com/Statistical-Methods-Seventh-isbn-0813815606/dp/B0012S4NIE
[23] SSSA, 2011. Soil and Water Management and Conservation SSSA Division S-06, Annual Newsletter, October 2011 P6.https://dl.sciencesocieties.org/publications/aj/articles/109/3/739
[24] Songa, W., W. K. Ronno, and D. L. Danial 1994. Production constraints of beans in the semi-arid Eastern Kenya with special reference to charcoal rot," in Proceedings of the Regional Workshop for Eastern, Central and Southern Africa held at Njoro, Kenya, Wageningen Agricultural University, October 1994.pp. 251–255. https://www.cabdirect.org/cabdirect/abstract/19951610199
[25] Wambugu, P. W., P. W. Mathenge, E. O. Auma and H. A. vanRheenen (2012). Constraints to On-Farm Maize (Zea mays L.) Seed Production in Western

Kenya: Plant Growth and Yield.International Scholarly Research Network, ISRN Agronomy, Volume 2012, Article ID 153412, 7 pages. doi:10.5402/2012/153412

[26] Waller R.A. and B.D. Duncan 1969. A bays rule for the symmetric multiple comparison problem. J. Amer. Assoc., 64, 1484-1503. https://www.jstor.org/stable/2286085?seq=1#page_scan_tab_contents

[27] Warraich EA, Basra SMA, Ahmad N, Ahmed R and Aftab M. 2002. Effect of nitrogen on grain quality and vigor in wheat (Triticum aestivum L.). International Journal of agriculture & Biology 4:517-520. http://www.fspublishers.org/published_papers/5383_..pdf

[28] Yari, L., M. Aghaalikhani, and F. Khazaei, 2010. Effect of Seed priming duration and temperature on seed germination behavior of bread wheat (Triticum aestivum L.). ARPN Journal of Agriculture & Biological Sciences, 5(1):1-6.http://www.arpnjournals.com/jabs/research_papers/rp_2010/jabs_0110_166.pdf

[29] Zakaria M.S., A. H. Fahmy and S. E. Yuosef 2007. Cotton Seed, Seed Viability and Seedling Vigour as Affected by Nitrogen, Potassium, Phosphorus, Zinc and a Plant Growth Retardant. The African Journal of Plant Science and Biotechnology, 1(1): 16-25. http://www.globalsciencebooks.info/Online/GSBOnline/images/0712/AJPSB_1(1)/AJPSB_1(1)16-25o.pdf

[30] Zakaria M.S., Ashraf HF and Serag EY. 2009. Direct and residual effects of nitrogen fertilization, foliar application of potassium and plant growth retardant on Egyptian cotton growth, seed yield, seed viability and seedling vigor. Acta Ecological Sinica, 29: 116-123. http://file.scirp.org/pdf/AS_2013122517125575.pdf

Response of nutrient management practices through organic substances on rice var. GR-11 in North Konkan Coastal zone of Maharashtra

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***Abstract*— *The management of soil organic matter is crucial to maintain a productive organic farming system. No one source of nutrient usually fulfills to maintain productivity and quality control in organic system.In addition, the inputs to supplement nutrient availability are often not uniform presenting additional challenges in meeting the nutrient requirements of crops in organic system.With this concept, a field experiment was conducted at the research farm of ASPEE Agricultural Research and Development Foundation, Tansa Farm, At Nare, Taluka Wada, Dist. Palghar, Maharashtra, during Kharif 2016-17 in rice.Different treatments comprising organic amendments such as Azotobacter, Banana Pseudostem sap 2%, Vermiwash 2% and Panchgavya 2% each applied alone or in all possible combinations were tried in organic crop production.These treatments were compared with absolute control (No biofertilizer+ No Spray). Recommended dose of chemical fertilizer 100:50:50 kg NPK ha^{-1}. A Rice variety 'GR-11' was taken.Results revealed a significant enhancement in grain yield of rice over absolute control due to the application of different organic amendments applied alone or in combinations. Rice grain yield increased by 35.5% over absolute control when organic amendments viz., Seedling deep in Azotobacter + Vermiwash 2% + Banana Pseudostem Sap 2% were applied together.The rice grain yield (5.7 t ha^{-1}) obtained under combined application of above three organic amendments was at par with the yield recorded under seedling deep in Azotobacter + Vermiwash 2% + Panchgavya 2%.An interesting observation recorded was that there was no serious attack of any insects pest or disease in organically grown crop.The study revealed that addition of four organic amendments viz. seedling deep in Azotobacter, vermiwash 2%, Panchgavya 2% and Banana Pseudostem Sap 2% could give the optimum yield of organic rice var. GR-11.*

Keywords*— *Azotobacter, Vermiwash, Noval fertilizer, Panchgavya.

I. INTRODUCTION

Organic farming production system aims at promoting and enhancing agro-ecosystem health, biodiversity, biological cycles and soil biological activities. The popularity of organic food and organic farming across the world has tempted rice producers in India to focus on the production of organic rice. Organic farming is an alternative agriculture which has been proposed as a solution to the problems associated with inputs of chemical fertilizers and pesticides. It is based on ecological approach to nutrient supply and crop protection rather than a chemical one. In organic farming, we constantly work to build the healthy soil that translates into healthy plants. Crop plants remove varying amounts of different nutrients from soil and to compensate the loss from the soil, organic amendments rich in nutrients must be added (Singh & Mandal, 2000). In organic farming, we feed to the soil micro and macro-organisms, which deliver a smorgasbord of minerals, vitamins and other nutrients to the crop at a metered pace.Through organic farming, incidences of diseases and insects may be reduced and soil and grain quality improved (Stockdale *et al.* 2001).With such background, an experiment was conducted to find out the feasibility of organic farming in riceand examine the impact of this on the yield and quality of grain.

II. MATERIALS AND METHODS

The experiment was conducted at ASPEE Agricultural Research and Development Foundation Farm, Village-Nare, Tauka- Wada, district- Palghar in *kharif* season during 2016-17 in Randomized Block Design (RBD) with three replications. The plot size was4.5 m x 2.5 m.The experimental site was located at 19.65^{0}N latitudes and 73.13^{0}E longitudes with average annual rainfall of 2600 mm.Fourteen treatments comprising different organic amendments such as seedling dip in Azotobacter, Vermiwash 2%, Panchgavya 2% and Banana Pseudostem Sap 2% each applied alone and in combination were tested inorganic crop production. Azotobacter are aerobic, free-living soil microbes which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen which is inaccessible to plants and releasing it in the form of ammonium ions into the soil (nitrogen fixation). In addition to being a model organism for studying diazotrophs, it is used by humans for the production of biofertilizers. The Liquid organic manure Panchagavya was freshely prepared at farm and vermiwash was colleted from vermicompost unit at farm. The noval fertilizer was brought from Navsari Agricultural University, Navsari. It was a sap extracted from banana pseudo-stem. This sap was rich source of major nutrients like nitrogen, phosphorus, potash and micro nutrients like iron, boron, molybdenum, magnesium, calcium, sulphur, zinc and copper. This sap was also worked as a growth promoters like gibbrelic acid and cytokinin.

Rice variety 'GR-11' was sown in first fortnight of June during 2016-17 after seed treatment with the fungicide thiram @ 3 g kg^{-1} seeds. Twenty five days old seedlings were transplanted at spacing of 20 cm x15 cm. The bed size was 4.5 m x 2.5 m. Nitrogen, phosphorus and pottassium were applied at the rate of 100:50:50 kg ha^{-1} in the form of urea, single super phosphate and muriate of potash, respectively. The entire quantity of P and K fertilizers along with 50 % N fertilizer was applied at the time of sowing. Remaining 50 % urea was applied in two equal splits one at tillering and another at panicle initiation stage as top dressing. Randomly five plants were selected from each plot for recorded regular biometric observations from 30 DAS till harvest. Data were compiled and analyzed using appropriate statistical methods.

III. RESULT AND DISCUSSION

Plant growth parameters

The plant growth parameters viz., plant height and number of tillers were markedly influenced by various organic amendments applied in rice. The maximum value of these parameters was recorded with treatment T_{12}seedling dip in Azotobacter + Vermiwash 2% + Banana Pseudostem Sap 2% which was at par with treatments T_{13} and T_{14}. The liquid organic manures contain small amount of essential nutrientsand growth boosters and these constituents are known to have positive effect on plant growth development and yield attributes of crop. This was the possible reason for increasing the growth and yield of rice under liquid manure treatments. To study the feasibility of foliar applied organic liquid manures on crops, several experiments were conducted by Venkataramana *et al.* (2010) with vermi wash and cow dung wash on mulberry at Vikarabad (AP) and Venkatalakshmi *et al.* (2009) with panchgavya on amaranthus at Coimbatore (TN). The results obtained are also under the study in conformity with the findings of Tharmaraj *et al.* (2011).

Yield parameters

Yield contributing parameters such as length of panicle, seeds per panicle, test weight, grain and straw yields were measured at harvest of the crop.The results in table 1 indicated that different treatments induced marked variations in length of panicle, seeds per panicle, test weight, grain and straw yields and harvest index. Highest values of all these parameters were found with seedling dip in Azotobacter + Vermiwash 2% + Banana Pseudostem Sap 2%.

The higher length of panicle (21.5 cm), seeds per panicle (131.4), test weight (21.4 g), grain (5956 kg) and straw (6208 kg) yields and harvest index (49%) were recorded in treatment T_{12}, seedling dip in Azotobacter + Vermiwash 2% + Banana Pseudostem Sap 2%) . In case of grain yield, treatment T_{12} was at par with treatment T_{13} and T_{14} while in case of straw yield, it was at par with treatment T_{13}. The results of experiment conducted at SWMRU, NAU, Navsari also proved that foliar application of banana sap @ 2% on mango increased the fruit retention and fruit yield (Anon., 2010). These results are also in conformity with the findings of Bokare (2013) in onion.

REFERENCES

[1] Anonymous (2010). RKVY project, Navsari Agricultural University, Navsari, Gujarat, India.

[2] Bokare, S. P. (2013). Effect of banana pseudostem sap and vermiwash spray on yield and quality of organically grown onion. M.Sc. (Agri.) thesis, Navsari Agricultural university, Navsari, Gujarat, India.

[3] Singh Y. V., Mandal B. K. (2000): Rate of mineralization of Azolla, other organic materials andurea in water logged soils. Trop Agr (Trinidad) 77 (1): 119-122.

[4] Stockdale E. A., Lampkin N. H., Hovi M., Keating R., Lennartsson E. K. M., Macdonald D. W.,Padel S., Tattersall F. H., Wolfe M. S., Watson C. A. (2001): Agronomi and environmental implicationsof organic farming systems. Adv Agron 70: 261-327.

[5] Tharmaraj, K., Ganesh. P., Kolanjinathan. K., Suresh Kumar. R. and Anandan A. (2011) Influence of vermicompost and vermiwash on physico chemical properties of rice cultivated soil. *Current Botany*, **2** (3): 18-21.

http://dx.doi.org/10.22161/ijeab/2.6.28 *ISSN: 2456-1878*

[6] Venkatalakshmi, K., Balasubramanian, A. and Sankaran N. (2009). Influence of seed treatment and foliar spray of panchagavya on growth, yield attributes and yield of *Amaranthus viride. Madras Agriculture Journal*, **96** (1-6): 135-138.

[7] Venkataramana, P.B., Narasimha Murthy, Krishna Rao, J.V. and Kamble. C.K. (2010). Efficacy of foliar sprays of vermi wash and cow dung wash on biochemical and yield attributes and yield of mulberry (*Morus alba* L.). *Karnataka Journal of Agriculture Science*, **23** (2): 358-360.

Response of nutrient management practices through organic substances on rice var. GR-11

Treatment Detail	**Plant height (cm)**	**No. of tillers**	**Lenght of panicle (cm)**	**Seed per panicle**	**Test weight (g)**	**Seed Yield (kg/ha)**	**Straw Yield (kg/ha)**	**Harvest Index (%)**
Control (No biofertilizer+ No Spray)	92.8	11.0	12.4	116.0	12.5	4230	4418	48.5
Banana Pseudostem sap 2%	102.9	15.1	17.6	124.5	17.0	5077	5253	49.2
Vermiwash 2%	103.0	15.5	18.0	125.4	17.7	5185	5374	49.1
Panchgavya 2%	101.6	14.7	17.2	124.0	16.4	4952	5151	49.0
Vermiwash 2% + Banana Pseudostem Sap 2%	100.8	14.2	16.6	123.2	15.8	4880	5107	48.9
Vermiwash 2% + Panchgavya 2%	99.7	13.3	16.0	122.0	15.2	4745	4920	49.6
Banana sap 2% + Panchgavya 2%	98.1	12.9	15.6	121.6	14.3	4500	4698	48.8
Seedling deep in Azotobacter	104.7	16.2	18.4	126.7	18.1	5214	5421	49.0
Seedling deep in Azotobacter + Banana Pseudostem sap 2%	108.0	16.7	19.3	127.9	19.1	5423	5683	48.7
Seedling deep in Azotobacter + Vermiwash 2%	110.2	17.2	19.7	128.5	19.6	5507	5741	49.0
Seedling deep in Azotobacter + Panchgavya 2%	105.4	16.4	18.8	127.3	18.6	5316	5562	48.9
Seedling deep in Azotobacter + Vermiwash 2% + Banana Pseudostem Sap 2%	118.5	19.7	21.5	131.4	21.4	5956	6208	49.0
Seedling deep in Azotobacter + Vermiwash 2% + Panchgavya 2%	115.4	18.5	20.8	129.6	20.6	5726	6016	48.3
Seedling deep in Azotobacter +Banana sap 2% + Panchgavya 2%	112.8	17.9	20.2	129.0	20.0	5624	5885	48.1
S.Em.	2.1	1.4	0.8	1.66	0.77	123.76	107.16	
C.D.	6.1	4.0	2.2	4.82	2.25	359.84	311.59	

Determination of Yield and Yield Components of Vetch and Cereal Mixture and Evaluation Using by GGE-Biplot Analysis

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Abstract— *This study was carried out to determine forage and seed yield and its effecting component in different vetch and cereal mixtures, ecological condition of Tekirdağ-Thrace region of Turkey. The study was conducted using a total 5 vetch and cereals varieties includes three different vetch species orakefe, Hungarian vetch species sarıefe, narbon vetch candidate variety 570, two different cereals (barley variety scarpia, oat variety sebat) and their mixture combinations, each species were sown as sole, double and triple mixtures (8 combinations for each genotype) a randomized complete block design with 3 replications was laid out on 2013-2015 growing season. Eight mixture combinations for each genotype (common vetch, Hungarian vetch, Narbon vetch, barley and oat) were evaluated for yield and major plant structural characteristics. Genotype-Trait (GT) biplot analyses were used. Applying type of analyses to the multiple trait data revealed that GT biplot graphically displayed the interrelationships among traits and facilitated visual comparison of mixtures and selection for each genotype. Wide variation was observed for traits plant structure and components (plant height, branch number and pod number/plant, 1000 seed weight, individual genotype ratio). It was found that; seed yield, 1000 seed weight, plant height, branch and pod number /plant were the highest value in pure stand NV570. In addition (570+sebat) had maximum dry forage yield, sarıefe as pure stand has the maximum plant height and seed yield value. On the other had sarıefe+scarpia combination showed very high value for pod number/plant, branch number/plant and fresh and dry forage yield. Pure stand scarpia was the best performer in seed yield. CV+NV+scarpia had the highest 1000 seed weight, fresh and dry forage yield. Maximum planth height was determined from sebat+scarpia, CV+O+B and CV+HV+B combinations. Favorable seed yield and 1000 seed weight value was produced under pure stand sebat seeding. Intercrop NV570+O combination had the higher fresh and dry forage yield. CV+HV+O, CV+NV+O and CV+B+O combination had the highest plant height of sebat.*

Keywords— GGE-Biplot, Forage Crops, Vetch, Cereal, Yield

I. INTRODUCTION

In the Thrace region, there are many wild relatives of cultivated plants such as wheat, cheakpea, broad bean, vetch as wel as many endemic species. http://www.fao.org/ag/agp/agpc/doc/counprof/Turkey/Turkey.htm. Thrace is topographically very heterogeneous. Most of this region is occupied by undulating plains drained by the river Ergene. Vetches (*Vicia* spp.) are legumes well adapted to winter growth in the Mediterranean environments throughout the world on a variety of soil types and are used in west Asia, North Africa, Avustralia, and Turkey for varied purposes such as dry matter, silage and green manure [1],[4]. A number of vetch species (Vicia spp.) have considerable potential as grain and forage legumes since they are well adapted to the different soil and climate conditions throughout the Mediterranean environments [3], [20], [25]. In the Mediterranean Basin, including Turkey, vetches are the most common annual forage crops cultivated for hay, pasturage or silage production either alone or mixed with cereals. Hungarian vetch (*Vicia pannonica* Crantz.) is a winter hardy species, which is widely used in regions with cool winter growing conditions [2],[3]. Intercropping has been practiced traditionally in tropical regions for centuries. Interest in the intercropping of cereal-legume has been growing in many temperate and tropical regions in recent years [15], [35]. This is due to the numerous benefits. Intercropping of cereals and legumes is widely used inlow-input agriculture because the mixture of nitrogen (N)-fixing and non-N-fixing crop species provides complementarities in the utilization of resources [16]. In Mediterranean countries, intercropping of cereals with legumes has been a common cropping system in short-seasonrain-fed environments, especially because of their increased productivity and sustainability [26]. However, vetch has a vine growing habit and if sown as monocrop, it lodges heavily [11]. Because of having weak and thin stem, the vetches spread. So harvest gets difficult and because of losing leaves 324, its forage and quality decrease. Vetch should be sown mixture with cereals in order to overcome spreading. As a result, forage yield and quality start to decrease due to the decomposition of herbage. Due to this, it is sown with winter-sown small grains, such as oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and triticale (x *Triticosecale* Wittmack). In mixtures, companion cereals provide structural support for common vetch growth, improve light interception and facilitate mechanical harvest, whereas common vetch in mixtures improves the quality of forage [28], [11], [18]. So far, there has been no report on what cereal is the best companion crop for vetch [10], [30]. [28] reported that the most suitable cereal for mixtures with common vetch is wheat. Because high cereal rate in botanic composition of legume + cereal mixture cause low protein content which is one of the most important factors to determine the forage quality [7], [4]. Use of cereals and grasses in mixtures with legumes not only increases forage yield, but also providesphysical support to, which facilitates mechanical harvestingand other benefits of these mixtures includegreater use of light, greater uptake of water and nutrients,enhanced weed suppression, and increased soil conservation, [24], [6]. Disadvantages of mixtures may include extra work in preparing and planting seed and lack of mixed crop tolerance to herbicides. Higher yields have been documented for intercropping oats and vetches [13]. Generally green herbage and dry matter yield and percentage of mixture decreased by increasing seed rate of legume in the mixture [19], [28], [7]. Mixtures containing 25-50 % legumeproduced more quality forage and yield per unit area than those of pure sowings [27], [5], [9]. [31] and [8] found the highest forage yield at the ratio 75:25 (oats: vetch) differences in soil fertility might be a factor in this response. As any of the species increased in the mixture yield gradually decreased, minimum yield was found when each species were sown alone and the yield was 55% lower than the maximum. Dry weight was affected by the common vetch ratio as was reported before [8], [31], [11]. The highest protein and digestibility contents were found in common vetch sown alone, as the proportion of oats increased there was a gradual reduction in both protein and digestibility, the lowest contents for both were with oats sown alone, with no difference ($P>0.05$) between oats cultivars. [11] also pointed out that protein content of rainfed oat-common vetch mixture depends on vetch proportion. Recently, many new researches have been conducted where vetch species were grown with different cereals in an intercropping system, whereas generally associated with oat; wheat and barley have been carried in recent years. However few related with more than double mixture have been carried in recent years. [32] developed a site regression methodology which contains genotype main effect plus genotype by environment (GGE) using biplot procedure as a graphical tool for analysis of multi environment trials dataset. A biplot is a plot that simultaneously displays the effects of genotypes and the environment [14]. The biplot according to GGE model is a biplot that displays the GGE of multi-environment trials dataset. It is constructed by plotting the fi rst two principal components (PC1 and PC2) derived from singular value decomposition of the environment-centered data [36]. However, it can also be equally used for all types of two-way data that assume a two way structure. The genotypes can be generalized as rows and the multiple traits as columns. [34] used a genotype by trait (GT) biplot, which is an application of the GGE biplot technique to study the genotype by trait data. The objectives of this study were to evaluate the forage production capacities of vetch and cereals mixtures, to assess the effects of different mixture levels on the yield, yield components of the mixtures. And also determining the most convenient mixture combinations and interactions in this intercropping systemvetch and cereal mixtures using by GT biplot technique, under the seacost condition of Northern Turkey.

II. MATERIALS AND METHODS

2.1 Field Experiments

Plant materials, common vetch (*Vicia sativa* L."Orakefe") Hungarian vetch (*Vicia pannonica* Crantz. "Sarıefe") Narbonne vetch (*Vicia narbonensis* candidate variety "570"), Oats (*Avena sativa* variety "Sebat" and Barley (*Hordeum vulgare* variety "Scarpia") were used as plant material of the research. Each species were sown as sole, double and triple mixtures.Experiments were conducted during the growing seasons 2013-2014 and 2014-2015 at the experimental area of field crops department of Namık Kemal University Tekirdağ, Turkey. Experimental area is located at 36° 15´N and 36° 30´ E direction and has a typical meditarrenean climate generally. Before seeding 50 kg/ha of N and 50 kg/ha of phosphorus were applied as a starter fertilizer (Using complex fertilizer 20-20-0). Seeding was made in October of both years at same rate. Field soil was loamy structure and had low organic matter which was poor for nitrogen and phospohorus. The plots were six rows of 5m long with 25 cm row spacing. Central two rows harvested for fresh forage, the other inner two rows harvested for grain yield. The sowing rate of Common vetch "Orakefe" (CV) 100kg/ha, Hungarian vetch "Sarıefe" (HV) 80kg/ha, Narbon vetch "570" (NV) Candidate variety 150kg/ha, Barley "Scarpia" (B) 180kg/ha and Oat "Sebat" (O) 180kg/ha were sown alone as well as double and triple mixtures. The mixtures were planned and seeding %60 legumes, % 40 cereals designated. Plot combination of each species was planned different ratio. Plant materials were sown Orakefe" (CV), Candidate variety Narbon vetch "570" Cultivar "Sarıefe"(HV), Cultivar "Sebat" (O), Cultivar "Scarpia" (B) as sole crop, double combinations (CV-NV, CV-HV, CV-O and CV-B) and triple combinations (HV-NV-CV; HV-B-CV and HV-O-CV) . Yield and yield characters were determined for each cultivar in all combination individually. The experimental design was randomized complete block design with three replications combined over years. All the data were subjected to analysis of variance (ANOVA) procedures using the MSTATC statistical software package. The weed Control by hand was carried out twice when density was high, in the preflowering and postflowering stages. The meteorological data of the experimental area during growing season from October to July was given at table 1. It has a longterm rainfall of 477,9 mm during growing season, while the average temperature of the first (13.8 °C) and second growing season (13,3 °C) was higher than the long term average value (12.5°C). The total precipitation was 524.2 mm and 598,1mm during the 2013-2014 and 2014-2015 growing seasons respectively.

2.2 Statistical analysis

The Anderson and Darling normality test was used to normality test of dataset using MINITAB version 14 (2005) software. Cluster analysis is a tool for classifying objects into groups. The GT biplot analysis was performed on the eight cluster groups and eight for legume base and six for cereal base quantitative traits. The genotype by trait (GT) biplot method [34] was used to show the vetch+cereal mixture by trait two-way data in a biplot. These statistical methods have been described in detail by [32] and [34]. All biplots presented in this study were generated using the software GGEbiplot package [33].

2.2.1 Principal component analysis (PCA*)*

Principal component analysis (PCA) of the quantitative data was performed to investigate the importance of different characters in explaining vetch and cereals mixtures and the most commonly is useful tools for screening mixtures, and thus guide in the choice of combination for high and quality production. Biplot was used to assess the GGE biplot method [32] to assess the patterns of relations among morphological attributes, mixtures and their combinations and conducted in the dimension of first two principal components (PC1 and PC2), using a singular-value decomposition procedure.

2.2.2 Cluster analysis

In order to determine the similarities or differences among mixtures, a cluster analysis was performed as well. The statistics of the cluster analysis based on the seven morpho-agronomic traits for eight different mixture combinations for legumes. In addition, six morpho-agronomic traits for eight different mixture combinations were examined for cereals.

III. RESULTS AND DISCUSSION

The mixture combinations and evaluated of characters for each genotype were given individual as well.The following can be seen from fig. 1. The combinations of each genotype characters Orakefe (CV), 570(NV) +CV, Sarıefe (HV), Sebat (O)+CV, Scarpia(B)+CV, HV+NV+CV, HV+B+CV, HV+O+CV were given an individual. The first one was given as common vetch variety and its combination can be seen from fig. 1. The GT biplot for Common vetch variety "Orakefe and its mixtures with cereals dataset, based on proposed method of [32] explained 66% of the total variation of the standardized data. This relatively moderate percentage reflects the complexity of the relationships among the measured traits. The first two principal components (PC1 and PC2) explained 44% and 22%, respectively. In the GT biplot, a vector is drawn from the biplot origin to each marker of the mixture to facilitate visualization of the relationships between and among the characters as well as mixture combinations

The vertex combinations in this invertigation are (CV), (HV+NV+CV), (HV+O+CV), (B+CV), (O+CV) and (HV+CV). These combinations are the best or poorest combination in some or all the parameters since they had the longest distane from the origin of biplot. Therefore, it seems that Orakefe (CV) had the hight value. Seed yield (SY), Pod number/plant (PNP) common vetch ratio (CVR) and plant height (PH) value. Sebat (O)+CV and Scarpia (B)+Sarıefe (HV)+CV mixture of this sector had good amount branch number/plant (BNP) and thousand seed weight (TSW). The after combination (HV+O+CV) and related sector was not good performance for parameter HV+NV+CV, HV+B+CV and NV+CV mixtures. Which fall in its sector showed good performance for dry forage yeild (DFY) and Fresh forage yeild (FFY). Cluster analysis was done and a dendogram generated from combinations. Distance matrices could distinquished clearly (similarity 66,67%) six cluster or another cluster dendogram was done and a dendogram generated from combination distance matrices could distiguised clearly (similarity %33) four clusters (Fig 2).

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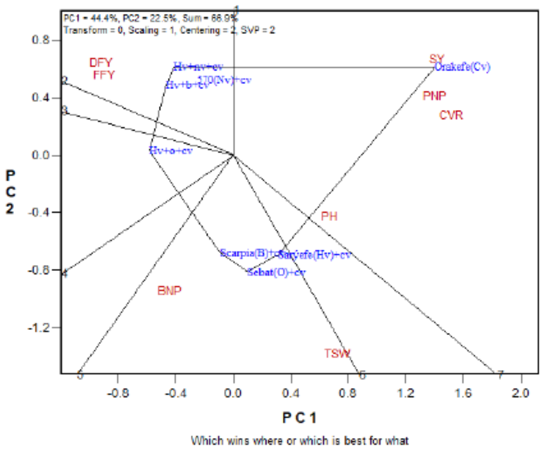

Figure 1. Polygon view, presents data of Common vetch variety "Orakefe" with 8 different mixture combinations in seven different traits.

Table 1. Correlation coefficient among seven Common vetch varieties Orakefe and its mixtures traits

Characters	BNP	PNP	SY	TSW	FFY	DFY	CVR
PH	0,081	0,056	-0,433**	0,630**	-0,239	0,093	0,373*
BNP	1	0,206	0,167	0,451**	0,162	0,117	-0,224
PNP		1	-0,191	0,137	0,241	0,273*	0,931**
SY			1	-0,097	-0,038	-0,452**	-0,401**
TSW				1	-0,123	0,053	-0,368*
FFY					1	0,698**	0,888**
DFY							0,245
CVR							1

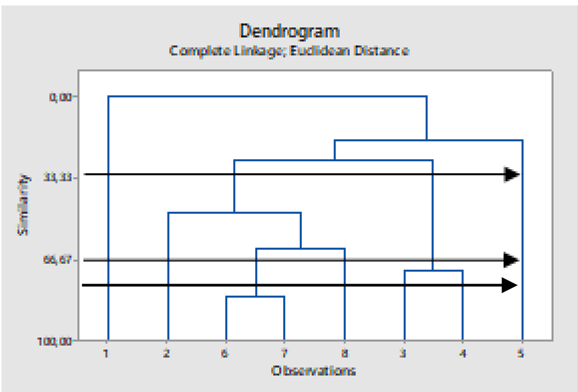

Figure 2. Clusters dendogram of Common vetch variety Orakefe with 8 different mixture combinations in seven different traits

The number of clusters was verified by multivariate analysis of variance test. This relatively moderate percentage reflects the complexity of the relationships among the measuret parameters. Thus reducing entry numbers of mixtures from 8 combinations to 6 clusters. Additional, 6 clusters are necessary to explain 66.67% similarity of the mixture combinations.

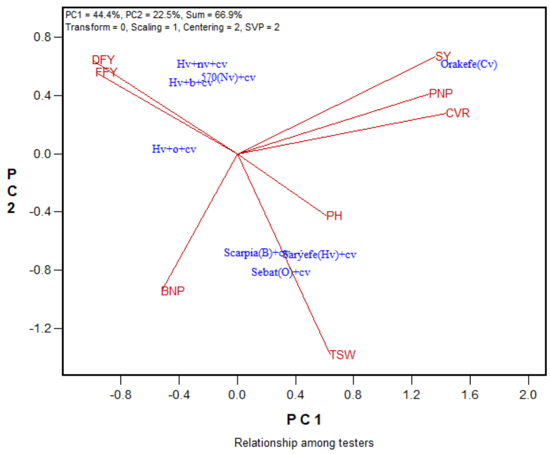

Figure 3. Vector view mixtures by trait biplot, showing the interrelationship among measured traits for Common vetch variety Orakefe with 8 mixtures combinations

In the Fig. 3, the correlation coefficient between any two traits is approximated by the cosine of the angle between the vectors. The correlation coefficients among the traits indicate that the biplot currently shows relationship among the traits that had relatively large loading on both PC1 and PC2. Therefore, the most prominent relations by this fi gure (8 mixtures and seven traits) are: a strong positive association among SY and PNP, among SY and CVR, among DFY and FFY had positive correlation as well. There was a near zero correlation between BNP and TSW with SY, PNP and CVR (Fig. 3) as indicated by the near perpendicular vectors (r= cos 90 = 0). Some of mentioned results can be verified using correlation coefficients of Table. 1.

The GT biplot for Narbon vetch candidate variety (NV570) and its mixtures with cereals based on method of [34] explained 66% total variation of standardized data. The principle component vectors (PC1 and PC2) explained 61.4 % and 18.1 %, sum of the vectors was 79.5%. In the GT biplot, this view helps identify mixtures with highest values for one or more traits. The scores of six traits seed yield (SY), plant height (PH), narbon vetch ratio (NVR), tausand seed weight (TSW), pod number /plant (PNP) and branch number /plant (BNP) fel in the NV570 sector. NV570 had heighest and nearest heighest values for this traits. Orakefe+NV570 combination was second to NV570 for these traits. Similarly combination of sebat+NV570 mixture was the highest in fresh and dry forage yield, other mixture combination. CV+B+NV570 had the heighest fresh forage yield. Since the biplot did not explain all variation of these predictions may not exactly reflect the observed values.

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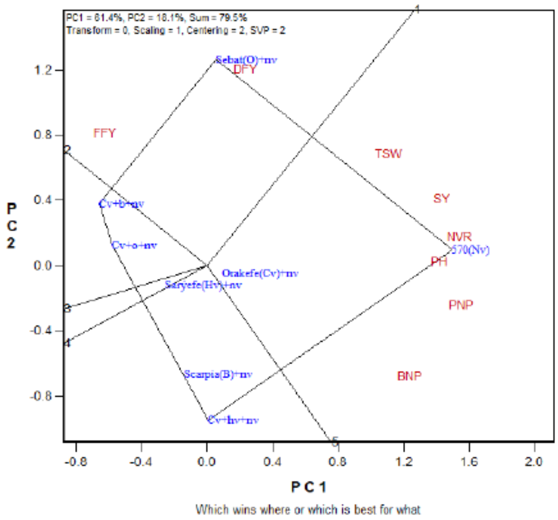

Figure 4. Polygon view, presents data of "Narbon vetch candidate variety" with 8 different mixture combinations in seven different traits

Fig. 5 displays the tree diagram. The figure provides a graphical view of the clusters. Cluster analysis was approved as a suitable method for data classifying and suggested by [22]. Based on the cluster analysis in Fig. 5, we can divide the 8 mixture combinations can divide into 6 clusters based on the studied agronomic characters. In other words, 6 clusters are necessary to explain 66.67% similarity of the mixture combinations.

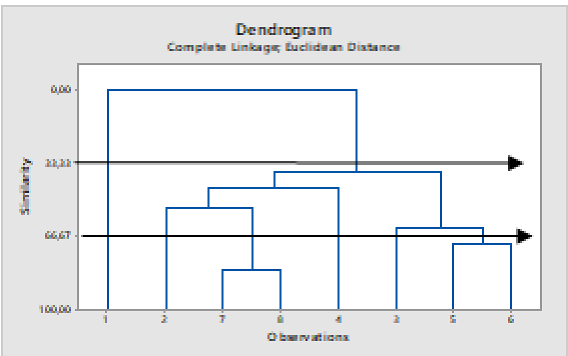

Fig. 5. The cluster dendogram of Narbon vetch candidate variety with seven different traits of eight mixture combinations.

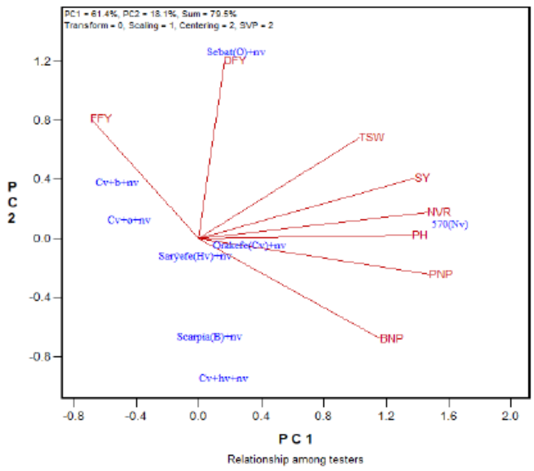

Figure 6. Vector view mixtures by trait biplot, showing the interrelationship among measured traits for Narbon vetch candidate variety with 8 mixtures combinations.

The correlation coefficients among the seven traits are presented in Table 2. The vector view of a GGE biplot provides a succinct summary of the interrelationships among the traits [34]. Figure 6 is the vector view of the GGE biplot, in which the mixture combination is connected with the biplot origin via traits. This view of the biplot aids understanding of the interrelationships among mixture combination. The cosine of the angle between the vectors of two traits approximates the correlation coefficient between them. Therefore, the most prominent relations were: (a) near-zero correlations between TSW and DFY, between PNP and TSW, and between BNP and DFY as indicated by the near-perpendicular vectors (r = cos90 = O); and (b) positive associations among PH, NVR, PNP and SY, and between PNP and BNP as indicated by acute angles.

Table 2. Correlation coefficient among seven traits of Narbon vetch candidate variety 570 and its mixtures combinations

Characters	PH	BNP	PNP	SY	TSW	FFY	DFY	NVR
PH	1	0,511**	0,651**	0,724**	0,168	-0,357**	-0,049	0,868**
BNP		1	0,561**	0,441**	0,018	-0,438**	-0,110	0,647**
PNP			1	0,804**	0,269	-0,385**	0,041	0,960**
SY				1	0,334*	-0,314*	0,088	-0,409**
TSW					1	0,074	-0,006	0,226
FFY						1	0,612**	0,948**
DFY							1	0,732**
NVR								1,000

The GT biplot for Hungarian vetch "Sarıefe" and its mixtures with cereals based on method of [34] explained

66% total variation of standardized data. The principle component vectors (PC1 and PC2) explained 41.8 % and 25.7 %, sum of the vectors was 67.5%. The biplot representing a polygon view (Fig. 7), having some vertex mixturess while the rest are inside the polygon. These vertex mixtures are supposed to be the most responsive since they have the longest distance from the biplot origin. Responsive mixtures are either best or poorest at one or all mixtures [34]. Thus it seems that Scarpia (B) +HV and CV+ Scarpia (B) +HV mixture combinations had the highest values for DFY, BNP, PNP, FFY and TSW. The other vertex mixture combination which fall in its sector were good for plant height (PH), Hungarian vetch ratio (HVR) and seed yield (SY). The other vertex mixtures (CV+HV and CV+NV+HV) and related sectors were not good performance for the measured traits.

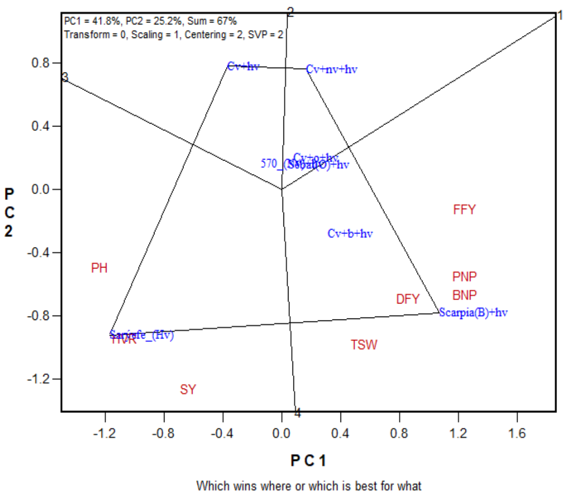

Figure 7. Polygon view, presents data of Hungarian vetch variety Sarıefe with 8 different mixture combinations in seven different traits.

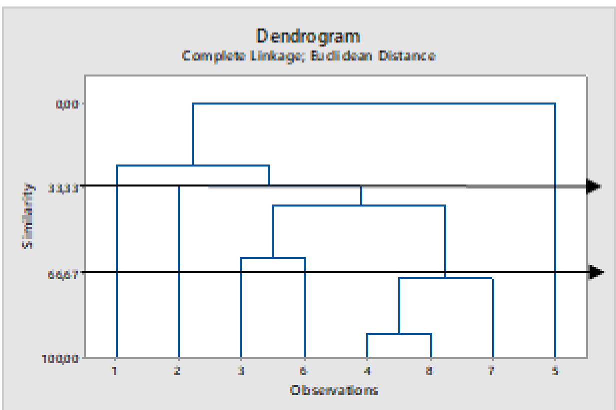

Figure 8. The cluster dendogram of Hungarian vetch variety Sarıefe with seven different traits of eight mixture combinations

Based on the cluster analysis in Fig. 8, we can divide the 8 mixture combinations can divide into 4 clusters based on the studied agronomic characters in similarity level 33.33 % of mixture combination. In other words, 4 clusters [1, 2, (3,6), (4, 7, 8), 5] are necessary to explain 33.33% similarity of the mixture combinations.

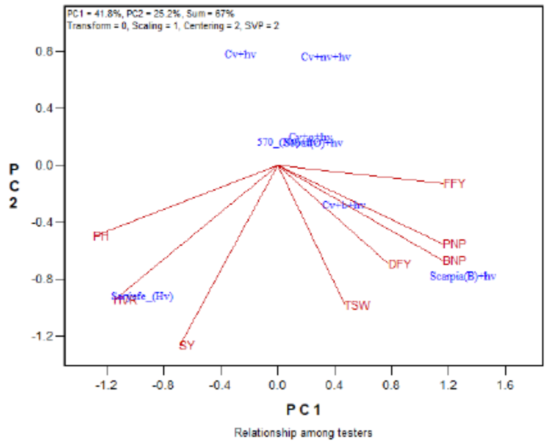

Figure 9. Vector view mixtures by trait biplot, showing the interrelationship among measured traits for Hungarian vetch variety Sarıefe with 8 mixtures combinations.

The mixtures-traits vectors and groups illustrate the specific interactions of each mixture with each trait (Fig 9). The biplot showed DFY that was highly correlated in terms of PNP (Fig.9). Positive correlations were found between FFY (Fresh forage yield), DFY (Dry forage yield), PNP (Pod number/plant) and BNP (Branch number/plant), as indicated by the acute angles (vector angles < 90°) of their respective vectors. There was a near zero correlation between BNP, PNP and FFY with PH (fig 7) as indicated by near perpendicular vectors (r=cos 90=0). The biplot of the relationship between traits negative correlation was observed between BNP and PNP with PH as indicated by the large obtus angles between their vectors. Having same result observed from Table 3.

Table 3. Correlation coefficient among seven traits of Hungarian vetch variety Sarıefe and its mixtures combinations

Characters	PH	BNP	PNP	SY	TSW	FFY	DFY	HVR
PH	1	-0,459**	-0,497**	0,339*	0,020	-0,035	0,144	0,848**
BNP		1	0,637**	0,167	0,029	0,348**	0,273	-0,350**
PNP			1	0,222	0,108	0,414**	0,419**	-0,278
SY				1	0,233	-0,030	0,128	-0,352**
TSW					1	0,085	0,081	0,084
FFY						1	0,939**	0,747**
DFY							1	-0,018
HVR								1

The GT biplot can be used to compare mixture combination on the basis of multiple traits and to identify mixture that are particularly good in certain aspects and therefore can be candidates for high production in mixture sowing. The GT biplot for oat variety "Scarpia" and its mixtures with cereals based on method of [34] explained 66% total variation of standardized data. The principle component vectors (PC1 and PC2) explained 39.9 % and 28.2 %, sum of the vectors was 68.8%. The perpendicular lines to the polygon sides facilitate comparison between neighboring mixtures. Specifically, comparison between CV+NV+ (Scarpia) B and (Scarpia) B neigboring vertex mixtures. According to [34] we have used the GT biplot to compare mixture combinations on the basis of multiple traits and to identify mixtures or groups of mixtures that are particularly good in certain aspects and therefore can be determined the best combination in vetch species+ cereal mixtures. The equality lines, which originate from the centre of biplot and are perpendicular to the sides of polygon, divide the graph into four sectors. The partitioning of GT interaction through GT biplot analysis shows that the first and second principal components (PC1 and PC2) together can explain 68% of the total variation. The yields of these mixtures were either the highest or lowest in one or more test environments. From the polygon view of biplot analysis, the genotypes fell into four sections and the test environments can be grouped in three sections (Section 1, 2 and 3). CV+NV+ B (Scarpia) combination were the highest tousand seed weight (TSW), fresh forage yield (FFY) and Dry forage yield (DFY). In addition Barley variety (Scarpia) was the highest seed yield (SY) and barley ratio value (Fig. 10). On the otherhand these vertex pure stant Scarpia and its mixtures, NV570+B, HV+B and CV+B were the highest value of Barley ratio and seed yield. (Fig 10). Heighest plant height of Scarpia (B) was recorded on O+B;CV+O+B and CV+HV+B mixture combinations.

Cluster analysis was given in fig 11. According to the result of Scarpia combination can diveded in to 3 clusters based on studied. Five mixture combinations were extracted from the 8 studied combinations. In fact, with this method, 8 mixture combinations were reduced to three in 33.33 % similarity level of the mixture combination (Fig. 11). Another explanation, Three clusters [(1, 2, 3, 7, 8), (4, 5), 6] are necessary to explain %33 similarity level of the mixture combinations.

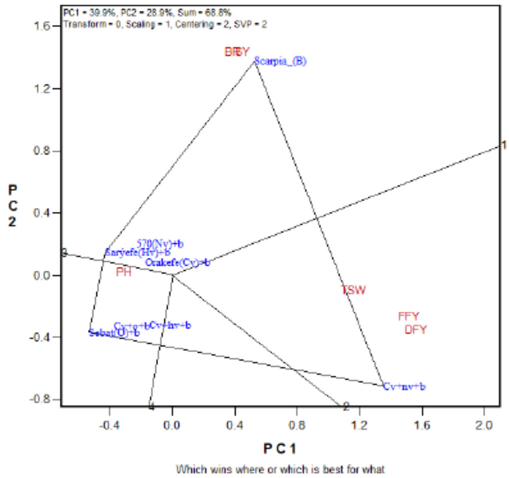

Figure 10. Polygon view, presents data of "Oat variety Scarpia" with 8 different mixture combinations in seven different traits

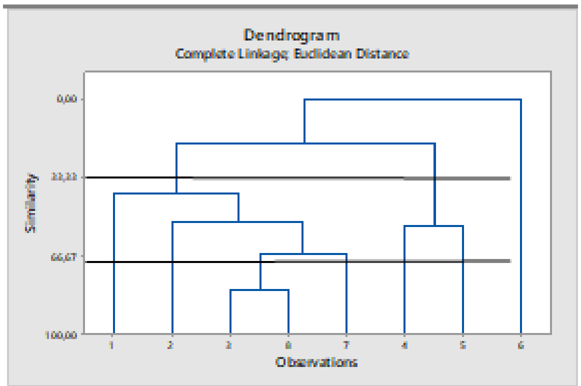

Figure 11. The dendogram of Oat variety Scarpia with seven different traits of eight mixture combinations using hierarchial cluster analysis (Ward's method and squared Euclidean distance

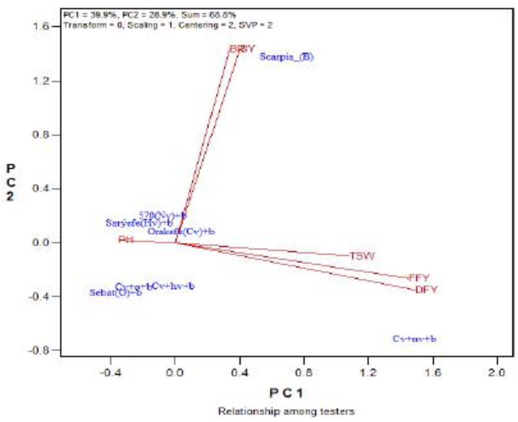

Figure 12, Vector view mixtures by trait biplot, showing the interrelationship among measured traits for "Oat variety Scarpia" with 8 mixtures combinations.

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The correlation between these traits supports this theory that plant height and Scarpia ratio can improve Scarpia seed yield. The correlation coefficient between Scarpia seed yield and TSW is positive. TSW is one of the improtant components for increasing yield production, so, this is normal and expected. There is a positive and significant correlation between TSW and FFY; DFY. It is seen a close and positive association between FFY and DFY traits in many studies. The GT biplot for Barley variety "Sebat" and its mixtures with cereals based on method of [34] explained 66% total variation of standardized data. The principle component vectors (PC1 and PC2) explained 39.9 % and 28.2 %, sum of the vectors was 68.8%. The biplot representing a polygon view (Fig. 1). Having some vertex hybrids while the rest are inside the polygon. These vertex hybrids are supposed to be the most responsive since they have the longest distance from the biplot origin. Responsive hybrids are either best or the poorest at one or all locations [34]. Thus, hybrid Sebat was the highest seed yield and thousand seed weight and sebat ratio (OR). Similarly, in the next sector where the vertex 570(NV)+O excelled for Fresh and Dry forage yield. In addition sector where the vertex combination CV+(HV)+O followed by CV+(NV)+O and CV+B+O exceled for plant height. None of the traits fell in the sectors with Sarıefe (HV)+O and Orakefe (CV)+O as the vertices genotypes, indicating that these mixture combinatios were not best in any of the trait, Sebat (O) and 570(NV)+O combinations seemed to be heighest- yielding for seed yield, thousand seed weight, Fresh and Dry forage yield in two mentioned combinations.

Table 4. Correlation coefficient among seven traits of Scarpia and its mixtures combinations

Characters	PH	SY	TSW	FFY	DFY	BR
PH	1	0,319*	0,267	0,102	0,017	-0,247
SY		1	0,326*	-0,099	0,011	0,139
TSW			1	0,288*	0,321*	0,029
FFY				1	0,886**	0,760**
DFY					1	-0,158
BR						1

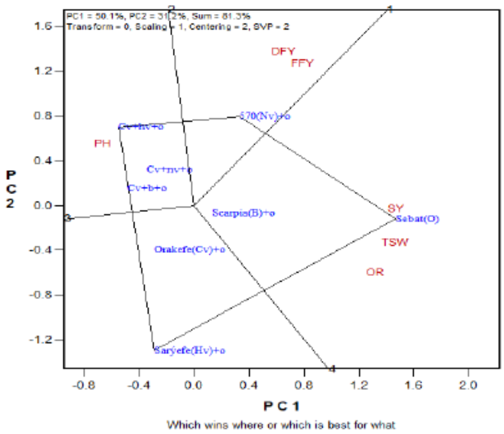

Figure 13. Polygon view, presents data of "Barley variety Sebat" with 8 different mixture combinations in seven different traits.

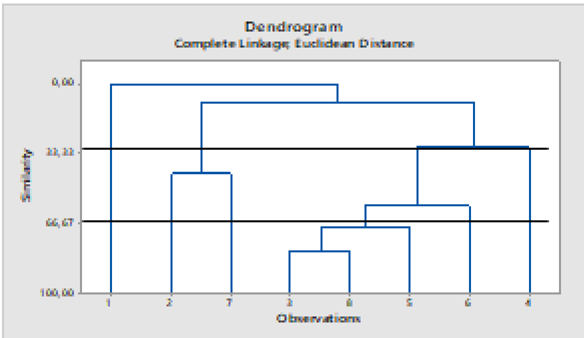

Fig. 14. The cluster dendogram of Barley variety Sebat with seven different traits of eight mixture combinations.

Analysis of the results showed that the cultivar was clustered in to four main clusters in 33.33 % similarity level (Fig. 14). In the second cluster were included 1 (NV)+O and 7 (CV+HV+O) and third clusters were included 3 (CV+O), 8 (CV+B+O); 5 (B+O); 6 (CV+NV+O) which were similar each other. Four mixture combinations were extracted from the 8 studied combinations. In fact, with this method, 8 mixture combinations were reduced to four in 33.33 % similarity level (Fig. 14).

In GGE-biplot, the cosine of the angle between any two location vectors stands for correlation intensity. Less than 90° indicates a positive correlation, more than 90° a negative correlation, and close to 90° no correlation [37], [29]. According to Fig. 15, there were positive relationships between SY and TSW, FFY and DFY, PH and FFY, PH and DFY. TSW and OR.On the other hand, the negative correlations were observed between SY and PH, TSW and PH, OR and PH (Fig. 15).

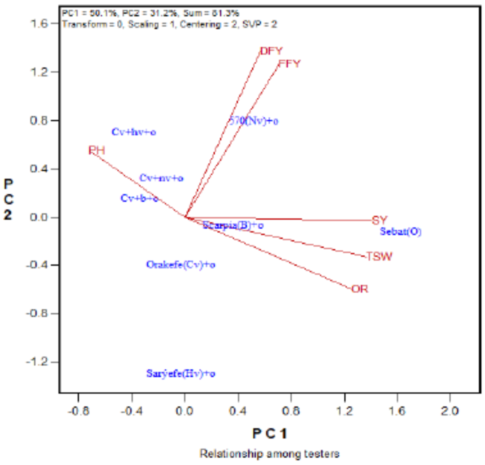

Fig. 15, Vector view mixtures by trait biplot, showing the interrelationship among measured traits for "Barley variety Sebat" with 8 mixtures combinations.

Table 5. Correlation coefficient among seven traits of Barley variety Sebat and its mixtures combinations

Characters	PH	SY	TSW	FFY	DFY	OR
PH	1	-0,334*	-0,097	0,085	0,165	-0,319*
SY		1	0,680**	0,357*	0,315*	0,737**
TSW			1	0,288*	0,321*	0,907**
FFY				1	0,886**	0,088
DFY					1	-0,095
OR						1

IV. CONCLUSION

The GT biplot analysis of 8 different combinations showed variable ranges of purestand and mixtures of vetches and cereals. According to polygon view of traits and combination; seed yield, pod number /plant and plant height were found to be heighest value from pure stand Orakefe. Seed yield, tausand seed weight, planth height, Branch and pod number /plant were the heighest value in pure stand NV570. In addition NV570+sebat had maximum dry forage yield. Sarıefe as purestand has the maximum planth height and seed yield value. On the other hand Sarıefe+Scarpia combination showed very high value for pod number/plant, branch number/plant, and fresh and dry forage yield. Purestand Scarpia was the best performer in seed yield. CV+NV+Scarpia had the highest tausand seed weight, fresh and dry forage yield. Maximum planth height was determined from Sebat+ scarpia, CV+O+B and CV+HV+B combinations. Favurable seed yield and tausand seed weight value was produced under purestand sebat seeding. Intercrop NV570+O combination had the higher fresh and dry forage yield. CV+HV+O, CV+NV+O and CV+B+O combination had the highest plant height of sebat.

REFERENCES

[1] Abd El Moneim, A.M., Cocks P.S., Swedan, Y., 1988. Yield stability of selected forage vetches (Vicia spp.) under rain fed conditions in West Asia. Journal of Agriculture Science, 111: 295-301.

[2] Acikgoz, E. 1982. Cold tolerance and its association with seedling morphology and chemical composition in annual forage legumes. II. Vetch (Vicia) species. Plant Breeding, 88: 278-286.

[3] Açikgoz, E. 1988. Annual forage legumes in the arid and semi-arid regions of Turkey. In: Nitrogen Fixation by Legumes in Mediterranean Agriculture. (Eds.: D.B. Beck and L.A. Materon). Martinus Nijhoff Publ., pp. 47-54.

[4] Açikgöz, E., 2001. Forage Crops. U.U. Güçlendirme Vakfı Yayın No: 182, 584 pp., Bursa.

[5] Alemu, D., W. Mwangi, M. Nigussie, and D. J. Spielman. 2007. An Analysis of Maize Seed Production and Distribution Systems in Ethiopia's Rift Valley. Ethiopian Institute of Agricultural Research (EIAR) Research Report 72. Addis Ababa: EIAR.

[6] Anil, L., Park, J., Phipps, R.H., Miller, F.A., 1998. Temperate intercropping of cereals for forage: a review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53, 301–317. Association of Official Analytical Chemists (AOAC), 1980. Official Methods of Analysis, 11th ed. AOAC, Washington, DC, p. 125.

[7] Anlarsal A., Yücel C., "The Effect Of Different Sowing Times And Seeding Rates On The Potential Of Forage Production Of Fenugreek Lowland Conditions ", Agricolture Mediterranea. , vol.125, pp.172-176, 1995.

[8] Ansar M., M. A. Mukhtar, R. S. Sattar, M. A. Malik, G. Shabbir, A. Sher and M. Irfan. 2013. Forage yield as affected by common vetch in different seeding ratios with cereals in Pothohar region of Pakistan. Pak. J. Bot. 45(SI):401-408.

[9] Buyukburc, U. and Y. Karadag, 2002. The amount of NO3-N transferred to soil by legumes, forage and seed yield, and the forage quality of annual legume + triticale mixtures. Turk J. Agric For., 26: 281-288.

[10] Caballero, R., Goicoechea, E.L., 1986. Utilization of winter cereals as companion crops for common vetch and hairy vetch. In: Proceedings of the 11th General Meeting of the European Grass. Fed. pp. 379–384.

[11] Caballero R., E. L. Goicoechea, P. J. Hernaiz. 1995. Forage yields and quality of common vetch and oat

sown at varying seeding ratios and seeding rates of vetch. Field Crops Research. 41:135-140.

[12] Droushiotis, D.N., 1989. Mixtures of annual legumes and small-grained cereals for forage production under low rainfall. J. Agric. Sci. 113, 249– 253.

[13] Ercoli, R., J. García, L. Salvini, A. Izzo and J. Bartozzetti. – 1997. Manual del dispositivo de seletividad de langostino con doble grilla Disela II. INIDEP, Inf. Téc. Int., Julio, 1997: 1-7.

[14] Gabriel, K. R. (1971). The biplot graphical display of matrices with application to principal component analysis. Biometrika 58, 453–467. doi: 10.1093/biomet/58.3.453.

[15] Geno, L., and B. Geno, 2001. Polyculture Production: Principle, benefits and risk of multiple cropping. A report for the Rural Industry Research and Development Corporation (RIRDC), Publication, No. 01134.

[16] Hauggaard-Nielsen H, Ambus P, Jensen ES 2003. The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutr Cycl Agroecosyst 65:289–300).

[17] http://www.fao.org/ag/agp/agpc/doc/counprof/Turkey/Turkey.htm

[18] Lithourgidis, A.S., Vasilakoglou, I.B., Dhima, K.V., Dordas, C.A, Yiakoulaki, M.D. 2006). Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. Field Crop Res 99: 106–113.

[19] Lunnan, T. 1989. Barley-pea mixtures for whole crop forage. Effects ofdifferent cultural practices on yield and quality. Norwegian J. Agric. Sci, 3.

[20] Martiniello, P. and A. Ciola. 1995. Dry matter and seed yield of Mediterranean annual legume species. Agron. J., 87: 985-993.

[21] Minitabm 2005. Analysis of variance. minitab 14 Help to-go-field. http:// www. Minitab.com/support/docs/re114/14 help files/ Statistics. Analysis of variance. Pdf. Retrived November 19, 2007.

[22] Mohammadi, S.A., Prasanna, B.M. 2003. Analysis of genetics diversity in crop plants: salient statical tools and considerations. Crop Sci 43: 1235-1248.

[23] MSTAT-C, 1988. A Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments. Crop and Soil Sciences Department, Michigan State University, East Lansing.

[24] Osman, A.E. and A.M. Osman, 1982. Performance of mixtures of cereal and legume forages under irrigation in the Sudan. J. Agric. Sci., 98: 17–21

[25] Papastylianou, I. 1995. Effect of rainfall and temperature on yield of Vicia sativa under rainfed Mediterranean conditions. Grass and Forage Science, 50: 456-460.

[26] Papastylianou, I. 2004. Effect of rotation system and N fertilizer on barley and common vetch grown in various crop combinations and cycle lengths. J. Agric. Sci. 142, 41–48.

[27] Qamar I.A., Keatinge J.D.H., Mohammad N., Ali A., Khan M.A. 1999. Introduction and management of common vetch/barley forage mixtures in the rainfed areas of Pakistan. 3. Residual effects on following cereal crops, Aust. J. Agr. Res. 50, 21–27

[28] Roberts, C.A., Moore, K.J., Johnson, K.D. 1989. Forage quality and yield of wheat-common vetch at different stages of maturity and common vetch seeding rate. Agron. J. 81, 57–60.

[29] Shiri, M.R. 2013. Grain yield stability analysis of maize (Zea Mays L.) hybrid under different drought stress conditions using GGE biplot analysis. Crop Breeding Journal, 3, 107— 112.

[30] Thompson, D.J., Stout, D.G., Moore, T. 1992. Forage production by four annual cropping sequences emphasizing barley irrigation in southern interior British Columbia. Can. J. Plant Sci. 72, 181–185.

[31] Tuna, C. and Orak, A. 2007. The role of intercropping on yield potential of common vetch (Vicia sativa L.)/ oat (Avena sativa L.) cultivated in pure stand and mixtures. J. of Agric. and Bio. Sci., 2 (No.2): 14-19.

[32] Yan, W., L.A. Hunt, Q. Sheng and Z. Szlavnics, 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Sci., 40: 597-605.

[33] Yan, W., P.L. Cornelius, J. Crossa and L.A. Hunt, 2001. Two types of GGE Biplots for analyzing multi-environment trial data. Crop Sci., 41: 656-663.

[34] Yan, W. and I. Rajcan, 2002. Biplot analysis of test sites and trait relations of soybean in Ontario. Crop Sci., 42: 11-20.

[35] Vandermeer, J.H. 1992. The Ecology of Intercropping. Publisher: Cambridge University Press.

[36] Yan, W. 2001. GGE biplot—a Windows application for graphical analysis of multi-environment trial data and other types of two-way data. *Agron J* 93(5): 1111-1118.

[37] Yan, W. and Kang, M. S. 2003. GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC Press, Boca Raton, FL.

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Forest Conservation Knowledge-Community Perception Within Protected Areas: The Case of Karagöl-Sahara National Park

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Abstract—Commitment of local communities to protected areas is essential for conserving forest and biodiversity. However, in many developing countries like Turkey, former management strategies kept human from protected areas using coercion. Fortunately, more recent regimes attempt to give local populations more control on the management but little is known about local residents' perceptions, beliefs and attitudes toward the management of these areas. This study, carried out around the Karagöl Sahara National Park, determined factors which support local communities' positive perceptions towards forest conservation in the park, analysed their assessment of current park management activities compared to former management approaches and draw the implications for effective participatory management of protected areas. We collected socio-demographic data from 100 residents on their awareness of conservation methods. The findings indicated that the positive behavior of local communities towards conservation of forest within Karagöl Sahara National Park was highly correlated with the current management strategy that involved more effectively local communities, the educational level of participants.

Participants' perceptions of forest conservation were strongly related to locally perceived benefits. Although 91 % of participants were favorable to the concept of forest and biodiversity conservation within the park. Our results suggested that understanding local residents' perceptions and using them as a starting point to improve the park–people relationship could help park management staff to involve more effectively local communities and improve their awareness about biodiversity conservation within the park.

Keywords— Karagöl-Sahara National Park, forest, IUCN, community conservation.

I. INTRODUCTİON

Protected areas are the cornerstone of biological conservation. As defined by the International Union for Conservation of Nature (IUCN), protected area is a clearly defined geographical space, recognized, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values (Dudley, 2008). One

basic objective of a system plan for protected areas is to effectively protect, develop and maintain representative samples of the various biotopes in the areas where they are installed (Abuzinada, 2003). Although protected areas have usually been set aside from human exploitation, it is now increasingly recognized that they should play a role in sustaining livelihood of adjacent local communities (Hamilton et al., 2000; Walpole and Goodwin, 2001; Charnley et al., 2007).

Responding to perceptions of many conservationists — especially those working in poorer countries — that wildlife conservation and protected areas were doomed unless local communities become an integral part of conservation efforts (Hackel, 1999; Hamilton et al., 2000; Yeo-Chang, 2009), new strategies, often referred to as "community conservation" have evolved over the past two decades (Infield and Namara, 2001).

The "community conservation" approach seeks to accommodate local peoples' needs and aspirations by empowering them, promoting their active participation in local resource management, and improving their economic welfare (Songorwa, 1999; Infield and Namara, 2001; Mehta and Heinen, 2001).

To assess the effectiveness of these new park management policies for the long-term conservation of the park resources, it is important to gain insights into the factors that determine local communities' current positive perceptions about conservation of biodiversity within the park and their impressions relating to the park managers' activities. Following insights from previous research that local people perception is influenced by the type of management and the benefits they perceived from protected areas (Ormsby and Kaplin, 2005), we hypothesized that people who developed positive perception about Karagöl Sahara National Park and its biodiversity are those who get high benefits from the park. We also made the assumption that people who have positive opinion about the current park management methods developed positive perception about forest conservation within it. Based on preliminary results

(Gillingham and Lee, 1999; Mehta and Heinen, 2001) and knowledge of cultural differences and gender in the area (Vodouhê et al., 2009), we also made assumption that local communities perceptions are function of their sex, origin, main activities, formal educational level and affiliation. The objectives of this study were to (1) determine factors which support local communities' current positive perceptions towards forest conservation in Karagöl Sahara National Park, and (2) analyse their assessment of current park management activities compared to former management approaches and draw the implications foreffective participatory management of protected areas.

II. METHODS

2.1. Study area

The location of Karagol-Sahara National Park and its immediate surroundings are shown in Figure 1. The research area was calculated by GIS techniques. This area is about 21,912 ha, and between 900 and 2700 m in altitude. Karagol-Sahara National Park covers 3466 ha area in this watershed. The area includes Ciritdüzü, Cevizli, Veliköy, Pınarlı, Meşeli, Yukarıkoyunlu, Aşağıkoyunlu, Köprülü, Kirazlı, Karakoy, Kocabey, Yavuzköy villages, kıfllas (low mountain pastures), and yaylas (high mountain pastures). There are many lakes, streams, mountains, and hills in the area.

In our study case, to identify factors which determine local residents' perceptions to conserve or not the forest and to manage the park, we used data related to participants' socioeconomic, demographic and perception towards forest conservation and park management.Our perception influences our attitude (Trakolis, 2001). Indeed, attitudes are formed in part bycommunities' and individuals' perceptions and experiences (Infield and Namara, 2001). The attitude itself can be considered an independent variable affecting behavior, however, and it can also affect the independent variable of motivation. In one sense, the attitude can also be considered an intervening variable since it is a derivative of motivation which determines behavior. We argue that people consider the implications of their actions before they decide to engage or not to engage in a given behavior (Ajzen and Fishbein, 1980). Therefore, knowing people's perceptions can produce useful information that could be incorporated into the decision-making process and lead to resolution of conflicts between local people and park authorities by improving attitudes and altering behavior.

2.2. Household surveys

Protected areas in developing countries are increasinglypopular destinations for wildlife tourists, and tourism has thepotential to generate sustainable local benefits, sufficient for localpeople to value, and therefore protect, their wildlife heritage as asource of incomeMost of the questions were closed-ended, although we includedsome open-ended questions to investigate participants' perceptiontowards forest conservation and park management. We alsorecorded data about participants' age, gender, profession, and educational level. We asked participantsabout their involvement in park activities, their link with localorganization in charge of park management, the benefits they obtainfrom park and their opinion on park management. We also collecteddata on participants' economic activities, their various sources ofincome and conservation awareness.

in our study case, to identify factors which determinelocal residents' perceptions to conserve or not the forest andto manage the park, we used data related to participants' socioeconomic,demographic and perception towards biodiversity conservationand park management. These variables were identified inprevious studies as significant predictors of perceptions aboutconservation.

III. RESULTS AND DİSCUSSİON

According to the survey data recorded in research field, socio demographic characteristics belong to local community are shown on Table 1.

Table.1: Socio-demographic features of participant

Gender
Woman
Man
Total
Age
17-20
21-30
31-40
41-50
50 +
Total
Marital Status
Single
Married
Total
Educational Status
Literate
Primary Education
Secondary Education
Faculty
Master- PhD
Total
Job
Retired
House wife
Laborer
Unemployed

Officer
Student
Freelancer
Total
Level of income
to 1000 TL
between 1001 and 2000 TL
between 2001 and 3000 TL
over 3000 TL
Total
Family size
between 1-3
between 4-6
Total

Table.2: Summary of the main questions of the questionnaire and some examples of answers.

Some Qestions
Age, gender, education level, main activity?
Do you know the main objective of the park? Yes/No
Why was the park created? Conservation/tourism/no opinion
Importance of the presence of the park to surrounding people? Road maintenance/incomes from tourism/development projects/infrastructure
Your expectation about the park management? More involvement of local people/more land for agriculture/more revenue from protected areas management

Our results suggest that people's positive perceptions of forestconservation were strongly influenced by their educationallevel and assessment about park management. Gender, age, family size had less influence.

These meaningful factors may be grouped into socio-demographiceducational level and perception factors (assessmentabout park management). Perceptions were relative to benefitsobtained from park due to the current management approach. Similarresults were found by Pyrovetsi and Daoutopoulos (1997) and Allendorf et al. (2006) who concluded thatindigenous people may express anti-environmental attitudes forvariety of reasons, including low education levels, lack of awarenessabout environmental issues and lack of participation.

Local communities also benefit frommany development projects and infrastructure due to the presenceof the park. The findings clearly suggest that benefits are strongincentive for people to perceive conservation positively. Correlationbetween benefits and positive perception of biodiversity conservationhas been confirmed to be positively significant in many cases(Mehta and Heinen, 2001; Baral andHeinen, 2007). The improvement of benefits that local communitiesgot from the park will be a powerful incentive to increase theirwillingness about forest conservation within this area..

Educational level is one of the variables which affect people'sperception about forest conservation in the Karagöl Sahara NationalPark. All participants who are unfavorable to forest conservationhave a weak formal education level. This result supports thepositive link between consciousness about forest conservationand education showed by previous authors. Higher level ofeducation also helps to understand the role of the park and theusefulness of the conservation of its biological diversity. Anotherimportant reason which explains the relative high impact ofeducation on people's positive perception on forest is thatthose with good level of education rarely practice agriculture as theirmain activity. They are involved in non agricultural jobs such asteaching or working in local or national non government organizations.

Thus, they have less contact with park staff and resources.Although benefits obtained directly from the park are the mainfactor which improve people awareness about biodiversity conservation,those who originated from villages near the park alsosupported the existence of the park.

This finding appears to confirm surveys around parksin Turkey where people's agreement on the necessityto protect forest for future generations conduct them to developpositive attitudes about protected areas. The reference to future generation insupporting biodiversity and forest conservation is very important and needssupport from the park staff for more generalization**.**

People who benefit from the Karagöl Sahara National Park, especially in terms of employment opportunities such as anti-poaching ranger and tourism guide, can hold more favorable attitudes towards the park, and extension of these benefits, in addition to locally relevant education, may have the greatest potential in shaping attitudes towards conservation.

The people's positive perception on park management isa good opportunity for biological diversity conservation. Previousstudies had showed that people's perceptions of the protected areasmanagement also strongly influenced their attitude about conservation.

Education is one of the factors which has positive impact on people perception of forest conservation. Education could be an important way to motivate people to develop or reinforce positive perception about biodiversity conservation.

REFERENCES

[1] Abuzinada, A.H., 2003. The role of protected areas in conserving biological diversity in the kingdom of Saudi Arabia. Journal of Arid Environments 54, 39–45.

[2] Allendorf, T., Swe, K.K., Oo, T., Htut, Y., Aung, M., Aung, M., Allendorf, K., Hayek, L.A., Leimgruber, P., Wemmer, C., 2006. Community attitudes toward three protected areas in Upper Myanmar (Burma). Environmental Conservation 33 (4), 344–352.

[3] Ajzen, I., Fishbein, M., 1980. Understanding Attitudes and Predicting Social Behaviour. Prentice-Hall, New Jersey

[4] Baral, N., Heinen, J.T., 2007. Resources use, conservation attitudes, management intervention and park–people relations in the Western Terai landscape of Nepal. Environmental Conservation 34 (1), 64–72.

[5] Charnley, S., Fischer, A.P., Jones, E.T., 2007. Integrating traditional and local ecological knowledge into forest biodiversity conservation in the Pacific Northwest. Forest Ecology and Management 246, 14–28.

[6] Dudley, N., 2008. Guidelines for Applying Protected Area Management Categories.IUCN, Gland, Switzerland. 86 pp.

[7] Gillingham, S., Lee, P.C., 1999. The impact of wildlife-related benefits on the conservation attitudes of local people around the Selous Game Reserve, Tanzania. Environmental Conservation 26, 218–228.

[8] Hackel, J.D., 1999. Community conservation and the future of Africa's wildlife. Conservation Biology 13, 726–734.

[9] Hamilton, A., Cunningham, A., Byarugaba, D., Kayanja, F., 2000. Conservation in a region of political instability: Bwindi impenetrable forest, Uganda. Conservation Biology 14, 1722–1725.

[10] Infield, M., Namara, A., 2001. Community attitudes and behaviour towards conservation: an assessment of a community conservation programme around LakeMburo National Park, Uganda. Oryx 35 (1), 48–60.

[11] Mehta, J.N., Heinen, J.T., 2001. Does community-based conservation shape favorable attitudes among locals? An empirical study fromNepal. EnvironmentalManagement 28, 165–177.

[12] Ormsby, A., Kaplin, B.A., 2005. A framework for understanding community resident perceptions of Masoala National Park, Madagascar. Environmental Conservation 32 (2), 156–164

[13] Pyrovetsi, M., Daoutopoulos, G., 1997. Contrasts in conservation attitudes and agricultural practices between farmers operating in wetlands and a plain in Macedonia, Greece. Environmental Conservation 24 (1), 76–82.

[14] Songorwa, A.N., 1999. Community-based wildlife management (CWM) in Tanzania: are the communities interested? WORLD 27 (12), 2061–2079.

[15] Trakolis, D., 2001. Local people's perceptions of planning and management issues in Prespes Lake National Park, Greece. Journal of Environmental Management 61, 224–227.

[16] Vodouhê, G.F., Coulibaly,O., Greene, C., Sinsin, B., 2009. Estimating local values of non-timber forest products to Pendjari Biosphere Reserve Dwellers in Benin. Economic Botany 63 (4), 397–412.

[17] Walpole, M.J., Goodwin, H.J., 2001. Local attitudes towards conservation and tourismaround Komodo National Park, Indonesia. Environmental Conservation 28 (2),160–166.

[18] Yeo-Chang, Y., 2009. Use of forest resources, traditional forest-related knowledge andlivelihood of forest dependent communities: cases in South Korea. Forest Ecologyand Management 257, 2027–2034.

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Chemical composition and anti-arthritic activity of *Anacyclus valentinus* extract on adjuvant-induced arthritis in rats

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Abstract— *Anacyclus valentinus L. is a common annual plant in Algeria, known for her various therapeutic effects. In addition, the plant is used as a food condiment. We reported our investigations on the chemical compositions and the antiarthritic activity of methanolic extract of A. valentinus (MEAV). The polyphenol extraction by maceration with methanol (80%) gave yields of 17.82%. The identification by LC-MS and colorimetric assays revealed the wealth of methanolic extracts on phenolic compounds including flavonoids (52.15 mg Equ/g) and lactones.*

Acute oral toxicity of extract was performed in line with OECD guidelines and the lethal dose 50 was assessed greater than 2500 mg/Kg. Regarding the anti-arthritic power, rheumatoid arthritis was induced by Freund's adjuvant in rats. The methanolic extract of A. valentinus presented a largest effect with weight gain, an arthritic score, thymus indices; spleen and serum parameters close to those of the control. The extract also inhibited edema and restored cartilage structure.

Keywords— ***Anacyclus valentinus, Arthritis, diclofenac sodium, Freund's complete adjuvant, paw volume***

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and systemic autoimmune joint disease characterized by pain, swelling and inflammation of peripheral joint and destruction of articular tissues and restricted joint movement [1]. RA is one of the most common inflammatory disorders affecting approximately 0.5–1.0% of global adult population [2]. The systemic ramifications of the disease, apart from morbidity and mortality, include cardiopathy, nephropathy, vasculopathy and pulmonary and cutaneous disorders [3]**.**

Conventional medicines used for RA include non-steroidal anti-inflmmatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), biological response modifiers, and corticosteroids [4]. However, these treatments are accompanied by frequently toxicity and severe side effects. Therefore, more patients look for natural agents with relatively less side effects. Plant derived drugs serve as lead molecules to develop more effective and less toxic medicines [5].

Anacyclus valentinus is a medicinal plant belonging to the family *Asteraceae*, locally known as «Guertoufa» [6] and mainly distributed in the Mediterranean basin and in northern Africa [7]. The plant is widely used in folk medicine for treatment of diverse diseases, such as diabetes [8] and cholesterol [9]. *A. valentinus* has an inhibitory effect on bacteria [10] and fungal [11]**.** It is also used in some parts of the country as a food condiment. Phytochemical studies on plants belonging to the genius *Anacyclus* made known the presence of terpenoids, steroids, coumarin and flavonoids like quercetin and luteolin [12]. Although the plant possesses many potential therapeutic activities in traditional system and containing rich phytochemical constituents, no work has been still done about the anti arthritic activity. Taking these facts into considerations the present study made an attempt to study the chemical composition and to evaluate the anti arthritic activity of methonolic extract of *A. valentinus* against Freund’s complete adjuvant induced arthritis in experimental rats.

II. METHODOLOGY

2.1. Plant material

The areal parts of *Anacyclus valentinus* were collected on October 2013, from local area of El- Bayadh, Algeria. The plant material was identified and authenticated by botanists from Department of Biology, University of Mascara, Algeria. The plant was shade dried powdered in grinder and passed through sieve of mesh size N°40.

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2.2. Preparation of extract

10 g of dried powder was macerated with 80% methanol at room temperature for 24h. The plant residue was re-extracted with addition of 80% methanol, and filtered again after 24 h. Combined filtrates were concentrated by evaporation, dried in desiccator and were stored in airtight containers until usage [13].

2.3. Analysis of extract

2.3.1. Total phenolic content determination (TPC)

The total phenolic content of the extract was determined by the Folin - Ciocalteau method [14]. Briefly 200 µl of the extract was added to 1000 µl of Folin-Ciocalteau reagent (diluted to 10% in distilled water) and 800 µl of sodium carbonate at 7,5 %. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 760 nm. Gallic acid in the concentration range of 0-170 µg/ml was used as standard for construction of calibration curve. The total phenolic content was expressed as mg/g in terms of gallic acid equivalents (GAE).

2.3.2. Total flavonoid content determination (TFC)

Aluminum chloride colorimetric method was used for estimation of total flvonoid content [15]. 500 µl of extract was mixed with 1500 µl of distilled water, methanol, 150µl of sodium nitrite (6%). After 5min, 150 µl ml of aluminium chloride (10%) was incorporated and set aside at room temperature for 6 min. The absorbance of the reaction mixture was measured at 510 nm with ultraviolet (UV) visible spectrophotometer. The total flvonoid content was expressed in terms of mg/g in terms of quercetin equivalents (QE).

2.3.3. LC/MS anlysis

The identification of the phenolic compounds from *A. valentinus* was carried out using liquid phase chromatography coupled with mass spectrometry (LC-MS). The methanolic extract was accurately weighed, and dissolved in methanol. 5.0 µL of sample was injected into Shimadzu LC/MS chromatography equipment. LC-MS analysis was performed on on a Phenomenex Luna 3u C18 column, whith UV detector. The mobile phase consisted of ultra-pure distilled water and acetonitrile. The solvent A consists of 50% water + 50% acetonitrile, while the solvent B is composed of 25% water + 75%. The flow rate was 0.4 mL/min. The column temperature was set at 40 ° C.

2.4. Drugs and chemical reagent

Diclofenac sodium was purchased from Pharmacy (Mascara, Algeria). Freund's complete adjuvant agent (FCA) (Sigma Aldrich), CRP and FR kits (Spinreact) were obtained from was obtained from the university.

2.5. Animals

Healthy adult male albino rats of Wistar strain "*Rattus norvegecus*" (weighing 150 – 200 g) were used. The experimental procedures and protocols were carried out in accordance with ethical guidelines. Animals were provided by the Experimental Station of the University of Mascara/Algeria. They were housed under standard conditions of temperature (24 ± 2 °C) with a 12:12 light: dark cycle and relative humidity (50 - 60 %). The animals were given standard diet and water *ad libitum*.

2.6. Acute toxicity

Acute oral toxicity study of methanolic extract of *A. valentinus* was performed in line with OECD guideline 423[16].

2.7. Induction of arthritis

Adjuvant arthritis was induced as described by [17]. Briefly, animals were injected intra-plantar with 0.1 ml of Complete Freund's Adjuvant (CFA) into the left hind paw of each rat. This consists of *Mycobacterium butyricum* suspended in heavy paraffin oil. Methanolic extract (300mg/Kg) and diclofénac sodium (20mg/Kg) were administered to rats in the various groups. For the comparison, two control groups were used arthritic and non-arthritic controls or vehicle control.

2.8. Drug administration

Two sets of experiments were performed. In the first set of experiments (preventive protocol), the effect of drugs was investigated when given 2h before induction of arthritis. The animals were grouped as fallow: **Group I:** arthritic control; **Group II:** non-arthritic control (Receiving 10 ml / Kg of sterile physiological water); **Group III:** treated with diclofenac sodium; **Group IV:** pre-treated with methanolic extract of *A. valentinus* (MEAV).

In the second set of experiments, effect of the drugs on established arthritis (curative protocol) was studied. Drugs were administered on day 9 after the induction. The animals were randomly grouped as fallow: **Group I**, **Group II** and **Group III** are the same as for the previous test; **Group IV":** treated with MEAV.

2.9. Parameter assessment

2.9.1. Paw swelling

The pad thicknesses of the injected paw were measured before and every other day after FCA injection using a calliper. The paw swelling at each time point was expressed as an increase in the footpad thickness (mm)

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[18]. For groups pre-treated, measurements were taken on the day of induction on the 5th, 10th and 15th day of treatment. While, for animals subjected to curative tests, the thickness of the paw was determined on the day of induction, on the 1st, the 7th and the 15th day of treatment.

2.9.2. Arthritic index

At the end of the experiments, morphological feature of arthritis was monitored, using the criteria as indicated in "TABLE 1" for each animal. The scores for each paw were then added to get the total arthritic index. The average of treated animals is compared with the control group [19].

Table.1: Shows the scores of arthritic index

Lesion site	Nature of lesion	Score
Ears	Absence of nodules and ridness	0
	Presence of nodules and ridness	1
Nose	No swelling of connected tissue	0
	Intensive swelling of connected tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
For paws	Absence of inflammation	0
	Inflammation at least 1 joint	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

2.9.3. Measurement of body weight

The body weight of rats was measured regularly using an analytical balance. The initial weight and final weight of each group were recorded.

2.9.4. Thymus index and spleen index assay

At the end of experiments, the animals were sacrificed and thymus and spleen were promptly removed and weighed. The indexes of thymus and spleen were expressed as the ratio of thymus and spleen wet weight versus body weight (mg/g), respectively [20].

2.9.5. Blood analysis

Blood samples were collected; centrifuged and supernatant serum was collected. Different parameters were estimated like alkaline phosphatase marker for bone destruction (ALP) [21], rheumatoid factor and C-reactive protein marker of inflammation. These latter were measured by latex agglutination method. Values were expressed as IU/l, IU/ml and mg/L respectively.

2.9.6. Radiographic analysis

For radiological studies, the affected paws of experimental rats were removed and preserved in 10% formalin. X-ray images were taken for these paws, and checked for the soft swelling, bony erosions and narrowing of the spaces between joints [22].

2.10. Statistical analysis

All the data are presented as mean ± SD. Statistical analysis was performed with ANOVA. *P* values below 0.05 ($p < 0.05$) were considered statistically significant.

III. RESULTS

3.1. Total phenolic content and flavonoid content

Total phenolic and total flvonoid content of the methanolic extract is shown in table 2. Following the table, MEAV which gave a yield of 17.82 % is very rich in phenolic compounds, especially flavonoids which occupy 45.16% of the totality of the phenolic compounds.

Table.2: Yield, total phenolic content and total flavonoid content of methanolic extract of A. valentinus

Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
17.82 ± 0.49	115.47 ± 0.13	52.15 ± 0.78

3.2. LC-MS analysis of MEAV

The chemical composition of MEAV was further investigated by LC-MS analysis (Fig.1, Table 3). The table showed the richness of MEAV in flavonoids. Many compounds have characteristic spectra of flavonols (kaempferol, quercetin, myricetin), flavones (apigenin, luteolin) and flavanols (catechin). These results confirm the colorimetric analysis.The analysis also revealed the presence of derivatives of phenolic acids (sinapic acid, ferulic acid-β-glucoside), sesquiterpenes (lycoperodine) and derivatives of thymol (9,10-dihydroxy-8-methoxy thymol).

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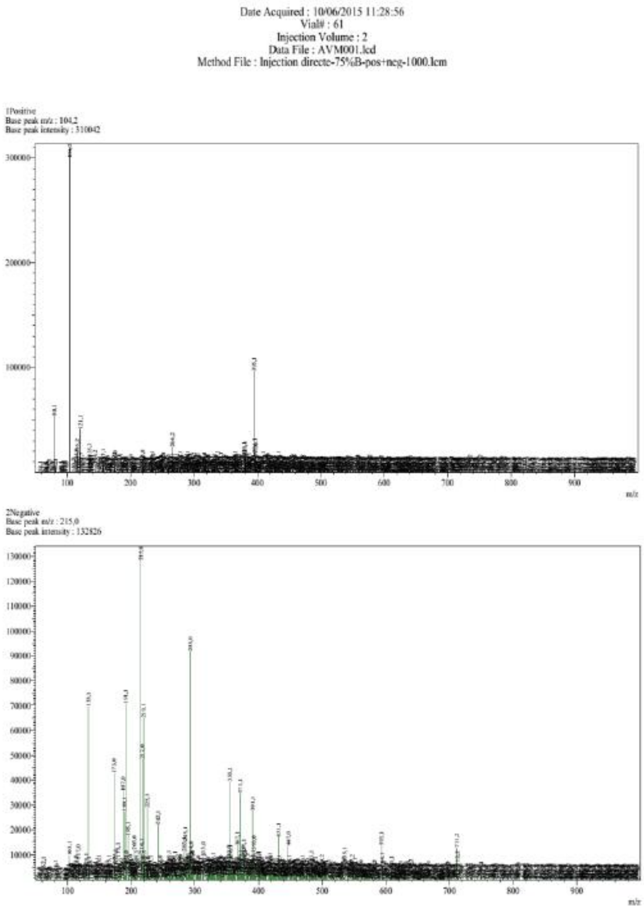

Fig.1: LC-MS-ESI spectrum in positive (up) and negative (low) form of MEAV

Table.3: Identification of MEAV by LC-MS

Pic N°	m/z Ions +/ -	Possible identification
4	266,2	Apigenin
1	/133,1	malic Acide
2	/173,0	Ascorbique Acide
3	/187,0	azelaïque Acide
4	/189,1	Catechin
5	/215,0	Lycoperodin 1
6	/217,0	9,10-dihydroxy-8-methoxy thymol
8	/225,1	sinapic Acide
10	/355,1	β-glucosid ferulic Acide
11	/371,1	Myricetin mono-acetate
		Di hydroxy-tetra methoxy flavone
13	/431,1	Apigenine -7-O- glucoside
		Kaempferol -3-O- rhamnoside
14	/447,0	Luteolin 3-O-β- glucopyranoside
		Quercetin 3-O- rhamnoside

3.3. Acute oral toxicity

There was neither change in behavioral pattern or any sign of toxicity during the observations up to 14 days. In addition, no mortality was recorded, the LD_{50} is therefore assumed to be greater than 2500 mg/Kg (the biological evaluation was carried out at doses between 200 and 2500 mg/kg). We can conclude that MEAV is safe when administered orally.

3.4. Effect of methanolic extract on adjuvant induced arthritis

3.4.1. Paw swelling

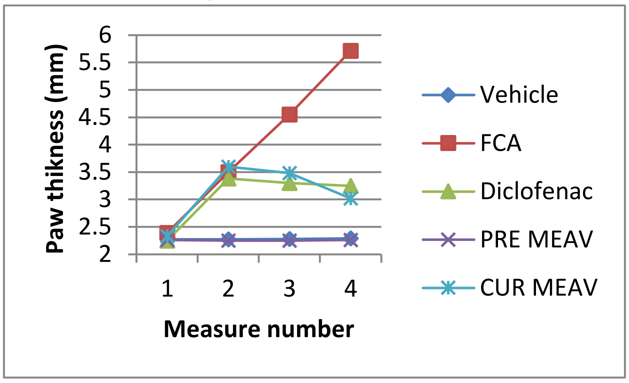

Fig.2: Effect of methanolic extract of A. valentinus (MEAV) on paw swelling

Paw swelling is one of the major factors in evaluating the degree of inflammation and therapeutic efficacy of the drug. Rats injected with FCA (group II) showed a significant increase in paw volume when compared to the normal rats (group I). Pre-treatment with MEAV at the dose of 300 mg/kg inhibited the formation of edema. However, the curative treatment with extract and diclofenac showed a significant reduction in rat paw thickness when compared with the group II (Fig.2).

3.4.2. Arthritic index

As shown in figure 3, after FCA immunization, the rats showed a significant ($P < 0.05$) increase of arthritis index. However, pre-treatment and treatment with MEAV at 300mg/Kg and diclofénac at 20 mg/kg significantly diminished the arthritis index compared with FCA group. Noting that, the lowest index was recorded in rats receiving MEAV as a preventive measure.

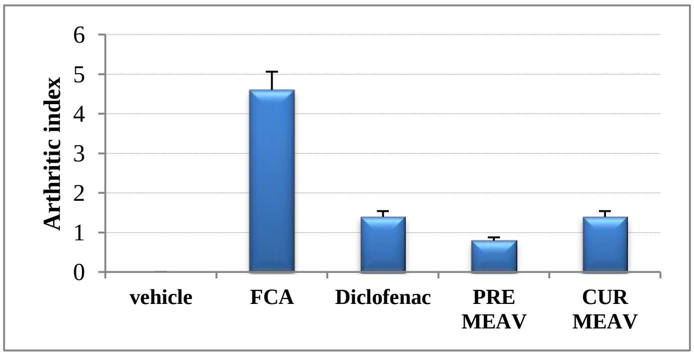

Fig.3: Arthritis index in rats receiving diclofenac and MEAV

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3.4.3. Measurement of body weight

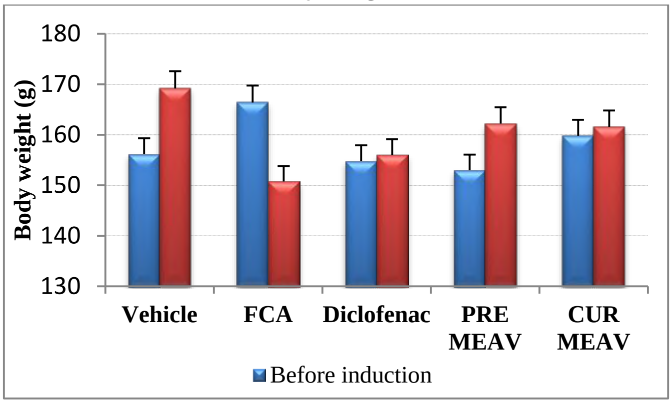

Fig.4: Mean changes in body weight

The relationship between the extent of joint inflammation and the weight loss were investigated. As shown in figure 4, the average body weights of the rats receiving normal saline (group I) was increased normally with a gain of 13g. On the other hand, a massive and significant weight loss appears in arthritic rats. However, administration of different drugs significantly attenuated body weight loss. They are ranked in descending order as follows: PRE MEAV > CUR MEAV > Diclofenac.

3.4.4. Thymus index and spleen index assay

The index of thymus and spleen was assayed the day of sacrifice. Results report an increase in thymus and spleen index of FCA control group compared to normal control group. There was significantly (< 0.05) reduction of index of thymus and spleen in MEAV treatments groups (Table 4). Diclofenac treatment also significantly decreased the spleen index and thymus index.

However, the most important result was registered for MEAV preventive treatment compared with normal group.

Table.4: Effect of MEAV on immune organs in arthritic rats

	Thymus Index	Spleen Index
Vehicle	0.683 ± 0.101	2.320 ± 0.212
FCA	1.295 ± 0.131	3.387 ± 0.151
Diclofenac	0.839 ± 0.128	2.732 ± 0.237
PRE MEAV	0.690 ± 0.177	2.478 ± 0.594
CUR MEAV	0.741 ± 0.117	2.751 ± 0.278

3.4.5. Blood analysis

Table 5 depicts the effect of MEAV on levels of blood marker in arthritic rat serum. A significant increase in the levels of ALP, RF and CRP was observed in arthritic rats. The administration of MEAV (300mg/kg) and diclofenac (20mg/kg) significantly decreased all these parameters compared to FCA control (< 0.05).

Table.5: Effect of MEAV on serum parameters in arthritic rats

	ALP (UI/L)	RF (UI/ml)	CRP (mg/L)
Vehicle	79.65 ± 3.90	8 ± 0	6 ± 0
CFA	114.17 ± 4.01	57.60 ± 14.31	38.40 ± 13.14
Diclofenac	93.48 ± 8.10	14.40 ± 10.43	7.20 ± 2.68
PRE MEAV	77.29 ± 8.44	9.60 ± 3.57	6 ± 0
CUR MEAV	81.28 ± 6.24	11.20 ± 4.38	7.20 ± 2.68

3.4.6. Radiographic analysis

The radiographic images of the joints of all groups of rats are shown in Fig.5. The articulation of normal control group presented a normal opacity, joint space and cartilage appeared to be normal.

As shown in this figure, it is evident from the radiographic images that adjuvant treated rats (group II) developed periosteal reaction, irregular joint space with opacity essentially concentrated at the level of the femoral condyle. The presence of knee arthritis (inflammation of the knee joint), joint instability and cartilage lysis were also observed.

Although diclofenac treatment (group III) reduced inflammation and improved the metabolic function of rats, the radiographic image of joint showed opacity essentially concentrated at the tibial condyle. A lysis of the articular cartilage is also observed.

In the extracts pre-treated group (group IV), the joint space appeared normal, no periosteal reaction was observed and joints appeared to be normal.Whereas, a slight bone lysis was determined at the joint of MEAV-treated rats (group IV"). This joint had a radiographic appearance of normal opacity.

Group I

Group II

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Group III

Group IV

Group IV”

Fig.5: X-ray photographs of left hind paw of each group of rats

IV. DISCUSSION

Adjuvant-induced arthritis is commonly used in experimental model for preclinical studies. Due to its short duration of testing, easy measurement and similarities to human RA, this model has been used frequently to evaluate therapeutic agents. In this model, rats developed a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodelling [23].These inflammatory changes eventually result in the complete destruction of joint stability and mobility in the arthritic rats. Also, soft tissue swelling around the ankle joints was appeared during the progress of arthritis in FCA injected rats, which was considered as oedema of the exacting tissues.

Paw swelling is a parameter of measuring the anti-arthritic activity of various drugs. The visual arthritic index was used to evaluate the severity of arthritis. The determination of paw swelling and arthritic index is simple, sensitive, and quick procedure for evaluating and assessing the degree of inflammation, the therapeutic and curative effects of drugs [24]. In the present study, treatment with MEAV markedly reduced paw swelling and arthritic index induced by FCA compared with FCA group. We further confirmed its protective effect. In fact, the preventive treatment gave results close to those of the normal control group.

Progression of disease status and response to anti-inflammatory therapy are indirectly linked with change in body weight. RA is associated with weight loss, known as rheumatoid cachexia which leads to the decreased physical activity, muscle strength and decreased daily performance [25]. Decrease in body weight was attributed to reduced absorption of ^{14}C- glucose and ^{14}C-leucine in rat’s intestine [26]. In the present investigation, the FCA treated rats showed less body weight gain as compared with diclofénac, extract pre-treated and extract treated arthritic rats. Thus, body weight gain may be due to the restoration of the absorption capacity of the intestine.

The spleen is a vital organ which serves as the available source of cells and antibody formation, known to be involved in immunological response in adjuvant arthritis.The RA model is an immune hyper-functional model, and marked splenomegaly, and lymphoid hyperplasia were associated with RA [27]. On the one hand, the increase in the weight of the spleen occurs following the release of the cytokines causing a perturbation of the histology of this organ. On the other hand, the variation in thymus weight is due to the suppression or imbalance of the immune system [28]. In the present study, it was found that MEAV significantly decreased the spleen index and thymus index induced by FCA compared with FCA group. This finding suggests that the extract may help in the recovery of the hyper-functioning of immune organs without causing damage.

Cytoplasmic cellular enzymes, such as alkaline phosphatase (ALP) are present in the body, but especially in the liver, bone, intestine, kidneys and white blood cells. The cellular localization of these enzymes assigns them a certain activity in the transfer of phosphoric esters through the membranes. They are involved in intestinal absorption and in the ossification process [29]. The attainment of the above-mentioned organs causes the liberation of ALP [30]. A significant ($P < 0.05$) reduction of ALP level was observed after administration of MEAV (300 mg/kg).

Serum rheumatoid factor (RF) is the immunological expression of an individual's immune system reaction to the presence of an immunoglobulin molecule that is recognized as "non-self." This response results in the presence of immune complexes. These, in turn, bind complement and may eventually lead to synovium, cartilage, and bone destruction. Higher the levels of serum rheumatoid factor, higher are the development of inflammation [31]. *A. valentinus* pre-treated and treated animals showed significantly lesser serum RF when compared to disease control animals.

CRP is an acute-phase protein and has been identified as an important biomarker for various inflammatory and degenerative diseases. The increment of this protein is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages [32]. Freund’s adjuvant induced arthritis rats increased the CRP level as

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evidenced in the inflammatory process. A significant ($P < 0.05$) reduction of CRP level was observed after administration of MEAV, suggested that decreased the inflammatory reactions.

Radiographic changes in RA are useful indicative measures which specify the severity of the disease. Soft tissue swelling, bony erosions and narrowing of joint spaces can be observed [33]. The observed changes in radiographic findings can be attributed to positive benefits provided by the methanolic extract of *A. valentinus* on the inflammatory mechanisms in the joint tissues.

It is well known that diclofenac had the anti-inflammatory and analgesic activity and often used to cure rheumatoid arthritis. However, this drug only inhibits primary response; do not affect the second response and immune function. In addition, it has obvious side effect and toxicity. Among all tests, the pre-treatment by MEAV proved to be the most effective in chronic inflammation when compared to the other groups. Its action is probably through the inhibition of the production of cyclooxygenase and prostaglandins. The extract, also has an immune-protective effect. In fact, several species of the Asteraceae family are known for their anti-inflammatory effect [34, 35]. These could be attributed to the phenolic constituents present in the plant detected after phytochemical analysis.

Quercetin, myricetin, kaempferol and apigenin identified in the extract have an effect on the synthesis of cyclooxygenase and lipoxygenase [36, 37]. Luteolin 3-O-β-glucopyranoside also identified has an inhibitory effect on the synthesis of thromboxanes and consequently on the synthesis of the main arachidonic acid actor of the pro-inflammatory process [38].

V. CONCLUSION

The results obtained in the present study indicates that phenolic compounds of *A. valentinus* not only directs towards the control of arthritis progression and/or the inflammation associated with joint synovitis, but also prevents cartilage and bone destruction of the arthritic joints. Extracts of *A. valentinus*, may have great potential as an alternative to the therapeutic agents currently available for treatment of RA. However, there is need to isolate and characterize the active compounds responsible for the observed anti-arthritic activity.

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REFERENCES

[1] L. Klareskog, A.I. Catrina, S. Paget (2009). Rheumatoid arthritis. Lancet 373, 659–672.

[2] P. Patel, D. Patel, N. Patel (2012). Experimental investigation of anti-rheumatoid activity of *Pleurotus sajorcaju* in adjuvant-induced arthritic rats. Chin J Nat Med; 10:0269–74.

[3] D.K. Praveen, M. Suchita (2013). Herbal sources of anti-arthritic potential: a comprehensive review. Int. J. Pharm. 4, 88–92.

[4] N.J. Gullick, D.L. Scott (2012). Drug therapy of inflammatory arthritis. Clin Med; 12:357-63.

[5] M. Rajkumar, C.R. Harish, K. Asres, C. Veeresham (2008). *Toddalia asiatica* (Linn) Lam-A comprehensive review. Pharmacogn Rev; 2:386-97.

[6] K. Maiza, R. A. Brac de la Perrière, V. Hammiche Pharmacopée traditionnelle saharienne : Sahara septentrional. *In:* Schröder, E., Balansard, G., Cabalion, P., Fleurentin, J., Mazars, G. (1993). Médicaments et aliments : L'approche ethnopharmacologique. Actes du 2ème Colloque Européen d'Ethnopharmacologie et de la 11ème Conférence internationale d'Ethnomédecine. Heidelberg, pp. 169-171.

[7] P.H. Julve (2015). ff- Baseflor. Index botanique, écologique et chorologique de la flore de France.

[8] A.L. Tadjeddine, N. Kambouche, H.Medjdoub, B. Meddah, A. Dicko *et al.*, (2013). Antidiabetic effect of *Anacyclus valentines* L. aqueous extract in normoglycemic and streptozotocin induced-diabetic rats. American Journal of Phytomedicine and Clinical Therapeutics. AJPCT 5 424-43.

[9] I. Hacheimi and O. Kadi (2009). Effet de l'extrait aqueux de *L'Anacyclus Valentinus* l sur le cholestérol.

[10] C. Selles, M.A. Dib, N. Djabou, F. Beddou, A. Muselli, B. Tabti, J. Costa and B. Hammouti (2013). Antimicrobial activity and evolution of the composition of essential oil from Algerian *Anacyclus pyrethrum* L. through the vegetative cycle, Natural Product Research, 11:46.

[11] K. Boungab, A. Tadjeddine , L. Belabid, Z. Fortas, F. Lazrag (2011) Antifungal activity of extracts of Anacyclus valentinus L. on phytopathogenic fungi. 4th International Conference on Alternative Methods in Crop Protection. Evolution of French and European regulatory frameworks. Innovative new means and strategies, New Century, Lille, France. 230- 238.

[12] G. Harald (1978). Comparative phytochemistry and systematics of *Anacyclus*. *Biochemical Systematics and Ecology*, 1978; 6: 11 –17.

[13] Sawsan Abuhamdah, Rushdie Abuhamdah, Suleiman Al-Olimat and Paul Chazot (2013). Phytochemical Investigations and Antibacterial Activity of Selected

Medicinal Plants from Jordan. *European Journal of Medicinal Plants 3(3): 394-404*.

[14] N. Boizot and J-P. Charpentier (2006). Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Cah. Tech. INRA. N°. special. pp. 79-82.

[15] V. Dewanto, X. Wu, K.K. Adom, R.H. Liu (2002). Thermal Processing Enhances the nutritional Value of Tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50 : 3010–3014.

[16] H. Khan, M. Saeed, A.U.H. Gilani, M.A. Khan, A. Dar, I. Khan (2010). The antinociceptiveactivity of Polygonatumverticillatum rhizomes in pain models. J Ethnopharmacol, 127(2):521 –527.

[17] E. Woode, E.Boakye-Gyasi, C.A. Danquah, C. Ansah, M. Duwiejua (2009). Anti-arthritic effects of Palisota hirsuta K. Schum. Leaf extract in Freund's adjuvant arthritis in rats. International journal of pharmacology. 5 (3): 181-190.

[18] Abdel-Moein N.M., Abdel-Moniem E.A., Mohamed D.A. and Hanfy E.A. (2011). Evaluation of the anti-inflammatory and anti-arthritic effects of some plant extracts. grasas y aceites, 62 (4). 365-374.

[19] H. Gerhard Vogel (2002). Drug discovery and evaluation, pharmacological assays, Second edition, p 802- 803.

[20] L.L. Zhang, W. Wei, S.X. Yan, X.Y. Hu, W.Y. Sun (2004). Therapeutic effects of glucosides of Cheanomeles speciosa on collagen-induced arthritis in mice. Acta Pharmacologica Sinica 2004; 25: 1495–1501.

[21] P.D. Eckersall & A.S. Nash (1983). Isoenzymes of canine plasma alkaline phosphatase : an investigation using isoelectric focusing and related to diagnosis, Res. Vet. Sci., 1983, 34, 310-314.

[22] Karnati Mamatha, Chandra Rodda H., Veeresham Ciddi, Kishan Bookya (2013). Anti -arthritic activity of root bark of *Oroxylum indicum* (L.) vent against adjuvant-induced arthritis. Pharmacognosy Research ; Vol 5 (2) : 121 – 128 .

[23] S.R. Yend, V.D. Sannapuri, N.S. Vyawahare, U.N. Harle (2010). Antirheumatoid activity of aqueous extract of *Piper nigrum* on Freund's Adjuvant induced arthritis in rats. *I*nt J Pharma Sci Res. 1(1): 129-33.

[24] A.D.Kshirsagar, P.V. Panchal, U.N. Harle, R.K. Nanda, H.M. Shaikh (2014). Antiinflammatory and antiarthritic activity of anthraquinone derivatives in rodents.
Int. J. Inflamm.12690596.

[25] R. Roubenoff, R.A. Roubenoff, J.G. J.J. Cannon, Kehayias, B. Zhuang, B. Dawson-Hughes, *et al.* (1994). Rheumatoid cachexia: cytokine driven hypermetabolism accompanying reduced body cell mass in chronic inflammation. J Clin Invest; 93:2379–86.

[26] E. Brunet-Guedj, B. Brunet, J. Girardier, B. Moyen (2006). Medecine du sport. 7th ed. Masson. pp. 263–7.

[27] A.C. Issekutz, K. Sapru (2008). Modulation of adjuvant arthritis in the rat by 2 methoxyestradiol: an effect independent of an anti-angiogenic action. Int. Immunopharmacol. 8, 708–716.

[28] C. Gebhard, S.F. Stämpfli, C.E. Gebhard *et al.* (2009). Guggulsterone, an anti-inflammatory phytosterol, inhibits tissue factor and arterial thrombosis. Basic Res Cardiol;104 (3):285-94.

[29] D. Ricklin, , G. Hajishengallis, *et al.* (2010). "Complement: a key system for immune surveillance and homeostasis." Nat Immunol 11(9): 785-797.

[30] A. Akhtar, Deshmukh A.A., Raut C.G., Somkuwar A.P., & Bhagat S.S. (2012). Prallethrin induced serum biochemical changes in Wistar rats. *Pesticide Biochemistry and Physiology*, 102: 160-168.

[31] S. Dubucquoi & S.Fily-Nalewajk (2008). Prise en charge de la polyarthrite rhumatoïde en 2008 : la biologie peut-elle répondre aux attentes des cliniciens ? Rev Fr Laboratoires ;404:51-7.

[32] M.B. Pepys, G.M. Hirschfield (2003). C-reactive protein: a critical update, Journal of Clinical Investigation 111:1805–1812.

[33] E.D. Harris (1990). Rheumatoid arthritis. Pathophysiology and implications for therapy. N Eng J Med.322(18): 1277-89

[34] K. Batanouny (2005). Guide to Medicinal Plants in North Africa. Centre for Mediterranean Cooperation. *International Union for Conservation of Nature and Natural Resources*, 1: 35-37.

[35] G. Benitez, M.R. Gonzalez-Tejero and J. Molero-Mesa (2010). Pharmaceutical ethnobotany in the western part of Granada province (southern Spain): Ethnopharmacological synthesis. J. Ethnopharm., 129: 87–105.

[36] Y.K. Kim, Y.S. Kim, S.U. Choi, S.Y. Ryu (2004). Isolation of flavonol rhamnosides from Loranthus tanakae and cytotoxic effect of them on human tumor cell lines. Arch. Pharm. Res. 27:44 - 47.

[37] Bahmani Mahmoud, Shirzad Hedayatollah, Majlesi Maedeh *et al.* (2014). A review study on analgesic applications of Iranian medicinal plants. Asian Pac J Trop Med; 7(Suppl 1): S43-S53.

[38] G. Odontuya, J. R. S. Hoult, and P. J. Houghton (2005). Structure-activity relationship for anti-inflammatory effect of luteolin and its derived glycosides. Phytother. Res. 19: 782 - 786.

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Analysis of Pesticide Residues in Curry Leaves and Red Gram in Tirupati Region, Chittoor by Gas Chromatography

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Abstract— *Medicine is food and food is medicine" is the best way to describe on how the ailments were cured by using the plants during the ancient period of time. The "Magical plant of Indian Spice" (Murraya koenigii) has served human kind not only as food enhancer but also serve as village or folk medication to cure many disorders, the tribal communities has used many parts of the Murrayakoenigiito cure them diseases. Pigeonpea [Cajanuscajan(L.) Millsp.]Pigeon pea (Arhar) commonly known as Red gram or tur is a very old crop in India. The term 'Pigeon pea' was coined in Barbados . The present study is to determine the pesticide residue levels in Curry leaf (Murraya koenigii) and Red gram (Peagion Pea) in Tirupati , Chittoor region.*

Keywords— *Curry leaves, Gas Chromatogrpahy, Pesticide Standards, Red Gram.*

I. INTRODUCTION

Murraya koenigii, commonly known as curry leaf or karivepaku or Kari patta in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species (Gabriel Charles and Vincent Onuegbu, 2014). Fresh leaves, dried leaf powder, and essential oil are widely used for flavoring soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning and ready to use other food preparations (Gupta et al.,1971) . It is called as 'Magical plant of Indian spice'. It is not only used as food enhancing purpose but also shows medication for many diseases(Surbhisinghal and meenakshibatt, 2016). The essential oil is also utilized by soap and cosmetic aromatherapy Industry. Peagion Pea which belongs to the family Fabaceae, is commonly known as Red gram ,in Andhra pradesh we called as Kandi pappu, other names like Tur, arhar, Dhal etc. This is very old crop and major cultivated crop in India (Gowda et al., 2011). These crops were contaminated with pesticides, not only these two, almost all crops are contaminated with pesticide which are sprayed on crops. So the present work is aimed to determine the pesticide residue levels in Curry leaves and Red gram in Tirupati region, Chittoor by using Gas Chromatography. Pesticides which are determined in this article are chlorpyrifos, profenofos, triazophos. Chlorpyrifos is a broad spectrum organophosphate pesticide, kills the insects and worms on crops, animals, buildings(Mackay et al.,2014). Profenofos is a non-systemic broad spectrum, foliar insecticide and acaricide. It shows excellent translaminar action and effective against chewing and sucking insects, mites on cotton, rice, maize, sugar beet, soybeans, vetables, tobacco, oil seeds etc., (Madhulika kushwaha et al. 2016). Triazophos is a broad spectrum, non systematic contact organosphosphorus pesticides used to control pests, insects, acarids, some nematodes in okra, cotton, maize, rice paddies etc.,(Aungpdradit et al. 2007).Pesticide residues in curry leafs was determined in different markets of Andhra Pradesh & Telangana by using LC-MS/MS.(Priyadarshini et al., 2017)

II. MATERIALS AND METHODS:

Market samples of curry leaf and Red gram were collected from local market Tirupati region Chittoor. Samples were extracted for pesticide residues following the validated QuEChERS method to give best results**.**

2.1 Sample extraction:

Curry leaf samples were analyzed for pesticide residues following the AOAC official method (QuEChERS) which is the best method in the laboratory. The samples were collected from local Tirupati market in polythene bag. Curry leaves was homogenized separately with robot coupe Blixer. 5g of sample was weighed and taken in 50 ml centrifuge tube and 10ml distilled water, 15 ml acetonitrile was added to sample tube. The sample was homogenized at 14000-15000rpm for 2-3 min. 6g sodium chloride was added to sample, mixed thoroughly by shaking gently followed by centrifugation for 3min at 2500-3000rpm to

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separate the organic layer. The top organic layer of about 9ml was taken into the 15ml centrifuge tube and added with 1.4g magnesium sulphate to remove the moisture content and added 1g PSA sorbent (for dispersive solid phase d-SPE cleanup), and 0.05g of GCB (Graphitized Carbon Black) shaken gently followed by centrifuge for 2min at 2500rpm. The sample tube was vortexed for 30 sec then followed by centrifuge for 5 min at 2500-3000rpm. 2ml supernant layer was transferred into 10ml tube for evaporation using water bath and reconstitute with 1ml of n-hexane. Now pour it in GC vials and inject it in GC having FPD detector. Same procedure were followed for Red gram. Weigh individual reference standard around 15mg in 25ml volumetric flask. Dissolve in n-hexane for standards to be used on GC-FPD and adjust to 500ppm removing required quantity and making up to 25ml. The working standards are prepared by serial dilutions from stock solution i.e 0.1, 0.25, 0.5, 0.75, 1ppm by suitable solvent n-hexane and used as standard check in analysis linearity studies(Swarupa Rani et al., 2016).

III. INSTRUMENT ANALYSIS

The chromatographic system was SHIMADZU AOC-20S 2010 plus, equipped with an Auto sampler and EB_1 Column 0.25mm×0.25µm, 30m length. The determination of pesticide residue analysis was carried out by following conditions - Injection volume 1µl, Run time 20min, Detector Flame photometric detector(FPD), Injection temperature 260^0c, Column flow 6ml/min, column programming 60^0c, Column $EB_{1,}$ Detector, temperature 280^0c the run time will change for standards.

IV. RESULTS AND DISCUSSION

The pesticide residue levels are calculated by following formula:

Residues(µg/g) :

$$\frac{\text{height or area of sample}}{\text{height or area of the sample}} \times \frac{\text{µl of sample injected}}{\text{µl of the standard injected}} \times$$

conc. of standard / weight of sample x final volume

- equation (1)

Pesticides are applied to Vegetables, leafy vegetables, fruits and various crops at various stages of cultivation and for protection against a range of pests during harvest before they become available to consumer.

To ensure the safety of food for consumers and regulate international trade, legislations such as European Union directives has established maximum residue limits for pesticides in food stuff. Thorough monitoring of pesticide residues is crucial for proper risk assessment of human exposure through food.

Compared with other available methods, the QuEchERS method is believe to give the best results.

4.1 GC determination of pesticide standards:

The pesticide residue was identified by comparing its retention time (Rt). The quantitative determination was carried out with help of a calibration curve. A good linearity was established by a correlation coefficient (R^2) value (0.97).

Correlation coefficient is a statistical tool used to measure the degree of this type relationship and here a high correlation value (A value very close to1.0) indicates a high level linear relationship between the concentration of standards and peak area. For quantification an external calibration curve with four different concentration matrix matching were made.

The standards are dissolved in n-hexane it was injected in GC as an blank. In blank chromatogram we observed only one peak i.e solvent peak as shown in Fig.1

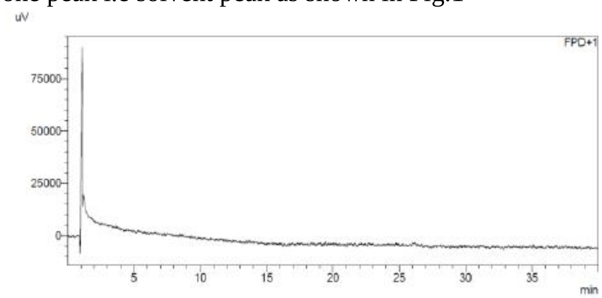

Fig.1: The blank chromatogram with solvent peak

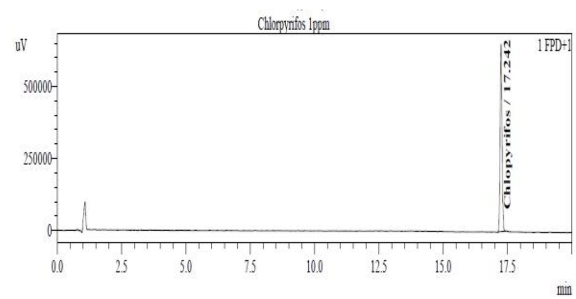

Fig.2: The Standard chromatograms of Chlorpyrifos

Peak	Retention time	Area	Height	Name
1	17.242	3383918	649767	Chlorpyrifos
Total		3383918	649767	

4.1.1 Linearity for the Chlorpyrifos:

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The linearity was established by analysing standard solutions of the insecticides at five concentrations. The concentrations were 0.1, 0.25, 0.5, 0.75, 1µg/ml. Graphs were constructed from the GC chromatograms based on the average peak area of the signal response versus the concentration plot of the analytes. The parameters such as the slope of the regression line, y-intercept and the correlation coefficient were evaluated.

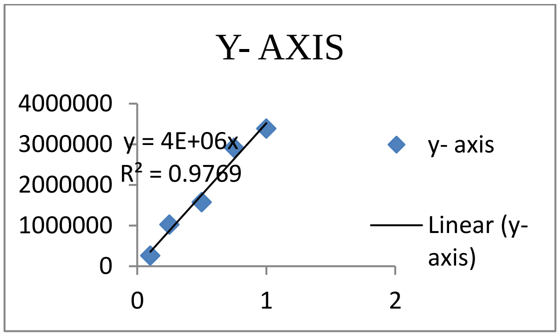

Fig.3: Linearity of chloropyrifos

Table : 1

Sl no.	Concentration(µg/ml)	Area
1	0.1	256874
2	0.25	1022717
3	0.5	1572715
4	0.75	2910537
5	1	3383918
6	Slope	4E
7	Y-intercept	06x+4E
8	Correlation coefficient	0.976

The linearity of the method was determined at five concentration levels ranging from 0.1-1ppm for Chlorpyrifos. The regression line equation for Chlorpyrifos was Y=4E+06X and the regression coefficient value was 0.967 respectively.

The regression data for the calibration curve showed good linear relationship over

a concentration range 0.1-1ppm for Chlorpyrifos with respect to peak area.

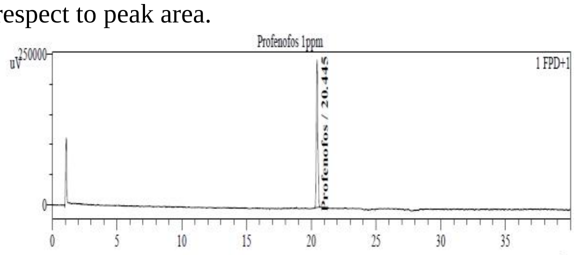

Fig.4: standard chromatogram of profenofos

Peak	Retention time	Area	Height	Name
1	20.445	1985369	243885	Profenofos
Total		198569	243885	

4.1.2 Linearity for Profenofos:

The linearity of the method was determined at five concentration levels ranging from 0.1-1 ppm for profenofos. The regression line equation for Profenofos was Y=2E+06Xand the regression coefficient value was 0.994 respectively.

The regression data for the calibration curve showed good linear relationship over a concentration range 0.1-1ppm for Profenofos with respect to peak area.

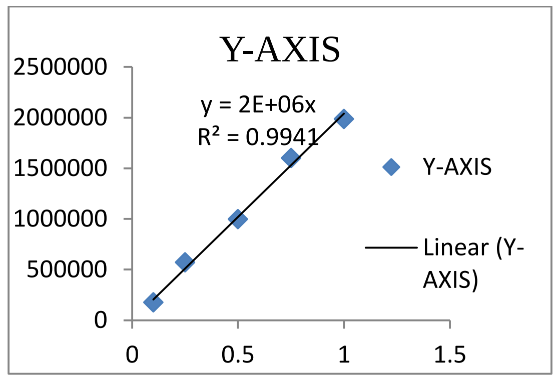

Fig.5: Linearity of profenofos

Table : 2

Sl no	Concetration(µl/ml)	Area
1	0.1	175354
2	0.25	570827
3	0.5	997618
4	0.75	1600129
5	1	1985369
6	Slope	2E
7	y-intercept	2E+06X
8	Correlation coefficient	0.994

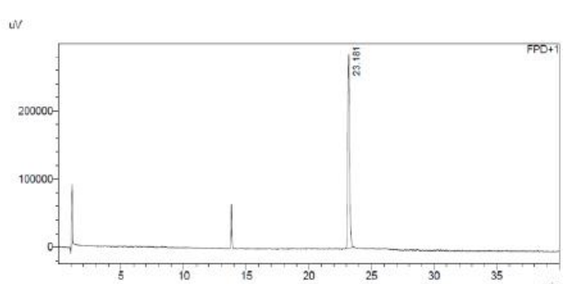

Fig.6: Standard chromatogram for triazofos

Peak	Retention time	Area	Height	Name
1	23.181	3032415	284816	Triazofos
Total		3032415	284816	

4.1.3 Linearity for Triazofos :

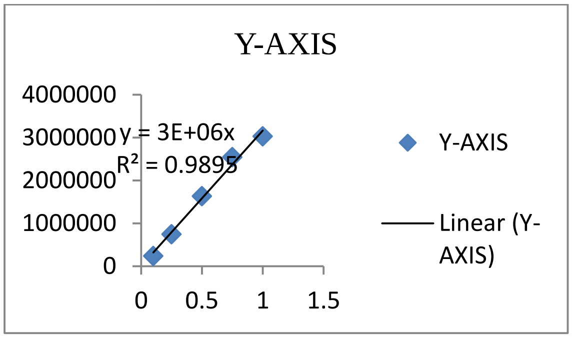

Fig.7: Linearity of triazofos

Table : 3

Sl no	Concentration	Area
1	0.1	237780
2	0.25	743255
3	0.5	1634619
4	0.75	2545213
5	1	3032415
6	Slope	3E
7	y-intercept	06X+3E
8	Correlation coefficient	0.989

The linearity of the method was determined at five concentration levels ranging from 0.1-1 ppm for Triazofos. The regression line equation for Triazofos was Y=3E+06Xand the regression coefficient value was 0.989 respectively.

The regression data for the calibration curve showed good linear relationship over a concentration range 0.1-1ppm for Triazofos with respect to peak area.

4.2 Chromatograms of curry leaves samples

4.2.1 Unwashed curry leaves

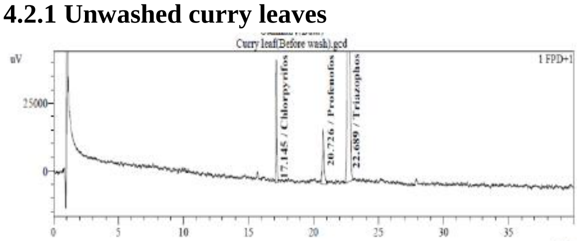

Fig.8: Sample chromatogram of unwashed curry leaves

Sample	Detected pesticide	Retention time	Area	Height
Unwashed curry leaves	Chlorypyrifos	17.145	233414	45295
	Profenofos	20.726	191030	20284
	Triazofos	22.697	4574265	452562

Observation:

The Curry leaf samples were analyzed in GC for the presence of pesticide residue to know the different pesticides eluted in washed curry leaves samples, were compared with standard chromatograms with their retention time i.e Chlorpyrifos RT was 17.242, Profenofos RT was 20.445 and Triazofos RT was 23.181. Then the presence of pesticide residue levels are compared with standard MRLs fixed by prevention of food Adulteration Act (PFA), Govt. Of India was followed by EUROPEAN UNION MRLs.

In Unwashed curry leaves the Chlorpyrifos was found to be 0.069 mg/kg, Profenofos was found to be 0.096mg/kg and Triazofos was found to be 1.508mg/kg. In this present study we observed that Triazofos was detected above the MRLs i.e is 1.508mg/kg respectively. But it should be 0.01mg/kg as per EUROPEAN UNION (EU).

4.2.2 Washed curry leaves:

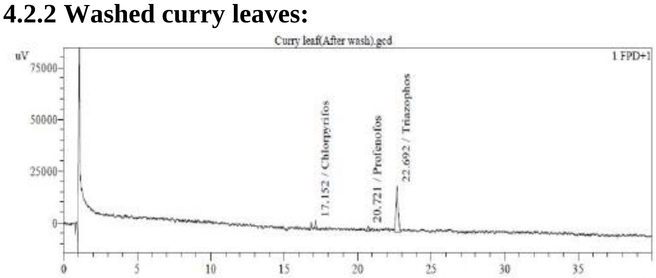

Fig.9: Sample chromatogram of washed curry leaves

Sample	Detected pesticide	Retention time	Area	Height
Washed curry leaves	Chlorypyrifos	17.152	23422	4212
	Profenofos	20.721	20501	2642
	Triazofos	22.692	240020	22350

Observation:

In washed Curry leaves the Chlorpyrifos was found to be 0.0069 mg/kg, Profenofos was found be 0.01mg/kg and

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Triazofos 0.079mg/kg. So, in washed curry leaves the pesticide residue levels are decreased when compared with unwashed curry leaves residue levels (Chlorpyrifos was 0.069 mg/kg, Profenofos was 0.096mg/kg and Triazofos was 1.508mg/kg). so water might have a capacity to minimize the pesticide residues.

4.2.3 Curry leaves soaked in salt water:

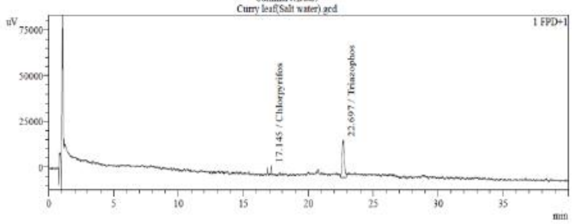

Fig.10: sample chromatogram of curry leaves soaked in salt water

Sample	Detected pesticide	Retention time	Area	Height
Curry leaves soaked with salt water	Chlorypyrifos	17.145	21996	5042
	Profenofos	Not detected	-	-
	Traizofos	22.697	228521	20446

Observation:

In this sample, the Chlorpyrifoswas found to be 0.0065 mg/kg , Triazofos 0.075mg/kg and Profenofos was not detected. The Curry leaves were soaked in salt water about half hour, the pesticide residue levels was reduced (Chlorpyrifos was 0.0065 mg/kg , Triazofos 0.075mg/kg and Profenofos was not detected) when compared with both washed(Chlorpyrifos was 0.0068 mg/kg , Profenofos was 0.01mg/kg and Triazofos was 0.079mg/kg) and unwashed (Chlorpyrifos was 0.0069 mg/kg, Profenofos was 0.096mg/kg and Triazofos was 1.508mg/kg) curry leaves samples. In soaked curry leaves the Profenofos was not detected, so the salts may have capacity to remove pesticide after soaking curry leaves in salt water and it may be advisable to the people.

4.3 The Chromatograms of Red gram sample was carried out by GC:

Major cultivation crops in pulses are red gram. Red gram is found to be contaminated with more number of pesticides the most commonly detected pesticide in cereals and pulses is Chlorpyrifos which has effect on health and safety of mammals. Poisoning with this compound can effect the central nervous system, cardiovascular and respiratory system elucidated the percent contamination of pesticides in the pulses.

4.3.1 Unwashed red gram:

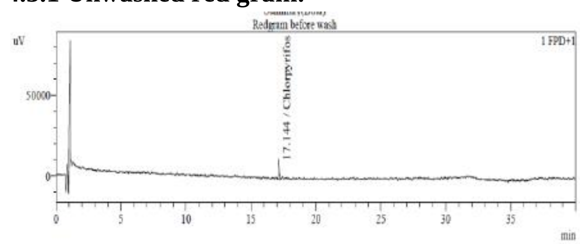

Fig.11: chromatogram of unwashed red gram sample

Sample	Detected pesticide	Retention time	Area	Height
Washed red gram	Chlorpyrifos	17.144	74396	13069

Observation:

In Unwashed red gram the Chlorpyrifos was found to be 0.024mg/kg. It was within the limits according to European union (EU) i.e Chlorpyrifos MRL is 0.05mg/kg

4.3.2 Washed red gram:

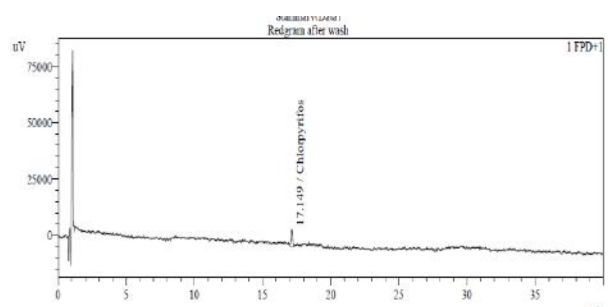

Fig.12: sample chromatogram of washed red gram

Observation:

In washed red gram the Chlorpyrifos was found to be 0.014mg/kg.it was with in the limits according to European union(EU). The Chlorpyrifos was decreased in washed red gram when compared to unwashed red gram sample i.e about 0.02mg/kg, in washed with tap water the residue levels was 0.014mg/kg.

REFERENCES

[1] Gabriel Charles Disegha, Vincent Onuegbulzionworu. Antifungal activities of curry leaf (murraya koengii) Extract on some selected fungi. 2014. *Chemistry and Materials Research*, 6(11), 1-7.

[2] Gupta GL and Nigam SS. 1971 chemical examination of leaves of *Murrayya koenigii*, *Planta Med*, 19 83-86.

[3] Surbsighal and Dr. Meenakshi bhatt. 2016, A Review on *Murraya koenigii*(curry plant) - Methi neem. *World journal of pharmacy and pharmaceutical sciences*, 5 : 397-408.

[4] Gowda CLL, Saxena KB, Srivastava RK, Upadhyaya HD, 2011 Pigeonpea: From an orphan to leader in food legumes in biodiversity in agriculture, domestication evolution and sustainability 15, 361-373.

[5] Mackay D, Giesy JP, Solomon KR. 2014, Fate in the environment and long-range atmospheric transport of the organophosphorus insecticide chlorpyrifos and its oxon. Rev environment contamination toxicology, 231, 35-76.

[6] Madhulika kushwaha, shalini verma, and subhankar chatterjee. 2016. Profenofos an acetylcholinesterase-inhibiting organophosphorus pesticide a short review of its usage toxicity and biodegradation. Journal of environmental quality. 45, 1478-1489.

[7] Aungpradit T, Suthivaiyakit P, Martens D, sutthivaiyakit S, Kettrup AAF. Photo catalytic degradation of triazophos in aqueous titanium dioxide suspension.2007 Microbialresearch, 13, 146-204.

[8] Swarupa S, Shashi vemuri and Venkateswar Reddy V. 2017 Pesticide usage pattern and farmers perception in curry leaf [*Murraya koeinigii*(L.) sprengel]. *Journal of environmental science, toxicology and food technology* (IOSR-JESTFT) 11, 66-72.

[9] Priyadarshini G, Shasi Vemuri, Narendra Reddy C, Swarupa rani S.2012. Determination of Pesticide Residues in curry leaf in different markets of Andhra Pradesh and Telangana, India. International journal of environment, Agriculture and Biotechnology, 2, 101-111.

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Determinants of Choice of Storage Systems for Root and Tuber Crops in Benue State, Nigeria

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Abstract— *Determinants of Choice of Storage Systems for root and tuber crops in Benue State of Nigeria were examined. The specific objectives were to determine the factors affecting choice of storage systems by root and tuber crop farmers; and determine the relationship between choice of storage systems and farm productivity. Data were collected from 288 root and tuber crop producers in eight Local Government Areas and 32 wards, using a multi-stage sampling technique. Structured questionnaire was used to collect the data. Data collected were analysed using logit model and Mann-Whiney U test. The results indicate that total output, gender, educational level, household size, and farm size significantly affect the choice of storage systems by root and tuber crop producers. The results also reveal that the output of farmers who utilized only the local storage systems exceeds those who utilized both the local storage systems in addition to the modern storage techniques. It was recommended that strategies and policies aimed at encouraging root and tuber crops farmers to adopt a particular storage technique should take into consideration their socio-economic characteristics. Also research efforts aimed at improving the effectiveness of the local storage systems using locally sourced materials should be encouraged.*

Keywords*— *Determinants, Choice, Local Storage Systems, Root and Tuber Crops, Benue State, Nigeria.

I. INTRODUCTION

Root and tuber crops play significant roles in the socio-economic lives of people of sub-Saharan Africa. Reports by African Ministerial Council on Science and Technology (AMCOST), 2006 and Food and Agricultural organization (FAO), 1998 revealed that these crops are important in household food security and income generation in many African countries. About 500 to 700 million people across the humid, tropical world which includes less-developed countries (LDCs) grow and consume these crops as their staple food (Ravi, Aked and Balagopalan, 1996).

In developing world especially Nigeria, Kana, Aliyu and Chammang (2012) reported that these root and tuber crops occupy a remarkable position in food security due to their high calorific value and carbohydrate content. They further pointed out that these crops are integral part of the food supply in the world, provide an important source of animal feed, and are also used as industrial raw materials.

In spite of the benefits of these root and tuber crops to the rural farmers in Benue State Nigeria, post-harvest losses have continued to dampen their potentiality. Oracca-Tetteh (1978) reported that though attempts have been made to increase their production by bringing more land into cultivation and use of improved seeds and chemicals, these have been less effective because any apparent gain in production has been lost to post-harvest losses.

Post-harvest losses in root and tuber crops have been attributed to the local methods for storing these crops. According to Tyler (1982); Mughogho (1989); and Omoruyi, Orhue, Ake-Obo and Akhimien (1995), produce stored under the traditional system usually do not keep long and farmers usually suffer great losses. This report is corroborated by Okoedo-Okojie and Onemolease (2009) who reported that indigenous storage of farm produce is less effective compared to modern storage methods.

Efforts gear towards addressing post-harvest losses in root and tuber crops cannot, however, be fully realized if knowledge of factors that influence root and tuber crop farmers' choice of storage systems is not known. Understanding these factors will spur innovative policy extension programme formulation towards developing the roots and tubers sub-sector in Nigeria. The broad objective of this study was to analyse the determinants of choice of storage systems for root and tuber crops in Benue State of Nigeria. The specific objectives include to:

i. determine the factors affecting choice of storage systems by root and tuber crop farmers; and
ii. determine the relationship between choice of storage systems and farm productivity.

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II. METHODOLOGY

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 10^0E. Benue State is the nation's acclaimed food basket because of the abundance of its agricultural resources. It is a major producer of food and cash crops (BNARDA, 2004). Farmers who are engaged in arable crop production like yam, cassava, sweet potato, maize, rice, vegetables, soybeans as well as livestock like poultry, goat, sheep, piggery, cattle, and fish abound. Also agribusiness entrepreneurs who are involved in yam distribution/marketing, yam chip and flour production abound in the State (Okeke, Nto and Mbanasor, 2015).

Sampling Technique and Data Collection

The population for the study consisted of root and tuber crop farmers in the State. As a result of the enormity of the population for the study, a sample of 288 root and tuber crop farmers from eight Local Government Areas and 32 wards known for root and tuber crops production were selected using multi-stage sampling technique.

The data for the study were collected using a well-structured questionnaire. Data were collected on the socio-economic characteristics of root and tuber crop farmers; types of local storage methods for root and tuber crop farmers; effectiveness of the various local storage methods; farm output from the various storage methods; and problems associated with the various storage methods.

Data Analysis

Logit model was used to determine the factors affecting choice of storage systems by root and tuber crop farmers, while Mann-Whiney U test was used to realized the relationship between choice of storage systems and farm productivity.

Model Specification

The Logit model for determining the factors affecting choice of storage systems was specified as follows:

$$\text{In}\left(\frac{P}{1-P}\right) = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 + b_9X_9 + b_{10}X_{10} + \mu$$

where,

In = Base of natural logarithm

P = the probability that a respondent uses local traditional storage methods

a = Constant of the equation

b_1-b_{10} = Coefficients of the predictor variables

X_1 = Size of labour force (number of workers)

X_2 = Annual income (₦)

X_3 = Output size (Kg)

X_4 = Age (years)

X_5 = Gender (male = 1; female =0)

X_6 = Marital status (married = 1; single = 0)

X_7 = Membership of cooperative society (member = 1; non-member = 0)

X_8 = Educational level (years)

X_9 = Household size (number)

X_{10} = Farm size (ha)

The Mann-Whitney model for determining the relationship between choice of storage systems and farm productivity was specified as follows:

$$U = n_1 n_2 + \frac{n_2(n_2+1)}{2} - \sum_{i=n_1+1}^{n_2} R_i$$

Where,

U = Mann-Whitney U test

n_1 = Sample size one (choice of storage methods)

n_2 = Sample size two (farm productivity)

R_i = Rank of the sample size

III. RESULTS AND DISCUSSION

Factors Affecting Choice of Storage Systems

The logit model was used to investigate the effect of socio-economic characteristics of root and tuber producers on their choice of storage systems. The estimated relationship is presented in Table 1.

From the analysis, the model chi-square was 56.04 and significant at 1%, thus rejecting the null hypothesis of 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 root and tuber producers and their choice of storage systems was supported.

The Pseudo R square was 0.3565, indicating a relationship of 35.65% between the predictors and the predictions. In other words, about 36% of the likelihood of farmers utilizing the local storage technique is explained by the independent variables. 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.

Analysis of the result shows that the coefficient of total output was significant at 5% and positively related to choice of local storage systems. The positive sign of the coefficient is at variance with the *a priori* expectation, implying that if the output of root and tuber producers increases, the producer is 0.0000773% more likely to utilize the local storage methods. The reason is obvious. Farmers with large scale production may have challenges managing the resulting output after harvest, especially in terms of

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available space for effective storage and treatment; hence they will rely on the local methods for storing their increased output. This finding is at variance with Ansah and Teteh (2016) who reported that with large scale production, farmers are expected to make higher investments and generate larger incomes, hence such farmers are able to adopt effective postharvest management techniques.

Table.1: Logit model of the factors affecting choice of storage systems

Variables	Estimated coefficient	Standard error	Z-value	Marginal effect
Labour force size	-0.0384	0.0796	-0.48NS	-0.00465
Annual income	-0.0000000855	0.000000106	-0.81NS	-0.0000000103
Total output	0.00000639	0.00000272	2.59**	0.000000773**
Age	-0.008396	0.0332	-0.25NS	-0.00102
Gender (1)	-1.2057	0.5755	-2.03**	-0.1603**
Marital status (1)	0.2468	0.7260	0.34NS	0.02958
Membership of cooperative (1)	0.5158	0.5173	1.00NS	0.06320
Educational level	0.2066	0.0773	3.01***	0.02498***
Household size	0.1043	0.0546	2.03**	0.01261**
Farm size	-0.5978	0.1821	-3.84***	-0.07228***
Constant	-1.4504	1.5913		
Log likelihood	-50.5779			
LR Chi2 (10)	56.04			
Prob>Chi2	0.0000			
Pseudo R2	0.3565			

Source: Field survey data, 2017

*** Significant at 1%; ** Significant at 5%

The result shows that the coefficient of gender was significant at 5% level and negatively related to choice of local storage systems. The negative sign of the coefficient is in agreement with the *a priori* expectation, implying that if a root and tuber producer is a male, he is 16.03% less likely to utilize the local storage methods. *Ceteris paribus*, owing to their easier accessibility to investment capital, root and tuber producers who are male are more likely to invest in improved storage techniques than their female counterpart. This is in agreement with Okeke, Mbanasor and Nto (2015) who reported that yam entrepreneurs who are male usually have opportunity for investment capital compared to women entrepreneurs who tend to devote more of their time and earnings into their families.

Analysis of the educational level shows that the coefficient was significant at 1% level and positively related to choice of local storage systems. The positive sign of the coefficient is at variance with the *a priori* expectation, implying that as

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the educational level of root and tuber producers increases, they are 2.498% more likely to utilize the local storage methods. Farmers' human capital which education enhances, plays a significant role in the decision to adopt improved production and postharvest management practices. However, root and tuber producers with high level of education who still prefer the local storage techniques are those with large household size. This large household size implies that these root and tuber producers will prefer the local storage methods due to high consumption expenditure which translates to low savings and investment in improved storage techniques. This finding is corroborated by Giroh, Gal and Minampah (2012) who revealed that a farmer with a large household size will likely channel more of his/her income to food consumption expenditure rather than to save and invest in farming enterprise.

The result shows that the coefficient of household size was significant at 5% level and positively related to choice of local storage systems. The positive sign of the coefficient is in consonance with the *a priori* expectation, implying that if the household size of root and tuber producer increases, the producer is 1.261% more likely to utilize the local storage methods. This is because root and tuber producers with large household sizes will invest in the cheaper local methods of storage than the expensive improved storage method as their high dependency ratio translates to more consumption expenditure, hospital bills, and school fees. This finding agrees with Okeke, Mbanasor and Nto (2015) who observed that entrepreneurs with large family size will find it difficult to save and invest owing to the high dependency ratio which translates to more consumption expenditure.

The coefficient of farm size was significant at 1% level and negatively related to choice of local storage systems. The negative sign of the coefficient is in consonance with the *a priori* expectation, implying that if farm size of root and tuber producers increases, they are 7.228% less likely to utilize the local storage methods. Farmers with large farms are more likely to adopt improved storage techniques when compared to farmers with smaller farm size. This is because of their desire to preserve the large output from such large farms. This is corroborated by Okoedo-Okojie and Onemolease (2009) who reported that farmers with large farms are positively disposed to use of farm innovations largely because having larger farms strengthens the farmer's capacity to produce more which he/she would be interested in preserving from loss, using improve storage techniques.

Relationship between Choice of Storage Systems and Farm Productivity

The relationship between choice of storage systems and farm productivity is presented in Tables 2 and 3.

Table 2 shows that the mean output rank of farmers who utilized both modern and traditional storage techniques was 111.37CEW while those who utilized only traditional techniques was 154.78CEW.

The test for significant difference of mean output rank between those farmers who utilized both traditional and modern storage techniques and those who utilized only traditional storage techniques is presented in Table 3.

The Man-Whitney U-test shows that there was a significant difference in the mean output rank between those farmers who utilized both modern and traditional storage techniques and those who utilized only traditional storage techniques at 1% significant level (U = 4216.500, P = 0.000).

*Table.2: Storage systems and farm output in cereal equivalent weights (CEWs)**

Storage technique	Mean output rank	Sum of output ranks
Modern and traditional technique	111.37	19267.50
Traditional technique	154.78	11608.50

Source: Field survey data, 2017

*CEW = Cereal Equivalent Weights: Yam (0.26 CEW/kg); Cassava (0.32 CEW/kg); Sweet potatoes (0.28 CEW/kg)

Table.3: Test for significant difference of mean output rank between modern/traditional techniques and traditional techniques

Item	Farm output in Cereal Equivalent Weight
Mann-Whitney U	4216.500***
Wilcoxon W	19267.500
Z	-4.378
Asymp. Sig (2-tailed)	0.000

Source: Field survey data, 2017

Grouping variable: storage techniques; *** significant at 1%

The low output of root and tuber producers who also utilized the modern storage techniques in addition to the local storage methods could be attributed to the high cost associated with these modern techniques. The lower cost of the traditional storage techniques in addition to their effectiveness ensure increase output. This finding is corroborated by Nwaigwe, Okafor, Asonye and Nwokocha (2015) who revealed that the advantages of these local storage methods include: the materials for construction are locally available and are at a very low cost; protect tubers from rodent attack; create favourable temperature for stored products; offers protection against weight loss owing to respiration and transpiration; does not require supplementary funds and provides protection against heat; no transport expenses involved, and keeps tubers in good condition.

IV. CONCLUSION

The results indicate that total output, gender, educational level, household size, and farm size significantly affect the choice of storage systems by root and tuber crops producers.

The results also reveal that the output of farmers who utilized only the local storage systems exceeds those who utilized both the local storage systems in addition to the modern storage techniques.

Based on these findings, it was recommended that strategies and policies aimed at encouraging the root and tuber crops producers in adopting a particular storage technique should take into consideration those factors that influence their choice of storage systems. Also, research efforts aimed at improving the effectiveness of the already existing local storage systems using locally sourced materials should be encouraged.

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REFERENCES

[1] AMCOST (2006). Technologies to reduce post-harvest food loss The African Ministerial Council on Science and Technology (AMCOST) of the African Union (AU), Pretoria, South Africa, (2006). From http://www.nepadst.org/platforms/foodloss.shtml (Retrieved November 8, 2015).

[2] Ansah,I.G.K. and Tetteh, B.K.D. (2016). Determinants of Yam Postharvest Management in the Zabzugu District of Northern Ghana. *Advances in Agriculture*, 1-10.

[3] Ayegba, O. and Ikani, D.I. (2013). An Impact Assessment of Agricultural Credit on Rural Farmers in Nigeria. *Research Journal of Finance and Accounting*, 4(18): 80-89

[4] BNARDA (2004). *The Impact of Benue State Agricultural and Rural Development Authority*. Pp. 42.

[5] FAO (1998). Storage and Processing of Roots and Tubers in the Tropics. The Food and Agricultural Organisation (FAO), Rome. From <http://www.fao.org/documents/show.htm> (Retrieved November 8, 2015)

[6] Giroh, D.Y., Gal, T.N. and Minampah, C.J. (2012). Analysis of the Determinants of Savings among Gum Arabic Collectors in Selected Local Government Areas of Adamawa State, Nigeria. *New York Science Journal*, 5(11): 1-6

[7] Kana, H.A., Aliyu, I.A. and Chammang, H.B. (2012). Review on Neglected and Under-Utilized Root and Tuber Crops as Food Security in Achieving the Millennium Development Goals in Nigeria. *Journal of Agriculture and Veterinary Sciences*, Volume 4: 27-33

[8] Mughogho M.J.K.(1989). Malawi: Food security issues and challenge for 1990's. In: M Rukuni (Ed.): *Food Security Policies in the SADC Region*. Harare. University of Zimbabwe and Michigan State University Food Security Research in Southern Africa Project. Department of Agricultural Economics and Extension.

[9] Nwaigwe, K.N., Okafor, V.C., Asonye, G.U. and Nwokocha, J.C. (2015). *Analysis of Tuber Storage Techniques in Africa: A Review*. ASABE Annual International Meeting, July 26-29, New Orleans, Louisiana.

[10] Okeke, A.M., Mbanasor, J.A. and Nto, P.O. (2015). Determinants of Investment Capacity among Yam Production Entrepreneurs in Benue State, Nigeria. *Journal of Poverty, Investment and Development*, 16: 1-8.

[11] Okeke, A.M., Nto, P.O. and Mbanasor, J.A. (2015). Analysis of the Factors Influencing Savings and Investment Behaviour among Yam Entrepreneurs in Benue State, Nigeria. *European Journal of Business and Management.* 7(27): 205-209.

[12] Okoedo-Okojie and Onemolease (2009). Factors Affecting the Adoption of Yam Storage Technologies

http://dx.doi.org/10.22161/ijeab/2.6.33 *ISSN: 2456-1878*

in the Northern Ecological Zone of Edo State, Nigeria. *J. Hum. Ecol*., 27(2): 155-160

[13] Olaoye, O.J., Ashley-Dejo, S.S., Fakoya, E.O., Ikeweinwe, N.B., Alegbeleye, W.O., Asholu, F.O. and Adelaja, O.A. (2013). Assessment of Socio-economic Analysis of Fish Farming in Oyo State Nigeria. *Global Journal of Science Frontier Research, Agriculture and Veterinary*, 13(9): 10-18.

[14] Omoruyi, S.A., Orhue, U.X., Ake-obo, A.A. and Akhimien, C.I. (1995). *Prescribed agricultural science for senior secondary schools*, Benin City: Idodo Umeh Publications Limited, pp. 4 – 6

[15] Onemolease EA 2005. Impact of the Agricultural Development Programme (ADP) Activities in Arable Crop Production on Rural Poverty Alleviation in Edo State, Nigeria. Ph.D Thesis (Unpublished), University of Benin, Benin City, Edo State, Nigeria p123

[16] Orraca-Tetteh, R. (1978). Post-harvest physiology and storage of Nigerian crops *Food and Nutrition Bulletin*, 1(1). The United Nations University Press, Tokyo, Japan.. From <http://www.unu.edu/unupress/ food/8F011e/8F011E0a.htm> (Retrieved November 8, 2015)

[17] Ravi, V., Aked, J. and Balagopalan, C. (1996). Review on Tropical Root and Tuber Crops: Storage Methods and Quality Changes. *Critical Reviews in Food Science and Nutrition*, 36(7): 661-709

[18] Tyler, P.S. (1982). Misconception of food losses *Food and Nutrition Bulletin* 4(2), The United Nations University Press, From <http://www.unu.edu/unupress/food/8F042e/8F042E05.htm> (Retrieved November 8, 2015).

[19] Ufuoku, U.A., Uzokwe, U.N. and Ideh, V. (2006). Comparative Analysis of Cooperative and Non-cooperative Fish Farmers in the Central Agroecological Zone of Delta State Nigeria. *Extension Farming Systems Journal*, 2(1): 97-104

[20] Yuguda, R.M., Girei, A.A., Dire, B., Salihu, M. (2013). Socio economic factors and constraints influencing productivity among cassava farmers in Taraba State, *Nigeria. International Journal of advances in Agricultural science and technology, vol 1 pg (1- 5)*

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Factors Affecting the Farmer's Response to the Development of Soybean Farming in East Java Indonesia

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Abstract— *The research was conducted to find out (1) factors influencing farmer's decision to cultivate soybean, and (2)the opportunity level of farmer response to soybean farming in Jember and Sampang. Research location was determined through purposive method and sampling conducted through simple random sampling method. Data analysis method used was logit regression model where the dependent variable (Y) was dummy variable with value of 1 (response) and 0 (non-response). Research result found G value of 130.198 (p-value = 0.0001) indicating that logistic regression model, as a whole, could explain farmers' decision in their response to soybean farming. Factors influencing farmers' response to soybean farming were acreage, education, income, and area status.*

Keywords— ***Response, Soybean, Logistic Regression Model, Opportunity, East Java.***

I. INTRODUCTION

Soybean is a strategic food commodity in Indonesia. Therefore, effort for self-sufficiency in soybean should be conducted continuously since it is not only to fulfill food needs but also to support agroindustry and save foreign exchange as well as decrease dependence on imported food (Amaruddin et al., 2002; Supadi, 2008). An excessive dependence on imported food to fulfill the needs could threat social, economic and political stability that in turn have potential to disturb the independence of the nation (Amang and Sawit, 1997; Suryana, 2002; Arifin, 2004; Husodo, 2004).

The government has targeted self-sufficiency in soybean in the future. Currently, the need or national demand for soybean reaches 2.2 ton per year and national production is only able to fulfill 35-40% of the need thus import is the only way to fulfill the shortage. High trend in soybean demand is a big opportunity to increase interest among farmers to cultivate the commodity as well as increase their household income. Currently, national soybean production is decreasing despite the positive but slow growth in soybean productivity (Ariani, 2005; Supadi, 2008). It means that the production level of soybean is decreasing due to the decrease in planting areas. Based on farmer's view, the decrease in soybean planting areas indicates less participation among farmer to cultivate soybean. However, the opportunity in the development of domestic soybean production is still open due to the extent of land availability, agricultural land ecosystem suitability to cultivate soybean and high market demand for soybean cultivation.

Jember and Sampang Regencies are two regencies that give contribution in the supply of soybean production in East Java Province. Soybean production in East Java in 2014 has increased to 355.46 thousand ton of dry grain or an increase of 26 thousand ton (7.89 percent) from those in 2013. The increase in soybean production is occurred due to the increase in harvest area and productivity of 4.26 thousand hectare (2.02 percent) and 0.90 quintal/hectare (5.75 percent), respectively. The increase in soybean production is related to the increase in harvest area in Jember Regency. It is due to the supporting weather, good crop maintenance by farmer and promising soybean price. As well as in Sampang Regency, soybean harvest area is also increasing due to the Program of Expansion of Planting Area (Perluasan Areal Tanam = PAT) in soybean planted in April 2014. The program is partly used land that usually planted with corn thus harvest area of corn is decreasing (Statistik Jawa Timur, 2015).

The increase in soybean production is closely related to farmer's behavior in soybean production process activity and level of farmer participation influences the effort. Therefore, it is interesting to conduct a research on the response of farmer to the development of soybean farming in both regencies as the representative of soybean production center in East Java. The research aimed to: (1) identify factors influencing farmer's decision to cultivate soybean, and (2) find out the opportunity level of farmer response to soybean farming in Jember and Sampang Regencies.

II. METHODS

The research was conducted in Jember and Sampang Regencies, East Java Province. Location was determined purposively since both areas had an increased trend in soybean harvest area in 2014. Sample for Jember Regency was 65 respondents with 45 farmers who respond and 20 farmers who do not respond to soybean farming. In Sampang Regency, the respondents consisted of 30 farmers who respond and 19 farmers who do not respond. The research used survey method conducted in 2015.

To identify factors influencing farmer response to soybean farming quantitative approach of econometric analysis, which was logistic regression analysis, was conducted. Logistic regression is statistical analysis method used to describe the relationship between independent variable and dependent variable having two or more categories with independent variable having categorical or interval scales (Hosmer and Lemeshow, 1989). Vasisht (2000) stated that logistic regression is a univariate or multivariate analysis used to predict dependent variable, which is a probability of an incident using one or more independent variables. Logistic regression approach was used since it could explain the relationship between dependent and independent variables that otherwise unable to be explained by regular regression.

According to Nawangsih and Bendesa (2013), some studies need to be done with logistic regression model, which is G test to test whether independent variables have significant influence on dependent variable, simultaneously. Wald test, on the other hand, is used to find out whether each independent variable has influence on dependent variable, partially. In addition, Hosmer-Lemeshow test is used to test model feasibility.

Logistic regression consists of two types, binary and multinomial logistic regression. Binary logistic regression has dependent variable that divided into two categories and logistic multinomial has dependent variable that divided into more than two categories. The research used binary logistic and independent variable of X with continue, discrete and categorical scales.

Logit model is a linear regression model where the dependent variable is dummy variable. Generally, the value of 1 is used if an incident "is occurred" and 0 if an incident "is not occurred". Logit model used in the research was as follow:

$$Yi = Zi = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 4X4 + \beta 5X5 + \beta 6D1 + \varepsilon$$

Where:

Y = Dummy of Farmer response

Y=1, if farmers conduct soybean farming and

Y=0, if farmers do not conduct soybean farming

X1 = Variable of acreage

X2 = Variable of farmer's age

X3 = Variable of farmer's education level

X4 = Variable of number of family member

X5 = Variable of farmer's income

D1= Dummy of area status; (1=if the area is Jember Regency, 0=if the area is Sampang Regency)

βo-βn = Regression coefficient

ε = error

III. RESULT AND DISCUSSION

3.1. Characteristics of Farmer

The following Table 1 shows result of characteristics of farmers who respond and do not respond to soybean farming.

East Java Province has a considerable contribution in the supply of domestic soybean production in national level, which is 37.22 percent in 2014 (Statistik Jatim, 2015) and the contribution is estimated to increase in 2015. Knowledge of farmer response is important as anticipation by treating it with the influencing factors. Since farmers are the main actor in the program of soybean farming development, their response to soybean farming is very important to be studied as a consideration for local government that conduct program policy related specifically to the characteristics of farmer in their socio-economic aspect.

Based on Table 1, it can be seen that the average of farmer's acreage was varied between farmers who respond and do not respond to soybean farming with farmers who respond had bigger acreage than those farmers who do not respond. Regarding age, farmers who cultivate soybean were older than those who do not respond indicating that younger farmers interested more to non-soybean farming. In addition, there was no difference in level of education for both farmer groups. It means that both farmer groups had similar level of education, which was elementary school. In variable of number of family member, it can be seen that the average number of family member in farmer who respond was 4 people that bigger than those of farmers who do not respond with average family member of 3 people. Regarding income level, the income of farmers who respond was lower than those farmers who do not respond to soybean farming. It means that non-soybean farming gained more income than soybean farming in the same planting season.

3.2. Factors Influencing Farmer Response

Factors influencing farmer response to soybean farming was analyzed using logistic regression (logit model). The analysis aims to see the opportunity of independent variables whether or not they have influence on dependent variable, in this case the decision of farmer to respond to cultivate soybean (1) and the decision of farmer for not doing (to not respond to) soybean farming (0).

Based on result of minitab analysis version 16 as indicated in Table 2, it can be seen that G value was 130.198 with p-value of 0.0001 (indicated testing number below 0.05). It means that logistic regression model, as a whole, could explain or predict the decision of farmer to do (respond to) soybean farming. The result was confirmed by the value of G that bigger than the value of Chi-Square of 17.66 (Pearson Method). The feasibility of logistic regression model (goodness of fit) in predicting was analyzed using Chi-square Hosmer and Lemeshow tests. The test result shows Chi-square value of 4.2808 with p-value of 0.831. It means that logistic regression model was fit to be used for next analysis since there was no significant difference between predicted classification and observed classification. Further, Wald test result indicates that, partially, factors influencing (p-value below 0.1) farmer response to soybean farming were X1 (acreage), X3 (education), X5 (income) and D1 (area status).

Acreage

Acreage in the model was a variable with a very significant influence on farmer response to cultivate soybean. The value of Wald test (Z test) for acreage variable was 2.41 and p-value of 0.016. It indicates that farmers with wider acreage would respond more to cultivate soybean than those farmers with narrow acreage. Coefficient of Odds ratio of 4761.89 implies that the opportunity for farmer with wider acreage could reach 4761.89 times than those farmers with narrow acreage. Rationally, the result analysis could be understood since, according to Sumarno and Adie (2010), soybean farming is in the category of high risk and according to Soekartawi (1988) only farmers with wider acreage are willing to take a risk since they would still be able to fulfill their family need when they fail. On the contrary, farmer with narrow acreage tended to avoid the risk. The fact is in line with Rao (1975 in Sabrani, 1988), Hammal (1983), and Dillon and Scandizzo (1978) stated that small farmers tend to avoid risk compare to farmers with wider acreage.

Education

The relationship between farmer response and education had negative sign with coefficient of Z = -2.20 and p-value of 0.028. It means that the lower the level of education of farmers, their response to soybean farming was increasing. Coefficient of Odds ratio was 0.47 meaning that famers with level of education of one year higher had response opportunity to soybean farming of 0.47 times than farmer with lower education. In other words, farmers with higher education had lower opportunity to do (response to) soybean farming. According to Rachmawati and Djuwendah (2015), level of the application of technology in soybean production was dominated more by farmers with elementary school level of education. It is in line with Hadi and Edyanto (2015) stated that the average of formal education level of soybean farmer was elementary school. The condition was in accordance with description data showing that level of education of soybean farmers was elementary school.

Income

Income based on estimation result through logistic regression shows Z coefficient of 2.57 and p-value of 0.010. It implies that income had significant influence on farmer response to soybean farming in significant level of 99 percent. The negative sign means that the bigger the income of farmers the smaller the opportunity of farmers to response to soybean farming. On the contrary, farmers with lower income had bigger opportunity to response to soybean farming. The value of regression coefficient of 0.0000076 indicates that if the difference in farmers' income was Rp. 100,000, Odd ratio would be 2.13. It means that the opportunity of response from famer with income lower than Rp. 100,000 was 2.13 times than those of farmers with income of (Rp. 100,000) bigger than them. Soybean farmers gained income of Rp. 2,023,916; whereas, non-soybean farmers gained income of Rp.4,684,962. It indicates that the income of soybean farmers was lower than those of non-soybean farmers. The amount of income gained by farmers will be taken into consideration when farmer's decision making on type of commodity to be cultivated. It is in line with Bishop and Toussaint (1989) that farmer's income could be influenced by their selection of production yield. The selection of production yield was conducted by farmers based on their expected income and the sales of their produce. Therefore, before selecting or cultivating a commodity, farmers would consider the amount of income gained from the commodity.

Area Status

As in the case of acreage, variable of area status had a very significant influence in confidence level of 95 percent. The coefficient of Z test was 2.05 with p-value of 0.041. Coefficient of Odds ratio of 196.94 implies that farmers in Jember Regency had opportunity to cultivate soybean of 196.94 times compare to those farmers in Sampang Regency. In other words, farmers in Jember

Regency had higher opportunity to cultivate soybean than those farmers in Sampang Regency.

IV. CONCLUSION

Factors that significantly influenced the decision making of farmers to cultivate soybean were acreage (X1), education (X3), income (X5) and area status (D1). The opportunity of soybean cultivation in Jember Regency was bigger than Sampang Regency.

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REFERENCES

[1] Amang, B dan Sawit, 1997. Perdagangan Global Dan Implikasinya Pada Ketahanan Pangan Nasional. Jurnal Agro-Ekonomika. No.2. Tahun XXVII. PERHEPI. Jakarta
[2] Amaruddin, Masyhuri, Sutrilah., 2002. Analisis Keunggulan Komparatif Dan Tingkat Proteksi Efektif Pada Komoditas Kedelai Di Pulau Jawa. Jurnal : Agrosains 2002, XV(2) UGM
[3] Ariani, M. 2005. Penawaran dan permintaan kacang-kacangan dan umbi-umbian di Indonesia. SOCA 5(1): 48−56.
[4] Arifin, B., 2004. Analisis Ekonomi Pertanian Indonesia. Penerbit Buku Kompas Jakarta
[5] Bishop dan Toussaint. 1989. Pengantar Analisis Ekonomi Pertanian. Jakarta: Mutiara
[6] Dillon, J.L. and P.L. Scandizzo. 1978. Risk attitude of subsistance farmers in Northeast Brazil: A sampling approach. *American Journal of Agricultural Economics 60(3):235-425.*
[7] Hadi,S. dan Ediyanto,R. 2015. Responsif Petani Terhadap Usahatani Kedelai Semakin Melemah di Kabupaten Jember. Agritrop Jurnal Ilmu-Ilmu Pertanian
[8] Hammal, K.B. 1983. Risk avertion, risk perception, and credit use. The case of small paddy farmers in Nepal. Research Paper No. 21. Kathmandu.
[9] Hosmer,D.W. dan Lemeshow. 1989. Applied Logistic Regression. New York; John Willey and Sons
[10] Husodo, S Y., 2004. Membangun Kemandirian Pangan: Suatu Kebutuhan Bagi Indonesia, Negara Berpenduduk Banyak dengan Potensi Pangan yang Besar. Yayasan Padamu Negeri. Jakarta
[11] Nawangsih, E. dan Bendesa, IKG. 2013. Perbandingan Ketepatan Model Logit dan Probit Dalam Memprediksi Kecenderungan Tingkat Hunian Kamar Usaha Akomodasi di Bali 2010. Jurnal Ekonomi Kuantitaif Terapan. Vol.6 No.1 Februari 2013. ISSN:2301-8968
[12] Rachmawati,E. dan Djuwendah,E. 2015. Tingkat Penerapan Teknologi Produksi Kedelai (Glysine max (L) Merril) di Kecamatan Conggeang dan Buahdua Kabupaten Sumedang. http://pustaka.unpad.ac.id/wp-content/uploads/2015/01/5-Tingkat-Penerapan-Teknologi-Produksi-Kedelai.pdf
[13] Sabrani, M. 1988. Perilaku petani peternak domba dalam alokasi sumberdaya. Disertasi. Universitas Gadjah Mada. Yogyakarta. (Tidak dipublikasikan).
[14] Statistik Provinsi Jawa Timur, 2015. No. 47/07/35/Th.XIII,1 Juli 2015
[15] Soekartawi. 1988. Prinsip Dasar Komunikasi. pp 46. UI Press. Jakarta.
[16] Sumarno dan Adie, M.M. 2010. Strategi Pengembangan Produksi Menuju Swasembada Kedelai Berkelanjutan. Iptek Tanaman Pangan Vol. 5 No. 1 – 2010
[17] Supadi, 2008. Menggalang Partisipasi Petani untuk Meningkatkan Produksi Kedelai Menuju Swasembada. Jurnal Litbang Pertanian, Vol 27 (3).
[18] Suryana, A., 2002. Benarkah Impor Pangan Kita Mencemaskan, Sekretariat Dewan Ketahanan Pangan. Jakarta
[19] Vasisht,A.K. 2000. Logit and Probit Analysis. New Delhi. Library Avenue.

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Table.1: Characteristics of Farmers who Respond and do Not Respond to Soybean Farming

No	Variable of characteristics	Mean value		T test (P-Value)	Description
		Response (St.Dev)	Non-response (St.Dev)		
1.	Acreage (Ha)	0.4720 (0.2589)	0.3295 (0.1525)	3.69 (0.0001)	Significant
2.	Age (year)	50.21 (13.23)	40.69 (6.35)	5.19 (0.0001)	Significant
3.	Education (year)	8.693 (2.746)	7.923 (2.120)	1.66 (0.101)	Not Significant
4.	Number of family member (people)	4.240 (1.113)	3.564 (0.788)	3.75 (0.0001)	Significant
5.	Income (Rp)	2.023.916 (499.464)	4.684.962 (2.240.665)	7.32 (0.0001)	Significant

Source: Result of data processing

Table.2: Result of Logistic Regression Model Test

Predictor	Coeff.	SE Coeff.	Z	P	Odds Ratio
Constant	6.92618	5.48496	1.26	0.207	
x1 (acreage)	70.638	29.3359	2.41	0.016	4761.89
x2 (age)	0.0206426	0.0956729	0.22	0.829	1.02
x3 (education)	-0.758026	0.345258	-2.20	0.028	0.47
x4 (number of family member)	-0.838059	0.897021	-0,93	0.350	0.43
x5 (income)	-0.0000076	0.0000030	-2,57	0.010	1,00
D1 (area status)	5.28292	2.57923	2,05	0,041	196,94

Log-Likelihood = -8.137
Test that all slopes are zero: G = 130.198, DF = 6, P-Value = 0.0001
Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	17.6646	107	1.000
Deviance	16.2746	107	1.000
Hosmer-Lemeshow	4.2808	8	0.831

Source: Result of Analysis

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An Update of Weed Flora of Vıneyards ın Northwestern Turkey

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Abstract— *The weed flora of vineyards in northwestern Turkey was determined in a survey carried out in 93 vineyards. Total of 68 species 53 dicotyledonous and 11 monocotyledonous belonging to 32 families were identified in grape growing areas. The majority of weed species were annual species with different vegetation periods. The dominant weed species in the region were Capsella bursa pastoris, Convolvulus arvensis, Senecio vulgaris, Stellaria media, Sorghum halepense, Euphorbia helioscopia. Meanwhile frequent families were Poaceae, Asteraceae, Brassicaceae, Fabaceae, Geraniaceae, Lamiaceae, Polygonaceae and Euphorbiaceae.*

Keywords— Weed flora, vineyards, Thrace Region, Turkey.

I. INTRODUCTION

Weeds compete with grapevines for water, light, and nutrients leading to approximately 37% yield reduction, 68% cane weight loss, and 3% berry weight loss. Weeds growing within the vine rows can also reduce harvest efficiency [1]. Becides some species can reduce vine growth by releasing growth inhibiting allelopathic chemicals [2]. Becides this effects weeds can host of many pathogens and pests including disease vectoring insects and nematodes [3; 4; 5; 6; 7; 8; 9;10;11].

Many factors such as climate changes and seed dispersal thorughout years results in significant change of weed flora. The populations of weed may increase as well. On the other hand due to higher rise in population througout years one weed may become as serious treat to production.

Grapevine is important crop plant in northeastern Thrace Region of Turkey. Tekirdag, Edirne and Kırklareli are main grape growing provinces with total vineyard areas covering a surface of 37.4; 19,5 and 7 ha respectively. and vineyards were mostly established with Semillion, Merlot, Trakya Ilkeren, Okuzgozu, Cardinal, Gamay, Yapıncak, Cinsault, Italia, Erenkoy Beyazı, Alphonse L. and Cabernet Sauvignon grape varieties grafted mostly on Kober 5BB rootstock.

Weed infestations are problematic in the region especially in newly established vineyards affecting growth of young rootstocks. In addition many weeds such as *Datura stramonium* and *Convolvulus arvensis* were found to host common grapevine diseases including *Grapevine Fanleaf Virus* and *Rhizobium vitis.* Root damage of Root Knot Nematodes (*Meloidogyne* spp.) were also observed in *Purtulaca oleraceae* and some other weeds.

Weed management in the region mostly relies on mechanical and chemical control. The application and selection of control methods depends on knowledge of species (broad leaved, narrow leaved) life cycles (seed germination period) and growth habitats (annual, biannual, perannual). Meaanwhile effective chemical weed control can only be achieved by correct timing the application to the right target while mechanism of action of herbicides varies among species.

The aim of this study was to determine cureent weed species coinfesting wine-growing areas in northeastern Thrace Region of Turkey. The research area covered more than 70.000 ha vineyard area.

II. MATERIAL AND METHOD

To determine weed species field observations were carried out in randomly selected 93 vineyards varying in size of 5 to 25 da.

Species was recorded in each vineyard and density was evaluated by counting plants or shoots of weeds at 1 m^2 frame. Frequency of occurrence (f%) of species was calculated as the number of vineyards where the species was recorded divided by the total number of vineyards visited.

Furthermore herbarium of each plant were prepared by slow drying in air flow incubator at 30°C and were deposited in Tekirdag Viticulture Research Institute. Plants were identified by using various keys [12]

III. RESULTS

Based on our taxonomic identifications weed flora of Thrace Region was mostly composed of dicotyledons with % 69. The rest were monocotyledons with 31%. A total of 68 species of weeds belonging to 31 families were recorded (Table 1; figs.1&2&3&4&5&6). Results of our

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taxonomic identification of families and species are given in Table 2 in alphabetical order.

Table.1: The list of families, the number of species and relative frequencies (%)

Family	No. of species	% f of total
Poaceae	12	17.6
Asteraceae	12	17.6
Fabaceae	5	7.35
Brassicaceae	5	7.35
Geraniaceae	3	4.41
Lamiaceae	2	2.94
Apiaceae	2	2.94
Euphorbiaceae	2	2.94
Malvaceae	2	2.94
Polygonaceae	2	2.94
Solanaceae	2	2.94
Fumariaceae	1	1.47
Convolvulaceae	1	1.47
Amaranthaceae	1	1.47
Violaceae	1	1.47
Caryophyllaceae	1	1.47
Chenopodiaceae	1	1.47
Cuscutaceae	1	1.47
Scrophulariaceae	1	1.47
Cucurbitaceae	1	1.47
Cyperiaceae	1	1.47
Hyacinthaceae	1	1.47
Papaveraceae	1	1.47
Plantaginaceae	1	1.47
Apocynaceae	1	1.47
Urticaceae	1	1.47
Primulaceae	1	1.47
Rubiaceae	1	1.47
Portulacaceae	1	1.47
Aristolochia	1	1.47
Zygophyllaceae	1	1.47
Boraginaceae	1	1.47
Total	68	100

Fig. 1: Centauria solstitialis from Asteraceae

Fig. 2: Datura stramonium from Solanaceae

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Table.2: List of families and species of weeds identified in vineyards

FAMILY	SPECIES
Amaranthaceae	Amaranthus retroflexus L.
Apiaceae	Daucus sativa L.
	Conium maculatum L.
Apocynaceae	Cynanchum acutum L.
Aristolochia	Aristolochia clematitis L.
Asteraceae	Senecio vulgaris L.
	Matricaria chamomilla L.
	Xanthium strumarium L.
	Xanthium spinosum L.
	Cirsium arvenseL.
	Lactuca serriola L.
	Taraxacum officinale L.
	Silybum marianum L.
	Sisymbrium altissimum L.
	Sonchus arvensis L.
	Conyza canadensis L.
	Tragopogon dubius L.
Boraginaceae	Heliotropium purpureum L.
Brassicaceae	Capsella-bursa pastoris L.
	Raphanus raphanistrum L.
	Lepidium draba L.
	Sinapis arvensis L.
	Thlaspi arvensis L.
Caryophyllaceae	Stellaria media L.
Chenopodiaceae	Chenopodium album L.
Convolvulaceae	Convolvulus arvensis L.
Cucurbitaceae	Ecballium elaterium L
Cuscutaceae	Cuscuta spp.
Cyperaceae	Cyperus rotundus L.
Euphorbiaceae	Euphorbia helioscopiaL.
	Euphorbia villosa L.
Lamiaceae	Lamium amplexicauleL.
	Lamium purpureumL.
Fabaceae	Vicia sativa L.
	Trifolium repens L.
	Vicia craccaL.
	Medicago sativa L.
	Medicago polymorpha L.
Fumariaceae	Fumaria officinalis L.
Geraniaceae	Geranium dissectum L.
	Geranium molle L.
	Erodium cicutarium L.
Hyacinthaceae	Muscari armeniacum L.

Fig. 3: Papaver rhoeas infested vineyard

Fig.4: Heavy infested vineyard in Tekirdag

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Table.2: List of families and species of weeds identified in vineyards

FAMILY	SPECIES
Malvaceae	Malva sylvestris L.
	Malva neglecta L.
Papaveraceae	Papaver rhoeas L.
Plantaginaceae	Plantago lanceolata L.
Poaceae	Bromus tectorum L.
	Agropyron repens L.
	Alopecurus myosuroides L.
	Poa annua L.
	Sorghum halepense L.
	Echinochloa crus-galli
	Setaria verticillata L.
	Cynodon dactylon L.
	Hordeum murinum L.
	Avena fatua L.
	Lolium perenne L.
Polygonaceae	Rumex crispus L.
	Polygonum aviculare L.
Portulacaceae	Portulaca oleaceae L.
Primulaceae	Anagallis arvensis L.
Rubiaceae	Galium aparine L.
Solanaceae	Solanum nigrum L.
	Datura stramonium L.
Scrophulariaceae	Veronica hederifolia L.
Violaceae	Viola arvensis L.
Urticaceae	Urtica urens L.
Zygophyllaceae	Tribulus terrestris L.

Fig. 5: Heliotrophium purpureum from Boraginaceae

Fig. 6: Cuscuta spp. from Cuscutaceae

The life forms of weeds in vineyards mostly composed of therophytes (71 %), followed by hemicryptophytes (22 %) and geophytes (7%) (Fig. 7).

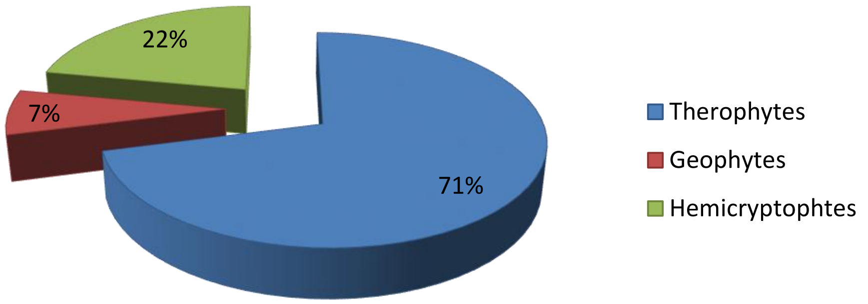

Fig. 7: Life forms of weed species (percentage %)

The species *Capsella-bursa pastoris* L., *Convolvulus arvensis* L., *Sorghum halepense* L., *Senecio vulgaris* L., *Lamium amplexicaule* L., *Papaver rhoeas* L., *Euphorbia helioscopia* L.,*Stellaria media* L., *Geranium dissectum* L.and *Agropyron repens* L.were the most frequent weeds in Thrace Region (Fig. 8.)

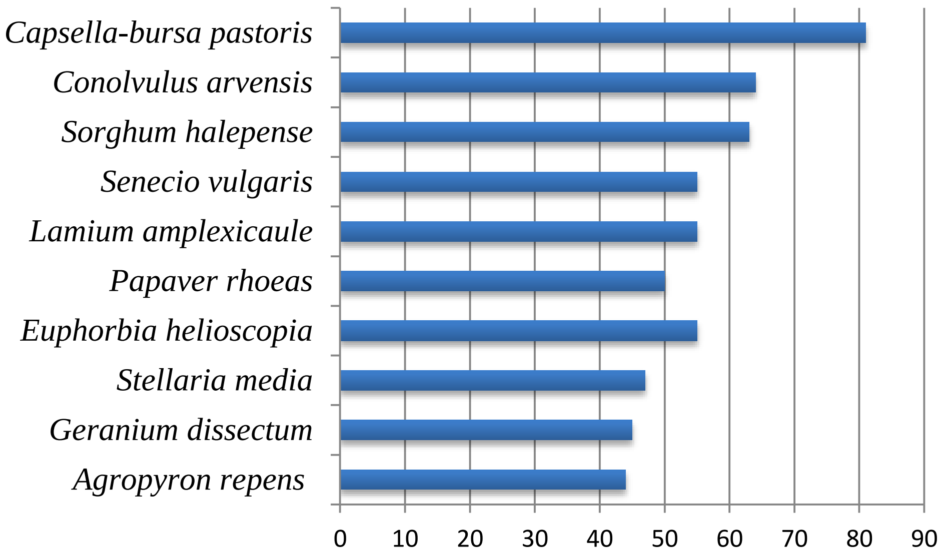

Fig.8: Occurance rate of some weed species in vineyards

The highest number of plants on raw were counted as 20 for *Hordeum vulgare* L., 20 for *Euphorbia helioscopia* L., 33 for *Agropyron repens* L., 25 for *Chenopodium album* L., 26 for *Lactuca serriola* L.

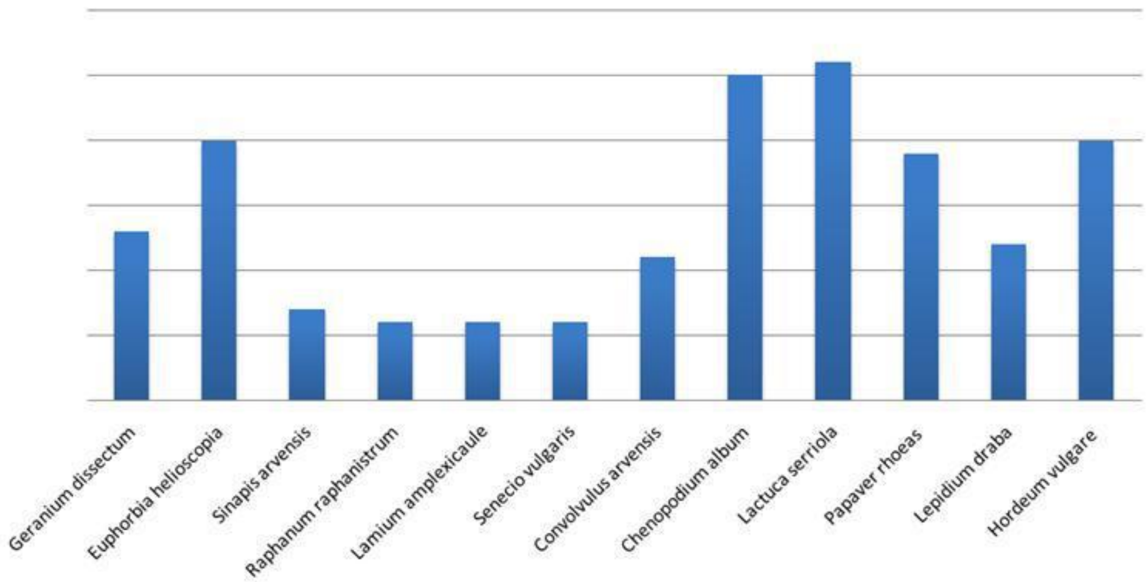

Fig.9: Relative density of some weed species in vineyards

IV. DISCUSSION

The weed flora of Thrace Region constitutes of 68 species from 31 families mostly from Poaceae, Asteraceae, Fabaceae and Brassicaceae. Similar results were obtained in a study carried out by researchers in western part of Turkey [13] Total of 36 weed species belonging to Poaceae, Brassicaceae, Polygonaceae and Amaranthaceae, Asclepiadaceae, Caryophyllaceae, Chenopodiaceae, Convolvulaceae, Cyperaceae, Fabaceae, Fumariaceae, Geraniaceae, Malvaceae, Papaveraceae, Poaceae, Portulacaceae, Solanaceae, Urticaceae, Zygophyllaceae families were identified in Aegean Region.

Meanwhile having similar climate conditions in neighbour country Iran 51 species (dicotyledonous 43 species) belonging to 22 families were reported. Poacea was the dominant family with a relative frequency of 17.4 %, and was followed by Fabaceae. [14]

Among all weed species in survey area *Capsella-bursa pastoris* L., *Convolvulus arvensis* L., *Sorghum halepense* L.were dominant species while *Datura stramonium* observed in a few locations. Becides some tall weeds were problematic to newly planted young rootstocks which has smaller sizes.

Dodder (*Cuscuta* spp.) are parasitic on several plants and is reported as virus vector. Grapevine Leafroll Associated

Virus-7 (GLRaV-7) is transmitted from one host to another by *C. reflexa* and *C. europea* [15]. *Cuscuta* spp. which only identified in 2 vineyards were not common in vineyards.

Recent flora of vineyards in the north eastern Turkey were not known. Due to agricultural practices many weed species may disperse to new area. On the other hand removed and newly established vineyards can alter the existing flora.

V. CONCLUSIONS

In this study we tried to evaluate the current weed status of local vineyards as well as the density of most important species, and distributions. Several perannual and annual weed species were identified in vineyards of north eastern part of Turkey. Most of the weeds were dicotyledonous. *Sorghum halepense* was a serious treat to young vinegrowth that needs to be controlled chemically of mechanically.

REFERENCES

[1] Wisler G.C. & Norris R.E. (2005). Interactions between weeds and cultivated plants as related to management of plant pathogens. Weed Science 53, 914–917
[2] Byrne, M. E. and Howell, G. S., (1978). Initial response of Baco physical conditions. Am. J. Enol. Vitic. 29, 192-198.
[3] Griffin, G. D. (1982). Differences in the response of certain weed populations to *Heterodera schachtii*. Journal of Nematology 14:174-182.
[4] Powell, C.A., Forer, L.B., Stouffer, R.F., Commins, J.N., Gonsalves, D., Rosenberger, D.A.et al. (1984). Orchard weeds as hosts of tomato ringspot and tobacco ringspot viruses.Plant Dis. 68:242–244
[5] Dissanayake N., Hoy J. W., and Griffin J. L.(1997). Weed hosts of the sugarcane root rot pathogen, *Pythium arrhenomanes*. Plant Dis. 81:587-591.
[6] Hollowell J. E., Shew B. B., Cubeta M. A., and Wilcut J. W. (2003). Weed species as hosts of *Sclerotinia minor* in peanut fields. Plant Dis. 87:197-199.
[7] Arli-Sokmen, M., Mennan, H., Sevik, M.A. et al., (2005). Occurrence of viruses in field-grown pepper crops and some of their reservoir weed hosts in Samsun, Turkey Phytoparasitica 33: 347
[8] Rich, J. R., J. A. Brito, R. Kaur, and J. A. Ferrell. (2008). Weed species as hosts of Meloidogyne: A review. Nematropica 39:157-185.Wisler, G.C. and R.F. Norris., 2005. Interactions between weeds and cultivated plants as related to management of plant pathogens. Sym. Weed Sci. 53:914-917.
[9] Golino, D.A., Sim, S.T., Osman, F., Aldamrat, R., Klaassen, V., Rowhani, A., (2009). Survey of wild grapes, weed and cover crop species for grapevine viruses. *Progrès Agricole et Viticole*, p. 110-111
[10] Elsner E. and Hanson D., (2014). Weed identification. Missiouri weed extension dep.
[11] Izadpanah, K., Zaki-aghl, M., Zhang, Y. P., Daubert, S. D., and Rowhani, A., (2003). Bermuda grass as a potential reservoir host for *Grapevine Fanleaf Virus*. Plant Dis. 87:1179-1182
[12] Naidu, V.S.G.R. (2012). Hand Book on Weed Identification Directorate of Weed Science Research, Jabalpur, India Pp 354.
[13] Kaçan, K , Boz, Ö (2015). The comparison and determination of the weed species in Conventional and Organic Vineyards. Ege Üniversitesi Ziraat Fakültesi Dergisi 52: 169-179
[14] Rostami, M. & Ahmadi, A.R. (2014). Analysis of weed flora in traditional vineyards of Malayer. In E. Tielkes (ed.) Book of abstracts, Tropentag 2014, International Research on Food Security, Natural Resource Management and Rural Development (pp. 36), Witzenhausen, Germany: German Institute for Agriculture in the Tropics and Subtropics.
[15] Mikona C., Jelkmann W., (2010). “Replication of *Grapevine leafroll-associated virus 7* (GLRaV-7) by *Cuscuta* species and its transmission to herbaceous plants”. Plant Disease 94: 471-476.

Study Physicochemical of the Raw Palm Oils of the Republic of Gabon and Congo

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Abstract— *The palm oil exists in several forms: palm oil raw, not refined, of red color because very rich carotenoids, called «red palm oil». In Africa, it is generally sold and consumed rough, i.e. not having undergoes the stages of refining. Taking into account the quantity and relevance of existing work on the palm tree with oil, we limited our study of Elaeis guinensis of Congo Basin to the analysis of the oils taken on the ground of the natural palm plantations and of the plantations of the palm plantation of M'vouna (Gabon) and of the Rural Campus of Loukoko (Congo) the study of our samples reveals the presence of carotenoids between 400 and 480 nm, and the presence of the chlorophyllian pigments with 600 and 670 nm for the great number of analyzed spectra. In the palm oil, the contents of carotenoids extend between 400 and 480 nm. The total content carotenoids varies from 897,1 µg/ml with 1229,3 µg/ml for the samples of the Palm plantation of M'vouna (Franceville, Gabon), whereas for the samples of Loukoko (Congo) that varies from 583,1 µg/ml with 1110,5 µg/ml on average. During precipitation partial of the raw palm oils of Gabon, three cases of figure ariseed (progressive, brutal and null solidification). The content total polyphenols varies from 439 to 2516 µg/ml. Of all the analyzed samples, the sample of Congo contains the maximum of phenolic compounds (2516 µg/ml). Minimum of 439 µg/ml, being obtained on the sample of Gabon. Their high content in carotenoids, fatty-acid unsaturates and the strong content total polyphenols, make our different oils more beneficial as well in the nutritional plan as therapeutic. The objective of work is the physicochemical characterization of the raw palm oils of the Republic of Gabon and Congo. The physicochemical characteristics of these oils are given according to the conventional normalized methods. They are the analyzes such as the spectroscopic evaluation of the color, the content carotene, partial solidification and the content polyphenols.*

Keywords— ***raw palm oils, color, carotene, solidification, polyphenols, Gabon, Congo.***

I. INTRODUCTION

Palm oil of Gabon east very appraisal in central Africa in particular in Cameroun. To our knowledge, the data on the palm oils are not complete, and there do not exist comparative studies carried out on the physicochemical properties of these artisanal raw palm oils in Gabon, in comparison with "traditional" oils more. This lack of data seems to be a handicap for the valorization of these oilseeds which could have food potentials of applications, cosmetic and energy, which remains unfortunately under-exploited. In this work, one will compare the physicochemical characteristics of oils of the raw palm oils of Gabon with those of oils of Congo of everyday consumption (nut oil of palm) for a better valorization in the human consumption and to arouse his interest.

The study of the absorption spectrum of an oil in the visible one makes it possible to determine the maximum wavelengths giving an indication on the pigments which are dissouts there.

The color of the palm oil is primarily related to the presence of carotenoids and chlorophyls. In the palm oil, the contents of carotenoids extend between 400 and 480 nm.

The composition and the total content of the pigments naturally present in oil, are important parameters because they are correlated with the color, which is a basic attribute to evaluate the quality of palm oil. The pigments are also implied in the mechanisms of self-oxidation and the photo-oxidation. Their presence in the palm oil is detected in a band which extends between 600 and 670 ppm for chlorophyls. With the light, chlorophyl supports the formation of the radicals of oxygen and accelerates oxidation but in the darkness, chlorophyl acts as an antioxydant.

Chlorophyl is a chlorine (four cores pyrroles in circle), chelating a magnesium atom in the center, as well as an alcohol with long chain, phytol. It has a structure comparable with that of heme (present in the blood red globules). It is the presence, in its structure, of many doubles combined connections which allows an

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absorption of the light radiation. The side chains of chlorine are variable and this involves a modification of the absorption spectrum between the various families of chlorophyls **(Hartmut and Lichtnetharler, 1987).**

The name "carotene" is derived from the root of carrot (Daucus carota), which was insulated like pigment coloured in 1831 (Tan, 1988). Roughly, the majority of 500 known carotenoids are naturally coloured (example tonalities yellow-orange-reds), and nearly about fifty these dyes have the activity of the vitamin has with various degrees.

Chemically, the carotenoids are combined hydrocarbons being able to be classified like carotenes (absence of oxygen atoms) or xanthophylls (presence of several oxygen atoms). The palm oil contains the highest concentration of the carotenoids agro-derivatives. The content carotenoids of the raw palm oil is of 500 - 2000 Mg/kg whereas other raw plant oils contain of them approximately 100 mg/kg **(Lecerf, 2013).**

The melting solubility and point constitute the two principal physical properties of the lipids. The distinction between oils and greases rests on their melting point. Oils are liquid greasy substances at the temperature of 15°C, while greases are more or less solid at this temperature (they are known as also "concrete"). Such a distinction can however lead to a certain ambiguity: the same greasy substance being able to be fluid or concrete according to the value of the room temperature of the country considered. It is of however certain interest to the nutritional plan, insofar as greases contain more saturated fatty-acids than oils **(FAO/OMS, 1977).**

The greasy substances being essentially made up of triglycerides, their temperature of solidification will be mainly function of their composition in fatty-acids.

Studies showed that the simple phenols and the phenolic acids, like the flavonoïdes, play a key role in the elimination and the detoxification of the free radicals, and make it possible to increase the resistance of the LDL (Low Density Lipoproteins) oxidation and to inhibit the peroxidation of the lipids **(Decker, 1995; Visioli et al., 1995).** The content of phenolic compounds of the palm oil is function of the variety of nuts, of their maturity at the time of harvest, the environment and the conditions of treatment.

II. MATERIAL AND METHODS

2.1. MATERIAL

The vegetable material consists of nut of palm and raw palm oil.

The study related to samples of palm oils collected on the ground to Gabon and Congo. These samples come from six (06) provinces of Gabon. The economic capital (Port-Gentil, in maritime Ogooue), was from the isolated start of the investigation, because considered as a place of flow of the production and not of manufacture. The investigation was not made either in the provinces of Nyanga and Ngounie, provinces too far away from the site of the University of Sciences and Technology of Masuku (USTM) and especially of difficult access. The investigation in Congo related to the town of Brazzaville and the area of the Pool, considered as the attic of the country.

It is thus in the Estuary (Libreville, N'Toum and Cocobeach), Haut-Ogooue (Palm plantation of association M'Vouna, Djamiti, Franceville); the Means-Ogooue (associations Nkolo of Atongowanga, Lambarene and Nyngone Georgette de Faisceaux, Lambarene); Ogooue Ivindo (Makokou); Ogooue Milk (Koula-Moutou) and Woleu-Ntem (Oyem), which we carried out of the investigations. The palm oils analyzed within the framework of this work all are extracted artisanalement and artisanalement stored with room temperature.

In Congo, the investigation was focused on the households of the town of Brazzaville and the palm plantation of the Rural Campus of Loukoko (RCL), in the area of the Pool.

2.2. METHODS

For the physicochemical characteristics of oils, the spectroscopic evaluation, partial solidification were given according to the normalized methods **(AFNOR, 1981; IUPAC, 1979)**. The evaluation of the content total carotenoids and total chlorophyls, and the content polyphenols were made according to the method described by **(Benabid, 2009)**.

Spectroscopic evaluation of the color

Into a graduated flask of 10 ml, one introduces 0,1 g of beforehand melted grease with 50°C and one supplements with chloroform to the feature of gauge. One measures then absorption, using a spectrophotometer of the type GENESYS 10 Bio (Photo 1), between 400 and 750 nm. The maximum ones of absorption for carotenoids are: 400,425,455 and 480 nm, and for chlorophyls: 600 and 670 nm, which are the principal pigments of plant oils.

Photograph 1: spectrophotometer of the type GENESYS 10 Bio

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Content carotene

The total carotenoids were determined by spectrophotometry UV with the wavelengths ranging between 400 to 760 nm.

The calculation of the content carotene in the palm oil, was carried out starting from the Optical Density (OD) with 460 nm according to:

Y = 0,0775x + 0,0029

$x = \frac{y-0,0029}{0,0775}$ with X: content carotene and Y: represent the Optical Density

The identification of the content of carotenoids was carried out by considering the reading of the Optical Densities (OD) with 460 nm and the equation:

% carotenoid = 4,57 OD (R^2 = 0.989) **(Davis and Al, 2009)**.

Partial solidification

Into a test-tube of 50 ml, one introduces 20 ml of oil of the fat contents, heated beforehand by the drying oven at the temperature of 70°C. In the course of time, the quantity of oil having precipitated (in ml) is raised.

Proportioning of polyphenols

The total polyphenols are proportioned thanks to their capacity to reduce the acids phosphotungstic and phosphomolybdic, contents in the reagent of Folin, out of oxides of tungstens and molybdenum. The latter present a bluish coloring, measured with 760 nm.

200µl (0,2 ml) of oil is added to 400 µL (0,4 ml) of a mixture water-methanol (25: 75; v/v). The unit is placed at the vortex during 1 minute in order to extract polyphenols in the medium methanolic. One adds then 200 µl (0,2 ml) of dichlorométhane, to allow the oily phase to become more; the goal is to facilitate the collection of 200µl of supernatant methanolic. 800µl of reagent of Folin diluted 10 times in water is then added and it mixture is left 2 minutes to the room temperature before addition of 1 ml of sodium carbonate (75g/L). This last mixture is heated 15 min with 50°C then analyzed to 760 nm **(Benabid, 2009)**.

The external calibration of the gallic acid uses the reagent of Folin (4mL), a sodium carbonate solution (5mL) and a solution mother, consisted of the methanol-water mixture and gallic acid.

Table.1: hereafter defines the various proportions of the calibration of the gallic acid:

Not range (mg/L)	Reagent of Folin (mL)	Sodium carbonate (mL)	Solution Mother (mix methanol-water and gallic acid) (v :v) en mL
0	4	5	(1 : 0)
50	4	5	(0,1 : 0,9)
100	4	5	(0,2 : 0,8)
250	4	5	(0,5 : 0,5)
500	4	5	(0 : 1)

Content polyphenols

The content of total polyphenols was given starting from the calibration curve (figure 1) of the gallic acid.

The calculation of the content polyphenol in the palm oil starting from OD with 450 nm was carried out in the following way:

Y = 0,0051x + 0,0492

$x = \frac{y-0,0492}{0,0051}$ with X: content polyphenol and y: represent the optical density

III. RESULTS AND DISCUSSION

Physicochemical characteristics of the oils studied by various methods **(AFNOR, 1981; IUPAC, 1979)** gave the following results:

Spectroscopic evaluation of the color

The majority chlorophyllian pigments in oils of green coloring and carotenoids majority in oils of red/yellow coloring absorb in the visible one between 400 and 700 nm **(Silou et al., 2004)**.

The measurement of absorption in this zone informs about the intensity of the color of oil or studied grease. This intensity is, in theory proportional to the quantity of pigments present in oil, when the law of Beer-Lambert is followed.

The palm oils of Gabon have identical spectra (figures 1) and present peaks between 425 and 480 nm, which reveal the presence of carotenoids.

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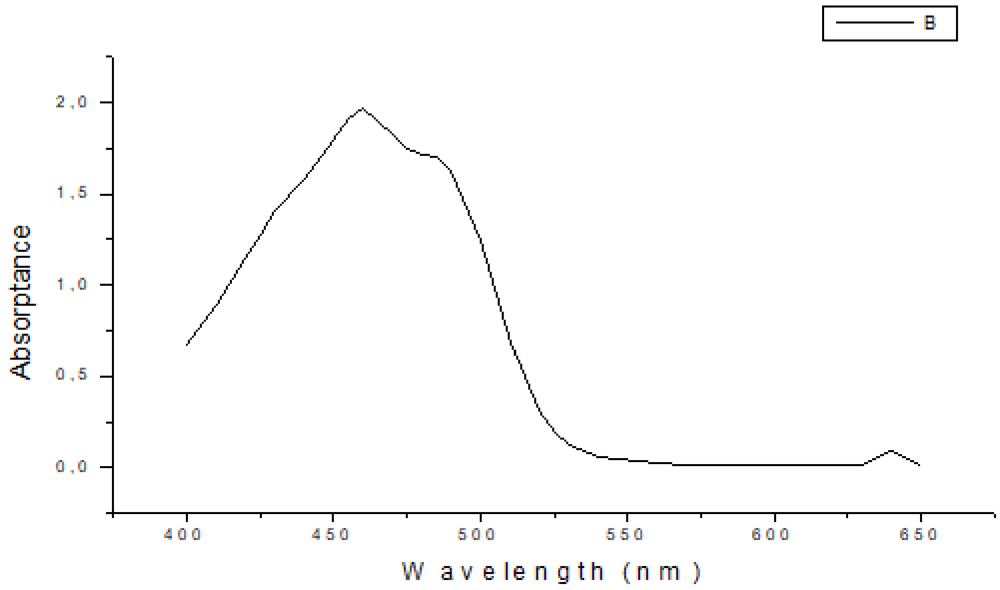

Fig.1: Curve of absorption in the visible of the HPR2 M'vouna (Gabon)

It is noted that the eight palm oil samples of Loukoko have similar spectra(figures 2), showing distinctive peaks which also reveal the presence of carotenoids. They are in conformity with those met in the literature for plant oils **(Helmy, 1990)**. It is also noted that all these eight absorption spectra take the same form. All this intuitively enables us to suggest the same hierarchy in the intensity of the color perceived for the samples of Loukoko taken in 2013 what confirms well the not distinctive observation from point of view of the coloring of oils of this area. However, not being able to conclude with the absence from the chlorophyllian pigments in these oils from Gabon and Loukoko (2013), we put forth the assumption that the pigments are there with the state of traces.

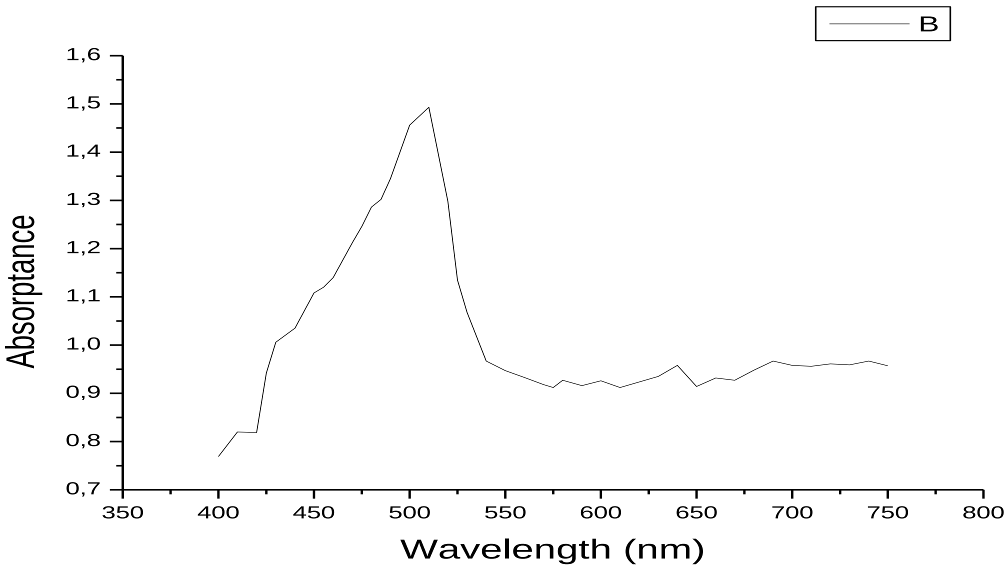

Fig.2: Curve of absorption in the visible ofHP 05/14 Loukoko (Congo)

It is noted that the five palm oil samples of Loukoko have spectra different from those of 2013.By studying the oils extracted from seeds of citrus and cucurbitaceous, **Helmy (1990)** identified the maximum ones of absorption with 400,425,455 and 480 nm for carotenoids, 600 and 670 nm for chlorophyls.The examination of these spectra shows distinctive peaks which reveal the presence of carotenoids between 400 and 480 nm, and the presence of the chlorophyllian pigments with 600 and 670 nm for these five spectra.

Content carotene

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The content carotene was calculated starting from the equation:

% carotenoid = 4,57 OD (R2 = 0,989) **(Davis and Al, 2009)**.

The calculation of the content carotene in the palm oil starting from C with 460 Nm was carried out in the following way:

% carotenoid = 4,57 OD (R2 = 0,989).

Q2 (10 ml) = C2 x10 = C1

Q2 (10 ml) = C2 x10, it is the quantity contained in 1 ml of the solution S.

Q1 (10 ml) = C1 X 10, it is the quantity contained in 1 ml of starting oil.

With:

C1: concentration of carotenes in the starting oil, in µg/mL or ppm

C2: concentration of carotenes in S (solution), in µg/mL or ppm

Q1: quantity of carotenes in µg in the starting oil

Q2: quantity of carotenes in µg in S1

Application: case of the oil of the palm plantation of M'vouna (HPR 1 M'vouna)

C2= 4,57 X OD

C2 = 4,57 X 2,547 = 11,64 µg/mL

Q2 (10 ml) = 11,64 x10 = 116,4 µg, it is the quantity contained in 1 ml of solution S.

Q1 (10 ml) = 116,4 X 10 = 1164 µg it is the quantity contained in 1 ml of starting oil.

Content carotenoids (HPR1 Palm plantation of M'vouna) = 1164 µg/ml

This returns 11,64 X 100 i.e. C2 X 100

The calculation of the content carotene in the palm oil starting from OD with 460 Nm was carried out in the following way:

Y = 0,0775x + 0,0029

X = (y-0,0029) /0,0775 with X: content carotene and there: represent the Optical Density (OD)

The total content carotenoids varies from 897,1 µg/ml (HPR2) with 1229,3 µg/ml (HPR5) for the samples of the Palm plantation of M'vouna (Franceville, Gabon), of 574,9 µg/ml (HP3) with 1330,8 µg/ml (HP6) for the samples of Lambarene (Gabon), whereas for the samples of Oyem it is of 878,4 µg/mL (HP 9) and 1190,9 µg/mL (HP 10) and finally for the samples of Loukoko (2013) that on average varies from 583,1 µg/ml (HPR 2/13/L) with 1110,5 µg/ml (HPR 9/13/L) (table A).

Table.A: Content carotene of the samples of Gabon and Loukoko (Congo)

References	**OD**	**C2 (µg/mL)**	Content carotenoid **ppm (µg/mL)**
HPR 1 M'vouna	2,547	11,640	1164
HPR 2 M'vouna	1,963	8,971	897,1
HPR 4 M'vouna	2,161	9,876	987,6
HPR 5 M'vouna	2,690	12,293	1229,3
HP 3 Lambarene	1,258	5,749	574,9
HP 4 Lambarene	2,416	11,041	1104,1
HP 6 Lambarene	2,912	13,308	1330,8
HP 7 Lambarene	2,424	11,078	1107,8
HP 9 Oyem	1,922	8,784	878,4
HP 10 Oyem	2,606	11,909	1190,9
HPR 1/13/Loukoko	1,586	7,248	724,8
HPR 2/13/ Loukoko	1,276	5,831	583,1
HPR 3/13/ Loukoko	2,140	9,780	978
HPR 4/13/ Loukoko	1,557	7,115	711,5
HPR 5/13/ Loukoko	2,249	10,278	1027,8
HPR 6/13/ Loukoko	1,507	6,887	688,7
HPR 7/13/ Loukoko	1,792	8,189	818,9
HPR 8/13/ Loukoko	1,658	7,577	757,7
HPR 9/13/ Loukoko	2,43	11,105	1110,5

As a whole, these results are similar to those found by Choo (1996) but higher than those of Sundram and Al (2003).

The carotenoids are very important functional compounds in the raw palm oil and their total content varies 574,9µg/ml with 1330,8µg/ml for the samples of Gabon with an average of 1046,49µg/mL and 583,1 µg/mL with 1110,5µg/ml for the samples of Congo, with an average for the whole of 822,33 µg/ml.

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The carotene rate of the palm oil 1,4,6,7 and 8 of Loukoko (respectively 724,8µg/ml; 711,5µg/ml; 688,7µg/ml; 818,9µg/ml and 757,7µg/ml) are similar to that brought back by Sundram (2003) and Tan (1986). The other samples of the two countries have rates of slightly high carotenoids; that could be related to the various agronomic, climatic, genetic factors (Hendson and Chai 1997; Hendson and Mohd 2005).

Partial solidification

An oil is defined as a liquid greasy substance at the natural state, and a grease, a greasy substance in a solid state. A liquid fraction and a solid fraction coexist in the palm oil, it is of this fact known as semi-solid. The relationship between the solid and the liquid is one of the criteria which enters the evaluation of the quality of oil, would be this only in an intuitive way on the level of the local consumer.

The refined palm oil must be completely colorless and limpid. Crude oil rich in vitamin has must be red and contain more liquid than of solid.

We evaluated, at the laboratory, the quantity of the solid deposited, after 4 days, starting from oils of reference which we used during the study. They are oils of references of the palm plantation of M'vouna, of the area of Franceville in the province of Haut-Ogooue (Gabon) and that of the Rural Campus of Loukoko (CRL) in Congo.

We followed the kinetics of formation of the solids starting from the liquid samples after extraction and during storage. The phenomenon observed seems to be random, it there a:

- ✓ of oils in which the solid settles gradually at various speeds (figure 3);
- ✓ of oils which take almost spontaneously masses some (figure 4);
- ✓ of oils which take brutally in mass after a relatively long rest (figure 5);
- ✓ of oils which remain completely liquid over the period of storage considered (figure 6).

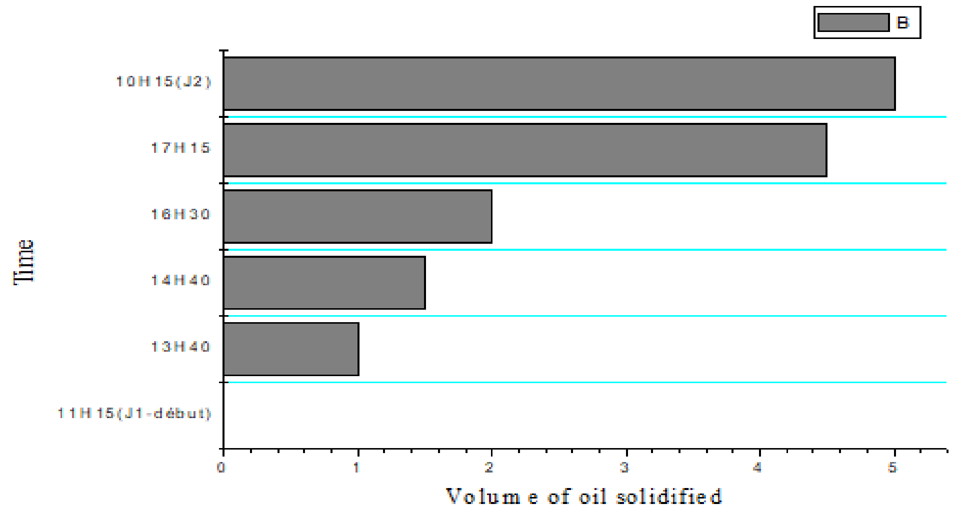

Fig.3: Progressive deposit of the solid in the palm oil (HPR1P. M'vouna)

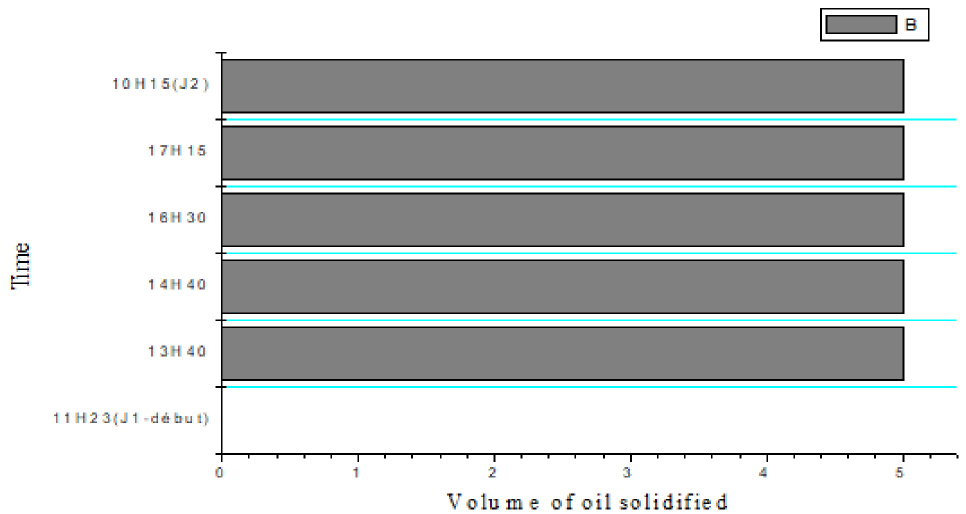

Fig.4: total deposit of the solid in the palm oil after a time HP3 P. M'vouna

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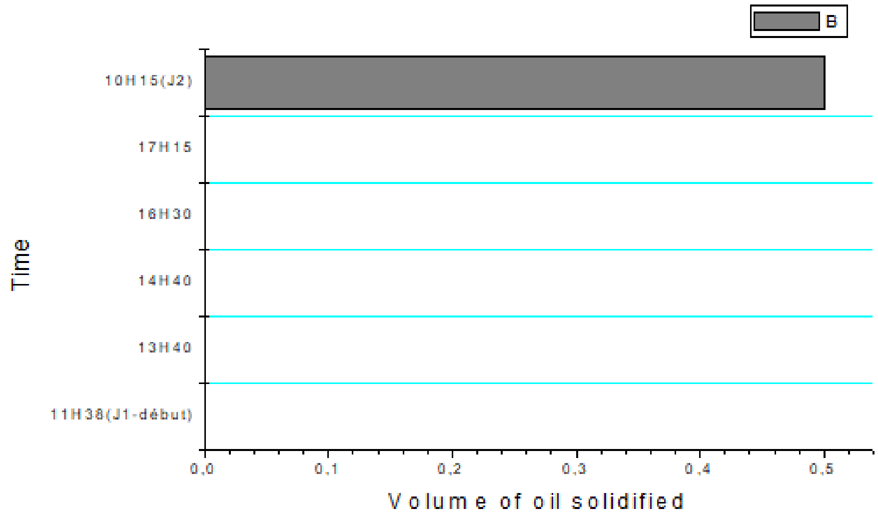

Fig.5: Deposit brutally masses some after a rest relatively long HP4 Lambarene

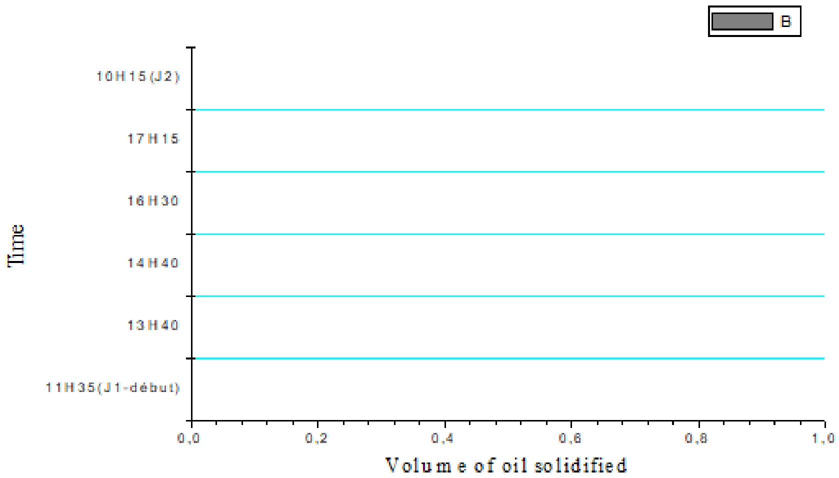

Fig.6: Absence of deposit over the period of storage considered HP 3 Lambarene

A work of qualitative evaluation of the content of solid (one month after extraction) of the various oils extracted the pilot unit of the Rural Campus of Loukoko (CRL), indicates a completely random behavior like illustrates it photograph 2.
We observed all the conceivable behaviors, as indicated higher for oils of Gabon, between completely solid oil with that completely liquid.

Photograph.2: Containers of 10L each one containing of oils resulting from different operations of extraction on the unit controls CRL (Congo).

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During precipitation partial of the raw palm oils of Gabon, three cases of figure ariseed. First case, the raw palm oil can be solidified gradually at the end of a certain time. Secondly, the raw palm oil can be solidified of only one blow in a very short time. And finally, the raw palm oil can not be solidified.

The raw palm oil is semi-solid, i.e. a liquid fraction and a solid fraction coexist. In the first case, the raw palm oil is solidified gradually and the solid fraction is lower than the liquid fraction then we can say than the palm oil presents less impurities than in the second case whose solid fraction is higher than the liquid fraction. In the third case, it on the other hand presents very few impurities and we can say thus that oil enough or much was filtered.

Proportioning of polyphenols

The antioxydant effects of polyphenols in the fruits, the wine, citrus fruits, the plants and certain oils as the olive oil were studied much but little work exist on the level of the raw palm oil. The effects of extracts polyphenolic of various varietal forms and various areas of the raw palm oil on oxidation were evaluated.

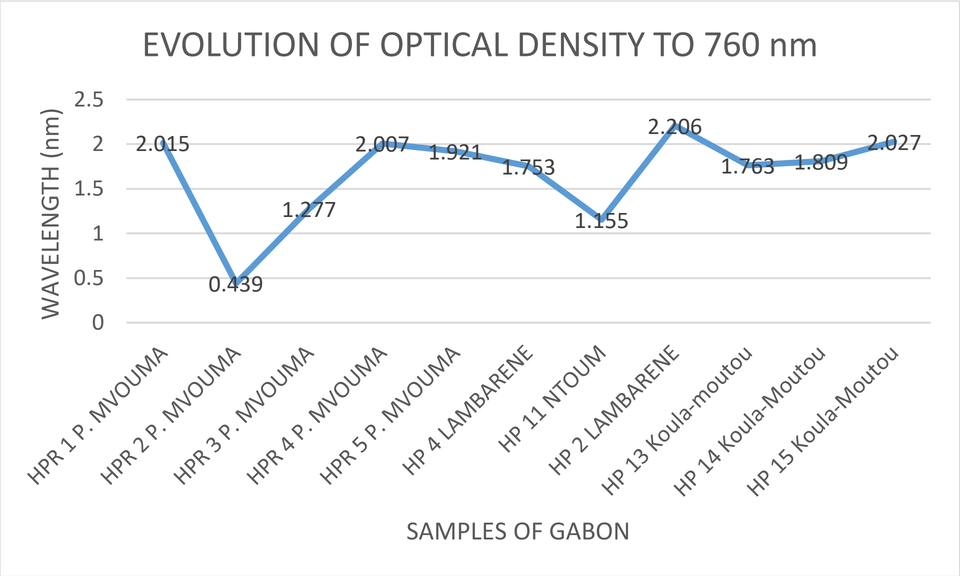

Fig.7: evolution of the optical density with 760 nm of the samples of Gabon

- ***Samples of Loukoko (2013)***

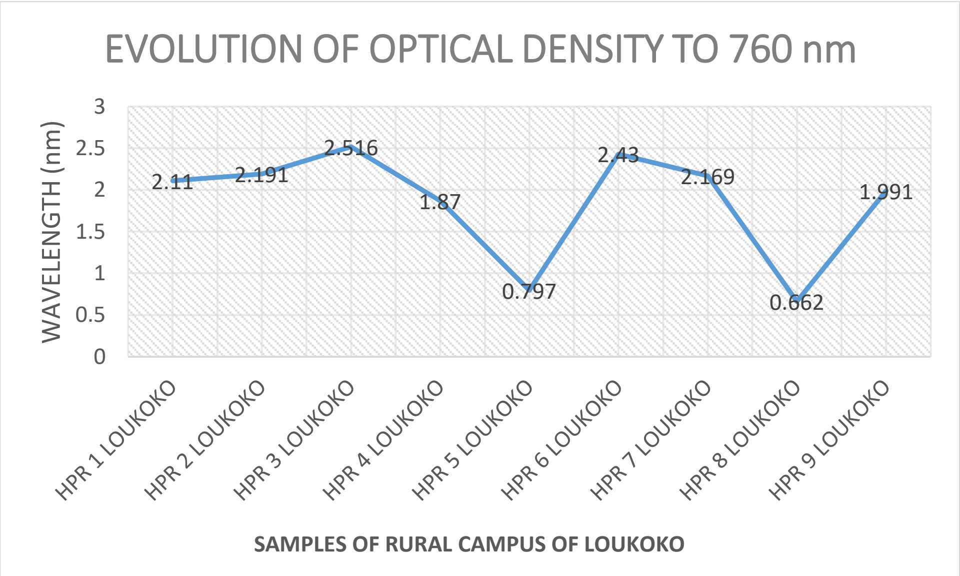

Fig.8: evolution of the optical density with 760 nm of the samples of Congo (Loukoko 2013)

- **Samples of Loukoko (2014)**

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The reading of the OD has was made with the wavelength of 760 nm.

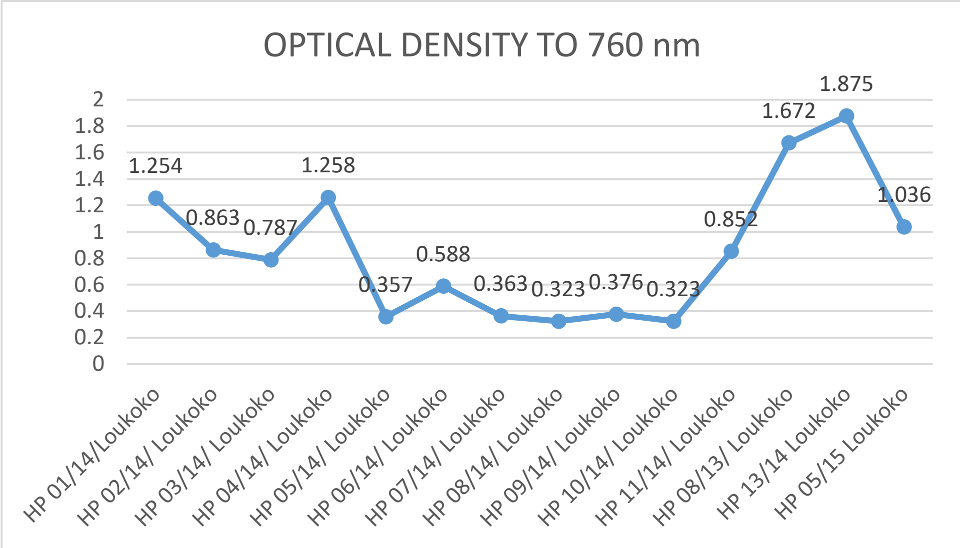

Fig.9: Evolution of the optical density with 760 nm of the samples of Congo (Loukoko 2014)

Content total polyphenols

The content polyphenols was given starting from the calibration curve of the gallic acid (see figure 10).

The calculation of the content polyphenol in the palm oil starting from OD with 450 nm was carried out in the following way:

Y = 0,0012x - 0,0004

$x = \frac{y+0{,}0004}{0{,}0012}$ with X: content polyphenol and there: represent the optical density

The content total polyphenols varies from 439 to 2516 µg/ml (figure 11). Of all the analyzed samples, the sample of Congo (HPR 3 LOUKOKO) contains the maximum of phenolic compounds (2516 µg/ml). Minimum of 439 µg/ml, being obtained on sample HPR 2 P M'vouna (Gabon).

S_1 (solution 1), C_1, Q_1	0.2 mL oils 0.4 mL water/methanol 0.2 mL CH_2Cl_2	Dilution 1/4	Dilution 1/40
S_2 (solution 2), C_2, Q_2	0.2 mL de S1 0.8 mL reactive of Folin (diluted 10 times) 1 mL carbonate of Na (75 g/L)	Dilution 1/10	

C1, C2: concentrations of polyphenols in S1 and S2 in µg/mL or ppm

Q1, Q2: quantities of polyphenols in µg in S1 and S2

Calibration curve

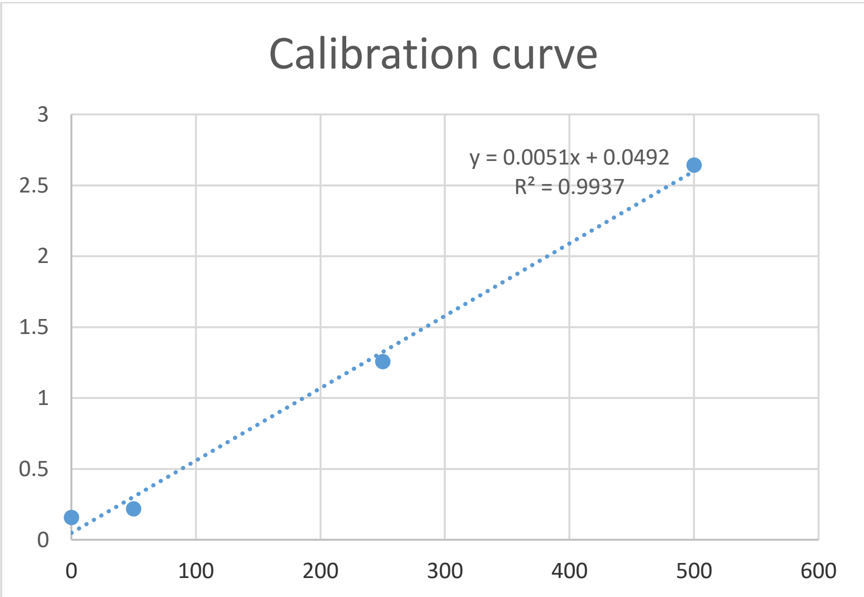

Fig.10: calibration curve of the gallic acid

$Y = 0.0051x + 0{,}0492$ ($R^2 = 0.9937$)

$OD = 0{,}0051C_2 + 0{,}0492$ (ppm)

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HPR 1 M'vouna (OD = 1,378)

$Y = 0.0051x + 0,0492$ ($R^2 = 0.9937$)

$OD = 0,0051C_2 + 0,0492$ (ppm)

$0,0051C_2 = OD - 0,0492$

$C_2 = \frac{OD - 0,0492}{0,0051}$

C2 = 260,55 ppm (µg /mL)

$C_1 = C_2 \times 10 = 2605.5$ µg /mL

$Q_1 = C_1 \times V_{S1}$

$Q_1 = 2605,5 \times 0,8 = 2084,4$

Q_1 ($V_{S1} = 0.8$ ml) = 2084,4 µg

2084,4 µg polyphenol are containedin 0,2 ml of starting oil

that is to say: 2084,4 X 5 in 1 ml

Content polyphenols in oil: 10422 µg/mL Is 10,4 mg/ml

Appreciably equal to 10,4 mg/g

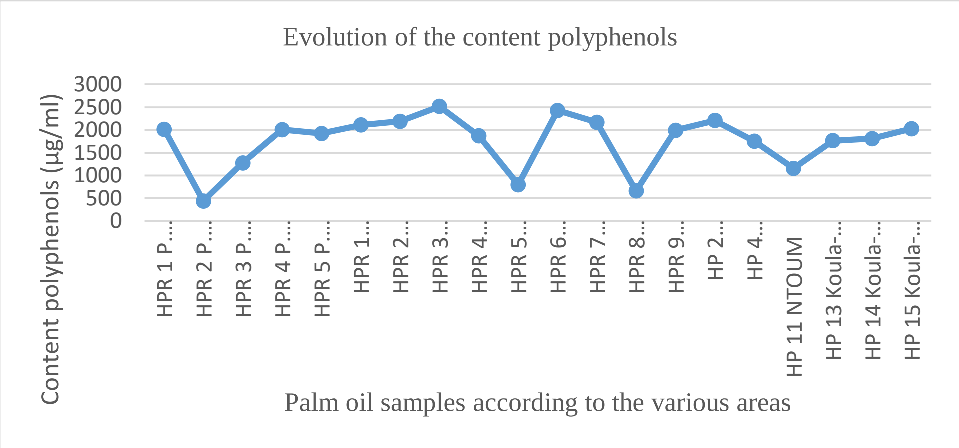

Fig.11: evolution of the content polyphenol of the various samples according to the areas

The contents total polyphenols were determined by the colorimetric method of Folin-Ciocalteu in the rough extracts ethanolic. The range of calibration was carried out starting from the gallic acid used like standard. The optical density was measured using a spectrophotometer of the type GENESYS 10 Bio (Photo 1).

The content total polyphenols varies from 3,1 to 14,6 mg/g (figure 9) with a significant growth recorded for the various samples of Loukoko HPR 3 L and HPR 6 L (respectively 2516 mg/g and 2430 mg/g). These results approach rather those found for the olive oil (Aguileraand*al.* 2005; Servile and *al.* 2003). The values found for our oils are as a whole higher than those reported by Aguilera and Al 2005.

The results presented to figure 11, show that the total polyphenol contents of the analyzed palm oils strongly vary campaign with another.

Thus marketing year 2013/2014, was characterized by the highest contents total polyphenols (superior with 10422 ppm). These contents similar to those are reported in work of Vossen (2007). The variations of the contents polyphenols observed can be due to the difference in degree of maturity of nuts before trituration (early harvest of nuts) but also depend on the varietal profile and the geographical area. It is the same for the fatty-acids and carotenoids.

Their high content in carotenoids, fatty-acid unsaturates and the strong content total polyphenols, make our different oils more beneficial as well in the nutritional plan as therapeutic. Indeed several work showed the undeniable role of polyphenols of the wine and the fruits, powerful antioxydant in the prevention of human pathologies, in particular of the cancerous tumors, the cardiovascular diseases, the inhibition of the oxidation of the LDL (Lan and Al 2007; Proteggente and Al 2002). These phenolic compounds correspond to a very broad range of chemical structures and are a good witness of the extraordinary capacity of biosynthesis of the plants, allowing their use in fields as varied as the agroalimentary one or pharmacology.

We noted a low variability of the various parameters: total fatty-acids, carotenoids and polyphenols in the raw palm oil consumed in Gabon and Congo. The various palm oil samples from Gabon, prove to be rich in saturated fatty-acids, carotenoids and total polyphenols.

IV. CONCLUSION

The raw palm oils produced in Gabon and Congo have considerable contents of carotene and natural polyphenols. Thus marketing year 2013/2014, was characterized by the highest contents total polyphenols (superior with 10422 ppm). The content polyphenols has a significant growth recorded for some samples of Loukoko (Congo) going up to 2516 mg/g. The carotenoids are very important functional compounds in the raw palm oil and their total content is of

1046,49µg/mL on average for the samples of Gabon and an average of 822,33 µg/ml for the samples of Congo. This strong rate of content carotenoids testifies to the perfect color orange red of the raw palm oil which indicates the presence of vitamin A. According to the conditions, the greasy substances are liquefied or solidified in varied and variable crystalline forms: it is what is called polymorphism. The whole of these data shows, contrary to many other vegetable fats, that the palm oil has many advantages: a strong consistency, a neutral taste, a relative thermal stability and a tartinability.

REFERENCES

[1] **AFNOR (Association Française pour la Normalisation), (1981)**.Corps gras, graines oléagineuses, produits dérivés).Recueil des Normes Françaises, AFNOR, Paris (France).2e Ed. p. 438.

[2] **Anguilera J., Rodriguez-Vargas S., Prieto J.A. (2005)**. The HOG MAP Kinase pathway is required for introduction of methylglyoxal-responsible genes and determines methylglyoxal resistance in saccharomyces cerevisial. *Mol microbial*56 (1). p. 228-239.

[3] **AOAC (Association of Official Analytical Chemists), (1999)**. Official Methods of Analysis. 16th Ed. Ed. P. Cunniff, AOAC International, Maryland, MD (USA). p. 956.

[4] **Benabid, H. (2009)**. Caractérisation de l'huile d'Olive Algérienne, Apports des méthodes chimiométriques. Thèse de Doctorat.Université Mentouri de Constantine (Algérie). p. 51-52.

[5] **Codex Alimentarius (2005),** Alinorm 01/17: Norme pour les huiles végétales portant un nom spécifique, Codex-Stan 210. p.14

[6] **Codex Alimentarius (1995).** Rapport de la quatorzième session du comité du codex sur les graisses et les huiles. *Alinorm* 95/17.21ème Session. Londres, Royaume Uni. p. 9-16

[7] **Davis AR., Fish W.M., Perkings Veazie P.** (**2008/2009**). A rapid spectrophotometric method to determine beta-carotene content in *Cucumis melo* germplasm. *Cucurbit genetic cooperative Report* 31/32. p. 5-7

[8] **Decker, E. A. (1995).** The role of phenolics, conjugated linoleic acid, carnosine, and pyrroloquinoline quinone as non-essential dietary antioxidants. *Nutr Rev*. 53. p. 49-58.

[9] **FAO/OMS (1977).** Rôle des graisses et huiles alimentaires en nutrition humaine. Rapport d'une commission mixte d'experts. Rome (Italie). p. 105

[10] **Hartmut, K. et Lichtnetharler, (1987).** Chlorophylls and Carotenoids: Pigments of photosynthetic. *Methods in Enzymology*.148, pp. 350-382

[11] **Helmy, E. H. (1990).** Studies on the pigments of some citrus, prune and cucurbit seed oils when processed with or without cotton seed oil.*J. Amer. Oils Chem. Soc.*. 67(6). p .376-380.

[12] **Hendson I.E., Mohd H.H. (2005).** The influence of climatic conditions on gas and energy exchanges above a young oil palm stand in north Kedah, Malaysia. *Journal of Oil Palm Research*, 17: 73-91.

[13] **Hendson IE., Chai SH. (1997).** Analysis of oil palm productivity. II. Biomass, distribution, productivity and turnover of the root system. *Elaeis*, 9:78-92.

[14] **IUPAC** (**International Union of Pure and Applied Chemistry).** (**1979**). Méthodes d'analyses des matières grasses et dérivés. 6ème Ed. Lavoisier Tec et Doc. Paris (France) p .190.

[15] **Lan S., Jun-Jie Y., Denys C., Kequan Z., Jerey M., Liangli (Lucy) Y. (2007).** Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry*, 100. 990–997.

[16] **Lecerf, J. M. (2013).** L'huile de palme: aspects nutritionnels et métaboliques. Rôle sur le risque cardiovasculaire.*OCL*. 20 (3). p. 147 – 159.

[17] **Proteggente A. R., Pannala A. S., Paganga G., Van Buren L., Wagner E., Wiseman S., Van De Put F., Dacombe C., Rice-Evans C.A. (2002**). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research*. 36 (2): 217-33.

[18] **Serville M. N., Demanez L., Demanez J. P. (2003)**. Acta Oto-rhino-laryngologica.*Belgica*. 58 (1). p.53-59.

[19] **Silou, Th. Biyoko, S., Heron, S., Tchapla, A., Maloumbi, M.G. (2004).**Caractéristiques physico-chimiques et potentialités technologiques des amandes d'Irvingia *gabonensis*, *Rivist. Ital. della sostanze grasse*, 81. p.49- 57.

[20] **Sundram K., Sambanthamurthi R., Tan YA. (2003)**. Palm fruit chemistry and nutrition. *Asia Pacific Journal of Clinical Nutrition*, 12 (3): 355-62.

[21] **Tan, B. (1988).** Oil Palm / Palm oil Conf : Tech. Progress and Prospect, A.S.H. Edition Ong, PORIM Press, Kuala Lumpur, Malaysia, p. 370.

[22] **Visioli, F. Bellomo, G. Montedoro, G. et Galli, C. (1995).**Low density lipoprotein oxidation is

inhibited in vitro by olive oil constituents. *Atherosclerosis*. 117. p. 25-32.

[23] **Vossen, P.-M.(2007)**. International olive oil council trade standard for olive oil. Organic Olive Production Manual. ANR Publications.105). p 23-24.

Required flows for aquatic ecosystems in Ma River, Vietnam

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Abstract— *Ecological flow requirements for the Ma River in dry season were assessed in three reaches of Ma – Buoi, Ma – Len and Ma – Chu. 5 indictor fish species was chosen based on biodiversity survey and roles of those species in aquatic ecosystem as well as local communities. Biological and hydrological data (dry season of 2016-2017) and 35 year recorded hydrological data were collected and analyzed as input data for a physical habitat model River HYdraulic and HABitat SImulation Model – RHYHABSIM. Model results shown that the optimal flows of the reaches were very much higher compare with the minimum annual low flow - MALF. In this study, $MALF_{7day}$ were applied to calculate the recommended minimum flows of the three reaches. The recommended required minimum flows for Ma – Buoi, Ma – Len and Ma – Chu reaches were 51 m^3/s, 49 m^3/s and 61 m^3/s, respectively. It must be stressed that this study only assessed whether or not there is enough habitat available for the river to sustain a healthy ecosystem.*

Keywords— Ma River, Minimum Annual Low Flow – MALF, Required flows, River HYdraulic and HABitat SImulation Model – RHYHABSIM, Weighted Useable Area –WUA.

I. INTRODUCTION

Flow management, in its basic sense, is the allocation of the resources, water, for specific uses and purposes. The different uses for an individual flow could include domestic used water, irrigation, fisheries, recreation, carrier of treated waste-water, and the maintenance of the natural/native biodiversity etc. At any point in time, the water quantity in a flow is affected by natural factors such as precipitation and geology, as well anthropogenic influences including the physical alteration of the stream, river, dams\weirs, and surface and groundwater abstraction [1].

Water abstraction plays an important part in most surface water systems, especially, the water that is present in a flow even during extended dry periods. Over exploitation of flow's water resources can significantly reduce a stream's base flow to the point where once permanent streams become ephemeral. This change can have severe consequences for the native flora and fauna of the flow (i.e. [2], [3], [4]).

In order to manage the freshwater resources, both an inventory of the water resource available and an assessment of the ecology of the natural (unaltered) freshwater ecosystem need to be undertaken. Habitat models such as habitat hydraulic models are one of the tools available to evaluate how changing flow regimes will affect the physical habitat for the biological communities [5]. These models combine the hydrological and biological variables in a system, simulating how available habitat for a particular species will change with differing hydrological responses to resource utilization [6], [7]. RHYHABSIM (short for River HYdraulic and HABitat SImulation Model) was developed by Ian Jewett in the 1980s and is continuingly being improved, intended for use by water managers [5], [8]. RHYHABSIM is able to model habitat responses to changing hydrological conditions, and has been identified as a management tool for assessing current ecosystem condition.

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Hydraulic-habitat models marry water depth and velocity predictions made by a hydraulic model with fish frequency-or density-based habitat suitability criteria (or curves) (HSC) for these hydraulic, and other physical, habitat variables (e.g., substrate) to predict weighted useable area (WUA; more correctly termed the area weighted suitability) [9], [10].

Habitat models, such as RHYHABSIM, attempt to quantify the flow required to maintain a healthy ecosystem, thus providing stream/river managers with important information from which to base their water management decisions (such as water abstraction) upon.

This paper looks at the application of RHYHABSIM as a tool to aid the management of freshwater ecosystems. Applying on a case study of Ma River, Vietnam, the model is used to predict the flows needed to provide the necessary habitat to sustain naturally recruiting populations of local fish species in dry season. The application of the model is evaluated with regard to its usefulness from a resource manager's perspective.

II. STUDY SITE AND METHODOLOGY

2.1 Study site

The Ma River is a river in Asia, originating in northwest of Vietnam. It runs for 400 km through Vietnam, Laos, and then back through Vietnam, meeting the sea at the Gulf of Tonkin.

The largest tributaries of the Ma River are the Chu River (or the Nam Sam River as it is called in Laos), the Buoi River, and the Cau Chay River. All of them join the Ma River in Thanh Hoa Province in North Central Vietnam. The Ma River creates the Ma River Delta (also called the Thanh Hoa Delta), the third largest in Vietnam.

Like the Red River (Song Hong) to the north, it has an irregular regime with maximum flow toward the end of the summer.

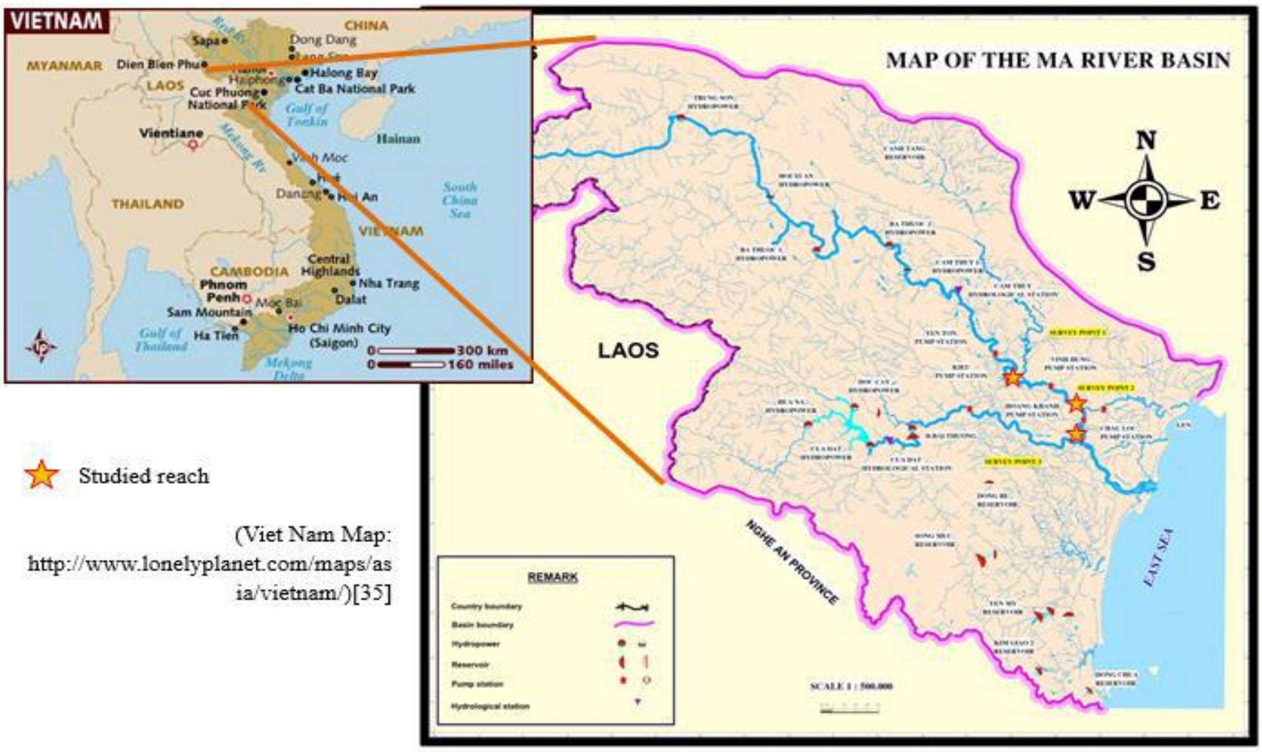

Fig. 1: Map of Ma River Basin

The Ma River delta differs, however, from that of the Red River because of its narrowness and the presence of sandy soil.

The average temperature in the Ma River basin is relatively high throughout the year. The average temperature recorded at the 14 meteorological stations within the Ma River basin varies spatially ranging from 20.9-23.0^{0}C, reflecting the topographical characteristics and altitudes of the locations. Annual rainfall is substantial with dominant winds from south and southeast during May to September months.

The river flow varies greatly in time and space. The river flow in cubic meters per second (m^3/s) varies quite greatly in Cam Thuy. The average discharge in April (111 m^3/s) is only one-third of the annual discharge (334 m^3/s) and one-seventh of the highest average discharge (in August). Data show that the highest discharges monitored at Cua Dai, Xuan Khanh and Cam Thuy are 442 m^3/s and 1,713 m^3/s, respectively, and 258 times higher than the lowest discharges at the same gauging station.

In the dry season, the runoff is only 4.76 billion m^3, making up 26% of the total annual runoff. The driest period is between February and April, which comprises

8% of the annual flow. March tends to have the lowest flow rates, contributing only 2.4% of the total [11]. Together with the demand for difference water uses, the requirement water for aquatic ecosystem in Ma River becomes an issue especially in dry season.

There are three reaches with 17 cross sections (4 to 8 cross sections per reach) were set up and investigated in the main flow of Ma River during dry season of 2016-2017. All the reaches are located in upstream of the distributaries of Ma River, the first one is Buoi River, second one is Len River and the last one is Chu River (Figure 1).

2.2 RHYHABSIM

RHYHABSIM uses a combination of a hydraulic simulation model to predict flow conditions, and biological models to quantify how the change in flow impacts available habitat for a number of fish species. Fish habitat predictions are quantified using an index called Weighted Usable Area (WUA), which incorporates the relative quantity and quality of available habitat at a given flow [12], [13]. WUA is expressed as an area of suitable habitat per length of river (m^2/m).

The most common use of RHYHABSIM modeling is to provide guidance when setting minimum flow limits for Ma River. This process uses the model results to help inform a minimum flow which balances in stream and out-of-stream uses. This is accomplished primarily by two steps:

- Identifying the point at which habitat loss decreases disproportionally to reduction in flow, known as the inflection point on the habitat × flow response (WUA) curve;
- Determining a flow-related baseline and assessing habitat relative to that baseline, usually the naturalized mean annual low flow (MALF);

The first step is often used where seasonal flow fluctuations (most notably low flows – dry season) are not the limiting factor in physical habitat for fish species. This is identified where the optimum flow for a given species is less than the mean annual low flow. Using the flow × WUA curve, the minimum flow is often chosen as the inflection point; where the relationship between flow and habitat is 1:1. At flows below this inflection point, a reduction in flow results in a proportionally greater reduction in habitat, thus increasing risk of habitat loss for the management species [14].

2.3 Stream Survey Methodology

The objective of the stream survey was to obtain the measurements needed to model the stream parameters that influence fish habitat: stream depth, velocity, discharge and substrate. The three reaches were surveyed according to standard RHYHABSIM protocol and methodology (provided in [15][16]). For this study, each survey site contained 4 to 8 cross-sections, with an even distribution of cross-sections between riffles, runs and pools. The survey took place in two parts – the initial (Feb, 2016), more intense survey, and follow-up visits. The initial visit was used by the model to establish the basic hydraulic parameters for the stream [16]. The follow-up visits (Feb, 2017), conducted at different stream discharge rates, were used to calibrate the model, which was then used to predict how the stream's physical attributes (velocity, width, depth and substrata) change with stream discharge.

At the initial survey for each of the 17 cross-sections, the following parameters were measured:

- Stream profile from the top of the stream bank
- the stream profile defined the confines of the stream.
- Flow velocity and discharge rate – velocity is particularly important, as it will vary across the cross-section, influencing the model results.
- The stream stage (water level) at one fixed point in the stream for each cross-section. The stream stage was measured at this point in the follow-up visits.
- The substrata across the profile of the streams. The substrate index is vegetation, mud/silt, sand, gravel, coarse gravel, cobbles, boulders and bedrock, classified as 1-8 respectively [4].

2.4 Indicator fish species

In this study, 5 following fish species were used to estimate required water flow for Ma River. 1) Common carp - *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes: Cyprinidae); 2) Common armorhead catfish - *Cranoglanis henrici* (Vaillant, 1893) (Siluriformes: Cranoglanididae); 3) Greenback mullet - *Chelon subviridis* (Valenciennes, 1836) (Perciformes: Mugilidae); 4) Dusky sleeper - *Eleotris fusca* (Forster, 1801) (Perciformes: Eleotridae); and 5) Tank goby - *Glossogobius giuris* (Hamilton, 1822) (Perciformes: Gobiidae).

These 5 species were chosen because of following reasons: different possibility catching along of research areas in Ma River; inhabit in different water column: benthopelagic with common carp, common armorhead catfish and tank goby, demersal with greenback mullet and dusky sleeper; adapt with different optimum current speed: 0.3-0.4 m/s with common carp and tank goby, 0.4-0.6 m/s with common armorhead catfish and greenback mullet, 0.2-0.3 m/s with dusky sleeper; and many different following detail characteristics.

The first species, common carp - *Cyprinus carpio*, is a very common in freshwater and brackish environment throughout the world with the body size range in 25 - 36 cm as adult. This fish has highly commercial value in fisheries, aquaculture and also in aquarium. Common carp inhabit warm, deep, slow-flowing and still waters such as lowland rivers and large, well vegetated lakes and they can adapt with wide variety of conditions but generally favor large water bodies with slow flowing or standing water and soft bottom sediments [17]. Both adults and juveniles feed on a variety of benthic organisms and plant material. They spawn along shores or in backwaters and larvae survive only in very warm water among shallow submerged vegetation. Under tropical conditions, common carp breeds throughout the year but seasonal spawned in temperate waters [18].

The next chosen species is common armorhead catfish - *Cranoglanis henrici*. This species distribute in Thailand, Philippines, Indonesia, China (Hainan island, Guangdong, Guangxi, Yunnan) and Vietnam [19]. They live at bottom and near bottom, preferring moderately and slowly running waters with much sandy and muddy bottom. They usually live in colonies and are found mainly in the downstream of rivers in Northern provinces. *C. catfish* in general and *C. henrici* in particular are famous for their tasty and nutritious meat. *C. henrici* is found in all river systems from the North to the South of central Vietnam, but not found in the South [20], [21] with the spawning season from May to July [22].

Greenback mullet, *Chelon subviridis*, form schools in shallow coastal waters and enters lagoons, estuaries, and fresh water to feed. Juveniles may enter rice fields and mangroves. Greenback mullet feed on small algae, diatoms and benthic detrital material taken in with sand and mud; fry take zooplankton, diatoms, detrital material and inorganic sediment [23]. Spawning occurs at sea with pelagic and non-adhesive eggs [24].

The fourth species, Dusky sleeper - *Eleotris fusca*, is found in rivers, estuaries and coastal regions throughout the Indo-west Pacific, from the eastern coast of Africa to the Hawaiian Islands where this species spawns during May to December with most proportion from August to November [25], [26]. They occur in the lower reaches of freshwater streams, usually on mud bottoms and feed on crustaceans and small fishes [27]. Dusky sleeper spawns eggs on submerged plants with small leaves, female tends and fans the eggs until hatching and loosely guards the fry for a few days thereafter [28]. Juveniles are found mainly among mangrove roots in the more saline areas of lagoons and estuaries [28].

The last chosen species, Tank goby - *Glossogobius giuris*, is found mainly in freshwater and estuaries, but also enter the sea; this fish species also occur in canals, ditches and ponds [29]. The species has a marine larval stage, but can breed in fresh water. It has been recorded breeding during the 'dry' season in northern Australia and in summer in South Africa [30] and from March to September in Manchar Lake, Pakistan [31].

III. RESULTS

3.1 Biological Data – the Habitat Suitability Curves

The profiles for the thee stream reaches are shown in Table 1 and Figure 2. The upper reach consisted of fast habitats (depth > 0.80 m and velocity ≥ 0.82 m/s). The lower reach had deep-slow mesohabitat in its part (depth ~ 2.51 m and velocity ~ 0.15 m/s) with area of 475.84 m^2 and average width of 189.28 m. The lowest reach and the shallow-fast (depth ~ 2.81 m and velocity ~ 0.16 m/s) with area of 640.52 m^2 and average width of 228.12 m.

The Habitat Suitability Curves (HSC) (Figure 3) showed that a depth of 5.0 m and velocity of around 0.4-0.5 m/s are optimum (these are Food producing criteria, Waters 1976). The curves for substrate type indicated that all the five selected species was associated with a wide variety of substrate classes, such as mud/silt, gravel, coarse gravel and sand. The curves for *C. henrici, C. subviridis, E. fusca, G. giuris* indicated preference for large boulders and boulders, whereas *C. carpio* for sand and mud/silt only.

3.2 Model results

Ma – Buoi Reach

The habitat surveys of this reach were carried out at a flow of 80.62 m^3/s, at the survey flow of 76.80-84.97 m^3/s. The average width of this reach was 131.81 m, depth 0.89 m, and velocity 0.90 m/s. Substrate assessments at all sites were similar, with >95% sand and the remaining substrate a mixture of gravel and mud.

Maximum habitat for *C. henrici, G. giuris* and *C. carpio* was provided by a flow of 90 m^3/s, and the amount of suitable habitat began to fall when flows fall below 20 m^3/s. Maximum *C. subviridis* and *E. fusca* habitat was provided by a flow of 80 m^3/s, with a reduction beginning when flows fell below 20 m^3/s (Figure 4.1, Table 2).

Ma – Len Reach

The habitat surveys of this reach were carried out at average flow of 74.59 m^3/s, at the survey flow of 70.48-79.92 m^3/s. The average width of the river was 189.28 m, depth 2.51 m, and velocity 0.15 m/s. Substrate assessments at all sites were similar, with 73.6% sand and the remaining substrate a mixture of gravel and mud of 6.9 and 19.5, respectively.

According to Figure 4.2, Table 2, optimal flow for all the indicator species are very high compare with the previous reach. *C. henrici, G. giuris* and *C. carpio* was provided maximum habitat by a flow of more than 80 m^3/s, and the amount of suitable habitat began to fall when flows fall below 30 m^3/s. Maximum *C. subviridis* and *E. fusca* habitat was provided by a flow of 100 m^3/s, with a reduction beginning when flows fell below 30 and m^3/s.

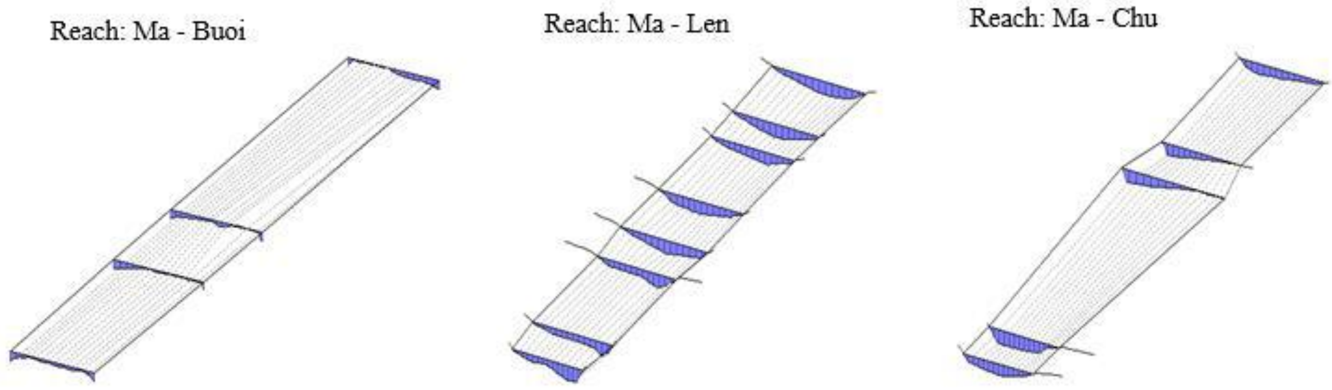

Fig. 2: Isometric view of the cross-sections in the three target reaches of Ma River. Blue color indicates water; solid line indicates the contour of the cross-section.

Table.1: Reach Hydraulic Geometry

Section	Flow (m^3/s)	Width (m)	Depth (m)	Velocity (m/s)	Area (m^2)
Ma-Buoi		**Reach length: 1,303.27 m**			
Section1	84.97	130.30	0.81	0.94	105.94
Section2	83.55	140.19	0.82	0.82	115.38
Section3	76.80	111.61	0.88	0.92	98.19
Section4	77.16	142.21	0.98	0.89	138.59
Reach	***80.62***	***131.81***	***0.89***	***0.90***	***117.64***
Ma-Len		**Reach length: 1,194.44 m**			
Section1	79.92	159.87	2.50	0.19	398.81
Section2	78.48	181.85	2.06	0.19	374.53
Section3	76.52	178.05	2.35	0.17	417.95
Section4	75.56	198.29	2.73	0.13	541.49
Section5	71.72	192.26	2.68	0.13	515.79
Section6	72.25	189.37	2.44	0.15	462.84
Section7	71.82	195.34	2.63	0.13	514.23
Section8	70.48	211.70	2.74	0.11	579.47
Reach	***74.59***	***189.28***	***2.51***	***0.15***	***475.84***
Ma-Chu		**Reach length: 1,587.82 m**			
Section1	105.84	190.47	3.34	0.16	635.61
Section2	101.61	190.25	3.96	0.13	754.10
Section3	107.97	281.72	2.32	0.15	654.26
Section4	116.51	214.63	2.13	0.22	456.71
Section5	116.59	231.44	2.71	0.18	627.63
Reach	***109.70***	***228.12***	***2.81***	***0.16***	***640.52***

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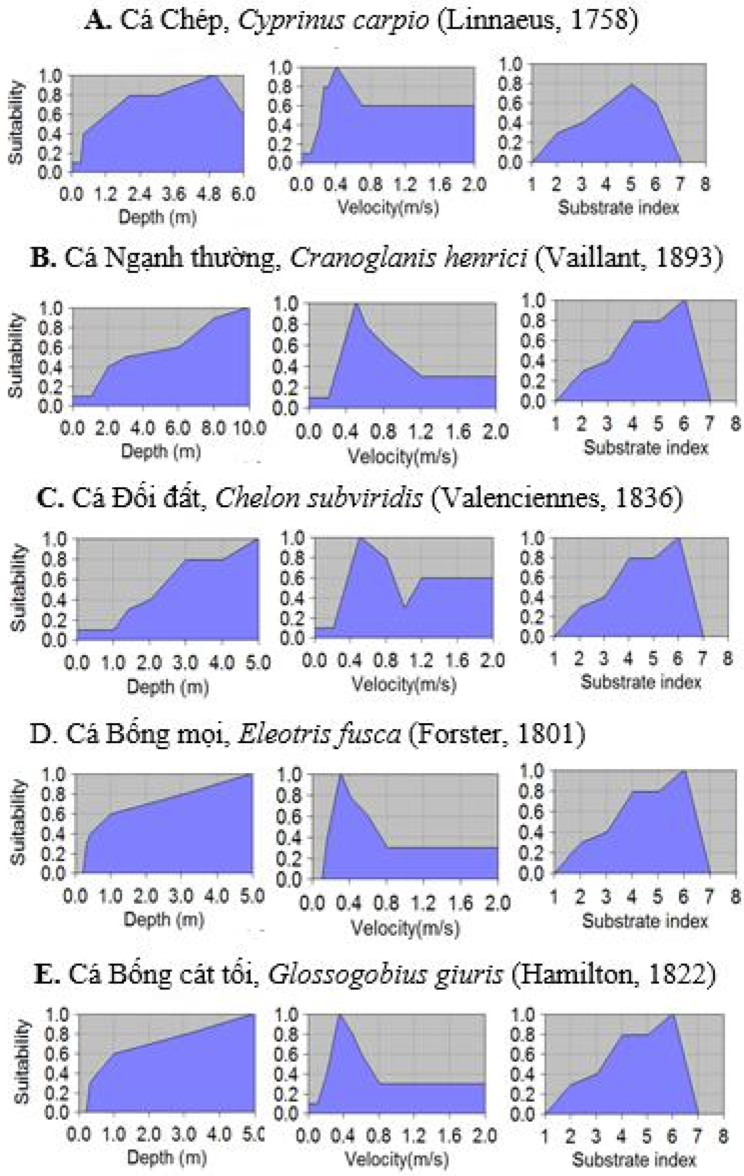

Fig. 3: The biological input to the model in the form of Habitat Simulation Curves.

Ma – Chu Reach

The habitat surveys of Ma-Chu reach were carried out at an average flow of 109.70 m^3/s, at the survey flow of 101.61-116.59 m^3/s. The average width of the river was 228.12 m, depth 2.81 m, and velocity 0.6 m/s. Substrate assessments at all sites were similar, with 79.4% sand and the remaining substrate a mixture of gravel and mud of 3.9 and 16.7, respectively.

Maximum habitat for *C. henrici*, *G. giuris* and *C. carpio* was provided by a flow more than 100 m^3/s, and for *C. subviridis* and *E. fusca*, it was > 130m^3/s. The amount of suitable habitat began to fall when flows fall below 50 m^3/s for all *C. henrici*, *C. subviridis*, *E. fusca* , *G. giuris* and *C. carpio* (Figure 4.3, Table 2).

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Table.2: Flow requirement for fish species at each reach in Ma River

Reach/ Target fish species	MALF (m^3/s)	MALF $_{7day}$ (m^3/s)	Optimum flow (m^3/s)	Declined Flow (m^3/s)
Ma - Buoi	**50.59**	**56.00**		
Cyprinus carpio			80-110	<20
Cranoglanis henrici			70-100	<20
Chelon subviridis			70-100	<20
Eleotris fusca			80-110	<20
Glossogobius giuris			80-110	<20
Ma - Len	**55.48**	**60.89**		
Cyprinus carpio			>80	<30
Cranoglanis henrici			>80	<30
Chelon subviridis			>80	<30
Eleotris fusca			>80	<30
Glossogobius giuris			>80	<30
Ma - Chu	**63.37**	**70.34**		
Cyprinus carpio			>100	<50
Cranoglanis henrici			>100	<50
Chelon subviridis			>100	<50
Eleotris fusca			>100	<50
Glossogobius giuris			>100	<50

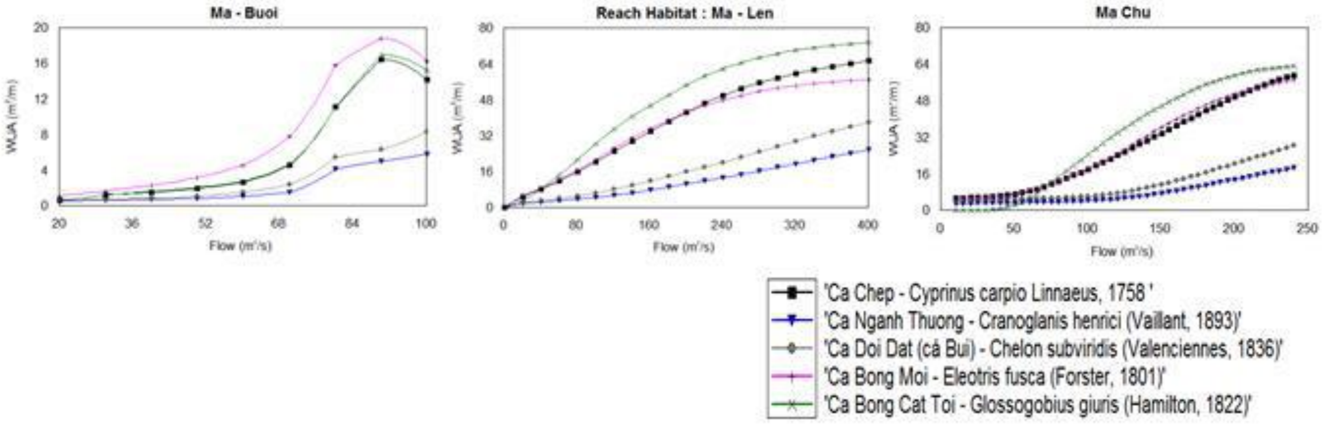

Fig. 4: Fishes' habitats in the three reaches of Ma River

Table.3: Recommended minimum flows

Reach	MALF $_{7day}$ (m^3/s)	WUA (m^2/m)@ MALF $_{7\ day}$	80% of WUA (m^2/m)@MALF $_{7\ day}$	Corresponding Minimum Flow (m^3/s) (approximate)
Ma - Buoi	56.00	2.80	2.24	**51.00**
Ma - Len	60.89	45.70	36.56	**49.00**
Ma - Chu	70.34	38.80	31.04	**61.00**

IV. DISCUSSIONS

The method of using mean annual low flow (MALF) as an indicator to determine appropriate minimum flows based on RHYHABSIM model outputs have been applied for a number of studies [32]. It states that where maximum habitat is greater than the mean annual low flow (MALF), it is acceptable to set the recommended minimum flow at 80% of the habitat available at the MALF. This situation often exists in the reach Ma – Buoi in Ma River, where annual summer low flows cannot provide optimum conditions; therefore setting minimum flows at the habitat optimum is unrealistic. The approach described in the Sustainable Low Flow Project recognizes this and uses both habitat data and historical flow data to arrive at minimum flow recommendations that are realistic, conservative, and attainable. It is important to consider natural flow conditions without the influence of abstraction when setting minimum flows.

In several cases, 7-day MALF value was applied instead of 1-day MALF. An analysis of the relationships between the 7-day MALF and the 1-day MALF shows that the ratio ranges from 1.0 to more than 1.7. More than 80% of catchments have a ratio of less than 1.2, and the median ratio is 1.08 [33]. However, low flows is a set the limit to habitat quantity, providing that the duration of low flows is sufficient to engender a biological response [33]. Therefore, in this study, the value of 7-day MALF was used.

The suggested minimum flow rules given in the proposed National Environmental Standard (New Zealand) on ecological flows [34] are:

- For rivers and streams with mean flows less than or equal to 5 m^3/s, a minimum flow of 90% of the mean annual low flow (MALF).
- For rivers and streams with mean flows greater than 5 m^3/s, a minimum flow of 80% of MALF.

HSI graphs indicate that the optimum quality of fish habitat occurs at lower flows than optimum habitat quantity (WUA). It is recommended that WUA be the primary consideration when addressing minimum flows. WUA combines habitat quality with area (quantity), and is considered to be more conservative. From a fisheries management perspective, a greater supply of suitable habitat is more important for fish productivity than a small supply of high quality habitat [32], thus WUA was used to arrive at a minimum flow figure.

Taking into account of evaluating 5 species, and it was done through 4 to 7 section analysis is an essential factor when recommending a minimum flow and allocation limit. It requires that ecological “bottom lines” are maintained (Ngaruroro, 2008).

Based on the results of RHYHABSIM and the MALF value which were calculated based on analyzed data of 35 year (1980-2015), recommended minimum flows of the three reaches were proposed (Table 3). The recommended minimum flows for reaches Ma – Buoi, Ma – Len and Ma – Chu are 51 m^3/s, 49 m^3/s and 61 m^3/s, respectively.

It must be stressed that this study only assessed whether or not there is enough habitat available for the river to sustain a healthy ecosystem. Even if the streams are achieving the needed flows for suitable habitat, they still could be underperforming according to the environmental goals set (i.e. not achieving a ‘good ecological condition’). Other factors could be influencing the biota, including pollution, predation, invasive species, sedimentation and alteration of stream morphology etc.

It should be recognized that optimal protection of in stream values cannot be achieved when social and economic considerations are accounted for. It is the goal of river management to achieve balance between all in stream values, while maintaining ecosystem health.

Monitoring and follow-up of the data is also important to assure the accuracy of the model results. Continual monitoring of the stream ecosystem is important to assure the accuracy of the model results. Monitoring of the actual flow recommendations, when they are in place, should include visual observations to decide if the flow limits set by the model and the following negotiation are actually meeting the hydromorphological demands of the streams such as covering riffles, providing enough depth in pools etc. The biological component should also be monitored to ensure that the flows are adequate. Monitoring will allow the data input and model output to be assessed and refined as conditions change both in the stream and as a result of management decisions. This will create a more solid basis for ongoing and future management decisions.

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REFERENCES

[1] C. J. Gordon, N.D., T.A. McMahon, Finlayson, B.L., Gippel and and R. J. Nathan, *Stream Hydrology: An introduction for ecologists*. 2004.

[2] B. Hunt, B., Weir, J. and Clausen, "A stream depletion experiment," *Ground Water*, vol. 39, no. 2, pp. 283–289, 2001.

[3] K. R. Nyholm, T., Christensen, S., and Rasmussen, "Flow depletion in a small stream caused by ground water abstraction from wells.," *Ground Water*, vol. 40, no. 4, pp. 425–437, 2002.

[4] P. Thorn and J. Conallin, "RHYHABSIM as a Stream Management Tool: Case Study in the River Kornerup Catchment, Denmark," *J. Transdiscipl. Environ. Stud.*, vol. 5, no. 1, pp. 1–2, 2006.

[5] I. G. Jowett, "Instream fl ow methods: A comparison of approaches: Regulated Rivers," *Res. Manag.*, vol. 13, pp. 115–127, 1997.

[6] K.. Bovee, "A guide to instream habitat analysis using the instream fl ow incremental methodology," *Instream Flow Inf. Pap. Fort Collins, U.S. Fish Wildl. Serv.*, vol. 12, 1982.

[7] T. W. Milhous, R. T., D. L. Wegner, "Users guide to the Physical Habitat Simulation System (PHABSIM).," *Instream Flow Inf. Pap. Fort Collins, U.S. Fish Wildl. Serv. (FWS/OBS/81/13).*, vol. 11, 1984.

[8] I. G. Jowett, *River hydraulic and habitat simulation, RHYHABSIM computer manual.*, vol. New Zealan. 1989.

[9] A. Bovee, K. D., B. L. Lamb, J. M. Bartholow, C. B. Stalnaker, J. G. Taylor and J. Henriksen, "Stream habitat analysis using the instream flow incremental methodology. U.S.," *Geol. Surv. Inf. Technol. Rep. 1998-0004, Fort Collins, Color.*, 1998.

[10] R. M. Jowett, I., T. Payne, "SEFA–system for environmental flow analysis: software manual version 1.21.," *Available: http://sefa.co.nz/. (March 2016).*, 2014.

[11] W. Bank, *Environmental Assessment (EA) Viet Nam Managing Natural Hazards Project (VN-Haz/WB5)*, vol. E2993. 2017, p. 18.

[12] J. Hay, "Review of Hawke"s Bay Regional Council"s Instream Habitat Modelling on the Lower Tukituki River.," *Prep. Hawke"s Bay Reg. Counc. Cawthron Rep. No. 1542*, 2008.

[13] J. Hay, "Instream Flow Assessment for the Lower Ruamahanga River," *Prep. Gt. Wellingt. Reg. Counc. Cawthron Rep. No. 1403.*, 2008.

[14] K. Johnson, "Tukituki River Rhyhabsim and Water Temperature Assessments Tukituki River Rhyhabsim and Water Temperature Assessments Minimum Flow and Water Temperature Assessments," 2013.

[15] B. Clausen and I. G. Jowett, "Guide for the RHYHABSIM program," pp. 1–14, 1998.

[16] I. G. Jowett, "Survey and analysis of instream habitat," in *Computer Manuel*, Revised by., NIWA, Hamilton. New Zealand., 1998.

[17] M. and J. F. Kottelat, *Handbook of European freshwater fishes*. Publications Kottelat, Cornol and Freyhof, Berlin, 2007.

[18] K. H. Alikunhi, "Synopsis of biological data on common carp Cyprinus carpio (Linnaeus), 1758 (Asia and the Far East)," *FAO Fish. Synop*, vol. 31, no. 2, p. 39, 1966.

[19] I. F. Pravdin, *A Guide of Fish Study*. Science and Technics Publishing House, Hanoi, 1963.

[20] N. H. D. Nguyen Dinh Vinh, Tran Thi Thuy Ha, Tran Duc Hau, "Morphological and Molecular Identification of species of Catfish Genus Cranoglanis from Lam River, Nghe An, Vietnam," *Biol. Forum–An Int. J.*, vol. 9, no. 2, pp. 37–43, 2017.

[21] H. H. and M. K. Ng, "Cranoglanis henrici (Vaillant, 1893), a valid species of cranoglanidid catfish from Indochina (Teleostei, Cranoglanididae)," *Zoosystema*, vol. 22, no. 4, pp. 847–852, 2000.

[22] T. T. B. Dong Quoc Trinh, "Study on several rproductive biological characteristics of armorhead catfish (Cranoglanis henrici Vaillant, 1893)," *J. Fish. Sci. Technol. Nha Trang Univ.*, vol. 2, pp. 78–82, 2013.

[23] I. J. and H. S. Harrison, "Order Mugiliformes. Mugilidae. Mullets," in *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 4. Bony fishes part 2 (Mugilidae to Carangidae). FAO, Rome*, K. E. C. and V. H. Niem, Ed. 1997, pp. 2069–2108.

[24] C. M. and D. E. R. Breder, *Modes of reproduction in fishes*. T.F.H. Publications, Neptune City, New Jersey, 1966.

[25] H. H. Ng, *Eleotris fusca. The IUCN Red List of Threatened Species 2012: e.T166077A1109943.* 2012.

[26] N. Y. and K. T. Ken Maeda, "Size and age at recruitment and spawning season of sleeper, genus eleotris (Teleostei: Eleotridae) on Okinawa Island.

http://dx.doi.org/10.22161/ijeab/2.6.37 *ISSN: 2456-1878*

Sourthern Japan," *Raffles Bul. Zool.*, vol. Supplement, no. 14, pp. 199–207, 2007.

[27] S. H. M. and M. A. Allen, G.R., *Field guide to the freshwater fishes of Australia*. Western Australian Museum, Perth, Western Australia., 2002.

[28] R. Pethiyagoda, *Freshwater fishes of Sri Lanka*. The Wildlife Heritage Trust of Sri Lanka, Colombo., 1991.

[29] W. J. Rainboth, *Fishes of the Cambodian Mekong. FAO species identification field guide for fishery purposes*. FAO, Rome, 1996.

[30] J. S. Larson, H., Britz, R. & Sparks, *Glossogobius giuris. The IUCN Red List of Threatened Species 2016: e.T166533A19011337*. 2016.

[31] Qambrani GR, Soomro AN, Palh ZA, Baloch WA, Tabasum S, "Reproductive Biology of Glossogobius giuris (Hamilton), in Manchar Lake Sindh, Pakistan.," *J Aquac Res Dev.*, vol. 6, p. 392, 2015.

[32] J. H. & L. K. John W. Hayes, Eric Goodwin, Karen A. Shearer, "Can Weighted Useable Area Predict Flow Requirements of Drift-Feeding Salmonids? Comparison with a Net Rate of Energy Intake Model Incorporating Drift–Flow Processes," *Trans. Am. Fish. Soc.*, vol. 145, no. 3, 2016.

[33] R. Henderson, *Relationships between 1-day and 7-day MALF in the Horizons Region Relationships between 1-day and 7-day MALF in the Horizons Region Horizons Regional Council (funded by EnviroLink (FRST), project managed by Jon Roygard and*, no. October. 2008.

[34] Mike Thompson, "Minimum flow recommendations for the Wellington region. Technical report to support the Proposed Natural Resources Plan," 2015.

[35] Lonelyplanet, "Map of Vietnam." [Online]. Available: http://www.lonelyplanet.com/maps/asia/vietnam/.

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Deductive and Multi-criteria Approach to Ecosystem Modeling and Habitat Mapping of Shea Butter Trees *(Vitellaria Paradoxa)* in the Tropical Savanna

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Abstract— *An ecosystem map for 14 local administrative units of Kwara state North Central Nigeria and Vitellaria paradoxa habitat in the broad Savanna region was produced using multi criteria and integrated GIS models as against the traditional single layer thematic approach. The criteria used in classifying and mapping the ecosystems are: climate (rainfall and temperature), physiography (slope, relief), vegetation/land cover and drainage system. The climate layer was extracted from WorldClim database using DIVA GIS, the topographic layer was produced from 90 m NASA/SRTM digital elevation model. NDVI was run on composite images to produce vegetation layers. All the input data layers were spatially modeled in ArcGIS to generate the 7 classes of ecosystems. The Georefrenced trees sample points from field survey was overlaid on classified images to produce distribution pattern of Vitellaria paradoxa and its habitat in Savanna wood land ecosystems.*

Keywords— Ecosystems, Mapping, Shea butter, Multi-criteria.

I. INTRODUCTION

The use of multi-criteria approach to ecosystem mapping is relatively new and its potential has not been fully explored especially in Sub Saharan Africa (Salako 2016). Most research on ecological classification and mapping in Africa especially Nigeria had used thematic or topical approaches such as vegetation zones and agro climatic zones in their analysis and description of ecosystem. These approaches, however, do not yield a complete understanding of the interrelationships among the various forces driving ecosystem composition and functions (Lugo et al, 1999), coupled with arbitrary drawing of the ecological zones by heavy reliance on expert judgment and instinct thus limiting their use in scientific analysis (Lugo et al. 1999 and Olson et al. 2001). Although multi data layers approach has been widely used in ecosystem mapping in South America and North America (Sayre 2008 and Sayre 2009),it was not until recent times that it was used for ecosystem mapping in Africa, few of such efforts are A New Map of Standardized Terrestrial Ecosystems of Africa (Sayre et al 2013) and Biogeoclimatic ecosystem zones of Mambilla Plateau, Nigeria (Salako et al 2016). 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 affected ecosystems (Gladstone and Thomas 1990) and its various components.

Geographical Information System and Remote Sensing (GIS/RS) and its capability for modeling has proven to be a very useful tool for large scale mapping of ecosystem and preparation of land cover map (Trisurat, *et al* 2000, Lu and Weng 2007), using GIS/RS techniques is faster and enable wider geographic coverage within short time frame (USGS/GAP, 2002, Lowry, *et al* 2005). Good ecosystem mapping has been the basis for identifying species habitat suitability, and framework for resources planning, conservation and managing species at risk (Province of BC 2006). It provides detail explanations on various environmental variables that characterize an area rather using single thematic layer approach such as vegetation (Guinea Savanna) or climate (Savanna Climate).

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Ecosystem mapping could be deductive by overlaying the available geospatial data to generate new ecosystems (Comers et al. 2003). This approach involve extensive use of remote sensing and GIS techniques to spatially combine several data layers to produce ecosystem map, and was relatively less expensive especially when working in a large area. Alternatively, ecosystem could be mapped by associating environmental attributes of point source data with known ecosystem occurrences at their locations; however this option is data intensive and costly especially when working in a large area (Sayre et al 2009). Effort is made in this study to combine the use of GIS modeling with use of field data to generate ecosystem map.

Vitellaria paradoxa (Shea butter) is a woody plant native to the Guinea Savannah of West Africa, It was reported to contain high fat and oil contents which make it an irresistible fuel wood for it burns longer and steadily compare to other tree species in its habitatand of immense socio economic and health values (Goreja 2004). Thousands of lives especially women in rural areas in West Africa depend on it for their sustenance not only because it is the major source of income but also serves as food and condiments.

Despite the importance of this tree to the socio economic lives of vast number of this people living in fragile environment, even though was classified as vulnerable in IUCN red list (IUCN 2013), It has received little or no global attention as endangered or threatened species. The rate at which this tree was been felled and burn as charcoal in recent times portend a dangerous trend and could result to the extinction of the tree in the next few decades except urgent action is taken. Also the actual location and mapping of Vitellaria paradoxa habitat within the broad Savanna ecosystem has not been well defined. These work objectives are (i) to develop a detail and explanatory ecosystem map of the selected area in Kwara State North central Nigeria, and (2) locate the distribution pattern of *Vitellaria paradoxa* and its habitat within the ecosystem, our comprehensive ecosystem classification can be used by land managers throughout Nigeria to manage and conserve Nigeria's diverse ecosystems, including predicting the potential effects of climate change.

II. MATERIALS AND MEETHODS

2.1 Study area

The study area is comprised of 14 local administrative units of Kwara State, Nigeria (Fig 1). With seasonal rainfall mostly in the months of June to September and total annual rainfall of 1200 mm in the southern axis and between 700-900mm in the northern axis and mean annual temperature of 26ºC, it broadly falls under Tropical Savanna climate. The vegetation is characterized by deciduous trees and long grass under story. However, there are clusters of dense forest in the south and south eastern corner of Ifelodun Oke Ero and Ekiti Local Government. Farming and marketing of agricultural products is the major occupation.

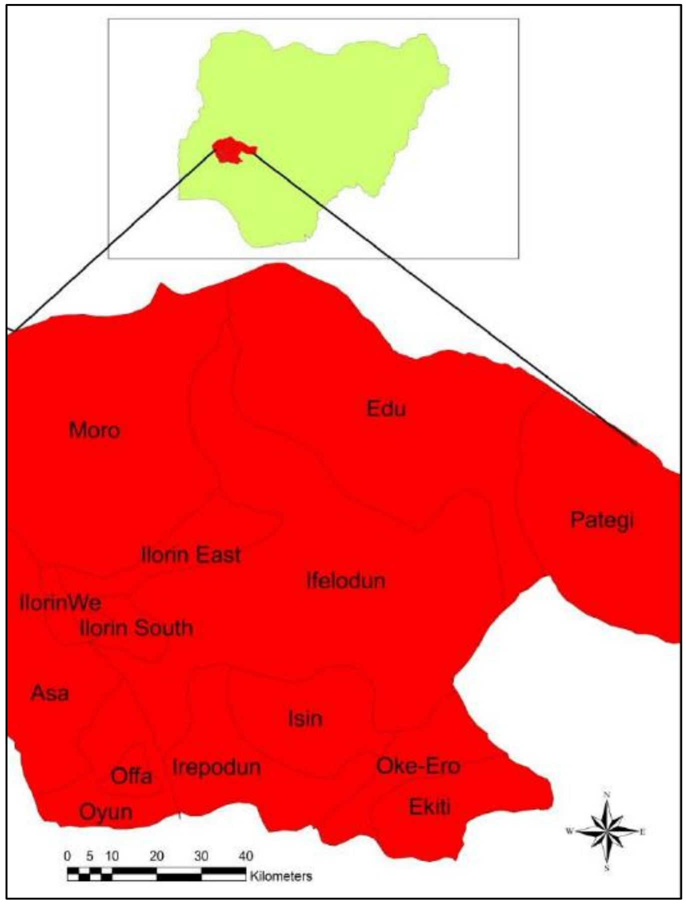

Fig.1: Map of the 14 selected local administrative units of Kwara state (Nigeria as Inset)

2.2Satellite Image Data and Processing

All spectral bands in Satellite images of Landsat 8 OLI on path 190 row 054 in 2016 with 90% cloud free were acquired form USGS. Landsat 8 was launched on February 11, 2013, it is the eighth satellite in the Landsat program; the seventh to reach orbit successfully, providing moderate-resolution imagery, from 15 meters to 100 meters, of Earth's land surface and polar regions, Landsat 8 comprises 2 sensors: The Operation Land Imagers (OLI) and Thermal InfraRed Sensor (TIRS) which see improved signal to noise (SNR) radiometric performance and for better land-cover characterization. OLI collects data from nine spectral bands at 30 m excepts bands 8- Panchromatic which is at 15 m

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while TIRS collects from 2 additional spectral bands at 100 m. OLI two new spectral bands include a deep blue coastal / aerosol band and a shortwave-infrared cirrus band (Table 1). Images were equally obtained Google Earth for features identification needed for classification.

Table.1: Landsat 8 OLI and TIRS details

Spectral Band	Wavelength	Resolution	Solar Irradiance
Band 1 - Coastal / Aerosol	0.433 – 0.453 µm	30 m	2031 W/(m²µm)
Band 2 – Blue	0.450 – 0.515 µm	30 m	1925 W/(m²µm)
Band 3 – Green	0.525 – 0.600 µm	30 m	1826 W/(m²µm)
Band 4 – Red	0.630 – 0.680 µm	30 m	1574 W/(m²µm)
Band 5 - Near Infrared	0.845 – 0.885 µm	30 m	955 W/(m²µm)
Band 6 - Short Wavelength Infrared	1.560 – 1.660 µm	30 m	242 W/(m²µm)
Band 7 - Short Wavelength Infrared	2.100 – 2.300 µm	30 m	82.5 W/(m²µm)
Band 8 – Panchromatic	0.500 – 0.680 µm	15 m	1739 W/(m²µm)
Band 9 – Cirrus	1.360 – 1.390 µm	30 m	361 W/(m²µm)
OLI Spectral Bands			

Spectral Band	Wavelength	Resolution
Band 10 - Long Wavelength Infrared	10.30 – 11.30 µm	100 m
Band 11 - Long Wavelength Infrared	11.50 – 12.50 µm	100 m
TIRS Spectral Bands		

2.3 Image processing and Vegetation layer

Unsupervised classification was run on false color composite images of Bands 5, 4, 3 (NIR/Red/Green bands) from Landsat 8 (OLI) vs. R/G/B) . This technique produces clusters or spectral classes based on spectral values or digital number (DN) of the composite images. We map the distribution of the *Vitellaria paradoxa* species by overlaying the georefrenced species point data from the field survey on the unsupervised classified image and further use the DN to establish direct relationship with the species distribution pattern. The spectral values (DN) associated with Vitellaria Paradoxa were used to identify possible occurrence of the tree species in other parts of the study area. However, it was observed that, *Parkia Biglobosa* and *Daniella oliveri* had almost the same spectral values with *Vitellaria paradoxa*, possibly due to the spatial resolution of the satellites images used (28.5-30m),we therefore grouped them into same class in our mapping. The map produced here however should not be interpreted to be entirely representing *Vitellaria paradoxa* distribution.

NDVI is a very simple vegetation index and has been used to quantitatively and qualitatively evaluate vegetation covers (Ghorbani et al. 2012) it compares the measure of infrared reflectance to that of the red reflectance 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. We run NDVI on the composite image bands 5,4,3and set the limit to 0.4 to eliminate field points that falls on non-trees feature The result was further reclass from 1 to 7, I means bare land, 4 shrubs and 7 dense forest. Only point data that falls within 4- 7 pixels were used in our tree species distribution analysis.

2.4 Field data

Malete Elemere covering about 500 ha of land was selected as our site for intensive field survey where over 50 sample plots of 10m2 were laid using systematic random techniques. The field data collected include the tree species name and their location (longitude, latitude, and altitude) was determined using hand held Garmin eTrex GPS. Special attention was paid on the identification of Shea Butter in a plot. The field data was used as training data for the identification of the Vitellaria paradoxa on the classified images.

2.5 Ecosystem Mapping Approach:

Our Mapping approach follows the deductive methods used in terrestrial ecosystem mapping of conterminous United

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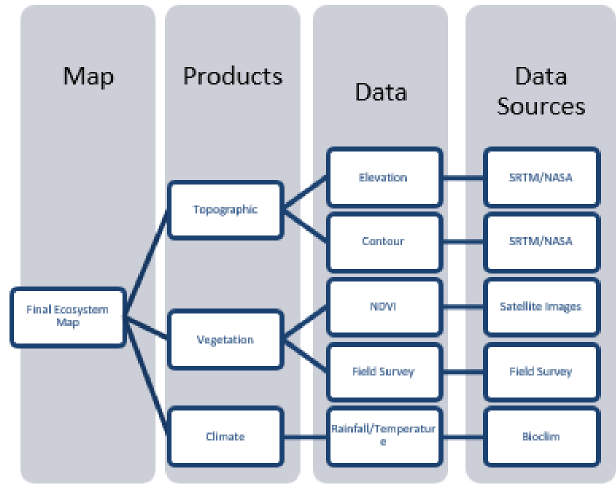
States (Sayre 2009). This approach involves mapping the physically distinct environmental areas which forms the fundamental building blocks of ecosystem. These physical distinct blocks include: the climate, vegetation, lithology, soil, topography/landform among others (Bailey 1996, Comers and others 2003, Sayre et al. 2009).In this study data were obtained and GIS layers created for each of the ecosystem building blocks- climate (temperature and rainfall), vegetation and landcover, Rivers, elevation, landforms, slope (fig. 2) which subsequently were spatially combined in a sequence using boolean fuzzy sum overlay in ArcGIS (Sayre et al 2009, Salako et al 2016).

*Climate Data/layers***:** To create the climate layers of Mean Annual Temperature (MAT) and Total Annual precipitation (TAP), the climate data CLM files Tiles 26 of Worldclim.org database was downloaded and the data extracted was converted into grid raster files in DIVA GIS and subsequently imported into ArcGIS 10.2 (Scheldeman and Zonneveld 2010) from which MAT and TAP of the study area was subset,

*DEM Layer***:** A 90 m Digital Elevation Model (DEM) from NASA/Shuttle Radar Topographic Mission- SRTM was acquired from which the study area was subsets and imported into GIS environment. The elevation data was processed by using the reclass menu in spatial analyst tool in Arc GIS 10.2 version to reclassify the study area into classes at an interval of 80 m (Fig 6). ESRI vector data on the study area drainage system was subset from national dataset and was overlaid on the contour (Fig 7) to explain the relationship between the relief and rivers system.

GIS Ecosystem modeling

i



ii

Fig. 2: Flow chart for ecosystem modeling (i) and mapping

III. RESULTS AND DISCUSSION

All the inputs layers that form the ecosystem building blocks were spatially combined to produce the ecosystem map for the study area. In naming the ecosystem classes, a combination of local knowledge of plant species, field works, high resolution satellite Image from Google Earth, literatures and classification system used in African Ecosystems Classification (Sayre *et al.*, 2013) were adopted.

3.1 Mapping climatic zones: Climate and its derivative bioclimate strongly influence the differentiation and distribution of ecosystems especially the moisture availability (Sayre et al 2009). Although on average the total annual rainfall in the study area exceeds 1000 mm which is considered to be wet enough for plant productivity, however, three distinct distributional patterns are identified; the southern half which comprises of Ilorin metropolitan area, Offa, Ekiti, Oke Ero and part of Ifelodun Local administrative units had over 1150 mm of total annual rainfall, this section receives the highest total annual rainfall and constitutes the wettest part of the study area. The middle part had annual total of slightly above 1100 mm, while the Northern part of Moro local administrative units receive marginally above 1000 mm (Fig.3) This shows that rainfall decreases northward in line with movement of Inter Tropical Convergence Zone (ITCZ) as typical of the tropics. Mean annual temperature follows an inverse relationship as temperature decreases southward. The highest mean annual temperature of over 27°C were recorded in the northern section and cover four local administrative units of Moro, Ifelodun, Edu and Pategi (Fig. 4)

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There is a difference of over 150 mm in total annual rainfall between the wettest and the less wet part and given the fact that temperature is rarely a limiting factor in the tropics excepts in higher altitude, the differential in moisture availability greatly defines the length of growing periods within the study area and account for vegetation cover and plant species variations.

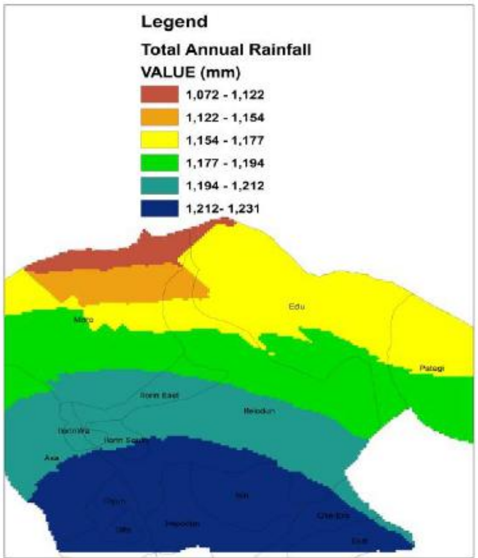

Fig.3: Total Annual Rainfall (mm)

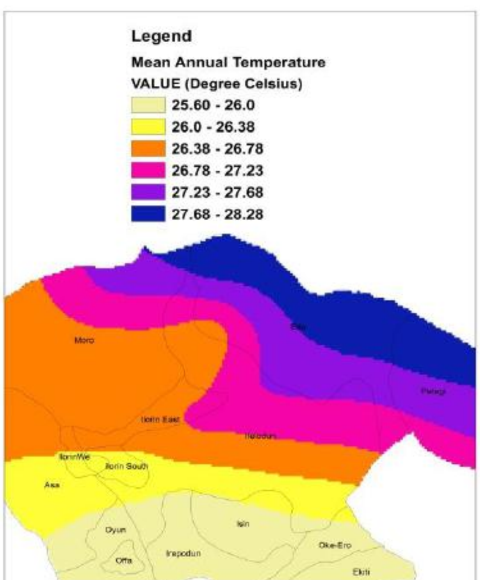

Fig.4: Mean annual Temperature (°c)

3.2 Landforms: The role of landforms in the distribution and differentiation of ecosystems cannot be over emphasized not only that altitude defines temperature distribution pattern but a number of organisms, vegetation are associated with certain landforms and topography (Treitz and Howarth 2000). Also depth of soil, rivers flow are strongly correlated with slope and landforms (Kruckeberg, 2002, Reza
And Abdullahi 2016)

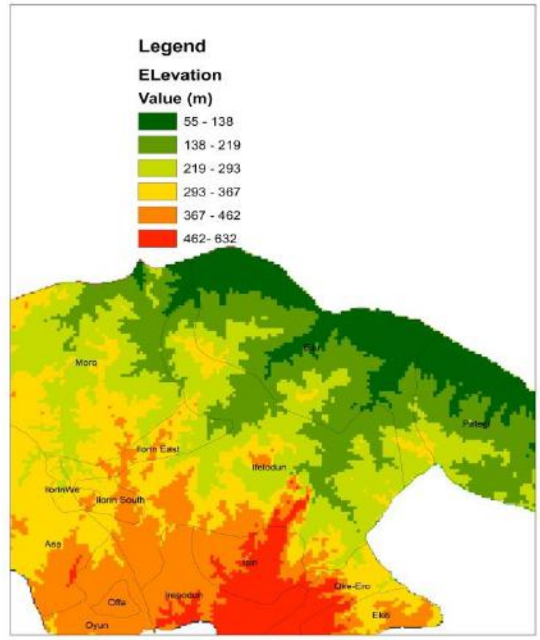

Fig. 5: The relief

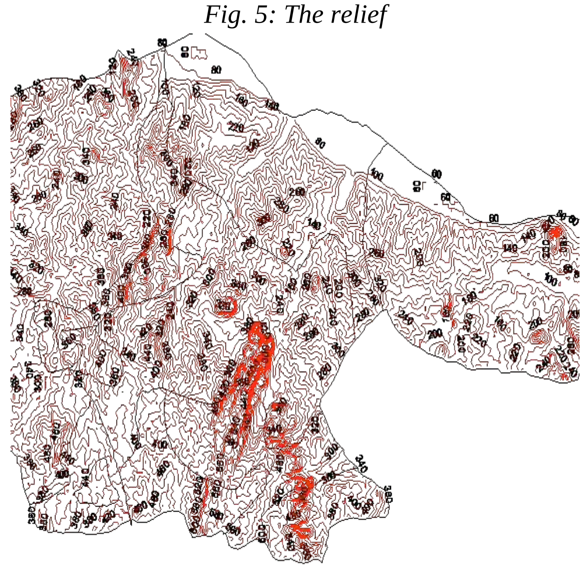

Fig.6: contour at 20 m

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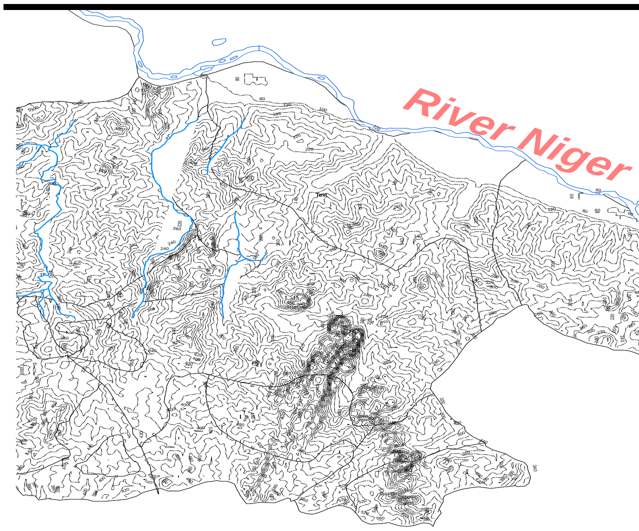

Fig.7: Contour overlaid with river system

From the DEM layers we classified our study area into local relief and slope. The low mountains with an average height of 550 m were found in the south eastern section which was bordered by high plain of 350 m. The northern segment are generally comprises low and flood plain ranges from 55 m to 150 m (Fig. 5 and 6) and river valley system (fig 7)

3.3 Vegetation index: Vegetation has been described as one of the best ways to describe life forms in an environment not only it creates their microclimate but also the direct product of various interacting physical processes on earth. Each vegetation structure reflects not only the climatic conditions but also other ecological conditions (Biodiversity Conservation). Our reclassified NDVI values ranges from 1 to 7, where 1 shows area of little or no vegetation and 7 depicts area of dense vegetation (Fig. 7). There is direct relationship between rainfall pattern, landforms and vegetation distribution in the study area. Dense and close canopy vegetation is found in wet, hilly, steep slope in the south eastern section.

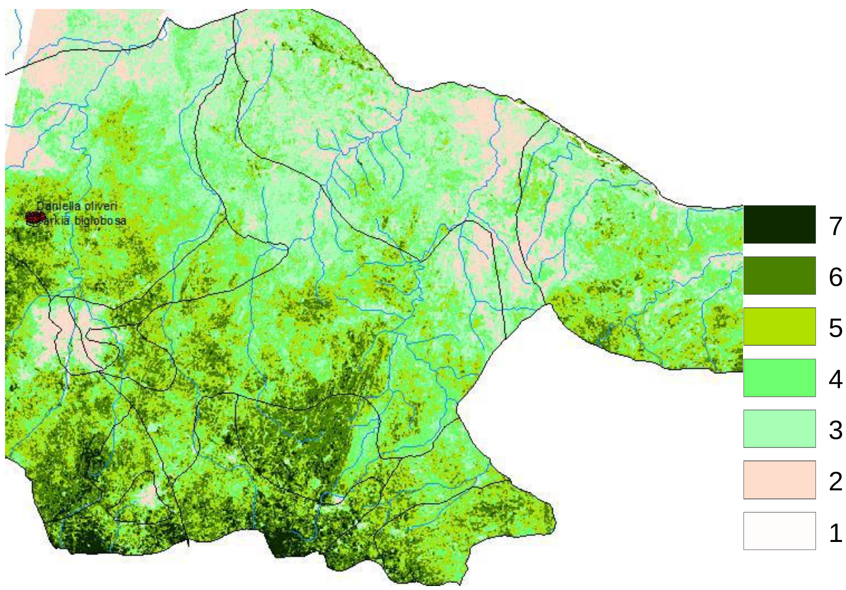

Fig.7: Vegetation Index

3.4 Ecosytem Units and Shea Butter habitat

A total of seven classes of ecosytem units were mappped in addition to urban/agricultural farm settlements land use ecosystem (Table 2) which can be described as diverse for it compose the terrestrial, aquatic, hilly and forest ecological units (Rezza and Abdullahi 2016) and the disribution of the ecosystem by local adminstrarive units are presened in (Table3).

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Table.2: Ecosystem Classes by area coverage in Hectares

S/N0	Class	Pixel samples	Area (Ha)	Percent of total area
1	Upland Forest Ecosystem	888	75,840	4.39
2	Low Land Forest Ecosystem	1,495	127,681	7.39
3	Savanna Grassland Ecosystem	8,677	741,068	42.94
4	Savanna Wood Land Ecosystem	2,388	203,948	11.82
5	River Basin/Valley Ecosystem	5,345	456,492	26.44
6	River Basin Ecosystem (Intensive)	1,389	118,628	6.88
7	Urban/Agric Land Use Ecosystem	27	2,306	0.14
	Total	20,209	1,725,963	100

Climate especially rainfall, vegetation and topography greatly defined the pattern of ecosystem in the study area (Treitz and Howarth 2000, Sayre eta l 2013). In the Upland forest ecosysytem, hardwoods and Palm trees are the dominant plant species with long grasses as undergrowth. Adjoining the upland forest ecosystem is the lowland forest ecosystem, the latter has similar plant compositions with the former excepts that it was found in the lower elevation (between 300 -350 m) and less dense.

Table.3: Ecosystems classes distributions by local administrative units in Km²

S/No	Class	Asa	Edu	Ekiti	Ifelodun	Ilorin East	Ilorin south	Ilorin West	Irepodun	Isin	Moro	Offa	Oke Ero	Oyun	Pategi
1	Urban/Agric land Use Ecosystem	1	58	0	1	0	0	4	0	0	2	2	0	0	110
2	River Basin Ecosystem (Intensive	0	474	0	49	4	0	0	0	0	57	0	0	0	643
3	River Basin Ecosystem	1	992	1 5	354	12	0	7	1	0	277	1	0	1	1238
4	River valley Ecosystem	25	770	180	369	10	8	12	4	0	311	3	4	1	999
5	Savanna Grassland Ecosystem	1508	436	178	1845	381	168	87	177	79	2349	60	87	142	376
6	Savanna Woodland Ecosystem	276	155	186	1122	83	21	4	178	177	609	42	157	210	126
7	Lowland forest Ecosystem	54	5	68	246	19	1	0	128	178	101	7	154	189	16
8	Upland Forest Ecosystem	16	0		61	0	0	0	370	128	0	0	82	0	0

These ecosystem units are restricted to Irepodun, Isin and to lesser extent Oke ero, however, Oyun has a significant land mass about 189 hectares under lowland forest ecosystem (Table 3). The dense vegetation that characterise upland forest ecosystem in the south easten section is a product of rugged topography and ample rainfall which make the length of growing period in this ecosystem units longer than the rest.

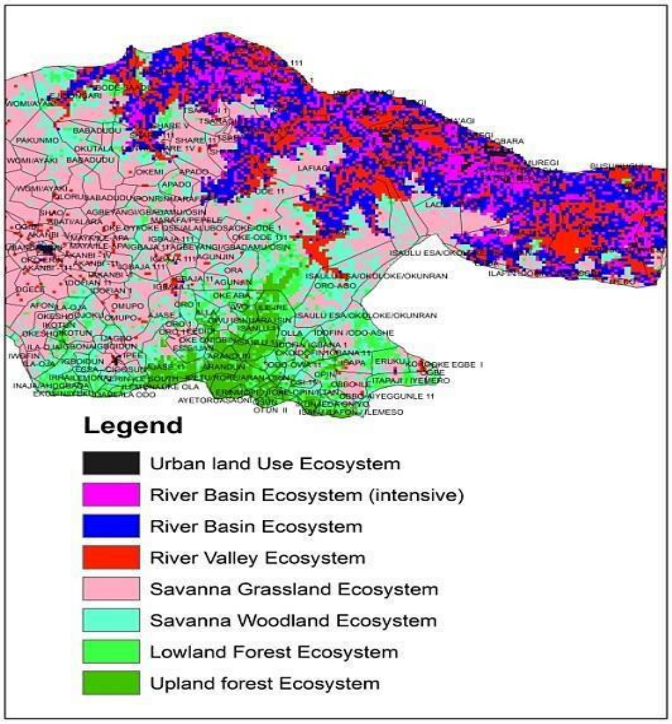

Fig. 8: Ecosystem classes in selected Local administrative units of Kwara State, Nigeria

Fig. 9i riparian vegetation in river basin ecosystem *ii cluster forest in river basin ecosystem*

iii Dense canopy in upland forest *iv Savanna woodland ecosystem*

River Ecosystems are mostly found in the northern segment in Edu and Pategi local administsrative units, these ecosytem generally consist of lowland and flood plain with elevation ranges from as low as 50 m to 150 m and are subdivided into three subclasses: i the river basin ecosystem (intensve) characterised by intensive subsitense agriculture, ii river valley ecosystem consists numerous tributaries of river Niger and riparian vegetation of cluster forests , iii river basin ecosystem include other areas within the river basin not dierectly related to the two erlier identified (Fig 8, i- iv). Aquatic plants are commonly found and the soil is conststanly covered by water thus made the ecosystem vulnerable to flooding and soil erosion

The Savanna Ecosystems is subdivided into savanna wood and grass land depending on the presence of woody plants. This ecosytem is dominated by grasses and bushes of smaller trees, It covered about 1 million hectares of land and it's the largest ecosytems classes in the study area. These ecosystems are found in all local admministsrative units but has areacover of over 60% of Moro and Asa, and about 30% of Ifelodun and Ilorin (Table 3). Plant communities include grasses, graminoid, and other herbaceous plants in addition to woody plants (trees and shrubs) in Savanna woodland ecosystem. The rainfall (average of 1000 mm total annual) is suffiecnt to support grasses and woody plants in moister areas. Geomorphological this ecosytem is classified as low (300 m) but punctuated by undulating lowland in its border with river basin ecosystem.

3.5 Shea Butter Habitat Map

The result of overlaying field sample points on ecosystems map reveals that Shea butter*(Vitellaria Paradoxa)* habitat is commonly found in the Savanna Woodland Ecosystem and within the boundary of Savanna Grassland and Savanna Wood land (Fig 10). The mean annual temperature of 26°c and total annual rainfall of 1120 mm in the ecosystems support the growth of deciduous trees and shrubs. As stated earlier this habitat is equally associated with Locust beans *(Parkia biglobosa)*. Other trees and shrubs species found in this ecosystem are: *Daniella oliveri, Azadiracta indica, Piliostigma thonningii and Acacia nilotica.* It was also observed that locust bean (Parkia biglobosa) has become alternative target for illegal burning for charcoal in the absence of Shea butter in a logging site. Savanna Woodland Ecosystem which constitute Shea butter habitat although cover about 12% of the total land cover yet it is the most threatened ecosystem in terms of bush burning, overgrazing and tree burning for charcoal with dire consequences on biodiversity (Meerman and Sabido 2001). There is an ongoing project to monitor and quantified changes that has happened to Shea butter and the ecosystem over the last 20 years and project possible consequences.

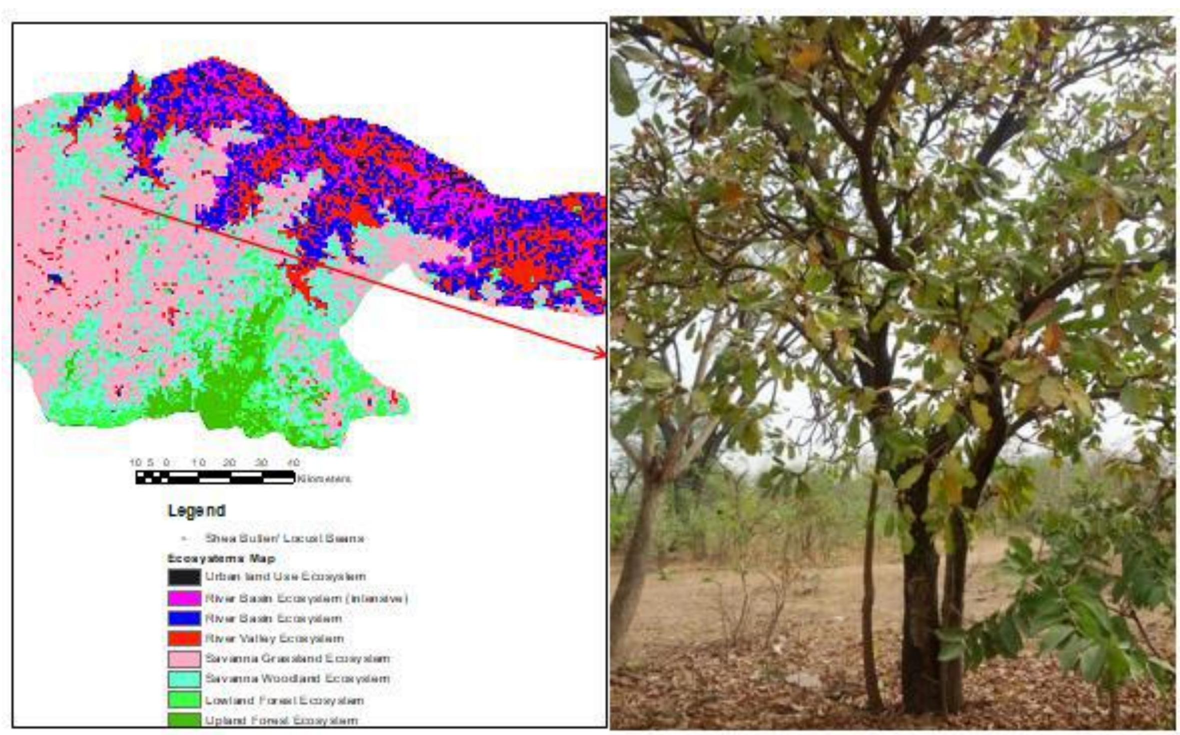

Fig.10: Shea Butter trees distribution in Savanna Woodland Ecosystem

IV. CONCLUSION

This mapped ecosystem is based on the current environmental variables without assessing human disturbance even though we acknowledged significant changes in land cover over times in our study area (Loveland et al 2002). Mapping ecosystem through spatial combination of the distinct environmental variable helps in predicting the possible changes that may occurs in the event of any alteration in any of the variables. For instance future climate change will alter the location and perhaps the areal extent of ecosystems and invariably the plant and animal habitat. Resource management is key to sustainable development, the distribution of the mapped ecosystems across the administrative units provide a good working tools to land and environmental managers for rational spatial resource planning (Grooves 2003, Sayre et al 2009).

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- Nigeria Tertiary Education Fund (Tetfund) which provided the fund for this work through Institutional Based Research (IBR) grants for 2015/2016 allocation for Kwara State University Malete, Nigeria.
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REFERENCES

[1] Bailey, R.G., 1996, Ecosystem geography: New York, Springer-Verlag, 204 p., 2 pls. in pocket.

[2] Biodiversity Conservationwww.biodiversity_worldwide-info/biodiversity-conservation

[3] Comer, Patrick, Faber-Langendoen, Don, Evans, Rob, Gawler, Sue, Josse, Carmen, Kittel, Gwen, Menard, Shannon, Pyne, Milo, Reid, Marion, Schulz, Keith, Snow, Kristin, and Teague, Judy, (2003) Ecological systems of the United States, A working classification of U.S. terrestrial systems: Arlington, Va., NatureServe, 75 p. (Also available online at http://www.natureserve.org/publications/ usEcologicalsystems.jsp.)

[4] Defries, R., & Townshend, J. (1994). 'NDVI derived land classifications at a global scale'. International Journal of Remote Sensing, 17, 3567– 3586.

[5] Ghorbani A, Mossivand A.M and Ouri A.E (2012) 'Utility of the Normalized Difference Vegetation Index (NDVI) for land/canopy cover mapping in Khalkhal County (Iran)', Annals of Biological Research, 2012, 3 (12):5494-5503

[6] Goreja W. G (2004) Shea Butter: The Nourishing Properties of Africa's Best-Kept Natural Beauty Secret Amazing Herbs Press Paperback

[7] Gladstone, W. and F. Thomas Ledig (1990).“Reducing pressure on natural forests through high-yield forestry. “Forest Ecology and Management 35(1): 69-78.

[8] Groves, Craig, 2003, Drafting a conservation blueprint; A practitioner's guide to planning for biodiversity: Washington, D.C., Island Press, 404 p.

[9] IUCN (2013) Red list 2013: threatened species across the regions of the world.

[10] *www.iucnredlist.org*

[11] Kruckeberg, A. (2002). Geology and Plant Life: The Effects of Landforms and Rock Types on Plants University of Washington Press, Seattle, WA, USA. 362 pages.

[12] *"LANDSAT 8 Satellite details 2013-008A NORAD 39084". N2YO. 24 January 2015. Retrieved*

[13] Lu, D. and Q. Weng (2007). A survey of image classification methods and techniques for improving classification performance. International Journal of Remote Sensing, Vol. 28(5): 823-870.

[14] Lowry, J. H, Jr., R. D. Ramsey, K. Boykin, D. Bradford, P. Comer, S. Falzarano, W. Kepner, J.Kirby, L.Langs, J. Prior-Magee, G. Manis, L. O'Brien, T. Sajwaj, K. A. Thomas, W. Reith, S. Schrader, D. Schrupp, K. Schulz, B. Thompson, C. Velasquez, Wallace, E. Waller and B. Wolk.(2005). Southwest Regional Gap Analysis Project: Final Report on Land Cover Mapping Methods, RS/GIS Laboratory, Utah State University, Logan, Utah.

[15] Loveland, T.R., Sohl, T.L., Stehman, S.V., Gallant, A.L., Sayler, K.L., and Napton, D.E., (2002)A strategy for estimating the rates of recent United States land-cover changes: Photogrammetric Engineering & Remote Sensing, v. 68, no. 10, p. 1091–1099.

[16] Lugo AE Brown SL Dodson R Smith TS Shugart HH (1999). The Holdridge life zones of the conterminous United States in relation to ecosystem mapping. Journal of Biogeography 26 : 025 1038

[17] Meerman J C and Sabido W (2001) Central American Ecosystems Map : Belize Vol.1 Program for Belize Belize City.

[18] Olson, D.M., Dinerstein, Eric, Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'amico, J.A., Itoua, Illanga, Strand, H.E., Morrison,

J.C., Loucks, C.J., Allnutt, T.F., Ricketts, T.H., Kura, Yumiko, Lamoreux, J.F., Wettengel, W.W., Hedao, Prashant, and Kassem, K.R.,(2001), Terrestrial ecoregions of the world; A new map of life on Earth: BioScience, v. 51, no. 11, p. 933–938.

[19] Province of BC (2006) Standard for mapping ecosystems at risk in British Columbia [electronic resource]: an approach to mapping ecosystems at risk and other sensitive ecosystems.ISBN 978-0-7726-5699-5.

[20] Rezza MIH andAbdullahi S.A (2016) Developing Ecosystem Maps Using Eco Geological Information for Sustainable Management of Natural Resources. Open Journal of ecology, 6, 3

[21] Salako, G., Olalubi, O., Sawyerr, H., Howe, G., Adebayo, A. and Adio, A. (2016) Using Multi Techniques Analysis in Biogeoclimatic Ecosystem Classification and Mapping of Mambilla Plateau in Taraba State Nigeria. Open Journal of Ecology, 6, 412-426.

[22] Sayre, R., P. Comer, J. Hak, C. Josse, J. Bow, H. Warner, M. Larwanou, E. Kelssa, T. kele,H. Kehl, R. Amena, R. Andriamasimanana, T. Ba, L. nson, T. Boucher, M. Brown, J. Cress, O. Dassering, B. Friesen, F. Gachathi, S. Houcine, M. Keita, E. Khamala, D. Marangu, F. Mokua, B. Morou, L. Mucina, S. Mugisha, E. Mwavu, M. Rutherford, P. Sanou, S. Syampungani, B. Tomor, A. Vall, J. Vande Weghe, E. Wangui, and L. Waruingi. (2013). A New Map of Standardized Terrestrial Ecosystems of Africa. Washington, DC: Association of American Geographers. 24 pages.

[23] Sayre, Roger, Comer, Patrick, Warner, Harumi, and Cress, Jill, (2009). A new map of standardized terrestrial ecosystems of the conterminous United States: U.S. Geological Survey Professional Paper 1768, 17 p. (Also available online.)

[24] Scheldeman, Xavier and van Zonneveld, Maarten. (2010). Training Manual on Spatial Analysis of Plant Diversity and Distribution. Bioversity International, Rome, Italy

[25] Treitz, P. and Howarth, P. (2000) Integrating Spectral, Spatial, and Terrain Variables for Forest Ecosystem Classification. Photogrammetic Engineering and Remote Sensing, 66, 305-317. [

[26] Trisurat, Y., A. Eiumnoh, S. Murai, M.Z. Husain and R.P. Shrestha. (2000). Improvements of tropical vegetation mapping using a remote sensing

[27] USGS/Gap (2002) Southwest Regional Gap analysis project (SWReGAP) (2002). Mapping land cover Large Geographic Areas. Integration of GlS and Remote sensing technologies

Bio-efficacy of some insecticides against cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

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***Abstract*— *Laboratory bioassay of eight insecticides namely Chlorpyriphos 20 EC, Ethiprole+ imidacloprid 80 WG, Pymetrozine 50 WP, Lamda cyhalothrin 4.9 CS, Imidacloprid 30.5 SC, Acephate 95 SG, Thiacloprid 240 SC and Fipronil 5 SC was done against cotton mealybug, Phenacoccus solenopsis Tinsley. Among insecticides, Lamda cyhalothrin 4.9 CS was the most toxic with the lowest LD_{50} 16.03 ppm followed by Chlorpyriphos 20 EC (LD_{50} 27.56 ppm), Ethiprole+ imidacloprid 80 WG (LD_{50} 44.82 ppm), Imidacloprid 30.5 SC (LD_{50} 80.68 ppm), Thiacloprid 240 SC (LD_{50} 87.13 ppm), Pymetrozine 50 WP (LD_{50} 181.45 ppm), Acephate 95 SG (LD_{50} 359.61 ppm), Fipronil 5 SC (LD_{50} 705.59 ppm).*

Keywords— *Bioassay, Lamda cyhalothrin 4.9 CS, insecticides, cotton mealybug, LD_{50},*

I. INTRODUCTION

Phenacoccus solenopsis was initially described by Tinsley [12] from specimens infesting the roots and stems of *Boerhavia spicata* and *Kallstroemia californica* within the nests of ants, *Solenopsis geminata*, in New Mexico, USA. It is a bisexual species and completed many generation in a year. Adult females are about 2 to 5 mm long and 2 to 4 mm wide. They are covered with a powdery, waxy secretion with six pairs of transverse, dark bands that are located across the pro- to meta-thoracic segments. A series of waxy filaments extend from around the margin of the body with the pair of terminal filaments longest. The ovisac is composed of fluffy, loose-textured wax strands [4]. They are polyphagous in nature and infest more than 154 plant species of 53 families comprising 20 field and horticultural crops, 45 ornamentals, 64 weeds and 25 bushes and trees [1, 2]. The insect weakens the plants by sucking the sap from leaves, twigs, stems, and sometimes from the roots and also from fruiting bodies. Later, the entire plants become stunted and shoot tips develop a bushy appearance and ultimately causing havoc loss of the farmers. Due to their short life cycle they are capable to increase their numbers and spread rapidly within very short span of time in favourable environmental condition. For this, other control measures except chemical control is time consuming. So, chemical control is the last resort to check the mealybug population within short period of time. Keeping in view, the present study aims to evaluate the efficacy of certain new and conventional insecticides against this pest in order to monitoring insecticide resistance and to identify the potential molecules for developing proper management strategy against this pest.

II. MATERIALS AND METHODS

The present experiment was conducted in the Regional Research and Technology Transfer Station (OUAT), Chiplima, Sambalpur, Odisha during February-March, 2016.

Source of the insecticides

Commercial formulations of Chlorpyriphos 20 EC (Sumitomo Chemical India Pvt. Ltd.), Ethiprole+ imidacloprid 80 WG (Bayer Crop Science Ltd), Pymetrozine 50 WP (Syngenta Korea Ltd.), Lamda cyhalothrin 4.9 CS (Safex Chemicals [India] Ltd.), Imidacloprid 30.5 SC (Coromandel Agrico Pvt. Ltd.), Acephate 95 SG (Rallis India Ltd.), Thiacloprid 240 SC (Bayer India Ltd.) and Fipronil 5 SC (Makhteshim- Agan India Pvt. Ltd.) were obtained from respective principal manufactures. The proprietary products were used to prepare stock solution in distilled water from which further concentrations were prepared subsequently by serial dilution (six to eight different concentrations were used for bioassay). Each treatment including untreated control was replicated thrice.

Bioassay test

Leaf dip method

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Laboratory bioassay was done by leaf dip method [6, 9], unsprayed chrysanthemum leaves were taken and after washed in fresh water those leaf discs were dipped in the test solutions for 5 minutes with gentle agitation. Then they were placed on tissue papers for drying. On drying, these were placed in petri dish and petioles of the leaves were wrapped by water-soaked cotton. On each leaf disc, 20 mealybugs (3rd instar) taken from unsprayed chrysanthemum plants were placed with a fine camel hair brush and the test containers were covered with lid.

Data analysis

The responses (mortality) of mealybugs were recorded after 24 hours post-exposure period. The mortality data were subjected to log-dose probit analysis to generate estimates of a lethal concentration. Probit analysis and lethal concentrations were calculated according to Finney's method by using Polo plus software. This type of bioassay provides an exposure that is more similar that the insects would experience under field conditions.

III. RESULTS AND DISCUSSION

The LD_{50} values obtained from probit analysis for mortality values after 24 hours of each insecticide applied are given in the table 1. According to the results of probit analysis of different tested insecticides, it is found that Lamda cyhalothrin 4.9 CS was the most toxic with the lowest LD_{50} 16.03 ppm to mealybug, *P. solenopsis* followed by Chlorpyriphos 20 EC (LD_{50} 27.56 ppm), Ethiprole+ imidacloprid 80 WG (LD_{50} 44.82 ppm), Imidacloprid 30.5 SC (LD_{50} 80.68 ppm), Thiacloprid 240 SC (LD_{50} 87.13 ppm), Pymetrozine 50 WP (LD_{50} 181.45 ppm), Acephate 95 SG (LD_{50} 359.61 ppm), Fipronil 5 SC (LD_{50} 705.59 ppm). As Fipronil 5 SC has the highest LD_{50} value that refers it is the least effective among all tested insecticides against *P. solenopsis*. Qiao-li *et al*., [8] studied the relative toxicity of some insecticides against 3rd instar nymphs of cotton mealybug with leaf dipping method and found that after 24 hours of treatment, relative toxicity of some insecticides from high to low was lambda-cyhalothrin, profenofos, chlorpyrifos, avermectins, phoxim, carbosulfan, spinosad, chlorfenapyr, beta cypermethrin, methomyl, emamectin benzoate, triazophos, petroleum oil, and rotenone respectively. Seni and Sahoo, [10] studied the bio-efficacy of some insecticides against papaya mealybug, *Paracoccus marginatus* and observed that after 24 hours, Chlorpyriphos 20 EC (LC_{50} 21 µl/l) and Thiamethoxam 25 WG (LD_{50} 44 mg/l) were the most toxic and Buprofezin 25 SC (LC_{50} 1000 µl/l) was the least toxic among the insecticides tested in bioassay test (Potato dip method) and in case of field trials, Thiamethoxam 25 WG, Spirotetramat 240 EC, Imidacloprid 17.8 SL, Dimethoate 30 EC, Lamda-cyhalothrin 5 EC and Buprofezin 25 SC were found effective for management of the papaya mealybug. Tanwar *et al*., [11] reported that chlorpyriphos was effective against mealybug both in laboratory bioassay and in the field. Nagrare *et al*., [7] tested some insecticides against *P. solenopsis* under the laboratory conditions and observed the better efficacy of chlorpyriphos followed by triazophos, diclorvos, endosulfan and spinosad. Banu *et al*., [3] also found effectiveness of chlorpyriphos against *P. solenopsis* and *Paracoccus marginatus* in laboratory condition. Mandal *et al*., [5] conducted laboratory bioassay to determine the relative toxicity of some insecticides against 3rd instar nymphs of cotton mealybug, *P. solenopsis* and found that after 24 hours of exposure, chlorpyriphos 20 EC was most effective followed by followed by dichlorvos 75 EC, triazophos 40 EC and spinosad 45 SC. Seni and Naik, [9] studied the Laboratory bioassay of eight insecticides at Chiplima, Odisha against mustard aphid, Lipaphis erysimi (Kalt.) using leaf dip method in 2016 and found that among insecticides, chlorpyriphos 20 EC was the most toxic (LD_{50} 5.38 ppm) followed by imidacloprid 30.5 SC (LD_{50} 22.14 ppm), ethiprole+ imidacloprid 80 WG (LD_{50} 22.94 ppm), thiacloprid 240 SC (LD_{50} 27.17 ppm), pymetrozine 50 WP (LD_{50} 25.59 ppm), lamda cyhalothrin 4.9 CS (LD_{50} 31.34 ppm), acephate 95 SG (LD_{50} 111.22 ppm), fipronil 5 SC (LD_{50} 234.15 ppm). The value obtain from this experiment can be used in future for monitoring surveys or for the immediate purpose of comparing the current results to that of a previously determined LD_{50} to determine the susceptibility of the target insects has shifted or not. The LD_{50}s can also be used to examine seasonal changes in insecticide susceptibility or compare responses among species or insecticide [6].

IV. CONCLUSION

Thus, the present study revealed that among all the tested chemicals Lamda cyhalothrin 4.9 CS, Chlorpyriphos 20 EC, Ethiprole+ imidacloprid 80 WG, Imidacloprid 30.5 SC, Thiacloprid 240 SC may be recommended for effective management of cotton mealybug, *P. solenopsis* and the value of LD_{50} of different insecticide against cotton mealybug be used in future for monitoring of any resistance development in cotton mealybug.

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Table.1: Dosage mortality response and LD_{50} values of different insecticides for Phenacoccus solenopsis after 24 hours of exposure

Insecticide	Heterogeneity	Slope	LD_{50} (ppm)	Fiducial limits	Relative toxicity
Chlorpyriphos 20 EC	0.68	3.621± 0.579	27.56	22.025- 34.793	25.60
Ethiprole+ imidacloprid 80 WG	1.03	3.466± 0.548	44.82	31.742- 64.481	15.74
Pymetrozine 50 WP	0.78	3.131± 0.494	181.45	142.584- 234.989	3.89
Lamda cyhalothrin 4.9 CS	0.64	3.076± 0.564	16.03	11.969- 20.566	44.02
Imidacloprid 30.5 SC	0.15	3.887± 0.634	80.68	64.731-100.367	8.74
Acephate 95 SG	1.46	2.917± 0.472	359.61	227.687-645.910	1.96
Thiacloprid 240 SC	0.85	3.582± 0.606	87.13	68.415- 109.113	8.10
Fipronil 5 SC	0.16	4.491± 0.791	705.59	572.615- 866.168	1

REFERENCES

[1] Alam, S. F., Seni, A. and Sahoo, A. K. 2011. Biology of the mealybug, *Phenacoccus solenopsis* Tinsley (Pseudococcidae: Hemiptera) on sprouted potato and brinjal plant. *The Journal of Plant Protection Sciences*, 3(2): 32-36.

[2] Arif, M. I., Rafiq, M., Ghaffar, A. 2009. Host plants of cotton mealybug (*Phenacoccus solenopsis*): a new menace to cotton agro-ecosystem of Punjab. *International Journal of Agricultural Biology*, 11: 163-167.

[3] Banu, J. G., Surulivelu, T. Amutha, M. Gopalakrishnan, N. 2010. Laboratory evaluation of insecticide and biopesticides against *Phenacoccus solenopsis* and *Paracoccus marginatus* infesting cotton. *Journal of Biopesticides*, 3(1): 343-46.

[4] Kosztarab, M. 1996. Scale insects of northeastern North America: identification, biology, and distribution. Martinsville, USA: Virginia Museum of Natural History, vii + 650 pp.; 34 pp. of ref.

[5] Mandal, D., Bhowmik, P., Halder, P., Chatterjee, M. L. 2013. Determination of relative toxicity and base line data of different insecticides against cotton mealybug (*Phenacoccus solenopsis* Tinsley). *The Journal of Plant Protection Sciences*, 5(1): 26-31.

[6] Miller, A. L., Tindall, K., Leonard, B. R. 2010. Bioassays for monitoring insecticide resistance. *Journal of Visualized Experiments*; 46: e2129, doi:10.3791/2129.

[7] Nagrare, V. S., Kranthi, S., Dhara, R. K., Jothji, B., Amutha, M., Deshmukh, A. J., Bisane, K. D., Kranthi, K. R. 2011. Compendium of cotton mealybugs. Institute for Cotton Research, Nagpur, India. P. 42.

[8] Qiao-li, L., Guang-wen, L., Yong-yue, L. 2014. Toxicity of 14 insecticides to cotton mealybug *Phenacoccus solenopsis* Tinsely with leaf dipping method. Guangdong Agricultural Sciences. 2014-06. http://en.cnki.com.cn/Article_en/CJFDTOTAL-GDNY201406027.htm dated 06. 12.2017.

[9] Seni, A, Naik, B. S. 2017. Bio-efficacy of some insecticides against mustard aphid, *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae). *Journal of Entomology and Zoology Studies*, 5(6): 541-543.

[10] Seni, A., Sahoo, A. K. 2015. Efficacy of certain insecticides on papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink (Hemiptera: Pseudococcidae). *Journal of Entomology and Zoology Studies*, 3(4): 14-17.

[11] Tanwar, R. K., Jeyakumar, P., Monga, D. 2007. Mealybugs and their management. Technical Bulletin No. 19, 2007, NCIPM, New Delhi, India. p. 12.

[12] Tinsley, J. D. 1898. An ants'-nest coccid from New Mexico. The Canadian Entomologist, 30:47-48.

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A Review of Housing Problems

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***Abstract*— *Housing problems occur both in rich and poor communities across the globe. The objective of this research is to review housing problems which lead to unwholesome environmental conditions. The method used is a review of academic articles, textbooks, internet materials, news articles and publicly available materials on housing problems. Previous authors whose works were reviewed have a convergent view on housing problems including overcrowding and congestion, poor accessibility, substandard and inadequate housing, high cost of building materials, high interest rate and lack of interest by financial institutions to facilitate loans to investors and uncoordinated policies by government. The paper made the following recommendations: (1) government should build low-cost houses to cater for the large number of people who, due to their low-income earnings could not afford a decent apartment; (2) formulation of economic, social and environmental policies that facilitate housing that is both affordable and sustainable by government; (3) improvement of sanitation in poor neighborhoods with poor housing conditions through urban renewal programme.*

Keywords*— *Housing Problems, Low-income Earners, Overcrowding, Review. Sustainable Development.

I. INTRODUCTION

Housing is a basic need of every human being just as food and clothing. It is very fundamental to the welfare, survival and health of man (Fadamiro, Taiwo and Ajayi, 2004). Hence, housing is one of the best indicators of a person's standard of living and his place in the society. In developing countries, poor housing delivery has been attributed to inadequate mechanisms and systems for land allocation, funding, mortgage institutions and infrastructure (Encarta, 2007). Shelter is central to the existence of men (Kehinde, 2010); He also stated that housing involves access to land, shelter and the necessary amenities to make the shelter functional, convenient, aesthetically pleasing, safe and hygienic. According to him, unsanitary, unhygienic, unsafe and inadequate housing can affect the security, physical health and privacy of man. Okafor (2016) asserted that housing all over the world has remained an interdependent phenomenon that faces mankind and it represents one of the most basic human needs which no doubt has a profound impact on the health, welfare and productivity of every individual irrespective of social-economic status,colour or creed.

Housing problem is one of the social problems bred by capitalism manifested as a particular form of housing need with the growth of the urban population and the transformation of a dwelling into a commodity; there is a sharp deterioration in the working people's living conditions and huge rise in apartment (The Great Soviet Encyclopedia, 2000). The housing problems and the housing needs are manifested in overcrowding, poor and inadequate social amenities, unsatisfactory and unwholesome environmental conditions and urban squalor, the absence of open space, the development of land area leading to overcrowding of buildings, inaccessibility within residential areas and in scarcity and high cost of building materials (Ananwa, 2006). According to the Human Development Nigeria (2000), housing problems result mainly from unprecedented growth of urban population. In Nigeria, according to this source, the states with largest proportions of urban divellers far in excess of the national average are Lagos (94%), Oyo (69%), Anambra (62%) and Rivers (60%). The inevitable outcome of this explosion is the aggregation of urban blight and squalor, resulting in the majority of urban divellers living under sub-human conditions in squatter settlements, especially those without employment and any visible means of livelihood (Mordi, 2002). In urban areas, the major housing problems are severe shortages of housing, overcrowding and the spread of slums and shantytowns (Uwejeya, 2012). According to Freeman (2002) and Kotkin (2013), the nature of housing problems in the United States has shifted from shortages to problems of quality, affordability and inability of certain groups in the population to obtain decent housing. Therefore, this research is focused on a review of housing problems.

1.1 Statement of the Problem

The importance of housing to man remains an incontrovertible fact; that housing is second to food, in the basic needs of man is generally becoming old fashioned as some schools of thought are now saying give us house and we will take care of food (Sustainable Cities Programme, Egypt, 2003). The problem of housing shortage grows

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worse by the day in many developing nations including Nigeria, conceivably major tract of housing crises notable in urban centers in most developing nations is that of inadequate supply relative to demand (Olutuah, 2000). According to him, the shortage, in both quantitative and qualitative terms is more acute in urban centers. Omojinmi (2000) observed that people whosleep in indecent urban Nigeria are more than people who sleep in decent houses; thus, it is ascertain that there is inadequacy in population in Nigeria (Arayela, 2003).

According to the United Nations Habitat (2010), 30 percent of the world's urban population lives in slums, deplorable conditions where people suffer from one or more of the following basic deficiencies in their housing: lack of access to improved water; lack of access to improved sewage facilities (not even an outhouse); living in overcrowded conditions; or living in buildings that are structurally unsound; living in a situation with no security of tenure (that is, without legal rights to be where they are, as renters or as owners). The same report said that 35 percent of the world's rural population lives in unacceptable conditions. Overall more than two billion people are in desperate need of better housing (Enoghase, Oladunjoye, Airahuobhor, Okwuke, Orukpe, Ogunwusi and Bakare, 2015).

1.2 Objective

The objective of this paper is to conduct a review of housing problems.

II. CONCEPTUAL FRAMEWORK: CONCEPT OF SUSTAINABLE DEVELOPMENT

This research is based on the concept of sustainable development. World Conference on Environment and Development (WCED) (1987) opined that sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs. Morelli (2010) and Greenwood (2011) saw sustainable development as meeting the resources and services needs for current and future generations without compromising the health of the ecosystems that provide them and more specifically as a condition of balance, residence and interconnection that allow human society to satisfy its needs while neither exceeding the capacity of its supporting ecosystems to continue to regenerate the services necessary to meet those needs nor by our actions diminishing biological diversity. Therefore, the concept of sustainable development needs to be built into housing programmes so as to provide houses for the present generation without compromising the ability of posterity to meet their own housing needs. This is only possible if the present housing problems are addressed and this underscores this review.

III. METHOD

These researchers made use of a review of academic articles, textbooks, internets materials, news articles and publicly available materials on housing problems. The researchers gathered 28 materials, but summarized the characteristics of 10 deemed more relevant to housing problems for the review. This enabled the researcher to make a synthesis of various researchers' views on housing problems and congestion that produced the results, recommendations and conclusion of the study.

IV. REVIEW OF RELATED LITERATURE

Ibimilua and Ibitoye (2015) conducted a study on housing policy in Nigeria and opined that housing problem is peculiar to both rich and poor nations as well as developed and developing countries. Furthermore, They stated that certain problems are associated with housing worldwide which include shortage of housing (qualitatively and quantitatively), homelessness, government shortsightedness about the needs of the people, access to building land, house cost in relation to specification and space standard, as well as high interest rate of home loans. Additionally, they reported that the reasons for shortage of housing in Nigeria include poverty, high rate of urbanization, high cost of building materials, as well as rudimentary technology of building.

Enisan and Ogundiran (2013) carried out a study on challenges of housing delivery in metropolitan Lagos and opined that It is an obligation for any good government to provide affordable accommodation to its citizens. They also stated that there is need for the government of the nation to ensure affordable accommodation to citizen irrespective of their location in the country. Additionally, they reported that the statistics of homelessness is the best we deserve; currently many cannot afford a decent home, nearly half of Nigeria's population lives in urban and semi-urban areas, with majority living in slums and substandard accommodation. To this end, they concluded that Nigerian government and other players in the housing delivery are not treading the same path; other countries tread in meeting up the housing needs of their citizenry. Faith (2014) studied the perceived impact of population growth on housing in Asaba and opined that increasing population places a serious demand on the available facility. She also reported that the rate at which facilities required are provided does not keep pace with the rate at which the population is growing, there is bound to be problem of deficit in the needed resources. Additionally, she stated that in different parts of the world, the explosive growth of the human population in the past few decades has been accompanied by a relatively slow rate of increase in housing, thus leading to housing problems. She concluded

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that the rapid rate of growth of both the urban and rural populations and inadequate funding has made it extremely difficult to provide sufficient housing for the ever-increasing population.

Okafor's (2016) research on the residential housing problem in Anambra State revealed that housing all over the world has remained an interdependent phenomenon that affects every facets of mankind and it represents one of the most basic human needs which no doubt has a profound impact on the health, welfare and productivity of every individual irrespective of socio-economic status, colour or creed. Furthermore, he was of the opinion that in spite of the importance of housing to mankind, there is however, a universal shortage of needed dwelling units especially in developing countries including Nigeria where population growth and urbanization are rapidly on the increase and where the gap between the housing supply and housing demand is so wide. Isma'il, Ezra, Abdulkadir, Muhammad and Hadiza (2015), in their study on urban growth and housing problems in Nasarawa State, Nigeria, noted that housing is a residential structure where man lives and grows. Furthermore, they were of the view that it is therefore universally acknowledged as one of the most basic human needs for survival on the surface of the Earth. They concluded that the demand for housing has been an issue of global concern as the housing provision still remains one of the most difficult problems facing humanity.

Abimaje, Akingbohungbe and Baba (2014) conducted a study on housing affordability in Nigeria and opined that the increasing urbanization in major cities of Nigeria as occasioned by rural urban migration has led to the over population of these towns and cities. They were of the view that this has constituted the focus of many studies. Furthermore, they concluded that only a few focused on affordability of housing that is a serious problem in these centers. Cheserek and Opata (2011) studiedenvironmental and housing problems of low-income households in Eldoret Municipality, Kenya and opined that rapid growth in cities has been accompanied by a rapid growth in the number of urban inhabitants who live in sub-standard and overcrowded conditions. They concluded that demand for residential housing has grown faster than the supply leading to increased prices of land and house-rent and to over-crowded housing.

Gbadebo and Olanrewaju (2015) conducted a study on problems and prospects of housing delivery in Osun State, Nigeria and opined that housing problem is a global phenomenon confronting developed and developing, rich and poor, nations. They were also of the view thathousing is paramount to human existence as it ranks among the top three needs of man. Furthermore, they pointed out that its provision has always been of great necessity to man. They concluded that in a unit of the environment, housing has profound influence on the health, efficiency, social behavior, satisfaction and general welfare of the community. Olotuah (2015) studied accessibility of low-income earners to public housing in Ado-Ekiti, Nigeria and opined that the poor quality of housing inhabited by the poor is a consequence of high level of shortages, in quantitative terms of housing to accommodate them and the lack of the resources to pay for quality housing available. He observed manifestation of severe overcrowding in inadequate dwellings found in urban centers in Nigeria, which are often of poor architectural standard, poor construction, with inadequate services supplied including drainage.

Ezeigwe (2015) conducted a study on evaluation of the causes of housing problems in Nigeria and opined that one of the basic needs of man is shelter and to most groups this means housing. He was also of the opinion that poverty and population increased due to urbanization, high cost of land, non-implementation of the housing policies. Furthermore, he pointed out that Failure on the side of the government, high cost of building materials and corruption which implies the least of the problems is corruption. Tawseef, Tawheed and Shamim (2013) carried out a study on urban housing problems: a micro-level study on residential houses of Tibetan Community in Srinagar City and opined that housing as a problem is not unique to lndia. According to them, there is hardly any country whether developed or under developed in the world today, which could justly claim to have solved this problem. They concluded that problems of housing in the poor or economically less developed countries particularly those of Asia, Far East and Africa assume a more painful complexion because such countries do not only have serious housing shortages, growing additional housing needs and poor housing stocks, but are woefully deficient in essential services and community facilities. Owolabi (2014) studiedcharacteristics of housing in Nigeria and stated that most areas of urban centers of Nigeria are faced with vast numbers of problems, which have resulted to overcrowding, high rents and slum settlements. Furthermore, he was of the opinion that this brought about inadequacies of basic infrastructural facilities and social services in terms of quantity and quality such as pipe borne water, electricity, roads, schools and health institutions.

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Table.1: Summary of Characteristics of some of the Studies on Housing Problems

S/N	Author(s)	Research Topic	Method(s)	Results	Recommendation(s)	Conclusion
1.	Ibimilua and Ibitoye (2015).	Housing Policy in Nigeria: An Overview.	Internet and Questionnaire.	The reasons for shortage of housing in Nigeria include poverty, high rate of urbanization, high cost of building materials, as well as rudimentary technology of building.	There should be access to land, finance and building materials.	Housing problem in urban places take the form of slum dwelling, overcrowding, and substandard housing units.
2.	Enisan and Ogundiran (2013).	Challenges of Housing Delivery in Metropolitan Lagos.	Literature research of authors' works.	Overcrowding and Unplanned human settlement; sprawl development arising from rapid population growth pose challenges to delivery of housing in Lagos.	The government of Lagos State needs to promote policies that will enhance reduction in the cost of building materials and place muchemphasis on accessibility to land.	The main component of housing delivery is availability of land resources.
3.	Faith **(**2014).	The Perceived Impact of Population Growth on Housing in Asaba.	Questionnaire.	Increase in human population which came out as a result places a serious demand on the available facility.	Public housing should be built and sold to migrants who may be low- middle or- high- income earners at a highly subsidized rate.	It is safe to conclude that both public and private participation are needed in the enhancement of the available housing stock in terms of quality and our National Housing Policy that stated that every Nigerian should have access to adequate shelter as a right as well as the achievement of the goals of our National Population Policy for sustainable development which aims at the attainment of a balance between the rate of population growth and resources.

4.	Okafor (2016).	The Residential Housing Problem in Anambra State (A Case Study of Onitsha Metropolis).	Internet and Questionnaire.	High cost of building materials and high cost of infrastructural development; middle and low income earners cannot afford the selling price of developed houses; therefore, investors cannot recoup their investment.	Less emphasis should be placed on direct provision of housing.	It is therefore important for both private and public sectors to carefully integrate ideas in line with the housing reforms to stimulate and generate sustainable development of the housing sector.
5.	Isma'il, Ezra, Abdulkadir, Muhammad and Hadiza (2015).	Urban Growth and Housing Problems in Karu Local Government Area of Nasarawa State, Nigeria.	Questionnaire, interviews and Observation.	This conforms to the view that inadequate housing in Nigeria is a manifestation ofPoverty because majority of the people earn low income and could not afford the rising cost of accommodation in towns and cities.	Provision of basic amenities and infrastructural facilities and utilities is very necessary to reduce housing problems in the Karu area.	This corroborated with the views of the residents in the area who identified the housing problems in order of prominence as overcrowding of houses leading to spread of diseases, poor accessibility resulting to congestion.
6.	Abimaje, Akingbohungbe and Baba (2014).	Housing Affordability in Nigerian Towns: A Case of Idah, Nigeria.	Questionnaire.	With the present high cost of building materials, labour, land which consequently has brought about increase in cost of housing and rent, these low income earners will not be able to afford adequate housing.	Emphasis should be placed on low and medium housing units using earth blocks, burnt bricks, compressed earth bricks and intermediate technology. This will reduce the cost of housing, thus making it more affordable to many people.	The greater percentage of the people in the study areas have no adequate accommodation and cannot afford adequate ones where available as they pay more than 30% of their income on housing.

7.	Cheserek and Opata (2011).	Environmental and Housing Problems of Low-Income Households in Eldoret Municipality, Kenya.	Both open and closed-ended Questionnaire.	Majority of low-income groups reside in slum areas due to poverty and partly as a result of low educational achievement that cannot enable them to obtain high-income jobs.	Low-income residents need to be educated on and be made aware of the benefits of personal hygiene and environmental quality in maintaining good health.	Low income people should be enlightened to put value for their money spent on housing and other services.
8.	Gbadebo and Olanrewaju (2015).	Problems and Prospects of Housing Delivery in Osun State.	Questionnaire.	Identified the increase and improvement of overall quality of housing, provision of infrastructure facilities, public and private sectors of the economy.	There should be housing legislation that will enhance high quality housing production to improve building industry, reduce housing shortage with improved standard.	It identifies high cost of building materials, lack of finance, problem of land acquisition, lack of research and manpower training.
9.	Olotuah (2015).	Accessibility of Low-Income Earners to Public Housing in Ado-Ekiti, Nigeria.	Questionnaire.	Low-income earners were almost schemed out Regarding access to the buildings.	The provision of infrastructural facilities and social services should be made by government in these housing schemes.	The accessibility of low-income earners in Ado Ekiti to two public housing schemes in the city was found to be highly deplorable.
10.	Ezeigwe (2015).	Evaluation of the Causes of Housing Problems in Nigeria.	Oral interview, observation and Questionnaire.	High cost of land, high cost of building materials and corruption.	The private sector to collaborate with the government in provision of housing for Nigerians, developers to invest in mass housing provision as this will help in reducing the deficit in the housing sector.	Housing deficit is one of the major problems suffered by urban and rural areas in the country and the analysis of the data generated from the study area shows that the main causative factor is poverty.

Source: Researchers'design, 2017

V. RESULTS AND DISCUSSION

Housing problem is peculiar to both rich and poor nations as well as developed and developing countries.The researchers in Table 1 are all very relevant to housing problems. From the table 1,Ibimilua and Ibitoye (2015), Faith (2014), Okafor (2016) and Abimaje*et al* (2014) have a convergent view that high rate of urbanization and increase in population are the leading factors of housing

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problems. Cheserek and Opata (2011), Enisan and Ogundiran (2013) and Olotuah (2015) had a unity of opinion that housing problems came as a result of overcrowding of people in their study areas. Human populations were unequal with the available housing infrastructure for their habitation. Ibimilua and Ibitoye (2015) and Enisan and Ogundiran (2013) viewed the key effect of housing problem as homelessness because majority of people in urban cities live in slums and sub standard accommodation while many others cannot afford the price of house rents. Ezeigwe (2015) and Ibimilua and Ibitoye (2015) observed that high cost of building materials and poverty contributed to housing problem. On the approach of proffering solution to housing problems, previous research by Enisan and Ogundiran (2013) suggested that government needs to promote policies that will enhance reduction in the cost of building materials. Isma'il*et al* (2015) studied that the demand for housing has been an issue of global concern as the housing provision still remains one of the most difficult problems facing humanity. Ibimilua and Ibitoye (2015), Enisan and Ogundiran (2013), Faith (2014), Okafor's (2016), Isma'il*et al* (2015), Abimaje*et al* (2014), Cheserek and Opata (2011), Gbadebo and Olanrewaju (2015), Olotuah (2015), Ezeigwe (2015), Tawseef*et al* (2013) and Owolabi (2014)made use of questionnaire, oral interview, observation and review of related literature which is a standard method for obtaining data and information in a research.

VI. RECOMMENDATIONS

Based on the review and results of the study, it is recommended as follows:

i. Government should build low-cost houses to cater for the large number of people who, due to their low-income earnings, could not afford a decent apartment
ii. Employers should also award building loan to their staff with no interest to enable them build their own houses. This will go a long way towards ensuring equal distribution of house ownership
iii. Government should develop economic, social and environmental policies that facilitate housing that is both affordable and sustainable.
iv. There should be urban renewals at slum area of cities to change the poor environmental conditions prevalent in the localities.

VII. CONCLUSION

This paper discussed housing problems by reviewing various works done by previous authors. From the review, many authors agreed that housing problem was universal. Generally, the authors concurred that housing problems in urban places take the form of slum dwelling, homelessness, overcrowding, squatter settlements and substandard housing units. Again, in the rural areas, poor housing quality, deficient environmental conditions as well as inadequate infrastructural facilities are the order of the day. This study therefore, concludes that for a sustainable development to be achieved both in urban and rural areas, adequate solutions should be provided for housing problems.

VIII. ACKNOWLEDGEMENT

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REFERENCES

[1] Abimaje, J., Akingbohungbe, D.O., and Baba, A.N. (2014).Housing Affordability in Nigerian Towns: A Case of Idah, Nigeria.*International Journal of Civil Engineering, Construction and Estate Management*, 1 (2):1-9.
[2] Ananwa, B. (2006). Housing Problem and Congestion in Onitsha.UnpublishedB.Sc, Thesis, Department of Geography and Regional Planning, Delta State University, Abraka, pp. 3-6.
[3] Arayela, O. (2003). Panacea for Increasing Housing Stock at Reduced Cost in Nigeria.*African Journal of Development Studies*, 3 (1):12-16.
[4] Aribigola, A. (2006). Rational Choice Model and Housing Decision in Akure, Ondo State, Nigeria.*Confluence Journal of Environmental Studies*, 1 (1): 53-63.

[5] Cheserek, G.J. and Opata, G.P. (2011).Environmental and Housing Problems of Low-Income Household in Eldoret Municipality, Kenya.*Journal of Emerging Trends in Economics and Management Sciences (JETEMS)*, 2 (4):1-5.
[6] Encart (2007).Evalaution of the Causes of Housing Problems in Nigeria: A Case study of Awka Capital City of Anambra State. *Journal of Economics and Sustainable Development*,6 (20):1-7.
[7] Enisen, O. and Ogundiran, A. (2013). Challenges of Housing Delivery in Metropolitan Lagos.*Research on Humanities and Social Sciences*, 3 (20): 1-9.
[8] Enoghase, S., Airahuobhor, A., Oladunjoye, P., Okwuke, E., Orukpe, A., Ogunwusi, B. and Bakare, S. (2015): Nigerias 17 Million Housing Deficit Challenges Buhari Daily Independent Online. Accessed at: http://dailyindependentnig.com/2015/04/nigerias-17m-housing-deficit- challenges- buhari/, 22-10-2017.
[9] Ezeigwe, P. (2015). Evaluation of the Causes of Housing Problems in Nigeria: A Case Study of Awka the Capital City of Anambra State. *Journal of Economics and Sustainable Development*, 6 (20):1-7.
[10] Fadamiro, J.A., Taiwo, A.A. and Ajayi, M.O. (2004).*Sustainable Housing Development and Public Sectors Intervention in a Developing Country*.Editorial on Scientific and Environmental Issues in Population, Environmental and Sustainable Development.Published by Grans, pp. 50-100.
[11] Faith, J. (2014).The Perceived Impact of Population Growth on Housing in Asaba.*Journal of Research in Arts and Social Sciences*, 8 (2):1-8.
[12] Freeman, L. (2002). America's Affordable Housing Crisis: a Contract Unfulfilled. *American Journal of Public Health*, 92 (5): 709-712.
[13] Gbadebo, M.A., and Olanrewaju, S.B. (2015).Problems and Prospects of Housing Delivery in Osun State.*Journal of Humanities and Social Science*, 20 (8):1-7.
[14] United Nations Environment Programme(UNEP) and United Nations Human Settlement Programme(UN-HABITAT) (2003) *Sustainable Cities Programme*, Alexandria, Egypt.
[15] Ibimilua, A.F., and Ibitoye, O.A. (2015).Housing Policy in Nigeria: An Overview.*American International Journal of Contemporary Research*, 5 (2):1-7.
[16] Ismail, M., Ezra, I., Abdulkadir, M.Y., Muhammad, A.T. and Hadiza, T.A. (2015).Urban Growth and Housing Problems in Karu.*Global Journal of Research and Review*, 2 (1):1-13.
[17] Kehinde, F. (2010). Housing Policy and Development in Nigeria.In: Omotosa, F., Agagu, A.A., and Abegunde, O. (ed), *Governance, Policies and in Nigeria. Port-Novo, Editions SonoudAfrique*
[18] Kotkin, J. (2013). Americas Emerging Housing Crisis. American Works. Accessed at: *http://www.forbes.com/joelkotkin/2013/07/26/Americas-emerging-housing-crisis*
[19] Mordi, S. (2002). Social Policy and Housing Needs in Nigeria. Paper presented at *A Two- Day Workshop on Your Right to Own a House at Asaba by the Delta State Ministry of Housing*, June, 26-27, 2007.
[20] Morelli, J. and Greenwood, R. (2011).Environmental Sustainability and Professional Responsibility.*Seventh Environmental Management Leadership Symposium*, 12-13 May. 2011, Rochester, NY.pp.22-24.
[21] Okafor, B.N. (2016). The Residential Housing Problem in Anambra State (A Case Study of Onitsha Metropolis). *International Journal of Civil Engineering, Construction and Estate Management*, 4 (2):1-18.
[22] Olotuah, A. (2015). Accessibility of Low-Income Earners to Public Housing in Ado-Ekiti, Nigeria.*Civil and Environmental Research*, 7 (7):1-6.
[23] Olutuah, A. (2000). Housing Needs in Low-income Civil Servants, AdoEkiti, Nigeria.*African Journal of Social and Policy Studies*, 1 (2):27-32.
[24] Omojinmi, I.O. (2000). Sina Technical Workshop on housing Co-operatives Nairobi, October 6-17, 2000.
[25] Owolabi, B.O. (2014). Characteristicesof Housing in Nigeria: A case Study of Oyo State, Nigeria. *Academia Journal of Environmental Sciences*, 2 (8):1-19.
[26] Tawseef, T., Tawheed, Y. and Shamim, A.S. (2013). Urban Housing Problems: A Micro-Level Study on Resiedntial Houses of Tibetan Community in Srinager City. *European Academic Research*, 1 (5):1-13.
[27] Uwejeya, D.O. (2012). Resource Exploitation and the Environment: The Case of Housing in Auchi and Igarra, Edo State Nigeria. In Efe S.I. and Atubi A.O (eds) *Environment and Socio-Economic impact of Natural Resource Exploitation in Auchi and Environs Edo State, Nigeria*. An Occasional Publication Series of the Department of Geography and Regional Planning, Delta State University, Abraka.
[28] World Commission for Environment and Development (WCED) (1987). *Our Common Future*.Oxford University Press.

Cu^{2+} removal from aqueous solution by *Platanus orientalis* leaf powders

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Abstract— *An investigation steeredto ascertain the adsorption potential of fallen Platanus orientalis leaf powder (FPOLP) ascost-effective adsorbentto remove*Cu^{2+}*from an aqueous solution. The FPOLP was physically activated in two different forms (oxidation) and (*N_2*) flowconditions. Batch operations for* Cu^{2+} *adsorption were performed to ascertain adsorption characteristics of FPOLP and activated samples. The results indicated that the optimum activation temperature and time were 500 °C and 180 min, respectively, while the best* Cu^{2+} *removal was achieved when the solution was controlled at pH 3 and the adsorbent dosage at 3 g/L.Additionally, an evaluation of the mechanism of adsorption fitted very well intopseudo-second-order. FTIR, scanning electron microscopy and BETmeasurements suggested that the new functional groups and the increased surface area related to the porous structure played a critical role in* Cu^{2+} *removal by the activated leaf powder. FPOLP has a great potential to remove* Cu^{2+} *in an aqueous solution.*

Keywords— Platanus orientalis leaf; Cu^{2+}***; kinetics; isotherms; adsorption.***

I. INTRODUCTION

Industrialization certainly has come with many associated challenges and consequences, some of which include environmental pollution. Heavy metals pollution, as one of the contributory factors to the unending environmental contamination, has attracted global concerns in the past few decades (Özer et al. 2004).Among the wide range of pollution, wastewaters which contain heavy metals such as copper end up being discharge into the environment without appropriate and adequate treatments, resulting in severe environmental pollution(Argun et al. 2008).In addition, the pollution is coupled with increasing demand for freshwater, *e.g. g*lobal water demand is estimated to increase by 55% as a result of industrialization and domestic use by 2050(UNWater 2015).Copper, as a very valuable metal used in our daily lives such as pipes, valves and fittings(WHO 2010). However, the process of its exploitation and refining result in the emission of sulfur dioxide gases (SO_2) into the atmosphere. This phenomenon poses health hazards to humans like capillary damage, irritation, mucosal and hepatic(Yao et al. 2010, Solomon 2009, Zeitoun et al. 2014).

Based on World Health Organization's guidelines, when copper (Cu)concentration exceeds 1 mg L^{-1} in drinking water, it becomes unsafe for humans (Gorchev and Ozolins 2011).In the manufacturing companies or industries, Cu effluents concentration above 3.0 mg L^{-1} are not safe for discharge (Robert O. Pickard 2011), however not much has been achieved in terms of a viable and safe disposal method for Cu sludge. Therefore, it is very necessary to find effective remedies to curtail therisks associated with this problem. When Cu sludge is appropriately treatment before discharge, it will prevent possible pollution of freshwater bodies or the environment. In the past few decades, several attempts aim at finding harmonious solution to the problems of wastewater purification have been made by a number of research works. The adopted approaches include but not limited to chemical precipitation ((USEPA 2013), reverse osmosis(Kurniawan et al. 2006), membrane filtration(Juang and Shiau 2000) and adsorption (Chen et al. 2010). Among the various methods listed, adsorption techniquesappear to have comparative advantage over other techniques, these include high efficiency, environmentally friendly, simple operation, minimum complication (Sohn and Kim 2005). These merits make adsorption more suitable for wastewater treatment(Koumanova and Allen 2005, Erdem et al. 2004,Anagement 2014).

The use of waste materials(Wang and Guo 2011) and agricultural by-products(Venkata Ramana et al. 2012)for use as adsorbents to treat heavy metals is increasingly gaining attention, such as Neem leaves(Ibrahim and Sani 2015),(Nwabanne and Igbokwe 2012), bael leaves (Chakravarty et al. 2010), *Platanus orientalis* leaves(Abadian et al. 2015),teak leaves(Goswami et al. 2013) and *Elaeis guineensis* leaves (Soliman et al. 2016). Large quantities of waste (litter) from *Platanus orientalis* are generated worldwide, most particularly in South-East Europe, Asia and Northern Iran,during winter season.This results in significant disposal challenges due to the shear amount of waste generated from shed leaves.

The focus of thisresearch is to usethe un-activated (FPOLP)and activated (AC) leaf powder of *Platanus orientalis*to investigate the uptake of Cu^{2+} from the aqueous medium.The kinetics and mechanisms involved in the adsorption are subsequently discussed thoroughly.

II. METHODS AND EXPERIMENTAL MATERIALS

2.1. Materials and chemicals

Samples of *Platanus orientalis*leaves(Fig. 1a) were obtained in December 2015, during pre-winter season from the West Mafanshang Campus of Wuhan University of Technology, Wuhan, Hubei Province, China. The dry leaveswere carefully cleaned using deionized produced from Milli-Q Direct 16 distillation machine with Vent Filter MPK01, and oven-dried.An amount of 300 grams of these leaves were roughly crushed, washed using deionized water several times to purify the sample. Subsequently, the leaves were put in 101-1AB, manufactured byTianjin TaisiteInstrument Co. Ltd at 80 °C for 48 h to improve its brittleness. The dried samples were then crushed into fine powder smaller than 75 µmusing a mechanical grinder (RK/XPM – Ø 120 ×3,Wuhan Rock Comminution Instrument Co., Ltd). The leaf powder was repeatedly washed until its turbidity disappeared. After that, the mixture was decanted and filtered followed by another 24 h drying at 80 °C. They processed sample was kept in a moisture-free apparatus for use.

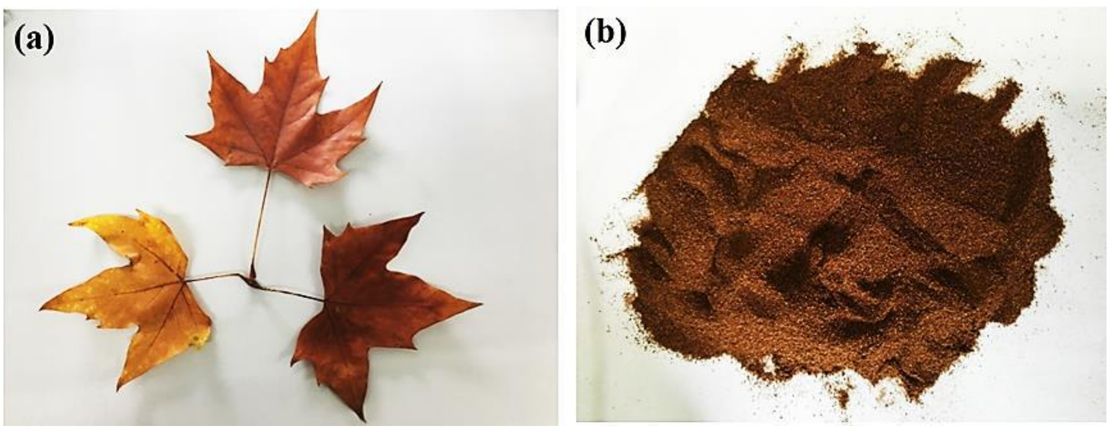

Fig. 1. (a) Leaves of fallen Platanus orientalis from West Mafangshan Campus,Wuhan University of Technology, Wuhan, Hubei province, China, and (b) ground FPOLP powder from (a).

A stock Cu^{2+}solutionwas prepared via $CuSO_4{\cdot}5H_2O$, dilutions were made tomeet the designed concentrationsfor theadsorption.Other chemicals used in the investigation were nitric acid (HNO_3), hydrochloric acid (HCl), potassium nitrate (KNO_3), potassium bromide (KBr), sodium hydroxide (NaOH), ammonium citrate tribasic ($C_6H_{17}N_3O_7$), ammonia solution ($NH_3{\cdot}H_2O$), ethanol absolute (CH_3CH_2OH) and bis (cyclohexanone) oxalyldihydrazone (BCO) and neutral red. All the above chemicals and reagents listed were of standards, produced byChina Sinopharm company,

2.2 Sample activation

Powdered FPOLPsamples were physically activated at different temperatures of 300, 400, 500 and 600 ºC for different time intervals at 60, 180 and 300 min, respectively, using both muffle furnace (SX2-5-12,Hubei Yingshan Jianli Electric Furnace Co., Ltd) and electric tube furnace (SK-G06123K, Tianjian Zhonghuan Exp. Electric Furnace Co., Ltd).An amount of 40 g sample was activated (oxidation) and labeled as AC1and another (under nitrogen) flow labeled as AC2, repectively. The samples were kept in crucibles and covered appropriately before carbonization to avoid direct contact of heat with samples.

2.3 Adsorption series

Different concentrations 25, 50, 75 and 100 mg L^{-1} were examined with varied adsorbent load 1, 2, 3 and 4 g L^{-1}. A fixed volume of 25 mL of various concentrations were put into 250 mL conical flask each to conduct the experiment. The preliminary pH of all solutions were accustomed in the range of 2 – 4 by adding either diluted NaOH or HCl. At each time, the explored mixtures were shaken in the thermostatic shaker bath (BS-S, Guohua Electrical Appliance Co., Ltd) at 150 rpm at designed temperatures for

120 min. After that, the suspensions were then filteredand both the adsorbate and residue used for subsequent analysis. The experimental kinetics regarding the amount of Cu^{2+} ions adsorbed $q_t$at a given period *s* (mg g^{-1}) were evaluated by employing the following relationship:

$$q_s = V\frac{(C_o - C_f)}{wt} \qquad (1)$$

where the parameters are initial concentration C_0andCu^{2+} concentrationC_f(mg L^{-1})ata given period s respectively, Volume of Cu^{2+}(L)and w_t adsorbent mass (g). The Cu^{2+}removal efficiency (RE) was examined via this relationship:

$$RE = \frac{(C_m - C_t)}{C_m} \times 100\% \qquad (2)$$

where $C_m$represents initial Cu^{2+}concentration and(Ct) Cu^{2+}Conc. (mg L^{-1}) at given period t.

2.5 Sample characterization

The interaction mechanisms between adsorbate and adsorbent are investigated throughsolid addition reported in (Wan Ngah and Hanafiah 2008). A 45 mL solution of 0.01mol/L KNO_3prepared was pipetted six 250 mL flasks. To achieve the desired pH 0 – 12, diluted Sodium hydroxide and Hydrochloric acid were intermittently employed using pH meter(Cyberscan 2100,EUTECH INSTRUMENT). The controlled pH_S were chronicledas pH_i (initial), 3 g L^{-1} mass is load in each KNO_3 solution and tightly covered followed by mechanical agitation at a speed 150 rpm for 240 min at 25 °C. After the shaking completed, the resultant mixtures were re-examined and noted as pH_f(final).The differences in pH, thus pH_i and pH_f values were used to determine the change in pH ($\Delta pH = pH_f - pH_0$). The ΔpH values were plotted against the pH_i, and the pHpzc was then determined.

FTIR study was conducted using Thermo-scientific NICOLET (iS10) Spectrometer to analyze the surface functional groups, 0.01g dried sample from various adsorbents was ground into fine particles and mixed well with 0.05g potassium bromide (KBr) prior to being suppressed as pellets. The spectrum ranging from 500 to 4000$cm^{-1,}$ it was allowed to standardize 32 times and the background set.

The SEM analysis was performed using analytical JSM-IT300 manufactured by JEOL Ltd. Japan.The BET was analyzed using ASAP 2020M indicating thespecific surface area (m^2/g) of FPOLP and AC1.

III. RESULTS AND DISCUSSION

3.1 Activation temperature effect

An investigation into acivation temperatue was conducted from 300 to 600 ºC using the muffle furnace for 180 min.After activation,3 g L^{-1}sample was added into 25 mL solution of concentration 100 mg L^{-1} Cu^{2+}adjusted at pH 3 for the adsorption experiment. The results in Fig.2 showed that only approximtely 23% of the Cu^{2+} was removed by using the unactivated powder sample (FPOLP). With the increment in activation temperature from 300 to 500 ºC, the Cu^{2+}*RE* was increased apparently from around 26% to greater than 97%, however with a slightly decrease to around 95% being observed at 600 ºC, indicating that 500 ºC was the optimum temperature for activation.

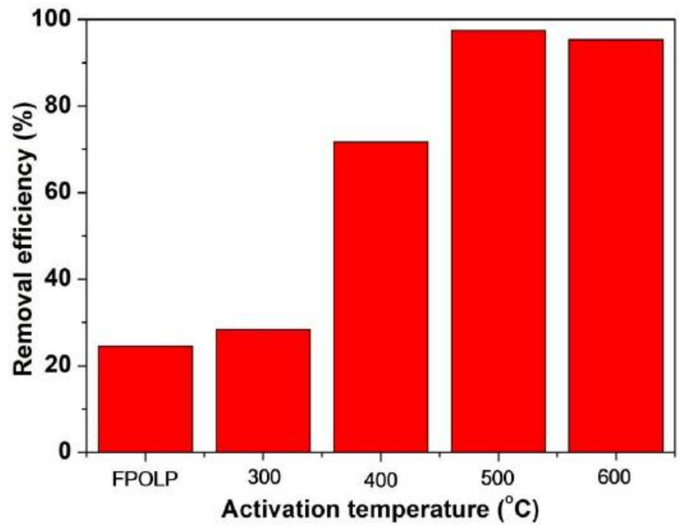

Fig.2: u^{2+} removal efficiency at pH 3 and different temperatures

3.2 Activation conditions and mass load

Investigation was conductedto understand the role of activation conditions for Cu^{2+}*RE*.The adsorption experimentwas conducted by100 mg L^{-1} concentration of Cu^{2+} at pH 3, 25 °C and 150 rpm using FPOLP, AC1 and AC2 by way of adsorbents were conducted. The AC2 was also activated under 500 ºC for 3 h but with nitrogen flow. FPOLP output(Fig. 3a) is in a way less efficient as compared to the AC1, but certainly more efficient than AC2. It is indicating that the use of electric furnace under (oxidation) condition was much more effective in producing activated samples for better Cu^{2+} adsorption, as compared to the tube resistance furnace which had nitrogen flow. The *RE* of Cu^{2+}increased very slowly when the amount of FPOLPloadwas varied beginning 1 up to3 g L^{-1},e.g. Cu^{2+} exhaustion increased to 33.38 % downfrom 21.98 %. Insignificant change was noted however when adsorbent mass was subsequently increased to 4 g L^{-1}. Similar Cu^{2+} removal behavior was observed for AC2, although with overall lower performance.The *RE*of Cu^{2+} usingAC1 increased slightly to a maximum of 95.75% from 94.74%

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with increasing adsorbent load from 1 up to 3 g $L^{-1}$with no apparent increment inremoving $Cu^{2+}$when adsorbent mass stood at 4 g L^{-1}.

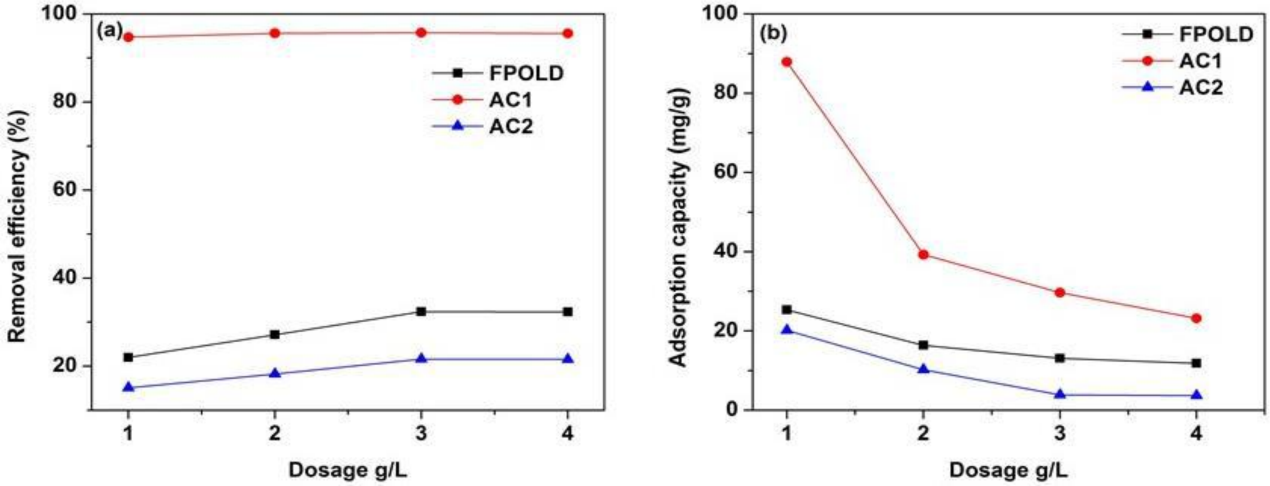

Fig.3: Activation conditions and mass load effect over Cu^{2+} (a) removal efficiency and (b) adsorption capacity using 100 mg $L^{-1}$$Cu^{2+}$ solution.

In contrast, Fig. 3bshowed the effects of sample dosage on the adsorption capacity (q_e).It is overall presented AC1 apparently greater compared to FPOLP, with AC2 having the lowest Cu^{2+} up take capacity, e.g., adsorption capacity of AC2 decreased gradually from 20.18 to 3.70 $mgg^{-1}$with varied adsorbent load of1 to 4 g L^{-1}. While FPOLP ability to adsorb Cu^{2+} reduced from 25.33 to 11.85 mg g^{-1}.Although showing the same declining trend for AC1, Cu^{2+} adsorption capacity of AC1 reduced from 87.93 to 23.19 mgg^{-1}. Therefore, activation under atmosphere conditions (oxidation)was used for subsequent investigations.

3.3Activation time influence

Investigation carried out to determine Cu^{2+}uptake efficiency using sample activated at 500 ^{o}Cunder atmosphere conditions (oxidation) forvarious times, *e.g.* 60, 180 and 300 min correspondingly. Noticeably, Cu^{2+}*RE* gradually diminished when Cu^{2+}concentration changed from 25 to 100 mg L^{-1} for all three samples investigated, *e.g.* the removal percentage of $Cu^{2+}$by the sample activated for 60 min decreased from around 70% to less than 60%. In addition, it is observed that Cu^{2+}*RE* increased with increasing time for adsorption from 60 to 180 min. This was followed by a decrease when activation time was prolonged to 300 min, especially whenCu^{2+}concentration was increased (e.g. 100 mg L^{-1}) indicating a detrimental effect due to longer activation time.This informed the activation duration for subsequent sample preparations.

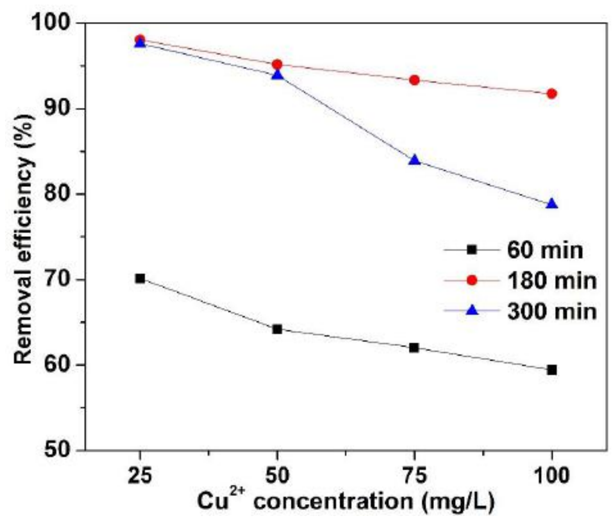

Fig.4: Influence of activation durationCu^{2+}removal efficiencyvia AC1 load

3.4 Influence of solution pH

Solution pH is a vital parameter that has appreciable influence on metal ions uptake (Reddy et al. 2012). An investigation into the effect of solution pH was conducted using3g L^{-1} AC1adsorbent (*i.e.* 75 mg AC1 sample was added into 25 mL solution) with Cu^{2+} concentration100mg L^{-1}. Fig. 5a shows a pH dependent adsorption since a change in pH from 2.0 up to 3.0 resulted in a significant improvement in Cu^{2+} adsorption for all investigated adsorbent loadbut began to decline slightlywith pH 4.It became evident therefore to use pH 3 as optimum since it yielded the highest removal efficiency ofCu^{2+} investigated.

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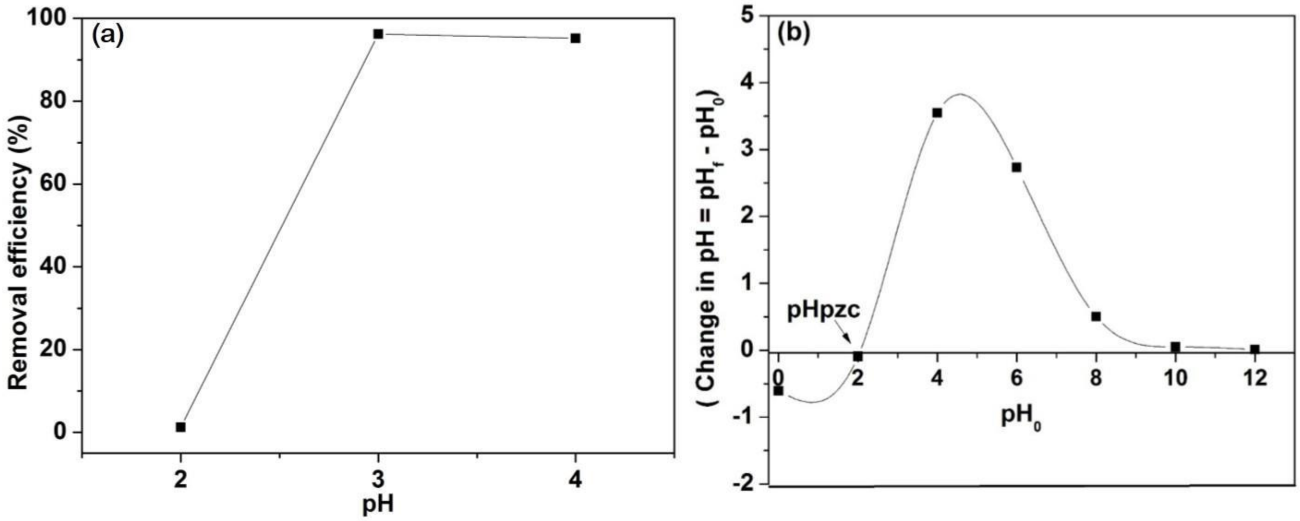

Fig.5: (a) pH effect onCu^{2+}removalAC1 at 25 ºC and 150 rpm; (b) pHpzc of AC1.

Investigation based on pH at point zero charge (pHpzc) of the adsorbent was performed to ascertain the mechanisms of interaction in view of the interdependent roles Cu^{2+} and adsorbent both play(Pellera et al. 2012).Fig. 5b indicated that the pHpzc of AC1 in this study was pH 2, indicating that at pH 2, the superficial charge of the loaded mass becomes nonalignedwhile the attraction force neutralized between the two interfaces. Once the solution pH is less than the pHpzc, the charges on the adsorbent surface generally becomes positive. This will most likely result in a repulsive behavior when the various ions come into contact with each other. In the field of magnetism, like poles repels while unlike poles attract. The inverse leads to a negatively charged surface, thus, (Cardenas-Peña et al. 2012). In this case, cation attraction most likely would take place. In this investigation, the pH 3 at which maximum adsorption took place was greater than the pHpzc,this could probably be responsible for the favourable adsorption due toelectrostaticenergy acting on both surfaces.

3.5 Role of contact time

As an important parameter, the role of contact time in this study was scrutinized as reported in Fig. 6. It was detected that, symmetry status was achieved beyond 40 minutes with a 96.6%. Moving forwad, Cu^{2+}removal at the end of the 120 minutes cycle,with a given original concentration of 100 mg L^{-1} at 25degreesin AC1 3 gL^{-1} evolved differenntly. This can be interpreted as two faces interaction *e.g*. the initial adsorption was risen from 75 to 92% in the early 20 minutes of the process (Fig. 6a), then, came much reduced rate of reaction until 40 minutes, consistent with other studies (Villaescusa et al. 2004).This is an indication of a high attraction force between the AC1surface and the Cu^{2+}ions. Since all active sites were unoccupied intially with high solute concentration gradient (Abadian et al. 2015). The reduction in rapid uptake towards the final lap of the investigation may only point to a conclusion that, diminishing unoccupied adsorbent sites and the concentration gradient(Pathania et al. 2013).The adsorption capacity (Fig.6b) followed a similar trend with that of the removal efficiency, showing an approximate 32 mg g^{-1} being recorded at 120 min.

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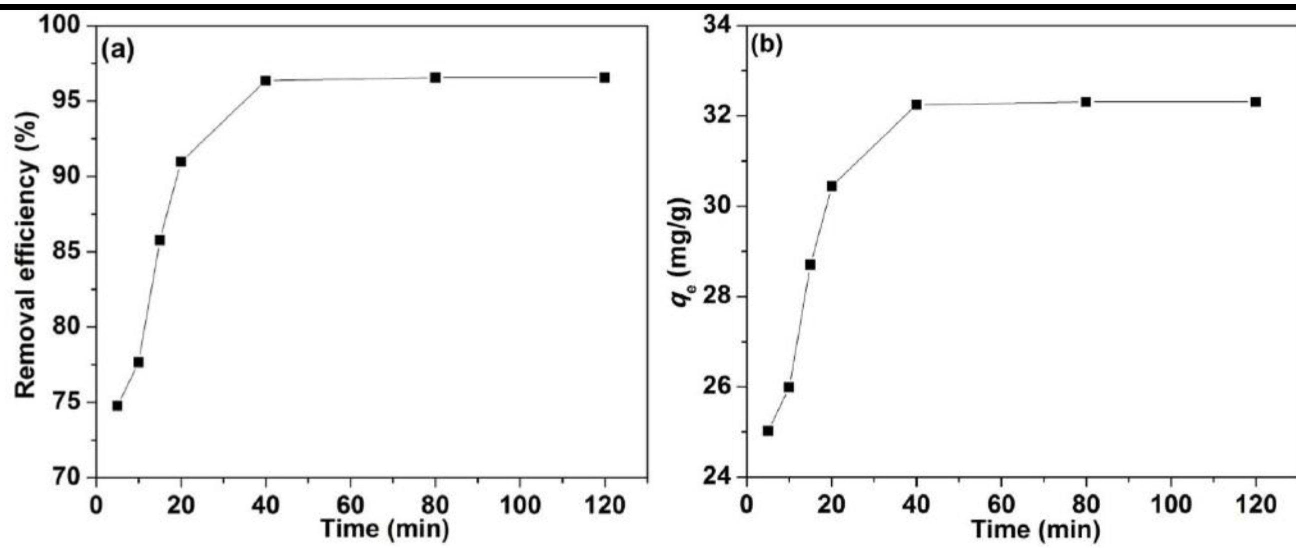

Fig. 6: Relationship of contact time with respect to Cu^{2+} concentration of 100 mg L^{-1} at 25°C, 150 rpm, adsorbent concentration 3 g L^{-1}(a) removal efficiency and (b) adsorption capacity.

3.6 Kineticsphenomenon

There exists some relationship between adsorbent-adsorbate in the adsorption of Cu^{2+} on AC1which can be expatiated using two different approaches. Firstly, Lagergren 1st order model relation is employed followed by 2nd order model formulation which represented by the parameters as follow:

$$log(q_e - q_t) = log q_e - t\frac{k_1}{2.303} \quad (3)$$

$$\frac{t}{q_e} = \frac{1}{k_{q_{e2}}} + \frac{t}{q_e} \quad (4)$$

where $k_1$andk_2 arerepresenting the Lagergren rate coefficientsrespectively,q_t andq_e (mg g^{-1}) are quantitiesCu^{2+}uptake at given time *a*andsymmetry.

Adsorption kinetics were evaluated by using 100 mg L^{-1} Cu^{2+}solution temperature at25°C employing AC1 asmade known in Fig. 7.Verification of Lagergren 1storder model revealedk_1 (0.138 min^{-1}) inFig. 7a) and q_e(23 mg g^{-1}) as products of intercept and slope determined from the graph of log ($q_e - q_t$) against time t (min^{-1})with r^2 = 0.96. The Lagergren 1storder model was not suitable for the first evaluation of Cu^{2+} adsorption onto AC1 as the calculated q_e (q_e, cal) was significantly lower than the experimental q_e (Q_e, exp) as indicated below, Table 1.

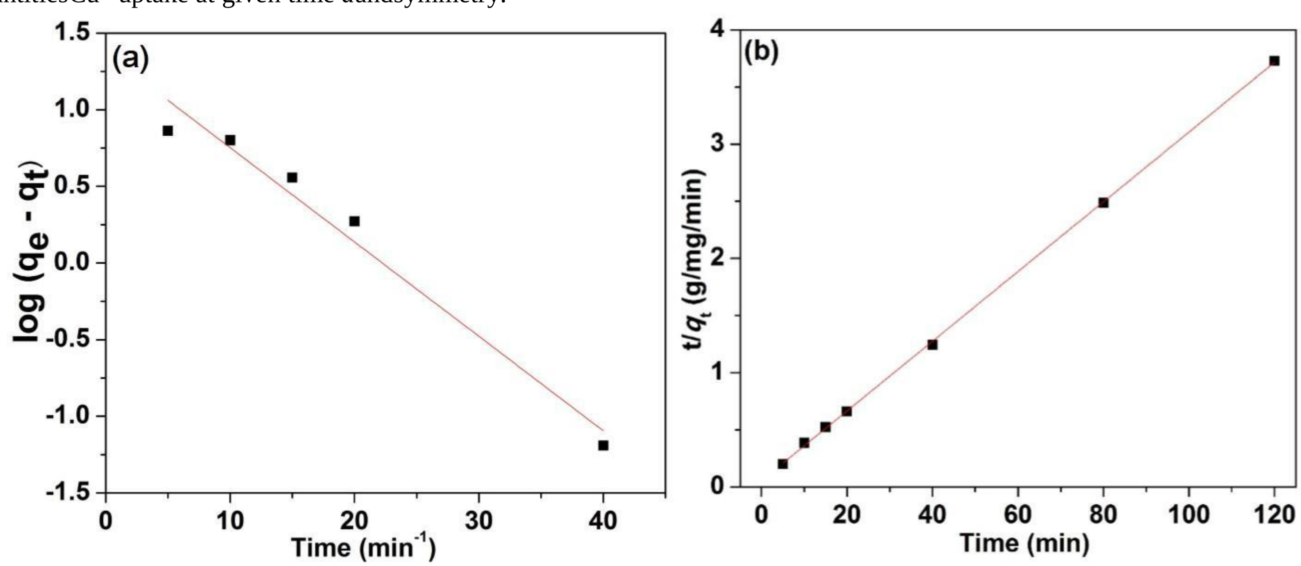

Fig.7: Kinetic modelgraphs on Cu^{2+}uptake adsorption by AC1: (a) Lagergren 1storder and (b) 2ndorder model correspondingly.

The plotted yields, helped to determine the value for k_2 which represented a demonstrating of pseudo 2nd order (Ho and McKay 1999)which stoodat0.0167 (g mg^{-1} min) whileq$_e$ harmoniously stood at 32.78 (mg g^{-1}). A plot of the coordinates t/q against t ispresented in Fig.7b, with coefficient of R^2recorded 0.99.It has been reportedly severally through adsorption investigations that, k_2 valueusually is near or close to the experimental equilibrium while k_1 in many scenarios are lower than k_2.Additionally, observation is that, the coefficient of correlation (r^2) is mostly lower than 0.999. adequate appreciation of kinetics of adsorption will greatly help to figure out appreciate strategy to employ in wastewater purification. As postulated already, in this investigation, the value for calculated qe (Cq$_e$) was in consonance with the experimental qe (Exq$_e$), this agrees with many survey findings(Simonin 2016).

Tab.1:. Kinetic models for Cu^{2+} removal by AC1

Experiment	Pseudo-first-order			Pseudo-second-order		
Exq$_e$(mg g^{-1})	k_1 (min^{-1})	Cq$_e$, (mg g^{-1})	r^2	k_2 (g mg^{-1} min)	q_e, cal (mg g^{-1})	r^2
32.17	0.138	23.00	0.96	0.0167	32.78	0.99

3.7 FT-IR spectral analysis

Fig. 8 shows the FT-Infraredbandsconsisting FPOLP, AC1 and AC1' (recovered residue). Recorded broad peaks between 3400 cm^{-1} and 3550 cm^{-1} on the adsorbents (Fig. 8a)may be assigned to high concentration of O−H functional group as a result of the vibration stretch effect, indicating availability of “free” hydroxyl elementswithin FPOLP surface as similarly (Li et al. 2007).Consequently, the peak magnitude reduced in AC1 sample after thermal treatment.The peaks at 2850 as well 2925cm^{-1}showed some attributes ofaromatic compounds(Odewunmi et al. 2015)even though there was significantly reduction in the AC1 band (Fig. 8b) after thermal treatment at 500 °C under an oxidized medium for 180 min.As AC1 had better Cu^{2+}adsorption capacity than FPOLP, the presumption is that these two peaks did not play roles in Cu^{2+}adsorption. The peaksranging from 1626 cm^{-1} to 1629 cm^{-1}may be attributed to thestretching modes of carbonyl groups in the forms of carboxylic acids (Jokar et al. 2016).

The peak at 1318 $cm^{-1}$and 1400 cm^{-1} were attributed different composition of aromatic nitriles (Li et al. 2012;Thusnavis K.P.V. K, and Sankara N. P. M. 2011), or the stretching effect of C−O andC-C stretch, respectively (Martin Chaplin 2013).After carbonization (Fig. 8b), band 1318 cm^{-1}disappearedhowever, band 1048cm^{-1}may be credited to the stretching vibration effect of primary alcohol (Martin Chaplin 2013). It had undergone shifting to new band at 1100 cm^{-1} (Fig. 8b) and further shifted to 1102 cm^{-1} (Fig. 8c) probably due to C-O-C after thermal treatments and subsequent adsorption for AC1 and AC1'. The peaks at 874cm^{-1} could be ascribed to C-H bend as a feature of alkene compounds observed in Fig. 8a and Fig. 8b were increased in Fig. 8c, indicating that this function group might contribute the combination with Cu^{2+}.

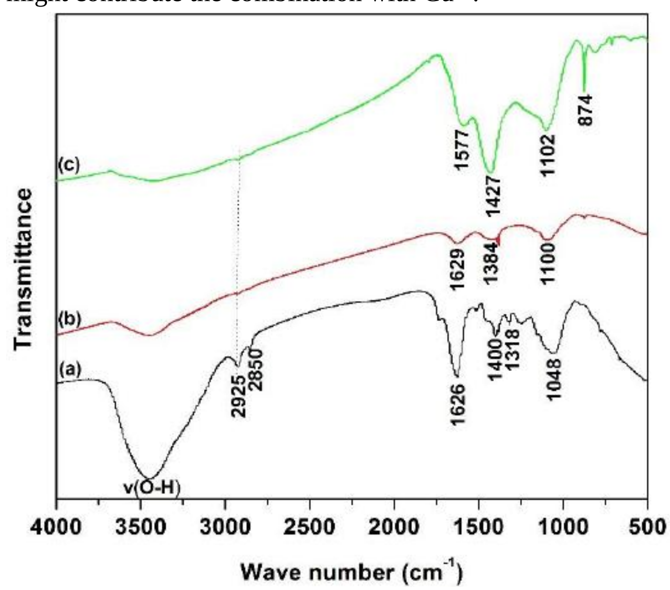

Fig. 8: FT-Infrared spectra (a)FPOLP, (b)AC1, (c)AC1'.

It should be noted that band shifts to lower wavelengthsindicates weakening bonds, whereas shifts to higher wavelengths indicated increment in bond strength (Dwivedi et al. 2011).Band 1577 cm^{-1}could be as a result of chemical interaction between Cu^{2+} and the surfaces of the leaf powder.Other research has shown that, activation under oxygen condition has high yielding adsorptive capacity when it comes into contact with Cu^{2+}(Klasson et al. 2009), this may partly account for the different adsorption potentials between FPOLP and AC1. Although the FT-Infrared examinationshowed some differencesin the samples before and after activation, the significant

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variationin Cu^{2+} adsorption ability could possibly be due activation influencean under oxidized environment.

3.8 SEM analysis

Fig. 9 shows the SEM images of FPOLPand AC1 after examination. It is observed that the surface of the uncharacterized FPOLP looked 'blocked' (Fig.9a). However, compared to AC1 which was carbonized in an oxidized condition, ended up having more pores on its surface (Fig. 9b).The BET measurements gave further insight into what could have possibly accounted for the adsorption differences even though under same controlled conditions, the specific surface dimensions were2.1330 and 25.3165 $m^2 g^{-1}$ for FPOLP and AC1 correspondingly.

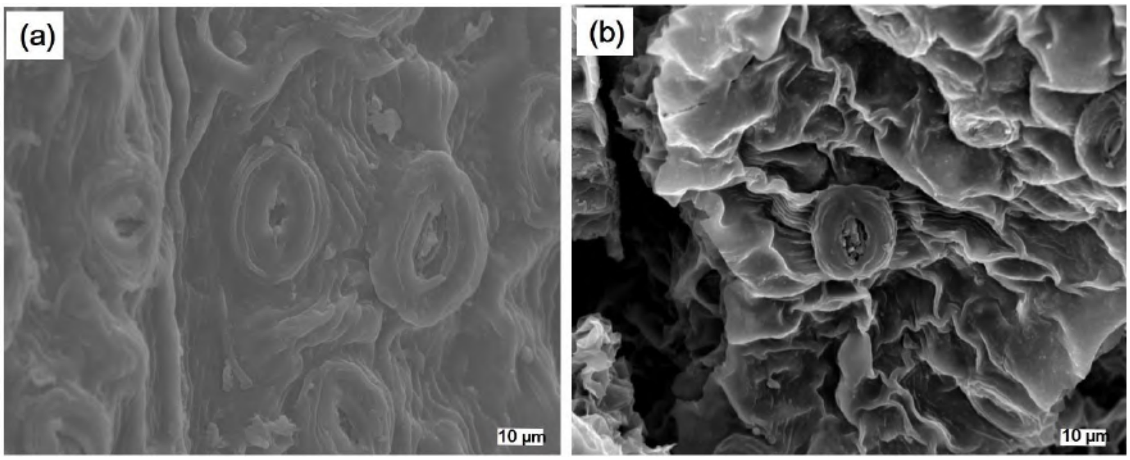

Fig.9: SEM images of (a) FPOLP and (b) AC1.

IV. CONCLUSIONS

This study revealed that the *Platanus orientalis* litter (leaves) has good potential to be used as raw material for the production of AC for effective treatment of Cu^{2+} from aqueous solution. The outcome of this investigation partly fulfilled the objective of the study which among others was to exploit the benefits that could be driven from the large quantum of waste generated annually from this plant. The removal of $Cu^{2+}$was not independent of solution pH sincemaximum uptake had been achieved atpH3.

As earlier stated, the evaluation of the mechanisms of interaction that occurred between the adsorbate and adsorbent, proved Lagergren 2ndorder as the suitable model for this investigation.

Different adsorbent mass load 1, 2, 3 and 4 gL^{-1} were investigated. Initial increment in adsorbent dosage to 3 from1 g L^{-1}led to a boost in Cu^{2+} removal from 94.74to 95.75% when it was loaded with AC1,but beyond that, no significant changes were recorded. Similarly, when an increment in AC2 mass load to 3 from 1 gL^{-1} resulted into an increase in Cu^{2+} removal efficiency from 15.08 to 21.61%. Therefore, activation of the leaves samples under an oxidized condition (pyrolysis) hadproven to be much more effective compared to that under N_2 condition. Additionally, SEM techniques were employed to examine the morphological attributes of the samples under investigation. The SEM images showed very good porosity of the AC1 which had the best adsorption capacity of 87.93 mg g^{-1}. Another aspect of this work considered was to evaluate the influence solution concentration would have in Cu^{2+}removal. The outcome of the investigation pointed to the fact that, when Cu^{2+} concentration was varied in the range of 25 and 100 mg L^{-1}, using 3 g L^{-1} mass of AC1, the depletion of Cu^{2+} reduced. It however brought about a rise in the adsorption capacity of all samples investigated.Results from pHpzc analysisrecorded at pH 2 indicated a lower figure compared with the optimum adsorption at pH 3.It explained the mechanism of interaction that took place when the Cu^{2+} solution was loaded with the AC1 and FPOLD. In addition, the FTIR, BET and SEM results indicated that the greater surface area obtained after activation contributed greatly to the higher adsorption capacity.

V. ACKNOWLEDGMENT

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REFERENCES

[1] Abadian, S., Rahbar-Kelishami, A., Norouzbeigi, R., Peydayesh, M.: Cu(II) adsorption onto Platanus orientalis leaf powder: Kinetic, isotherm, and thermodynamic studies. Res. Chem. Intermed. 41, 7669–7681 (2015).

[2] Anagement, M.: Adsorption Studies of Heavy Metals by Low-Cost Adsorbents. J. Appl. Sci. Environ. Manag. (2014).

[3] Argun, M.E., Dursun, S., Karatas, M., Gürü, M.: Activation of pine cone using Fenton oxidation for Cd(II) and Pb(II) removal. Bioresour. Technol. 99, 8691–8698 (2008).

[4] Brahmi, M., Ahmed, W., Olfa, F., Abdennaceur, H.: Henna wood as an adsorptive material for bentazon. African J. Biotechnol. 13, 3597–3606 (2014).

[5] Cardenas-Peña, A.M., Ibanez, J.G., Vasquez-Medrano, R.: Determination of the point of zero charge for electrocoagulation precipitates from an iron anode. Int. J. Electrochem. Sci. 7, 6142–6153 (2012).

[6] Chakravarty, S., Mohanty, A., Sudha, T.N., Upadhyay, A.K., Konar, J., Sircar, J.K., Madhukar, A., Gupta, K.K.: Removal of Pb(II) ions from aqueous solution by adsorption using bael leaves (Aegle marmelos). J. Hazard. Mater. 173, 502–509 (2010).

[7] Chen, H., Zhao, J., Dai, G., Wu, J., Yan, H.: Adsorption characteristics of Pb(II) from aqueous solution onto a natural biosorbent, fallen Cinnamomum camphora leaves. Desalination. 262, 174–182 (2010).

[8] Dwivedi, A.D., Dubey, S.P., Gopal, K., Sillanpää, M.: Strengthening adsorptive amelioration: Isotherm modeling in liquid phase surface complexation of Pb (II) and Cd (II) ions. Desalination. 267, 25–33 (2011).

[9] Erdem, E., Karapinar, N., Donat, R.: The removal of heavy metal cations by natural zeolites. J. Colloid Interface Sci. 280, 309–314 (2004).

[10] Gorchev, H.G., Ozolins, G.: Guidelines for Drinking-water Quality. Who. 564 (2011).

[11] Goswami, A.K., Kulkarni, S.J., Dharmadhikari, S.K., Phutke, M.: Adsorption of Copper (II) ions from SyntheticWaste Water By Teak Leaves. Int. J. Sci. Eng. Technol. Res. 2, 1356–1359 (2013).

[12] Ho, Y.S., McKay, G.: Pseudo-second order model for sorption processes. Process Biochem. 34, 451–465 (1999).

[13] Ibrahim, M.B., Sani, S.: Neem (Azadirachta indica) Leaves for Removal of Organic Pollutants. 1–9 (2015).

[14] Jokar, M., Farahani, T.S., Ramezanzadeh, B.: Electrochemical and surface characterizations of morus alba pendula leaves extract (MAPLE) as a green corrosion inhibitor for steel in 1M HCl. J. Taiwan Inst. Chem. Eng. 63, 436–452 (2016).

[15] Juang, R.-S., Shiau, R.-C.: Metal removal from aqueous solutions using chitosan-enhanced membrane filtration. J. Memb. Sci. 165, 159–167 (2000).

[16] Klasson, K.T., Wartelle, L.H., Rodgers, J.E., Lima, I.M.: Copper(II) adsorption by activated carbons from pecan shells: Effect of oxygen level during activation. Ind. Crops Prod. 30, 72–77 (2009).

[17] Koumanova, B., Allen, S.J.: Decolourisation of Water / Wastewater Using Adsorption (Review). J. Univ. Chem. Technol. Metall. 40, 175–192 (2005).

[18] Kurniawan, T.A., Chan, G.Y.S., Lo, W.H., Babel, S.: Physico-chemical treatment techniques for wastewater laden with heavy metals. Chem. Eng. J. 118, 83–98 (2006).

[19] Li, F.T., Yang, H., Zhao, Y., Xu, R.: Novel modified pectin for heavy metal adsorption. Chinese Chem. Lett. 18, 325–328 (2007).

[20] Li, X., Deng, S., Fu, H.: Inhibition of the corrosion of steel in HCl, H 2SO 4 solutions by bamboo leaf extract. Corros. Sci. 62, 163–175 (2012).

[21] Martin Chaplin: Infrared Spectroscopy. (2013).

[22] Nwabanne, J.T., Igbokwe, P.K.: Mechanism of Copper (II) Removal from Aqueous Solution Using Activated Carbon Prepared from Different Agricultural Materials. Int. J. Multidiscip. Sci. Eng. 3, 46–52 (2012).

[23] Odewunmi, N.A., Umoren, S.A., Gasem, Z.M.: Watermelon waste products as green corrosion inhibitors for mild steel in HCl solution. J. Environ. Chem. Eng. 3, 286–296 (2015).

[24] Özer, A., Özer, D., Özer, A.: The adsorption of copper(II) ions on to dehydrated wheat bran (DWB): Determination of the equilibrium and thermodynamic parameters. Process Biochem. 39, 2183–2191 (2004).

[25] Pathania, D., Sharma, S., Singh, P.: Removal of methylene blue by adsorption onto activated carbon developed from Ficus carica bast. Arab. J. Chem. (2013).

[26] Pellera, F.M., Giannis, A., Kalderis, D., Anastasiadou, K., Stegmann, R., Wang, J.Y., Gidarakos, E.: Adsorption of Cu(II) ions from aqueous solutions on biochars prepared from agricultural by-products. J. Environ. Manage. 96, 35–42 (2012).

[27] Reddy, D.H.K., Seshaiah, K., Reddy, A.V.R., Lee, S.M.: Optimization of Cd(II), Cu(II) and Ni(II)

biosorption by chemically modified Moringa oleifera leaves powder. Carbohydr. Polym. 88, 1077–1086 (2012).

[28] Robert O. Pickard: Guidelines for discharging wastewater from industrial facilities. Sewer Use Progr. 311, (2011).

[29] Simonin, J.P.: On the comparison of pseudo-first order and pseudo-second order rate laws in the modeling of adsorption kinetics. Chem. Eng. J. 300, 254–263 (2016).

[30] Sohn, S., Kim, D.: Modification of Langmuir isotherm in solution systems - Definition and utilization of concentration dependent factor. Chemosphere. 58, 115–123 (2005).

[31] Soliman, A.M., Elwy, H.M., Thiemann, T., Majedi, Y., Labata, F.T., Al-Rawashdeh, N.A.F.: Removal of Pb(II) ions from aqueous solutions by sulphuric acid-treated palm tree leaves. J. Taiwan Inst. Chem. Eng. 58, 264–273 (2016).

[32] Solomon, F.: Impacts of copper on aquatic ecosystems and human health. Mining.com Mag. 25–28 (2009).

[33] Thusnavis K.P.Vinod Kumar, M. Sankara Narayana Pillai, G.R.: Seed Extract of Psidium guajava as Ecofriendly Corrosion Inhibitor for Carbon Steel in Hydrochloric Acid Medium. J. Mater. Sci. Technol. 27, 1143–1149. (2011).

[34] UNWater: Water for a sustainable world Water for a sustainable. (2015).

[35] USEPA: Technology : Chemical Precipitation. (2013).

[36] Venkata Ramana, D.K., Harikishore Kumar Reddy, D., Yu, J.S., Seshaiah, K.: Pigeon peas hulls waste as potential adsorbent for removal of Pb(II) and Ni(II) from water. Chem. Eng. J. 197, 24–33 (2012).

[37] Villaescusa, I., Fiol, N., Martínez, M., Miralles, N., Poch, J., Serarols, J.: Removal of copper and nickel ions from aqueous solutions by grape stalks wastes. Water Res. 38, 992–1002 (2004).

[38] Wan Ngah, W.S., Hanafiah, M.A.K.M.: Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: A review. Bioresour. Technol. 99, 3935–3948 (2008).

[39] Wang, B., Guo, X.: Reuse of waste beer yeast sludge for biosorptive decolorization of reactive blue 49 from aqueous solution. World J. Microbiol. Biotechnol. 27, 1297–1302 (2011).

[40] WHO: Chemical fact sheets. Guidel. Drink. Water Qual. 296–461 (2010).

[41] Yao, Z., Qi, J., Wang, L.: Equilibrium , kinetic and thermodynamic studies on the biosorption of Cu (II) onto chestnut shell. 174, 137–143 (2010).

[42] Zeitoun, M.M., Sayed, E.-, Mehana, E.: Impact of Water Pollution with Heavy Metals on Fish Health: Overview and Updates. Glob. Vet. 12, 219–231 (2014).

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Agro-Morphological Variability Assessment of Common Bean (*Phaseolus vulgaris* L.) Genotypes in High Hill Jumla, Nepal

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Abstract— *Common bean (Phaseolus vulgaris L.) is the most important leguminous crop widely grown from Terai to high hills of Nepal covering 10,529 hectare land producing 15550 mt with the productivity of 1477 kgha^{-1} and Jumla occupies almost 21% of the total bean cultivated area of the country. For the experiment, twelve genotypes of common bean were collected from different locations of Nepal. The experiment was conducted at Agricultural Research Station Vijayanagar, Jumla Nepal from July, 2016 to October, 2016 in Randomized Complete Block Design with three replications and twelve treatments. Different agro-morphological characters were observed and wide variability was recorded in growth habit, flower color, seed color, seed shape, seed length (0.76-1.7cm), pod color, pod length(9.1-14.3 cm), plant height (57.1-116.5 cm), days to flowering (38-52 days) and maturity (74-90 days), number of pods plant^{-1} (9-22 seeds), number of seeds pod^{-1} (4-8 pods), hundred grain weight (18.3-54.7gm) and yield (2339-4164KgHa^{-1}). Significant differences were found among genotypes for days to flowering, pod length, no of seeds pod^{-1} and yield hectare^{-1}.The coefficients of determination illustrate the positive and significant association of grain yield hectare^{-1} with number of seeds pods^{-1}, therefore these traits should be considered for genetic improvement through breeding.*

Keywords— Common bean, agro-morphological, genotypes, variability, breeding.

I. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important leguminous crop widely grown from Terai to high hills of Nepal. It is important cash crop of mid-western development region grown during summer in upland under rain fed condition. It is annual crop, tolerant to drought. The performance of bean is well in drier areas where the climatic condition to cultivate maize is suitable (Alghamdi, 2007). Since, bean is a warm season grain legume, it cannot be grown well under temperature below 20°C. The optimum mean temperature for common bean is 20-25°C. High temperature hindrances with seed setting while low temperature has adverse effects on growth (Alghamdi & Ali, 2004).In Nepal, it covers 10,529 ha land producing 15550 mt with the productivity of 1477 kgha^{-1} (ABPSD, 2015). Only at Jumla, a district of high hill, it occupies almost 21% of the total cultivated area of the country (DADO Jumla). The crop has more importance in subsistence agro-farming system where it is grown without or with little external input in marginal land. However, farmers today, consider bean as a cash crop and grow a number of landraces with varying morphology (Neupane and Vaidya, 2002).

The primary center of origin and diversity for common beans is considered to be the Americas, where various researches have suggested that most groups are derived from the Andean common bean gene pool (Gepts et al. 1986). However, common beans are widely distributed and very diverse all around the world, whole in Europe and Asia (Blair et al. 2010).

Common bean is consumed in various ways: as split and whole dal, as a dry bean, as green pod vegetable. Bean flour is used for making *roti* (bread) *and dhindo (*thick porridge*), khichdi* (rice and beans cooked together). It is used as a folk medicine for diarrhoea, dropsy, dysentery, diabetes and kidney problems (Duke, 1991). Bean seed is rich in protein contents, fibre, polyphenols, flavonoids, carotenoids, saponins, oligosaccharides, condensed tannins, lectins, trypsin inhibitors and phytic acid which are considered to be the most important phytochemical responsible for antioxidant biological activities and preventive against cardiovascular or chronic degenerative(Camacho et al. 2006). Also it is source of essential vitamins (A, B_{12} and C), minerals and dietary fiber, which make it an excellent component of the human nutrition (Filgueira 2013).

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Various studies of common bean in high hills of Nepal particularly in Jumla district have indicated that it contains the greatest genetic diversity of *Phaseolus vulgaris*. Jumla, which is famous as a potential area of bean production is deprived of most of the activities regarding the characterization of the beans. The maintenance of this diversity is fundamental to develop and support breeding programs and enhance productivity. Morphological and agronomic traits of the plant are preferred for the primary characterization of the genotypes as it is being cheaper and easier to assess. Several characters are used to characterize genetic resources, particularly morphological and agronomic (Singh 2001). The present work is aimed to assess the genetic divergence among the bean genotypes evaluated and the extent of phenotypic and genetic variability with respect to agro-morphological traits.

II. MATERIALS AND METHODS

The experiment was conducted in the field, at the Agricultural Research Station Vijayanagar, Jumla Nepal, geographically located at 29^0 17'N latitude and 82^0 1'E longitude with an elevation of 2290 m amsl. Climatic condition in Jumla ranges from cool summer to freezing winter with low humidity and low rainfall.

Twelve genotypes of common bean were collected for the experiment from different locations of Nepal. PB lines were introduced in Jumla from National Grain Legume Research Program Khajura, Nepalgunj which have been cultivated by farmers of Jumla and KBL lines were collected from the Jumla which have been cultivated in Karnali region since very long time.

The experiment was conducted from July, 2016 to October, 2016 in Randomized Complete Block Design with three replications and twelve treatments. The plot size for each treatment was six square meter with row to row distance 50 cm and plant to plant distance of 10 cm adjusting four rows each measuring 3m length. The seed was sown on 9 July, 2016. The seed rate and fertilizer dose used was 120 kg/ha and 100:60:40 NPK kg/ha respectively. Nitrogen was provided in two splits, half as basal dose and next half was top dressed at vegetative growth stage just after first weeding (one month after sowing). Data were recorded for agro-morphic characters like days to 50% flowering (DF), Days to 75% maturity (DM), Plant height (PH), pod length (PL), number of pods plant^{-1} (NOPP), number of seeds pod^{-1} (NOSP), seed size, 100 seed weight and grain yield. The morphological characters were flower color, growth habit, pod color, seed shape and seed color (IBPGR, 1982).

Statistical Analysis

The data recorded were entered in microsoft excel 2016 and analysis was done in R Studio 3.1.1. Duncan Multiple Range Test (DMRT) was done in 5% level of significance for mean comparison. Correlation and regression analysis were done using microsoft excel. T test and cluster analysis was performed using agricolae package and h-clust respectively. Data was normalized before calculation of Euclidean distance (Wiley, 2014).

III. RESULTS AND DISCUSSIONS

Table.1: Agro-morphological traits of 12 genotypes of common bean evaluated at Jumla, Nepal.

Genotype	Growth Habit	Flower Color	Seed color	Pod Color	Seed Shape	Seed Length(cm)
PB0001	Bushy	Light pink to white	Dark red with white streaks	Green	Kidney	1.45
PB0002	Trailing	Dark Pink	Creamy white with purple streaks	Green with dark purple streaks	Elongated	1.39
PB0048	Trailing	Very light pink to white	Dark red	Green	Elongated	1.70
KBL-1	Trailing	Dark Pink	Black	Green	Elongated	1.09
KBL-2	Trailing	White	Dark Red	Reddish Green	Elongated	1.09
KBL-3	Trailing	Very dark pink	Dark purple with white streaks	Green	Elongated	1.25
KBL-4	Trailing	Dark Pink	Creamy to very light brown	Green	Flattened elongated	1.08
KBL-5	Bushy	Pink	Light brown	Green	Elongated	1.36
KBL-6	Trailing	White	White	Green	Elongated	0.76
KBL-7	Trailing	Whitish Pink to dark	Creamy to very light	Green	Oval	1.06

		pink	brown			
KBL-8	Bushy	Very Light Pink to white	White with dark red streaks	Green	Kidney	1.52
KBL-9	Trailing	Dark Pink	Light to dark grey	Green	Elongated	1.04

Three genotypes PB0001, KBL-5 and KBL-8 are bushy in nature whereas all others are trailing type. The flower color shows variations from white, light pink to very dark pink and the seed color varies widely among genotypes. The color of pods at maturity ranges from yellow, purple stripe on white to caramine red. The seed shape of two genotypes PB0001 and KBL-8 were kidney shaped, KBL-7 was oval shaped, KBL-4 was flattened elongated and all other genotypes possessed elongated seed. The seed length ranges from 0.76 to 1.7 cm in which PB0048 possessed highest seed length 1.70 cm and KBL-6 showed lowest seed length, 0.76cm (Table 1).

Table.2: Mean value of agro-morphological characteristics of 12 genotypes of common bean evaluated at Jumla, Nepal.

S.N	Genotypes	FD	MD	PH	NOPP	PL	NOSP	HGW	YLD
1	PB0001	47	87	57.7	13	9.5	4	43.3	3691^{ABC}
2	PB0002	51	84	109.1	16	10.7	6	36	3610^{ABC}
3	PB0048	45	84	110.6	18	10.7	4	47.3	3222^{ABCD}
4	KBL-1	49	85	116.5	20	9.9	7	23	3683^{ABC}
5	KBL-2	46	84	103.1	16	9.7	8	24.3	4164^{A}
6	KBL-3	52	90	100.5	15	10.4	6	29	3538^{ABC}
7	KBL-4	50	90	82.7	17	10.5	6	26.3	3796^{AB}
8	KBL-5	38	74	62.5	15	14.3	6	31	2620^{BCD}
9	KBL-6	47	84	78.6	22	9.1	6	18.3	3779^{AB}
10	KBL-7	45	88	97.5	12	10.8	7	39	2339^{D}
11	KBL-8	38	79	57.1	9	12.3	5	54.7	2517^{CD}
12	KBL-9	45	87	90.3	17	11.3	7	24.3	3278^{ABCD}
CV		9.03	5.45	30.4	25.89	13.04	16.14	34.59	21.14
LSD		6.92	-	-	-	2.33	1.58	-	1177.85
F-test		**	NS	NS	NS	*	*	NS	*

Note: SN-serial number, FD-days to 50% flowering,MD-days to 75% maturity, PH-plant height in cm, NOPP-number of pods plant^{-1}, PL-pods length in cm, NOSP-number of seeds pod^{-1}, HGW-hundred grain weight in gram, YLD-Yield kgha^{-1}, CV- coefficient of variation, LSD-least significant difference. Means followed by the same letters in a column are not significantly different by DMRT at 5% level of significance, NS-non significant.

Regression Analysis

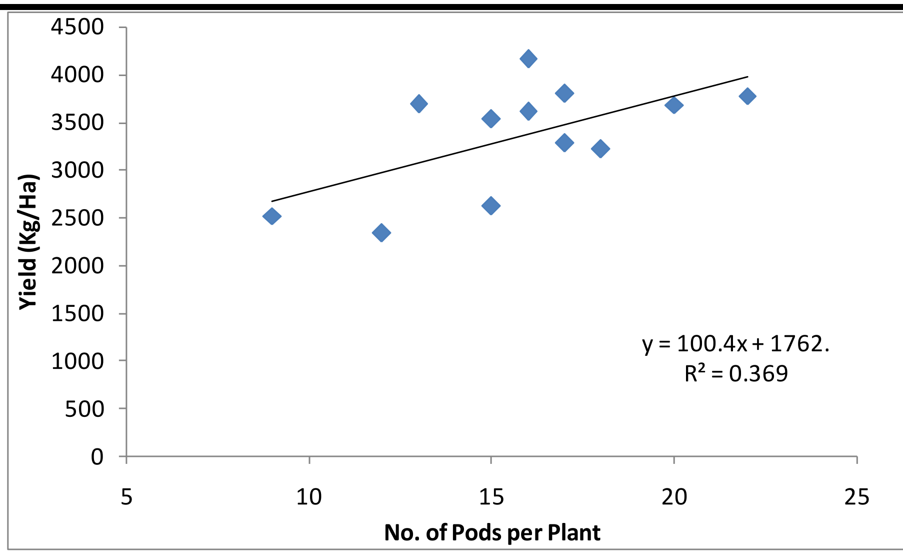

Fig.1: Linear Relationship Between No. of Pods Plant^{-1} and Yield(KgHa^{-1}) of Bean Genotypes at Jumla, Nepal.

There is a positive linear relationship between No. of Pods Plant^{-1} and Yield (KgHa^{-1}). According to linear regression equation, with unit increased in No. of Pods Plant^{-1}, Yield would have been increased by 100.4 times. According to coefficient of determination, about 36.90% variation in yield was due to No. of Pods Plant^{-1} and remaining portion due to other variables as shown in Figure 1.

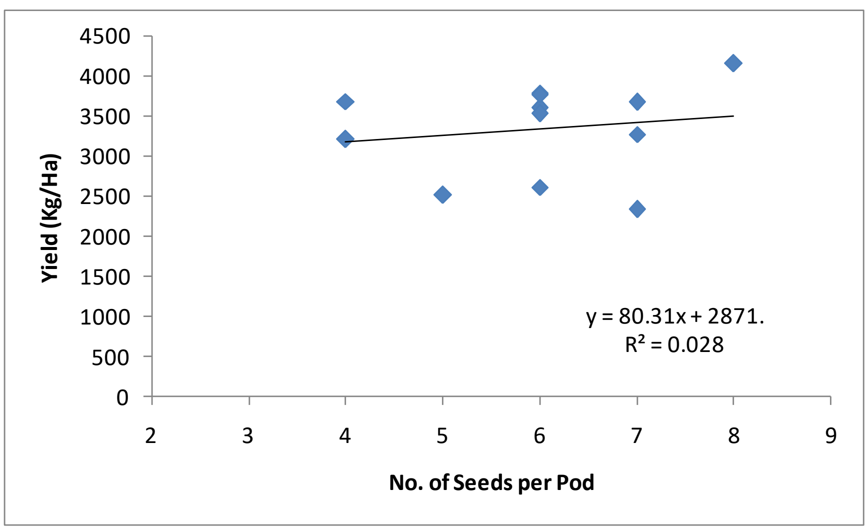

Fig.2: Linear Relationship between No. of Seeds pod^{-1} and Yield (KgHa^{-1}) of Bean Genotypes at Jumla, Nepal.

The positive and significant ($P<0.05$) linear relationship between No. of Seeds pod^{-1} and Yield (KgHa^{-1}) as shown in Figure 2, showed that with unit increased in No. of Seeds pod^{-1} increased in yield by 80.31 times. According to the coefficient of determination about 2.80% (R^2=0.028) variation in yield was due to No. of Seeds pod^{-1} and remaining portion due to other variables.

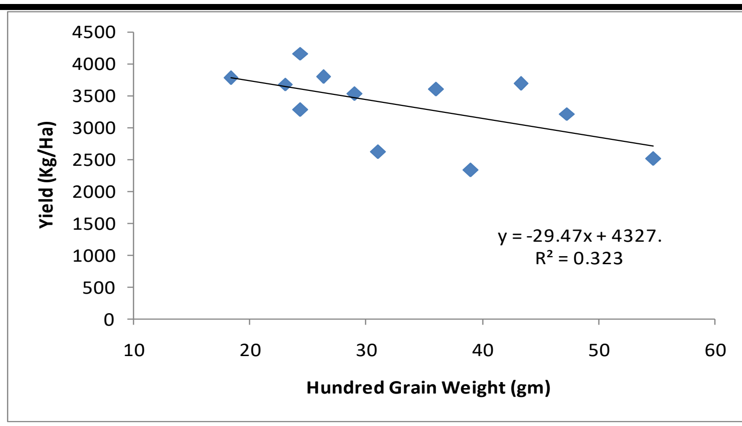

Fig.3: Linear Relationship between Hundred Grain Weight (gm) and Yield(KgHa^{-1}) of Bean Genotypes at Jumla, Nepal.

There is a negative linear relationship between hundred grain weight (gm) and Yield (KgHa^{-1}). The contribution of hundred grain weight to the yield was approximately 32.30% (R^2=0.323) and remaining portion might be due to other variables as shown in Figure 3.

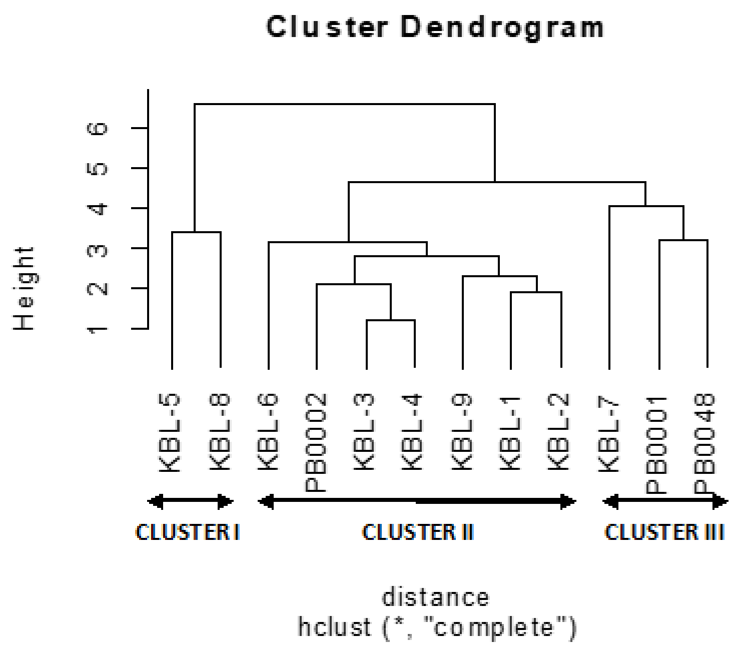

Fig.4: Dendrogram showing clustering pattern of 12 common bean genotypes based on Euclidean distance values obtained from agro-morphological data.

The germplasm used in this experiment were grouped in cluster I, II and III in (Figure 4) which holds 2, 7 and 3 genotypes respectively. The mean value of various traits for each character showed that genotypes with minimum days to flowering, minimum days to maturity, maximum pod length and minimum yield hectare^{-1}were found in cluster I. Likewise, genotypes of cluster II constitute maximum days to maturity, maximum plant height, maximum no. of pods plant^{-1}, minimum hundred grain weight and maximum yield hectare^{-1}. Genotypes with similar pod length and similar days to flowering and days to maturity were kept in cluster III.

IV. CONCLUSION

Genotypes on intra cluster showed similar agro-morphological traits whereas inter clusters' genotypes showed higher agro-morphic diversity, mainly for days to flowering, pod length, no of seeds pod^{-1} and yield hectare^{-1}.

Therefore, for further improvement, effective phenotypic selection can be done from these diversified genotypes however, it is now essential to undertake more research work on characterization of common bean from Jumla, Nepal.

V. ACKNOWLEDGEMENT

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REFERENCES

[1] ABPSD (2015) Statistical Information of Nepalese agriculture, 2015. Ministry of Agricultural Development, Kathmandu, Nepal.
[2] Alghamdi, S. S., Kh and A. Ali. 2004. Performance of several newly bred faba bean lines. *Egypt. J. Plant Breed.*, 8: 189-200.
[3] Alghamdi, S. S. 2007. Genetic behavior of some selected faba bean genotypes. *African Crop Sci.Conference proceedings. African Crop Science Society. El-Minia, Egypt*, 8: 709-714.
[4] Blair, M. W. et al. Extensive diversity and inter-genepool introgression in a world-wide collection of indeterminatesnap beans accessions. *Theoretical and Applied Genetics*, v. 120, n. 7, p. 1381-1391, 2010.
[5] Duke, J. A. 1981. Handbook of Legumes of World Economic Importance. USDA, Bellsville, Maryland, New York and London.
[6] Filgueira, F. A. R. *Novo manual de olericultura*:agrotecnologia moderna na produção e comercialização de hortaliças. 3. ed. Viçosa: UFV, 2013.
[7] Gepts, P. et al. Phaseolin-protein variability in wild forms and landraces of the common beans (*Phaseolus vulgaris*): evidence for multiple center of domestication. *Economic Botany*, v. 40, n. 4, p. 451-456, 1986.
[8] Krishi Bikash Karyakram Tatha Tathyanka Ek Jhalak 2071/072, District Agriculture Development Office (DADO) Jumla, 2015.
[9] Neupane, R. K. and Vaidya, M. L. 2002. Proceedings of the first stakeholders' meeting on development of improved production technology of *Phaseolus* beans to the hills of mid western Nepal, 22 June 2002 ARS, Jumla. NGLRP Rampur.
[10] Singh SP (2001) Broadening the genetic base of common bean cultivars: a review. Crop Science 41: 1659-1675.
[11] Wiley J. (2014) Wiley StatsRef: Statistical Reference online: onlinelibrary.wiley.com.

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Assessment of Loan default Trend on the Amount of Loan Granted to Farmers in Kwara State, Nigeria 1984- 2014

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Abstract— *This study was carried out to assess the growth trend in the amount of loan granted to farmers in the study area for the period 1984-2014. Secondary data was used and sourced from central bank of Nigeria statistical bulletin, Nigeria bureau of statistics and Kwara state bureau of statistics. The result showed that there was an acceleration in the amount of loan defaulted by farmers for the period 1984-2014 in the study area and 73% variations was noticed in the exponential time trend for the period. It was concluded that a rigorous effort to speedy the increase and availability of loan process to make more funds available should be inaugurated to increase farmers ability to purchase improved inputs so as to increase productivity in the agricultural sector as a whole and proper monitoring should be put in place.*

Keywords*— *Loan, Default, trend, loan granted, Farmers.

I. INTRODUCTION

Agricultural loan involves giving out credit in cash to farmers for the purpose of farming. There is no doubt that agricultural credit is one of the fundamental ingredients for sustainable agricultural production; as such its accessibility and demand is one of the prerequisites for attaining the national goal of reducing poverty and economic development. Agricultural household models have suggested that farm credit is not only necessitated by the limitations of self-finance, but also by uncertainty pertaining to the level of output and the time lag between inputs and outputs (Kohansal and Mansoori , 2009).

A number of studies (Kohansal and Mansoori, 2009; Okarie, 2004) show that growth rate of investment in agriculture is less than other economic sectors. On this note, financing agriculture is one of the most important factors necessary for the improvement of the quality and quantity of farm harvest leading to increase in farmer's income as well as reducing poverty.

Generally, there is an acceptance of the important role of farm credit and a wide appreciation by most governments of the need for credit in agriculture. However, past trends and problems of agricultural loans are clear but future changes are uncertain. The recognition credit plays in agriculture is also fundamental to development of household food security, which can help farmers to maximize their economic potentials. Failures to recognize the extent of the consequences in which farmers access loan may be costly and can result in misguided policies and programs by the government, which may further bring about complex situation of hunger.

A lot of studies (Agnet, 2004; Asante, *et al.* 2011; Ewuola and Williams, 1995; Ijere, 1998; Nnadozie and Uzoigwe, 2002; Oladeebo and Oladeebo, 2008; Osuntokun, 1980 and Udoh, 2008) have noted the indispensability of credit in the process of socio-economic transformation and none of these studies assesses default trend on the amount granted to famers in Kwara state.

II. METHODOLOGY

Secondary data were used in the assessing the trend of loan granted over the period 1984 to 2014 and it was sourced from the central bank of Nigeria statistical bulletin, Nigeria bureau of statistics and Kwara state bureau of statistics. Trend and growth analysis is used to understand if there is a drift in growth of a variable over a period of time. In modeling time trend for this study the exponential trend or log-linear as employed by Tanko, *et al.* (2010); Maikasuwa and Ala, (2013) was modified and used for the analysis.

The exponential trend equation for loan default was specified as follows:

$$y_t = e^{\beta_0 + \beta_1 t_1 + u_t} \quad \text{.........................1}$$

By taking the natural logarithm of both sides to form an amenable ordinary least square equation as follows:

$$\ln y_t = \beta_0 + \beta_1 t_1 + u_t \quad \text{...........................2}$$

Where: Y= amount of loan defaulted
"t"= time trend variable
β_0 = Intercept of the trend equation
β_1= Trend coefficient
u_t = Error term

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To determine if there is acceleration, deceleration or stagnation in loan default in the study area, Quadratic equation time trend variable was fitted as follows:

$LnY_t = \beta_0 + \beta_1 t_1 + \beta_2 t^2{}_1 + U_t$................................3

All variables as previously defined β_0, β_1, β_2 are parameters to be estimated. In the specification of equation above, the linear and quadratic time variable t^2 allows for the possibility of determining whether there was acceleration, deceleration or stagnation in loan default in the study area during the period of 1984-2014. In determining the pattern of growth our main concern was on β_2 which reveals a measure of growth pattern following Oyenweanku, (2004); Tanko, *et al.* (2010); Maikasuwa and Ala, (2013). β_2 is > 0 and statistically significant it is acceleration, if β_2 is < 0 and statistically significant it is deceleration and if β_2 is positive or negative and not statistically significant then there is stagnation in the growth.

III. RESULTS AND DISCUSSIONS

The result of growth trend was done with the estimated regression coefficient of the time trend variable as in equation 3. The data used is a secondary yearly data using an independent variable for the analysis as time lag (years of study) 1984 to 2014, while the dependent variable is amount of loan granted for the period 1984 to 2014. The adjusted R^2 value of 0.68 shown in the Table 1 implies that time trend, as a variable was very important as it accounted for 68% of variations noticed in loan granted in the study area. The coefficient of the variable (0.0217) was significant suggesting that there has been an increase in loan defaulted for the period 1984-2014.

Table.1: Estimated exponential and quadratic growth trend of loan granted in the study area

Period	β_0,	β_1	β_2	Adjusted R^2	F- value	Significant level
Exponential trend	-417.7036	0.0217		0.68	7.74	0.00***
	[56.08]	[0.028]				
Quadratic trend	32277.33	-31.4861	0.0081	0.73	2.517	0.018**
	[12989.48]	[12.9927]	[0.0033]			

Source: field survey, 2016, *[]=Standard error. ***=1%*, **=5%

To investigate for the existence of acceleration, deceleration or stagnation in trend of loan defaulted in the study area. The result of the quadratic equation in time trend variable was fitted in equation 3. The result in table 1 reflected and revealed that the equation has a good fit giving the R^2 value of 0.73 and the associated F-statistics at 2.517, which is statistically significant at ($P<0.05$). Table 1 also showed the slope coefficient of t^2 that the value of β_2 (0.0081) is positive and statistically significant at ($P<0.05$) level of significance. The significant positive value is a confirmation that there is an acceleration in the amount of loan defaulted over the period 1984-2014. It therefore entails the full implementation of proper use of loan granted in order to achieve the needed demand of funds in agriculture thereby translating it to a viable sector of the economy.

IV. CONCLUSION AND RECOMMENDATION

The study concluded that there exists a positive (increase) default trend in the loan defaulted over the years studied. Efforts should be made to increase availability of loan and make more funds available to agriculture so as to boost agricultural activities in the area which will enable farmers have access to improved seeds, farm implements etc.

REFERENCES

[1] Agnet, A. (2004, November 2,). *Making farm credit work for the small-scale farmers*. Retrieved from <http://www.agnet.org/library/nc/145b/>>

[2] Asante, B. O., Sefa, , V., and Sarpong, D. (2011). Determinants of small scale farmers' decision to join farmer based organizations in Ghana. *African Journal of Agricultural Research*, 6(10): 2273-2279.

[3] Ewuola, S., and Williams, S. (1995). Effects of Institutional and Borrower Characteristics on Loan Recovery: A Study of Ondo State Agricultural Credit Corporation. *Agro search*, *1*(2): 109-116.

[4] Ibrahim, F., Mohammed, U., Nmadu, J., Yakubu, I., and Ibrahim, P. (2010). Forecasting and growth trends of sugar cane production: Meeting the goals of commercial Agriculture in Nigeria. *11th annual conference of nigerian asocitaion of agricultural economics* (pp. 40-43). Minna, Niger state: NAAE.

[5] Ijere, M. (1998). *Agricultural Credit and Economic Development*, lagos: Longman.

[6] Kohansal M.R, and Mansoori, H. (2009). Factors Affecting Loan Repayment Performance of Farmers in Kharasan-Razavi Province of Iran. *Conference on International Research on Food Security.* Hamburg: University of Hamburg.

[7] Maikasuwa, M., and Ala, A. (2013). Trend analysis of area and productivity of sorghum in Sokoto State,

Nigeria, 1993-2012. *European Scientific Journal, 9*(16): 69-75.

[8] Nnadozie, A., and Uzoigwe, J. (2002). Effectiveness of Local Sanctions on Agricultural Loan Recovery Under Community Banking in Enugu State. *Journal of the Science of Agriculture, Food Technology and the Environment, 2*(1): 56 – 62.

[9] Okarie, A. (2004). Major Determinant of Agricultural Smallholder Loan repayment in Developing Economy: empirical evidence from Ondo state Nigeria. *The Journal of Socio-Economics*, 21 (4): 223-234.

[10] Oladeebo, J. O., & Oladeebo, O. (2008). Determinants of loan repayment among smallholder farmers in Ogbomoso agricultural zone of Oyo State, Nigeria. *Journal of Social Sciences*, 17(1): 59-62.

[11] Osuntokun, T. (1980). Improving The Structure of Agricultural Industry with Agricultural Credit. *Ondo State Component of the 4th National Development Plan 1980-85,* Akure: Ministry of Finance, Akure .

[12] Oyenweanku, C. (2004). Satgnation, acceleration and deceleration in agricultural production in Nigeria, 1970-2000. *Journal of Agriculture and food Science, 2* (2): 131-140.

[13] Tanko, L., Jirgi, A., & Igwe, K. (2010). Trend analysis of area, production and productivity of rice in Nigeria. *Proceedings of the 11th Annual Conference of National Association of Agricultural Economist (NAAE)* (pp. 44-48). MInna, Niger state: National Association of Agricultural Economist.

[14] Udoh, E. (2008). Estimation of loan default among beneficiaries of a state Government owned agricultural loan scheme, Nigeria. *Journal of Central European Agriculture*, 9(2): 343-352.

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Effect of Feeding Graded Levels of Fermented Sweet Orange (*Citrus Sinensis*) Fruit Peel Meal on the Growth and Nutrient Digestibility of Broiler Chicken

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Abstract— *A sixty-three day feeding trial was conducted with one hundred and eighty (180) day-old Anak 2000 broiler chicks with an average weight of 54.26g. They were randomly assigned in groups of 30 each to 6 dietary groups T_{0}, T_{10}, T_{20}, T_{30}, T_{40}, and T_{50} which had 24-hour fermented sweet orange fruit (Citrus sinensis) peel as a replacement for maize at 0, 10, 20, 30, 40, and 50% respectively, to determine their growth performance. Each treatment group had 3 replicates with equal no of chicks in a completely randomized design. The birds were provided with water and feed ad libitum. Body weight and feed intake were taken, water intake measured, feed conversion ratio, body weight gain and water: feed ratio calculated. A five (5) day Digestibility trial was done in the night week with three (3) chickens per treatment to determine nutrient digestibility. Replacement of maize with sweet orange peel significantly retarded ($p<0.05$) final live body weight gain, and the feed conversion ratio of broiler chickens and elevated the water : feed ratio. Nutrient digestibility was not significantly affected ($p>0.05$). The study showed that utilisation of 24-hour fermented sweet orange peel as a dietary replacement for maize at levels of 10%-50% did not support the growth of broiler chicken. It is recommended that sweet orange peel be fermented for longer duration beyond 24 hours to further evaluate its potential feed value in broiler chicken diet.*

Keywords— Fermentation, orange peel, Growth, chicken.

I. INTRODUCTION

Animal protein intake in Nigeria from meat, egg and milk sources as in some developing countries is low with attendant effects of malnutrition and lowered human productivity. This is partly due to the high costs of these finished products as a result of high feeding costs and disease problems with its toll on animal population in spite of growing human population. In addition, climate change is also exerting negative environmental effect on the animals. Monogastric animals especially birds compete with man for conventional feedstuffs especially cereal grain because of increase in human population and food industries particularly, and lowered cereal and legume grain farm outputs occasioned by climate change. This has thus increased the demand for protein of animal origin (Oluwafemi, 2009). Large scale commercial poultry production is one quick and effective way of correcting the problem of animal protein intake deficiency, and broiler chickens are usually the most universal and important as producers of table meat. Agro-allied by-products have in the past 40 years become important feed components in monogastric diets in Nigeria, to reduce the demand pressure on convectional feedstuffs. Whereas, oil seed cakes are used often as protein component in monogastric diets, agro by-products from fruits and tubers are used as replacement for maize or grain, the energy component. The use of some of these by-products are associated with problems like presence of anti-nutritional factors harmful to the animal health and high fibre content which reduces the feed value of these agricultural by-products, thereby making their processing before use in animal feed critical. Cassava products contain cyanogenic glucoside which can be reduced by cooking, frying, drying and fermentation (Udedibe *et al*., 2004). Sweet orange peels have been reported to contain limonene, saponin, tannin, flavonoid, phytate, oxalate (Oluremi *et al*., 2007b) which could be reduced by fermentation. Processing is done to reduce moisture content to prevent spoilage and increase shelf life, remove harmful compounds, improve nutrient availability and digestibility with overall effect on the enhancement of the nutritional value of the by-product. The usefulness of a nutrient to an animal depends on its digestibility, and digestibility is affected by anti-nutritional factors (Longe, 2006; Panda, 2006). Thus, digestibility of any given feed by broiler chicken is a prerequisite for good performance.

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The objective of this study was to determine the effect of replacing maize with graded levels of fermented sweet orange fruit peel on the performance of broiler chickens.

II. MATERIALS AND METHODS

Experimental site

The feeding trial was carried out at the Poultry unit in the Teaching and Research Farm, Federal University of Agriculture Makurdi, Benue State, Nigeria.

Processing of Sweet orange fruit peel and Preparation of Experimental Diets

Fresh sweet orange peels were gathered from orange fruits sellers within the Makurdi metropolis. They were packed into empty feed sacks, tied at open end, and allowed 24hours to ferment. Thereafter, they were spread on concrete platform and sun-dried to less than 10% moisture within 48hours. The sun-dried peels were ground, added to other feedstuffs and manually mixed to compound the experimental diets. Six diets coded T_0, T_{10}, T_{20}, T_{30}, T_{40} and T_{50} for each of starter broiler (Table 1) and finisher broiler (Table 2) were formulated, with sweet orange peel replacing dietary maize at 0, 10, 20, 30, 40 and 50% respectively.

Experimental animals, Design and Management

One hundred and eighty (180) day-old Anak 2000 broiler chicks were purchased from TUNS Farm in Nigeria and used for the feeding trial. They were randomly allocated using the Table of random numbers (Little and Hills, 1977) to six dietary treatments balancing for live weight. Each treatment had three replicates with 10 chicks each. The experiment was completely randomized design.

The experimental birds were brooded with kerosene lantern and charcoal as sources of illumination and heat respectively. They were raised in deep litter system, fed and served drinking water *ad libitum* for sixty-three days. Newcastle vaccine was given at day-old and week 5, and infectious bursal disease (gumboro) vaccine at week 4. Coccidiostat was given at preventive dose at alternate weeks because coccidiosis was endemic in the research environment. Neomycin chick formula (antibiotics) was given to the birds on arrival, in addition with Vitalyte (antistress) which was periodically given pre- and post-weekly weighing of the experimental birds and administration of vaccine. Drinkers were washed, litter materials in the feeder were removed daily and litter on the floor was maintained dry to secure the health of the birds.

Data collected and Statistical analysis

Feed intake was determined weekly and daily feed intake computed. Daily water intake was obtained from known quantities of water served 24 hourly less the unconsumed water, and the evaporative water loss using the procedure of Shoremi *et al*. (1998). Body weight of each replicate was taken weekly and body weight gain calculated by difference (current weight less former weight). Feed conversion ratio was calculated as feed intake: body weight gain ratio and water: feed ratio computed.

In the last week of the feeding trial, one chicken per replicate with average weight similar to that of the treatment group was transferred into the metabolic cage. After a 2-day adjustment period, weighed treatment diets were served daily for 5 consecutive days, fresh faecal outputs collected daily, weighed, oven dried, and re-weighed. Dried faecal outputs per replicate were pooled, milled and analysed along with the experimental diets to determine their proximate constituents (AOAC, 1995).

The data obtained were analysed using the analysis of variance (Steel and Torrie, 1980) and where significant difference was observed among treatment means, least significant difference was used for mean separation.

III. RESULTS AND DISCUSSION

The effect of replacement of maize with fermented sweet orange fruit peel in the diet of broiler chicken on growth performance is in Table 3. The live weight, feed intake, body weight gain, feed conversion ratio and water: feed ratio obtained were significantly different ($p<0.005$) among the treatments whereas, water intake was not ($p>0.05$). The final live weight, feed intake, body weight gain tended to decrease whereas, feed conversion ratio and water: feed ratio became poorer as the percent maize replacement with sweet orange peel increased from 0% to 50%. The utilisation of sun-dried 24 hours fermented sweet orange fruit peel as a replacement for maize depressed the growth of broiler chickens and this negative effect became more severe at higher levels. This showed that sweet orange fruit peel as processed in this study still had a low feed value. It has been reported that sweet orange peel has higher crude fibre content than maize (Oluremi *et al*. 2007a) and anti-nutritional factors (Oluremi *et al*. 2007b). The negative effect of alternative feed resource includes high fibre content which affects nutrient bio-availability, and anti-nutritional factor which are deleterious to animal health and growth (Dafwang, 2006). Dietary fibre stimulates water consumption and this probably caused increased water: feed ratio obtained.

The effect of the experimental diets on nutrient digestibility of broiler chickens is in Table 4. The digestibility of crude protein, crude fibre, ether extract and nitrogen free extract was not significantly affected ($p>0.05$) among the treatment means. It was also observed that the digestibility value for each nutrient was average ranging between 60% and 70%. The only exception being the coefficient of digestibility of crude fibre where for chickens in T_{30} a value of 57% was

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obtained. The broiler chickens in the control group (T_0) did not show any superiority in nutrient digestibility compared to the chickens in the sweet orange peel based dietary treatments. The nutrient digestibility of broiler chickens may not have been a major determinant of the pattern recorded for the growth rate as given by the body weight gain which significantly declined ($p<0.05$) as the percent replacement level of maize by sweet orange peel increased in the diet. It is therefore apparent that some of the anti-nutritional factors present in the peel as reported by Oluremi *et al.* (2007b) may have retarded the growth rate of the broiler chickens.

The study has revealed that the utilisation of sweet orange peel fermented for 24hours as a replacement for maize at 10, 20, 30, 40 and 50% did not support the growth of broiler chickens. It is recommended that sweet orange fruit peels fermented for longer duration beyond 24 hours be evaluated for their effect on the growth response of broiler chickens.

Table.1: Composition of Broiler starter diets containing 24-hour fermented Sweet orange peel

Ingredients	Experimental Diets					
	T_0	T_{10}	T_{20}	T_{30}	T_{40}	T_{50}
Maize	49.00	44.10	39.20	34.30	29.40	24.50
Sweet orange peel meal	0	4.90	9.80	14.70	19.60	24.50
Soybean meal	42.50	42.50	42.50	42.50	42.50	42.50
Brewers dried grain	3.00	3.00	3.00	3.00	3.00	3.00
Blood meal	1.50	1.50	1.50	1.50	1.50	1.50
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.28	0.28	0.28	0.28	0.28	0.28
Lysine	0.22	0.22	0.22	0.22	0.22	0.22
Vitamin/mineral premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients						
Crude protein (%)	25.06	25.03	25.00	24.97	24.94	24.91
Crude fibre (%)	4.46	5.01	5.56	6.11	6.66	7.21
Ether extract (%)	3.85	3.77	3.70	3.63	3.55	3.48
Calcium (%)	1.10	1.10	1.10	1.10	1.10	1.10
Phosphorus (%)	0.88	0.86	0.85	0.83	0.82	0.81
Methionine (%)	0.82	0.81	0.80	0.79	0.78	0.77
Lysine (%)	1.63	1.62	1.61	1.59	1.58	1.57
Energy (kcalME/kg)	2820.06	2800.25	2780.45	2760.64	2740.84	2721.04

Table.2: Composition of Broiler finisher diets containing 24-hour fermented Sweet orange peel

Ingredients	Experimental Diets					
	T_0	T_{10}	T_{20}	T_{30}	T_{40}	T_{50}
Maize	51.58	46.42	41.26	36.11	30.95	25.79
Sweet orange peel meal	0	5.16	10.32	15.47	20.63	25.79
Soybean meal	26.57	26.57	26.57	26.57	26.57	26.57
Maize offal	9.00	9.00	10.00	11.00	12.00	12.00
Brewers dried grain	6.00	6.00	6.00	6.00	6.00	6.00
Blood meal	3.00	3.00	3.00	3.00	3.00	3.00
Bone ash	3.00	3.00	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin/mineral premix*	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients						
Crude protein (%)	21.27	21.23	21.20	21.17	21.14	21.11

Crude fibre (%)	4.95	5.52	6.10	6.68	7.26	7.84
Ether extract (%)	3.81	3.73	3.66	3.58	3.50	3.42
Calcium (%)	1.09	1.09	1.08	1.08	1.08	1.08
Phosphorus (%)	0.83	0.81	0.78	0.78	0.77	0.75
Methionine (%)	0.69	0.68	0.66	0.66	0.65	0.64
Lysine (%)	1.21	1.19	1.18	1.17	1.15	1.14
Energy (kcalME/kg)	2857.97	2837.12	2816.26	2795.45	2774.49	2753.74

Table.3: The effect of 24-hour fermented Sweet orange peel meal on Performance response of Broiler chicken

Performance indices	Experimental Diets						SEM
	T_0	T_{10}	T_{20}	T_{30}	T_{40}	T_{50}	
Initial live weight (g)	54.95	54.62	54.67	54.50	52.07	54.67	
Final live weight (kg)	2.01^{a}	1.88^{b}	1.73^{c}	1.61^{d}	1.50^{e}	1.33^{f}	0.03
Body weight gain (g/day)	41.73^{a}	38.11^{b}	33.81^{c}	30.43^{d}	27.98^{e}	22.38^{f}	0.54
Feed intake (g/day)	114.45^{a}	105.73^{b}	100.98^{bc}	97.95^{cd}	100.63^{bc}	94.34^{d}	1.91
Feed conversion ratio	2.74^{a}	2.77^{a}	2.99^{ab}	3.22^{b}	3.59^{c}	4.23^{d}	0.10
Water intake (ml/day)	205.37	212.99	216.42	225.61	217.75	213.22	10.12^{ns}
Water:Feed ratio	1.79^{a}	2.01^{ab}	2.13^{b}	2.30^{b}	2.16^{b}	2.25^{b}	0.10

^{a,b,c,d,e,f}Means with different superscripts in the same row are significantly different (P<0.05); ^{ns}Not significantly different (P>0.05); SEM=Standard error of mean; T_0=0% maize replacement with sweet orange peel meal; T_{10}=10% maize replacement with sweet orange peel meal; T_{20}=20% maize replacement with sweet orange peel meal; T_{30}=30% maize replacement with sweet orange peel meal; T_{40}=40% maize replacement with sweet orange peel meal; T50=50% maize replacement with sweet orange peel meal

Table.4: The effect of 24-hour fermented Sweet orange peel meal on nutrient digestibility by Broiler chicken

Nutrient	Experimental Diets						SEM
	T_0	T_{10}	T_{20}	T_{30}	T_{40}	T_{50}	
Crude protein	64.18	64.48	63.02	60.23	62.46	60.78	1.10^{ns}
Crude fibre	61.27	62.83	62.64	57.49	60.42	60.21	1.15^{ns}
Ether extract	65.26	66.88	66.47	63.78	62.69	63.74	0.87^{ns}
Nitrogen free extract	64.10	66.70	66.53	64.24	63.63	64.63	0.87^{ns}
Metabolisable energy	70.11^{ab}	69.96^{ab}	79.44^{a}	53.32^{b}	62.33^{ab}	62.33^{ab}	2.73

^{a,b}Means with different superscripts in the same row are significantly different (P<0.05); ^{ns}Not significantly different (P>0.05); SEM=Standard error of mean; T_0=0% maize replacement with sweet orange peel meal; T_{10}=10% maize replacement with sweet orange peel meal; T_{20}=20% maize replacement with sweet orange peel meal; T_{30}=30% maize replacement with sweet orange peel meal; T_{40}=40% maize replacement with sweet orange peel meal; T50=50% maize replacement with sweet orange peel meal

REFERENCES

[1] AOAC (1995). Official Methods of Analysis. 16th Edition. Association of Official Analytical Chemists, Washington D.C. USA.

[2] Dafwang, I.I. (2006). Meat, Eggs and Milk from Farm Wastes: Explorations of Animal Nutrition Research and Extension. An Inaugural Lecture. 15th April 2006, Ahmadu Bello University, Zaria Nigeria. 61pp

[3] Little, T.M. and Hills, F.J. (1977). Agricultural Experimentation; Design and Analysis. John Wiley and sons, New York.

[4] Longe, O.G. (2006). Poultry: Treasure in a chest. An Inaugural Lecture, University of Ibadan on 24th August, 2006: No 180.

[5] Oluwafemi, R.A (2009). Palm Kernel Cake Utilization in Monogastric Animal Feeding – Implications for sustainable livestock development. International Journal of Veterinary Medicine 6(2): 68

[6] Oluremi, O.I., Andrew, I.A. and Ngi. J. (2007a). Evaluation of Nutritive potential of some Citrus fruits (*Citrus sinensis*) Varieties as Feeding stuffs in Livestock production, Pakistan Journal of Nutrition 6(6): 653-656.

[7] Oluremi, O.I.A., Ngi. J. and Andrew, I.A. (2007b). Phyto-nutrients in Citrus fruit peel meal and Nutritional implication for Livestock feeding. Livestock Research for Rural DevelopmentVol. 19, Article 89. http://www.cipav.org.co/lrrd/19/7olur19089.htm.
[8] Panda, A.K (2009). Phosphorous: The Essential Mineral Response of Broilers Fed with β-Mannanase in high and low energy palm kernel based diets. Proceedings 34th Annual Conference of the Nigerian Society for Animal Production (NSAP), March 15-18th, 2009 Pp 420-421.
[9] Shoremi, O.I.A., Ayoade, J.A. and Akinwale V.O. (1998). Maize replacement value of cassava peel soaked in water. Applied Animal Research 14: 183-187.
[10] Steel, R.G.D. and Torrie, J.H. (1980). Principles and Procedures of Statistics. A Biometrical Approach. 2nd Edition. MacGraw Books Co., USA.
[11] Udedibe, A.B.I., Anyaegbu, B.C., Onyechekwu G.C., and Egbuokporo, O.C., (2004). Effect of Feeding different levels of fermented and unfermented cassava tuber meals on the performance of broilers. Nigerian Journal Animal Production. 31: 211-217.

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Production Function Analysis of non-member of Dairy Cooperative Society for Milch cow in district Etawah of U.P.

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***Abstract*— *This study covered Cobb douglas production function, Tukey and Kramer analysis on Non members dairy cooperative society for milch cow in district Etawah of U.P. In study researchers have taken post- stratified into Landless, Marginal, small, medium and large herd size categories. The study effect of various factors of production in (Rs.) like Feeding cost included (dry fodder + green fodder), expenditure of concentrate included (grain + khali + mineral material and chunni / choker) and miscellaneous expenses included (labor charge and fixed cost) on milk produced by the cow of dairy cooperative society non members in annual in different categories of farmers. Further, the researchers have found out the comparative analysis of all the categories of dairy cooperative society non members. At last Tukey and Kramer test was applied on all the category of dairy cooperatives society members in milch cow to get into the depth of the problem under investigation. This study is helpful to find out the elasticity of different factors of milk production and comparative analysis in all categories of members dairy cooperative society in milch Cow by Cob douglas production function analysis.*

Keywords*— *Elasticity of fodder, Elasticity of concentrate, Elasticity of miscellaneous, Return to scale, Classification Code: Agriculture Management.

I. INTRODUCTION

FAO predicting a 2% increase of world milk production from 805 million tonnes in 2015 to 827 million tons in 2020. Most of this increase is expected to come from developing countries such as India, China, Pakistan and Turkey, where it will be used to meet growing demand. The FAO forecasts also show some supply growth in Europe, Australia and the US, although at much lower rates while they predict New Zealand‟s 2015 production to be roughly the same as last year. As consumption levels in developed countries such as Europe, Oceania and North America are unlikely increase fast enough to use up the additional milk supplies, this will lead to an increase in exports during 2015.

Uttar Pradesh is the highest milk producing state 23.33 Million Tonnes and hold a share of more than 17% in the total milk production in India. Apart from being the largest milk producer, Uttar Pradesh also has the largest number of cows and buffaloes, which is more than 1.8 Crore. in 2014-15. Kherigarh, Ponwar, Gangatiri and Kenkatha are some of the cow breeds found in Uttar Pradesh. These cow breeds are mainly found in Uttar Pradesh and known for producing milk in high quantity. Uttar Pradesh has more than 40 dairy cooperatives, which supply milk to many states in the country. On the basis of per capita milk consumption, Uttar Pradesh continued to remain the leading milk producer, followed by Rajasthan and Gujarat, whereas, the per capita demand was maximum in Punjab followed by Haryana.

Milk is an essential as well as popular food of the Indian diet. It is highly nutritious and occupies 15 percent of the total consumed dietary protein in the industrialized world. Grossly speaking milk constitutes 3.1 percent protein 4.0 percent fat, 5.0 percent lactose 0.74 percent minerals and sizeable amount of vitamins, milk is also a close substitute for nonvegetarian food.

"As per an assessment made by the Planning Commission Report- 2012, the domestic demand for the milk by 2020-21 is expected to be 172.20 million tons. India would have sufficient production to meet such demand. The international body on the farm sector in its latest „Food Outlook‟ report also estimates global milk production in 2020 grow by 2% to 827 million tones. The National Dairy Development Board (NDDB) had published a report in "Perspective 2010" in which to enable the co-operatives to meet the new challenges of globalization and trade liberalization. Like other major dairying countries of the world, the Indian co-operatives are expected to play a predominant role in the dairy industry in future as well.

However, India is in the meantime, attaining its past glory and is once again becoming "Doodh Ka Sagar". But what percentage of this Sagar is handled by the co-operatives is just a little over 7 per cent. Since liberalization of the dairy sector in 1991, established of the dairy factories in the country but their share of total milk is hardly 5 per cent. Therefore, the total share of the organized sector in India, both co-operatives as well as the private sector is hardly 12 per cent. Besides, growth in milk production is likely to continue at present * (Department of Animal Husbandry, Dairying& Fisheries, Ministry of Agriculture, GOI-2014-15) rate of 4.4 % in the near future. Who will handle this increment in milk production in India? Demand for milk at current rate of income growth is not sufficient; India needs to grow at-least 7 per cent per annum to full fill the demand. The study analyzed various factors of production in (Rs.) like Feeding cost included (dry fodder + green fodder), expenditure of Concentrate included (grain + khali + mineral material and chunni / choker) and miscellaneous expenses included (labor charge and fixed cost) on milk produced by the cow of dairy cooperative society members in annual in different categories of farmers i.e, landless, marginal, small, medium and large on the basis of land holding capacity. Analyses of Cobb Douglas production function, researchers find out elasticity of fodder, concentrate and miscellaneous factors of milk production. Further, the researchers have identified percentage of data variation on different category members of dairy cooperative society. At last Tukey and Kramer test was applied on all the category of dairy cooperatives society members in milch cow to get into the depth of the problem under investigation. This study is helpful to find out the comparative analysis in all categories of members dairy cooperative society in milch Cow.

"Etawah" in Uttar Pradesh is famous for its Bhadawari breeds of buffalo and Jamunapari breed of goats. The said breed of buffalo were also known for consuming less fodder relative to production of high fat content milk. However, all the milch animals such as buffalo, cow and goats are grazed in the ravines and the forest area between Jamuna and Chambal rivers of Etawah district of U.P. The numbers of milch livestock of Etawah district during 2012 were reported as total number of female adult cows 1, 10,825 total number of adult females' buffaloes 92065 and total female adult goats were 2, 41, 61.

II. REVIEW OF LITERATURE

Murithi, Festus Meme,(2002), study was motivated by the need to find means of increasing milk supply in Kenya in order to meet an expected rise in demand. The study was concerned with the efficiency of resource use in smallholder milk production. The major objective of the study was to determine whether tnere are possibilities of increasing milk production through re-allocation of the resources used in milk production~ The problems encountered by farmers involved in milk production were also examined. The data used in the study were collected from 60 smallholders who are members of five Dairy Co-operative Societies which are affiliated to the Meru Central Farmers Co-operative Union. A Cobb-Douglas milk production function was fitted using the inputs used in milk production. The results showed that concentrates significantly

Influenced milk yields. The test for efficiency of resource use revealed that there was inefficiency in the use concentrates. Profit maximization I~equires that the marginal value product of an input be equated to the price. If this condition is fulfilled in the study area with respect to concentrates, the average milk yeild per animal per year would increase by 73% above the current levels. An important conclusion of the study is that there could be substantial in milk output and consequently gains in farm profits if the amount of concentrates fed to the animals is increased above the cur-r-errt level s. It is recommended that:- (i) effot'ts be intensified to educate the benefits of increased feeding of concetrates to the (i i) animals, constraints which contribute to the unavailability of concentrates when farmers need them be removed, (iii) farmers be educated on how they can the excess animal feeds which is produced in the winter season to feed the animal and educated on how best season, they can utilize the farm by-products while they are of high nutrition value to feed the animals.

Sharma, P.K. & Singh, C.B. (1984), conducted a study in the intensive cattle development project and observed an increasing trend of human labor employment per household. The dairy enterprise on an average generated 250 days of employment on both category of beneficiary and non beneficiary households . The family labor income of Rs.1076 obtained from cross bred cow was much higher than that of a buffalo and local cow. Further the beneficiary households recorded higher income from different types of milch animal as compared to that of non beneficiary households. Therefore, they concluded that the project has been able to generate additional gainful employment in the study area and thus it can go a long way in boosting up income and employment levels specially an small cattle holdings. Sharma, P.K. & Singh, C.B. (1986), studied the impact of I.C.D.P. Karnal on production, consumption and

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marketed surplus of milk in rural Karnal. The study revealed that production of milk was relatively higher on the beneficiary households than that of nonbeneficiary households of cattle owners with rise in production of corresponding increase in milk being marketed by the beneficiary households. The overall marketed surplus of milk on beneficiary and non beneficiary households was about 44 and 28 per cent respectively. The project could, therefore be expected to provide a better source of income through milk production. Interestingly a positive impact of project was seen as consumption of milk. The per capita per day milk consumption of 729 and 623 gm on beneficiary and non beneficiary households respectively. It was much higher than the national average of 121 gm only

Hirevenkanagoudar, L.V. et .al., (1988),studied the impact of dairy development programmes of the Karnataka Dairy Development Cooperation (KDDC) on the selected economic aspects of small and marginal farmer and agricultural labours. The study revealed that over 56 per cent of KDDC beneficiaries were getting 50-75 per cent of their family income from dairy enterprises whereas, 60-87 per cent of nonKDDC farmers getting 25 per cent of their income from dairy enterprises. All KDDC farmers were selling milk to dairy co-operative societies. Mostly small farmers, marginal farmers and agricultural labors in the KDDC programme and 60 per cent of the non KDDC category through that dairy co-operative societies were the best agencies for milk marketing. More than 64 per cent of KDDC farmers had repaid 75 per cent to 100 per cent of the dairy loan, whereas only 10-25 percent at nonKDDC farmer had repaid 75-100 per cent of their dairy loan.

Dass, B. et. al., (1990), studied performance of dairy co-operative. involved in production of dairy co-operative involved in production and distribution of milk in Tarai region of district Nainital (Uttar Pradesh) during the year 1986. The study revealed that the co-operative societies had a positive and significant impact on the size of milch breed, level of milk production and marketed surplus of milk per member household. The size of milch herd increased by 55 per cent, the level of milk production by 65 per cent and marketed surplus of milk by 72 per cent in the societies group as compared the non-societies group. The income generated through dairying was 30 per cent of the total cash income in the societies group as against 21 per cent in the non-societies group.

Jitendra, K. & Shankara, M. (1992), studied the impact of dairy co-operative and income and employment in chittor district,Andhra Pradesh. It was found that agricultural labour and non-agricultural labour earned more income from dairying than small farmers who were earned more in crop production. The employment created to members (121.5 days in area-I and 112.2 days in area-II) was significantly more compared in non-members (76 days in area-I and 53.5 days in area-II) in the study area. Thus, the dairy co-operative have contributed in generating more income and employment to the dairy farmers.

Prajneshu,(2008), the set of Cobb-Douglas production functions is usually fitted by first linear zing the models through logarithmic transformation and then applying the method of least squares. However, this procedure is valid only when the underlying assumption of multiplicative error-terms is justified. Unfortunately, this assumption is rarely satisfied in practice and accordingly, the results obtained are of doubtful nature. Further, nonlinear estimation procedures generally yield parameter estimates exhibiting extremely high correlations, implying thereby that the parameters are not estimated independently. In this paper, use of expected-value parameters has been highlighted and the advantages of their use have also been discussed. Finally, the developed methodology has been illustrated by applying it to the wheat yield time-series data of Punjab.

Venkatesh P. and Sangeetha V.,(2011), a study was conducted to examine the cost structure and resource use efficiency of dairy farms in the Madurai district of Tamil Nadu. The dairy farmers were selected by using multi stage random sampling technique. Tabular analysis and Cobb-Douglas production function were used in this study. Total costs per lactation per animal estimated were of the order of Rs.12776.09, Rs 11791.20 and Rs.12079.28 and returns per rupee of investment 0.78, 1.08 and 0.95 respectively on small, large and pooled farms. Feed cost was the higher input cost in dairy farming (61.6%). The cost of production milk per litre was less in case of large farms (Rs. 4.62) compared to small farms (Rs. 5.39). Results indicated the inverse relationship with the size and the herd of the total costs, due to economies of scale. Functional analysis showed barring human labour on small farms all the selected input variables such as green fodder, dry fodder, concentrates and health care were positive and significant impact on the production of milk indicating the potentiality of their further use.

Meena G. L. et.al.,(2012), study was undertaken in Alwar District of Rajasthan with the objectives to examine the input-output relationships and assess the resource use efficiency in milk production. The study covered 75 cooperative member milk producers and 75 non-cooperative member milk producers. The results of Cobb-Douglas production function revealed that concentrate had positive and significant influence on returns from buffalo milk across all the household categories for both the member and non-member groups. Green fodder and dry fodder were also influenced the returns from milk significantly across all the household categories for both the member and non-member groups with the sole exception of large category of non-member group. D_1 (winter) and D_2 (Rainy) dummy variables were found to be positive and statistically significant. The results of Chow's test clearly revealed that the production functions between member and non-member groups differed significantly. The results of the resource use efficiency revealed that green fodder was over-utilized in small and medium categories for both the member and non-member groups, dry fodder was over-utilized by medium category of member group, concentrate was over-utilized by only medium category of member group and by small & medium categories of non-member group while it was under-utilized by large category of non-member group and labour was over-utilized by only small category of member group.

Makwana D. Girish et.al.(2016), suggested the dairy sub-sector occupies an important place in agricultural economy of India. As milk is the second largest agricultural commodity in contributing to GNP. Currently, more than 80 % of the milk produced in the country is marketed by the unorganized sector (private organization) and less than 20 % is marketed by the organized sector. But, both organized and unorganized sector in the dairy industry of the district face a lot of constraints relating to production and marketing constraints as well as – infrastructural , technical , socio-psychological, economical with high or low severity to expansion of milk production in the district, availability of green fodder and concentrate , knowledge of balance feeding, irregular sale of milk ,lack of time of marketing, less knowledge about of marketing strategies, no or less provision for advance payment for milk by society or vendors, delay in payment by unorganized sector, in ability to market for value added products, transportation. Processing availability of veterinary facilities , lack of awareness of animal health care and training facilities for scientific dairying etc. facing by cooperative and non-cooperative members in Kheda district of Gujarat.

III. RESEARCH METHDOLOGY

District Etawah milk producers' cooperative union was purposively selected from the state of Uttar Pradesh. Exhaustive lists of all the milk producers' cooperative societies in Etawah district milk producers' cooperative union were prepared. Researchers have selected randomly 150 non member of dairy cooperative society & 150 members of dairy cooperative society from 10 Villages of 2 blocks selected in district Etawah. All the milk producing household members and non members were classified into five categories, viz., Landless, Marginal, Small, Medium and Large farmers on the basis of land holding capability. Thus, in all, 300 households were interviewed during the year 2008-09. The primary data were collected to help of well structured pre-tested schedule by the personal inquiry method. The data collected were subjected to tabular analysis in order to study the comparative economics of milk production. Cobb-Douglas type Production Functional analysis was applied on cow milk production with three variables like-fodder, concentrate and miscellaneous of different categories landless, marginal , small, medium and large member farmers of dairy cooperative society.

The study effect of various factors of production in (Rs.) in case of milk cooperative societies non members in annual in different categories.

$$y = a x_1^{b_1} x_2^{b_2} x_3^{b_3} \quad \ldots\ldots (1)$$

$$\log y = \log a + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 \quad \ldots(2)$$

Where

Y	=	Production of milk in (Rs.)
X_1	=	Feeding cost included (dry fodder + green fodder)
X_2	=	Expenditure of Concentrate included (grain + khali + mineral material and chunni / choker)
X_3	=	Miscellaneous expenses included a labor charge and fixed cost.
b_i	=	Respective elasticity's of milk production
a	=	constant

Having estimated the cost of milk production, it is desirable to ascertain the reliability of these fodder costs, concentrate cost and miscellaneous expanses estimates. The most commonly used "t" test was applied to ascertain whether the cost of milk is significantly different from zero or not at some specified probability level.

"t" cal=b_j / standard error of b_j..

If calculated "t" value is greater than the table value of "t" at a specified probability level and "n-k-1" degree freedom, bj is said to be statistically significant.

IV. REASEARCH AND FINDINGS

Table.1: Return to Scale for the Dairy Cooperative Society Non-Members (Cow):

S.N.	Category	β1	β2	β3	Total β1+ β2+ β3	Return to Scale ≥1
1	Landless	-2.6880	8.5009	3.3698	9.1827	≥1
2	Marginal	119.4263	-4.5425	-2.3184	112.5654	≥1
3	Small	1.9451	5.3532	1.0665	8.3648	≥1
4	Medium	3.0623	5.7530	-5.5220	3.2933	≥1
5	Large	31.014	4.0267	-5.7890	29.2517	≥1

β1= Elasticity of Fodder

β2= Elasticity of Concentrate

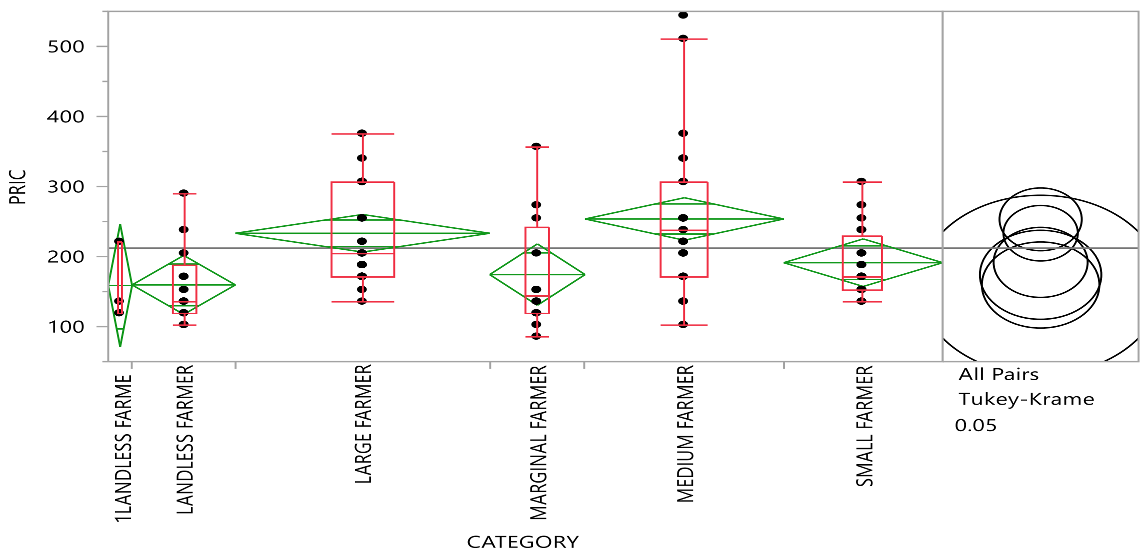
β3= Elasticity of Miscellaneous expanses

The above table no 1 reveal that Elasticity of milk production for all the five categories of non member farmers of dairy cooperative society in cow namely Landless, marginal, small, medium and large farmers. The last column indicates their economies of scale. Their respective value were observed 9.1827, 112.5654, 8.3648, 3.2933 and 29.2517 i.e., out of these five categories none of the any category non member farmers were observed had decreasing return to scale.

The all five categories i.e., landless, marginal, small ,medium and large exhibited increasing return to scale and analysis further reveals that return to scale was the highest for marginal farmers followed by large, landless and small and medium non member farmers of dairy cooperative society in case of cow.

5.12.6 Summary of all categories of Non Members Dairy Cooperative Society for Milch Cow:

Oneway Analysis of PRICE By CATEGORY



Quantiles

Level	Minimum	10%	25%	Median	75%	90%	Maximum
1LANDLESS FARMER	119	119	119	136	221	221	221
LANDLESS FARMER	102	102	119	136	187	268.6	289
LARGE FARMER	136	170	170	204	306	329.8	374
MARGINAL FARMER	85	90.1	119	144.5	242.25	331.5	357
MEDIUM FARMER	102	136	170	238	306	428.4	544
SMALL FARMER	136	137.7	153	170	229.5	270.3	306

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
1LANDLESS FARMER	3	158.667	44.160	71.04	246.29
LANDLESS FARMER	13	159.538	21.214	117.45	201.63
LARGE FARMER	32	233.219	13.521	206.39	260.05
MARGINAL FARMER	12	174.250	22.080	130.44	218.06
MEDIUM FARMER	25	253.640	15.297	223.29	283.99
SMALL FARMER	20	191.250	17.103	157.31	225.19

Std Error uses a pooled estimate of error variance

LSD Threshold Matrix

Abs(Dif)-HSD	MEDIUM FARMER	LARGE FARMER	SMALL FARMER	MARGINAL FARMER	LANDLESS FARMER	1LANDLESS FARMER
MEDIUM FARMER	-62.87	-38.91	-4.30	1.32	18.09	-40.85
LARGE FARMER	-38.91	-55.57	-21.39	-16.28	0.57	-59.67
SMALL FARMER	-4.30	-21.39	-70.30	-64.17	-47.48	-105.05
MARGINAL FARMER	1.32	-16.28	-64.17	-90.75	-74.28	-127.91
LANDLESS FARMER	18.09	0.57	-47.48	-74.28	-87.19	-141.51
1LANDLESS FARMER	-40.85	-59.67	-105.05	-127.91	-141.51	-181.50

Positive values show pairs of means that are significantly different.

Connecting Letters Report

Level				Mean
MEDIUM FARMER	A			253.64000
LARGE FARMER	A	B		233.21875
SMALL FARMER	A	B	C	191.25000
MARGINAL FARMER		B	C	174.25000
LANDLESS FARMER			C	159.53846
1LANDLESS FARMER	A	B	C	158.66667

Levels not connected by same letter are significantly different.

Ordered Differences Report

Level	Difference	Std Err Dif	Lower CL	Upper CL	Upper CL	p-Value
MEDIUM FARMER	94.97333	46.73414	-40.850	230.7967	230.7967	0.3319
MEDIUM FARMER	94.10154	26.15387	18.091	170.1125	170.1125	0.0065*
MEDIUM FARMER	79.39000	26.86124	1.323	157.4568	157.4568	0.0439*
LARGE FARMER	74.55208	46.18323	-59.670	208.774	208.7744	0.5912

				4		
LARGE FARMER	73.68029	25.15622	0.569	146.7918	146.7918	0.0471*
MEDIUM FARMER	62.39000	22.94601	-4.298	129.0780	129.0780	0.0805
LARGE FARMER	58.96875	25.89087	-16.278	134.2154	134.2154	0.2131
LARGE FARMER	41.96875	21.80206	-21.395	105.3321	105.3321	0.3933
SMALL FARMER	32.58333	47.35591	-105.047	170.2138	170.2138	0.9829
SMALL FARMER	31.71154	27.24935	-47.483	110.9063	110.9063	0.8528
MEDIUM FARMER	20.42125	20.41636	-38.915	77.79	79.7573	0.9168
SMALL FARMER	17.00000	27.92899	-64.170	230.7967	98.1700	0.9902
MARGINAL FARMER	15.58333	49.37194	-127.906	170.1125	159.0730	0.9996
MARGINAL FARMER	14.71154	30.61918	-74.277	157.4568	103.7001	0.9967
LANDLESS FARMER	0.87179	48.99069	-141.510	208.7744	143.2534	1.0000

Summary of all categories of Non Members Dairy Cooperative Society for Milch Cow:

The analysis are revealed that mean of small farmers was observed Rs. 316.667 they were the most benefited in nonmember cow followed by large farmers Rs. 288.00, Landless Rs. 221.48, Marginal farmers Rs. 213.69 and least for Medium farmers Rs. 212.00. This indicated fact that small farmer interestedness in milch animals especially in cow is the highest.

Tukey test was applied to get into the depth of the problem under investigation. This indicated that there is non-significance difference between small and large farmers for milch cow.

Further there is non significance difference between Large, Landless, Marginal and Medium farmers for milch cow. Further indicated the fact that P value for medium and Large farmers, medium and Landless and Large and medium farmers were observed significant at 5 % level of Probability (0.006. .0043 and 0.047).

Conclusion

The studyreveal that Elasticity of milk production for all the five categories of non member farmers of dairy cooperative society in milch cow namely Landless, marginal, small, medium and large farmers. Their economies of scale of out of these five categories none of the any category non member farmers were observed had decreasing return to scale.

The all five categories i.e., landless, marginal, small ,medium and large exhibited increasing return to scale and analysis further reveals that return to scale was the highest for marginal farmers followed by large, landless and small and medium non member farmers of dairy cooperative society in case of milch cow.

The analysis are revealed that mean of small farmers was the most benefited in nonmember cow followed by large farmers , Landless , Marginal farmers and least for Medium farmers . This indicated fact that small farmer interestedness in milch animals especially in cow is the highest. Further there is non significance difference between Large, Landless, Marginal and Medium farmers for non member of dairy cooperative society of milch cow.

REFERENCES

[1] http://planningcommission.nic.in/reports/genrep/ar_eng1112.pdf

[2] Dass, B., Shukla, O.S. et.al. (1990). "Performance of Dairy Cooperative involved in Production and

http://dx.doi.org/10.22161/ijeab/2.6.45 *ISSN: 2456-1878*

Distribution of Milk in Tarai region of District Nainital (Uttrakhand):" Indian Journal of Agri. Econ. 45 (3):

[3] Hirevenkanagoudar, L.V. Hanuman Thappa, H.S. & Jalihal, K.A. (1988). "Impact of Dairy Development on the Weaker Sections: A Study", Kurukshetra 36 (5): 7-11.

[4] Jitendra Kumar and Shankara Murthy (1992). "Impact of Dairy Co-operative on Income and Employment in Chittor District Andhra Pradesh- An Economic Analysis" Ind. Coop. Review 29 (4): 382-387.

[5] Murithi, Festus Meme.2002 Efficiency of resource use in smallholder milk production: the case of Meru central dairy farmers, Kenya,http://erepository.uonbi.ac.ke:8080/xmlui/handle/123456789/21808.

[6] Makwana D. Girish et.al.(2016), "Constraints of milk production :A study on cooperative and non-cooperative dairy farms in Kheda district of Gujarat" indian journal of applied research ,vol. 6, issue 5, pp464-466

[7] Meena G. L.*et.al*, 2012. Milk Production Function And Resource Use Efficiency In Alwar District of Rajasthan, *International Journal Of Scientific & Technology Research Volume*1, ISSUE 8, 115-119

[8] Prajneshu.2008. Fitting of Cobb-Douglas Production Functions: Revisited, *Agricultural Economics Research Review*, Vol. 21 , 289-292.

[9] Sharma, P.K. and Singh, C.B.(1986). "Milk Production Consumption and Marketable Surplus of Milk in Rural Karnal". Dairy guide 8(8):22-25

[10] Venkatesh P. and V. Sangeetha.2011.Milk Production and Resource Use Efficiency in Madurai District of Tamil Nadu: An Economic Analysis, *Journal of Community Mobilization and Sustainable Development* Vol. 6(1), 025-030.

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Review Study on Larvicidal and Mosquito Repellent Activity of Volatile Oils Isolated from Medicinal Plants

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Abstract – *Mosquito is a vector for serious human diseases like dengue fever, hemaorrhagic dengue fever and chikungunya, .yellow fever, malaria, filaria and encephalitis among these dengue, hemaorrhagic dengue and chikungunya are highly endemic diseases in Southeast Asian and African countries, causing millions of deaths each and every year. Mosquito repellents thus play a major role in preventing man-mosquito contact and there by minimize the chance of infections and its adverse effects. The development of resistance to chemical insecticides, results rebounding vectorial capacity. Synthetic repellents are chemicals which used worldwide for protection against mosquito-borne diseases and it adversely affects the environment by contaminating water, soil and air. There is an urgent need to find alternatives to the synthetic insecticides. Plants are rich source of alternative agents for control of mosquitoes and its vectors. Extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal and mosquito repellent activities. Literature has documented that essential oils and extracts have been traditionally used as effective repellents. The essential oils whose repellent activities have been demonstrated, as well as the importance of the synergistic effects among their components are the main focus of this review study. Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has been linked to the presence of monoterpenes and sesquiterpenes. The present review study focused the larvicidal potential and mosquito repellent activity of different volatile oils of medicinal plants. From an economical point of view synthetic chemical is still more frequently used as repellents than essential oils; these essential oils have the potential to provide efficient and can be used as a cheap, eco-friendly, safer for humans and the environment and also efficient alternative to the chemical larvicides.*

Keywords— Essential oils, Larvicidal activity, Medicinal Plants, Mosquito repellents, Volatile oils.

I. INTRODUCTION

Mosquitoes transmit serious human diseases, causing millions of deaths every year and the development of resistance to chemical insecticides, resulting in rebounding vectorial capacity. Mosquito has approximately 3500 species and present in tropical and subtropical regions of the world [1].Major genera of mosquitoes that act as vector for various disease including dengue, chikungunya, malaria, yellow fever, filariasis, Japanese encephalitis, Lyme disease, andepidemic polyarthritis [2, 3]. Among these mosquito borne diseases dengue, fever dengue hemaorrhagic fever, yellow fever and chikungunya are endemic in Southeast Asian countries [4]. These are transmitted by *Aedes aegypti* (Linn.) [5] *Anopheles stephensi* and *Culex quinquefasciatus.* Mosquito repellents thus play a major role in preventing man-mosquito contact and there by minimize the chance of infectious diseases. Synthetic repellents, such as DEET (Diethyl-meta-toluamide or N, N- diethyl-3-methyl benzamide (IUPAC Name)), are used worldwide for protection against mosquito-borne diseases. However, DEET has an unpleasant odor, can damage plastics and synthetic rubber and exhibits a high level of skin penetration [6]. Moreover, concerns have been raised over the safety of DEET and other synthetic compounds [7]; one of the methods available for controlling the mosquitoes is use of synthetic insecticides. Synthetic insecticides adversely affect the environment by contaminating water, soil and air. There is an urgent need to find alternatives to the synthetic insecticides.

Plants and its isolated compounds are alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target-insects and are eco-friendly [8].Traditionally plant based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemical may act as insecticides, repellents, anti-feedants, moulting hormones, juvenile hormone mimics, growth inhibitors, anti-moulting hormones as well as attractants. Plant based pesticides are less toxic, delay the development of resistance because of its new structure and easily biodegradable [9].

Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities [10]. About 2000 species of terrestrial plants have been reported for their insecticidal properties [11]. Medicinal plants are more potent, eco-safe and low-cost which is easily available and have become increasingly popular as safe and biodegradable mosquito repellents [12], it is less or no adverse effect to the environment.

Plant based products does not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant derived compounds, such as saponine [13], steroids [14, 15], isoflavonoids [16], essential oils [17], alkaloids and tannins [18] has potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquito [19, 20].

1.1. Mode of action of essential oils

Essential oils being complex mixtures of volatile organic compounds are generally produced as secondary metabolites in plants. They are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones and phenols). Essential oils have high repellency against arthropod species [21]. Literature has documented that essential oils and extracts have been traditionally used as effective repellents. The metabolites like the monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are the common constituents in a number of essential oils presenting mosquito repellent activity [22]. Literature cites that hairs on the mosquito antennae are temperature and moisture sensitive. The repellent molecules thus interacts with the female mosquito olfactory receptors thereby blocking the sense of smell which therefore comes as an hurdle in the recognition of host by the mosquitoes [23].

Elucidation of the mode of action of essential oils and their constituents is of practical importance for insect control because it may give useful information on the most appropriate formulation and delivery means. Volatile oil can disrupt communication in mating behavior of insect by blocking the function of antennal sensilla and unsuccessful mating could lead to a lower fecundity and ultimately lower the population of insect pest [24]. Rapid action of essential oils or its constituents against insect pests is an indicative of neurotoxic actions. The present review study carried out to validate the larvicidal potential and mosquito repellent activity of different volatile oils of medicinal plants.

II. LITERATURE SURVEY

Manimaran et al., evaluated larvicidal efficacy of the most promising oils such as mentha (*Mentha piperita*), clove (*Myrtus caryophyllus*) and calamus oils (*Acorus calamus*) which recorded low LC_{50} and LC_{90} values with 95% confidence lower and upper limits. Eucalyptus oil showed the least larvicidal activity with $LC_{50}$and LC_{90} values for larvicidal activity against *Anopheles stephensi*. Some plant oils also tested against *Culex quinquefasciatus*, the most promising oils were calamus, mentha and lemon oils which recorded LC_{50}andLC_{90}values with 95% confidence lower limits and upper limits for larvidial activity. Citronella oil showed least larvicidal activity with $LC_{50}$and LC_{90} values. Among the oils against *Aedes aegypti*, Mentha, citronella and clove oils showed the most potent larvicidal activity and recorded $LC_{50}$and LC_{90}values with 95% confidence lower and upper limits. Eucalyptus oil showed the least effective larvicidal activity with $LC_{50}$and LC_{90}values [25].

Cavalcanti et al., reported the most active essential oils against third instar larvae of *A. aegypti* were those of *O. gratissimun* (LC_{50} 60 ppm), *O. americanum* (LC_{50} 67 ppm), *L. sidoides* (LC_{50} 63 ppm), and *C. citrates* (LC_{50} 69 ppm). They finally concluded the essential oils of *O. americanum* and *O. gratissimun* showed as potent as *L. sidoides* and *C. citrates* in the larvicidal activity against *A. aegypti* and caused 100% mortality at a concentration of 100 ppm [17]. Sukumar et al., reported that *C. citrates* causes significant growth inhibition and mortality in later developmental stages of *A. aegypti* [26].

One research study revealed that the essential oils from *Mentha longifolia* L., and *Lavandula dentata* L. were evaluated for their insecticidal and repellent activity against adult females of *Culex pipiens* L. This study concludes *Lavandula dentata* oil showed higher repellent activity than *Mentha longifolia* oil against adults of *Culex pipiens*; paraffin oil significantly prolonged the time of protection for the two oils. The longest time of protection was recorded 165 min for *L dentata* oil, at 1 µl/cm^2, when the tested oils were applied in paraffin oil. The researcher suggested the two oils were effective as repellent substances when compared with commercial materials and also recommended further studies to isolate the most effective mosquito control agents from these oils [27].

Using a GC/MS, 24, 17, 20, 21, and 12 compounds were determined by Nataya Sutthanont et al., from essential oils of *Citrus hystrix*, *Citrus reticulata*, *Zingiber zerumbet*, *Kaempferia galanga*, and *Syzygium aromaticum*, respectively. The author analysed the principal constituents found in peel oil of *C. hystrix* were *β*-pinene and *d*-limonene, followed by terpinene-4-ol. Compounds in *C. reticulata* peel oil consisted mostly of *d*-limonene and *γ*-terpinene. The oils obtained from *Z. zerumbet* rhizome had *α*-humulene and zerumbone as major components. The most abundant compounds in *K. galanga* rhizome oil were 2-propeonic acid, pentadecane, and ethyl-*p*-ethoxycinnamate. The main component of *S. aromaticum* bud oil was eugenol, with minor amounts of *trans*-caryophyllene. The researcher

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assessed larvicidal efficacy and demonstrated that all essential oils were toxic against both pyrethroid-susceptible and resistant *Ae. aegypti* laboratory strains at LC_{50}, LC_{95}, and LC_{99} levels. They conclude and documented the promising larvicidal potential of essential oils from edible herbs, which used in controlling vectors of mosquito borne disease [28].

Pavela et al., documented twenty samples of essential oils obtained from *Mentha aquatica*, *M. longifolia*, *M. spicata*, *M. suaveolens*, *M. piperita*, *M. piperita* var. *crispa*, *M. villosa*, and *Pulegium vulgare* were tested for larvicidal activity against *Culex quinquefasciatus*. Essential oils obtained via hydro-distillation and subsequently analysed by gas chromatography-mass spectrometry (GC-MS). The researcher analysed the concentrations causing 50% or 90% larval mortality. Finally they conclude essential oils of *M. longifolia* and *M. suaveolens*, which were containing a majority of piperitenone oxide, and also had the highest larvicidal effects [29].

Table.1: Essential Oils that have shown Larvicidal and Mosquito Repellent Activity.

Vector's Scientific name	Plant Species	Family Name	Plant Parts Used	Reference
Aedes aegypti	*Mentha piperita*	Lamiaceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*Myrtus caryophyllus*	Myrtaceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*Acorus calamus*	Acoraceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*Cinnamomum*	Lauraceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*zeylanicum*	Cardiopteridaceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*Citronella mucronata*	Myrtaceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*Eucalyptus tereticornis*	Rutaceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*Citrus limon (L.)*	Rutaceae	Commercial	Manimaran et al., (2012)
Aedes aegypti	*Citrus sinensis*	Rutaceae	Dried Fruits	Choochote et al., (2007)
Aedes aegypti	*Zingiber piperitum*	Compositae	N.A	Gillij et al., (2008)
Aedes aegypti	*Baccharis spartioides*	Verbenaceae	N.A	Gillij et al., (2008)
Aedes aegypti	*Aloysia citriodora*	Poaceae	Fresh aerial parts	Oyedele et al., (2002)
Aedes aegypti	*C. citratus*	Lamiaceae	Leaves	Prajapati et al., (2005)
Aedes aegypti	*O. basilicum*	Lamiaceae	Shoot	Prajapati et al., (2005)
Aedes aegypti	*Rosmarinus offinalis*	Lauraceae	Bark	Prajapati et al., (2005)
Aedes aegypti	*Cinnamomum*	Lamiaceae	Commercial	Trongtokit et al., (2005)
Aedes aegypti	*zeylanicum*	Myrtaceae	Commercial	Trongtokit et al., (2005)
Aedes aegypti	*Pogostemon cablin*	Rutaceae	Leaves	Trongtokit et al., (2005)
Aedes aegypti	*Syzygium aromaticum*	Poaceae	Leaves	Trongtokit et al., (2005)
Aedes aegypti	*Z. limonella*	Caryophyllaceae	Flowers	Tunon et al., (2006)
Aedes aegypti	*C. nardus*	Caryophyllaceae	Flowers	Tunon et al., (2006)
Aedes aegypti	*D. caryophyllum*	Lamiaceae	Leaves with	Cavalcanti et al., (2004)
Aedes aegypti	*D. caryophyllum*	Lamiaceae	Bloom	Cavalcanti et al., (2004)
Aedes aegypti	*Ocimum gratissimum*	Geraniaceae	Leaves with	Cavalcanti et al., (2004)
Aedes aegypti	*O. americanum*	Poaceae	Bloom	Cavalcanti et al., (2004)
Aedes aegypti	*Pelargonium sidoides*	Poaceae	Leaves	Sukumar et al., (1991)
Aedes aegypti	*C. citratus*	Rutaceae	Leaves	Nataya et al., (2010)
Aedes aegypti	*C. citratus*	Rutaceae	Leaves	Nataya et al., (2010)
Aedes aegypti	*Citrus histrix*	Zingiberaceae	Fruits	Nataya et al., (2010)
Aedes aegypti	*Citrus reticulata*	Zingiberaceae	Fruits	Nataya et al., (2010)
Aedes aegypti	*Zingiber zerumpet*	Myrtaceae	Rhizome	Nataya et al., (2010)
Anapheles stephensi	*Kaemferia galanga*	Lamiaceae	Rhizome	Manimaran et al., (2012)
Anapheles stephensi	*Syzygium aromaticum*	Myrtaceae	Flower	Manimaran et al., (2012)
Anapheles stephensi	*Mentha piperita*	Acoraceae	Commercial	Manimaran et al., (2012)
Anapheles stephensi	*Myrtus caryophyllus*	Lauraceae	Commercial	Manimaran et al., (2012)
Anapheles stephensi	*Acorus calamus*	Cardiopteridaceae	Commercial	Manimaran et al., (2012)
Anapheles stephensi	*Cinnamomum*	Myrtaceae	Commercial	Manimaran et al., (2012)
Anapheles stephensi	*zeylanicum*	Rutaceae	Commercial	Manimaran et al., (2012)
Anapheles stephensi	*Citronella mucronata*	Rutaceae	Commercial	Manimaran et al., (2012)
Anapheles stephensi	*Eucalyptus tereticornis*	Lamiaceae	Commercial	Prajapati et al., (2005)
Anapheles stephensi	*Citrus limon (L.)*	Lamiaceae	Commercial	Prajapati et al., (2005)
Anapheles stephensi	*Citrus sinensis*	Lauraceae	Leaves	Prajapati et al., (2005)

Culex. quinquefasciatus	*O. basilicum*	Lamiaceae	Shoot	Pavela et al., (2014)
Culex. quinquefasciatus	*Rosmarinus offinalis*	Lamiaceae	Bark	Pavela et al., (2014)
Culex. quinquefasciatus	*Cinnamomum*	Lamiaceae	Stage of full	Pavela et al., (2014)
Culex. quinquefasciatus	*zeylanicum*	Lamiaceae	Bloom	Pavela et al., (2014)
Culex. quinquefasciatus	*Mentha aquatica*	Lamiaceae	Stage of full	Pavela et al., (2014)
Culex. quinquefasciatus	*Mentha longifolia*	Learuophullaceae	Bloom	Pavela et al., (2014)
Culex. quinquefasciatus	*Mentha spicata*	Lamiaceae	Stage of full	Pavela et al., (2014)
Culex. quinquefasciatus	*Mentha suaveolence*	Lamiaceae	Bloom	Manimaran et al., (2012)
Culex. quinquefasciatus	*Mentha piperita*	Myrtaceae	Stage of full	Manimaran et al., (2012)
Culex. quinquefasciatus	*Mentha villosa*	Acoraceae	Bloom	Manimaran et al., (2012)
Culex. quinquefasciatus	*Mentha pulegium*	Lauraceae	Stage of full	Manimaran et al., (2012)
Culex. quinquefasciatus	*Mentha piperita*	Cardiopteridaceae	Bloom	Manimaran et al., (2012)
Culex. quinquefasciatus	*Myrtus caryophyllus*	Myrtaceae	Stage of full	Manimaran et al., (2012)
Culex. quinquefasciatus	*Acorus calamus*	Rutaceae	Bloom	Manimaran et al., (2012)
Culex. quinquefasciatus	*Cinnamomum*	Rutaceae	Stage of full	Manimaran et al., (2012)
Culex. quinquefasciatus	*zeylanicum*	Lamiaceae	Bloom	Ansari et al., (2000)
Culex. quinquefasciatus	*Citronella mucronata*	Lamiaceae	Commercial	Prajapati et al., (2005)
Culex. quinquefasciatus	*Eucalyptus tereticornis*	Lauraceae	Commercial	Prajapati et al., (2005)
Culex. quinquefasciatus	*Citrus limon (L.)*	Graminae	Commercial	Pushpanathan et al., (2006)
Culex. quinquefasciatus	*Citrus sinensis*	Zingiberaceae	Commercial	Pushpanathan et al., (2008)
Culex. quinquefasciatus	*Mentha piperita*	Lamiaceae	Commercial	Rajkumar and Jebanesan
Culex. quinquefasciatus	*O. basilicum*	Solanaceae	Commercial	(2005)
Culex. quinquefasciatus	*Cinnamomum*	Poaceae	Commercial	Rajkumar and Jebanesan
Culex. quinquefasciatus	*zeylanicum*	Lamiaceae	Commercial	(2005)
Culex. quinquefasciatus	*C. citratus*	Rutaceae	Fresh leaves	Tawatsin et al., (2001)
Culex. quinquefasciatus	*Zingiber officinalis*	Lamiaceae	Leaves	Tawatsin et al., (2001)
Culex. quinquefasciatus	*Moschosma*	Myrtaceae	Bark	Trongtokit et al., (2005)
	polystachyum		N.A	Trongtokit et al., (2005)
	Solanum		Rhizomes	Trongtokit et al., (2005)
	xanthocarpum		Fresh Leaves	
	C. winterianus		Fresh Leaves	
	O. americanum		Leaves	
	Z. limonella		Leaves	
	Pogostemon cablin		Leaves	
	Syzygium aromaticum		Commercial	
			Commercial	

N.A – Not Available, Commercial – Essential oil purchased commercially.

One study revealed that the essential oil of *Allium macrostemon* bulbs and its two major constituents demonstrate strong larvicidal activity against *Aedes albopictus* mosquito larvae. The results suggested that the essential oil of *A. macrostemon* and the two major constituents may be recommended effectively in mosquito control, they also suggested further evaluation for safety in humans and to enhance their activity [30].

Rajkumar and Jebanesan, analysed essential oil obtained by steam distillation of leaves of *Clausena dentata*. Researcher observed the appearance of essential oil is colourless and a pleasant odour. The mean protection period of *Clausena dentata* essential oil at different concentrations against bite of *Aedes aegypti* was assessed by them. The results suggested that the organic solvent ethanol used in dilution of essential oil in protection period test did not showed positive irritant reaction. Both protection period and skin-irritant tests recommend essential oil could be used as natural repellent that prevent man-dengue vector contact [31].

Manzoor et al., investigated five essential oils from various parts of five plant species such as *Acorus calamus, Mentha arvensis, Ocimum basilicum, Saussrea lappa* and *Cymbopogan citratus* for their larvicidal property against *Aedes aegypti* (L.) and *Culex quinquefaciatus* (Say) larvae and their results are the highest larvicidal activity was documented in the essential oil from *O. basilicum* against *Ae. Aegypti* (L.) and *Cx. quinquefaciatus* (Say) with LC_{50} values. Finally they concluded that essential oils had potential for controlling mosquito larvae [32].

Essential oil hydrolates of four plants such as *Zanthoxylum limonella, Zingiber officinale, Curcuma longa* and *Cymbopogon citratus* were evaluated for their larvicidal activity against two laboratory reared mosquito species - *Aedes albopictus* and *Culex quinquefasciatus*. The researcher revealed that the hydrolate of *Z. limonella* was most effective against both *Ae. albopictus* and *Cx. quinquefasciatus* with LC_{50}values. The larvicidal activity of hydrolates of *Z. officinale*, *C. longa* and *C. citratus* were also found promising with LC_{50}valuesagainst *Ae. albopictus* and *Cx. quinquefasciatus* [33].

Larvicidal bioassay carried out by Sarita Kumar et al., with the seed oil against early IVth instars of *Ae. aegypti* caused LC_{50} and LC_{90} values after an exposure to 24 hours. The larvicidal effect of the celery seed oil augmented by 1.2-fold; after an exposure to 48 hours; revealed LC_{50} values. The seed oil did not cause immediate larval mortality; researchers suggested a delayed toxicity against the larval stage [34].

Larvicidal activity of essential oils from *Blumea mollis* [35] and *Zingifer officinalis* [36] has been reported against *Cx. quinquefasciatus*. Larvicidal activity of essential oils from *Melaleuca leucadendron*, *Litsea* cubeba and *Litsea salicifolia* [37], *Ocimum suave* and *O. kilimandscharicum* [38] have been reported against *Anopheles arabiensis, A. gambiae* and *Cx. quinqefasciatus*. Larvicidal activities of essential oils from *Zanthoxylum armatum* [39] and *Ocimum canum* [40] have been reported against *Cx. Quinquefasciatus, Ae. aegypti* and *An. Stephensi* [41].

Enan suggested that toxicity of constituents of essential oil is related to the octopaminergic nervous system of insects. Relatively few studies have been done on insecticidal activity or fumigant toxicity of caryophyllene oxide. Its high toxicity may result from the inhibition of the mitochondrial electron transport system because changes in the concentration of oxygen or carbon dioxide may affect respiration rate of insect, thus eliciting fumigant toxicity effects [42, 43]. Several reports indicate that essential oils and monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme (AChE) activity. Effects of furanocoumarins and pthalides isolated from *Angelica acutiloba Kitagawa var*. *sugiyame Hikino* against *Droshophila melanogaster* revealed the hypothesis that the insecticidal properties of the plant extracts are connected with the AChE (Acetylcholinesterase) inhibition [44].

Further studies on ethanolic extract from the fruits of *Pimpinella anisoides* V Brig. exhibited activity against AChE and BChE (Butyrylcholinesterase), with IC_{50} values. The most abundant constituents of the extract were *trans*-anethole that exhibited the high activity against AChE and BChE with IC_{50} values [45]. It is confirmed that the insecticidal activity of essential oils and/or mono terpenes is due to several mechanisms that affect multiple targets.

III. CONCLUSION

The most attractive aspect of using essential oils and/or their constituents for pest control is their favorable mammalian toxicity because many essential oils and their constituents are commonly used as culinary herbs and spices and as medicines. It is found that the use of biopesticides will help in preventing the discarding of thousands of tons of pesticides on the earth and provide the residue free food and a safe environment to live [46]. The present review study shows a range of essential oils and phytochemicals from varieties of families that exhibit interesting insecticidal properties against several insects and pests [47].

Essential oils are complex mixtures of various molecules. Their biological effects might be either the result of a synergism of all molecules or could reflect only those of the main molecules. Almost literature cases analyses only the main constituents of essential oils. In that sense, for biological purposes, it could be more informative to study the entire oil rather than some of its components because the concept of synergism seems to be important.

Conclusion of our review study suggests that the development of natural or biological insecticides will help to decrease the negative effects of synthetic chemicals. Negative effects refer to residues in products and insect resistance. The utility of plant's essential oils and phytochemicals analysed by many research studies and also others support the Biopesticidal nature of the plant derived essential oils. These oils can be used as a cheap, eco-friendly safe and efficient alternative to the chemical larvicides.

REFERENCES

[1] Ghosh A, Chowdhury N and Chandra G, (2011). Plant extracts as potential mosquito larvicides. Indian J Med Res, 135(46):581-598.

[2] Kalita B, Bora S and Sharma AK, (2013). Plant essential oils as mosquito repellent - a review. International Journal of Research and Development in Pharmacy and Life Sciences, 3(4):741-747.

[3] Ghoh A, Chowdhury N and Chandra G, (2012). Plant extracts as potential mosquito larvicides. Indian J Med Res, 135(23):581-598.

[4] Maillard M, Marston A, Hostettmann K, Search for molluscicidal and larvicidal agents from plants in Baladrin M, (1993). Human Medicinal Agents from Plants. American Chemical Society. Washington DC, 534: 256-273.

[5] Wattal BL, Joshi GC and Das M, (1981). Role of agriculture insecticides in precipitating vector resistance. J Communicable Diseases, 13: 71-73.

[6] Qiu H, Jun HW and John WM, (1998). Pharmacokinetics, formulation, and safety of insect repellent N, N-diethyl-3-methyl benzamide (DEET): a review. J Am Mosq Control Assoc, 14:12-27.

[7] Abagli AZ and Alavo TBC, (2011). Essential oil from bush mint, Hyptis suaveolens, is as effective as DEET for personal protection against mosquito bites. Open Entomol J, 5:45-48.

[8] Sukumar K, Perich MJ and Boobar LR, (1991). Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc, 7:210-237.

[9] Ignacimuthu S, (2000). The root of botanicals in combating mosquitoes. Abstracts: Proceedings of

symposium on recent trends in combating mosquitoes, Loyola College, Chennai, India, 19.

[10] Markouk M, Bekkouche K, Larhsini M, Bousaid M, Lazrek HB, Jana M, (2000). Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. J Ethnopharmacol, 73: 93-297.

[11] Feinstein L, (1952). Insecticides from plants. In: Insects: The year book of agriculture, USA, Washington, 222-229.

[12] Ansari MA, Mittal PK, Razdan RK and Sreehari U, (2005). Larvicidal and mosquito repellent activities of Pine (Pinus longifolia, Family: Pinaceae) oil. J Vector Borne Dis, 42:95-99.

[13] Wiseman Z, Chapagain BP, (2005). Larvicidal effects of aqueous extracts of Balanites aegyptiaca (desert date) against the larvae of Culex pipiens mosquitoes. Afr J Biotechnol, 4 (11): 1351- 1354.

[14] Chowdhury N, Ghosh A, Chandra G, (2008). Mosquito larvicidal activities of Solanum villosum berry extract against the dengue vector Stegomyia aegypti. BMC Complement Altern Med, 8:10.

[15] Ghosh A, Chowdhury N, Chandra G, (2008). Laboratory evaluation of a phytosteroid compound of mature leaves of day jasmine (Solanaceae: Solanales) against larvae of Culex quinquefasciatus (Diptera: Culicidae) and non-target organisms. Parasitol Res, 103: 221-277.

[16] Joseph CC, Ndoile MM, Malima RC, Nkunya MH, (2008). Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from Neorautanenia mitis. Trans R Soc Trop Med Hyg, 98 (8): 451-455.

[17] Cavalcanti ESB, Morais SM, Lima MAA, Santana EWP, (2004). Larvicidal activity of essential oils from Brazilian plants against Aedes aegypti L. Mem Inst Oswaldo Cruz, 99:541-544.

[18] Khanna VG, Kannabiran K, (2007). Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre, and Eclipta prostrata against Culex qinquifaciatus mosquito larvae. Afr J Biotechnol, 3: 307-311.

[19] Yang YC, Le EH, Lee HS, Lee DK, Ahn YJ, (2004). Repellency of aromatic medicinal plant extracts to Aedes aegypti. J Am Mosq Control Assoc, 20 (2): 146-149.

[20] Shivakumar MS, Srinivasan R and Natarajan D, (2013). Larvicidal Potential of some Indian Medicinal Plant Extracts Against Aedes aegypti (L.). Asian J Pharm Clin Res, 6(3):77-80.

[21] Bhupen Kalita, Somi Bora and Anil Kumar Sharma, (2013). Plant essential oils as mosquito repellent - a review. International Journal of Research and Development in Pharmacy and Life Sciences, 3(1)741-747.

[22] Nerio LS, Olivero-Verbel J and Stashenko E, (2010). Repellent activity of essential oils: A review. Bioresource Technology; 101(1):372-378.

[23] Tripathi AK, Upadhyay S, Bhuiyan M and Bhattacharya PR, (2009). A review on prospects of essential oils as biopesticide in insect-pest management. Journal of Pharmacognosy and Phytotherapy; 1(5):52-63.

[24] Ahmed KS, Yosui Y and Lachikawa T,(2001). Effect of neem oil on mating and oviposition behavior of azuki bean weevil, Callosobrucus chinensis L. (Coleoptera: Bruchidae). - Pakistan Journal of Biological Sciences, 4(11):1371-1373.

[25] Manimaran A, Mary Jee Jee Cruz M, Muthu C, Vincent S and Ignacimuthu S, (2012).Larvicidal and knockdown effects of some essential oils against Culex quinquefasciatus Say, Aedes aegypti (L.) and Anopheles stephensi (Liston).Advances in Bioscience and Biotechnology,3:855-862.

[26] Sukumar K, Perich MJ, Boobar LR, (1991). Botanical derivatives in mosquito control: A review. J Amer Mosquito Control Association, 7: 210-237.

[27] Al-Sarar AS, (2014).Chemical Composition, Adulticidal and Repellent Activity of Essential Oils from Mentha longifolia L. and Lavandula dentata L. against Culex pipiens L. J Plant Prot and Path, Mansoura Univ, 5(7):817-826.

[28] Nataya Sutthanont, Wej Choochote, Benjawan Tuetun, Anuluck Junkum, Atchariya Jitpakdi, Udom Chaithong, Doungrat Riyong, and Benjawan Pitasawat, (2010). Chemical composition and larvicidal activity of edible plant-derived essential oils against the pyrethroid-susceptible and -resistant strains of Aedes aegypti (Diptera: Culicidae). Journal of Vector Ecology, 35(1):106-115.

[29] Pavela R, Kaffková K and Kumšta M, (2014). Chemical composition and larvicidal activity of essential oils from different Mentha L. and Pulegium species against Culex quinquefasciatus Say (Diptera: Culicidae). Plant Protect Sci, 50:36–42.

[30] Liu XC, Liu Q, Zhou L and Liu ZL, (2014). Evaluation of larvicidal activity of the essential oil of Allium macrostemon Bunge and its selected major constituent compounds against Aedes albopictus (Diptera: Culicidae). Parasites and Vectors, 7:184.

[31] Rajkumar S and Jebanesan A, (2010). Prevention of Dengue fever through plant based mosquito repellent Clausena dentata(Willd.) M. Roem (Family: Rutaceae) essential oil against Aedes aegyptiL. (Diptera: Culicidae) mosquito. European Review for Medical and Pharmacological Sciences, 14:231-234.

[32] Manzoor F, Samreen KB and Parveen Z, (2013). Larvicidal activity of essential oils against Aedes aegypti and Culex quinquefasciatus larvae (Diptera: Culicidae). The Journal of Animal and Plant Sciences, 23(2):420-424.

[33] Bipul Rabha, Reji Gopalakrishnan, Indra Baruah and Lokendra Singh, (2010). Larvicidal activity of some essential oil hydrolates against dengue and filariasis

vectors. E3 Journal of Medical Research, 1(1):014-016.

[34] Sarita Kumar, Monika Mishra, NaimWahab and RadhikaWarikoo, (2014).Larvicidal, repellent, and irritant potential of the seed-derived essential oil of Apium graveolens against dengue vector, Aedes aegypti L. (Diptera: Culicidae). www.frontiersin.org , 2(147):1.

[35] Senthilkumar A, Kannathasan K and Venkatesalu V, (2008). Chemical constituents and larvicidal property of the essential oil of Blumea mollis (D. Don) Merr. against Culex quinquefasciatus. Parasitology Research, 103:959-962.

[36] Pushpanathan T, Jebanesan A and Govindarajan M, (2008). The essential oil of Zingiber officinalis Linn (Zin-giberaceae) as a mosquito larvicidal and repellent agent against the filarial vector Culex quinquefasciatus say (Diptera: Culicidae). Parasitology Research, 102, 1289-1291.

[37] Noosidum A, Prabaripai A, Chareonviriyaphap T and Chandrapatya A, (2008). Excito-repellency properties of essential oils from Melaleuca leucadendron L., Litsea cubeba (Lour.) persoon, and Litsea salicifolia (Nees) on Aedes aegypti (L.) mosquitoes. Journal of Vector Ecology, 33:305.

[38] Kweka EJ, Mosha F, Lowassa A, Mahande AM, Kitau J, Matowo J, Mahande MJ, Massenga CP, Tenu F, Feston E, Lyatuu EE, Mboya MA, Mndeme R, Chuwa G and Temu EA, (2008). Ethnobotanical study of some mosquito repellent plants in North Eastern Tanzania. Malaria Journal, 7:152.

[39] Tiwary M, Naik SN, Tewary DK, Mittal PK and Yadav S, (2007). Chemical composition and larvicidal activities of the essential oil of Zanthoxylum armatum DC (Rutaceae) against three mosquito vectors. Journal of Vector Borne Diseases, 44:198-204.

[40] Singh NP, Kumari V and Chauhan D, (2003). Mosquito larvicidal properties of the leaf extract of a herbaceous plant, Ocimum canum (Family: Labitae). Journal of Communicable Diseases, 35:43-45.

[41] Cheng SS, Liu JY, Tsai KH, Chen WJ and Chang ST, (2004). Chemical composition and mosquito larvicidal activity of essential oils form leaves of different Cinnamonum osmophloem provenances. Journal of Agricultural and Food Chemistry, 52:4395-4400.

[42] Enan E, (2001). Insecticidal activity of essential oils: octopaminergic sites of action. - Comparative Biochemistry and Physiology Part C, 130: 325-337.

[43] Emekci M, Navarro S, Donahaye E, Rindner M, Azrieli A, (2004). Respiration of Rhyzopertha dominica (F.) at reduced oxygen concentrations. - Journal of Stored Products Research, 40: 27–38.

[44] Miyazawa M, Tsukamoto T, Anzai J, Ishikawa Y, (2004). Insecticidal Effect of Phthalides and Furanocoumarins from Angelica acutiloba against Drosophila melanogaster. - Journal of Agricultural and Food Chemistry, 52: 4401-4405.

[45] Menichini F, Tundis R, Loizzo MR, Bonesi M, Marrelli M, Statti GA, Menichini F and Conforti F,(2009). Acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from Pimpinella anisoides V Brig. (Apiaceae). - Fitoterapia, 80(5):297–300.

[46] Devi N and Maji TK, (2011). Neem Seed Oil: Encapsulation and Controlled Release - Search for a Greener Alternative for Pest Control. - In: M. Stoytcheva (Ed.): Pesticides in the Modern World - Pesticides Use and Management. InTech, pp.191-322.

[47] Asgar Ebadollahi, (2013).Plant Essential Oils from Apiaceae Family as Alternatives to Conventional Insecticides.Ecologia Balkanica, 5(1):149-172.

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Interaction of Metallic Iron with Solutions Containing Humic Acids and Cu(II)

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Abstract**—*****Humic acids are responsible for the heavy metal movement in the environment. In order to diminish soil pollution with heavy metals the treatment of groundwater with metallic iron has been proposed. Investigations with model solutions containing humic acids and Cu(II) have shown that metallic iron is an effective decontaminant for humic acids containing solutions. The application of the mechanical brush-up of the passive layers from surface using rotating systems loaded with iron pieces gives satisfactory results. The decontamination rate depends mainly on solution pH and the iron surface renewal rate. The presence of Cu(II) ions in the solution or metallic copper in the load increase the decontamination rate*.

Keywords—Humic acids; Copper ions, Metallic iron; Decontamination.

I. INTRODUCTION

The pollution of the environment with heavy metals is steadily increasing due to intensive agricultural and industrial activities. Among the anthropogenic pollutants, heavy metals make up a significant part. This pollution is caused mainly by atmospheric fallout from various sources, the most important being industrial and traffic emissions. Differently from organic pollutants, which may be destroyed to harmless substances, heavy metals are indestructible. Soil pollution by heavy metals has become a widespread serious problem in many parts of the world. Soils contaminated with heavy metals represent a permanent threat to soil ecosystems. Accumulated by plants heavy metals enter the food chain causing damage in animals and humans. However, the mobile species of heavy metals are most dangerous since dissolved in ground water they easily enter the living organisms [1, 2].The mobility of heavy metals is strongly influenced by the presence of other soil constituents and organic matter. The natural organic matter of soils is composed basically from humic substances. According to the solubility criteria, humic substances are divided into humic acids (which are not soluble in acidic solutions, but soluble at pH>2), fulvic acids (which are soluble in water at all pH values) and humans (which are insoluble in water at any pH value). Humic acids and fulvic acids are two major components of humic substances. These acids are organic polyelectrolytes containing various functional groups such as carboxylic, phenolic, and hydroxyl groups, and some functional groups containing nitrogen, sulfur, and phosphorus which form strong complexes with heavy metals [3-6].Humic and fulvic acids, being soluble and capable of forming strong complexes, are responsible for heavy metal spreading in soils.

The implementation of effective techniques and processes for the treatment of contaminated wastewater and groundwater in order to remove or minimize the concentration of pollutants is essential for the environment protection and human health. Widely used methods for effluent treatment such as precipitation, co-precipitation, coagulation, electrocoagulation, sorption etc are ineffective in the case of polluted groundwater due to high stability of heavy metal complexes with humic substances and high stability of the latter [7, 8]. Among the number of methods proposed for the treatment of the wastewaters of complicated chemical composition, zerovalentiron (Fe0) appears to be one of the most relevant [9-12]. Decontamination systems using zerovalent iron have a number of advantages: they are compact, cost effective; compatible with environment; the formed precipitate could be easily immobilized in building materials (concrete, bricks, expanded clays, etc.).

The iron dissolution proceeds with formation of Fe^{2+} or Fe^{3+}. The latter gives an anamorphous precipitate. It is well known that this precipitate acts as a good sorbent for organic and inorganic substances. Due to the formation of iron hydroxides during decontamination the co-precipitation of contaminants with iron oxides also plays an important role. The possible mechanisms of aqueous contaminant removal by metallic iron materials are thoroughly discussed in [12-15]

The main reason for the limited use of iron for the decontamination of polluted groundwater and wastewaters in practice is the passivation of iron surface during the treatment process. To keep the surface active, different means have been proposed, i.e. the usage of salts and complexing agents, contact with more

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electronegative metals. In recent years, the main focus has been devoted to the production of nanoscale iron particles, which enhance the speed and efficiency of the pollutant removal in comparison with micro-scale metallic iron [16, 17]. Usually they are synthesized in non-aqueous solvents using a catalyst and borohydride as a reducing agent. The synthesis and activity maintenance of zerovalent iron nanoparticles is expensive, the practical application is complicated.

This study deals with the mechanical brush-up of the iron surface with the purpose to keep them active during the decontamination process of humic acids containing solutions.

II. EXPERIMENTAL

1.1. Decontamination experiments

For the experiments model solutions containing humic acids and chemically pure copper sulphate were used. Solution pH was adjusted with diluted (1:10) H_2SO_4 or 5 % NaOH solutions. Decontamination was performed in the rotating systems loaded with metallic carbon steel or in the mixture with copper cylinders of 1 cm in diameter and length each (surface area of 4.71 cm^2). These pieces were loaded in a rotating barrel and poured with 1 L of solutions containing humic acids or their mixture with copper sulfate.

1.2. Analysis of solutions

The concentration of humic acids was determined as COD (chemical oxygen demand) in the oxidation reaction with $K_2Cr_2O_7$ after removal of Fe ions from the solution. In order to oxidize Fe(II) to Fe(III) the aliquot of solution after addition of alkali was kept in an open flask for 1 day and mixed occasionally. The Fe(III) hydroxides formed were removed using filtering through glass filters.

Total amounts of Fe(II) and Fe(III) in solutions were determined after Fe(III) reduction to Fe(II) with hydroxylamine; Fe(II) in solutions was determined photometrically at $\lambda = 490$ nm using o-nitrophenantroline as an indicator.

The concentration of Cu(II) in solutions was determined photometrically at $\lambda = 440$ nm using the indicator diethyldithiocarbamate after removal of iron ions with ammonium hydroxide. The formed precipitate, presumably consisting of Fe(II) and Fe(III) hydroxides, was removed using filtering through glass filters.

UV-visible spectra were recorded with a Perkin Elmar Lambda 35 UV/VIS spectrometer at 20ºC in 1 cm length quartz cells.

1.3. Analysis of the precipitate

The precipitate was also examined by means of the dual beam system Helios NanoLab 650 (FEI) in secondary electrons mode at 500 eV electron landing energy. EDS spectra were obtained on the same equipment using an Xmax 20 mm^2 (Oxford Instruments) X-ray detector at 20 kV.

III. RESULTS AND DISCUSSION

A. Decontamination of solution

Investigations showed that without renewal of iron surface the removal of Cu(II) from solutions stops after ~0.5 h and that of humic acids after ≤1 h. In order to find out the rotating rates for the most complete removal of passive layers from iron surface the experiments with different rotating rates during 1 h were carried out. Data depicted in Fig. 1 indicate that with increase of rotating rate the degree of both Cu(II) and humic acids increases and reaches its maximal values at 10 and 15 revolutions per minute (rpm) respectively. Further investigations were carried out at 2 different rotating rates, i.e. at 2 and 20 rpm when iron dissolution is insufficient and when the iron dissolution rate at the least affects the decontamination process resprctively.

It is well known that the humic acids form strong complexes with heavy metals including Fe(II) and Fe(III) [3-6]. Their solubility depends on the ratio between the concentrations of humic acids and metal ions and pH. Preliminary investigations using metallic iron for the humic acid removal from solution have shown that at the initial stage the dissolved iron makes soluble compounds. Later with an increase in dissolved iron concentration an insoluble precipitate forms. The effectiveness of the removal of humic acids from solutions can be easily evaluated visibly by its changes in color from brown to colorless. Humic acids demonstrate especially high absorbance in the UV wave-rang. Later with increase in wavelength it decreases (Fig. 2). Remarkably, the maximal absorbance is observed after some time, susceptibly, when the maximal amount of soluble iron-humic acid complex is formed. However, the COD (chemical oxygen demand), which reflects the humic acid concentration in the solution, steadily decreases.

Further investigations were focused mainly on the influence of pH and iron surface renewal rate (rotating rate) on the decontamination process. The enhancing effect of pH on the solubility of humic substances and hence on their mobility in soils is well known. On the other hand, increasing pH markedly slows down iron dissolution. However, the iron dissolution in the presence of humic substances is poorly investigated especially in the case when the iron surface is renewed mechanically.

The data presented in Fig.3 showed a significant influence of both pH and rotating rate on the decontamination process. As it could be expected an increase in pH decreases the rate of both metallic iron

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dissolution and its reaction with solution constituents; meanwhile an increase in rotating rate enhances the removal of the inactive iron compound from the surface and the increase in available sites for the reaction of solution components. In this case the decontamination rate remarkably increases.

Similar effects of pH and rotating rate are seen in the case when Cu(II) ions are present in the solution (Fig. 4). However, in this case the humic acid removal rate remarkably increases and the concentrations of Fe ions in solution become considerably lower. Besides, Cu(II) ions from the solution are completely removed after 0.5 h of treatment. Such an enhancing effect of Cu(II) ions on the decontamination process could be explained by either binding a part of humic acids with Cu(II) ions or an increase in metallic iron dissolution rate due to the formation of a galvanic pair of reduced copper on the iron surface. The recorded UV/Vis spectra also demonstrate a dramatic increase in absorbance in solutions containing Cu(II) indicating the formation of strong bonds between the humic acids and Cu(II).

In order to check the influence of Fe-Cu galvanic pair on the decontamination process the experiments were carried out with a load composed of iron and Cu pieces. The results of the investigations are presented in Fig 5 and Table 1. The humic acids removal rate is higher than that in the case of a simple Fe load and lower than that when Cu(II) is added into the solution. The content of Fe ions in this case is also lower than that in the case of Fe load and higher than that in the case of the addition of Cu(II) to the solution.

A strong effect of Cu(II) on the decontamination process is also seen in the case when Cu(II) is added to the solution in the form of a strong complex such as EDTA (Fig.6). The decontamination of the solution containing Cu(II)-EDTA complexes is a hard-to-solve problem. The application of metallic iron at optimal pH~3 enables to achieve a rather high degree of the removal of this complex. However, in alkaline solutions the effectiveness of decontamination is low.

Despite the uncomplimentary assessment of the kinetic investigations [18], the kinetic peculiarities of humic acid removal were evaluated by testing the experimental data according to the first order (1) and second order (2) kinetic equations:

$$c = c_0 \cdot e^{-k_1 t} \qquad (1)$$

$$\frac{1}{c} - \frac{1}{c_0} = k_2 t \qquad (2)$$

where$c$ is the concentration of humic acid at time t, mg L^{-1}, c_o is the initial concentration of humic acid mg L^{-1}; k_1 is the first order reaction rate constant, h^{-1}, and k_2 is the second order reaction rate constant, L mg^{-1}□h^{-1}. The plotting ln$c$ or 1/c versus t enables to determine -k_1 ork_2, respectively.

In kinetic experiments a coincidence regarded as reasonably satisfactory was obtained applying the first order and the second order rate equations. The parameters are presented in Table 1. The values of the regression coefficients $R^2 > 0.9$ indicate the possible run of process according to the first and second order reaction models. The presented values of k_1 and k_2 indicate that the reaction between the humic acids and Fe most rapidly proceeds at pH 6 and higher rotating rates, while it is slower at pH 8 and lower rotating rates, except for the case depicted in Fig. 4, curve 3, when the highest k_1 and k_2 are obtained at pH8.

The estimated better fit of kinetic data to the first and second order equations points to a rather complicated mechanism of humic acids and their Cu(II) complex removal from the solution. Actually, thetheoretical aspects of the decontamination of hazardous substances using iron have not been thoroughly investigated yet and most likely they are more complicated than the complexation or precipitation of insoluble compounds. Firstly, not only iron but also the hydrogen evolved during iron dissolution can act as a reducingagent. The reductive destruction proceeds when iron is applied to decontaminate solutions containing hazardous compouds such as chlorinated solvents, dioxines, pesticides, dyes etc. Metallic iron easily destroys unsaturated bonds in many organic compounds. Being of complicated organic composition, humic acids also could undergo reductive destruction.

Depending on pH and th epresence of an oxidizing agent, for instant oxygen, the iron dissolution reaction proceeds with formation of Fe^{2+}or Fe^{3+}. The dissolved iron undergoes further spontaneous reactions to produce hydroxides and/or polyhydroxides.

$$2Fe^{2+} + 1□2\ O_2 + 3H_2O\ □\ 2FeOOH + 4H^+, \qquad (3)$$

$$2Fe^{2+} + 1□2\ O_2 + 2H_2O\ □\ Fe_2O_3 + 4H^+. \qquad (4)$$

It is well known that this amorphous precipitate acts as a good sorbent for organic and inorganic substances. Due to the formation of iron hydroxides during the decontamination process, the coprecipitation of contaminants also plays an important role. The formation of polynuclear iron-hidroxococmplexes is also possible. These complexes distinguish themselves by high sorption capacity. The possible mechanisms of aqueous contaminant removal by metallic iron materials are thoroughly discussed in [15-18].

B. Investigations of precipitate

Differently from other precipitates, the distinctive feature of the precipitate formed using treatment with metallic

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iron is its compactness. The precipitate is magnetic and easily removable from solutions.

Sure enough, investigations of the chemical composition of precipitate (Table 2) have shown that it is composed from three main elements: consituents of humic acids, i.e. oxygen and carbon, and iron from steel. Precipitate also contains small amounts of impurities from carbon steel or humic acids such as Mn, Si, Ca. When the solutions contain Cu(II) ions (Fig. 4, Table 2) the content of Cu in the precipitate is also significant. However, the precipitate formed under different conditions also differs in composition. As a rule, the amount of carbon in the precipitate decreases with increase in the amount of Fe. This effect correlates with the effectiveness of decontamination. The most rapid and complete treatment of solutions containing humic acid sgives the precipitate containing a low amount of Fe and a high amount of carbon as it is in the case depicted in Fig.4, curve 1.

The morphology of the precipitate was observed by scanning electron microscopy (SEM) (Fig. 7). All the samples investigated have shown a typical grainy constitution characteristic of compact compounds.

IV. FIGURES AND TABLES

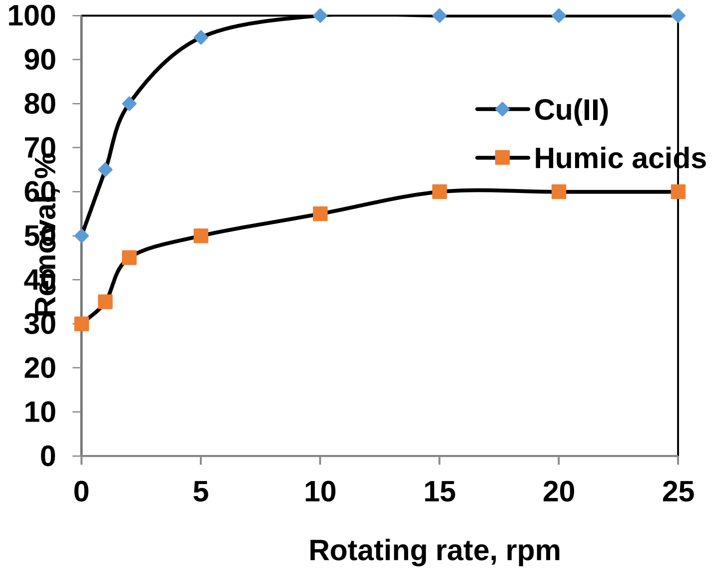

Fig. 1. Cu(II) and Humic acids removal from their 100 mg L^{-1} solutions. Load – 100 Fe pieces of 4.71 cm2 surface; initial pH 3; rotating rate 20 rpm

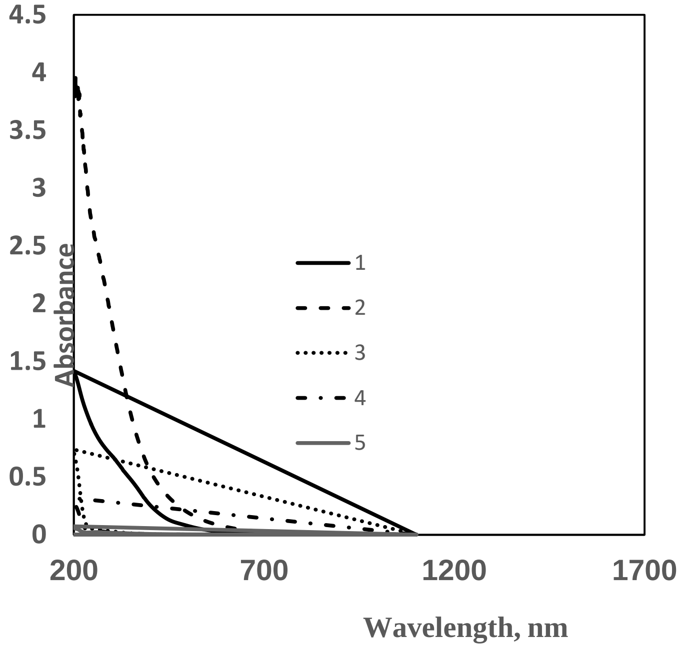

Fig.2: UV/vis spectra of 100 mg L-1 humic acids containing solution in dependence on treatment time with metallic iron (h): 1- 0; 2 – 1; 3 – 4; 5 – 6. Load – 100 Fe pieces of 4.71 cm2 surface; initial pH 3; rotating rate 20 rpm

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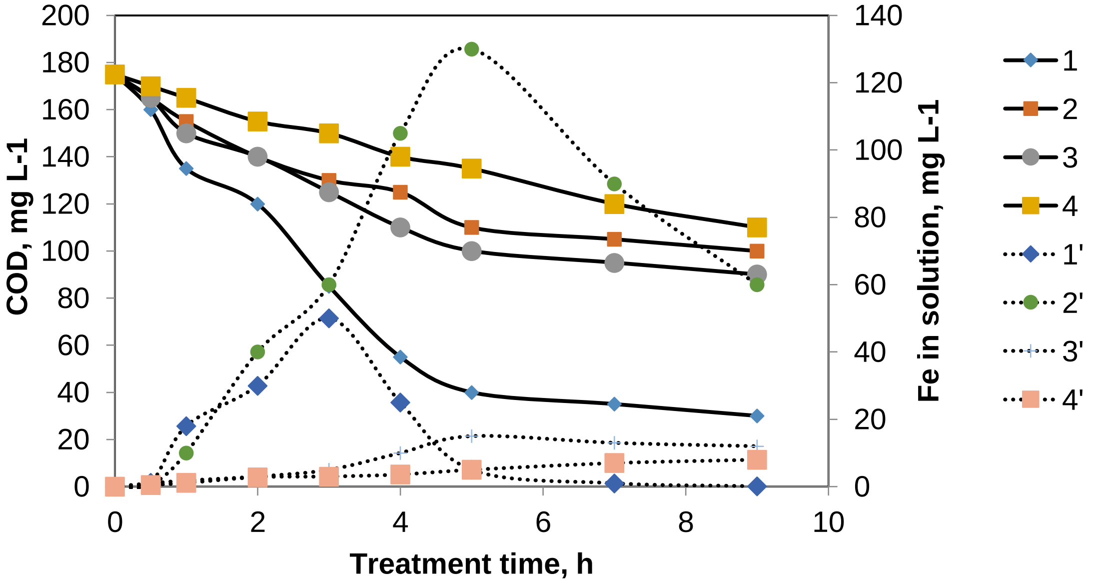

Fig.3. Humic acids as COD (1, 2, 3 and 4) and Fe_{total} (1', 2', 3' and 4') concentration changes with treatment time in solution containing initially 100 mg L-1 humic acids. Load – 100 Fe pieces of 4.71 cm^2 surface; initial pH 6 (1, 1', 2 and 2') and 8 (3, 3', 4 and 4'); rotating rate 20 rpm (1, 1', 3 and 3') and 2 rpm (2, 2', 4 and')

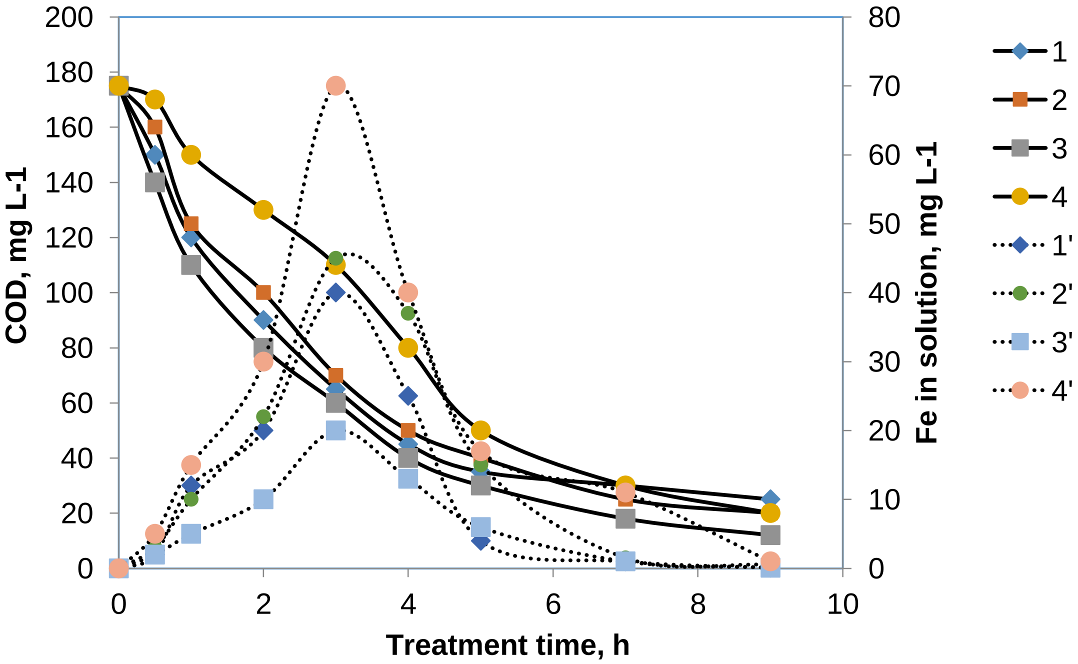

Fig.4. Humic acids as COD (1, 2, 3 and 4) and Fe_{total} (1', 2', 3' and 4') concentration changes with treatment time in solution containing initially 100 mg L^{-1} humic acids and 64 mg L^{-1} Cu(II). Load – 100 Fe pieces of 4.71 cm^2 surface; initial pH 6 (1, 1', 2 and 2') and 8 (3, 3', 4 and 4'); rotating rate 20 rpm (1, 1', 3 and 3') and 2 rpm (2, 2', 4 and 4')

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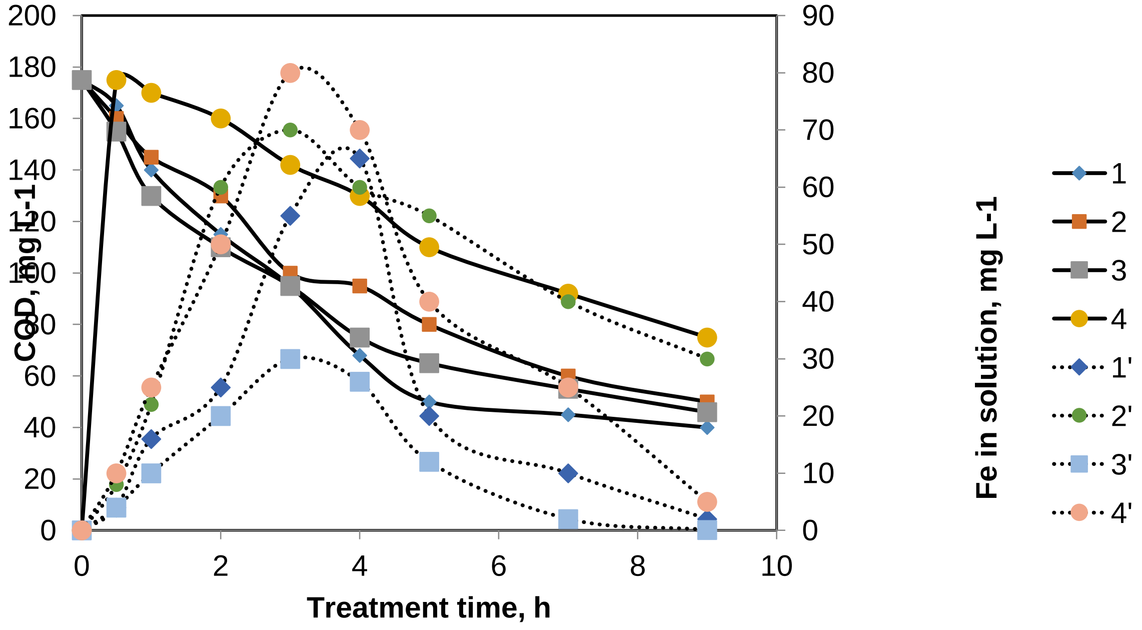

Fig. 5. Humic acids as COD (1, 2, 3 and 4) and Fe_{total} (1', 2', 3' and 4') concentration changes with treatment time in solution containing initially 100 mg L^{-1} humic acids. Load – 100 Fe pieces of 4.71 cm2 surface and 50 Cu pieces 4.71 cm^2 surface ; initial pH 6 (1, 1', 2 and 2') and 8 (3, 3', 4 and 4'); rotating rate 20 rpm (1, 1', 3 and 3') and 2 rpm (2, 2', 4 and 4')

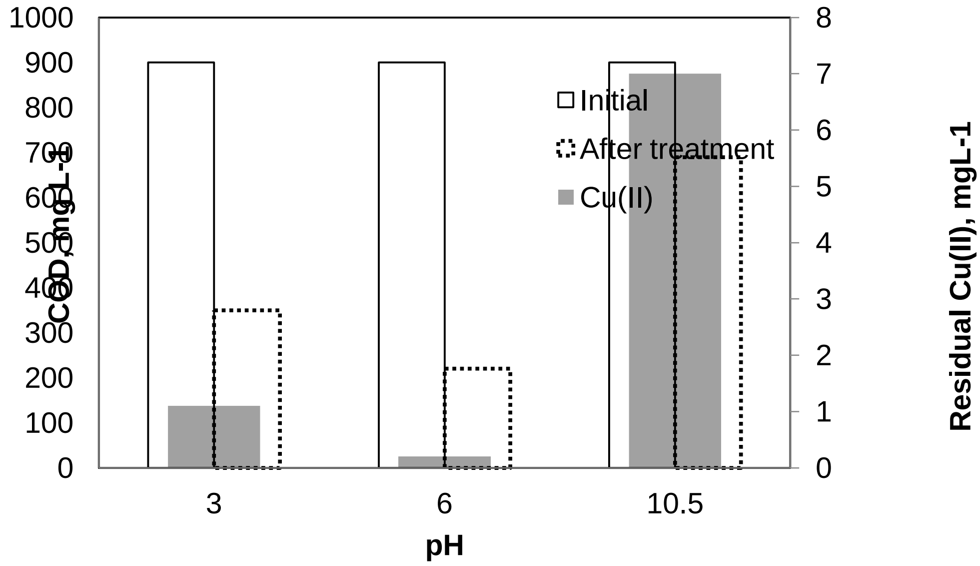

Fig. 6. Influence of pH on organic matter and Cu(II) removal from solutions containing humic acids 200 mg L^{-1}, EDTA – 2 mmol L^{-1} and Cu(II) – 1 mmol L^{-1}. Load – 100 Fe pieces of 4.71 cm^2 surface; rotating rate 20 rpm

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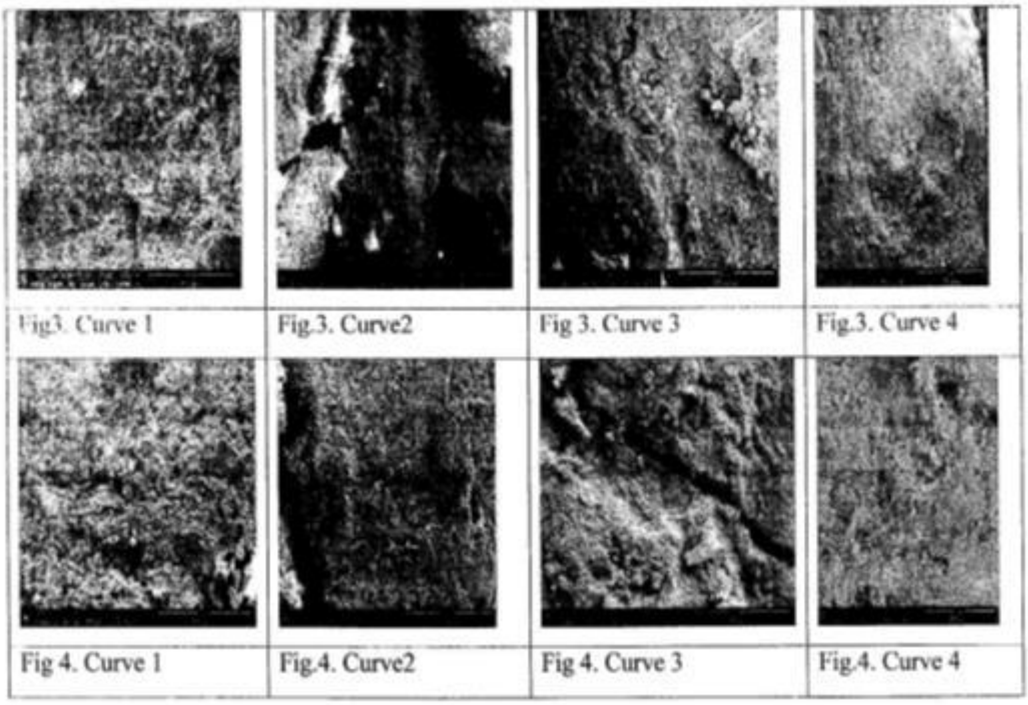

Fig, 7: SEM images of precipitate. Experiment conditions confirm those given in captions to Figures

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Table.1: The first and second order rate constants and correlation coefficients in kinetic experiments

Treatment conditions as in	First order reaction parameters		Second order reaction parameters	
	k_1, h^{-1}	R^2	k_2, L mg^{-1}□h^{-1}	R^2
Fig.3 Curve 1	0.256	0.96	0.001	0.959
Curve 2	0.63	0.934	0.003	0.962
Curve3	0.77	0.924	0.001	0.952
Curve4	0.051	0.997	0.001	0.996
Fig.4 Curve 1	0.227	0.927	0.004	0.982
Curve 2	0.255	0.977	0.005	0.973
Curve 3	0.3	0.987	0.008	0.941
Curve4	0.256	0.982	0.004	0.901
Fig.5 Curve 1	0.18	0.986	0.002	0.963
Curve 2	0.142	0.988	0.001	0.986
Curve 3	0.149	0.958	0.001	0.995
Curve 4	0.123	0.94	0.001	0.975

Table.2: Chemical composition of precipitates

Treatment conditions	Content of elements in atomic %									Remarks
	C	O	Na	Si	S	Ca	Mn	Fe	Cu	
Fig3. Curve 1	35,39	46,33	0,27	0,14	0,14	0,05	0,1	17,56	0,01	Nonmagnetic
Fig3. Curve 2	31,18	49,01	0,31	0,15	0,13	0,04	0,1	19,05	0,03	Nonmagnetic
Fig3. Curve 3	36,76	45,55	0,47	0,19	0,18	0,08	0,11	16,68	0	Magnetic
Fig3. Curve 4	40,99	42,73	0,3	0,23	0,14	0,07	0,07	15,37	0,1	Magnetic
Fig. 4 Curve 1	40,88	42,89	-0,02	0,13	0,15	0,03	0,07	12,91	2,96	Magnetic
Fig. 4	38,5	43,98	0	0,11	0,6	0,01	0,02	14,28	2,49	Magnetic

Curve 2										
Fig. 4 Curve 3	37,37	44,37	0,01	0,17	0,15	0,05	0,08	14,7	3,09	Magnetic
Fig. 4 Curve 4	41,5	41,66	0,03	0,23	0,19	0,06	0,06	12,49	3,82	Magnetic
Fig. 5 Curve 1	43	41,44	0,09	0,13	0,07	0,05	0,08	14,94	0,19	Magnetic
Fig. 5 Curve 2	38,39	44,32	0,11	0,14	0,08	0,05	0,1	16,63	0,17	Magnetic

V. CONCLUSION

Metallic iron is an effective decontaminant for solutions containing humic acids. The application of mechanical brush-up of the passive layers from the surface using rotating systems loaded with iron pieces gives satisfactory results.

The decontamination rate depends mainly on solution pH and the iron surface renewal rate.

The presence of both Cu(II) ions in the solution or metallic copper in the load increases the decontamination rate.

REFERENCES

[1] J. Kumpiene, G.Guerri, L.Landi, G.Pietramellara, P.Nannipieri, G.Renella, Microbial biomass, respiration and enzyme activities after in situaided phytostabilization of a Pb-and Cu-contaminated soil. Ecotoxicology and Environmental Safety 72 (2009) 115–119.

[2] R. BouKheir, B. Shomar, M.B. Greve, M.H. Greve, On the quantitative relationships between environmental parameters and heavy metals pollution in Mediterranean soils using GIS regression-trees: The case study of Lebanon. Environmental Pollution 206 (2015) 227-235.

[3] J. Burlakovs, M. Klavins, L. Osinska, O. Purmalis, The Impact of Humic Substances as Remediation Agents to the Speciation Forms of Metals in Soil. APCBEE Procedia 5 (2013) 192 – 196.

[4] Wang-Wang Tang, Guang-Ming Zeng, Ji-Lai Gong, Jie Liang, Piao Xu, Chang Zhang, Bin-Bin Huang, Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: A review, Science of the Total Environment 468–469 (2014) 1014–1027.

[5] Medhat A. Shaker, Hassan M. Albishri, Dynamics and thermodynamics of toxic metals adsorption onto soil-extracted humic acid, Chemosphere 111 (2014) 587–595.

[6] Qi Wang, ZhiyiXie, Fangbai Li, Using ensemble models to identify and apportion heavy metal pollution sources in agricultural soils on a local scale, Environmental Pollution 206 (2015) 227-235.

[7] Kai Fang, Dongxing Yuan, Lei Zhang, Lifeng Feng, Yaojin Chen, Yuzhou Wang, Effect of environmental factors on the complexation of iron and humic acid, Journal of Environmental Sciences 27 (2015) 188-196.

[8] C. Colombo, G. Palumbo, V. Michele Sellitto, H. Goo Cho, C. Amalfitano, P.a Adamo, Stability of coprecipitated natural humic acid and ferrous iron under oxidative conditions, Journal of Geochemical Exploration 151 (2015) 50–56.

[9] Y. Han, W. Yan, Bimetallic nickel-iron nanoparticles for groundwater decontamination: Effect of groundwater constituents on surface deactivation. Water Res. 66 (2014) 149-159.

[10] J. Kumpiene, S. Ore, G. Renella, M. Mench, A. Lagerkvist, Ch. Maurice, Assessment of zerovalent iron for stabilization of chromium, copper, and arsenic in soil, Environmental Pollution 144 (2006) 62-69

[11] J. Kumpiene , I. Castillo Montesinos, A. Lagerkvist, Ch. Maurice, Evaluation of the critical factors controlling stability of chromium, copper, arsenic and zinc in iron-treated soil, Chemosphere 67 (2007) 410–417

[12] O. Gyliene, T. Vengris, O. Nivinskiene, R. Binkiene, Decontamination of solutions containing Cu(II) and ligands tartrate, glycine and quadrol using metallic iron. J. Hazard. Mater. 175 (2010) 452–459.

[13] C. Noubactep, ‘A critical review on the process of contaminant removal in Fe0-H2O systems’, Environ. Technol., , 29(2008) 909-920.

[14] R. Rangsivek, M.R. Jekel, ‘Removal of dissolved metals by zero-valent iron (ZVI):Kinetics, equilibria, processes and implications for stormwater runoff treatment, Water Res.39 (2005) 4153–4163.

[15] D. Karabelli, C. Uzum, T. Shahwan, A. E. Eroglu, T. B. Scott, K. R. Hallam, I. Lieberwirth, Batch Removal of Aqueous Cu2+ Ions Using Nanoparticles of Zero-Valent Iron: A Study of the Capacity and Mechanism of Uptake, ,Ind. Eng. Chem. Res. 47 (2008) 4758–4764.

[16] Tingyi Liu, Zhong-Liang Wang, Yanqiu Sun. Manipulating the morphology of nanoscale zero-

http://dx.doi.org/10.22161/ijeab/2.6.47 *ISSN: 2456-1878*

valent iron on pumice for removal of heavy metals from wastewater, Chemical Engineering Journal 263 (2015) 55–61

[17] Seol Ah Kim, Seralathan Kamala-Kannan, Kui-Jae Lee, Yool-Jin Park, Patrick J. Shea,Wang-Hyu Lee, Hyung-Moo Kim, Byung-Taek Oha, Removal of Pb(II) from aqueous solution by a zeolite–nanoscalezero-valent iron composite. Chemical Engineering Journal 217 (2013) 54–60.

[18] C. Noubactep, On the validity of specific rate constants (kSA) in Fe0/H2O systems', J. Hazard. Mater. 164 (2009) 835-837.

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Mass Culturing of Stem and Bulb Nematode (*Ditylenchus dipsaci*) for use in screenİng and İmpression Training on Carrot Discs

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Abstract— *Stem and bulb nematode, Ditylenchus dipsaci, is a very important plant parasitic nematode, has a very wide host range and causes economic yield losses in many cultural plants in worldwide. Its races are very diverse and found in most temperate areas of the world. D. dipsaci is a migratory endoparasite, has the ability to enter into a dormancy stage. Genetic resistance offers one of the best control methods within the integrated pest management strategies for D. dipsaci. However mass rearing of stem and bulb nematode requires for the resistant studies including screening for this nematode species. The aim of study is focused on alternative rearing methods using carrot discs as a food source, culturing medium and provide a clearly outlined and visually informative guide. Carrot discs enable the rearing of high numbers of individuals of D. dipsaci for timely use in experiments and for screening purposes in under sterile conditions to provide a clean, same and pure source of inoculum. The carrot disc method has been shown to be suitable for stem and bulb nematode multiplication.*

Keyword—Resistant,stem and bulb nematode,culture,Technique.

I. SUMMARY

Nematologists have a long time were looking to find the best methods for culture and increase population density of plant parasitic nematodes (5)., but these techniques generally has not easy. Although,the three most commenly used techniques for culturing migratory endoparasitic nematodes, including alfa alfa callus (1,3,6),root expland (4),and carrot discs (5) have been published. We present in this study tried to use carrot cultur method to increase population density of nematodes. *Ditylenchus dipsaci* or stem nematode, attacks more than 1200 type of wild and cultivated plants.many agriculture plants are hosts for this species of nematode.*D.dipsaci* live mostly as a migratory endoparasite in different parts of plants like stem,leaves and flowers.In vitro rearing of *Ditylenchusdipsaci* nematodes on carrot discs was used for grown nematodes. This technique is workable to migratory nematodes (*Ditylenchus*spp) for serial manufacture of nematode for experimental aim, direct studies of nematodes, screening trial and increase nematodes. The most advantage this method to save timethat,allow a quick mass production of nematode. Plant parasitic nematode can only develop in fresh root tissue this method help *Ditylenchusdipsaci* nematode to be produce on sterilised carrot discs in petri dishes in an incubator more than in root plant under laboratory condition. Nematodes feeding two the type of plant tissue that necessary for their culture their can feed as migratory endoparasites and ectoparasites.Sedentary endoparasites nematode need individuate tissue for reproduction but it is the opposite about migratory endoparasites nematodes like *Ditylenchusdipsaci* that do not require for it and reproduce easily on undifferentiated tissue like as carrot disk and it has been indicated to be suitable tissue for their multiplication.This technique provides a basic facility for migratory endoparasites nematodes to be reared carrot discs in petri dishes inside an incubator more than in root of plant under greenhouse condition (8).Also, This study provides a protocol for the use carrot to produce and extract stem and bulb nematodes from carrot disk culture.

II. MATERIAL AND METHOD

We choose infected roots or soil with *Ditylenchusdipsaci* for extraction of the nematode to be culture.Nematodes were inoculated on the carrot disc.The suggested temperature for rapid multiplication between 19°c and 23°c depending on nematode reproduction population must be sub culture every 4-6 weeks, the development rate decreased when Temperatureincreased up to 30° or decrease down to 18 °c.The first to start a carrot culture, weselected clean carrots without overly thick and cracks, Washes selected carrots under distilled water, sterilise all of equipment and materials by autoclaving at 121°c for 20 minutes and sterilise the workingand tools surface with 96% ethanol and hold the carrot with 96% ethanol and flame over spirit lamp repeat this for three times.Also, westerilised all of the equipment including: forceps,peeler and knife with ethanol and flaming eachbefore used them every time. We peeled the carrot with the sterilised peeler and Cut the peeled carrot into 5 mm thick section of 3-4 cm diameter and transfer the cut carrot disc into sterilise petri dishes by using the sterilised forceps. Disk must not be less than 3 cm diameter, then transfer the carrot discs into sterilise glass petri dishes (5-6 cm diameter). We stored them in the dark incubator to keep underground condition for 3-4 weeks at 19-23°c.Also,it was necessary control carrot discs best signal of healthy cultures during incubation every week. After 3-4 weeks nematodes were started to exit the carrot and when nutrient in carrots go to become empty it was indicator for harvesting the nematodes .The other words, incubation time and the initial inoculum effected how soon the nutrients become empty. For selection of nematodes we placed a concentrated of the nematode extract into a small (3-5 cm diam.) andaccounted under light microscope.The number of nematode for inoculation depends how many carrots are to be required. However,it was suggested to inoculate at least 100-150 nematodes on one carrot disc and not inoculate less than 100 of them.It was recommended to inoculate at least five replicates from petri dishes .For mass multiplication we increasednumber of inoculated nematode on one carrot disc and selected female and male of the *Ditylenchusdipsaci* nematode and placed them in a glass petri including sterile distilled water.The aim was to deliver 50-80 nematodes per disc in a maximum of two 50 microliter smaller drops of nematode suspension transfer the nematodes.The number of nematodes in the suspension were determined the number to transfer. It was suggested use about 80-150 nematodes for mass multiplication. After that, we placed the carrot disk in an incubator for 5-6 week, after thanthe nematodes were harvested and stored in the fridge at 5°c,the nematode can remain viable for up to 1-2 week. For a more completed collection, the first cut carrot disc to small pieces and placed in the petri dishes with sterile water and let the nematodes migrate freely into the petri, thenextracted nematodes counted under binocular microscope and placed in the flask.

III. RESULT

The number of nematodes extracted from the 45,60,90 and 120 days callus culture are listed (Table 1). 2 or 3 thousands of nematodes can be extracted out of one carrot discs after 45-60 days of culture. However, the number of nematode is different use of nematode species and geographical population The greatest number of nematodes was recoverd from the 45 old day with temperature of 19 °c to 23°c,which yielded a greater than 1,2 and 3 fold in number of nematodes that was observed from the 60, 90 and 120 days with same temperature.The lowest number of nematodes recovery was from the 120 days with 25°c to 30°c in the carrot disk taht was only slightly increased from the initial inoculum levels.The number of nematodes extracted from callus after 45 days with 19°c to 23°c showed potential yields using this technique for us (Table 1).The other word,extracted of more than 3400 nematodes were observed formthe 45 days with 19°c to 23°c. Therfore, temperature between 19 °c to 23°c could increase the cultivation potential of *Ditylenchus dipsaci* nematode.Also,These result indicated that, when as the number of days increased and the temperature dropped under 19°c or increased up to 23°c, the number of nematodes decreased.Becuace of that we observedthat The stem and bulb (ditylenchus dipsaci) nematode was suited for culture carrot disk and the yield of eggs and increased population density of this migratory endoparasite nematode was much better by this technique than other extraction and culture methodes (2,5,7,8,9).Therfore, the carrot callus method was suitable and easy to use and produce high number of Ditylenchus dipsaci(Fig 1).

Table.1: The effect of different days and temperature on the development of the Ditylenchus dipsaci nematode.

Nematode number	15-18°c	19-23°c	25-30°c
45 day-old cultur	2200	3400	1400
60 day-old culture	2000	3000	1100
90 day-old culture	1100	1400	900
120 day-old culture	600	800	500

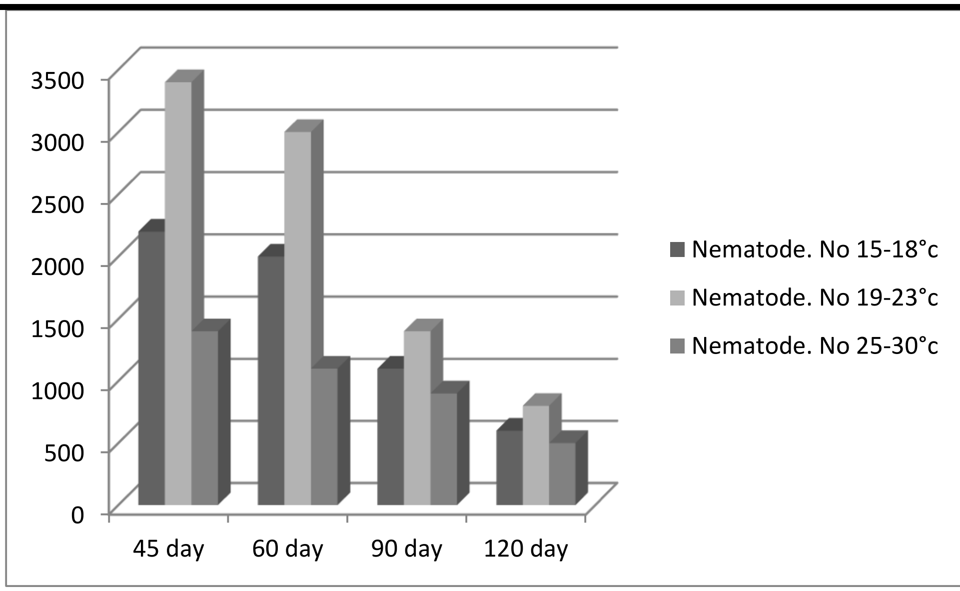

Fig.1: Mixed life stage and eggs number ofDitylenchus dipsaci nematode from 45,60,90 and 120 days old carrot callus culture.

IV. DISCUSSION

This protocol presented is now routinly used in our laboratory.The condition of carrots was importent, work (7) has shown that carrots used in carrot disk culture must be freshly harvested from the field and greenery intact before use.Also, carrots that are badly cut must be avoided.Culture can remain available for up to 2 months and are easly generated.Once the nematode are established on the callus can be divided to small infested piece and transferred directly to make a new carrot culture.The yield of eggs and increased population density of this migratory endoparasite nematode was much better by this technique than other extraction and culture methodes (2,5,7,8,9).

REFERENCES

[1] Bingefors, S., and K. B. Eriksson. 1963. Rearing stem nematode inoculum on tissue culture: Preliminary report. Landbrukshogskolan Annaler 29: 107-118.

[2] Huettel, R. N. 1985. Carrot disc culture. Pp. 212 in B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant nematology, laboratory manual. University of Massachusetts Agricultural Experiment Station, University of Massachusetts, Amherst.

[3] Krusberg, L. R. 1961. Studies on the culturing and parasitism of plant-parasitic nematodes, in particular Ditylenchus dipsaci and Aphelenchoides ritzemabosi on alfalfa tissues. Nematologica 6:181-200.

[4] Lauritis, J. A., R. V. Rebois, and L. I. Graney. 1982. Gnotobiotic cultivation of Heterodera glycines Ichinohe on Glycine max (L.) Merr. Journal of Nematology 14:422-424..

[5] Lawn, D. A., and G. R. Noel. 1984. Monoxenic culture of Pratylenchus scribneri on carrot disks. Proceedings of the First International Congress of Nematology. P. 47 (Abstr.)

[6] Myers, R. J., W. A. Feder, and P. C. Hutchens. 1965. The rearing ofRadopholus similis (Cobb) Thorne on grapefruit, okra, and alfalfa root callus tissues. Proceedings of the Helminthological Society of Washington 32:94-95

[7] Moody, E. H., B. F. Lownsbery, and J. M. Ahmed. 1973. Culture of the root-lesion nematode Pratylenchus vulnus on carrot disks. Journal of Nematology 5:225-226.

[8] O'Bannon,J. H., and A. L. Taylor. 1968. Migratory endoparasitic nematodes reared on carrot discs. Phytopathology 58:385.

[9] Young, T. W. 1954. An incubation method for collecting migratory endo-parasitic nematodes. Plant Disease Reporter 38:794-795

A Review of Rainfall Erosivity as a Natural Factor of Gully Erosion

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Abstract— *In this 21st century which is climate change-driven with more extreme rainfall events, gully erosion is increasingly becoming a global environmental problem influenced by both natural and anthropogenic factors. This paper is a literature review of rainfall erosivity as one of the natural factors of gully erosion. The central objective of the paper is to show in a global context how rainfall erosivity has influenced gully erosion. The method used is a review of academic/journal articles, textbooks, internet materials, conference papers and publicly available materials on gully erosion and rainfall erosivity as one of its factors. Previous authors whose works were reviewed on rainfall erosivity as a factor of gully erosion have a convergent view that rainfall is the primary cause of water-induced erosion and its power to do so is known as erosivity. They were of the view that a more intense rainfall of short duration can cause gully erosion more than a less intense one for a longer period of time. The authors also have a unity of opinion that areas with high rainfall regime such as the tropics are more prone to soil erosion than areas with low rainfall events. Recommendations to reduce the influence of rainfall as a factor of soil erosion include planting of trees and grasses that reduce the impact of rainfall to detach soil particles, conservation practices such as terracing, strip cropping and contour ploughing, a shift from rain-fed agriculture to dry season farming and grants to the affected people and landholders to manage gully erosion using adaptive measures based on their indigenous knowledge.*

Keywords*— *Gully Erosion, Rainfall Erosivity, Review, Model, Soil Loss, Sustainable Development.

I. INTRODUCTION

Gully erosion menace is a global phenomenon that provokes geographical instability (Dada, 2002; Abraham, 2010). It remains one of the most challenging environmental problems in the globe (Zegeye, Abiy and Tebebu, 2010; Ogbonna, 2012). The United States Global Change Research Information Office (2001) stated that approximately 90 percent of cropland in the United States is currently losing soil above the sustainable rate; the erosion rates in Asia, Africa and South America are estimated to be as high as those in the United States. In Nigeria, gully erosion is the most serious problem affecting a large population of urban and rural environments in South Eastern Nigeria (Ezeigwe, 2015). Agulu-Nanka in Anambra State is an area badly affected by gully erosion; up to 250 tonns per hectare have been lost (Kalu, 2001).

Gully erosion is a striking feature on the land surface and also an agricultural problem in the world (Igbokwe, 2008). Shit and Maiti (2002) asserted that gully erosion is one of the major devastating catastrophe that speed up soil erosion. Gully erosion is a highly visible form of soil erosion that affects geomorphological landscapes which restricts land use and threaten roads, fences, buildings and human life (Umah, Justin and Braimoh, 2016). Ofomata (2000) placed gully erosion under actual erosion which is the manifestation of the physical loss of the land due to erosion. Gullies are generally defined by their channel depth for which permanent gullies can range from 0.5-30m (Soil Science Society of America, 2000). Gully erosion is the removal of soil by runoff water and often persists in narrow channels and over short periods removes the soil from a narrow area to considerable depth (Poesen, Vandekerchove, Nactergaele, Wiideness and Verstraeten, 2002).Gully erosion is described as an accelerated process under which soil is bodily displaced and transported away faster than it can be formed (Igbokwe, Akinyede, Dang, Onoa, Nnodu and Anike, 2008). It is the removal of soil from narrow channels via the accumulation of surface runoff which tends to produce more sediment than other forms of soil erosion such as rilling (Shit and Maiti, 2012).

The factors that influence gully erosion could be natural or anthropogenic. Rainfall is one of the most significant factors affecting gully erosion; duration, intensity and frequency of rainfall cause gully erosion (Romkens, Helming and Prasad, 2002). Pathak, Wani and Sudi (2005) asserted that rainfall is an important factor of gully erosion; intense rains coupled with soils prone to sealing and crusting generate high runoff volume and concentrated flow.

Ehiorobo and Audu (2012) reported that gully erosion occurs due to extreme flow of field with a very high speed and energy to remove and transmit soil particles down-hill slope.

Rainfall erosivity is defined as the aggressiveness of rain to induce erosion to soil (Lal's, 2001). It is a major contributor to gully erosion (Romkens, Helming and Prasad, 2002). The higher the amount of rainfall, the higher the quantity of soil particles that are dissolved, displaced and moved away which causes gully erosion (Chimelu, Okeke, Nwosu, Ibe, Ndukwu and Ugwuobi, 2013). From Lal's (2001), Romkens *et al's* (2002) and Chimelu *et al's* (2013) definitions of rainfall erosivity, it can be distilled that the power of rainfall to cause erosion is known as erosivity and it is this that really influences the removal of soil particles in the rainsplash-sheet-rill-gully erosion processes with its accompanying adverse impacts. Therefore, the issue of gully erosion deserves priority attention to mitigate its dire consequences on the land status and environmental quality. This paper focuses on reviewing rainfall erosivity as a natural factor of gully erosion.

1.1 Statement of the Problem

Rainfall erosivity causes gully erosion in that the duration, intensity and frequency of rainfall induces the occurrence of gully development (Romkens *et al*, 2002). It affects all processes of erosion be it splash, sheet, rill and gully erosion. Gully erosion is the worst stage of all types of erosion and it affects several soil functions and hence soil quality (Poesen, 2011; Abdulfatai, Okunlola, Akande, Momoh and Ibrahim, 2014). It is the most destructive form of erosion damaging farmlands and difficult to resolve (Moges and Holden, 2009). It is one of the major causes of the resevoir situation in the Nile River Basin (Zegeye *et al*, 2010). Narain (2005) saw gully erosion as a threat to soil productivity. It causes water bodies' eutrophication (Istvanovics, 2009). Abdulfatai *et al* (2014) emphasized that many lives have been lost to gully erosion. According to them, some have fallen into the gullies and sustained various degrees of injury while some instances were also reported where people drowned in some of the gully sites.

The higher the amount of rainfall, the higher the quantity if soil particles that are to be dissolved, displaced and moved away (Chimelu *et al*, 2013). In the view of Yang, Kanae, Oki, Koike and Musaike (2003), changes in precipitation volume and intensity caused by climatic changes increases available rainfall for detaching and carrying sediments. They estimated that the global average erosion is projected to increase approximately by 9% in 2090 due to climatic changes.

1.2 Objective

The objective of this paper is to review rainfall erosivity as a natural factor of gully erosion.

II. CONCEPTUAL FRAMEWORK: SUSTAINABLE DEVELOPMENT

This research is based on the concept of Sustainable Development. In the opinion of World Conference on Environment and Development (WCED) (1987), sustainable development is the development that meets the needs of the present without comprising the ability of future generations to meet their own needs.

Morelli (2010) saw sustainable development as meeting the resources and service needs for current and future generations without compromising the health of the ecosystems that provide them and more specifically as a condition of balance, resilience and interconnection that allows human society to satisfy its needs while neither exceeding the capacity of its supporting ecosystems to continue to regenerate the services necessary to meet those needs nor by our actions diminishing biological diversity. Rainfall erosivity is a major factor of gully erosion which this research tends to review so as to build sustainability into the management of the menace.

III. METHOD

This research made use of a review of academic articles, textbooks, internet materials, conference papers and thesis both published and unpublished on gully erosion and rainfall erosivity as one of its natural factors. The researchers gathered 52 materials: journal articles, textbooks, seminar and conference papers for this research but summarized the characteristics of 10 that were considered more relevant to rainfall erosivity as a natural factor of gully erosion for the review.

IV. LITERATURE REVIEW OF RAINFALL EROSIVITY AS A FACTOR OF GULLY EROSION

Desir and Marin (2005) saw rainfall as one of the drivers of soil erosion owing to its potential for detaching soil particles and subsequent displacement. Rainfall is an important physical parameter that affects gully erosion in tropical regions (Thomas, 2009). Rainfall is the main source of energy for detachment and transport of soil particles from soil profile (Jain and Kothiyari, 2009). Rainfall erosivity is the potential ability of rainfall to cause soil loss (Silva, 2004). Morgan (2005) stated that erosivity is an important characteristic of rainfall because all things being equal, more erosion is caused by rainstorm of high intensity than by several storms of low intensity.

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Erosivity is related to the kinetic energy of rainfall which can be related to the amount of rainfall and intensity. Gonzalez-Hidalgo, Pena-Monne and Luis (2007) opined that a few very intense rainfall events are responsible for the largest part of soil erosion and sediment delivery in West Mediterranean. Rainfall erosivity is the impact of the kinetic energy of raindrops on soil; higher velocity and larger size of the raindrops result in higher kinetic energy and higher soil loss (Isikwue, Ocheme and Aho, 2015). Labriere, Locatelli, Frevcon and Bernoux, (2015) stated that the large amount and high intensity of rainfall in the humid tropics causes soil erosion in the region to reach dramatic levels.

Wischmeier and Smith (1978) asserted that rainfall erosive energy indicates the volume of rainfall and runoff but a long and slow rain may have some erosive energy value as a shorter rain at much higher intensity as erosion increases with intensity (I_{30}) which indicates prolonged peak rates of detachment and runoff. The equation of Wischmeier and Smith (1959) is:

$$R = \frac{1}{n} \sum_{j=1}^{n} \left[\sum_{k=1}^{m} (E)\,(I_{30}) \right]$$

where R is the average annual rainfall, E is the kinetic energy ($mJha^{-1}$), I_{30} is the maximum 30 minutes rainfall intensity (cmh^{-1}), J is the average of the number of years used to produce the average, K is the index of number of storms in a year, n is the number of years used to obtain the average and m is the number of storms in each year.

The Universal Soil Loss Equation (USLE) by Wischmeier (1959) included a method for calculating storm kinetic energy E and erosivity (R). The storm kinetic energy can be computed as the sum of each intensity group, I_i as :

$$E = (210 + 89\log I_i)\,D$$

where E is the kinetic energy, I_i is the rainfall intensity and D is rain depth for intensity group.

The soil loss by and large depends on rainfall energy known as erosivity index (EI_{30}) which is represented by R factor in (USLE) which is the numerical descriptor of the ability of rainfall to erode soil (Wischmeier and Smith, 1959). According to them, EI_{30} is a function of total rainfall, rainfall distribution, storm intensity, storm frequency and terminal velocity of the rainfall.

Rainfall erosivity expressed as R-Factor in RUSLE is the summation of the total storm energy (€) of an erosive rainfall event times its corresponding maximum intensity over a time span of 30 minutes (I_{30}) within a certain period (Brown and Foster, 1987). Wischmeier and Smith (1958) reported that when factors other than rainfall are held constant, soil loss is directly proportional to R factor of USLE. Brodie and Rosewell (2007) found out that rainfall intensity is a contributing factor to the amount of suspended particles washed from urban areas during storms. Hence, they postulated that the square of rainfall kinetic (I^2) provide a measure of the rainfall kinetic energy (KE) available for wash off process.

Bols (1978) proposed a formular for calculating the R factor in Indonesia in a model:

$$R = \frac{2.5P^2}{100(0.073P + 0.73)}$$

where P = Annual Precipitation in millimetres and R is in [$MJmmha^{-1}hr^{-1}yr^{-1}$]

Fornis, Vermeuleub and Nieuwenhuis (2005) studied soil erosion process, specifically detachment of soil particles by raindrop impact and suggested that kinetic energy is a common indicator of raindrops ability to detach soil particles from the soil mass. The aggressiveness of rainfall or its capacity to cause detachment can be expressed in terms of drop size, rainfall intensity and kinetic energy in the Hindu-Kush Himalayas (Ramprasad, Kothyari and Pandi, 2000). Fuhrer, Benistoton, Fischlon, Frei, Goyette, Jasper and Pfister (2006) predicted increasing trends of water erosion for Switzerland under future climate change due to more frequent and heavy rainfall during winter.

Simmons and Rickson (2008) stated that climate change in the United Kingdom is expected to affect the intensity, amount, frequency and type of rainfall with an increasing trend for heavy (greater than10mmd^{-1}) and extreme (greater than 95th percentile) rainfall events. Salako (2008) noted a decreasing trend of rainfalls in West Africa where rainfall erosivity is considered to be high. He made it known that high intensities of rainfall can result in high rainfall erosivity in regions with high amount of rainfall compared with those of relatively low amounts of rainfall.

When flow velocity fall due to a reduction in slope gradient, flow transport capacity is reduced and deposition of eroded material may occur (Morgan, 2005). According to him, the velocity of rainfall and its intensity are related: for instance a 1mm diameter raindrop has a thermal velocity of 4ms^{-1} while a 5mm diameter raindrop has a terminal velocity of 9ms^{-1}; that means the greater the diameter of raindrop, the greater the terminal velocity. Defra (2005) pointed out that the slope steepness along with the soil texture affects the level of risk of soil erosion by overland flow. He noted that very light soil with low organic matter content on gentle slope in low rainfall areas can erode severely and that the length of a storm drop affects raindrop detachment rates. According to him, significant soil erosion events in England are generally associated with rainfall intensities of >4mm per hour and rainfall quantities >15mm per day.

Wang, Zheng, Romkens and Darboux (2010) opined that erosivity is expended in several components: rainfall power (the product of total amount of incident rainfall energy and intensity) runoff power (the product of flow rate or flow gradient), seepage power (the product of seepage gradient and flow gradient). Ando, Antwi, Watatsuiki and Atakora (2012) showed in their study that the monthly rainfall amount throughout Ghana attained the erosive limit of 20-25mm which makes them erosive; the erosion rates are therefore expected in the major rainy season, especially in July when soils are largely bare.

Lee and Lee (2006) presented a model for estimating soil loss using remotely sensed geospatial data which improves soil loss. They calculated rainfall erosivity factor, R using Toxopeus equation Korea Institute of Construction Technology (KICT) (1992) which is well known for its superiority in Korea :

$$R = 38.5 + 0.35 \times Pr$$

where R is rainfall erosivity factor and Pr is the annual average rainfall.

Average annual soil loss (A) in erosion is seen as a multiplier of rainfall erosivity (the R-factor); the resistance of the environment which encompasses K (soil erodibility), LS (the topographic factor), C (plant cover) and P (erosion control practices) (Wischmeier and Smith, 1978). The equation is given as:

$$A = R \times K \times (LS) \times C \times P$$

For Southern Africa, Hudson (1986) reported that the only intensities above 1inch/hr (2.54cm/hr) caused significant splash erosion. His index KE>1 considers only the energy of rain falling at intensities >25.4mm. He calculated kinetic energy using:

$$KE = 29.8 - \frac{127.5}{I} \quad \frac{[J]}{[m^2 \times mm]}$$

where I is storm intensity (mm/h).

Rainfall erosivity is limited due to lack of long term series rainfall data and lack of correlation with measured soil losses (Gabrie, 2006

Table.1: Summary of Characteristics of some of the Studies that described Rainfall Erosivity as a natural factor of Gully Erosion

S/N	Author(s)	Topic of Research	Method(s)	Result	Recommendation(s)	Conclusion
1	Andoh, Antwi, Wakatsuiki and Atakora Eric (2012).	Estimation of Soil Erodibility and Rainfall Erosivity Patterns in the Agroecological zones of Ghana.	Experimentation (laboratory analysis)	The key result is that erosivity is least in dry season and greatest in the major rainy season.	There should be sustainable utilization of the country's land resources.	It was concluded that the monthly rainfall amount in the study area attained the erosive limit of 20-25mm and is therefore erosive in nature.
2	Brodie, and Rosewell (2007).	Theoretical Relationship between Rainfall Intensity and Kinetic Energy Variants associated with Storm Water Particle Wash off.	Experimentation (Laboratory analysis)	Average rainfall intensity is based on a rainfall duration that varies from event to event	Variants KE and M should be effectively interchangeable if used in particle wash off estimation.	It was concluded that average storm event rainfall intensity is an appropriate substitute for the constant stimulated rainfall intensity.
3	Gonzalez-Hidalgo, Pena-Monne De Luis (2007).	A Review of Daily Soil Erosion in Western Mediterranean Areas.	Literature review	One of the results is that the amount of total soil eroded tremendously varied from one place to another.	Erosion values should be presented within a temporal context; complete daily data should be published and requested by editors.	It was concluded that inspite of the high variability between sites, years and different approaches, over 50% of soil eroded annually belongs to just three

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S/N	Author(s)	Topic of Research	Method(s)	Result	Recommendation(s)	Conclusion
						daily erosion events.
5	Labriere , Locatelli, Laumonier, Freycon and Bernoux. (2015).	Soil Erosion in the Humid Tropics: A Systematic Quantitative Review.	Literature review, physical measurement, experimentation (laboratory analysis)	Soil loss was maximum on bare soils and strikingly exceeded that of all other land use types. Minimum soil loss was found in forests.	Implementation of sound practices of soil and vegetation measurement.	It was concluded that soil erosion in the humid tropics is concentrated both spatially and temporally and that low and ground layers of vegetation are essential in mitigating soil erosion.
6	Lee and Lee (2006).	Scaling Effect for estimating Soil Loss in the RUSLE Model using remotely sensed geo-spatial data in Korea.	Use of Geographic Information System (GIS)	The L- and S- factor are very sensitive to the spatial resolution while the remaining factors are not sensitive.	Since the spatial resolution is very sensitive, caution needs to be taken in selecting the grid size for estimating soil loss using numerical modeling approach.	The conclusion was made that the L- and S- factor is sensitive. The optimum resolution for soil loss came out to be 125m, but it may be dependent on the selection of model and quality of geo-spatial data.
7	De Silva Alexandre Marco (2004).	Rainfall Erosivity Map for Brazil.	Literature review, Geographic Information System (GIS).	North-Western region had the highest values of erosivity while the North-Eastern region showed the lowest annual values of erosivity.	Adoption of conservative practices especially in the development of technology.	The conclusion made was that the major part of the study area revealed annual erosivity values classified as strong.
8	Simmons and Rickson (2008).	Increasing Rainfall and Soil Erosion.	Observation of climate change modeling.	The key result is that the interception of long term rainfall data sets and increasingly accurate global and regional scale climate models led to the general acceptance that climate change has resulted in significant shifts in rainfall characteristics.	Effective on-farm and catchment scale and water management programmes should be devised to mitigate the menace.	It was concluded that changes in rainfall patterns have been observed and are predicted for the future.

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S/N	Author(s)	Topic of Research	Method(s)	Result	Recommendation(s)	Conclusion
9	Wang, Zheng, Romkens and Darboux (2013).	Soil Erodibility for Water Erosion: A Perspective and Chinese Experiences.	Literature review	For many soils, the erodibility factor USLE and RUSLE equations cannot be obtained in a reliable and satisfactory manner from the nomograph; soil erodibility can be predicted from a combination of physical properties and chemical parameters.	Due to the large difference between dry and wet season, seasonal K values should be calculated to obtain reasonable and accurate USLE-based predictions.	All conducted researches came to similar conclusions and proposed that the increases in soil erodibility were due to decreases in the percentage of water stable aggregation and increases in the surface water content due to snow melt.
10	Wischmeier and Smith (1978).	Predicting Rainfall Losses- A Guide to Conservation Planning.	Literature review of research works, laboratory analysis.	Present soil loss equations are substantially less accurate for prediction of specific events than for the prediction of long term averages.	The practice of tillage, mulching and planting on the contour should be encouraged.	The USLE is designed to predict long-time average soil losses for specified conditions. 90% of erosion on the steeply rolling wheat land was estimated to derive from runoff.

Source: Researchers' design, 2017.

V. RESULTS AND DISCUSSION

Gully erosion, greatly influenced by rainfall erosivity is the last stage in the rainsplash-sheet-rill-gully erosion processes, and it attracts the attention of most researchers on erosion because of its grave adverse environmental impacts. Erosivity is an important characteristics of rainfall as it the aggressiveness of rain to cause soil erosion (Lal's, 2001). It is the potential ability of rain to cause soil erosion whose extreme type in gully erosion. Various authors such as Chiemelu, *et al*.(2013), Labriere, *et al*.(2015) viewed that a high amount and intensity of rainfall indicates a high quantity of soil particles to be eroded. However, Gonzalez-Hidalgo *et al.* (2007) have a slightly contrary view that a few, very intense rainfall event is responsible for most soil erosion; that inspite of the high variability between sites, years and different approaches, over 50% of soil is eroded annually as can be seen in Table 1. According to Ramprasad *et al.* (2000) rainfall can be expressed in terms of drop size, rainfall intensity and kinetic energy in the Hindu-Kush Himalayas. Moreover, various authors concluded that a high kinetic energy is a common indicator of a high rainfall erosivitiy and indeed more gully erosion.

Salako (2008) and Fuhrer *et al* (2006) opined that climate variability is an important factor to determine rainfall erosivity as there is a decrease in rainfall where rainfall erosivity is considered to be high. Also, climate change affects the intensity, amount, frequency and type of rainfall in the United Kingdom thereby affecting the soil erodibility rate. From Table 1, Wischmeier (1978) stated that if one of the factors (of soil erodibility, topography, plant cover and erosion control practices) tends to zero, the rate of rainfall erosivity will also diminish. From the results of the study by Simmons and Rickson (2008) in Table 1, it can be deduced that the interception of long term rainfall data sets and increasingly accurate global and regional scale climate models leads to the general acceptance that climate change has resulted in significant shifts in rainfall characteristics.

Generally, it can be distilled from the results of the studies reviewed that regions with a high rainfall regime tend to have a high rate of soil erosion because of the significant impact of rainfall intensity and rainfall energy on the soil in question. For example, the South Eastern part of Nigeria which lies on the tropics with high rainfall regime is being devastated by gully erosion exemplified by the Agulu-Nanka-Oko badland in Anambra State, Nigeria.

VI. RECOMMENDATIONS

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The adage that: “prevention is better than cure” rightly applies to gully erosion. Many terrified gullies across the globe, exemplified by the bad lands of Loess Pleteau China and Agulu-Nanka-Oko in Anambra State, Nigeria would not have developed if adequate management strategies were taken at their early stage of formation. In the light of this background and based on this review, the following recommendations are made to reduce the impacts of the forces of raindrops and runoff (rainfall erosivity) that detach and transport soil particles.

1 Plant cover (vegetation) reduces the energy of raindrops and running water by imparting roughness to the flow of water thereby reducing its velocity, hence it will lessen the havoc. Also, planting of the trees with buttress roots should be encouraged to reduce the force of running water.
2 A shift from rain-fed agriculture to dry season farming is very important to avoid tillage of soils during the rains which predisposes them to the forces of raindrops and runoff that detach and transport soil particles in the rainsplash- sheet-rill-gully erosion processes.
3 Both the affected people and landholders should be adequately empowered through grants by governments, donor agencies and non-governmental organizations (NGOs) prevent formation of gullies because they know when rills begin to occur in their localities and as such can stop their expansion into gullies by using some adaptive measures based on their indigenous knowledge.
4 Training the affected people and landholders on some adaptive measures to soil erosion is a major component in the management of gully erosion. These stakeholders should be trained to enhance those adaptive measures such as tree planting, crop rotation, use of compost as manure and ploughing across the slope, rather than along it which obtains in some communities today, for the management of rills wherever it occurs to prevent them from developing into gullies.
5 Ministries, institutions and agencies responsible for the provision of rainfall data daily, monthly and yearly should make efforts to do so, update them and make them accessible to the public as this is not the situation in most countries of the world. With availability of data, researchers should be encouraged by government and donor agencies to conduct studies to develop regression equation (models) for computing rainfall erosivity and soil losses for different localities across the globe.
6 Diversion of water away from erosion prone gullies, thus dispersing the erosive power of the water over well vegetated areas. Diversion banks are a simple way of achieving this. Moreso, natural drains, footpaths and culverts should be properly managed through maintenance to minimize the erosive power of runoff.

VII. CONCLUSION

This study was conducted in the view of the fact that rainfall erosivity is a natural factor influencing the formation of gullies that leads to the destruction of the environment which is the destruction of man. From the review of studies on rainfall erosivity as a natural factor of gully erosion which is the most visible, extreme and last stage in the rainsplash-sheet-rill-gully erosion processes and based on the results, this study concludes that locally grown solutions in terms of adaptive measures by the affected people and landholders should be used to reduce the impact of rainfall erosivity in detaching and transporting soils to form gullies and triggering the expansion of existing gullies.

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REFERENCES

[1] Abdulfatai, I.A., Okinlola, I.A., Akande, W.G., Momoh, L.D., and Ibrahim, K.O. (2014). Review of Gully Erosion in Nigeria: Causes and Possible Solutions. *Journal of Geosciences and Geomatics,* 2(3):125-129.
[2] Abraham, C.M. (2010). Implications of Gully Development on Agricultural Production in Akwa Ibom State. Unpublished Ph.D. Thesis submitted to

the University of Uyo, Uyo Akwa Ibom State, Nigeria.
[3] Ando, H.F., Antwi, B.O., Wokatsuiki, T., and Atakora, E.T. (2012). Estimation of Soil Erodibility and Rainfall Erosivity Patterns in the Agroecological Zone of Ghana. *Journal of Soil Science and Environmental Management*, 3(11):275-279.
[4] Bols, P.L. (1978). *The Iso-erodent Maps of Java and Madura: Report of Belgian Technical Assistance Project ATA 105, Bogor, Indonesia:* Soil Research Institute, Bogor.
[5] Brodie, L., and Rosewell, C. (2007). Theoritical Relationship between Rainfall Intensity and Kinetic Energy Variants Associated with Storm Water Particle Wash Off. *Journal of Hydrology*, 340:40-47.
[6] Brown, L.C., and Foster, G.R. (1987). Storm Erosivity using Idealized Intensity Distributions. *Transactions of American Society of Agricultural Engineers*, 30:379-386.
[7] Chimelu, N., Okeke, F., Nwosu, K., Ibeh, C., Ndukwe, R., and Ugwuoti, A. (2013). The Role of Surveying and Mapping in Erosion Management and Control: Case Study of Omagba Erosion Site, Onitsha, Anambra State, Nigeria. *Journal of Environment and Earth Science*, 3(11):129-137.
[8] Dada, S. (2002). An Investigation on the Physical and Socioeconomic Determinants of Soil Erosion in the Harashe Highlands, Eastern Ethiopia. *Land Degradation and Development*, 14:69-81.
[9] Defra, (2005). *Controlling Soil Erosion: A Manual for the Assessment and Management of Agricultural Land at Risk of Water Erosion in Lowland England.* Published by the Department for Environment, Food and Rural Affairs, London.
[10] Desir, G., and Marin, C. (2005). Factors Controlling the Erosion Rates in a Semi-arid Zone (Bardenas Realest NE Spain). Catena, 71:31-40.
[11] Ehiorobo, J.O., and Audu, H.A. (2012). Monitoring of Gully Erosion in an Urban Area using Geographic Information Technology. *Journal of Emerging Trends in Engineering and Applied Sciences*, 3(2):270-275.
[12] Ezeigwe, P.C. (2015). Evaluation of Socioeconomic Impacts of Gully Erosion in Nkpor and Obosi. *Environmental Research*, 7(7):34-38.
[13] Fornis, R.L., Vermeuleub, H.R., and Niuwenhuis, J.D. (2005). Kinetic-energy Rainfall Intensity Relationship for Central Cebu, Philippines for Soil Erosion Studies. *Journal of Hydrology*, 300:20-32.
[14] Fuhrer, J., Beniston, M., Fischlin, A., Frei, C., Goyette, S., Jasper, K., and Pfister, C. (2006). Climate Risks and their Impacts on Agriculture and Forests in Switzerland. *Climate Change*, 71:79-102.
[15] Gabrie, D. (2006). Assessing the Modified Fournier Index and the Precipitation Concentration Index for some European Countries. In: *Boardman, J. And Poesen, J. (eds). Soil Erosion in Europe. Wiley and Sons, Ltd.* 2(14):675-684.
[16] Gonzalez-Hidalgo, J.C., Pena-Monne, J.L., and De Luis, M. (2007). A Review of Daily Soil Erosion in Western Mediterranean Areas. *Catena*, 71:193-199.
[17] Hudson, N.W. (1986).*Soil Conservation.* Batsford Limited, London, United Kingdom, p.324.
[18] Igbokwe, J.I. (2008). Gully Erosion Mapping/Monitoring in Parts of South Eastern Nigeria. Paper Presented at Department of Surveying and Geoinformatics, Nnamdi Azikiwe University, Awka, Anambra State. Accessed at: http:/www.nasrda.or/doc/jibokwe.pdf on 02 11 2017.
[19] Isikwue, M.O., Ocheme, J.E., and Aho, M.I. (2015). Evaluation of Rainfall Erosivity Index for Abuja. Nigeria Lombardi Method. *Nigeria Journal of Technology*, 34(1):52-63.
[20] Istavonics, V. (2009). Eutrophication of Lakes and Reservoirs. *Encyclopaedia of Inland Waters*, 1:157-165.
[21] Jain, S.K., and Kothyari, U.C. (2010). Estimation of Soil Erosion and Sediment Yield using Geographic Information System (GIS). *Hydrological Sciences Journal*, 45(5):771-786.
[22] Kalu, A.C. (2001). Soil Erosion and Landslides: 21st Century Issues and Challenges to Rural Development in Nigeria. Unpublished MURP Seminar Paper: Department of Urban and Regional Planning, Abia State University, Uturu Abia State, Nigeria.
[23] Korea Institute of Construction Technology (KICT) (1992). The Development of Selection Standard for Calculation Method of Unit Sediment Yield in River. KICT, 89-WR-113. Research Paper (in Korean), 1992.
[24] Labriere, N., Locatelli, B., Laumonier, Y., Freycon, V., and Bernoux, M. (2015). Soil Erosion in the Humid Tropics: A System of Quantitative Review. *Agriculture, Ecosystems and the Environment*, 203:127-139.
[25] Lal's, R. (2001). Soil Conservation for Sequestration. *Proceedings of the 10th International Soil Conservation Organisation Meeting*, 24th-29th May 1999, West Lafayette, pp.459-465.
[26] Lee, G.S., and Lee, K.H. (2006). Scaling Effect for Estimating Soil Loss in the RUSLE Model using Remotely Sensed Geospatial Data in Korea. *Hydrology and Earth System Sciences*, 3:135-137.

[27] Moges, A., and Holden, N.M. (2009). Land Cover Change and Gully Erosion Development in Umbulo Watershed, Southern Ethiopia. *Mountain Research and Development*, 29(3):265-276.

[28] Morelli, J. (2010). Economic Sustainability and the Preservation of Environmental Management. *Journal of Environmental Management*, 14(8):771-778.

[29] Morgan, R.P.C. (2005). *Soil Erosion and Conservation*, (3rd Edition). Blackwell Publishing Ltd.

[30] Narain, P. (2008). Dry Land Management in Arid Ecosystem. *J. Indian Soc. Soil Sci*, 56:337-347.

[31] Ofomata, G.E.K. (2000). Missing Links in the management of Soil Erosion Problems in Nigeria. In: *Environmental Pollution and Management in the Tropics*, Snaap Publishers.

[32] Pathak, P., Wani, S.P., and Sudi, R. (2005) *Gully Control in SAT Watersheds. Global Theme on Agroecosystems*. International Crops Research Institute for the Semi-Arid Tropics, p.28.

[33] Poesen, J. (2011). Challenges in Gully Erosion Research. *Landform Analysis*, 17:5-9.

[34] Poesen, J., Vandekerchove, L., Nachergaele, D.D., Wisdenes, G., and Verstraeten, G. (2002). Gully Erosion in Dry Land environment. In: *Dry Land Rivers. Hydrology and Geomorphology of Semi-arid Channels*, pp.229-262.

[35] Ramprasad, B.K., Kothyari, B.P., and Pande, R.K. (2000). Evaluation of Rainfall Erosivity in Bheta Gad Catchment, Kuman Hills of Littar, Pradesh, Central Himalayas. *The Environmentalist*, 20:301-308.

[36] Romkens, M.J., Helming, K., and Prasad, S.N. (2002). Soil Erosion under Different Rainfall Intensities, Surface Roughness and Soil Water regimes. *Catena*, 46(2-3):103-173.

[37] Salako, F.K. (2005). Rainfall Variability and Kinetic Energy in Southern Nigeria. *Climate Change*, 86:151-164.

[38] Shit, P.K., and Maiti, R. (2002). Rill Gully Erosion. In: *Bad Land Topography: Field Measurement and Monitoring (A Case Study on the Western Part of West Bengal, India)*. LAP LAMBERT Academic Publishing, Germany, p.136.

[39] Silva, A.M. (2004). Rainfall Erosivity Map for Brazil. *Catena*, 57:251-259.

[40] Simmons, R.W., and Rickson, R.J. (2008). Increasing Rainfall and Soil Erosion. In: *Water and Food*, pp.89-96.

[41] Soil Science Society of America (2001). Glossary of Science Terms – Soil Science Society of America, Modison, WI. Accessed at http:/www.soils.org/sssaglows, 10 10 2017.

[42] Thomaz, E.L. (2009). The Influence of Traditional Steep Land Agricultural Practices on Runoff and Soil Loss. *Agriculture, Ecosystems and Environment*, 130:23-30.

[43] Umah, K.A., Justin, U., and Braimoh, J. (2016). Investigating the Impacts/Causes of Gully Erosion in Auchi, Nigeria. *Journal of Geography, Environment and Earth Science*, 4(4):1-13.

[44] United States Global Change Research Information Office (2001). Accessed at [http//www.gcrio.org/geo/soil/html], 20 10 2017.

[45] Wang, B., Zheng, F., Romkens, M.J.M., and Darboux, F. (2013). Soil Erodibility for Water Erosion: A Perspective and Chinese Experiences. *Geomorphology*, 187:1-10.

[46] Wischmeier, W.H., and Smith, D.D. (1958). Rainfall Energy and its Relationship to Soil Loss. *Geophysical Union*, 39:285-291.

[47] Wischmeier, W.H. (1959). A Rainfall Erosion Index for a Universal Soil Loss Equation. *Soil Science Society of America Proceedings*, 23, pp. 246-249.

[48] Wischmeier, W.H., and Smith, D.D. (1978). Predicting Rainfall Erosion Losses – A Guide to Conservation planning. United States Department of Agriculture, *Agricultural Handbook*, p.58.

[49] World Commission for Environment and Development (WCED) (1987). *Our Common Future.* Oxford University Press.

[50] Yang, D., Kanae, S., Oki, T., Koike, T., and Musaike, K. (2003). Global Potential Soil Erosion with Reference to Land Use and Climatic Changes. *Hydrological Processes*, 17:2913-2928.

[51] Zegeye, A.D., Abiy, A.z., and Tebebu, T.Y. (2010). Surface and Subsurface Flow Effect on Permanent Gully Formation and Upland Erosion near Lake Tana in the Northern Highlands of Ethiopia. *Hydrology and Earth System Sciences*, 14(11):2207-2217.

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Phenotypic Screening of Drought-Tolerant Lines for Brown Planthopper, Blast and Phytic Acid Content Assay of Rice (*Oryza sativa* L.)

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Abstract**—*Advanced drought tolerant lines were analysed for blast disease, brown planthopper (BPH), and phytic acid content. Thirsty lines of BC2F4 derived from OMCS2000/ IR75499-73-1 were used to screen for BPH and blast resistance. Three good resistant lines were screened against blast (45, 54, and 310) under greenhouse condition. As eight lines were identified to be resistant to BPH. The results further reveal that BC2F4-45 was the best line resistant to both BPH and blast disease. These lines will be useful in reducing grain phytic acid and improving the nutritional value of rice grain. Based on an assay for high phosphate germination stage of rice, the lowest content was found in the I5 variety (line 45). Hence, this line provides the urgent objective for breeders in cultivars of these crops to genetically enhance a healthy and functional diet. These characters will then need to be incorporated into high yield under drought stress with others such as disease and insect resistance.*

Keywords**—*Brown planthopper, blast, phytic acid content, screening, phenotype, drought-tolerant, rice***

I. INTRODUCTION

Rice is a supreme commodity to mankind an important staple food for more than half of the world population, may provide 60-70 % body calorie intake to the consumers. Vietnam is one of the world's richest agricultural regions and is the second-largest (after Thailand) exporter worldwide and the world's seventh-largest consumer of rice. Rice production in India as well as in Vietnam must be doubled by 2025 to meet the requirement of the increasing population. This demand can be met only by enhancing the production and productivity of rice[1, 2].

A recent estimate on climate change predicts the water deficit to deteriorate further in years to come [3]and the intensity and frequency of drought are predicted to become worse [4]. Among biotic stresses, the disease has considered being the most devastating worldwide in rice, blast by *Pyriculariagrisea*. Similarly, groups of insects, brown planthopper [BPH], *Nilaparvatalugens*), has been the most damaging pest[5]. Brown planthopper is the most dangerous insect pest for rice and it causes severe yield losses by direct feeding and viral transmission of serious diseases. At high population density, hopper burn or complete drying of the plants is observed. From 2005 to 2006, more than 485000 ha of rice in the southern Vietnam was severely affected by viral diseases seemingly spread by BPH, resulting in the loss of 828000 tons of rice valued at US$120 million.

During water stress conditions or severity of drought, a major biotic stress- rice blast disease, caused by the filamentous ascomycete fungus *Magnaportheoryzae* (anamorph *Pyriculariagrisea*.) becomes a serious threat to rice production and leads to significant yield loss, as high as 70-80 % during an epidemic [7,8]. In Vietnam, this disease occurs particularly in a year with the long-wet season and causes the yield loss of up to 20%. Therefore, development of durable blast resistant varieties has been recognized as desirable means of disease management [9]. Thus drought-tolerant lines promoted at the advanced stage should possess tolerance of blast.

Besides, the major storage compound of phosphorus in plants tissue is phytic acid, (inositol hexakisphosphate)[10]. This compound can soak up irons and in foods and animal system and it decreases the absorption capacity of minerals like zinc, manganese, copper, molybdenum, calcium, magnesium, iron as well as protein [11]. Phosphorus content in phytic acid is also controlling inorganic phosphate concentration in developing seeds and seedling [12]. Loreti et al. [13] showed that during germination, phytates are broken down and release phosphorous, minerals, and myo-inositol which promotes rice germination and seedling

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stages. The low phytic acid trait addresses an urgent goal for the genetic improvement of rice because of anemia syndrome in rice. These characters will then need to be incorporated into high yield under drought stress with others such as disease and insect resistance. Therefore, this study was conducted based on the traits released behind major-effect drought-yield, to understand how the lines being interacted in stable tolerance to biological stress such as pests, diseases and improve promising nutritional drought tolerant lines.

II. MATERIALS AND METHODS

1. Plant materials

Thirty lines of BC_2F_4 from OMCS2000/ IR75499-73-1-B were screened for drought tolerance using phenotyping and molecular markers by Ha et al. [14]. These lines will be screened for BPH and blast resistance before they are introduced to farmers.

2. Screening for brown planthopper resistance

The seeds were presoaked and sown in rows in 60 x 45 x 10 cm seed boxes along with resistant and susceptible checks. A total of 10 seedlings per row were maintained per line with. There were three replications for each line and these were infected at 12 - 14 d old with the 2nd to 3rd instar hopper 4-6 nymphs per seedling. Seeds of susceptible check TN1 were sown in two border rows and in half of the middle row. Approximately one week after infestation hopper burn 'symptom' was observed. When more than 90% of susceptible check shows wilting, the plants were scored individually based on the scoring system proposed by the International Rice Research Institute [15] and each seedling was scored as 0 = no visible damage, 1 = partial yellowing of the first leaf, 3 = first and second leaves partially yellowing, 5 = pronounced yellowing or some stunting, 7 = mostly wilted plant but still alive, 9 = the plant completely wilted or dead.

3. Evaluation of blast resistance

Seeds were soaked for 1 day and sown in a 15 x 30 x 4-cm plastic tray containing sieved topsoil media. The rice plants were inoculated with blast pathotype spore suspension (1 x 10^5 spores/mL) 21 days after. Plants were incubated in a dark dew chamber for 24 h at 25°-28°C. After 24 h, the plants were returned to the greenhouse with a controlled water sprinkler to maintain the humidity around the plants. Disease reactions were recorded as the number of plants infected by a pathotype observed after 7 days of inoculation with the blast spores. Five infected leaves were recorded for each replication.

4. Phytic acid content assay

Seeds of rice varieties (0.05 g) were grind to a fine powder, mixed in 2 ml of 0.4 M HCl and incubated at 4 °C for overnight. The solution was mixed and 100 µl of the mixture was transferred to a cuvette. A volume of 1 ml was maintained by adding 900 µl distilled water. After that, 1ml of Chen' reagent ((6N H_2SO_4: 2.5% ammonium molybdate: 10% ascorbic acid: distilled water (1:1:1:2)) were added to a cuvette, covered with parafilm and mixed well by inversion. A blank was used as control having 1ml Chen' reagent and 1ml water [16]. The samples were then incubated at 37 °C for 1.5 hours. The absorbance of the reaction was measured at 820nm. The phytic acid content was determined using the known molarities of phosphate standard curve in triplication of 1mM KH_2PO_4 ranging from 25, 50, 100, 150, to 200 µl. Fig 1 showed the standard curve of phosphate for the Microtiter Plate PI assay followed by Chen's method.

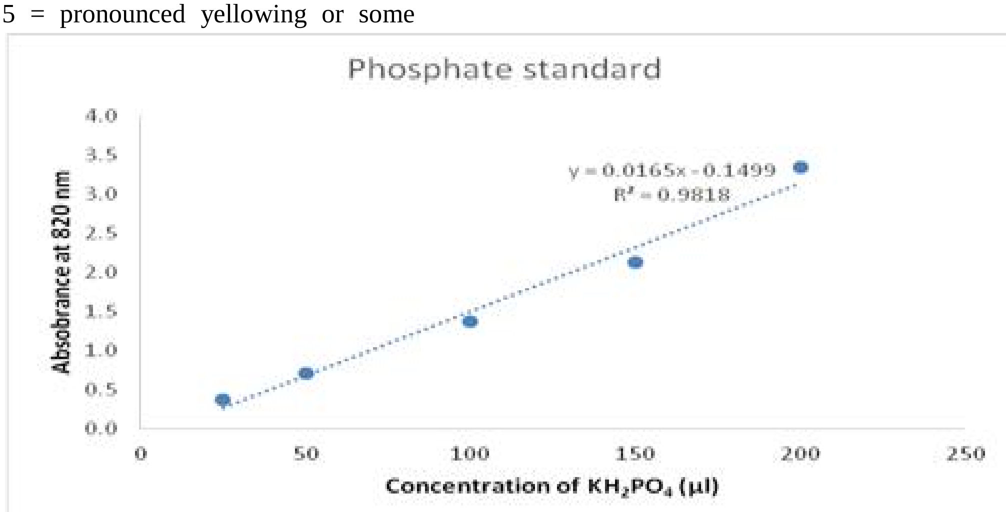

Fig. 1: Standard of phosphate

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Table 1: The list of lines/varieties in this research.

Codes	Varieties/lines
I8	F7 (OM6162/Swanasub1)
I34	BC2F4-54
I5	BC2F4-45
I49	F7 (IR75499-29-2-B/IR64 Sub1)

5. Statistical analysis

All experiments and data provided in this paper were repeated three times. Statistical analysis was carried out by using Minitab software. The data are presented as the means ± the standard deviation. Comparisons with $P < 0.01$ were considered significantly different.

III. RESULTS AND DISCUSSION

1. Screening for blast and Brown planthopper resistance

Development of the disease resistance or stress-tolerant plants is an important objective in rice breeding programs because the production of rice can be constantly affected by several major abiotic and biotic stresses. The phenotypic evaluation showed clear distinction between resistant and susceptible typesand clearly revealing moderately resistant types as well.

The isolate 2(U61-i0-k101-z05-ta102) of*Pyriculariagrisea* was isolated using the method described by Hayashi et al. [17] in this study. The Table 6.4 shows the reaction of BC_2F_4 lines derived from OMCS2000/ IR75499-73-1-B to brown planthopper and blast resistance. Two of resistant checks had the best level of 3 for BPH and a level of 3 for blast.

For blast, IR 24 which was a susceptible check variety and had a score of 9 which indicate susceptibility. Most lines had level 5, which is at moderately susceptible level. One line (BC_2F_4-310) had level of 1 and three lines (BC_2F_4-45, BC_2F_4-54 had level of 3. These lines were resistant to blast disease. One line was highly susceptible, level of 7. This result showed that one of these lines was better than the resistant check but the reminded of the lines were better than the susceptible check and parent varieties.

Among all the insect pests, brown planthopper, is one of the most destructive pests of rice causing severe yield losses [18](Sai Harini et al., 2013). The screening of lines/varieties resistant to BPH is an important experiment because new varieties should be tested before they are introduced to farmers. For brown planthopper, most of the lines had levels in the arrange 1-7. Three lines had the level of 1 and five had the level of 3. These lines are resistant to BPH. Three lines were highly susceptible, scores of 7. The nineteen lines had level 5, which is at moderately susceptible level. This result showed that three of these varieties were better than the resistant check (BC_2F_4-89, BC_2F_4-45, and BC_2F_4-95). Though many chemicals were recommended for the control of this pest [18], due to its feeding behavior at the base of the plant, the farmers are unable to control this pest effectively. Thus, farmers resort to blanket application of insecticides which often disrupts the ecological balance of rice ecosystem due to which this pest has already developed resistance against many insecticides in different Asian countries [19, 20]. The use of genetic resistance is the most effective measure for BPH management. Cultivation of resistant varieties is an economical, efficient and environmentally sound strategy for population management of insect-pests.

Table.2: Reaction of BC_2F_4 lines derived from OMCS2000/ IR75499-73-1-B against brown planthopper and blastresistance.

N_0	Name of variety	BPH (level)	Reaction	Blast (level)	Reaction
Susceptible	TN1	9	S	-	-
Resistance	PtB33	3	R	-	-
Susceptible	IR24	-	-	9	S
Resistance	Tetep	-	-	3	R
P1	OMCS2000	5	MS	5	MS
P2	IR75499-73-1-B	3	R	5	MS
1	BC_2F_4-17	5	MS	5	MS
2	BC_2F_4-25	5	MS	5	MS
3	BC_2F_4-45	1	R	3	R
4	BC_2F_4-54	5	MS	3	R

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N_0	Name of variety	BPH (level)	Reaction	Blast (level)	Reaction
5	BC_2F_4-56	5	MS	5	MS
6	BC_2F_4-68	5	MS	5	MS
7	$BC_2F_4$77	5	MS	5	MS
8	BC_2F_4-79	3	R	5	MS
9	BC_2F_4-89	1	R	5	MS
10	BC_2F_4-95	1	R	5	MS
11	BC_2F_4-99	7	S	5	MS
12	BC_2F_4-100	5	MS	5	MS
13	BC_2F_4-105	3	R	5	MS
14	BC_2F_4-112	7	S	5	MS
15	BC_2F_4-120	3	R	3	R
16	BC_2F_4-123	7	S	5	MS
17	BC_2F_4-130	5	MS	7	S
18	BC_2F_4-145	5	MS	5	MS
19	BC_2F_4-152	3	R	5	MS
20	BC_2F_4-155	5	MS	5	MS
21	BC_2F_4-158	3	R	5	MS
22	BC_2F_4-175	5	MS	5	MS
23	BC_2F_4-179	5	MS	5	MS
24	BC_2F_4-200	5	MS	5	MS
25	BC_2F_4-211	5	MS	5	MS
26	BC_2F_4-256	5	MS	5	MS
27	BC_2F_4-358	5	MS	5	MS
28	$BC_2F_4$278	5	MS	5	MS
29	BC_2F_4-289	5	MS	5	MS
30	BC_2F_4-310	5	MS	1	R

R: Resistance; S: Susceptible; MS: Medium Susceptible

2. Phytic acid content

Study of low phytic acid content in riceis important to improve promising nutritional lines.The present study revealed that highest content of phytic acid was observed in the I49 variety with 38.701 a ± 0.093, followed by I34 variety (33.610 ± 0.153). Besides that, the lowest content was found in the I5 variety (25.630 d ± 0.182) (Table 3). According to Khattak et al. [21] and Beleia,[22] phytates play an important part in mineral metabolism and mayreduce the availability of Fe, Zn, Ca, Mg, Cu, Mn, and Mo as well as protein. Therefore, low-phytic acid rice has higher bioavailable Zn^{2+} and Fe^{3+}, and this means that the low phytic acid content could serve theprinciple objective for breeding by improving nutritional value.

Table.3: The phytic acid content in the drought tolerant lines.

Varieties	Phytic acid content (µg/mL)
I8	30.721 c ± 0.061
I34	33.610 b ± 0.153
I5	25.630 d ± 0.182
I49	38.701 a ± 0.093

IV. CONCLUSION

The screening of varieties resistant to BPH and blast is an important experiment because new varieties should be tested before they are introduced to farmers. Advanced drought- tolerantlines indicate that BC_2F_4-45 was the best line resistant to both BPH and blast disease and had low phytic acid content. This variety will able to provide disease control at essentially no cost to the farmers.

REFERENCES

[1] M. Hossain. Agricultural policies in Bangladesh: Evolution and impact on crop prodution. In state, market and Development: Essays in hornor of Rehman Sobhan, ed, A.A. Abdullan and A.R. Khan. Dhaka: University Press Litmited, 1996.

[2] N. Mishra, N.C. Hazarika, K. Narain and J. Mahanta. Nutritive value of non-mulberry and mulberry silkworm pupae and consumption pattern in Assam, India. Nutr. Res. 23 (10),2003, 1303–1311.

[3] R. Wassmann, S.V.K. Jagadish, S.Heuer, A.Ismail, E. Redona, R.Serraj, R.K. Singh, G.Howell, H.Pathak, and K. Sumfleth. Climate change affecting rice production: the physiological and agronomic basis for possible adaptation strategies. Advances in Agronomy. 101, 2009, 59–122.

[4] B. Bate, Z.W. Kundzedwicz, S. Wu and J. Paulutikof, eds. Climate change and water: IPCC Technical paper VI. Geneva, Seizerland: IPCC Secretariat, 2008.

[5] L.B. Jiang, K.F.Jao, D.J. Wangand J.C. Wu. Effects of different treatment methods of fungicide jinggangmycin on reproduction and vitellogenin gene (N1vg) expression into the brown planthopper Nilaparvatalugens (Stål) Hemiptera: Delphacididae. Pesticide Biochem Physiol. 102, 2012, 51–55.

[6] P.V. Du, R.C.Cabunagan, P.Q.Cabauatan, H.S. Choi, I.R. Choi, H.V. Chien, N.H. Huan. Yellowing syndrome of rice: Etiology, current status, and future challenges. Omonrice 15, 2007, 94–101.

[7] H.K. Manandhar, K. Shrestha and P.Amtya. Seed-borne diseases. In: Plant diseases, seed production and seed health testing in Nepal (S.B. Mathur, P.Amaty, K. Shrestha and H.K.Mananhar, eds). Danish Government, Institute of Seed Pathology for Developing Countries, Compenhagen, Denmark. 1992, 59–74.

[8] G.S. Khush and K.K. Jena. Current status and future prospects for research on blast resistance in rice (Oryza sativa L.). In: Wang GL, Valent B (eds) Advances in genetics, genomics and control of rice blast disease. Springer, Dordrecht.2009, pp 1–10.

[9] S.H. Hulbert, C.A. Web, S.M. Smithand Q. Sun. Resistance gene Complexs: Evolution and utilization. Annu Rev Phytopathol. 9, 2001, 285–312.

[10] J. Shi, H. Wang, J. Hazebroek, et al. The maize low-phytic acid 3 encodes a myo-inositol kinase that plays a role in phytic acid biosynthesis in developing seeds. The plant Journal. (42), 2005, 708–719.

[11] A. Beleia, L.T.T Thao and E.I. Ida. Lowering phytic phosphorus by hydration of soybean. Journal of Food Science. 58, 1993, 375–377.

[12] R. N. Trethewey and A. M. Smith. Starch mobilization in leaves. In: Advance in Photosynthesis (Eds): R.C. Legood, T.D. Shake, S. Von-Cammerer. Photosynthesis: Physiology and metabolism. Dordrecht, The Netherlands, Kulwer Academic Publishers. 9, 2000, 205–231.

[13] E. Loreti, P. Vernieri, A. Alpi and P. Perata. Repression of α-amylase activity by anoxia in grains of barley is independent of ethanol toxicity or action of abscisic acid. Plant Biology. 4, 2002, 266–272.

[14] P.T. T Ha, D.T. Khang, P.T.Tuyen, T.B. Toan, N.N. Huong, N.T. Lang, B.C. Buu, T.D. Xuan. 2016. Development of new drought tolerant breeding lines for Vietnam using marker-assisted backcrossing. International Letters of Natural Sciences. Vol. 59, 2016, 1–13.

[15] IRRI. Standard Evaluation System for Rice. IRRI, Manila, Philippines.1996.

[16] P. S. Chen, T.Y. Toribara and H. Warner. Microdeteminations of phosphorous. Analytical Chemical. 28, 1956, 1756–1758.

[17] N. Hayashi and Y. Fukuta. Proposal for a new international system of differentiating races of blast (*PyriculariapryxaeCavara*) using LTH monogenic lines in rice (*Oryza sativa* L.) JIRCAS working report No. 63. Tsukuba city, Ibaraki prefecture Japan, Japan International Research Center for Agricultural Science. 11–5, 2009.

[18] A. Sai Harini, S. Sai Kumar, B. Padma, S. Richa, D.M.Ayyappa and S. Vinay. Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods. Africa journal of biotechnology. 12 (19), 2013, 2525–2525.

[19] P.S. Sarao. Integrated Management of insect-pests of rice and basmati. Prog Farm, 51, 2015, 9–12.

[20] K. Gorman, Z. Liu, I. Denholm, K.U. Bruggen, K.U and R.Nauen. Neonicotinoid resistance in rice brown planthopper, Nilaparvatalugens. Pest Manag Sci. 64, 2008, 1122–1125.

[21] M. Matsumura, H. Takeuchi, M. Satoh, S. Sanasa-Morimura, A. Otuka, T. Watanabe, D.V. Thanh. Current status of insecticide resistance in rice planthoppers. In: K.L. Heongand B. Hardy.

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Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia. Los Baños, the Philippines: International Rice Research Institute.2009, 233–244.

[22] A.B. Khattak, A. Zeb and N. Bibi, et al. Influence of germination techniques on phytic acid and polyphenols content of chickpea (*Cicerarietinum* L.) sprouts. Food Chemistry. 104, 2007, 1074–1079.

Water Quality Impact of Flow Station Effluent in a Receiving Creek.

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***Abstract*— *The physicochemical quality of a crude oil flow station effluent and water of an effluent receiving creek were investigated. Samples were characterized by laboratory analysis. High concentrations of some toxicants exceeding environmental standards were observed in the effluent and water samples, which include BOD_5 (544mg/L), COD (650mg/L), salinity (3162mg/L), copper (2.3mg/L), TDS (18900 mg/L), lead (0.51mg/L), and cadmium (0.04mg/L). The study underscored the need for proper treatment and monitoring of effluent to ensure compliance with statutory standard, before discharge into the environment to safeguard the ecosystem, as continued discharge of improperly treated effluent may compound the ecological problem of the receiving water environment*

Keywords*—*concentrations, degradation, impact, outfall, pollution, quality.

I. INTRODUCTION

Many activities of man have led to environmental pollution. Foremost among such is industrial activities. In addition to causing various devastating ecological and human disaster, industries contribute greatly to environmental degradation, and pollution problems of various magnitude, as a result of waste discharges (Nkwocha, and Okoye, 2007; Otaraku and Nkwocha, 2010; Nkwocha et al., 2013). Petroleum compounds are one of the major sources of water pollution. These are compounds discharged during the extraction, processing and refining of crude oil. In the petroleum industry, effluent waters (produced or process waters) are waste associated with oil and gas formation, and run-offs from production facilities/ Facilities such as flow stations, compressor stations, hold basins or discharge points are associated with handling oil-feed produced/formation water or process waters (SPDC, 2000). Effluent waters from crude oil and gas companies, refineries and petrochemical industries contain quantities of oil, organic components and heavy metals such as chromium, copper, iron, zinc, manganese lead, mercury, and cadmium at concentrations beyond tolerable limits, thus, requiring treatment. The discharge of untreated and fairly treated waste into ecosystem brings about structural, chemical and biological changes which affect the biota (Ogbeibu and Oribhubour, 2001).

Over the last three decades, a large number of developing countries have introduced industrial environmental standards. However, it is generally recognized that the implementation of those standards and instruments have typically been seriously lacking (Aluyor and Badmus, 2003). In Nigeria, there is a general concern for industrial pollution, especially in the oil and gas industry. The Federal Environmental Protection Agency (FEPA) has established effluent limitation guidelines for all categories of industries FEPA, 1991). The Directorate of Petroleum Resources (DPR) has also established similar standards for the oil and gas industry DPR, 1991), and regulates environmental standards in the oil and related industries.

However, despite the general concern about pollution in the oil and gas industry, there has not been much study on flow stations and related facilities, as to whether their effluent comply with legally accepted toxicants levels. Furthermore, the impact of these toxicants on the quality of the effluent receiving water body has not been investigated. This study is designed to evaluate the quality of a flow station effluent and its impact on the physicochemical quality of the watercourse receiving the effluent.

Study area

The flow station investigated (Nembe-1) is one of the many flow stations located in the Nembe district of Niger Delta region. It is owned and operated by one of the multinational oil companies based in Nigeria. It has a capacity of 60,000 bpd, and was established in 1977. In this flow station, drains from leaking vessels, liquid scrubbers, valves and other operational equipment are routed to a treatment facility. The basic treatment involves oil recovery, after which the resultant effluent is discharged into the surrounding creek.

II. MATERIALS AND METHODS

Effluent quality sampling

Samples were collected at the effluent discharge point with 2 litre plastic bottles that were pre-washed with nitric acid and thoroughly washed with distilled water. Samples for

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oil and grease were collected in dark air tight bottles. Collected samples were preserved in ice-chests and immediately transported to the laboratory for analysis. Sampling was done weekly for four weeks.

Water quality sampling

Samples for physicochemical quality analysis of the receiving water were collected 500m upstream and downstream respectively from the outfall using same procedure as for the effluent. Sampling was done fortnightly for 4 weeks.

Laboratory analysis of samples

The procedures of standard methods (APHA, 1995) were used for the laboratory analysis of samples. Temperature was determined at the point of sample collection using a mercury thermometer. An HACP pH meter was used for pH determination.

TDS was analyzed by weighing the deposits after evaporation of the filtrate of a known volume of sample, while the residue was used to estimate the TSS.

Oil and grease was analyzed by acidifying a known volume of sample with HCl, this was followed by extraction with trichlorofluoroethane and distillation. BOD was determined electro-analytically using an Oxyscan light oxygen meter, while COD was determined by oxidation with potassium dichromate.

A Perkin Elmer 3100 atomic absorption spectrophotometer (Boston, MA 02118-2512, USA) was used for the determination of heavy metals including cadmium (Cd), chromium (Cr), copper (Cu) zinc (Zn), iron (Fe) and lead (Pb).

III. RESULTS AND DISCUSSION

Effluent quality:

The weekly variations of the investigated effluent parameters compared with the limit set by DPR are presented in Figs 1-8. The effluent pH ranged between 7.2-7.6 with a mean value of 7.4 for the 4 weeks. These values were within the permissible limit of 6.5-8.5 set by DPR (1991) for effluents meant for discharge into inland waters. The effluent temperature ranged from 29.4 -30.9ºC with a mean value of 30ºC. DPR set a limit of 30 ºC. Other parameters that complied with the effluent limitation set by DPR include TDS, oil and grease. However, .BOD and COD with ranges of 20.8-544mg/Land 64.0-650mg/L respectively, exceeded DPR limit of 125 mg/L in the 1st week. High oxygen demanding effluents when discharged into natural water bodies have been linked with oxygen depletion of the water environment, and attendant health hazards on the aquatic organisms (Kiely, 2004; Abowei and Sikoki, 2005). The salinity of the effluent was higher than DPR recommended maximum level of 2000mg/L in the 1st and 2nd weeks. Pollution of the aquatic ecosystem poses a serious threat to aquatic organisms and ultimately the entire ecosystem (Otokonefor and Obiukwu, 2005).

Results of the heavy metal analysis of samples of the effluent are shown in Table 1. The concentration of these dissolved metals impacts ecological influence and affects the aquatic environment.. Hence, characterization of the metals in the crude oil effluent returning to the aquatic environment becomes a very important factor in the bid to combat ecological and structural degradation.

The levels of chromium and zinc were within the limits recommended by DPR.

Cadmium exceeded FEPA (1991) limit of 0.01mg/L throughout the period under investigation. Others that exceeded FEPA limits set for them include lead (2nd week), and copper (3rd and 4th weeks). Heavy metals are some of the most toxic, persistent, and widespread contaminants in aquatic systems (Carvalho et al., 1999) and their impact in various components of the ecosystem, particularly fishes, is a well-documented phenomenon (Ramaiingam et al., 2000; Jayakumar, 2000; Misra et al., 2002; Al-Saleh and Shenwari, 2002).

Water quality

The results of the analysis of the physicochemical properties of the impacted water body are presented in Tables 2 and 3. An upstream and downstream pH of 7.1 lies within the WHO acceptable standard for drinking water - pH 6.5-8.5 (WHO, 1993). The water did not fall within the values at which water is considered too acidic and unsafe for drinking and domestic purposes (Abara et al., 2005). Similarly, the temperature values (30.2 and 30.5 ºC) would not cause any threat to life since the values are almost within the acceptable limit (that is, 30 ºC). Elevated water temperatures cause a reduction in dissolved oxygen concentration of the water and attendant hazard to aquatic organisms (Bhatia, 2005; Obasi et al., 2004). Upstream and downstream TDS values of 17,662 mg/L and 18,950mg/L respectively, were by far higher than the acceptable limit of 250mg/L for potable water (WHO, 1993).

The high values also reflected in the salinity of the samples. The high values of the TDS and salinity of the upstream samples relative to the downstream implies that there may be other contributors/ apart from the effluent. Contamination by sea water and the impact of other oil exploration and production activities in the area are possible sources.

The other parameter that was elevated in the sample of the impacted water body is zinc. The concentration of zinc is worthy of note as it increased from 0.32mg/L upstream to 0.40mg/L downstream after impact, relative to WHO (1993) standard of 0.3mg/L. Toxicity identification studies have indicated that zinc may be the primary cause of toxicity in certain contaminated aquatic ecosystem (Bay et

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al., 2003). High concentration of zinc has been observed to be specifically toxic to an aquatic insect Ramotra elongate (1.658-2.853mg/L), and in the microtox test system (1.35mg/L) (Sukla and Omka, 1983).

IV. CONCLUSION

This study has shown that the quality of the flow station effluent discharged into Nembe-1 creek did not comply with statutory environmental standard. High levels of some toxicants exceeding the limit set by DPR and FEPA were observed in the effluent and impacted water body. Though, the contributions from other oil related activities around the study area may have added to the observed impact. The long term effect of effluent discharge into the creek is not known. A study by Reddy et al. (2002) has shown that hydrocarbon may remain buried in sediment for up to 30 years without major degradation. Continued discharge of improperly treated effluent may further compound the environmental problem of the area. An easy resolution of the problem entails proper treatment and monitoring of effluent to ensure compliance before release into the environment

Table.1: Weekly variations of effluent heavy metal concentration (mg/L) compared with DPR standard

Parameter	Week 1	Week 2	Week 3	Week 4	Mean	DPR
Cadmium	0.04	0.03	0.02	0.01	0.03	<0.01*
Chromium	<0.01	<0.01	<0.01	<0.01	0.01	0.5
Zinc	0.06	<0.08	0.53	0.5	0.65	5.0
Iron	0.48	0.26	0.65	1.0	0.66	20*
Lead	<0.001	0.51	<0.01	<0.01	0.01	0.05*
Copper	0.66	0.88	1.10	2.3	0.89	<1.0*

***FEPA limit (no limit set by DPR).**

Table.2: Water sample physicochemical quality analysis Unit in mg/L Values are means of determinations

Sampling Station	pH	Temp. ºC	Oil/Grease mg/L	TDS mg/L	BOD_5 mg/L	COD mg/L	Salinity mg/L
Upstream	7.1	30.2	__	17662	37.6	84	11128
Down Stream	7.1	30.5	__	18950	29.8	73,4	11360

Table.3: Water sample heavy metal analysis Unit in mg/L Values are means of determinations

Sampling Station	Cadmium	Chromium	Zinc	Lead	Copper
Upstream	0.02	<0.01	0.32	0.03	<0.01
Downstream	0.02	<0.01	0.4	0.04	<0.01

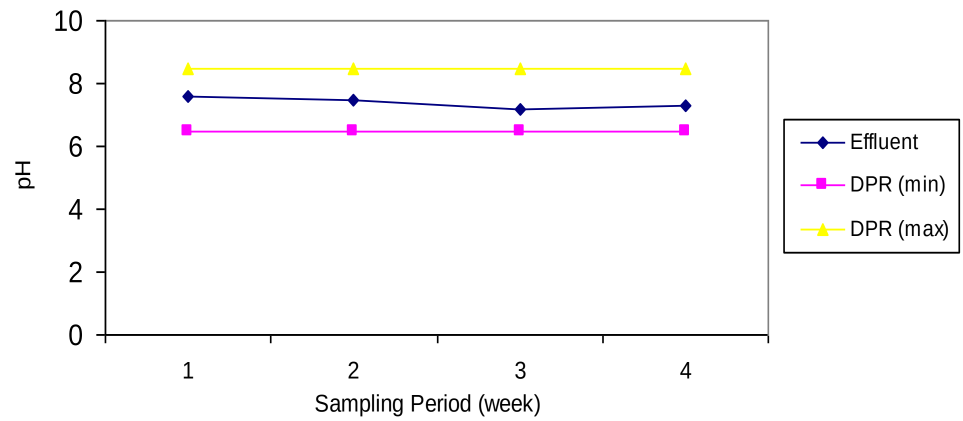

Fig. 1: Weekly variation of effluent pH compared with standard

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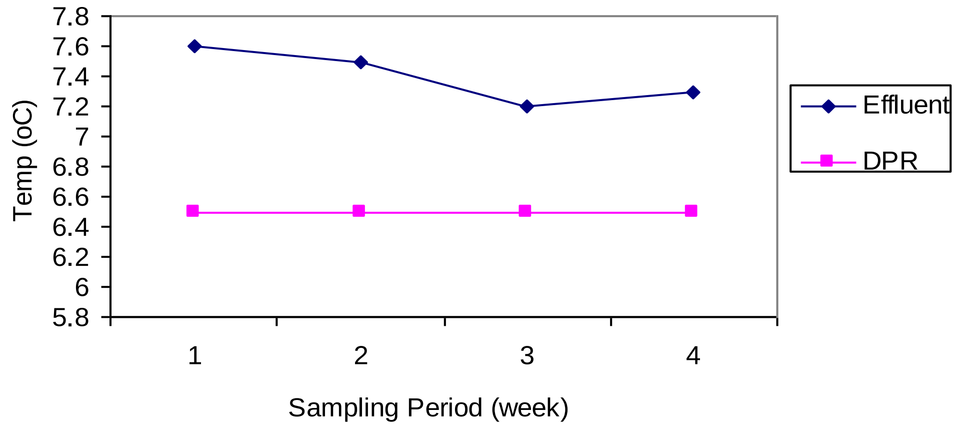

Fig, 2: Weekly variation of effluent temperature compared with standard

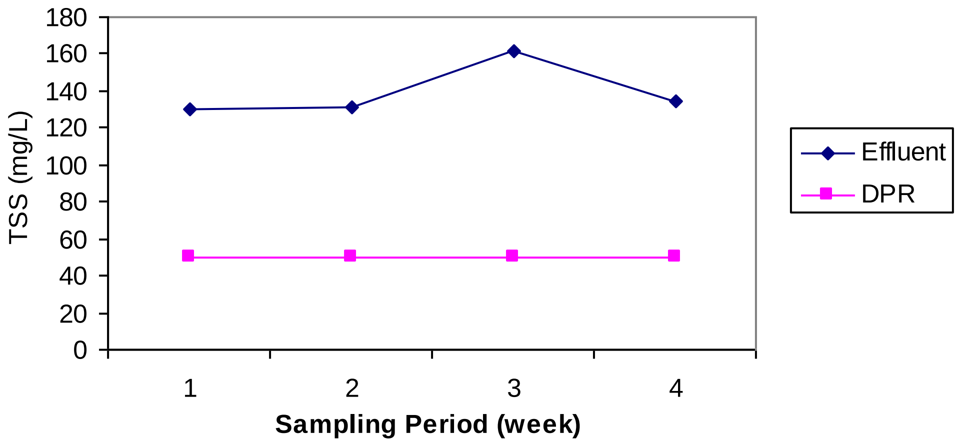

Fig. 3: Weekly variation of effluent TSS compared with standard

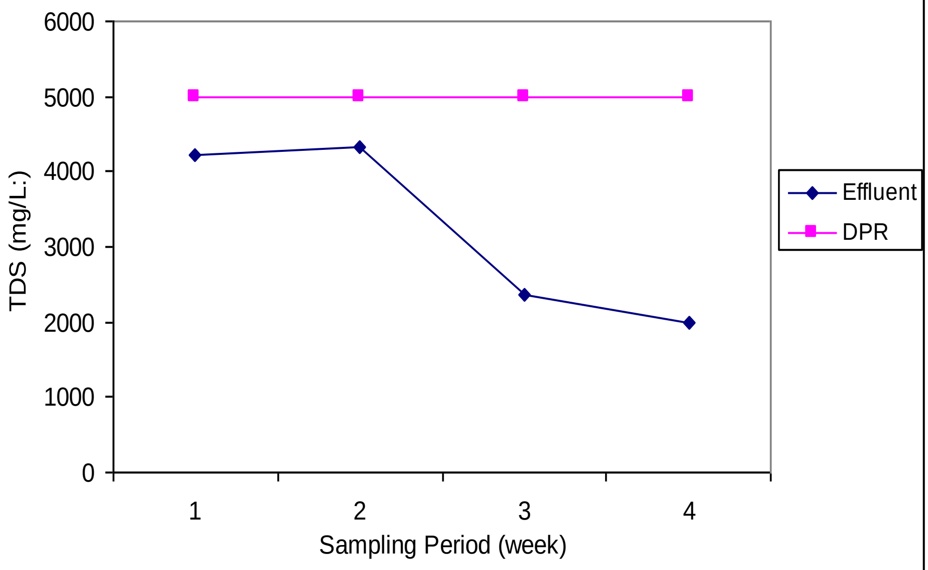

Fig. 4: Weekly variation of effuent TDS compared with standard

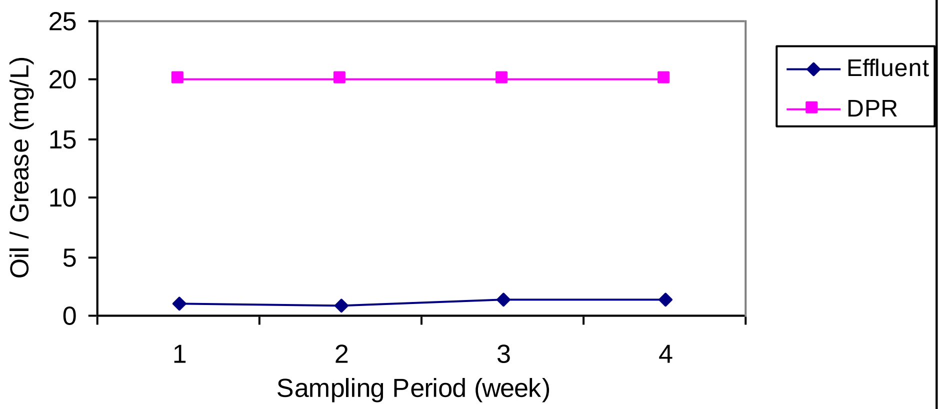

Fig. 5: Weekly variation of effluent oil and grease compared with standard

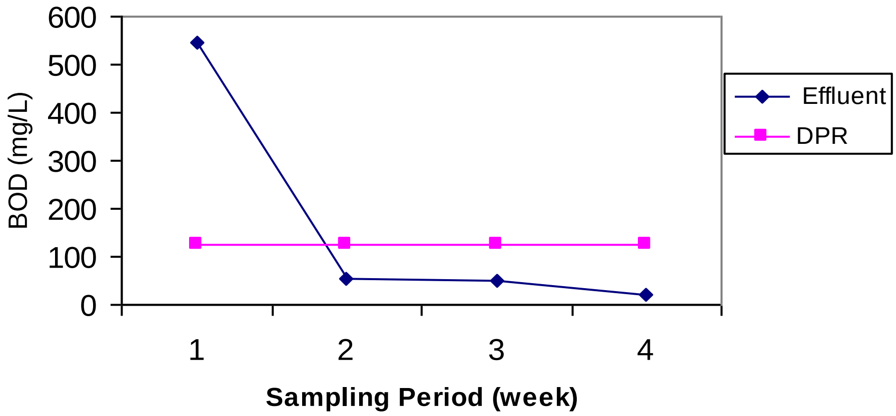

Fig. 6: Weekly variation of effluent BOD compared with standard

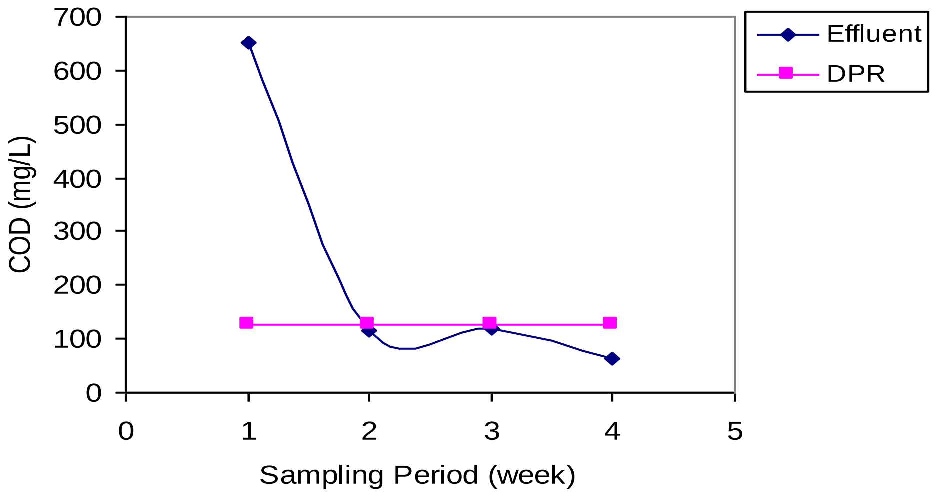

Fig. 7: Weekly variation of effluent COD compared wth standard

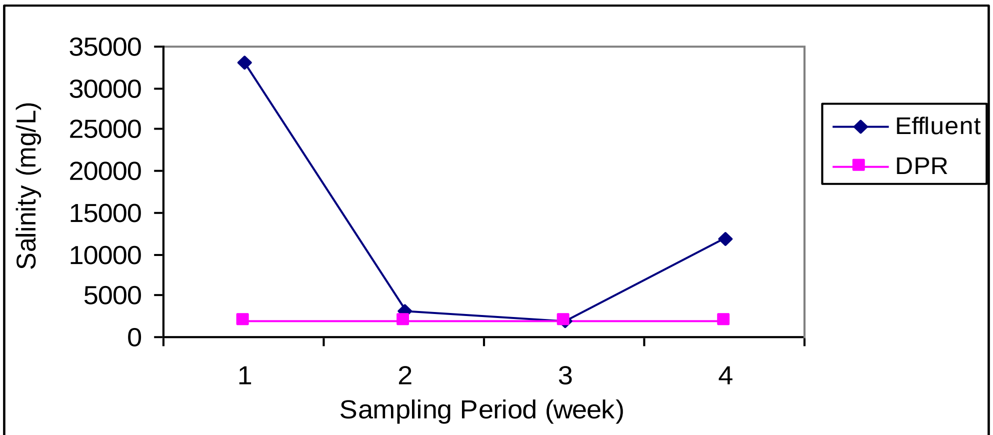

Fig. 8: Weekly variation of effluent salinity compared with standard

REFERENCES

[1] P.N. Abara, S.O. Obiekezie, R.A. Onyegba, and V.O. Nwaugo. Physicochemical characterization of Ivo River, Ebonyi State, Nigeria, International Journal of Natural and Applied Sciences, I (2): 145-149, 2005.

[2] J.F.N. Abowei, and F.D. Sikoki. Water pollution, Double Trust Publication, Company, Port Harcourt, pp.53. 2005.

[3] I. Al-Saleh, and N. Shenwari.Preliminary report on the levels of elements in four fish species from the Arabian Gulf of Saudi Arabia Chemosphere 48:749-755, 2002.

[4] E.O. Aluyor, and O.A.M. Badmus.Time series analysis of pollution levels in industrial waste water effluents A case of a beverage industry. Nigerian Journal of Biomedical Engineering, 2(1), 19-32, 2003.

[5] APHA Standard Method for the Examination of Water and Wastewater 19th edition American Public Health Association, Washington DC., 1995.

[6] S. Bay, B.H. Jones, K. Schiff, and L. Washburn. Water quality impact of storm water discharge into Santa Monica Bay Marine. Environmental Research 56:205-223, 2003.

[7] S.C. Bhatia. Environmental Pollution Control in Chemical Process Industries Khana Publishers, Delhi, pp.31-67, 2005.

[8] R.A. Carvalho, M.C. Brenfield, and P.H. Santskhi. Comparative bioaccumulation studies of colloidally complexed and tree ionic heavy metals in juvenile brown shrimp Penaius astecus (Crustacea Decapoda Penaedae). Limnol. Oceanogr. 44 (2): 403-413, 1999.

[9] Department of Petroleum Resources - DPR , Lagos. Environmental Guideline and Standard for the Petroleum Industry in Nigeria 1991.

[10] Federal Environmental Protection Agency - FEPA, National Environmental Protection (effluent limitation) Guidelines and Regulations 1991.

[11] Jayakumar, (2001). Levels of heavy metals in commercial fresh water fishes of Cohabatore, M. Phil. Dissertation thesis submitted to Bharathiar University.

[12] G. Kiely. Environmental Engineering, McGraw Hill Inc. New York, pp.493-790, 2004.

[13] S.M. Misra, K. Borana, S. Pani, A.Bajpai, and A.K. Bajpai. Assessment of heavy metal concentration in Grass Carp (Ctenopharyn godon idella), Poll. Res. 21 (1): 60-71, 2002.

[14] A.C. Nkwocha and J.I. Okoye. Quality evaluation of industrial liquid Effluent, Continental J. Applied Sciences 2, 51-55, 2007.

[15] A.C. Nkwocha, J.I. Okoye, F.E. Okpalama. and B.A. Oguama. Assessment of nutrient concentration of effluent discharges from a vegetable oil processing plant, Icastor Journal of Engineering Vol. 6. No 2, pp. 65-69, 2013.

[16] R.A. Obasi, O.F. Balogun, and O. Ajayi. The physicochemical investigation of river Ireje in Ekiti State, South West Nigeria, Journal of Applied Sciences 7(2) 4124-4, 2004.

[17] A.E. Ogbeibu, and B.J. Oribhubour. The ecological impact of stream Regulatiou using benthic micro invertebrates as indicators, Journal of Aquatic Sciences, 16 (2):132-138, 2001.

[18] I.J. Otaraku, and A.C. Nkwocha. Assessment of physicochemical quality of petroleum refinery

http://dx.doi.org/10.22161/ijeab/2.6.51 *ISSN: 2456-1878*

waste water effluent, Icastor Journal of Engineering 3 (2):165-174, 2010.

[19] T.V. Otukunefor, and C. Obiukwu. Impact of refinery effluent on the physicochemical properties of a water body in the Niger Delta, Applied Ecology and Environmental Research 3(1) pp. 61-72, 2005.

[20] V. Ramalingam, Vimaladevi, R. Naramdarji, and P. Prabakaran. Effect of biochemical changes in fresh water fish (Cirhinus mrigala) Poll. Res. 19(1):81-84, 2000.

[21] C.M. Reddy, T.I. Eglinton, A. Hounshell, H.K. White, L. Xu, R.B. Gaines, and G.S. Frysinger. The West Falmonth oil spill thirty years: the persistence of petroleum hydrocarbons in marsh sediments- Environmental Science and Technology 36:4754-4760, 2002.

[22] G.S. Shukla,, and M.R. Omka. Cadmium and zinc toxicity to an aquatic insect, Ramotra elongata (Fabr). Toxicology letters 15: 39-41, 1983.

[23] Shell Petroleum Development Company (2000). Technical Library, Petroleum Technology hand out

[24] WHO. Potable Water Quality Requirements (Standard).World Health Organization Geneva, Switzerland, 1983.

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Healthcare wastes management practices by public health facilities in Oshimili-South LGA of Delta State, South-South Nigeria

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Abstract— Background: *The sustainable management of Healthcare waste has continued to generate increasing public interest due to the health problems associated with exposure of human beings to potentially hazardous wastes arising from healthcare.*

Objectives: *To ascertain the healthcare wastes management practices by public health facilities in Oshimili-South LGA of Delta State.*

Methods: *A cross-sectional descriptive study. A multistage sampling technique was used in the selection of LGAs, healthcare facilities and respondents. There was Characterization and measurement (quantification) of waste. The study instrument was English language structured self administered questionnaire. The data obtained were analyzed using SPSS version 17.*

Results: *Practice of wastes reduction 92 (24.2%), wastes recycling 46(12.1%) and wastes weighing 72(18.4%). Fifty nine point four percent (59.4%) of respondents use personal protective equipments when handling wastes. Most 214 (62.2%) reported that burning was their final healthcare wastes disposal methods. The doctors, pharmacists and laboratory scientists all reported 100% use of protective gadgets. While the nurses (56.9%) and the wastes handlers (50.9%) use protective gadgets. On the average, 2.40kg/bed/day of waste was generated by the health facilities.*

Conclusion: *The study revealed that there was poor practice of waste segregation and recycling in all the facilities. There is need for more training.*

Keywords— *Healthcare, waste, management, practices.*

I. INTRODUCTION

In Nigeria, healthcare waste falls under the category of infectious waste.[1] These constitute a special category of waste because they contain potentially harmful materials like microbial culture and stock of infectious agents, pathological waste, waste from surgery or autopsy that were in contact with infectious agents, sharps (hypodermic needles, syringes, scalpel blades), waste from human blood and products of blood and laboratory waste. Other hazardous materials used by healthcare institutions that become part of their waste streams includes chemotherapeutic agents, antineoplastic chemicals, solvents, formaldehyde, photographic chemicals, radionuclides, mercury, anesthetic gases and toxic, corrosive and miscellaneous chemicals. Additional waste such as incinerator exhaust, laundry-related and kitchen waste are also Generated.[2]

HCWs management is a serious environmental impacting issue that must be addressed using series of management instruments that will help to alleviate the inherent havoc that these categories of waste have been causing and can cause to unsuspecting communities and inhabitants.[3] The sustainable management of Healthcare waste has continued to generate increasing public interest due to the health problems associated with exposure of human beings to potentially hazardous wastes arising from healthcare.[4,5]

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The problems in healthcare waste management stem from a failure of both practice and technology. Lack of waste segregation, unsafe waste handling, dumping of untreated wastes, preferential procurement of toxic products, extensive use of disposable materials, inadequate procedure for clean-ups and containment of spills, weak inventory controls of time-sensitive pharmaceuticals and reagents, and inappropriate classification of non-infectious waste as bio-hazard waste are examples of poor practices that lead to high rates of healthcare waste generation in health facilities.[6] The general options for management of solid waste range from prevention, minimization, reuse, recycling, energy recovery and disposal. That is integrated waste management. Methods of disposal of solid waste includes: sanitary landfills, incineration, composting, dumping, manure pits and burial.[7] The nature and quantity of healthcare waste generated, as well as institutional practices with regards to sustainable methods of healthcare waste management, including waste segregation and waste recycling are often poorly examined[8] and documented in several countries of the world despite the health risk posed by the improper handling of healthcare waste. It is also of serious concern that the level of awareness, particularly of health workers regarding healthcare waste has not been adequately documented.[8]

The Waste Management Authority in Lagos discovered that the specialized form of waste from hospitals and diagnostic laboratories was not being properly managed at any level in the state. The comingling of general or domestic waste with infectious waste (specialized waste) was very common[9], and healthcare waste being generated from healthcare facilities carries a higher potential of infection and injury than any other type of waste and poses danger if not properly managed. The authority saw the need for inter-sectoral collaboration and has been working with other state agencies including: the Ministry of Health; Ministry of Environment; Health Facility Monitoring and Accreditation Agency (HEFAMAA); the Environmental Protection Agency; John Snow Incorporated and AIDSTAR-ONE (NGO); in the development of consistent regulatory approach to ensure proper management of HCWs across the state. The authority medical waste management unit has also identified adaptations of various initiatives and "best practices". The introduction and free distribution of color coded bin liners and safety boxes to all accredited hospitals both (public and private) was one key strategy to jump-start the HCW management programme. Other efforts include participation of private service providers (PSP); annual HCW summit with stakeholders; training of hospital personnel and healthcare workers, emphasizing on waste segregation and containerization at source. They also organize training and workshops for waste handlers, effective monitoring and enforcement and strict legislation on infectious and hazardous waste management.[9]

The WHO Safe Healthcare Waste Management Policy Paper 2002[10] recommended that preventing the health risks associated with exposure to HCW for both workers and the public is achieved by promoting environmentally sound management policies for HCW; The Basel Convention is on management of hazardous and other wastes. It emphasizes the need to reduce exposure to toxic pollutants associated with the combustion process through the promotion of appropriate practices for high temperature incineration. Its strategies includes identification and development of recycling options wherever possible; research and promotion on new technology or alternative to small-scale incineration; risk assessment to compare the health risk associated with incineration and exposure to HCW; effective scale-up promotion of non-incineration technologies for the final disposal of HCW to prevent the disease burden from unsafe HCW management; promotion of the principles of environmentally sound management of HCW.[10]

In Nigeria, there is often the lack of manpower resource to properly manage huge amounts of medical waste generated.[21] Lack of relevant training and equipment for the waste handlers is a common feature particularly in public hospitals. They are highly exposed to risk of infection due to none provision of protective equipments.[27] It has also been found that health workers in Nigeria are unaware of relevant regulations and the existence of a hospital waste management policy.[11,12]

The study seeks to ascertain management practices of health facility wastes along with types and amount of wastes generated. This will bring to fore the gaps and lapses in HCWM.

Studies on healthcare wastes and healthcare wastes management practices have been conducted all over the world, amongst which is a study done in 2004 on healthcare waste management in the city of Ulaanbaatar, Mongolia.[13] In this study, information was obtained to describe the existing procedures practiced in handling and treatment of healthcare wastes produced. In addition, a cross-sectional study was conducted to characterize and quantify the healthcare wastes generated in Ulaanbaatar city of Mongolia.[13] A total of 56 healthcare facilities operating in the city were selected for the study: 15 large (13 public and 2 private). Results showed that an average total of about

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2.65 tones were produced per day in public health facilities, in which 0.78 tons are medical and 1.87 tons were general waste. The medical waste per patient per day was 1.4-3.0 times higher in the inpatient sections than in the outpatient sections.[13]

A study carried out in Karachi, Pakistan[14] on healthcare waste management in hospitals in Karachi, reported that healthcare waste management practices in hospitals in Karachi was far below the desirable level due to lack of understanding about its significance by stakeholders, and absence of professional input and capability to deal with the issues. Also reported, was general slackness on the part of facility managers, inadequately trained staffs, inappropriate tools and equipments, and limited awareness about the contagious nature of the waste. The healthcare waste is collected and disposed with municipal solid waste, which is dangerous to the health of the public.[14]

A critical analysis of healthcare waste management in a developed and developing Country, comparing England to India was done. The study employed a range of methods such as, audits and questionnaire surveys to examine healthcare waste management practices in the Andhra Pradesh State India. Compliance with the regulatory notifications for biomedical waste management and handling rules 1998, under the environmental protection act (1986) of the government of India was used as the standard.[15] Whilst in King George Hospital, England, the published practices of a case study organization in compliance with policies such as the hazardous waste regulations 2006 were utilized. The study reported that the management of healthcare waste in India was far below recommended practice and standard, despite the introduction of rules and regulations, wastes generated by government hospitals were still largely being dumped in the open, waiting for collection along with the general waste. Unlike India, the management of healthcare waste is very stringent in England.[15]

Another study carried out in Irrua specialist teaching hospital, Irrua, Edo State, Nigeria, on healthcare waste management, revealed that the average amount of healthcare waste was 0.62 kg/person/day at the out-patient units and 0.81 kg/bed/day in the in-patient wards. The proportion of respondents (healthcare workers including waste handlers) who had received specific training in the management of healthcare waste was 11.5% (6/52). The number who understood the importance of healthcare waste management practice was found to be 0. This study highlighted the pitfalls of healthcare waste management practices in Nigeria.[16] A similar study on healthcare waste disposal practices of 432 private dental practitioners in the city of Bangalore, India was carried out. Results revealed that Dentist in Bangalore were poorly aware regarding healthcare waste disposal methods and the existence of legislation governing healthcare waste disposal. These practitioners were not motivated enough to comply with the guidelines.[17]

A study in Lagos State Nigeria, to determine the variations and similarities in the healthcare waste disposal practices within two General Hospitals (Orile-Agege General Hospital and General Hospital, Lagos) located in separate Local Government Areas of Lagos State was done. The study used physical observation, waste quantification or estimation and documented information provided in the hospital records. Primary and secondary data were gathered, the primary were collated through the field survey over a month each, where oral interviews, field video and photography, discussion with workers, quantification of various sectional waste using two scales, one with +1/-0.1 accuracy with a minimum range of 0.02 and capacity of 3.0kg and the second with +1/-0.5 accuracy with minimum of 1kg to a maximum capacity of 120kg. The study revealed similarities in many areas.[3] Total waste segregation was not practiced on the two sites, no waste reduction or minimization measures exist either. Many sections do not keep proper records on the quantities of the materials used for treating patients; neither do they normally quantify their generated waste daily. Co-disposal of domestic and healthcare wastes like highly infectious wastes (like from DOTS and VCT) is the normal practice existing in the hospitals and co-collection of the generated wastes by wastes collectors was observed to be the norm and non observance of most colour codes recommended for keeping waste was the practice.[3]

A cross-sectional study in Nigeria Federal Capital Territory (FCT), Abuja in 2006 on characterization and management of solid medical wastes in five selected hospitals, showed that the average waste generation rate per bed/day was 2.78kg of solid waste, 26.5% of the total waste was hazardous in nature. Waste segregation was not practiced by any of the hospitals surveyed, 18.3% of the hospitals incinerated waste in a locally built brick incinerator; 9.1% bury; 36.3% burn waste in open pits while 36.3% dispose of a waste into municipal dumpsites. It was also found that waste management officers do not have formal training in waste management techniques; and hospital administrators pay very little attention to appropriate management of medical waste.[18]

A similar study was done in four (2 private and two public) hospitals with capacity ranging from 40 to 600 beds in metropolitan Lagos. Results showed that medical waste management practices in all hospitals indicate absence of full compliance with the protocol for handling medical wastes as stipulated in the relevant sections of the guidelines and standards for environmental pollution control in Nigeria. Three hospitals demonstrated high priority for segregation of infectious medical wastes. Average generation rate of medical wastes in the investigated hospitals range from 0.562kg/bed/day to 0.670kg/bed/day. Infectious waste accounts for between 26 to 37% of this volume. Only two of the hospitals investigated carry out treatment of their infectious and sharp wastes by incineration before final disposal. Burning and burial of medical waste is an unusual but common practice among the hospitals. All hospitals employ the services LAWMA for final collection and disposal of their medical wastes at Government approved sites.[19]

II. METHODS

The study was carried out in Oshimili-South Local Government Area (LGA) of Delta North Senatorial District of Delta State; it is a semi-urban area with headquarters in Asaba. It has an area of 603 square kilometers and a population of 149, 603 at the 2006 census.[20] It consists of ten towns or communities namely Asaba, Oko, Cable point, Central core area, Isieke, Ezenei, Umuagu, Umueze, Umuonaje and Zappa.[21] This LGA has good access roads and pipe borne water supply. Located in this LGA is the Federal Medical Center, Asaba, the Delta State University, Anwai Campus, the Federal College of Education (Technical). This area is connected to the National grid of the Power Holding Company of Nigeria. The main religion in this area is Christianity and the language spoken by the people are Igbo, English language and Pidgin English. There are eleven (11) public health facilities in the LGA. There are nine primary health care centers, one general hospital at Okwe and one tertiary health care facility (Federal Medical Centre, Asaba) in Oshimili-South LGA. The PHCs include PHC Umuagu, Okwe, Awai, Anala, Ogbele, Amakoma, Akwaebune, Umueze and West-End. The Federal Medical Centre (FMC) has 175 doctors, 42 pharmacist, 43 laboratory scientist and technicians, 400 nurses, 19 workers in radiology, 7 in physiotherapy, 7 in pathology and 132 health/ward assistance/health care wastes handlers. This make a total of eight hundred and fifty (855) health staff in the FMC. The general hospital has a total of 120 health staff, amongst whom, are doctors, nurses, laboratory staffs, pharmacist, ward assistants and waste handlers. The nine PHCs have a total of 248 medical and environmental health staff.

The study was a cross-sectional descriptive study among healthcare workers in public health facilities and they included medical doctors, pharmacists, nurses, midwives, laboratory scientists, laboratory technicians and healthcare waste handlers/ward orderlies. Non medical workers such as the administration and accounting staffs were excluded. Also excluded from this study are private health facilities and those who refused to give consent to participate in this study.

Characterization and measurement (quantification) of waste:

Waste items from the FMC, the General Hospital and the nine PHCs were categorized according to wastes types. The cooperation of staff of each shift was sought to place all the wastes generated inside the labeled colour coded polythene bags that were provided by the researcher as appropriate by repeatedly reminding them. Red coloured bags were used for highly infectious waste like body parts, placenta, body fluids, blood bags, culture plates etc. Yellow bags for waste moderately infectious but hazardous waste like swabs, wound dressings not too soaked, infusion sets, catheters etc. Black bags labeled 'general waste' for general (non-hazardous) waste like paper, food debris, cans etc and other labeled black bags for chemicals and drugs.

After twenty four (24) hours, the bags were collected by the research team members. In the renal unit, theatre and labour rooms, wastes were weighed per shift because the nature of the waste required immediate disposal. Wastes were collected on alternate days for a week per hospital and per PHCs. The wastes were sorted and weighed in polythene bags using a top loader scale Camry Emperors 100kg with a capacity of 100kg and intervals of 100gm. Smaller amounts of wastes were weighed using same make of scale but with a capacity of 20kg and intervals of 50gm. Scales were standardized everyday by a known weight. The characterization was done according to the categories stated above. Total waste per bed per day at each of the study sites was calculated by division of the total waste generated per day by the total number of occupied beds of each site.

Ethical approval for this study was given by the Ethical Committee of the University of Benin Teaching Hospital, Benin City, Edo State. Consent to participate in this study was sought from the respondents after detailed explanations to them about what the study entails, as well as assuring them of confidentiality of information to be given. The questionnaires were them self administered.

The sample size was calculated based on one proportion sampling with population greater than 10,000;

$n = Z^2 pq/d^2$

Where

n = the minimum sample size

z = standard normal deviate, set at 1.96 corresponding to 95% significance level.

P = 50% proportion of characteristics (attributes) in the population because there are no reports of previous studies on this in this area.

$q = 1 - p$

$q = 1 - 0.5 = 0.5$

d = precision or degree of accuracy i.e. acceptable margin of sample error set at 5% or 0.05.

Substituting the above figures in the formula, the desired sample size, n, for the study will be

$n = (1.96)^2 \times 0.5 \times 0.5 / 0.05^2 = 385$. This therefore means that a minimum sample size of 385 is required for the study to be valid.

Adjusting by 10% for non-response

n = 424 as now the sample size for the study.

A multistage sampling technique was used in the selection of LGA and participants in this study. There were nine primary health care centers, one general hospital at Okwe and one tertiary health care facility (Federal Medical Centre, Asaba) in Oshimili-South LGA. An English language structured self-administered questionnaire with open and closed ended questions with sections on social demographic data and wastes management practices was used for this study. The data obtained were analyzed using SPSS version 17. Test of associations were done using chi square statistics at 95% confidence levels.

III. RESULTS

In all, eleven health care facilities participated in this study. The FMC, General Hospital and nine PHCs.

Table.1: Socio-demographic characteristics of respondents: (N= 402)

Characteristics	Frequency	Percentages (%)
Age (years)		
10 – 19	7	1.9
20 – 29	82	22.1
30 – 39	138	37.2
40 – 49	106	28.6
50 – 59	38	10.2
Marital status		
Single	110	27.7
Married	245	60.9
Separated	20	5.0
Divorced	16	4.0
Widowed	11	2.7
Designation		
Doctors	46	11.4
Nurses	130	32.3
Pharmacists	20	5.0
Lab. Scientists	20	5.0
Wastes handlers/Health assist.	120	29.8
Others e.g. labourers etc	66	16.4
Religion		
Christianity	398	99.7
Islam	1	0.3

A total of 402 health workers from the two hospitals and the nine PHCs participated in the study giving a response rate of 95%. The age group 30-39 years constituted 138 (37.2%) of respondents. The mean age of respondents was 36.8$\pm$ 10years with standard deviation of 9.1 years. The age of respondents ranged from 18 – 55 years. Majority 245 (60.9%) of respondents were married, while 110 (27.7%) of the respondents were single. Nurses constituted the highest proportion of respondents in the study with a frequency of 130 (32.2%), followed closely by Healthcare Waste Management Workers (health/ward assistants/environmental health workers) who were 120 (28.9%) of the respondents. Majority 398 (99.7%) of the respondents were Christians.

Table.2: Hospital policy regarding healthcare waste management

Variables	Frequency (%)		Total
	Yes	No	
Awareness of HCWM policy	294(77.4)	86(22.6)	380(94.5)
Availability of focal person	233(72.1)	90(27.9)	323(80.3)
Employees information on policy	301(88.5)	39(11.5)	340(84.6)
Employees practice of policy	283(85.8)	47(14.2)	330(82.1)
Awareness of other hospital safety policies	233(57.5)	165(42.5)	388(96.5)
Penalty for not following policy	263(59.9)	136(40.1)	339(84.3)

The study revealed that 294(77.4%) of respondents are aware of a HCWM policy in their health facility; 233(72.1%) respondents know people in charge of the policy; 283(85.8%) of respondents practice this policy. Awareness of other policies like policy on universal precaution was 233(57.5%), and if there are penalties if the policies are not obeyed were 263(59.9%).

Table.3: Practices before final disposal

Variable	Frequency (%)		Total
	Yes	No	
Practices before final disposal			
Wastes reduction	92(24.2)	286(75.8)	380(94.5)
Wastes recycling	46(12.1)	333(87.9)	379(94.3)
Daily weighing of wastes	72(18.4)	320(81.6)	392(97.5)
Use of personal protective equipments	233(59.4)	159(40.6)	392(97.5)

Practice of wastes reduction 92(24.2%), wastes recycling 46(12.1%) and wastes weighing 72(18.4%) before final wastes disposal. It also revealed that only 233(59.4%) of respondents use personal protective equipments when handling wastes.

Table.4: Hospital waste disposal methods

Variable	Health facilities (%)			Total
	FMC	GH	PHC	
Final HCW disposal methods				
Incineration	10(2.9)	1(0.3)	19(5.5)	30(8.7)
Burying	29(8.4)	3(0.9)	49(14.2)	81(23.5)
Open dumping	4(1.2)	1(0.3)	3(0.9)	8(2.3)
Burning	53(15.4)	9(2.6)	152(44.2)	214(62.2)
Dumping into the river	0(0)	0(0)	11(3.2)	11(3.2)
Total	96(27.9)	14(4.1)	234(68.0)	344(100)

Most 214(62.2%) of the total respondents believe that the final healthcare wastes disposal methods of their health care facility is by burning. Fifty three (15.4%) of the FMC respondents; 9(2.6%) of the General hospital respondents; and 152(44.2%) of the PHCs respondents indicated burning as their final wastes disposal methods. This is closely followed by burying as a final wastes disposal method with FMC 29(8.4%); General hospital 3(0.9%); and the PHCs 81(23.5%) indicating burying as final disposal method.

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Table.5: Training on healthcare waste management (HCWM): (N=365)

Variable	Training on HCWM Yes (%)	No (%)	Total	Test/p-value
Designation of respondents				
Doctors	17(41.5)	24(58.5)	41(11.2)	
Nurses	68(58.1)	49(41.9)	117(32.1)	$X^2 = 17.304$
Pharmacists	3(27.3)	8(72.7)	11(3.0)	df = 5 p= 0.004
Lab. Scientists	11(78.6)	3(21.4)	14(3.8)	
Waste handlers/Health assist	61(59.8)	41(40.2)	102(27.9)	
Others e.g. Labourers etc.	32(40.0)	48(60.0)	80(21,9)	
Total	**192(52.6)**	**173(47.4)**	**365(100.0)**	
Health facilities				
FMC	58(55.2)	47(44.8)	105(28.8)	$X^2 = 1.309$
General hospital	19(59.4)	13(40.6)	32(8.8)	df = 2 p= 0.520
PHCs	115(50.4)	113(49.6)	228(62.5)	
Total	192(52.6)	173(47.4)	365(100.0)	

Indicates that laboratory scientists were found to have had more training on HCWM (78.6%), followed by wastes handlers/health assistants (59.8%), nurses (58.1%), and doctors (41.5%). The General Hospital had more training on HCWM for their health staff (59.4%), followed by the FMC (55.2%), and the PHCs (50.4%).

But however, statistically, designation of respondents, and difference in healthcare facilities were both not found to be significantly associated with training on HCWM. In other words, there was no difference in training on HCWM in the different designation and also in the different healthcare facilities.

Table.6: Use of protective gadgets and equipments

N = 392

Variable	Use of protective gadgets Yes (%)	No (%)	Total	Test/p-value
Designation				
Doctors	46(100.0)	0(0)	46(11.7)	
Nurses	74(56.9)	56(43.1)	130(33.2)	$X^2 = 73.549$
Pharmacists	15(100.0)	0(0)	15(3.8)	df = 5
Lab. Scientists	15(100.0)	0(0)	15(3.8)	p= 0.0001
Wastes handlers/Health assist.	56(50.9)	54(49.1)	110(28.1)	
Others e.g. Labourers etc.	27(35.5)	49(64.5)	76(19.4)	
Use of protective gadgets/ equipments				
FMC	106(89.8)	12(11.2)	118(30.1)	$X^2 = 92.920$
General Hospital	30(88.2)	4(11.8)	34(8.7)	df= 2
PHC	97(40.4)	143(59.6)	240(61.2)	p=0.001

There is a statistically significant difference in the use of protective gadgets and equipments among the different designations of health staffs and also among the different healthcare facilities. In other words, there is a statistically significant association between the designation of respondents, healthcare facilities of respondents and the use of protective gadgets.

The doctors (100.0%), pharmacists (100.0%), and laboratory scientists (100.0%), use protective gadgets all the time, while the nurses (56.9%) and the wastes handlers/health assistants (50.9%) use protective gadgets less often.

The respondents of the FMC (89.8%) and those of the General hospital (88.2%), use protective gadgets and equipments while respondents of the PHCs (40.4%) use these gadgets less often.

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Table.7: HCWM practices before final disposal

Variable	Practices before final disposal Yes (%)	No (%)	Total	Test/p-value
Weighing of waste before disposal				N = 392
FMC	32(27.1)	86(72.9)	118(30.1)	$X^2 = 52.75$
General Hospital	19(55.9)	15(44.1)	34(8.7)	df = 2
PHC	21(8.7)	219(91.3)	240(62.2)	p=0.0000
Practice of waste reduction				N = 380
FMC	40(36.4)	70(63.6)	110(28.9)	$X^2 = 18.46$
General Hospital	12(36.4)	21(63.6)	33(8.7)	df =2 p= 0.0000
PHC	40(16.9)	197(83.1)	237(62.4)	
Wastes recycling				N = 379
FMC	7(6.4)	103(93.6)	110(29.0)	$X^2 = 7.356$
General Hospital	2(6.1)	31(93.9)	33(8.7)	df=2 p=0.025
PHC	37(15.7)	199(84.3)	236(62.3)	
Disposal of all waste medical and Non-medical in same container				N = 391
FMC	48(41.7)	67(58.3)	115(29.4)	$X^2 = 30.32$
General Hospital	12(38.7)	19(62.3)	31(7.9)	df=2 p=0.0000
PHC	170(69.4)	75(30.6)	245(62.7)	
Use of special wastes containers for sharps				N = 360
FMC	94(79.7)	24(20.3)	118(30.1)	$X^2 = 1.38$
General Hospital	23(67.6)	11(32.4)	34(8.7)	df = 2
PHC	37(15.7)	199(84.3)	236(62.3)	p=0.0000

There is a statistically significant difference among those that weigh their wastes before disposal and those that do not in the different healthcare facilities. Weighing of wastes before disposal is most commonly practiced by respondents of the General hospital (55.9%), and least practiced by respondents of the PHCs (8.7%) and FMC (27.1%).

There is a statistically significant difference among those that practice wastes reduction before disposal and those that do not in the different healthcare facilities. Though, there is poor practice of wastes reduction in all the health facilities, it is worst with the PHCs (16.9%).

There is no statistically significant difference among those that practice wastes recycling, or use of special wastes containers for sharps and those that do not in the different healthcare facilities. The practice of wastes recycling is generally poor in all the health facilities studied. Though, no statistically significant difference, the use of special wastes containers for sharps is higher in the FMC (79.7%) and the General hospital (67.6%) as compared to the PHCs (15.7%).

Table.8: Final wastes disposal methods of Health Facilities *N = 344*

Variable	Health Facilities FMC (%)	GH (%)	PHC (%)	Total	Test/p-value
Final wastes disposal methods					
Incineration	10(33.3)	1(3.3)	19(63.3)	30(8.7)	
Burying	29(35.8)	3(3.7)	49(60.5)	81(23.5)	$X^2 = 13.078$
Open dumping	4(50.0)	1(12.5)	3(37.5)	8(2.3)	df = 8
Burning	53(24.8)	9(4.2)	152(71.0)	214(62.2)	p= 0.109
Dumping into the river	0(0)	0(0)	11(100.0)	11(3.2)	

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Indicates that 214(62.2%) of the respondents from the different healthcare facilities believe that their facilities final wastes disposal methods is by burning.

Though, there was no statistically significant difference between the final HCW disposal methods in the different healthcare facilities.

Table.9: Characterization and quantification of waste Amount of various categories of waste generated from hospitals per day

	General (kg)	**Sharps (kg)**	**Infectious (kg)**	**Pathological (kg)**	**Radiological (kg)**	**Pharmceuticals (kg)**	**Total (kg)**
Health facilities							
FMC	132.2	1.6	12.2	7.5	0.0	2.4	155.9
General hospital	23.7	0.7	10.2	2.3	0.0	1.2	38.1
PHCs	332.2	12.3	4.5	0.5	0.0	2.6	352.1
Total	488.1	14.6	26.9	10.3	0.0	6.2	546.1

Shows that the amount of various categories of waste differed. General waste had the highest quantity followed by infectious waste in all the health facilities.

Table.10: Mean waste generated per bed daily

	Mean number of Occupied beds/day (N)	**Mean total waste generated (kg)/day (kg)**	**Estimated waste/bed/day (kg)**
Health facilities			
FMC	86	155.9	1.81
General hospital	17	38.1	2.24
PHC	112	352.1	3.14
Total	**201**	**546.1**	**7.19**

Reveals that total amount of waste generated per facility increased with total number of occupied beds. However, the PHCs generated more wastes per bed/day (3.14kg/bed/day), while the FMC generated the least amount of waste/bed/day (1.81kg/bed/day). On the average, 2.40kg/bed/day of waste was generated by the health facilities.

Table.11: Composition of waste from the health facilities.

	Percentage composition of waste categories generated per day			
	General (non Hazardous) Kg(%)	**Hazardous infectious Kg(%)**	**Hazardous non infectious Kg(%)**	**Total Kg(%)**
Health facilities				
FMC	132.2(84.8)	21.3(13.7)	2.4(1.5)	155.9(100.0)
General hospital	23.7(62.2)	13.2(34.6)	1.2(3.1)	38.1(100.0)
PHCs	332.2(94.3)	17.3(4.9)	2.6(0.7)	352.1(100.0)
Total	**488.1(89.3)**	**51.8(9.5)**	**6.2(1.1)**	**546.1(100.0)**

This shows that the waste generated consisted of 89.3% general waste, 9.5% of hazardous infectious waste (sharps, infectious and pathological waste) and 1.1% of hazardous non infectious waste (pharmaceutical and radiological waste). Waste composition did not vary with hospital type.

IV. DISCUSSION

Segregation is the essence of waste management and together with waste identification, the key to minimization and effective management of healthcare waste. The most appropriate way of identifying the categories of healthcare waste is by sorting the waste into colour-coded plastic bags or containers.[22] In this study, only 43.3% of the respondents practiced waste segregation by disposing waste into colour coded plastic bags or containers, while 58.8% of the respondents deposit both medical and non-medical waste into same plastic or containers. Segregation of medical waste from non-medical waste was almost completely lacking in this study except from segregation of sharps, and this is in keeping with several studies like the study in two general hospitals in Lagos, Nigeria,[3] Another study in the Federal Capital Territory (FCT), Nigeria[18]; studies in the Fars Province of Iran[23] and the University Hospital in Fars, Iran[24]; other studies in Jos[25] and PortHarcourt,[26] Nigeria, that all reported poor segregation of medical from general wastes. Contrary to findings in this study, are reports of high priority segregation of infectious medical waste from general waste as reported in studies done in Lagos, Nigeria in two private and two public hospitals with bed capacity of 40 to 600 beds.[19] Similarly, a study in the United States (US) of America, reported a 95.4% rate of segregation of medical from general waste among US hospitals. Also reported in this study, is that 96.1% of US hospitals use labeled or colour-coded bags or both.[27]

Most (77.4%) of the healthcare workers are aware of HCWM policy in their healthcare facility. This is not in keeping with the study in Johannesburg, South Africa[22], the study in Bangalore, India[45] and the study in Egypt[28] where awareness of wastes management policy were either non-existent or very low. In this study, awareness of HCWM policy in the different health facilities; awareness of policy by the different designation of respondents (doctors, pharmacist, nurses, laboratory scientist and waste handlers/ward orderlies); abiding by the policy when aware of it; and if respondents participate in policy implementation, were all found not to be significantly associated with healthcare wastes management policy implementation. Awareness of respondents of HCWM policy was highest among the FMC respondents (81.3%). This could be attributed to the fact that the FMC is a tertiary healthcare facility with more highly educated healthcare workers as compared to the general hospital and PHCs. Awareness of HCWM policy was also highest among doctors (78.6%) as compared to nurses (72.7%), pharmacist (71.4%), laboratory scientist (64.3%), and wastes handlers/health assistants (62.4). Though, there was no statistically significant difference in awareness of HCWM policy among the different designations.

This study revealed that there is poor practice by healthcare workers regarding waste reduction (24.2%), waste recycling (12.1%), and weighing of waste (18.4) before final disposal. This shows a higher level of practice of waste reduction, recycling and weighing of waste than that of the study in two general hospitals in Lagos[3] that reported 0% practice of waste reduction, recycling and weighing of waste before final disposal. Most (62.2%) of the total respondents reported that the final healthcare wastes disposal methods of their health care facility is by burning. This is of a higher level of than that of the study in the FCT, Nigeria where 36.3% was reported.

Only 52.6% of the healthcare workers had a form training on HCWM. The study revealed that there was no statistically significant difference in training on HCWM in the different designation and also in the different healthcare facilities studied. These findings are not in keeping with the study in Karachi, Pakistan,[14] FCT, Nigeria,[18] Fars University Hospital, Iran[24] and the study in Rawalpindi, Pakistan[29] that all reported poor knowledge of HCW due poor training of healthcare workers.

Proper training and management of healthcare workers with regard to HCWM is necessary, observance of standard precaution together with use of Personal Protective Equipments (PPEs) is also required. The minimum PPEs consist of overalls, waterproof long boots, heavy industrial gloves and facemasks. HIV, hepatitis B and C infections are some of the deadly hazards healthcare workers are exposed to. These are risk not only to the workers but also to their family members.[24] In this study, use of PPEs was best among the doctors (100.0%), pharmacists (100.0%), and laboratory scientists (100.0%). Poor practice was observed among the nurses (56.9%) and the wastes handlers/health assistants (50.9%). This may be explained by the fact that the doctors and laboratory scientist are in direct contact with patients and their specimens and therefore are more cautious. The pharmacy staff probably may not be that exposed to these hazards, but in this study, they seem to be

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aware and very cautious of their safety. The poor usage amongst the nurses may be due carelessness on their part since knowledge of risk of HCW is high in this study. Poor usage amongst the waste handlers and ward assistants may be due to poor literacy, poor level of knowledge and poor risk perception. This seemingly poor usage of PPEs among sanitation staffs was also found among sanitation staff of in the study in Rawalpindi, Pakistan[29] and also lack of knowledge of use of PPEs in the study at the Fars University, hospital in Iran.[24]

Average waste generation per bed was 2.40kg/bed/day and this was similar to what was obtained in the FCT, Abuja, Nigeria where it was stated to 2.78kg/bed/day.[18] Lower values have been reported from Lagos,[19] Ilorin,[20] and Irrua,[16] However, per capital waste generation was much higher in Iran and the United States where it was 4.45kg and 6.93kg/bed/day respectively.[27,23] This varying amount of waste generated per bed daily between countries may be due to differences in economic status and development and the probability of using more disposable items and more consumables with higher economic status. The proportion of wastes constituents in this study (general waste made up of 89.3%, hazardous infectious 9.5% and hazardous non-infectious 1.1%; of the infectious, sharps consisted of 2.7%) were within the range reported from previous studies in Nigeria; Abuja[18] and Akure.[31]

V. CONCLUSION

The study showed that there is poor practice of waste segregation in all the facilities. None of the facilities segregated their wastes into different categories; wastes were not properly handled and were collected and disposed in mixed form. Also found was poor practice of waste reduction, recycling and waste weighing before final disposal. Practice was not influence by duration of work experience but was found in the case of use of personal protection equipments to be influenced by designation of the health worker.

A daily waste generation per bed was 2.40kg/bed/day. Waste composition was 89.3% general waste, 9.5% of hazardous infectious waste (sharps, infectious and pathological waste) and 1.1% hazardous non-infectious waste (pharmaceutical, chemical and radiological waste).

Most of the healthcare facilities did not have waste management plan, nor do they have waste management teams or clear defined procedures for waste management.

REFERENCES

[1] Federal Environmental Protection Agency (FEPA). Guidelines and standard for industrial Effluents, Gaseous emissions and hazardous waste management in Nigeria.1991. Accessed at www.vertic.org/media/.../Nigeria/NG_Environment_Agency_Act.pdf. On 11/06/2012.

[2] United States Environmental Protection Agency. Guides to Environmental Pollution Prevention: Selected Hospital Wastes streams. 1990. USEPA/625/7-90/009.

[3] Olubukola BO. Comparative Analysis of Healthcare waste management practice in two General hospitals in Nigeria.2009. Accessed at http://www.eco-web.com/edi/index.htm on 06/06/2011 at 10:21pm.

[4] Tudor TL, Noonan CL, Jenkin LET. Healthcare waste management; A case study from the Cornwall NHS, UK Waste Manage 2005; 25: 606-615.

[5] Ferreira AP, Veiga MM. Hospital waste operational procedures: A Case study in Brazil. Waste manage 2003; 21:377-382.

[6] UN/GEF. Global healthcare waste project. Accessed at www.gefmedwaste.org/ on 23/10/2012.

[7] Park K. Park's textbook of preventive and social medicine. m/s Bnarside bhant publishers 2009; 20 ed (13), 659-661.

[8] Farzadika M, Moradi A, Mohammadi MS. Hospital waste management statusin Iran: A case study in the teaching hospitals of Iran university of medical sciences. Waste Manage Res 2009; 27:384-389.

[9] Lagos Waste Management Authority 2011. Accessed at www.lawma.gov.ng/ on 14/01/2012.

[10] WHO. Safe healthcare waste management: policy paper, 2002. Accessed at www.who.int/water_sanitation_health/medicalwaste/hcwmpolicy/en/ on 02/12/2011.

[11] Townend WK, Cheeseman CR. Guidelines for the evaluation and assessment of the sustainable use of resources and waste management at healthcare facilities. Waste Manage Res. 2005; 23: 398-408.

[12] Adegbita MA, Nwafor SO, Afon A, Abegunde AA, Bamise CT. Assessment of Dental Waste Management in a Nigerian tertiary hospital. Waste Manage. Res. 2010; 28:769-777.

[13] Shinee S, Gombojav E, Nishimura A, Hamgjima N, Ito K. Healthcare waste management in the Capital city of Mongolia. Waste management 2007; 12: 22.

[14] Ahmed R, Ahmed N. Healthcare waste Management in Karachi, Pakistan.2008. Waste Management, 2004;

20(4): 34-52.Accessed at http//:www.waste.nl on 16/06/2011.

[15] Ijaya KG, Kavita D, Vidya KB. A critical Analysis of healthcare waste management in developed and developing Countries: Case study from India and England.2007. Accessed at www.swif.ait.ac.th/intlconf/ on 16/06/2011.

[16] Abah SO, Ohimain EI. Health care waste management in Nigeria: A case study. JPHE 2011; 3(3). 99-110.

[17] Sudhaka V, Chandrashekar J. Dental Healthcare Waste disposal among private Dental Practices in Bangalore City, India. Waste manage 2008; Accessed at www.ncbi.nlm.nih.gov/pubmed/18356854 on 17/06/2011.

[18] Bassey BE, Benka-Coker, MO, Aluyi SA. Characterization and management of solid Medical Waste in the Federal Capital Territory, Abuja Nigeria 2006. Accessed at www.ncbi.nlm.nih.gov/pmc/articles/pmc1831969 on 17/06/2011.

[19] Longe EO, Williams A. A preliminary study of medical waste management in Lagos metropolis, Nigeria. Iran.J. Environ. Health. Sci. Eng., 2006; 3(2):133-139.

[20] Mokuolu O. Improving the management of solid hospital waste in a Nigerian tertiary hospital. Free online files, September 2009. Available at http://www.thefreelibrary.com/health+general+community/2009/September/25- p5384

[21] Kane A, Lloyd J, Zaffran M, Simonsen L, Kane M. Transmission of hepatitis B, hepatitis C and human immunodeficiency viruses through unsafe injections in the developing world: model-based regional estimates. Bull needle stick accidents. World Health Org 1999; 41: 151-4.

[22] Cocchiarella L, Scott D, Deitchman SD, Young DC. Biohazardous waste management: What a the physician needs to know. Report of the council on scientific affairs. Arch Fam Med. 2000; 9: 26-9.

[23] Askarian M, Vakili M, Kabir G. Results of a hospital waste survey in private hospitals in Fars province, Iran. Waste Management, 2004; 24(4): 347-52.

[24] Askarian M, Vakili M, Kabir G. Hospital waste management status in university hospitals of the Fars province, Iran. Int. J. Environ. Health Res, 2004; 14(4): 295-305.

[25] Ngwuluka N, Ochekpe N, Odumosu P, John SA. Waste management in health care establishment within Jos metropolis, Nigeria. Afr J of Env Sc and Tech 2009; 3(12): 459-465.

[26] Ogbonna DN, Chindah A, Ubani N. Waste management options for healthcare wastes in Nigeria: A case study of Port Harcourt hospitals. Journal of Public Health Epidemiology 2012; 4(6): 156-169.

[27] Rutala WA, Odette RL, Samsa GP. Management of infectious wastes by US hospitals. JAMA, 1989; 262: 1635-40.

[28] Ramokate T, Basu D. Health care waste management at academic hospital: knowledge and practice of doctors and nurses. SAMJ, 2009; 99(6): 444-5.

[29] Chaudhry MA, Hyat A, Qureshi SM, Najmi SAA. Health hazards of hospital waste to sanitary workers at combined military hospital, Rawalpindi. Pakistan armed forces medical journal, 2004; 2(11).

[30] Coker AO, Sikiru KA, Sridhar MKC, Sangodoyin AY. Characterization and management of hospital solid wastes in Nigeria: Sanitation and water for all, proc. of the 25th WEDC Conf, Addis Ababa Ethiopia. 1999; 331-4.

[31] Babatola JO. A study of hospital waste generation and management practices in Akure, Nigeria. African Research Review 2008; 2(3): 292-305.

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Nutritional and Bread-Making Quality of Wheat as Influenced by Mineral Fertilization in a Compost-Amended Regosol soil

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***Abstract*—** *This experiment was conducted to assess the effect of different levels of nitrogen (N), phosphorus (P) and potassium (K) fertilization on growth, grain yield, nutritional and bread-making quality of wheat in a compost-amended regosol soil. Wheat cultivar Minaminokaori was grown in containers containing a mixture of regosol and aerobic compost (2:1 v/v). This study comprised a non-fertilized control (F_1) and six levels of NPK fertilizers: F_2 (80 kg N + 40 kg P_2O_5 +40 kg K_2O ha^{-1}), F_3 (110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}), F_4 (140 kg N + 80 kg P_2O_5 + 70 kg K_2O ha^{-1}), F_5 (170 kg N + 100 kg P_2O_5 + 85 kg K_2O ha^{-1}), F_6 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}) and F_7 (230 kg N + 140 kg P_2O_5 + 115 K_2O kg ha^{-1}). A significant improvement in agronomic performance, grain nutritional and bread-making quality of wheat was observed with the F_6 treatment, it increased the grain yield (151.6%), crude protein (65.3%), water-soluble pentosan (40.5%), and dry gluten (4-fold) compared to the control. The F_6 treatment also increased grain total N, P, K, Mg, Ca, Zn and inorganic phosphorus contents by 65.2, 33.6, 8.9, 19.7, 165.9, 26.1 and 80.0%, respectively, compared to control. However, it slightly increased grain phytate P content. The results from this study suggest that agronomic performance, grain yield, nutritional and bread-making quality of wheat can be improved with an appropriate dose of NPK in a compost-amended regosol soil.*

Keywords*— *Crude protein, Gluten, NPK, Pentosan, Phytate P, Wheat.

I. INTRODUCTION

Wheat is an important cereal crop in the world and is primarily grown for its grain, which is consumed as human food. It contributes about 20 % of the total dietary calories and proteins worldwide (Shiferaw *et al*., 2013), and has gotten more attention for food security especially with fast growing populations in developing countries. Enhancing crop productivity and achieving food security is possible through a wise fertilizer application, particularly in regosol soil which is deficient in nutrient. NPK fertilization in balanced share at proper time has a great impact on wheat growth and yield (Malghani *et al*., 2010).

N is a key component of proteins, nucleic acids, enzymes, coenzymes, and chlorophyll and therefore contributes to the biochemical processes of the plant (Benin *et al*., 2012). N fertilization during anthesis has a positive effect on synthesis of high grain protein content than earlier application (Wuest and Cassman, 1992). An adequate level of N fertilization increases the number of fertile tillers (Wilhelm, 1998), number of spikes, number of grains per spike, and grains yield (Al-Abdulsalam, 1997). Application of P enhances seed maturity and development (Ziadi *et al*., 2008), and adequate supply of P can increase grain yield of wheat by 20 % (Ascher *et al*., 1994). More than 70 % of grain total P is stored as phytic acid in seeds (Rosa, 1999). K plays a significant role in plant biochemical functions such as activation of various enzymes, synthesis of protein and carbohydrates, enhancement of fat content, improving drought tolerance, and resistance to frost and lodging (Marschner, 1995). An optimum rate of K fertilization increases number of productive tillers, number of grain per spike, 1000 grain weight, grain yield, and protein content of wheat (Alam *et al*., 2009).

Phytic acid (phytate) is the main phosphorus storage form in most cereals, legumes and nuts (Lopez *et al*., 2002). It is considered as an anti-nutritional factor that complex with proteins and chelates with some nutritionally important micronutrients (Fe, Zn, Mg, and Ca) and resulting in a significant decrease in the bioavailability of these nutrients (Raboy, 2001). Pentosans are the major non-starch polysaccharides and is divided into water-soluble pentosan and water unextractable solids. Water-soluble pentosan has a positive effect on dough rheological characteristics and macaroni production processing (Menger, 1976). Pentosan added to the dough increased dough development time, water-binding capacity and viscosity of dough (Jelaca and Hlynka, 1971).

Determination of grain nutrients, pentosan, gluten and phytate P content of wheat under different soil fertility is important as they influence nutritional quality and bread-making characteristics of flour. Therefore, the present experiment was conducted to study the effects of different levels of NPK on yield, grain minerals, crude protein, pentosan, gluten and phytate P content of wheat in a compost-amended regosol soil.

II. MATERIALS AND METHODS

The experiment was conducted in a greenhouse of the Faculty of Applied Biological Sciences, Hiroshima University. Conditions in the greenhouse were 65% humidity, 20-25 ºC day/15-18 ºC night temperatures, and natural sunlight. Containers (1.5 m length, 30 cm width, and 18 cm in depth) were used, and filled with a mixture of regosol and aerobic compost (2:1). Chemical analysis of this mixture showed that it contained; 0.2 % total N, 6.8 mg kg^{-1} available P and 79.6 mg kg^{-1} available K. Soil pH (H_2O) was adjusted to 6.5 by adding 1 ton ha^{-1} of dolomitic calcium magnesium carbonate. This investigation comprised a control (F_1, non-fertilized) and six levels of NPK fertilizers: F_2 (80 kg N + 40 kg P_2O_5 +40 kg K_2O ha^{-1}), F_3 (110 kg N + 60 kg P_2O_5 + 55 kg K_2O ha^{-1}), F_4 (140 kg N + 80 kg P_2O_5 + 70 kg K_2O ha^{-1}), F_5 (170 kg N + 100 kg P_2O_5 + 85 kg K_2O ha^{-1}), F_6 (200 kg N + 120 kg P_2O_5 + 100 kg K_2O ha^{-1}) and T_7 (230 kg N + 140 kg P_2O_5 + 115 K_2O kg ha^{-1}). The source of NPK was urea, single super phosphate, and potassium sulfate, respectively. All P, K and half dose of N were applied before sowing, and the remaining N was applied in two equal splits at tillering and anthesis stages. Minaminokaori, a commonly grown wheat cultivar in Japan was sown in the third week of November, then, ten-day-old seedlings were transplanted into the containers with 10 cm distance, following a randomized complete block design with 4 replicates. During the experiment all agronomic management practices were performed uniformly as required.

2.1. Growth, Yield, and Yield component:

Twenty plants (5 from each replicates) were randomly taken and the following yield components were evaluated: Number of tillers per plant was obtained by counting all tillers produced in each plant before harvest. Number of spikes were counted in each plant by the time of harvest. Number of grains per spike was obtained by counting grains in 10 spikes which were randomly collected from each treatment. To measure 1000 grain weight, 500 grains were counted and weighted with a prescribed accuracy, and then the value was multiplied by 2. To measure grain yield, mature spikes were collected, oven dried at 80˚C for 48 hours, threshed, and the grain yield was recorded and expressed in kg per hectare. Crop growth rate (CGR) was calculated as plant's dry weight increase per unit of time (Nogueira *et al*., 1994).

$$CGR= (W_2 - W_1) / (T_2 - T_1)$$

Where: W_1 and W_2 = total dry weight of plant at first and second sampling; T_1 and T_2= time of first and second sampling.

2.2. Grain mineral content

Samples of mature seeds were ground finely with a vibrating sample mill (TI-100, Heiko, Japan) and grain minerals contents were measured. The grain powdered samples were digested by sulfuric acid and heating, then they were diluted with distilled water, and K content was measured using flame photometer (ANA 135, Tokyo Photoelectric, Tokyo, Japan). Total P was determined in the same digested samples by UV-Spectrophotometer (U-3310, Hitachi Co. Ltd. Tokyo, Japan), following the molybdenum reaction solution method suggested by Chen *et al*. (1956). Grain Ca, Mg and Zn concentration were measured by an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu, Japan). Total N was measured using the Kjeldahl method after digestion with concentrated H_2SO_4 and H_2O_2 (10:5, v/v). Grain inorganic Phosphorus (Pi) was extracted in trichloroacetic acid (12.5%) + $MgCl_2$ (2 mmol /l) while stirring overnight, and Pi was measured colorimetrically, using a spectrophotometer following the molybdenum reaction regent method (Raboy and Dickinson, 1984).

2.3. Determination of grain starch and crude protein

To measure grain starch content, ethanol (80%) was added to the powdered samples to remove sugars and then starch was extracted with perchloric acid. Anthrone reagent was added to the test tubes containing extracted samples and then heated in a boiling water bath for 7.5 minutes. The absorbance of the extract was measured at 630 nm (Nag, 2016). To determine grain crude protein, the observed total N content from the Kjeldahl method was multiplied by 5.47 (Fujihara *et al*., 2008).

2.4. Determination of total pentosan and water-soluble pentosan

Total pentosan was measured using the orcinol-hydrochloric acid method, where finely ground samples were hydrolyzed with 2 N hydrochloric acid in a boiling water bath for 2.5 hours, and then centrifuged. A specific quantity of supernatants was transferred to the new test tubes and reaction regents ($FeCl_3$ and orcinol) were added and vortexed. The tubes were heated in boiling water for 30 minutes, cooled, and the absorbance was measured using a spectrophotometer. Grain water-soluble pentosan was extracted by hydrolyzing powdered samples in distilled water with shaking for 2 hours at 30 °C. Then, 4 N hydrochloric acid was added to the aliquots of the supernatant and placed in boiling water for 2 hours, and allowed to cool, and grain water-soluble pentosan content was measured by a spectrophotometer, using $FeCl_3$-orcinol reagents (Hashimoto *et al*., 1986).

2.5 Determination of gluten and phytate P content

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Gluten content was measured according to (AACC) international approved method by hand washing with 30 minutes resting time, and the result was expressed as dry gluten percentage. Grain phytate P was measured according to the method suggested by Raboy and Dickinson (1984), where aliquots of flour were extracted in extraction media (0.2 M HCl: 10 % Na_2SO_4) overnight at 4 °C with shaking. Extracts were centrifuged and phytate was obtained as a ferric precipitate and assayed for P colorimetrically using ammonium molybdate reaction reagent.

2.6 Statistical analysis

All the collected data were subjected to analysis of variance using SPSS statistics package, Student Version 19, and means (n = 4) were separated using the Duncan Multiple Range Test at p = 0.05.

III. RESULT AND DISCUSSION

3.1. Agronomic performance and yield

Crop growth rate (CGR) was significantly affected by different levels of NPK fertilization (Figure 1). There was a linear increase in CGR with an increase in NPK levels but further increase in NPK (F_7) did not enhance CGR significantly compared to F_5. This result is in agreement with Laghari *et al*. (2010) and Asghar *et al*. (2010) who found that further increase in NPK levels had a non-significant response. The result of this study indicated that NPK fertilization significantly increased the number of tillers per plant. The highest number of tillers was observed in plants where a high NPK (F_7) was applied (Table 1). There was a progressive increase in the number of tillers with increased levels of NPK. Similar findings were reported by Kausar *et al*. (1993) and Niamatullah *et al*. (2011) indicating that high level of NPK significantly increased the number of tillers in wheat. The mean number of spike per plant was ranged from 5.5 to 13.35. NPK fertilization enhanced the number of spike per plant and the highest number of spike per plant was observed with F_6 treatment (Table 1). The high rate of NPK (F_7) did not increase the number of spikes per plant because of prolonged vegetative growth which resulted in the production of more number of infertile tillers. These findings are in agreement with Hussain *et al*. (2002) who reported a decrease in the number of fertile tillers due to the application of a high rate of NPK. Similarly, Ali and Yasin (1991) found that high dose of N and P reduced the number of the spike in wheat. The maximum number of grains per spike was obtained with F_7 treatment. However, the number of grains produced by F_6, F_5, F_4, and F_3 treatments were statistically similar (Table 1). The reason for high number of grains per spike in F_7 might be due to higher rate of N and P which enhanced seed set in the spike (Hussain *et al*., 2002; Alam, *et al*., 2007; Malghani *et al*., 2010). The 1000 grain weight as an important yield contributing parameter was higher in plants which were supplied with a high rate of NPK. F_7 and F_6 treatments recorded the higher value for 1000 grain weight compared to control (Table 1). The moderate fertilizer levels (F_5, F_4, F_3, and F_2) produced statistically similar 1000 grain weight. Significant effects of NPK fertilization on 1000 grain weight of cereals were also reported by Maqsood *et al*. (2001) and Asghar *et al*. (2010).

Grain yield was significantly affected by different levels of NPK fertilization. Application of F_6 and F_7 resulted in increased number of fertile tillers, number of grains per spike and maximum 1000 grain weight which eventually contributed to the production of a higher grain yield. F6 treatment resulted in 151.64 % increase in grain yield over control. There was a slight decrease in grain yield of plants with F_7 treatment, compared to F_6 that might be due to the N interaction with P and K (MacLeod, 1969). Niamatullah *et al.* (2011), Khursheed and Mahammad (2015), and Abdul-Aziz *et al*. (2016) also concluded that grain yield of wheat and cereal crops can be increased with application of N, P, and K fertilizers.

3.2. Grain mineral content

Grain minerals contents were significantly influenced by NPK fertilization. Plants with F_6 treatment recorded higher grain total N, P, K, Pi, Mg, and Zn content. It was observed that further increase in NPK dose (F_7) only increased Ca content but the contents of N, P, K, Pi and Zn were slightly reduced in F_7 treated plants (Table 2). Gain Zn content was enhanced with application of high and moderate levels of NPK fertilization. In general, F_6 treatment increased grain total N, P, K, Mg Ca, Zn and Pi contents by 65.2, 33.6, 8.9, 19.7, 165.9, 26.13 and 80%, respectively, compared to control. Laghari *et al*. (2010) also found that NPK fertilization increased mineral uptake particularly, N, K and P in wheat. Application of high N fertilizer enhanced K accumulation in wheat grains (Sheoran *et al*., 2015). Saha *et al*. (2014) found that application of P fertilizer enhanced P content in wheat grains. While, application of very high dose of N did not enhance grain total P content of wheat (Akhtar *et al*. (2011). In this study the decrease in grain mineral content with F_7 treatment might be due to prolonged vegetative growth and excessive biomass production that reduced grain mineral content in wheat grains.

Table 1. Effect of NPK fertilization on number of tillers plant-1, number of spikes plant-1, number of grains spike^{-1}, 1000 grain weight, and grain yield of wheat. The same letter indicates no significant difference ($p \leq 0.05$).

NPK levels	Number of tillers	Number of spikes	Number of grains	1000 grain	Grain yield
	(plant^{-1})	(plant^{-1})	(spike^{-1})	(g)	(ton ha^{-1})
F_1	5.55^{e}	5.45^{d}	36.85^{b}	42.03^{c}	2.77^{c}
F_2	13.96^{d}	9.60^{c}	37.15^{b}	45.82^{ab}	4.52^{bc}
F_3	14.10^{d}	9.67^{c}	39.30^{ab}	46.29^{ab}	5.03^{b}
F_4	18.30^{c}	10.80^{bc}	40.35^{ab}	46.33^{ab}	5.22^{b}
F_5	19.40^{bc}	11.30^{bc}	40.41^{ab}	46.91^{ab}	5.53^{b}
F_6	21.30^{ab}	13.35^{a}	41.00^{ab}	47.57^{a}	6.97^{a}
F_7	23.40^{a}	12.20^{ab}	43.10^{a}	47.90^{a}	6.9^{a}

Table 2. Effect of NPK fertilization on grain mineral content in wheat grain. The same letter indicates no significant difference ($p \leq 0.05$).

NPK levels	N	P	K	Pi	Mg	Ca	Zn
	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g $^{-1}$)	(mg g $^{-1}$)	(µg g^{-1})	(µg g^{-1})
F_1	16.01^{d}	3.42^{c}	4.72^{b}	0.247^{c}	1.32^{e}	71.04^{c}	74.66^{b}
F_2	18.07^{cd}	4.01^{b}	4.68^{ab}	0.349^{b}	1.35^{de}	74.61^{c}	81.47^{ab}
F_3	21.10^{bc}	4.11^{b}	4.89^{ab}	0.369^{b}	1.43^{cd}	107.89^{bc}	87.97^{a}
F_4	21.40^{bc}	4.25^{ab}	4.92^{ab}	0.383^{ab}	1.48^{bc}	147.98^{ab}	88.87^{a}
F_5	22.93^{ab}	4.31^{ab}	4.98^{ab}	0.396^{ab}	1.52^{abc}	150.43^{ab}	90.99^{a}
F_6	26.45^{a}	4.57^{a}	5.14a	0.445^{a}	1.58^{a}	188.95^{a}	94.17^{a}
F_7	24.95^{ab}	4.35^{ab}	5.00^{ab}	0.407^{ab}	1.54^{ab}	197.14^{a}	92.29^{a}

3.3. Grain starch and crud protein content

Analysis of data showed that NPK fertilization did not affect grain starch content significantly. Control (F_1) recorded a higher value for grain starch, while with increase in NPK level grain starch content was slightly decreased. Kindred *et al.* (2008) observed that application of N fertilizer decreased the starch content of wheat grain. There is a negative relationship between crude protein and starch as the grain yield and crude protein increases with higher rate of fertilizers, the starch content decreases (Hlisnikovsky and Kunzova, 2014). A decrease in grain starch content due to fertilizer application was also reported by Crista *et al*. (2012) indicating that starch content was higher in plants with no fertilizer application. The highest grain crude protein (%) was observed with F_6 treated plants followed by F_7 and F_5.

However, the lowest crude protein was recorded in F_1 where no fertilizer was applied. There was a linear increase in crude protein with increase in NPK level up to F_6, but further increase in NPK rate (F_7) slightly reduced crude protein content of wheat grain (Table 3). F_6 treatment increased grain crude protein by 65.3% compared to control. Application of excessive NPK fertilizers reduced grain crude protein content. This result agrees with Sameen *et al.* (2002) who found a reduction in crude protein content of wheat cultivar V-94091 and V-94105 due to the highest rate of NPK fertilizers. Similarly, Crista *et al*. (2012) found that NPK fertilization enhanced the synthesis of the raw protein in wheat. N fertilizer plays a significant role in total N and crude protein accumulation in wheat grains, while application of high dose of N beyond the optimum level may have a negative effect on grain crude protein (Abedi *et al*., 2011).

3.4. Grain total and water-soluble pentosan content

Grain total pentosan content was decreased with increase in NPK level the highest value of total pentosan recorded with F_1 treatment where no fertilizer was applied. Plants supplied with different levels of NPK fertilizers, recorded statistically similar total pentosan content. The effect of NPK fertilization on the grain total pentosan and water-soluble pentosan contents of wheat has not been reported sufficiently by earlier researchers. The major proportion of total pentosan is water-unextractable pentosan which forms physical barriers for the gluten network during dough development. Unlike to total pentosan, grain water-soluble pentosan content was enhanced with application of NPK, and F_6 treatment recorded the highest value of grain water-soluble pentosan content. The F_6 treatment increased grain water-soluble pentosan content by 40.5% compared to control. Water-soluble pentosan plays a key role in bread-making quality of dough. It increases the viscosity and the stability of dough foam structure, which helps in a bigger loaf volume and a finer homogeneous bread crumb (Courtin and Delcour, 2002).

3.5. Grain dry gluten content

Gluten is responsible for the unique elasticity and stickiness of wheat dough, the properties that make it so useful in bread-making. In this study, grain dry gluten content was increased with increase in NPK level, and among the various levels of NPK F_6 and F_7 treatments recorded a higher dry gluten content. The F_6 treatment increased grain dry gluten by 4-fold compared to control. A slight decrease was observed with application of the highest NPK level (F_7) that might be because of decrease in protein content due to excess NPK application, as explained earlier in case of crude protein content. Mineral fertilization increased the gluten content of wheat compared to a control, but various levels of P and K did not affect grain gluten significantly (Gaj *et al*., 2013).

3.6. Grain phytate P content

Analysis of variance showed that F_6 and F_7 treatments recorded a higher grain phytate P content compared to F_1 (Table 3). Phytate is the major storage form of phosphorous in cereals, therefore the content of phytate P mostly depends on total grain P. Phytate P can contribute to the nutritional deficiencies when seeds are used as food (Rosa 1999; Raboy 2001). It binds with proteins and important minerals such as Fe, Ca and Zn, and reduces their availability (Raboy, 2001). There was no sufficient review on the effect of NPK fertilization on phytate P content of wheat grain. The increase in grain phytate P content with a higher dose of NPK fertilization might be associated with grain total P content which is enhanced by a higher rate of P fertilizer.

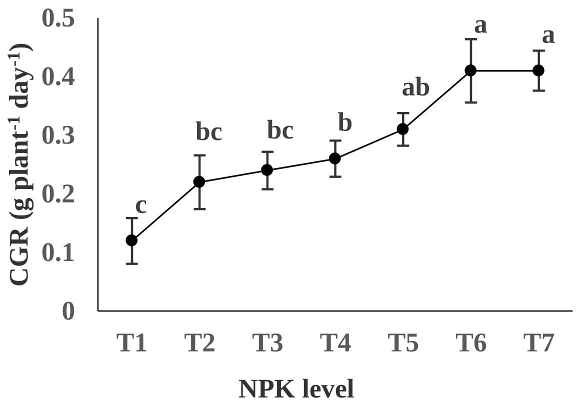

Fig. 1: Effect of NPK fertilization on crop growth rate (CGR). The same letter indicates no significant difference ($p \leq 0.05$).

Table 3. Effect of NPK fertilization on grain starch, crude protein, total pentosan, water-soluble pentosan and phytate P content of wheat. The same letter indicates no significant difference ($p \leq 0.05$).

Treatment	Starch	Crude protein	Total pentosan	Water-soluble pentosan	Dry gluten	Phytate P
	(%)	(%)	(mg g^{-1})	(mg g^{-1})	(%)	(mg g^{-1})
F_1	65.08^a	8.87^d	8.58^a	1.11^b	3.7^c	3.14^c
F_2	64.23^a	10.00^{cd}	7.78^{ab}	1.23^b	6.7^{bc}	3.26^{bc}
T_3	62.98^a	11.69^{bc}	7.75^{ab}	1.34^{ab}	8.5^b	3.52^b
F_4	62.93^a	11.85^{bc}	7.00^b	1.41^{ab}	12.3^b	3.64^{ab}
F_5	63.00^a	12.72^{ab}	6.94^b	1.49^a	16.5^{ab}	3.75^{ab}
T_6	63.02^a	14.66^a	7.24^b	1.56^a	18.9^a	3.86^a
F_7	63.00^a	13.82^{ab}	6.93^b	1.51^a	18.7^a	3.82^a

IV. CONCLUSION

The present results showed that different levels of NPK fertilization significantly influenced growth, yield, and grain nutritional and bread-making quality of wheat in a compost-amended regosol soil. F_6 treatment resulted in the production of higher grain yield, increased grain minerals contents, crude protein, water-soluble pentosan, and dry gluten content. F_6 treatment reduced the total pentosan, but did not significantly affect the level of grain starch content. By increasing the rate of NPK fertilization beyond F_6 treatment, only vegetative growth was enhanced. Therefore, F_6 treatment might have been an appropriate and economical rate of NPK to obtain maximum grain yield and improve grain nutritional and bread-making quality of wheat cultivar Minaminokaori in a compost-amended regosol soil.

REFERENCES

[1] Abdel-Aziz, Heba M. M.; Hasaneen, Mohammed N. A.; Omer, Aya M. (2016). Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil. Span. J. Agric. Res., doi:http://dx.doi.org/10.5424/sjar/2016141-8205.

[2] Abedi, T., A. Alemzadeh, Kazemeini S. A. (2011). Wheat yield and grain protein response to Nitrogen amount and timing. Aust. J. Crop Sci., 5: 330-336.

[3] Akhtar, M., A. Naeem, J. Akhter, S. A. Bokhari, Ishaque W. (2011). Improvement in nutrient uptake and yield of wheat by combined use of urea and compost. Soil Environ, 30: 45-49.

[4] Al–Abdul Salam, M. A. (1997). Influence of nitrogen fertilization rates and residual effect of organic manure rates on the growth and yield of wheat. Arab Gulf J. Sci. Res., 15: 647–60.

[5] Alam, M. R., M. Akkas Ali, M.S.H. Molla, M. A. Momin, Mannan M. A. (2009). Evaluation of different levels of K on yield and protein content of wheat in the high Ganges river floodplain soil. Bangladesh J. Agril. Res. 34: 97-104.

[6] Alam, M. Z., S. A. Haider, Paul N. K. (2007). Yield and yield components of Barley (Hordium vulgare L.) cultivars in relation to N fertilizer. J. Applied Sci. Res., 3: 1022-1026.

[7] Alam, S. M., Azam S., Ali S., Iqbal M., (2003). Wheat yield and P fertilizer efficiency as influenced by rate and integrated use of chemical and organic fertilizers. Pak. J. Soil Sci., 22: 72-76.

[8] Ali, R. and Yasin M. (1991). Response of wheat to N and phosphorus fertilization. Pakistan J. Agri. Res., 12: 130–3.

[9] Ascher, J.S., R.D. Graham, D.E. Elliott, J.M. Scott, Jessop R. S. (1994). Agronomic value of seed with high nutrient content. In D.A Saunders and G.P. Hettel, eds. Wheat in heat-stressed environments: irrigated, dry area and rice-wheat farming systems. CIMMYT, Mexico.

[10] Asghar, A. A. Ali, W. H. Syed, M. Asif, T. Khaliq, Abid A. (2010). Growth and yield of maize (*Zea mays* L.) cultivars affected by NPK application in different proportion. Pak. J. Sci., 62: 211-216.

[11] Benin, G., Elesandro Bornhofen, Eduardo Beche, Eduardo Stefani Pagliosa, Cristiano Lemes da Silva, Pinnow C. (2102). Agronomic performance of wheat cultivars in response to nitrogen fertilization levels. Acta. Sci., Agron., 34: 275-283.

[12] Chen, P.S., T.Y. Toribara, Warner H. (1956). Microdetermination of phosphorus. Anal Chem., 28: 1756–1756.

[13] Courtin, C. M., Delcour, J. A. (2002) Arabinoxylans and endoxylanases in wheat flour bread-making. *J. Cereal Sci*., 35: 225-243.

[14] Crista, F., Isidora Radulovi, Florin Sala, Laura Crista, Berbecea A. (2012). Influence of NPK fertilizer upon winter wheat grain quality. Res. J. Agric. Sci., 44: 30-35.

[15] Fujihara, S., H. Sasaki, Aoyagi Y., Sugahara T. (2008). Nitrogen-to-protein conversion factors for some cereal products in Japan. J Food Sci., 73: 204-209.

[16] Garg, B. K., Burman U., Kathju S. (2004). The influence of phosphorus nutrition on the physiological response of mothbean genotypes to drought. J. Plant Nutr. Soil Sci., 167: 503–508.

[17] Hashimoto, S., Shogren M. D., Pomeranz Y. (1986). Cereal pentosans: Their estimation and significance. I. Pentosans in wheat and milled wheat products. Cereal chem., 64: 30-34.

[18] Hlisnikovsky, L., Kunzova E. (2014). Effect of mineral and organic fertilizers on yield and technological parameters of winter wheat (Triticum aestivum L.) on Illimerized Luvisol. Polish J. Agron., 17: 18–24

[19] Hussain, M. I., Shamshad H. Shah, Sajad Hussain, Iqbal K. (2002). Growth, Yield and Quality Response of Three Wheat (Triticum aestivum L.) Varieties to Different Levels of N, P and K. Int. J. Agri. Biol., 4: 361-364.

[20] Jelaca, S. L. and Hlynka I. (1971). Water-binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. Cereal Chem., 48: 211-222.

[21] Kausar, K., Muhammad Akbar, E. Rashad, Abid Nisar A. (1994). Physiological response of nitrogen, phosphorus and potassium on growth and yield of wheat. Pak. J. Agric. Res., 14:126-130.

[22] Khursheed, M. Q., and Mahammad M. Q. (2015). Effect of Different Nitrogen Fertilizers on Growth and Yield of Wheat. Zanco J. Pure of Applied Sci., 27: 19-28

[23] Kindred, D. R., Tamara M. V., Richard W. M., Stuart S. J., Reginald C. A., James M. Brosnan, Sylvester-Bradleya R. (2008). Effects of variety and fertilizer nitrogen on alcohol yield, grain yield, starch and protein content, and protein composition of winter wheat. J. Cereal Sci., 48: 46–57.

[24] Laghari, G. M., F. C. Oad, S. D Tunio, A. W. Gandahi, M. H. Siddiqui, A. W. Jagirani, Oad S. M. (2010). Growth yield and nutrient uptake of various wheat cultivars under different fertilizer regimes. Sarhad J. Agric. 26: 489-497.

[25] Lopez, H. W., Leenhardt F., Coudray C., Remesy C. (2002). Minerals and phytic acid interactions: is it a real problem for human nutrition? Int J Food Sci Technol 37: 727–739.

[26] MacLeod, L. B. (1969). Effects of N, P, and K and Their Interactions on the Yield and Kernel Weight of Barley in Hydroponic Culture1. Agron. J., 61:26-29.

[27] Malghani, A. L., Asmat Ullah Malik, A. Sattarb, Fiaz Hussaina, G. Abbasc and J. Hussain, (2010). Response of growth and yield of wheat to NPK fertilizer. Sci. Int. (Lahore), 24:185-189

[28] Maqsood, M., Abid A. M., Iqbal A., Hussain M. I. (2001). Effect of variable rate of nitrogen and phosphorus on growth and yield of maize (golden). Online J. Biol. Sci. 1:19-20.

[29] Marschner, H. (1995). Mineral nutrition of higher plants, PP: 148-73. Academic press inc., San Diego. USA.

[30] Menger, A., (1976). Effect of raw materials and processing conditions on pasta quality. English Abs. From Getreide, -Mehl-und-Brot, 30: 149-155.

[31] Nag, A. (2016) Techniques in agricultural, Environmental and Food Engineering, 3rd ed. PHI learning private limited, Delhi- 110092, pp 39-41.

[32] Niamatullah, M., M. Khan, M. Q. Khan, M. Sadiq, K. U. Zaman, C.S. Hayat and S. Rehman, (2011). Impact of NPK application on the number of productive tillers and cost benefit analysis of wheat in Hill–Torrent irrigated area of D. I. Khan division, Khyber Pakhtoon Khwa. J. Anim. Plant Sci., 21: 211-214

[33] Nogueira, S. S. S., V. Nagai; N.R. Braga; M. Do C.S.S. Novo, Camargo M. B. P. (1994). Growth analysis of chickpea (Cicer arietinum L.). Sci. agric., Piracicaba, 51:430-435,

[34] Raboy, V. and D. B. Dickinson (1984). Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. Plant Physiol., 75: 1094-1098.

[35] Raboy, V., (2001). Seeds for a better future: 'Low phytate' grains help to overcome malnutrition and reduce pollution. Trends in Plant Science 6: 458–462.

[36] Rosa M., G. Estepa, E. G. Hernandez and B. G. Villanova, 1999. Phytic acid content in milled cereal products and breads. Food Res. Int., 32, 217-221.

[37] Saha, S., Bholanath Saha, Sidhu Murmu, Sajal Pati, Partha Deb R. (2014). Grain yield and phosphorus uptake by wheat as influenced by long-term phosphorus fertilization. Afr. J. Agric. Res., 9: 607-612.

[38] Sameen, A., Abid Niaz, Anjum, F. M. (2002). Chemical Composition of Three Wheat (Triticum aestivum L.) Varieties as Affected by NPK Doses. Int. J. Agri. Biol., 4: 537-539.

[39] Sheoran, H. S., B. S. Duhan, K. S. Grewal, Sheoran S. (2015). Grain yield and NPK uptake of wheat (Triticum aestivum L.) as influenced by nitrogen, vermicompost and herbicide (Clodinafop propargyl). Afr. J. Agric. Res., 10: 3952-3961.

[40] Shiferaw, B., M. Smale H. J. Braun, E. Duveiller, M. Reynolds, Muricho G. (2013). Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Sec., 5:291–317.

[41] Wilhelm, W.W. (1998). Dry matter partitioning and leaf area of winter wheat grown in a long term fallow tillage comparison in U.S. Central Great Plans. Soil and Tillage Res., 49: 49–56.

[42] Wuest, S. B., Cassman K. G. (1992) Fertilizer-nitrogen use efficiency of irrigated wheat: II. Portioning efficiency of preplant versus late-season application. Agron. J., 84: 689-694.

[43] Ziadi, N., G. Belanger, A.N. Cambouris, N. Tremblay, M.C. Nolin and Claessens A. (2008). Relationship between phosphorus and nitrogen contents in spring wheat. Agron. J. 100: 80-86.

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Performance of Some Soybean Genotypes (Glycine max L.) to Germination and Seedling Characters as Affected by Planting Dates and Phosphorus Fertilization

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***Abstract*— *To investigate the performance of some sunflower genotypes to phosphorus fertilizer rates and planting dates to germination characters and seedling parameters. A laboratory experiment accompanied in seed lab during April and May 2017.The experiments included six sowing dates at 1th May, 15th May and 31th May, three soybean cultivars namely Crawford, Giza 22 and Giza 111 and three rates of phosphorus fertilizer viz. 0, 37.2 and 74.4 kg P_2O_5/ha.The tallest shoot, great percentages of germination, the lowermostpercentagesof dead seedand the highestcoefficient of velocitypercentage from sown on mid-May. In addition, the lowest days of germination time was produced from sown on first May. Whereas, the tallest root, the highest weight of fresh shoot and root as well as shoot dry weight from sown on end-May.The results clearly revealed that the highest percentage of germination, soot length and root length obtained from sown cv. Giza 111. The uppermostenergy of germination, shoot and root dry weight were recorded from sown Giza 22 cultivar. In addition, sown Crawford cultivar produced the highestdead seedpercentage and the lowest mean germination time.The results indicated that the lowest mean germination time and maximum percentage of coefficient of velocity, tallest shoot and root, weight of fresh shoot, shoot and dry root were obtained fromfertilizationof phosphate at the rate of31 kgP_2O_5/fed. It summarized that seed Giza 111 cultivar recorded the best in seed viability when sown on first May and fertilized with phosphorus fertilizer at the rate of 74.4 kg P_2O_5/ha.*

Keywords*— *Soybean cultivars, planting dates, phosphorus fertilization rates, seed germination characters and seedling parameters.

I. INTRODUCTION

Soybean (Glycine max (L.) Merrl) is considered the most important oil crop in Egypt. To overcome the shortage of edible oil in Egypt, it could be achieved sown soybean at proper agronomic management, such as, sowing times of different soybean cultivars and phosphorus fertilizer rates and their effect on seed quality. The increase of seed production may attributed to agriculture management that could potentially improve seed viability.The JS-335 variety recorded the highest increase in germination percentage and seedling vigour index. Germination percentage, seedling vigour index and seedling dry weight decreased progressively as sowing delayed **(Vidyapeeth, 2002**). Sown at different dates affect seed productivity andcreatinga goodseed quality **(Rahman et al., 2005).** Sowing date is an important factor regulating soybean seed quality. G-2 cultivar had higher percentages of germination and seed vigour than cultivar PB-1 or BS-5 genotypes. The highest germination percentage and good vigour produced from all the cultivars on November and December sown.Whilst, sown in September increased germination percentage and seedling vigour during Kharif II. Sown during November to December in Rabi season and August to September in Kharif-II season produced the highest germination percentage**(Rahman et al., 2013).**The Giza 21 varietytopped other varieties in the seedling vigor index; however, Giza 35 varietyproduced the latest germination time **(Kandil et al., 2015).**

A significant variation in the percentage of germination and vigor index due to different sowing dates. Sown on 2nd December maximized the percentage of germination and vigor index that recordeda good quality soybean seed (**Kundu et al., 2016)**.Fertilization with K, Zn, and P enhanced seed viability and seedling vigor (**Sawan et al., 2011).**Extremein P and K fertilization rates can result in lower seed viability and vigor **(Krueger et al., 2013).**Therefore, the goal of this research is aimed to investigate soybean seed cultivars viability as influenced by sowing date and phosphorus fertilization rates.

II. MATERIALS AND METHODS

2.1. Research time and site:

The laboratory experiment intended to study the effect of three sowing dates at 1th May, 15th May and 31th Mayfor three soybean cultivars (Crawford, Giza 22, Giza 111) under and three phosphorus fertilizer rates (0, 37.2 and 74.4 kg P_2O_5/ha)to germination and seedling parameters.Afactorial experiment in RCBD with four replicationwas used. The experiment includes three factors, the first three sowing date at 29th April, 14th May and 30th May The second factor included three soybean cultivarsi*i.e.*Crawford, Giza 22, Giza 111) from ARC, Ministry of agriculture, Egypt. The third cultivar includesthe three phosphorus fertilizer rates (0, 37.2 and 74.4 kg P_2O_5/ha. Twenty-five seeds of uniform size in each treatment for each cultivar allowed germinating on a filter paper in 9 cm diameter Petri dishes. Seeds were germinate in a germination chamber in 20-25ºC.Thus, the whole experiment comprised 108 Petri dishes. Each filter paper moistened with a distilled water according to **ISTA Rules, 2016**.

2.2. Studied Characters:

Soybean seed of both seasons subjected for determination of germination characters and seedling parameters in the laboratory experiment. Germination characterswere estimated as follows:

1- The final germination percentage was determined after 8 days from sowing as equation described by **(Ellis and Roberts, 1981& Ruan *et al*. 2002).**

$$\mathbf{FGP} = \frac{\text{Number of germinated seeds}}{\text{Total Number of seed tested}} \times 100$$

2-The Germination Index was calculated according to **Karim *et al*. (1992)** equation**.**

$$\mathbf{GI} = \frac{\text{\% Germination in each treatment}}{\text{\% Germination in the control}} \times 100$$

3. The energy of germination was recorded on the fourth day according to **Ruan *et al*., (2002)**equation**.**

$$\mathbf{EG} = \frac{\text{Number of germinated seeds after four days}}{\text{Number of seed tested}} \times 100$$

4-Average of coefficient of velocity (CV) was calculated using the following formula as described **by Scott et al., 1984:**

5-The mean germination time (MGT): It was determined according to the equation of **Ellis and Roberts (1981)**:

$$MGT = \sum dn \div \sum n$$

6-Percentage of dead seed = Number of dead seed /total number of seeds

7-Shoot length (cm)of five seedlings from the seed to the tip of the leaf blade was measured.

8-Root length (cm)of five seedlings from the seed to the tip of the root was measured.

9-Weight of the fresh shoot (g) of five seedling shoots was weighted.

10-Weight of fresh root (g)of five seedling roots was weighted.

11-Weight of dry shoot (mg)of five seedling shoots was weighted after oven dryingat 75 º C for 48 h.

12-Root dry weight (mg)of five seedling shoots was weighted after oven dryingat 75 º C for 48 h.

2.3. Experimental analysis:

Collected data, statistical analysis of variance technique using the MSTAT–C statistical package programmed as described by a procedure of Gomez and Gomez (1991). For comparisons between treatment means, the lest significant differences test (LSD) for 5 and 1 % level of probability was used according to Snedecor and Cochran (1980).

III. RESULTS AND DISCUSSIONS

3.1. Effect of sowing dates:

The outcomes that obtainable in Tables (1, 2 and 3) exposed that sown dates significantly affected germination anddead seedpercentage, mean germination time, andcoefficient of velocitypercentage, root length (cm), weight of shoot fresh (g), shoot dry weight (gm),root fresh weight (gm), except energy of germination percentage, shoot length (cm) androot dry weight (gm) insignificantly affected. The tallest shoot (highest percentage of germination (84.66 %), the lowest dead seedpercentage (15.33 %) and the highestcoefficient of velocitypercentage (30.07 5) from sown on mid-May. In addition, the lowest mean of germination time (2.2 day) was obtained from son early on first May. Whereas, the tallest root (6.21 cm), the highest weight of fresh shoot (0.72 g) and root (0.15 g) as well as shoot dry weight (0.18 g) from sown on end-May.Sown on 2nd December maximized the percentage of germination and vigor index that recorded a good quality soybean seed (**Kundu et al., 2016)**.

Table.1: Average of germination, energy of germination and dead seed percentages, mean germination time and coefficient of velocity percentage as influenced by sowing date, soybean cultivars and phosphate fertilization rates.

Characters / Treatment	Germination %	Energy of Germination %	Dead seed %	Mean germination time	Coefficient of velocity
A-Sowing date:					
1 May	67.81	37.48	32.00	2.20	29.19
15 May	84.66	40.63	15.33	2.74	30.07
31 May	84.55	49.14	15.44	2.93	28.22
F. test	*	N.S	*	*	*
LSD at 5%	6.01	---	5.96	0.03	0.88
B-Soybean Cultivars:					
Giza 111	81.22	41.33	18.77	2.64	29.65
Giza 22	78.33	43.25	21.48	2.62	29.10
Crawford	77.48	42.66	22.51	2.60	28.72
F. test	*	*	*	*	N.S
LSD at 5%	6.01	10.79	5.96	0.03	---
C-Phosphorus fertilization:					
0 kg P_2O_5/ha	77.66	42.55	22.14	2.65	28.03
37.2 kg P_2O_5/ha	82.88	44.25	17.11	2.67	28.95
74.4 kg P_2O_5/ha	76.48	40.44	23.51	2.54	30.50
F. test	NS	NS	NS	*	*
LSD at 0.05	---	---	---	0.02	1.43

3.2. Performance of soybean cultivars:

Average of germination and energy of germination percentages and dead seedpercentage, mean germination time, andcoefficient of velocitypercentage, shoot length (cm), root length (cm), shoot fresh weight (gm), shoot and root dry weight (gm) significantly affected by studied soybean cultivars,except, coefficient of velocitypercentage, root and shoot fresh weight (gm) insignificantly influenced. The results clearly revealed that the highest percentage of germination (81.22 %), soot length (6.52 cm) and root length (6.26 cm) was recorded from sown Giza 111 cultivar. The highest energy of germination (43.25 %), shoot (0.16 g) and root (0.2 g) dry weight were recorded from sown Giza 22 cultivar. In addition, sown Crawford cultivar produced the highestdead seedpercentage (22.51 %) and the lowest mean germination time (2.6 day). The JS-335 variety recorded the highest increase in germination percentage and seedling vigour index. Germination percentage, seedling vigour index and seedling dry weight decreased progressively as sowing delayed **(Vidyapeeth, 2002**). Sown at different dates affect seed productivity andproducing good quality seed **(Rahman et al., 2005).**The Giza 21 variety topped other varieties in the seedling vigor index; however, Giza 35 variety produced the latest germination time**(Kandil et al., 2015).**

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Table.2: Average of shoot and root length (cm), weight of freshshoot and root (g),weight of dryshoot and root (g)as influenced by sowing date, soybean cultivars and phosphate fertilization rates.

Characters / Treatment	Shoot length (cm)	Root length (cm)	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
A-Sowing date:						
1 May	5.83	6.19	0.53	0.11	0.14	0.01
15 May	6.56	6.06	0.66	0.12	0.14	0.01
31 May	6.97	6.21	0.72	0.15	0.18	0.02
F. test	N.S	*	*	*	*	N.S
LSD at 5%	--	1.18	0.04	0.03	0.01	---
B-Soybean Cultivars:						
Giza 111	6.52	6.26	0.61	0.12	0.15	0.01
Giza 22	6.33	6.10	0.65	0.13	0.16	0.02
Crawford	6.05	6.09	0.64	0.12	0.15	0.01
F. test	*	*	N.S	N.S	*	*
LSD at 5%	0.09	1.18	---	--	0.01	0.01
C-Phosphorus fertilization:						
0 kg P_2O_5/ha	6.32	6.01	0.60	0.13	0.13	0.01
37.2 kg P_2O_5/ha	6.46	6.16	0.64	0.12	0.16	0.01
74.4 kg P_2O_5/ha	6.63	6.28	0.67	0.13	0.17	0.03
F. test	*	*	*	N.S	*	*
LSD at 0.05	0.09	1.32	0.03	---	0.01	0.01

3.3. Effect of phosphorus fertilizer rates:

Average of mean germination time andcoefficient of velocitypercentage**,** shoot length (cm), root length (cm), shoot fresh weight (gm),shoot and root dry weight (gm) significantly affected due to different phosphate fertilization rates, except, germination and energy of germination and dead seedpercentage and root fresh weight (gm) insignificantly affected as shown in Tables (1 and 2). The results indicated that the lowest mean germination time (2.54 day)and maximum percentage of coefficient of velocity(30.5 %), tallest shoot (6.63 cm) and root (6.28 cm), shoot fresh weight (0.67 g), shoot (0.17 g) and root (0.03 g) were obtained fromfertilizationof phosphate at a rate of74.4 kg P_2O_5/ha.Fertilization with K, Zn, and P enhanced seed viability and seedling vigor (**Sawan et al., 2011).**Extreme in P and K fertilization rates can result in lower seed viability and vigor**(Krueger et al., 2013).**

3.4. Interaction Effects:

3.4.1. Interaction between sowing dates and soybean cultivars:

Means ofmean germination time,coefficient of velocitypercentage,shoot length (cm), weight of fresh shoot and root (g) significantly influenced by the interactiveamong sowing dates and soybean cultivars, and insignificantly influenced germination, energy of germination and dead seedpercentages, root length (cm), weight of dry shoot and root (g). The results graphically illustrated in Figs. 1, 2, 3, 4 and 5clearly showed thatthe lowest mean germination time (3.11 day), the highest percentageof coefficient of velocity (30.7 %), the tallest shoot (7.7 cm)the highest fresh weight of shoot (0.79 g)and root (0.18 g). However, the lowest values were obtained from sown Giza 22 cultivar on end May. The JS-335 variety recorded the highest increase in germination percentage and seedling vigour index. Germination percentage, seedling vigour index and seedling dry weight decreased progressively as sowing delayed **(Vidyapeeth, 2002**).

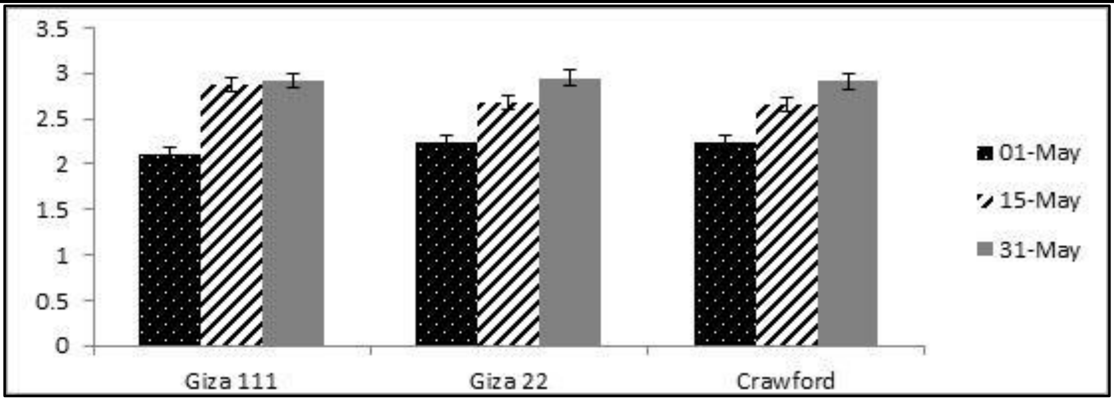

Fig.1: Average of main germination time as influenced by the interactive among soybean cultivars and sowing date.

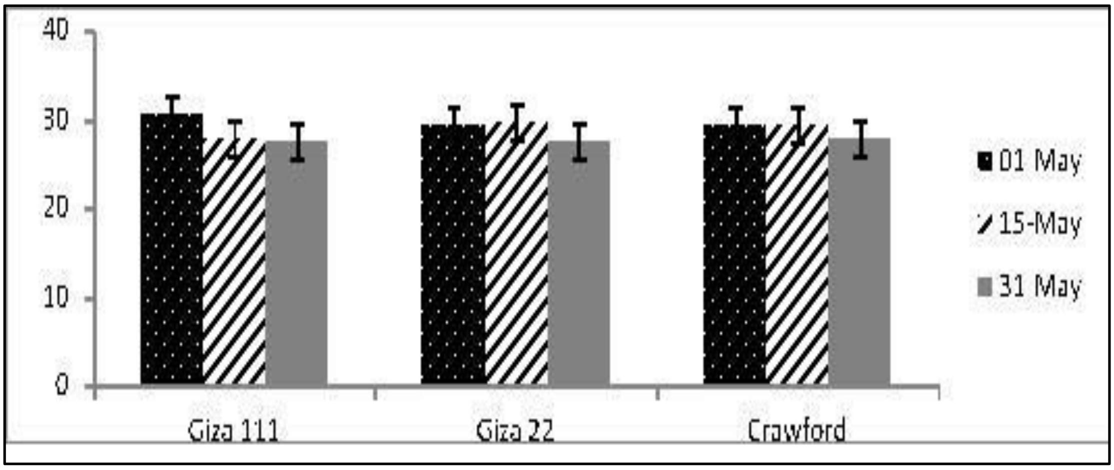

Fig.2: Average of coefficient of velocity as affected by interaction between soybean cultivars and sowing date.

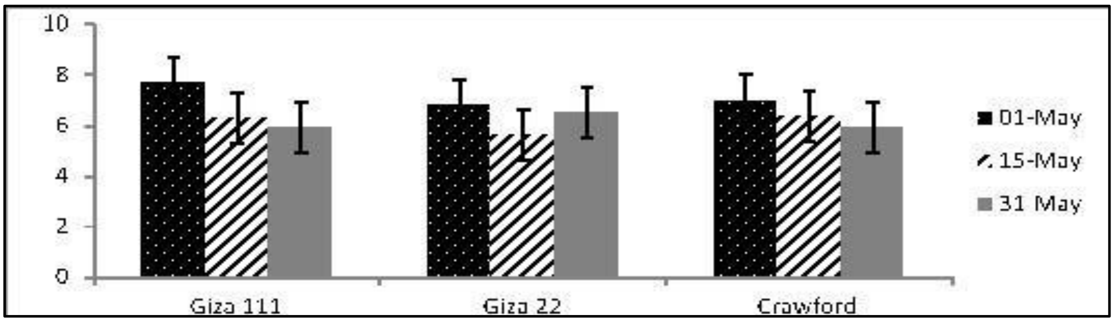

Fig.3: Average of shoot length (cm) as influenced by interaction between soybean cultivars and sowing date.

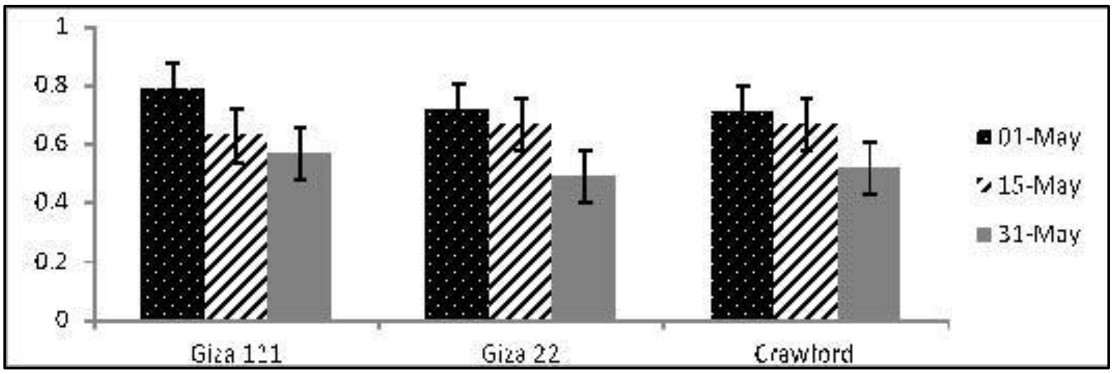

Fig. 4: Average of shoot fresh weight (g) as influenced by the interactive among soybean cultivars and sowing date.

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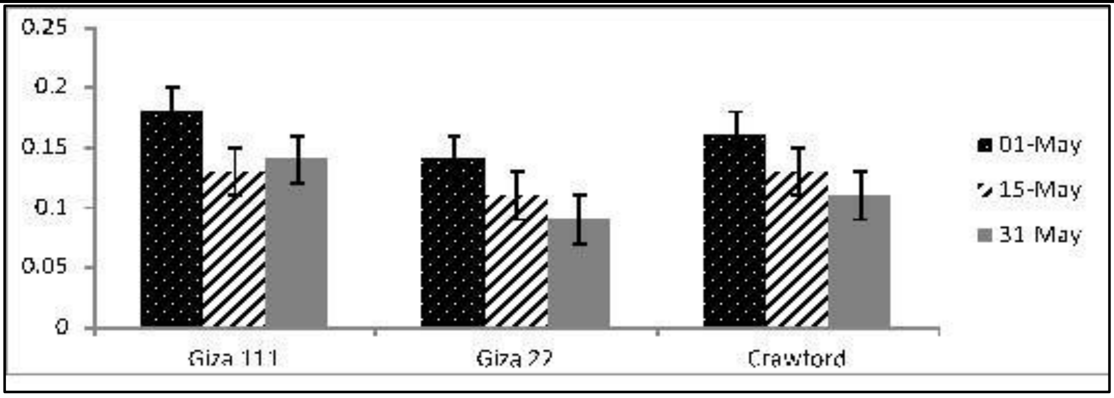

Fig.5: Average of root fresh weight (g)as influenced by interaction between soybean cultivars and sowing date.

3.4.2. Interaction between sowing dates and phosphorus fertilizer rates:

Means of energy of germination and coefficient of velocity percentage, root length (cm) andweightof fresh root (g), significantly affected by the interaction between sowing dates and phosphorus fertilizer rates but, insignificantly influenced mean germination time,shoot length (cm), weight of fresh shoot (g),dry weight of shoot and root (g).The results graphically illustrated in Figs. 6, 7, 8 and 9 clearly showed thatthe highest percentages ofenergy of germination (51.8 %), coefficient of velocity (31.7 %), the tallest root (6.89 cm) and the great root fresh weight (0.17 g). However, the lowest values were produced from sown on end May without phosphorus fertilizer supplying.Extreme in P and K fertilization rates can result in lower seed viability and vigor**(Krueger et al., 2013).**

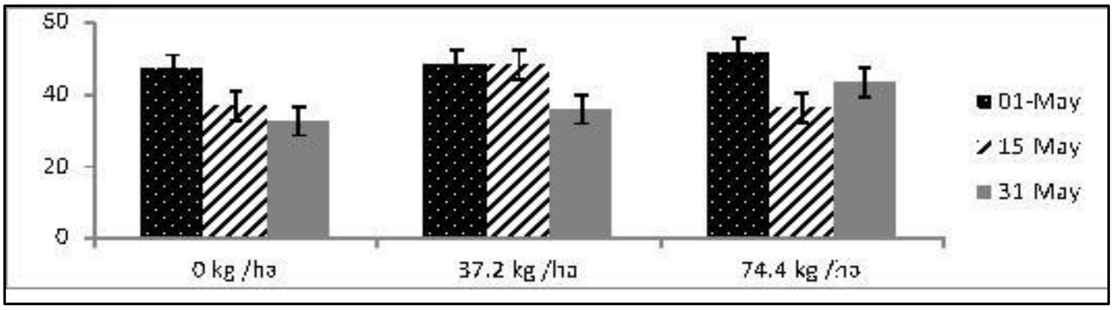

Fig. 6. Average of energy of germination as affected by interaction between phosphate fertilization and sowing date.

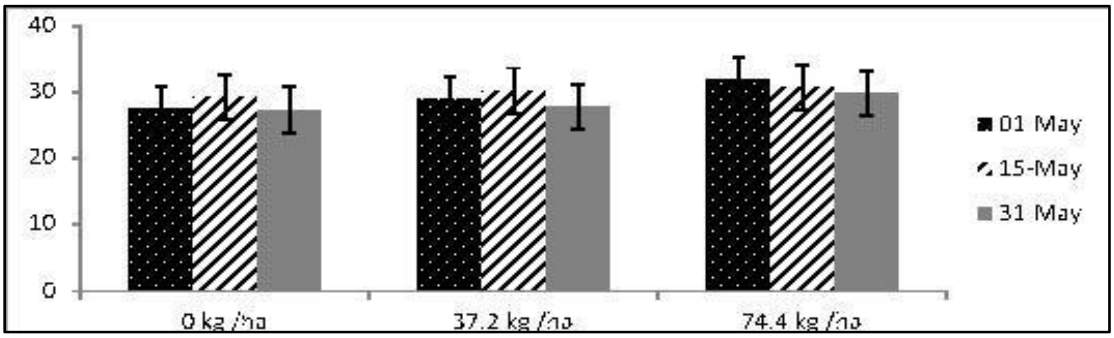

Fig. 7. Average of coefficient of velocity as affected by interaction between phosphate fertilization and sowing date.

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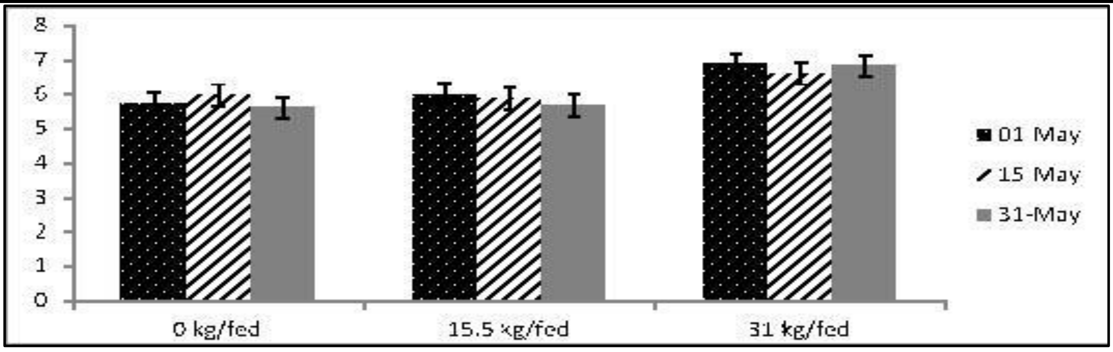

Fig. 8. Average of root length as influenced by the interactive among sowing date and phosphate fertilization rates.

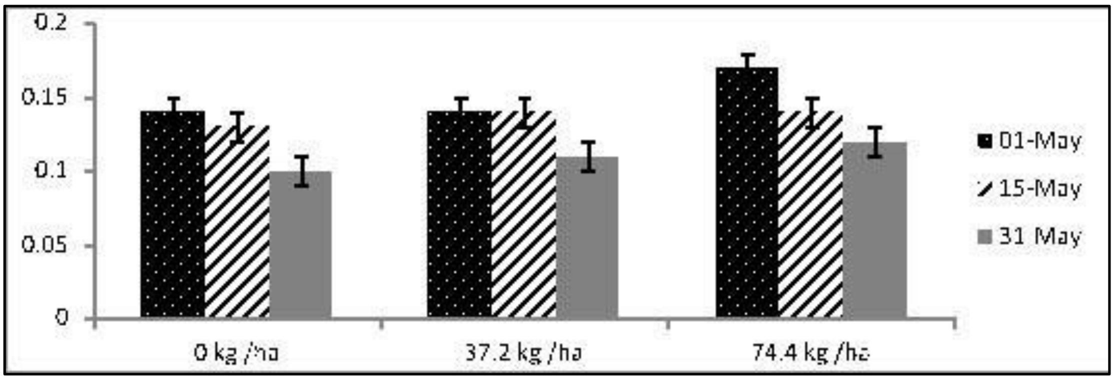

Fig. **9**. *Average of root fresh weight (g)as influencedby interaction between sowing date and phosphate fertilization rates.*

3.4.3. Interaction between soybean cultivars and phosphorus fertilizer rates:

Means ofgermination and dead seedpercentages,shoot and root length (cm), shoot fresh and dry weight (g)significantly affected by the interaction between soybean cultivars and phosphorus fertilizer ratesbut, insignificantly influenced mean germination time, energy of germination root fresh and dry weight (g).The results graphically illustrated in Figs. 10, 11, 12, 13, 14 and 15 clearly showed that highest % of germination (86.0 %), the lowest dead seed % (14.0 %), tallest shoot (7.1 cm) and root (7.0 cm), weight of fresh shoot (0.72g) and dry (0.19 g) were obtained from sown Giza 111 cultivar and phosphorus fertilization at the rate of74.4 kg P_2O_5/ha. However, the lowest values were recorded from sown Crawford cultivar and without phosphorus fertilization.

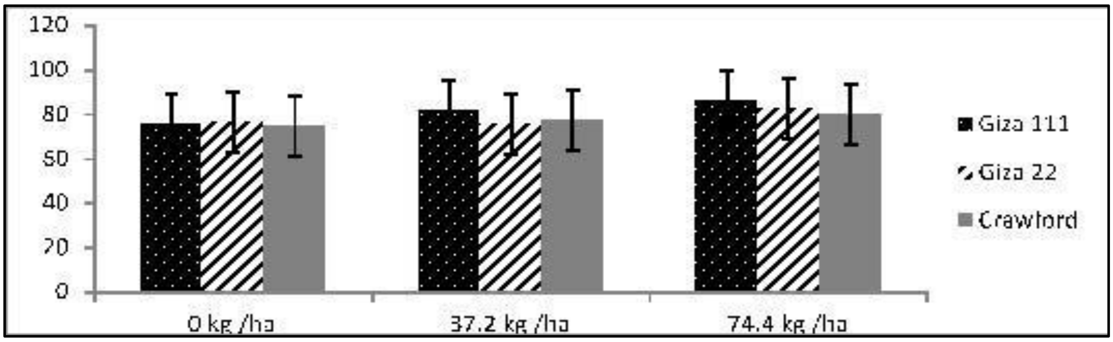

Fig. **10**. *Average of germination % as influenced by the interactive among soybean cultivars and phosphate fertilization rates.*

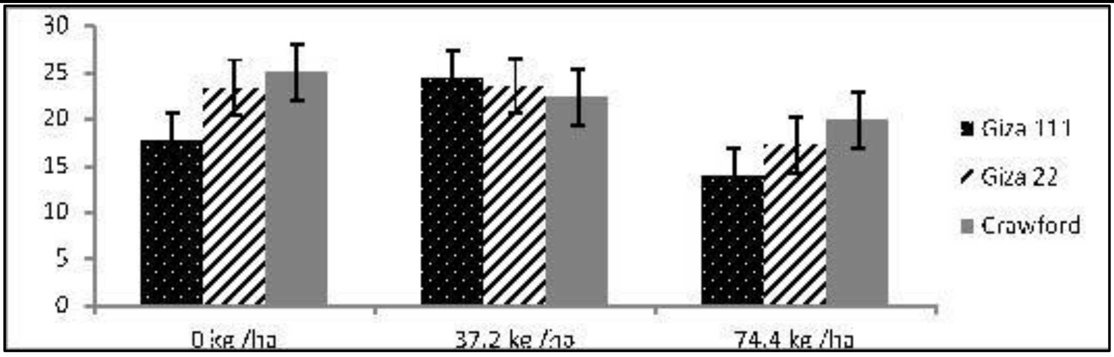

Fig.11. Average of dead seed% as influenced by interaction among soybean cultivars and phosphate fertilization rates.

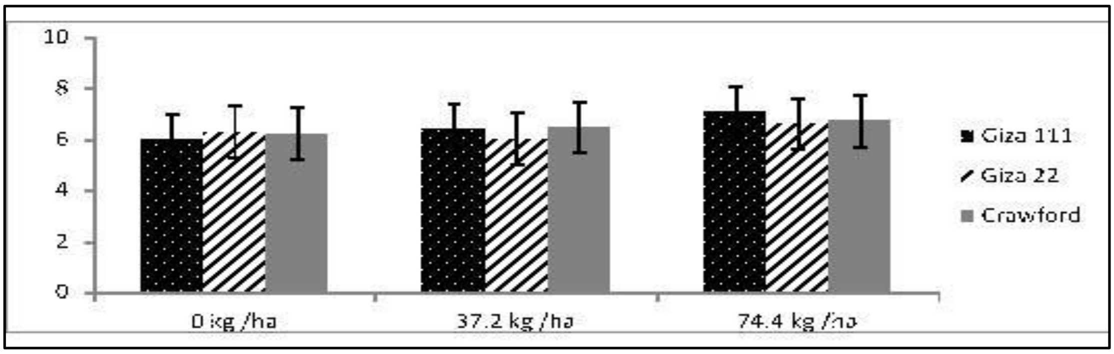

Fig.12. Average of shoot length (cm) as affected by interaction between soybean cultivars and phosphate fertilization rates.

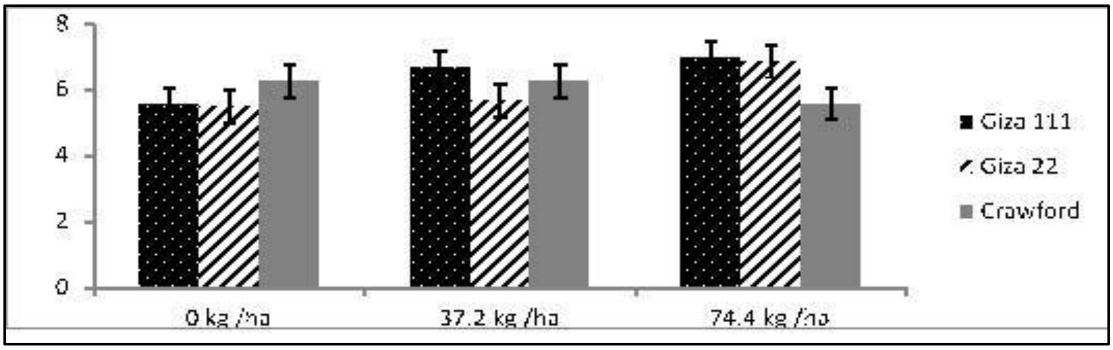

Fig. 13. Average of root length (cm) as affected by interaction between soybean cultivars and phosphate fertilization rates.

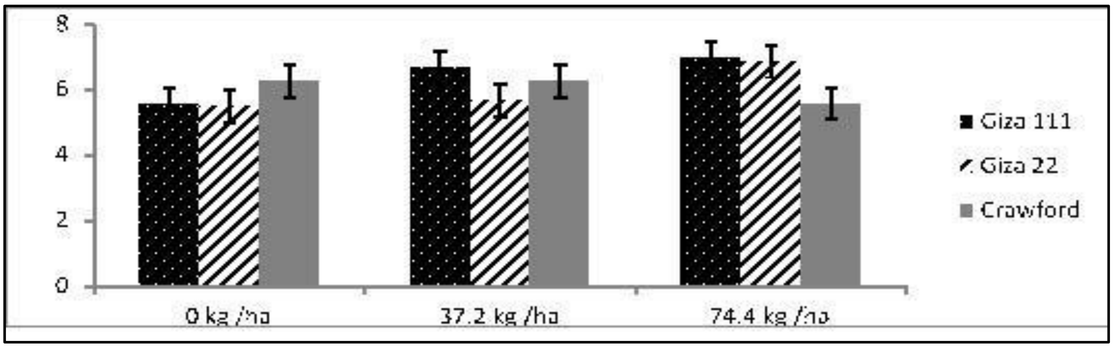

Fig. 14. Average weight offreshshoot (g) as affected by interaction between soybean cultivars and phosphate fertilization rates.

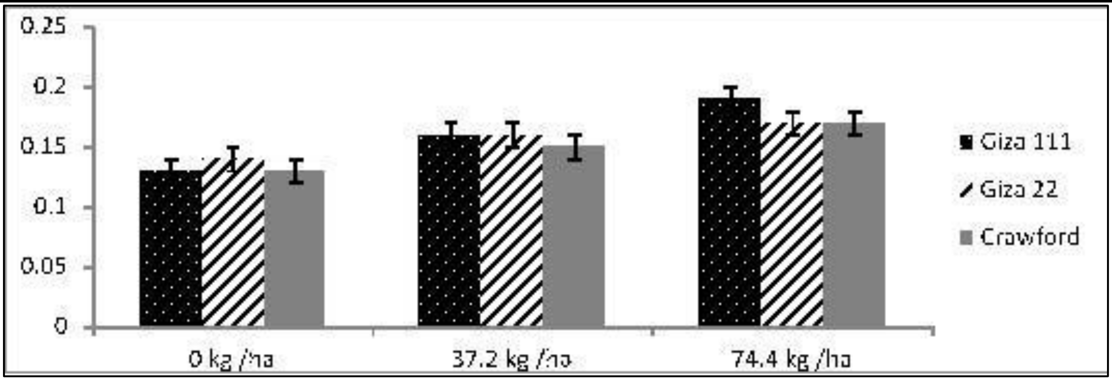

Fig. 15. Average weight ofdryshoot (gm) as affected by interaction between soybean cultivars and phosphate fertilization.

3.4.4. Interaction among sowing dates, soybean cultivars and phosphorus fertilizer rates:

Means of percentage of germination and energy of germination dead seed percentages, mean germination time, shoot and root length (cm), shoot fresh and dry weight (g),root fresh and dry weight (g) insignificantly affected by the interaction among sowing dates, soybean cultivars and phosphorus fertilizer rates.

IV. CONCLUSION

It could be concluded that seed Giza 111 cultivar recorded the best in seed viability when sown on first May and fertilized with phosphorus fertilizer at a rate of 74.4 kg P_2O_5/ha.

REFERENCES

[1] Abdel-Baki, A. A. and J. D. Anderson 1973.Viability and leaching of sugars from germinating barley. Crops Sci., 10: 31 – 34.https://dl.sciencesocieties.org/publications/cs/abstracts/10/1/CS0100010031

[2] Ellis, R.A. and E.H. Roberts 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol., 9: 373-409.http://agris.fao.org/agris-search/search.do?recordID=XE8182678

[3] Gomez, K.A. and A.A. Gomez 1991.Statistical Procedures in Agricultural Research, John Wiley and Sons, New York.http://pdf.usaid.gov/pdf_docs/PNAAR208.pdf

[4] Islam, M.M. and M. A. Karim 2010. Evaluation of Rice *Oryza sativa L*. genotypes at germination and early seedling stage for their tolerance to salinity. The Agric., 8 (2): 57 – 65.http://www.banglajol.info/index.php/AGRIC/article/view/7578

[5] ISTA Rules 2016.International seed testing association. ISTA Germination Sec. Chapter 19: pp. 19 – 41.https://www.seedtest.org/upload/cms/user/OGM15-05-Proposed-Changes-to-the-ISTA-Rules-for-2016.pdf

[6] Kandil A.A., A.E. Sharief and Kh. R. Ahmed 2015. Performance of some soybean Glycine max (L.) Merrill. Cultivars under salinity stress to germination characters. International Journal of Agronomy and Agricultural Research (IJAAR). 6(3): 48-56.http://www.innspub.net/wp-content/uploads/2015/03/IJAAR-V6No3-p48-56.pdf

[7] Kandil A.A., A.E. Sharief and M.S Sheteiwy 2013. Seedling Parameters of Soybean Cultivars as Influenced with Seed Storage Periods, Conditions and Materials. International Journal of Agriculture Sciences, 5(1): 330-338. https://bioinfopublication.org/files/articles/5_1_1_IJAS.pdf

[8] Karim, M.A., N. Utsunomiya and S. Shigenaga 1992. Effect of sodium chloride on germination and growth of hexaploid triticale at early seedling stage. Japanese Journal of Crop Science,61: 279 – 284.https://www.jstage.jst.go.jp/article/jcs1927/61/2/61_2_279/_article

[9] Krueger, K., A. S. Goggi, A. P. Mallarino and R. E. Mullen 2013. Phosphorus and Potassium Fertilization Effects on Soybean Seed Quality and Composition. Crop Science, 53(2): 602-610. DOI: 10.2135/cropsci2012.06.0372.

[10] Kundu, P. K., T. S. Roy, Md. S. H. Khan, K. Parvin4 and H. E. M. K. Mazed 2016. Effect of Sowing Date on Yield and Seed Quality of Soybean. Journal of Agriculture and Ecology Research International, 9(4): 1-7.DOI : 10.9734/JAERI/2016/29301

[11] Rahman, M. M., Hampton, J. G. and Hill, M. J. 2005. Soybean seed yield as affected by time of sowing in a cool temperature environment. Seed Science and Technology, 7: 1-

15.https://www.seedtest.org/en/seed-science-and-technology-_content---1--1084.html

[12] Rahman, M. M., M. M. Rahman and M. M. Hossain 2013. Effect of Sowing Date on Germination and Vigour of Soybean (Glycine max (L.) Merr) Seeds. The Agriculturists 11(1): 67-75.DOI: http://dx.doi.org/10.3329/agric.v11i1.15245

[13] Ruan, S., Q,Xue and K.Tylkowska 2002. Effects of seed priming on germination and health of rice *Oryza sativa L.* seeds. Seed Science and Technology, 30: 451-458.http://www.uaiasi.ro/CERCET_AGROMOLD/CA3-15-05.pdf

[14] Russell, D.F. 1986.MSTAT-C computer based data analysis software Crop andSoil Science Department, Michigan State University USA.https://msu.edu/~freed/mstatc.htm

[15] Sawan, Z.M., A.H. Fahmy and S. E. Yousef 2011. Effect of potassium, zinc and phosphorus on seed yield, seed viability and seedling vigor of cotton (Gossypium barbadense L.). Archives of Agronomy and Soil Science, 57(1): 75-90. http://dx.doi.org/10.1080/03650340903222328

[16] Snedecor GW and WG. Cochran 1980. Statistical Methods. 7ThEd. Iowa State University Press, Iowa, USA, ISBN-10: 0-81381560-6, Pp: 507. https://www.amazon.com/Statistical-Methods-Seventh-isbn-0813815606/dp/B0012S4NIE

[17] Vidyapeeth, M.P.K. 2002. Effect of sowing dates on seed yield, yield yield component characters and seed quality of soybean (Glycine max (l.) Merrill) in Kharif season. M. Sc. Thesis Department of Agricultural Botany, Ahmednagar Maharashtra, India. http://krishikosh.egranth.ac.in/bitstream/1/5810004414/1/28_C.pdf

[18] Waller R.A. and B.D. Duncan 1969. A bays rule for the symmetric multiple comparison problem. J. Amer. Assoc., 64, 1484-1503.https://www.jstor.org/stable/2286085?seq=1#page_scan_tab_contents

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Behaviors of Some Soybean Cultivars (Glycine max L.) Yield to Planting Dates and Different Phosphorus Fertilizer Rates

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Abstract— *Soybean production components such as planting date, cultivars and phosphorus fertilizer rates affected soybean yield. Two field experiments conducted in extensive field at El-Gahrbia district, Egypt during 2015 and 2016 seasons. The goal of this research was aimed to investigate the influence of sowing date at 1st May, 15th May and 31st May and three phosphorus fertilization rates, i.e. 0, 37.2 and 74.4 kg P_2O_5/ha on the performance of three soybean (Glycine max (L.) Merrl) cultivars i.e. Crawford, Giza 22 and Giza 111 on growth, yield and seed quality. The tallest plants, the thick stem, highest branches number/plant, pods number/plant, seed number/pod, weight of 1000 seed and seed yield/ha were recorded from sown early on first May in the both seasons. The tallest plants, the thick stems and highest number of branches/plant were recorded from sown Crawford cultivar. Whereas maximum number of pods/plant and number of seed/pod were found from sown Giza 111 cultivar in the both seasons. Increasing phosphorous fertilizer rates significantly increased all studied cultivars of seed yield and yield components. Tallest plant, the thick stem, the highest branches number/plant, pods number/plant, seed number/pod, weight of1000 seed and seed yield/ha were produced from phosphorous fertilizer at a rate of 74.4 kg P_2O_5/ha in the first and second seasons, respectively.*

Keywords— Soybean cultivars, planting dates, phosphorus fertilization rates, seed yield and yield components.

I. INTRODUCTION

Soybean (Glycine max L. Merrill) considered one of the greatest essential oil and protein legume crops as its high quality protein, 85 percent cholesterol free oil content **(Malik et al., 2007).** Soybean has gained increased attention in Egypt agriculture, because there is a great shortage source of vegetable oil**.** Sowing date is one of the major factors that influence growth and yield of legumes and positively correlates phonological phase of the environmental factors. The rationale for planting early is to avoid the high temperature of July and August for most flowering and seed filling **(Heatherly, 2005).**Decreases in seed yield produced when planting delayed until after the critical dates in late May and early June **(De Bruin and Pedersen, 2008 and Egli and Cornelius, 2009).**Maximum oil content obtained when planting in May, and oil content decreased as planting was delayed further **(Muhammad et al., 2009)**.Seed yield/ha increased by 19.7% when sown on 5th May compared with those sowed on 5th June and increased seed yield by 17.9% paralleled with those sowed on 20th April, and augmented seed yield by 10.3% paralleled with those sowed on 20th May **(Kandil et al., 2012).** The best sowing date on mid-June, but sowing lately on June avoids due to a reduction in yield and yield components **(Yagoub and Hamed, 2013).** The greatest seed yield obtained with an early sowing in mid-February and mid-March compared to late-April planting dates **(Grichar and Biles, 2014).** Significantly, higher yields obtained for earlier planting dates and yield decreased as planting delayed **(Hankinson, 2015).**Sowing soybean on July maximized seed yield and yield components **(Nwofia et al., 2016).**

Soybean cultivars differ markedly to dark period required to induce flowering. New genotypes are an important source to enhance and increase the commercial varieties productivity **(Dong et al., 2001).** Significant variances among studied genotypes for measurements viz., number of days to 50% flowering, plant height, pods number/plant, branches number/plant, 100-seed weight and grain yield **(Malik et al., 2007).**Sown cv. Giza 21 exceeded H-32 line by 16.63% and H-30 line by 14.6%, Giza 22 cv. by 13.7%, H-2L12 line by 6.5% and Giza111cv.by 5.3% of seed yield/ha **(Kandil et al., 2012).**The TGx1485-1D cultivar recorded maximized seed yield per unite land area,while the TGx1987-10F cultivar produced the lowest **(Nwofia et al., 2016).**Pods number and seed per plant differed among cultivars, and the cultivar "BRS 284" showed the best results **(Junior et al., 2017).**

Low phosphorus in soil is a major constraint for soybean growth and seed production. Increase phosphorus fertilization from 75 to 375 kg P_2O_5/ha, soybean yield increased up to 20% compared with the control level. Further P fertilization increase up to 975 kg P_2O_5/ha did not increase seed yield, but it has influence to seed quality **(Antunović et al., 2012).** To improve growth and development of plants due to supply phosphorus increased with the supply of assimilates to the seed, which finally gained more weight **(Devi et al., 2012).**Phosphorus supplement at the rate of 60 kg P_2O_5/ha produced the tallest plants and increased weight of 100 seeds and seed yield/ha**(Matusso and Cabo, 2015).**Phosphorus application at a rate of 60 kg/ha P produced the highest seed yield/ha and 60 kg/ha P recommended for greatest soybean production per unite area**(Maga et al., 2017).**

Sowing Giza-21, Giza-111, H-2 L12 and H-32 genotypes on 5th May or 20th May maximized seed yield**(Kandil et al., 2012).**The interaction between Genotype x environment played a significant role and given most genetic materials adapted to a range of environments. TGX 1910-14F and TGX1440-1E cultivars were stable under different phosphorus rates **(Ikeogu and Nwofia, 2013).** The higher pods numberper plant, seeds number of pods on the main stem and greatest seed yield/ha produced by April 30 and Sahar cultivar **(Sadeghi and Niyaki, 2013).**Soybean genotypes on early sown createdmore seed yield and quality as related to later planting dates. The highestpods number/plant and seeds number/plant recorded from 28th January and Faisal cultivar (**Rehman et al., 2014).** Higher amount of seed protein content produced from middle and late sowing dates. The seed yield decreased with a delay of sowing dates**(Junior et al., 2017).**Sowing on21th March, Williams-82 cultivar and 14th March, Williams-82cultivar recorded the largestpods number per plant and the number of seeds/plant **(Shah et al., 2017).**

Early planting of Pan 520RR and Highveld Top cultivars with increasing phosphorus rates up to maximized seed yield/ha **(Mabapa et al., 2010).**Consequently, the goals of the study were to check growth, yield and yield component responses of some soybean genotypes to sowing date and phosphorus fertilizer levels.

II. MATERIALS AND METHODS

2.1. Research time and site:

Three separate field experiments carried out in the extensive field for each season at Mehalla El-Kobra, El-Gahrbia district, Egypt during 2015 and 2016 seasons. Each experiment assigned in split plot design in RCBD with four replicates. The three separate experiments in each season include the sowing date at 1thMay, 15th May and 31th May. The three soybean (*Glycine max* (L.) Merrl) cultivars i.e. Crawford, Giza 22 and Giza 111 assigned in main-plots. Three phosphorus fertilization rates, i.e. 0, 37.2 and 74.4 kg P_2O_5/ha assigned in sub-plots. Then combined analysis done between sowing dates to get the main effect of planting dates and interaction between cultivars, phosphorus fertilization rates and planting dates. Each plot contained of five rows, 60 cm apart and four m long. The preceding crop was wheat. The analyses of chemical and physical properties of the experimental soil (0 to 30 cm depth) carried out according to the methods reported by Page et al. (1982). The soil was loamy clay in texture, the pH was 7.9, 7.6, organic matter was 4.8, 4.9 %, 7.7, 7.5, E.C. dS/m^{-1}, available nitrogen was 15.7 and 16.3 ppm and available phosphorus was 39.6 and 39.1 ppm of both seasons, respectively. Soybean seeds of the studied cultivars obtained from the Field Crops Research Institute, A. R. C., and Giza, Egypt. Seeds of studied varieties infected by specific *rhizobia*. After plotting and before the planting, sulfate of potassium (48 % K_2O) at a rate of 120 kg/ha and phosphorus fertilizers of the form calcium super-phosphate (15.5 % P_2O_5) at above rates supplied to experimental plots. Nitrogen at 36 kg N/ha of ammonium nitrate (33.5 % N) added before the first irrigation for each plot..

2.2. Studied Characters:

Ten guarded plants randomly taken from each plot to measure plant height (cm), stem diameter, branches number/plant, pods number/plant, weight of1000-seed (g) and seed weight/plant (g). The two inner of the central area harvested to find seed yield/m^2 in each plot and then calculated in kg per hectare.

2.3. Experimental analysis:

The analysis of data collected and statistically by the analysis of variance technique using the MSTAT–C statistical package programmed as described by a procedure of **Gomez and Gomez (1991)**. Lest significant differences test (LSD) at 5 and 1 % level of probability was used to compare between treatment means according to **Snedecor and Cochran (1980).**Combined analysis between planting dates done according to **Waller and Duncan (1969).**

III. RESULTS AND DISCUSSION

3.1. Planting dates effects:

Means of plant height (cm), stem diameter (cm), seed number/pod, and seed yield/ha (kg) as influenced by sowing date, in both seasons, except, branches number/plant, pods number/plant, weight of 1000 seed (g) insignificantly influenced only in the second one as exposed in Tables (1 and 2). The tallest plants (125.5, 136.9 cm), the thick stems (1.02, 1.22 cm), highest number of branches/plant (5.1, 6.1), number of pods/plant (123.3, 135.0), number of seed/pod (2.70, 2.72),1000 seed weight(203.30, 204.4 g) and seed yield/ha (3017.5, 30.49.7 kg/ha)were recorded from sown early on first May in the first and second seasons, respectively. However, the lowest values rom sown lately on end May. Decreases in seed yield produced when planting delayed until after the critical dates in late May and early June **(De Bruin and Pedersen, 2008 and Egli and Cornelius, 2009).**The highest percentages of oil was obtained when planting in May, and oil content decreased as planting delayed further **(Muhammad et al., 2009)**. **Grichar and Biles (2014), Hankinson (2015) and Nwofia et al. (2016)** recorded similar conclusions**.**

3.2. Cultivars performance:

Averages of plant height (cm), branches number/plant and pods number/plant significantly affected by soybean studied cultivars, in both seasons. However, stem diameter (cm) in the second season and number of seed/pod, in the first one significantly affected, weight of 1000 seed (g) and seed yield in kg/ha in both seasons insignificantly influenced only in the second one as revealed in Tables (1 and 2).The tallest plants (126.1, 136.5 cm), the thick stems (0.98, 1.26 cm) and highest number of branches/plant 4.6, 6.3) were recorded from sown Crawford cultivar. However, the highest number of pods/plant (100.7, 146.9) and number of seed/pod (2.77, 2.76) were obtained from sown Giza 111 cultivar in both seasons, respectively. Significant variances among studied genotypes for measurements viz., number of days to 50% flowering, plant height, pods number/plant, branches number/plant, 100-seed weight and grain yield **(Malik et al., 2007).**The TGx1485-1D cultivar recorded maximized seed yield per unite land area, while the TGx1987-10F cultivar produced the lowest **(Nwofia et al., 2016).**Pods number and seed per plant varied between genotypes, and the cv. BRS 284 showed the best results **(Junior et al., 2017).**

3.3. Phosphorus fertilizer rate effects:

Means of plant height (cm), stem diameter (cm), branches number/plant, pods number/plant, weight of 1000 seed (g) and seed yield/ha (kg) significantly affected by phosphorous fertilizer rates in both seasons, except, number of seed/pod insignificantly influenced only in the second one as exposed in Tables (1 and 2). Increasing phosphorous fertilizer rates significantly increased all studied cultivars of seed yield and yield components. The tallest plants (128.7, 136.5 cm), the thick stem (0.79, 1.24 cm), the highest number of branches/plant (5.3, 6.2), number of pods/plant (110.7, 146.8), number of seed/pod (2.79, 2.75), 1000 seed weight (204.8, 211.3 g) and seed yield (2936.9, 2988.7 kg/ha) were produced from phosphorous fertilizer at a rate of 74.4 kg P_2O_5/ha.Low phosphorus in soil is a major constraint for soybean growth and seed production. Increase phosphorus fertilization from 75 to 375 kg P_2O_5/ha, soybean yield increased up to 20% compared with the control level. Further P fertilization increase up to 975 kg P_2O_5/ha did not increase seed yield, but it has influence to seed quality **(Antunović et al., 2012).** To improve growth and development of plants due to supply phosphorus increased with the supply of assimilates to the seed, which finally gained more weight **(Devi et al., 2012).**Similarly, results in good covenant with those reported by **Kamara et al.(2007).Matusso and Cabo (2015) and Maga et al. (2017).**

3.4. Interaction Effects:

3.4.1. Interaction between sowing dates and soybean cultivars:

Averages ofplant height (cm), number of branches/plant, number of seed/pod andseed yield/ha (kg)significantly affected by the interaction between sowing date and studied soybean cultivars in both seasons, but, insignificantly affected stem diameter (cm), pods number/plant and weight of 1000 seed (g)**.** The results showed that tallest plants (132.1, 140.7 cm) and number of branches/plant (5.9, 7.5) were obtained from sown Crawford cultivar in first May in both seasons as shown in Figs 1 and 2. While the lowest values were produced from sown Giza 111 cultivar lately on end May. Moreover, the highest number of seed/pod (2.83, 2.87) and seed yield/ha (3117.1, 3142.0 kg/ha) were produced from sown Giza 111 cultivar on first May in both seasons as shown in Figs. 3 and 4.Whilst, the lowest values were produced from sown Giza 22 cultivar on mid-May. Decreases in seed yield produced when planting delayed until after the critical dates in late May and early June **(De Bruin and Pedersen, 2008 and Egli and Cornelius, 2009).**

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Table.1: Average of plant height (cm), stem diameter(cm), branches number/plant and pods number/plant as influenced by date of sowing, soybean cultivars and phosphate fertilization rates in both seasons.

Characters / Treatments	Plant height (cm)		Stem diameter (cm)		Branches number/plant		Pods number/plant	
	2014	2015	2014	2015	2014	2015	2014	2015
A-Sowing date:								
1 May	125.5	136.9	1.02	1.22	5.1	6.1	123.3	135.0
15 May	123.6	127.9	0.91	1.24	5.0	5.9	90.4	129.6
31 May	123.7	131.1	0.85	1.21	4.4	6.6	78.0	131.6
F. test	*	*	*	*	*	N.S	*	*
LSD at 5%	3.6	6.6	0.07	0.13	0.1	---	6.6	11.2
B-Soybean Cultivars:								
Giza 111	123.5	127.6	0.93	1.17	5.3	6.1	100.7	146.9
Giza 22	123.3	131.8	0.92	1.20	4.5	6.2	94.0	142.7
Crawford	126.1	136.5	0.98	1.26	4.6	6.3	97.0	106.5
F. test	*	*	*	N.S	*	*	N.S	*
LSD at 5%	3.6	6.7	0.02	---	0.1	0.1	---	11.2
C-Phosphorus fertilization:								
0 kg P_2O_5/ha	119.3	127.1	0.87	1.20	4.3	5.1	81.1	118.0
37.2 kg P_2O_5/ha	124.9	132.4	0.94	1.22	4.8	6.4	99.9	131.2
74.4 kg P_2O_5/ha	128.7	136.5	0.97	1.24	5.3	6.2	110.7	146.8
F. test	*	*	*	*	*	*	*	*
LSD at 5%	2.9	5.7	0.06	0.02	0.2	0.2	6.3	10.4

Table.2: Average of seed number/pod, of 1000 seed weight and seed yield in kg/ha as influenced by sowing date, studied soybean cultivars and phosphorus fertilization rates in both seasons.

Characters / Treatments	**Seed number/pod**		**1000 seed weight**		**Seed yieldin kg/ha**	
	2014	2015	2014	2015	2014	2015
A-Sowing date:						
1 May	2.70	2.72	203.0	204.4	3017.5	3049.6
15 May	2.63	2.63	200.9	204.4	2333.0	2369.7
31 May	2.87	2.70	185.3	198.5	2116.0	2139.
F. test	*	N.S	*	N.S	*	*
LSD at 5%	0.16	---	10.9	---	32.4	36.1
B-Soybean Cultivars:						
Giza 111	2.77	2.76	202.5	206.7	2516.8	2553.6
Giza 22	2.68	2.57	191.6	199.8	2460.9	2502.0
Crawford	2.74	2.72	195.1	200.8	2489.0	2503.6
F. test	*	*	N.S	N.S	N.S	N.S
LSD at 5%	0.16	0.13	---	---	---	---
C-Phosphorus fertilization:						
0 kg P_2O_5/ha	2.74	2.64	191.5	195.6	2088.0	2085.3
37.2 kg P_2O_5/ha	2.66	2.66	193.0	200.4	2442.2	2485.2
74.4 kg P_2O_5/ha	2.79	2.75	204.8	211.3	2936.4	2988.7
F. test	**	N.S	*	*	*	*
LSD at 5%	0.25	---	8.0	5.3	64.2	40.1

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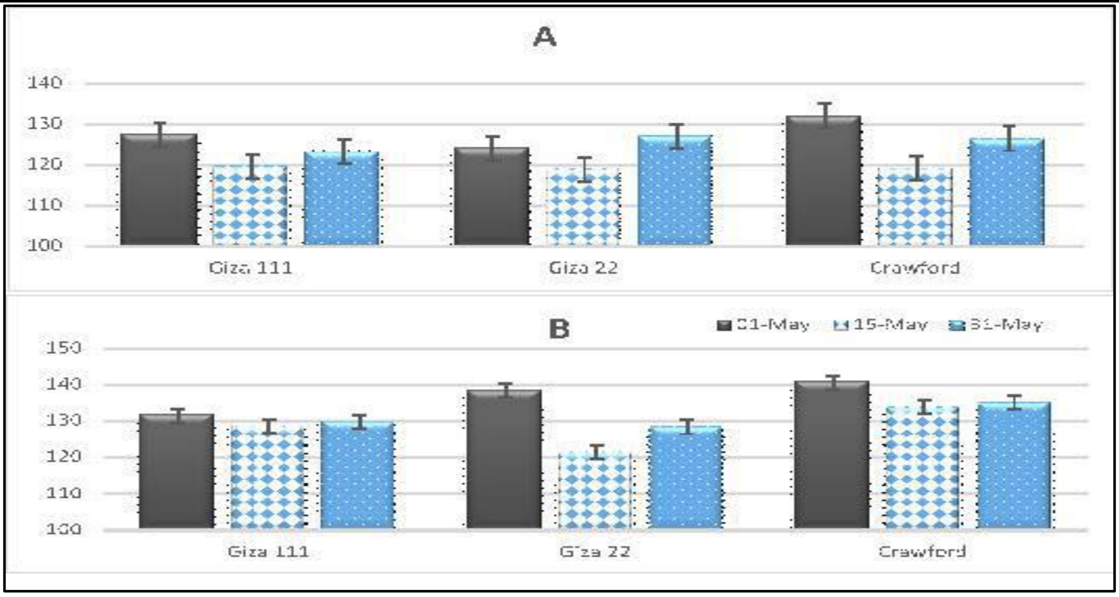

Fig.1: Average of plant height (cm) as influenced by the interactiveamong soybean cultivars and sowing date during two season (A) 2014 and (B) 2015 seasons.

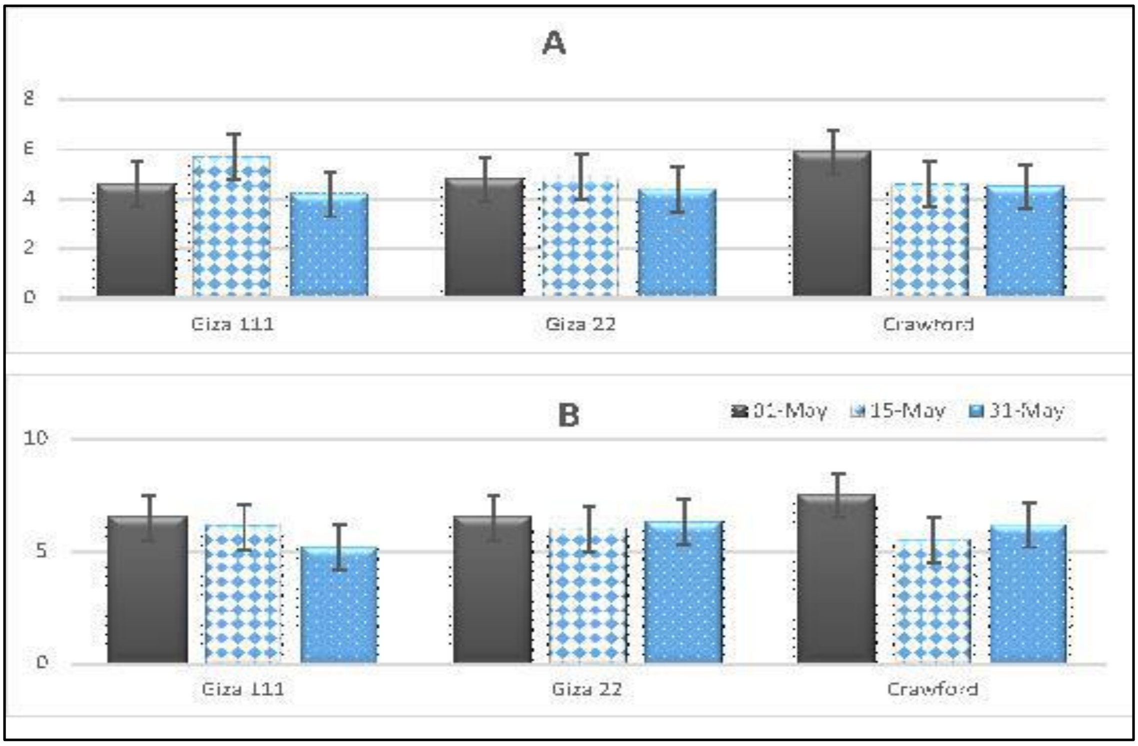

Fig.2: Average of number of branches/plant as affected by interaction between soybean cultivars and sowing date during 2014 and 2015 seasons.

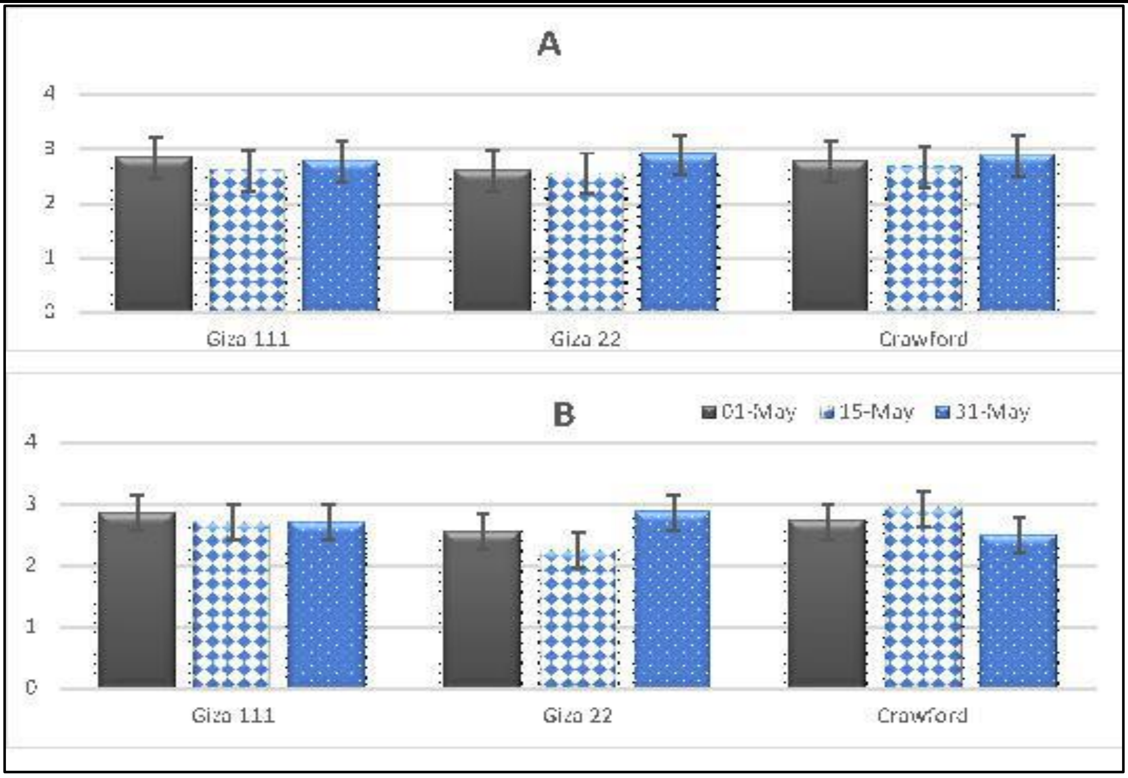

Fig.3: Average of number of seed/pod as affected by interaction between soybean cultivars and sowing date during 2014 and 2015 seasons.

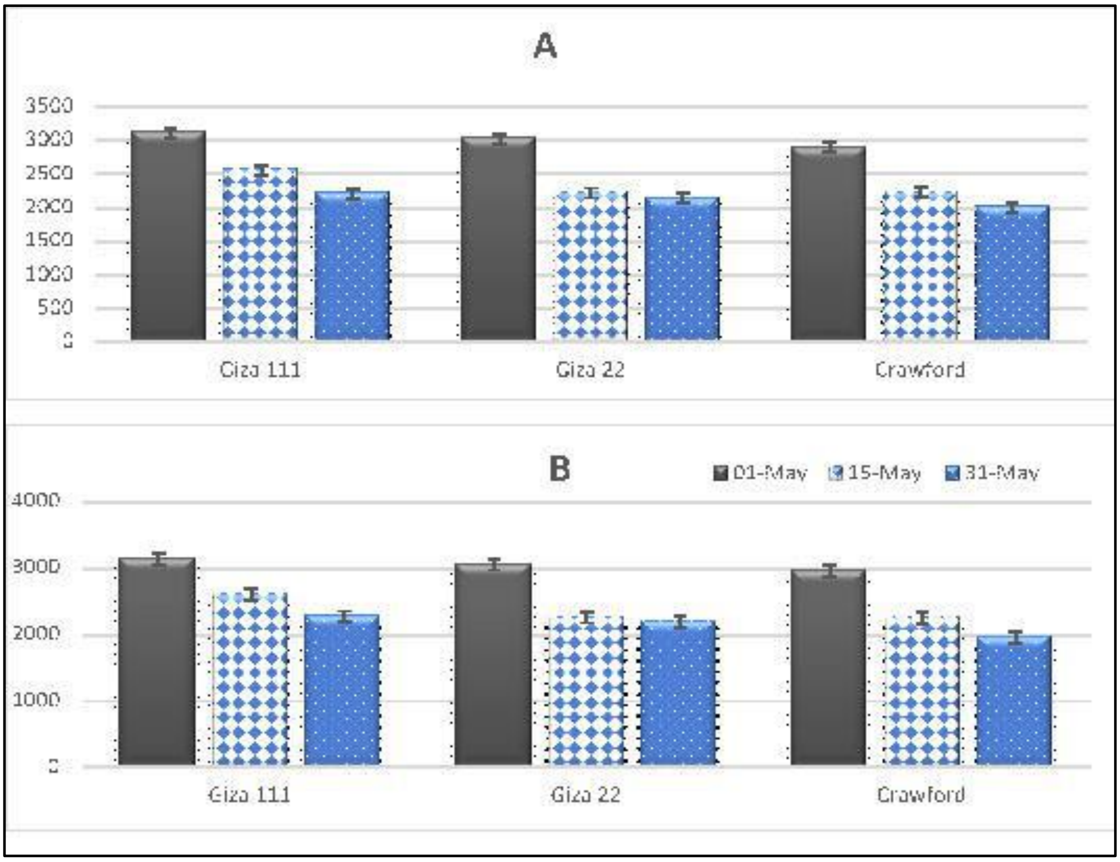

Fig.4: Average of seed yield/ha (kg) as affected by interaction between soybean cultivars and sowing date during 2014 and 2015 seasons.

3.4.2. Interaction between sowing dates and phosphorus fertilizer rates:

Mean of branches number/plant, pods number/plant, seed number/pod and seed yield/ha (kg)significantly affected by the interaction between sowing date and phosphorus fertilizer rates, but, plant height (cm), stem diameter (cm) and 1000 seed weight(g) insignificantly affected in both seasons. The highest number of branches/plant (5.5, 6.7), number of seed/pod (138.2, 158.0), number of seed/pod (2.88, 2.83) and seed yield kg/ha(3511.2, 3546.2 kg/ha) were obtained from sown early in first May and phosphorus fertilizer at a rate of 74.4 kg P_2O_5/ha in both seasons as shown in Figs. 5, 6, 7 and 8. However, the lowest values were produced from sown on end May and without phosphorus fertilization. To improve growth and development of plants due to supply phosphorus increased with the supply of assimilates to the seed, which finally gained more weight**(Devi et al., 2012).**Phosphorus application at the rate of 60 kg/ha P produced the highest seed yield/ha and 60 kg/ha P recommended for greatest soybean production per unite area**(Maga et al., 2017).**

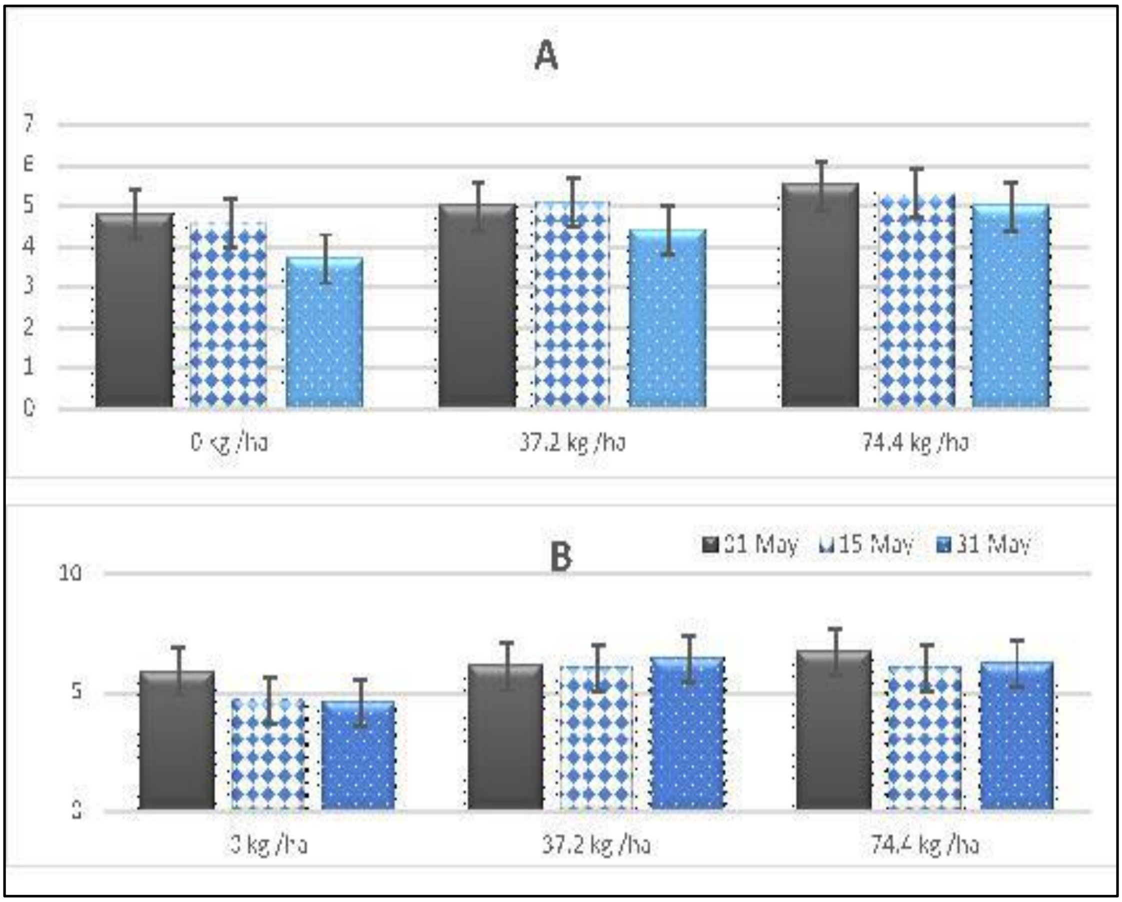

Fig.5: Average of number of branches/plant as influenced by interaction between phosphate fertilization and sowing date during 2014 and 2015 seasons.

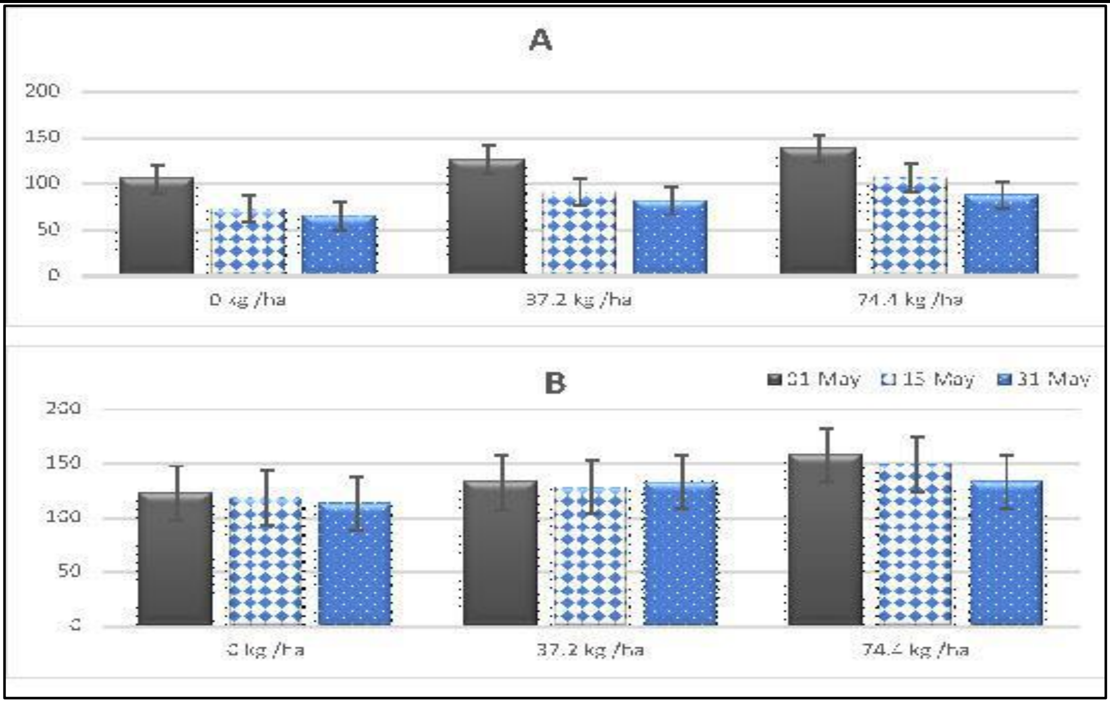

Fig.6: Average of number of pods/plant as influenced by interaction between phosphate fertilization and sowing date during 2014 and 2015 seasons.

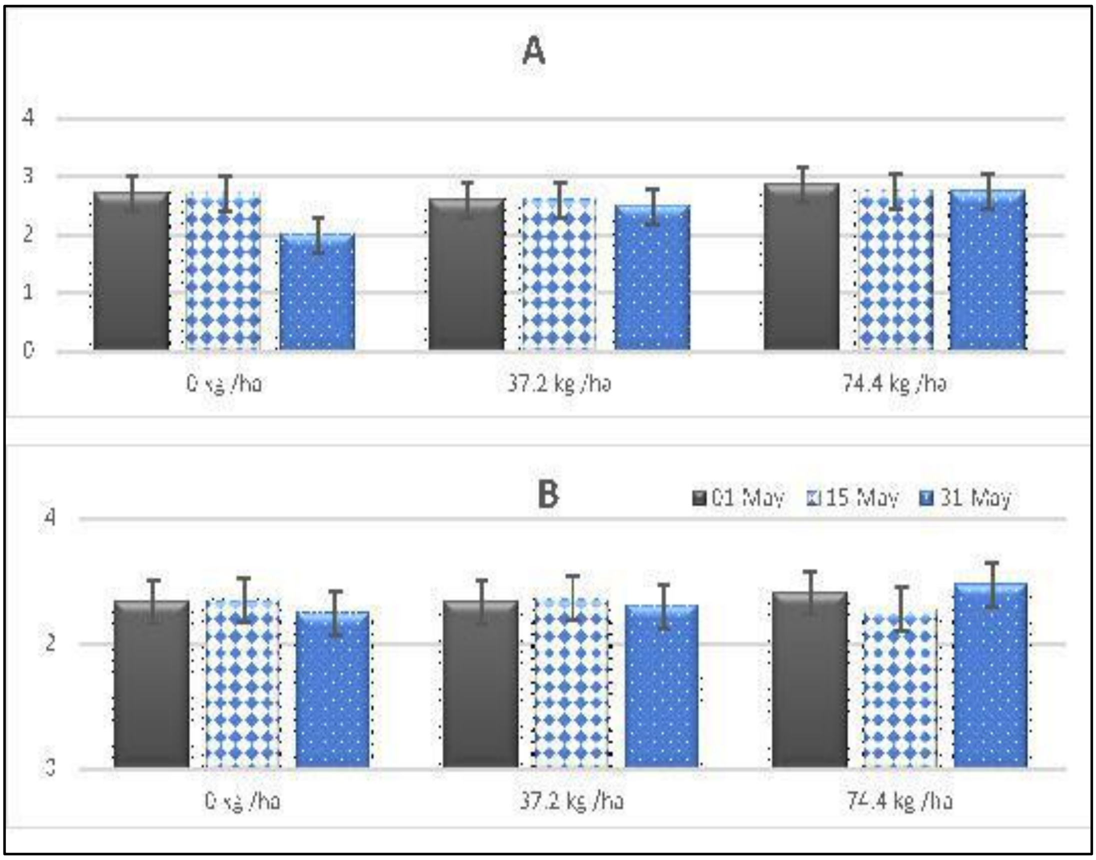

Fig.7: Average of number of seed/pod as affected by interaction between phosphate fertilization and sowing date during 2014 and 2015 seasons.

3.4.3. Interaction between soybean cultivars and phosphorus fertilizer rates:

Averages of 1000 seed weight (g)significantly influenced by the interface between studied soybean cultivars and phosphorus fertilization rates in both seasons, but, insignificantly affected on plant height (cm),stem diameter (cm),number of branches/plant, number of pods/plant, number of seed/pod and seed yield/ha (kg).

The results clearly indicated that the heaviest 1000 seed weight (205.7, 213.7 g)was produced from sown Giza 111 cultivar when fertilized by phosphorus fertilizer at a rate of 74.4 kg P_2O_5/ha in both seasons as shown in Fig.9.However the less weight of 1000 seed was recorded from sown Crawford cultivar and without phosphorus fertilization.Sowing Giza-21, Giza-111, H-2 L12 and H-32 genotypes on 5th May or 20th May maximized seed yield **(Kandil et al., 2012).** The interaction between Genotype x environment played a significant role and given most genetic materials adapted to a range of environments. TGX 1910-14F and TGX1440-1E cultivars were stable under different phosphorus rates **(Ikeogu and Nwofia, 2013).**

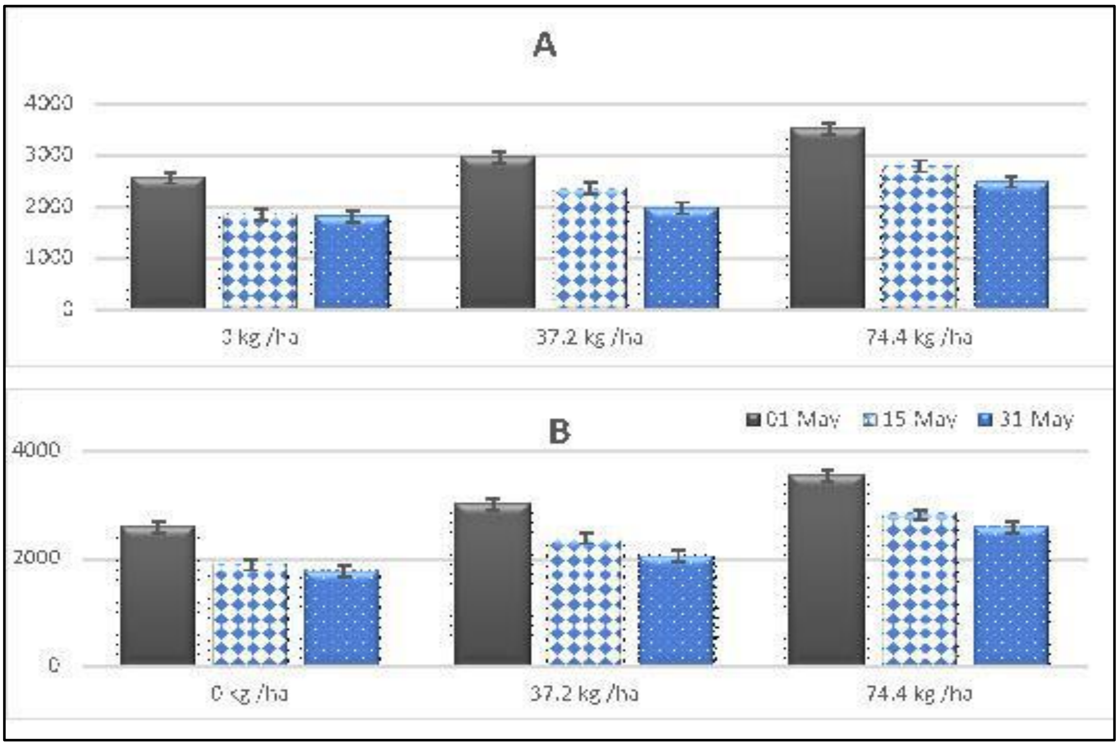

Fig.8: Average of seed yield/ha (kg) as influenced by the interactive among phosphate fertilization and sowing date during 2014 and 2015 seasons.

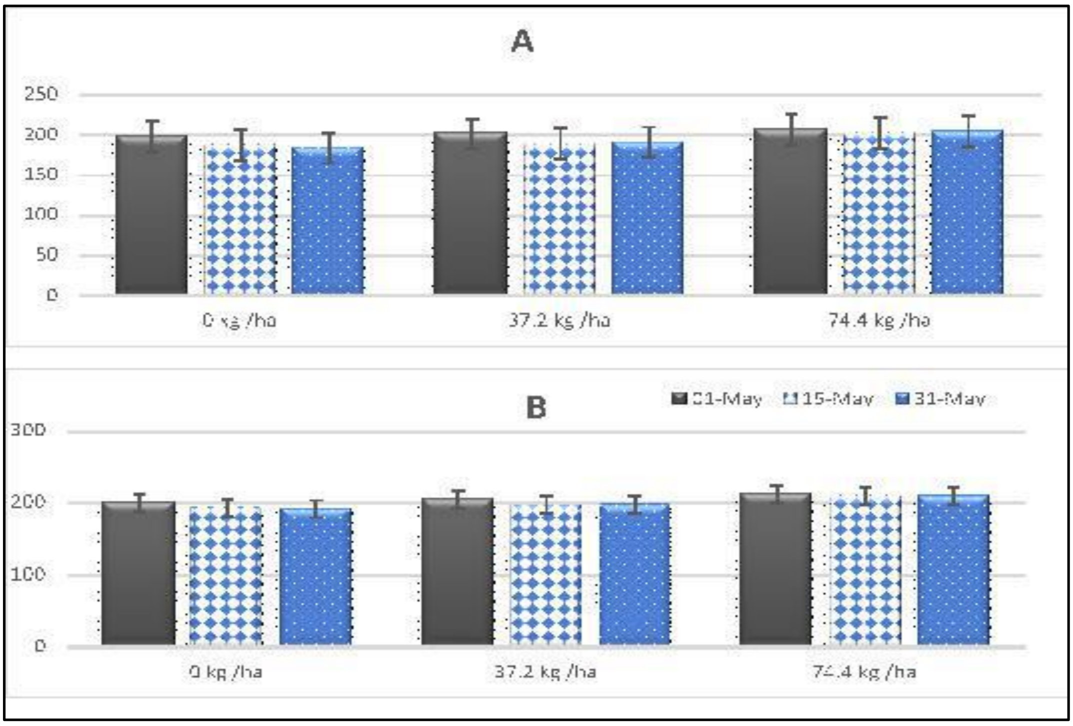

Fig.9: Average of 1000 Seed weight (gm).as affected by the interaction between phosphate fertilization and sowing date during 2014 and 2015 seasons.

3.4.4. Interaction between sowing dates, soybean cultivars and phosphorus fertilizer rates:

Means of plant height (cm),stem diameter (cm),number of branches/plant,number of pods/plant, number of seed/pod,1000 seed weight (g)and seed yield/ha (kg)insignificantly affected by the interaction between sowing dates, soybean cultivars and phosphorus fertilizer rates.

REFERENCES

[1] Antunović, M., M. Rastija, A. Sudarić, I.Vargaand J.Jović2012. Response of Soybean to Phosphorus Fertilization under Drought Stress Conditions.11th Alps-Adria Scientific Workshop Smolenice, Slovakia, Pp: 117-120. DOI: 10.1556/Novenyterm.61.2012.Suppl.2

[2] De Bruin, J.L. and P. Pedersen 2008. Soybean Seed Yield Response to Planting Date and Seeding Rate in the Upper Midwest. Agronomy Journal, 100(3): 696–703.doi:10.2134/agronj14.0277

[3] Devi, K.N., L.N.K Singh,T.S. Devi, H.N. Devi, T.B. Singh, K.K. Singh and W.M. Singh2012. Response of Soybean (Glycine max (L.) Merrill) to Sources and Levels of Phosphorus. Journal of Agricultural Science, 4 (6): 44 – 53.DOI: http://dx.doi.org/10.5539/jas.v4n6p44

[4] Dong, Y.S., B.C. Zhuang, L.M. Zhao, H. Sun and M.Y. He. 2001. The genetic diversity of annualwild soybeans grown in China. Theor. Appl. Genet., 103(1): 98-103.http://www.cgris.net/973/The%20genetic%20diversity%20of%20annual%20wild%20soybeans%20grown%20in%20China.pdf

[5] Egli, D.B. and P.L. Cornelius 2009. A Regional Analysis of the Response of Soybean Yield to Planting Date. Agronomy Journal 101(2): 330–335.doi:10.2134/agronj2008.0148

[6] Grichar, W. J.and S.P. Biles 2014. Response of Soybean to Early-Season Planting Dates along the Upper Texas Gulf Coast. International Journal of Agronomy Volume 2014, Article ID 252563, 7 pages, http://dx.doi.org/10.1155/2014/252563

[7] Hankinson, M.W. 2015. Planting Date and Starter Fertilizer Effects on Soybean Growth and Yield. M.Sc. Thesis, Ohio State University, USA. https://etd.ohiolink.edu/!etd.send_file?accession=osu1429734801&disposition=inline

[8] Heatherly, L. G. 2005. Midsouthern USA soybean yield affected bymaturity group and planting date. CropManagement, 4 (1): doi:10.1094/CM-2005-0418-01-RS

[9] Ikeogu, U. N. and Nwofia G. E. 2013. Yield parameters and stability of soybean [Glycine max.(L.) merril] as influenced by phosphorus fertilizer ratesin two ultisols. J. Plant Breed. Crop Sci., 5(4): 54-63. DOI: 10.5897/JPBCS12.014

[10] Kamara, A.Y., R. Abaidoo, J. Kwari and L. Omoigui2007. Influence of phosphorus application on growth and yield of soybean genotypes in the tropicalsavannas of northeast Nigeria, Archives of Agronomy and Soil Science, 53(5): 539-552.http://dx.doi.org/10.1080/03650340701398452

[11] Kandil, A.A., A.E. Sharief, A.R. Morsy and A.I. Manar El-Sayed 2012.Performance of some Promising Genotypes of Soybean UnderDifferent Planting Dates Using Biplots Analysis. Journal of Basic & Applied Sciences, 8:379-385. http://www.lifescienceglobal.com/images/Journal_articles/JBASV8N2A22-Sharief.pdf

[12] Mabapa P. M., J. B. O. Ogola, J. J. O. Odhiambo, A. Whitbread andJ. Hargreaves 2010. Effect of phosphorus fertilizer rates on growth and yieldof three soybean (*Glycine max*) cultivars in LimpopoProvince. African Journal of Agricultural Research, 5(19): 2653-2660. http://www.academicjournals.org/article/article1380963071_Mabapa%20et%20al.pdf

[13] Maga, T.J., T. S. Ter and K. J. Aorga 2017. Responses of soybean [*glycine max* (l.) Merrill] to phosphorusFertilizer timing and rates in Makurdi, Nigeria. International Journal of Scientific Research, 6(5):383-386. https://www.worldwidejournals.com/international-journal-of-scientific-research-(IJSR)/file.php?val=May_2017_1493736099__217.pdf

[14] Malik, M. F. A., M. Ashraf, A. S. Qureshi and A. Ghafoor 2007. Assessment of genetic variability, correlation and path analyses for yield and its components in soybean Pakistan Journal of Botany, 42(2): 971-976. https://pdfs.semanticscholar.org/17a7/426350783f03e3c62267776261bc0106d760.pdf

[15] Materusse, M. J. M. and F. G. D. Cabo 2015.Response of Soybean [Glycine max (L.) Merrill] to Phosphorus Fertilizer Rates in Ferralsols. Acad. Res. J. Agri. Sci. Res. 3(10): 281-288.DOI: 10.14662/ARJASR2015.057

[16] Muhammad, A., S.K. Khalil, K.B. Marwat, and A.Z. Khan. 2009. Nutritional quality and production of soybean land races and improved varieties as affected by planting dates. Pak J Bot 41(2): 683–689.http://www.pakbs.org/pjbot/PDFs/41(2)/PJB41(2)683.pdf

[17] Nwofia, G.E., R.E. Edugbo and E.U. Mbah 2016. Interaction of Genotype x Sowing Date on Yield and Associated Traits of Soybean [Glycine max (L.) Merrill] over Two Cropping Seasons in a Humid

Agro-ecological Zone of South-Eastern Nigeria. The Journal of Agricultural Sciences, 11(3):64-177 http://dx.doi.org/10.4038/jas.v11i3.8170

[18] Page, A.L., R.H. Millerand D.R. Keeney 1982. Methods of Soil Analysis: Part 2, Chemical and Microbiological Properties.(Eds) Agronomy Series No 9, American Society of Agronomy, Madison, WI. file:///C:/Users/Ali%20Sharief/Downloads/books-agronomymonogra-methodsofsoilan2-frontmatter.pdf

[19] Rehman, M.T.Khaliq, A. Ahmad, S.A.Wajid,F.Rasul, J. Hussain andS.Hussain 2014. Effect of Planting Time and Cultivar on Soybean Performancein Semi-Arid Punjab, Pakistan. Global Journal of Science Frontier Research: DAgriculture and Veterinary, 14 (3): 41-45. http://www.academia.edu/23666181/Effect_of_Planting_Time_and_Cultivar_on_Soybean_Performance_in_Semi-Arid_Punjab_Pakistan

[20] Sadeghi, S.M. and S. A. N. Niyaki 2013.Effects of planting date and cultivar on the yield and Yield components of soybean in north of Iran. ARPN Journal of Agricultural and Biological Science, 8(1): 81-85. http://www.arpnjournals.com/jabs/research_papers/rp_2013/jabs_0113_518.pdf

[21] Shah,T., N. Z.Kalsoom, A. Ahmad and A. Jalal 2017. Yield and Quality Traits of Soybean Cultivars Response to Different Planting Windows.International Journal of Statistics and Actuarial Science, 1(3): 55-59. doi: 10.11648/j.ijsas.20170103.11

[22] Waller, R.A. andD.B. Duncan 1969. A Bayes Rule for the Symmetric Multiple Comparison Problem. Journal of the American Statistical Association 64: 1484-1504.ttps://www.jstor.org/stable/2286085?seq=1#page_scan_tab_contents

[23] Yagoub, S. O. and M. H. A. Hamed 2013.Effect of Sowing Date on Two Genotypes of Soybean (Glycine max. Merrill.) Grown under Semi-desert Region. Universal Journal of Agricultural Research 1(3): 59-64.DOI: 10.13189/ujar.2013.010303

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Seedling Parameters as affected by Soaking in Humic Acid, Salinity Stress and Grain Sorghum Genotypes

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Abstract— *To study the effect soaking in humic acid and salinity stress on sorghum seedling parameters, a laboratory experiment accompanied in Seed Science Laboratory Faculty of Agriculture Mansoura University from June 2017 to July 2017. This exploration intended to study performance of seedling parameters of five grain sorghum cultivars viz. Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305 to soaking in humic acid under salinity concentrations of 3, 6, 9, 12 and 15 dS/m^{-1}beside the control, and humic acid soaking. The results showed that seed soaking in humic acid recorded the tallest shoot and root, weight of fresh shoot and root, weight of dry shoot and the lowest percentages of relative dry weight and highest salinity tolerance index. Seed soaking in humic acid exceeded shoot and root length (cm), weight of fresh shoot and root, weight of dry shoot and tolerance index by 14.3, 92.6, 8.7, 4.5, 4.7 and 40.8%, respectively compared without soakingin humic acid.The tallest shoot and the fresh shoot weight were produced from germinating Giza 15 cultivar. In addition, the tallest roots and highest values of stress tolerance index were recorded from sown Mecca hybrid and Giza 15 cultivars without significant differences. It could be stated that sown Mecca hybrid surpassed H-305 cultivar in root length by 36.3 %. Moreover, Giza 15 cultivar surpassed H-305 in shoot length, shoot fresh weight and stress tolerance index by 30.6, 10.1 and 29.1 %, respectively. The results clearly revealed that accumulative salinity concentrations from 3, 6, 9, 12 and 15 dSm^{-1} significantly produced the shortest shoot, root, weight of fresh shoot, root, weight of dry shoot, and root, highest percentages of seedling height reduction, and stress tolerance index, except the percentage of relative dry weight increased with salinity levels increased. The shortest shoot and root (cm), were recorded from the highest salinity concentrations of 15 dSm^{-1}. The gradually increases in salinity till of 15 dSm^{-1} significantly diminished the length of shoot and root, the fresh weight of shoot and root, the dry weight of shoot and root, seedling height reduction percentages, and stress tolerance index by 51.7, 17.7,4.7, 59.5, 38.7 and 57.4, respectively compared the control treatment. Accordingly, sown Mecca hybrid or Giza 15 cultivar with soaking in humic acid under salinity of 6 dSm^{-1}maximized seedling parameters and could recommended to cultivated in saline new reclaimed soils.*

Keywords—Sorghum varieties, humic acid, salinity levels.

I. INTRODUCTION

Salinity is harmfully influenced crop yield of various crops extents of Egypt and the world. Irrigation water sources in Egypt was limit. There is a shortage in cereal production with annually increases of population. To overwhelmed the lack of cereal productivity, it could be achieved by growing grain sorghum cultivars tolerant of salinity to get economical yields from saline reclaimed soils, particularly in early seedling stages is very important. Soil salinization is a great determinate factor of crop productivity, especially in dried area**(Ahmed, 2009).**Many physiological variations induced in plants affecting their growth, development and seedling parameters in arid and semi-arid districts(**Saroj and Soumana, 2014).**

Humic acid activate many processes accompanied emergence of primary root and shoot emergence. Soaking in potassium humate at concentration of 100 m.L^{-1} for 24 hours the length of root and shoot and the weight of dry shoot were increased with **(Ali and Elbordiny, 2009).**The root and shoot length increased in the seedlings which preserved with potassium humate over control **(Patil, 2010).** The seeds primed at level of 750 mg l for 12 h recorded the greatest seedling growth parameters. Increasing concentration up to 500 and 750 mgL-1had highest influenced of seedling growth parameters **(Asgharipour and Rafiei et al., 2011).** Germination percentage and tallest root produced from seed of wheat priming with humic acid compared with those primed in water**(Ali et al., 2014).** Seedling growth inhibiting by salt and varying due top retreatment of humic acid **(Çavuşoğlu and Ergin, 2015).**

The recommended cultivar for new reclaimed saline soils were Soavecultivar **(Almodares et al., 2007).** Root length affected by salt stress more than shoot length. 235461 and 69239 genotypes recorded the more salt tolerant during seedling growth **(Geressu and Gezahagne, 2008).**The most salt tolerance genotypes recorded from 235461, 69239, 223550, 69029 and 23403 genotypes during seedling stage. Nevertheless, sensitive to salt genotypes recorded from 22885, 233247, 237264, 237265 and 237267 **(Asfaw, 2011).**Sorghum genotypes differed from response to salinity, the medium tolerant recorded by Hegariand JS-263 medium cultivars, while Noor cultivar produced the medium sensitive and the sensitive produced from FJ-115 and PSV-4 cultivars **(Kausar et al., 2012).**The STI, GMP and MP genotypes were better to cultivate under saline and non-saline conditions. In contrast, cluster of the highest tolerance index and stress sensitivity index is sensitive to saline or non-saline conditions **(Hefny et al., 2013).**The highest seedling growth characters recorded from Meko, Gambella-1107, ICSV-111 and Melkam genotypes and were more salt tolerant cultivars. However, germinating ESH-2 and Gobye genotypes were salt sensitive throughout growth stage. **(Tigabo et al., 2013).** The most salt tolerant cultivars produced from germinated Shallu, Desert Maize, and 1790E genotypes, while the least salt tolerance genotypes produced from Schrock and RTx430 cultivars**(Sun et al., 2014).**

Wad Ahmed cultivar recorded the more salt tolerant, but Arfagadamak and Butana cultivars were the more salt sensitive **(El Naim et al., 2012).**The seedling characters decreased as the salinity concentrations increased in all studied cultivars. Salinity stress reduced root and shoot length and weight of dry seedling of all genotypes**(Chauhan et al., 2012).** The shortest shoot androot, the lowest weight of dry root and chlorophyll contents were produced from increasing of salinity levels of all studied cultivars**(Movafegh et al., 2012).**The control and salinity level of 5 dS.m^{-1} produced the tallest root and shoot. As salinity increased, the shoots and root length decreased. The highest shoot length recorded by KFS2 cultivar, so this genotype was salt tolerant cultivar **(Tabatabaei and Anagholi, 2012)**. Seedling growth of sorghum decreased due to salinity. Seedling dry weight decreased due to increasing in salinity concentration **(Behzadnejad and Tohidinejad, 2014).**Radical and shoot length and weight decreased with increasing salinity concentration**(Dadar et al., 2014).**The plumule and radical fresh and dry weight were decreased as the NaCl concentrations increased **(Sam et al., 2014).** Plumule and radical length reduced as NaCl level was amplified. The sensitive cultivar to salinity produced from Barbarei cultivars compared to Tabat and Wad-Ahmed cultivars, Tabat cultivar recorded the more-salt tolerant cultivars**(Siddig and Idris, 2015).**Therefore, the goals of this study aimed to study the behavior of seedling parameters of some grain sorghum cultivars soaking in humic acidunder salinity stresses.

II. MATERIALS AND METHODS

2.1. Treatments and Experimental Design:

A laboratory experiment carried out in the Faculty of Agriculture, Mansoura University from June 2017 to July 2017 in Agronomy Department, Seed Science Laboratory. The aimed of the investigation conducted to screening forfive (Sorghum bicolor L.(Moench) cultivars *i.e.* Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305 under salinity stress, sodium chloride (Na Cl) at the levels of 0 (as control), 3, 6,9, 12 and 15 dS/m^{-1} soaked or non-soaked in humic acid. RCBD design in factorial experiment in four replications was used. The first factor include two treatments with and without soaking in humic acid. The second one includes the five-grain sorghum cultivars *i.e.* Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305.Six different concentrations of NaCl include 0, 3, 6, 9, 12 and 15 dS/m^{-1}were allocated as third factor. The experiment included240 Petri dishes,then, the Petri dishes take placed in a growth chamber for 14 days at 28 ± C º for germination according to **ISTA, 2016** roles.

2.2. Studied characters:

Eight seedling trails were studied as follows:

1-The length of shoot (cm): It measured as average of five shoot length in centimeters (cm).

2-The length of root (cm): Itwas calculated as means of five root lengthf in centimeters (cm).

3-Weight of fresh shoot (g): Means of five shoots were weighted in gram (g).

4-Weight of fresh root (g): Means of five seedlings roots wasweightedin gram (g).

5-Weight of dry shoot (g): Mean of five seedlings shoots was weightedafter oven drying at 75 ºc for 48 h.

6-Weight of dry root (g):Mean of five seedlings roots was weighted after oven drying at 75 ºc for 48 h.

Physiological Parameters:

7-The percentage of seedling height reduction: It calculated as described by the following equation **(Islam and Karim, 2010).**

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$$\mathbf{SHR\ (\%)} = \frac{\text{Plant height at control} - \text{Plant height at saline condition}}{\text{Plant height at control condition}} \times 100$$

8-The percentage ofrelative dry weight**:**It calculated according (**Islam and Karim, 2010)**equation**:**

$$\mathbf{RDW\ (\%)} = \frac{\text{Total dry weight under saline condition}}{\text{Total dry weight under control condition}} \times 100.$$

To calculate the germination stress tolerance index (GSI), promptness index (PI) estimated using following **(Ashraf et al.,2006)**formula**:**

$$\textbf{Promptness index (PI)} = nd1\ (1.00) + nd2\ (0.75) + nd3\ (0.50) + nd4\ (0.25)$$

Where nd1, nd2, nd3 and nd4 = Number of seeds germinated on the 1st, 2nd, 3rd and 4th day, respectively.
The germination stress tolerance index (GSI) calculated in terms of percentage as follows:
9-Germinations stress tolerance index (GSTI) = It calculated according the following formula:

$$\mathbf{GSTI} = \frac{\text{PI of stress seeds}}{\text{PI of control seeds}} \times 100$$

2.3. Statistical Analysis:
Rendering to the system of variance (ANOVA)was used for the factorial in RCBD as published by Gomez and **Gomez (1991)**of the subjected data was statistically analyzed**.** LSDmethod was used as defined by **Snedecor and Cochran (1980).**The data as **(Russel, 1986)**.method was statistically analyzed using RCBD design by MSTAT-C computer package.

III. RESULTS AND DISCUSSIONS

3.1. Humic acid soaking effect:
The results presented in Tables (1 and 2) clearly showed that soaking in humic acid significantly affected shoot and root length (cm), weight of fresh shoot and root,weight of dry shoot, relative dry weight and germination stress tolerance index, however insignificantly affected root dry weight and percentage of seedling height reduction. The results showed that seed soaking in humic acid recorded the tallest shoot (5.75 cm) and radical (8.67 cm), the highest weight of fresh shoot (0.39 g) and root (0.36 g), the dry shoot weight (0.34 g) and lowest percentages of relative dry weight (79.95) and the highest salinity tolerance index values (80.59). It could noticed that soaking seed in humic acid exceeded the length of shoot and root (cm), weight of fresh shoot and root, weight of dry shoot and tolerance index by 14.3, 92.6, 8.7, 4.5, 4.7 and 408 %, respectively compared without humic acid soaking. Root and shoot length increased in the seedlings treated with potassium humate over control **(Patil, 2010).** Seeds primed at level of 750 mg l for 12 h recorded the greatest seedling growth parameters. Increasing concentration up to 500 and 750 mgL-1 had highest influenced of seedling growth parameters **(Asgharipour and Rafiei et al., 2011).** Germination percentage and tallest root produced from seed of wheat priming with humic acid compared with those primed in water**(Ali et al., 2014).** Seedling growth inhibiting by salt and varying due to pretreatment of humic acid **(Çavuşoğlu and Ergin, 2015).**

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Table.1: Means of shoot length, root length, weight of fresh shoot and dry as affected by humic acid soaking, sorghum cultivars and salinity concentrations.

Treatment	Shoot length	Root length	Weight fresh Shoot	Weight fresh root
A.Humic acid				
Without	4.93	7.64	0.35	0.33
Soaking	5.75	8.67	0.39	0.36
F-test	*	*	*	*
B.Sorghum cultivars				
Dora	4.97	6.61	0.37	0.35
H.306	5.24	7.95	0.38	0.36
Giza 15	6.37	7.81	0.39	0.32
Mecca hybrid	5.72	10.47	0.36	0.32
H-305	4.42	6.77	0.35	0.33
F-test	*	*	*	NS
L.S.D at 5%	0.42	0.71	0.02	-
C. Salinity Level				
0 dsm^{-1}	7.42	8.72	0.38	0.36
3 dsm^{-1}	7.03	8.67	0.37	0.34
6 dsm^{-1}	5.48	8.01	0.36	0.34
9 dsm^{-1}	4.41	7.50	0.37	0.33
12 dsm^{-1}	4.33	7.47	0.36	0.33
15 dsm^{-1}	3.58	7.18	0.36	0.32
F-test	*	*	*	*
L.S.D at 5%	0.46	0.78	0.02	0.02

3.2. Cultivars performance:

The outcomesof accessible results in Tables (1 and 2) clearly revealed that studied sorghum cultivars significantly influenced the length of shoot and root (cm), the weight of fresh shoot, seedling height reduction and relative dry weight percentages as well as stress tolerance index, however insignificantly affected root fresh and dry weight and shoot dry weight. The results clearly indicated that the tallest shoot (6.37 cm) and shoot fresh weight (0.39 g) were produced from sown Giza 15 cultivar.In addition, thetallest roots (10.47, 7.81 cm) and highest values of stress tolerance index (69.47, 70.83) were recorded from sownMecca hybrid and Giza 15 cultivars without significant differences. The maximum percentages of seedling height reduction(44.18 %) were foundfrom germinating H-306 cultivar and highest relative dry weight from sown Dora cultivar. The shortest roots (4.42 cm) and shoot (6.66 cm) the lowest values of shoot fresh weight(0.35 g), relative dry weight (79.44 %) and stress tolerance index (50.17) were obtained from sown H-305 genotypeIt could be stated that sown Mecca hybrid surpassed H-305 cultivar in root length by 36.3 %. Moreover, Giza 15 cultivar surpassed H-305 in shootlength, shoot fresh weight and stress tolerance index by 30.6, 10.1 and 29.1 %, respectively.The recommended cultivar for new reclaimed saline soils were Soave cultivar **(Almodares et al., 2007).** Root length affected by salt stress more than shoot length. 235461 and 69239 genotypes recorded the more salt tolerant during seedling growth **(Geressu and Gezahagne, 2008).** NM-92 cultivar was more salinitytolerant **(Ahmed, 2009).** The more-salt tolerance recorded from 235461, 69239, 223550, 69029 and 23403 genotypes during seedling stage. Nevertheless, sensitive to salt genotypes recorded from 22885, 233247, 237264, 237265 and 237267 **(Asfaw, 2011).** Wad Ahmed cultivar was recorded the more salt tolerant, but Arfagadamak and Butana cultivars were the more salt sensitive **(El Naim et al., 2012).** Shoot and root growth, weight of fresh and dry shoot and root were clearly demonstrated varietal differences **(Khan et al., 2014).**

3.3. Salinity level effects:

The outcomes of accessible results in Tables (1 and 2) clearly showed that studied sorghum cultivars significantly influenced all studied trials. Increment in salinity levels from 3, 6, 9, 12 and 15 dSm^{-1} significantly abridged the length of shoot and root (cm), weight of fresh shoot and root (g), weight of dry shoot and root, percentages of seedling height reduction and stress tolerance index, except, the relative dry weight increased with increasing salinity levels. The highest values of shoot (7.42 cm) and root length (8.72 cm), shoot (0.38 g) and root (0.36 g) fresh weight (g), shoot (0.34 g) and root (0.32 g) dry weight (mg), and stress tolerance index(100.0) were recorded from without salinity. The

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lowest values of shoot (3.58 cm) and root length (7.18 cm), shoot (0.36 g) and root (0.32) fresh weight (g), shoot (0.31 g) and root (0.29 g) dry weight (mg), seedling height reduction, (and stress tolerance index were produced from the highest salinity concentrations of 15 dSm^{-1}.The results revealed that accumulative salinity up to 15 dSm^{-1} significantly abridged the length of shoot and root (cm), the weight of fresh shoot and root (g), weight of dry shoot and root, the percentages of seedling height reduction besides stress tolerance index by 51.7, 17.7, 4.7, 59.5, 38.7 and 57.4, respectively compared the control treatment. The decrease in the dry weight which related with salt adaptation and lessening in photosynthetic rates per unit leaf area **(Netado et al; 2004).** Salt stress known to perturb a multitude of physiological processes **(Noreen and Ashraf, 2008).**The seedling characters decreased as the salinity concentrations increased in all studied cultivars. Salinity stress reduced root and shoot length and weight of dry seedling of all genotypes **(Chauhan et al., 2012).** The shortest shoot and root, the lowest weight of dry root and chlorophyll contents were produced from increasing of salinity levels of all studied cultivars **(Movafegh et al., 2012).** The STI, GMP and MP genotypes were suitable for cultivation under salinity and without salinity. In contrast, cluster of the highest tolerance index and stress sensitivity index is sensitive to under salinity and without salinity **(Hefny et al., 2013).** Salt stress harmfully disturbs plants and crops at all stages **(Hussain et al., 2013).** Similar conclusions were described by **Almodares et al. (2014), Behzadnejad and Tohidinejad (2014), Dadar et al. (2014) and Sam et al. (2014).**

Table.2: Averages of root fresh weight and root dry weight as affected by humic acid soaking, sorghum cultivars and salinity levels.

Treatments	Weight of dry shoot	Weight of dry root	Seedling height reduction	Relative dry weight	Stress tolerance index
A. Humic acid					
Without	0.32	0.32	39.49	84.41	47.67
Soaking	0.34	0.33	41.45	79.95	80.59
F-test	*	NS	N.S.	*	*
B. Sorghum cultivars:					
Dora	0.34	0.33	42.81	86.81	65.12
H.306	0.34	0.33	44.18	80.03	65.06
Giza 15	0.34	0.33	36.76	82.11	70.83
Mecca hybrid	0.31	0.32	36.42	82.51	69.47
H-305	0.32	0.31	42.12	79.44	50.17
F-test	NS	NS	*	*	*
L.S.D at 0.05	-	-	5.34	2.30	7.50
C. Salinity Level					
0 dsm^{-1}	0.34	0.32	100.00	100.00	100.00
3 dsm^{-1}	0.33	0.32	13.58	92.16	71.51
6 dsm^{-1}	0.33	0.32	22.51	87.02	66.53
9 dsm^{-1}	0.32	0.31	31.14	79.85	53.58
12 dsm^{-1}	0.32	0.30	35.13	72.79	50.56
15 dsm^{-1}	0.31	0.29	40.49	61.28	42.60
F-test	*	*	*	*	*
L.S.D 0.05	0.02	0.02	5.86	2.52	8.21

3.4. Interaction effects:

3.4.1. Interaction between humic acid soaking and studied sorghum cultivars effects:

The results graphically demonstrated in Fig. 1, 2, 3, 4 and 7showed the interaction effect between humic acid soaking and studied sorghum cultivars on shoot and root length, shoot and root fresh weight and stress tolerance index, respectively. This interaction significantly influenced shoot and root length, shoot and root fresh weight and stress tolerance index.The results showed that the tallest shoot (7.61 cm) and root (11.4 cm),shoot (0.49 g) and root fresh weight(0.37 g) and highest values of stress tolerance index (90.05) were recorded from sown Giza 15 cultivar with soaking in humic acid. However, the lowest values of shoot and root length and stress tolerance index were obtained from sown H-305 cultivar without soaking in humic acid. In addition, the interaction between humic acid soaking and studied sorghum

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cultivars significantly seedling height reduction affected as graphically demonstrated in Fig.5, the highest seedling height reduction (45.22 %) was obtained from sown H-306 cultivar with soaking in humic acid and the lowest values (36.14 %) produced fromsownH-305 cultivar with soaking in humic acid. Meanwhile, the interaction between humic acid soaking and studied sorghum cultivars significantly influenced relative dry weight as graphically demonstrated in Fig.6, the results rev that the highest relative dry weight (89.72 %) was recorded from sown Dora cultivar without soaking in humic acid. While, the lowest percentages of the relative dry weight was recorded from sown H-305 cultivar with soaking in humic acid. Cultivars resistant to levels of salinity due to the interaction between salt and its levels as well as the ways of seed soaking **(Wei and Qing-Xiang, 2011).**

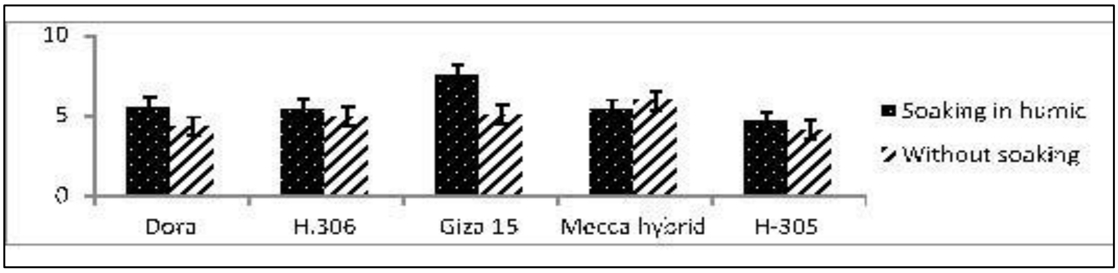

Fig. 1.Averages ofshoot length as influenced by humic acid soaking and studied sorghum cultivars.

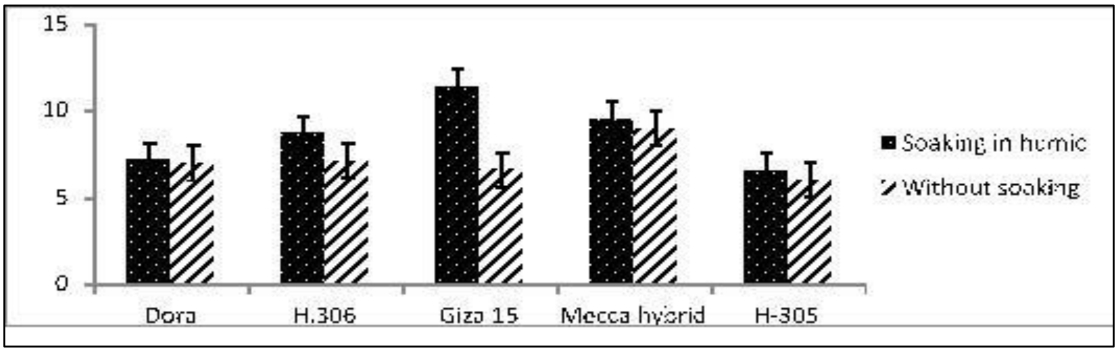

Fig. 2. Averages of root length as affected by humic acid soaking and studied sorghum cultivars.

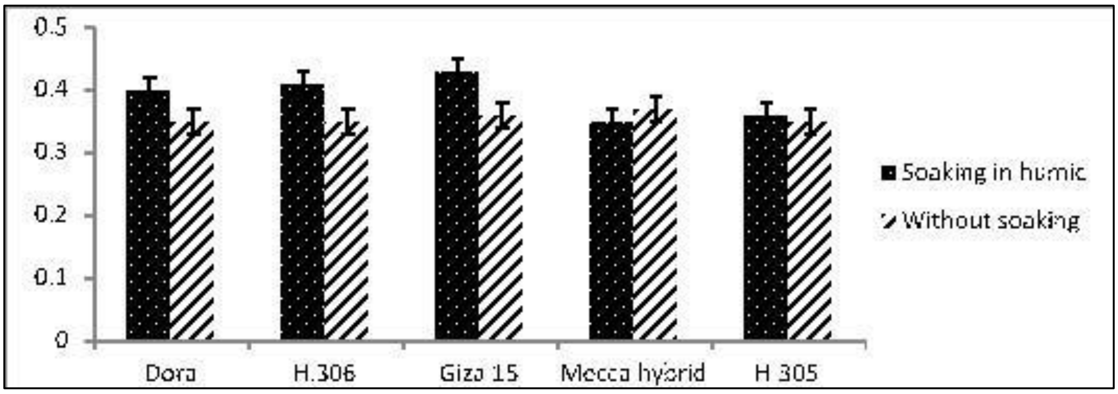

Fig. 3.Averages of shoot fresh weight as influenced by humic acid soaking and studied sorghum cultivars.

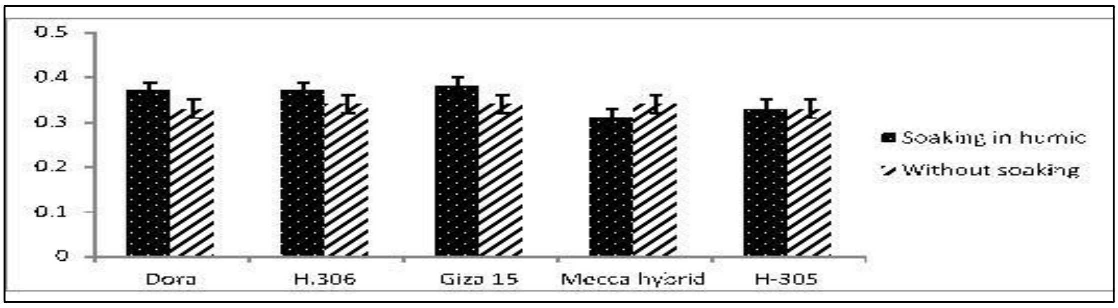

Fig. 4. Averages of root fresh weight as affected by humic acid soaking and studied sorghum cultivars.

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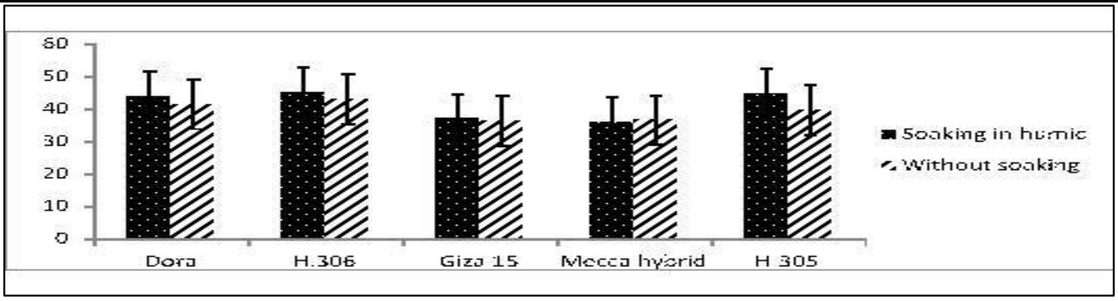

Fig. 5. Averages ofseedling height reduction as influenced by humic acid soaking and studied sorghum cultivars.

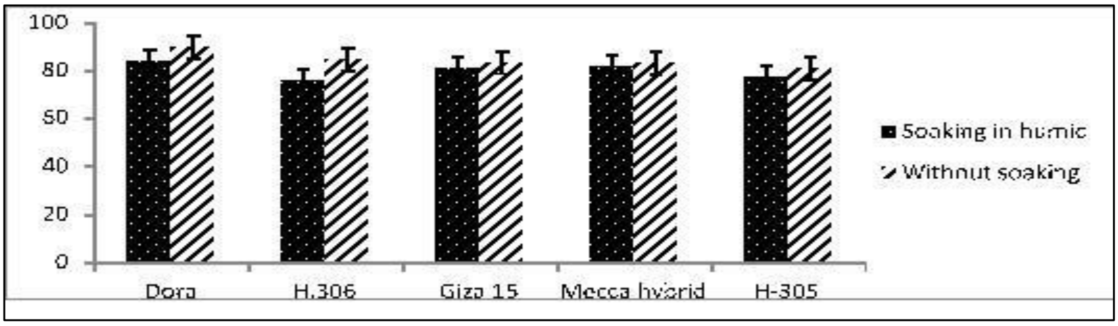

Fig. 6. Averages of relative dry weight as affected by humic acid soaking and studied sorghum cultivars.

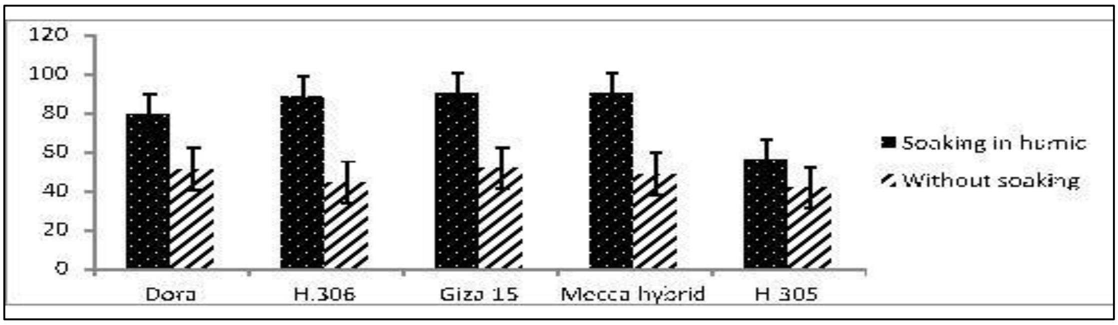

Fig. 7. Averages of germination stress tolerance index as influenced by humic acid soaking and studied sorghum cultivars.

3.4.2. Interaction between studied sorghum cultivars and salinity levels effects:

The outcomes graphically demonstrated in Fig.8revealed that the interaction between studied sorghum cultivars and salinity levels significantly affected root length. The resultsshowed that the tallest root (11.02 cm) was produced from sown Mecca hybrid at without salinity.Theweightof fresh **s**hoot as graphically demonstrated in Fig.9 as influenced by the interaction between studied sorghum cultivars and salinity levels.The highest fresh**s**hootweight (0.41 g) was produced from sown Giza 15 cultivar at without salinity.The results graphically demonstrated in Fig.10 showed the effect of the interaction between studied sorghum cultivars and salinity levels on weightof freshroot. The maximum freshrootweight (0.37 g) was recorded from sown H-306cultivar at the control treatment. The results graphically established in Fig.11 and 12, showed the effect of the interaction between studied sorghum cultivars and salinity levels onweightof dry**s**hoot and root, respectively.The results showed that the highest weightof dryshoot (0.36 g) and weightof dryroot(0.36 g)obtained from sown Dora cultivar and without salinity, respectively.The results graphically illustrated in Fig.13 indicated thatthe interaction effect between studied sorghum cultivars and salinity levels onthe percentage of seedling height reduction. The results showed that the less percentages of seedling height reduction (8.41 %) was produced from sown H-306 cultivar at salinity level of 3 dSm^{-1}.The results graphically confirmed in Fig.14 revealed theinteractioneffect between studied sorghum cultivars and salinity levels onthe percentages of relative dry weight. The results showed that the great percentages of relative dry weight (95.41 %) was produced from sown Dora cultivar at salinity level of 3 dSm^{-1}.The results graphically illustrated in Fig.15 showed the interaction effect between studied sorghum cultivars and salinity levels onstress tolerance index. The highest stress tolerance index (100 80.13 and 79.5 %) was produced from sown Mecca hybrid at without salinity and at

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salinity level of 3 dSm^{-1} without significant difference as well as between Giza 15 cultivar at salinity level of 6 dSm^{-1}, respectively.The more-salt tolerance recorded from 235461, 69239, 223550, 69029 and 23403 genotypes during seedling stage. However, sensitive to salt genotypes recorded from 22885, 233247, 237264, 237265 and 237267 **(Asfaw, 2011).** Sorghum genotypes differed from response to salinity, the medium tolerant recorded by Hegariand JS-263 medium cultivars, while Noor cultivar produced the medium sensitive and the sensitive produced from FJ-115 and PSV-4 cultivars**(Kausar et al., 2012).** The highest seedling growth characters recorded from Meko, Gambella-1107, ICSV-111 and Melkam genotypes and were more salt tolerant cultivars. However, germinating ESH-2 and Gobye genotypes were salt sensitive throughout growth stage**(Tigabo et al., 2013).** Inter cultivars genetic variation and concentration x cultivars interaction showed significant differences for all the parameters studied **(Khan et al., 2014).** The shoot length, shoot fresh and dry weight and radical decreased as the NaCl concentrations increased **(Sam et al., 2014).** Similarly, many investigators such as **Dadar et al. (2014), and Siddig and Idris (2015)** reported results**.**

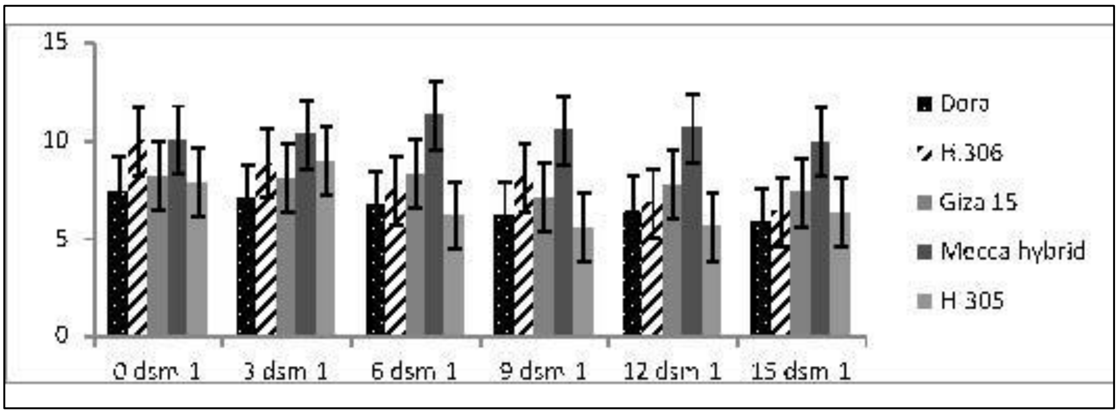

Fig. 8. Averages of root length as influenced by studied sorghum cultivars and salinity concentrations.

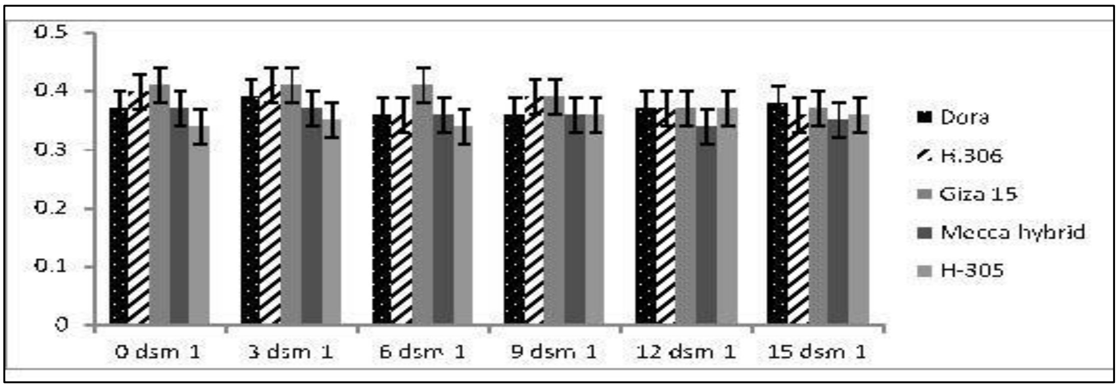

Fig. 9.Averages of shoot fresh weight as influenced by studied sorghum cultivars and salinity concentrations.

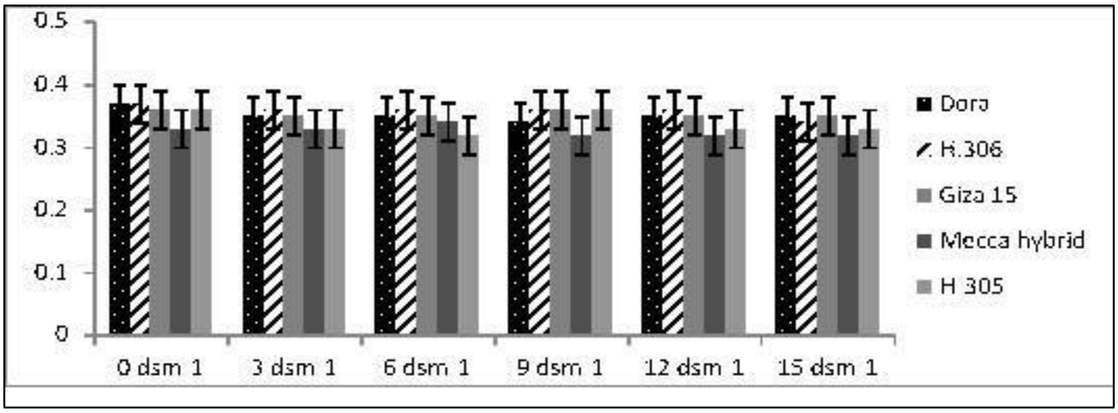

Fig. 10. Means of root fresh weight as influenced by studied sorghum cultivars and salinity concentrations.

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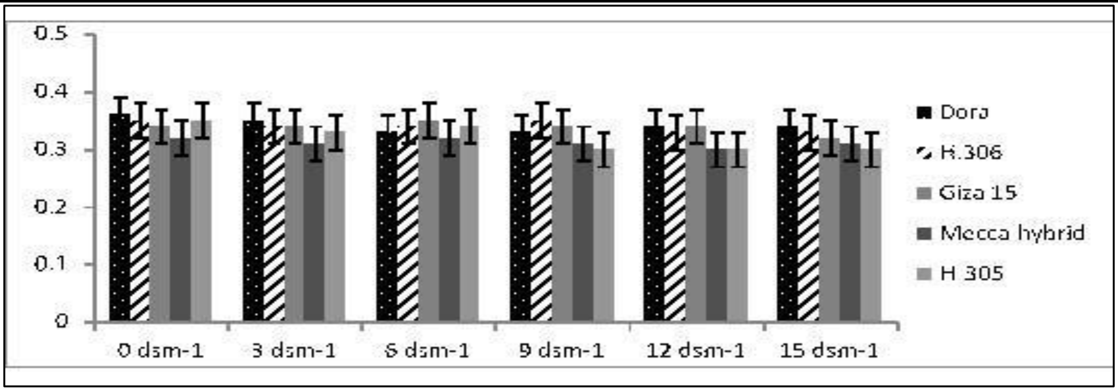

Fig. 11.Averages of shoot dry weight as influenced by studied sorghum cultivars and salinity concentrations.

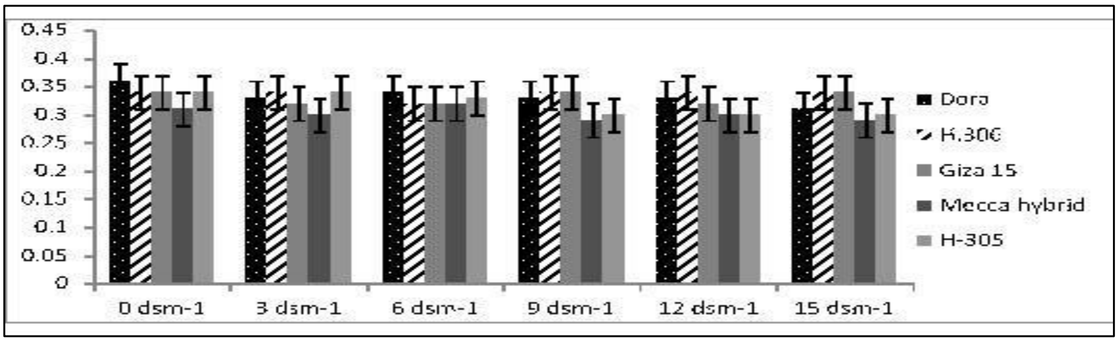

Fig. 12. Averages of root dry weight as influenced by studied sorghum cultivars and salinity concentrations.

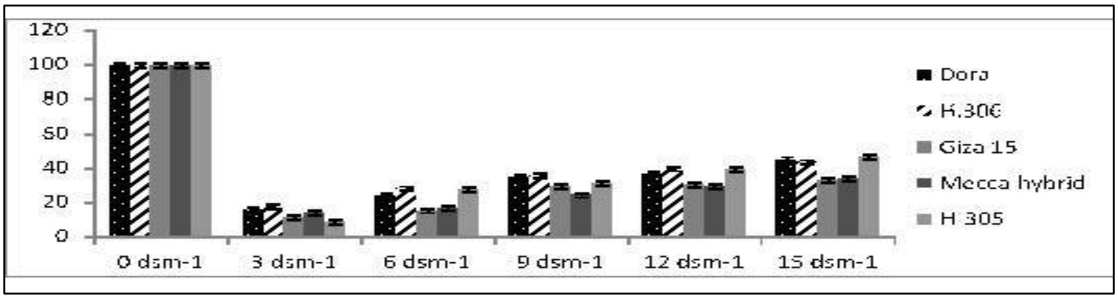

Fig. 13.Averages of seedling height reduction as influenced by studied sorghum cultivars and salinity concentrations.

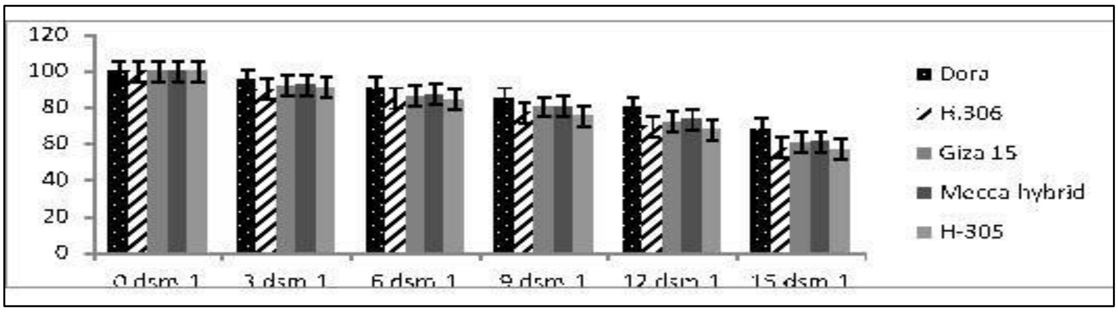

Fig. 14. Averages of relative dry weight as influenced by studied sorghum cultivars and salinity concentrations.

3.4.3. Interaction between humic acid soaking and salinity levels effects:

The results revealed that the interaction between humic acid soaking and salinity levels insignificantly influenced the length of shoot and root, weight of fresh shoot and root, the percentages of seedling height reduction and relative dry weight besides stress tolerance index.

3.4.4. Interaction between humic acid soaking x cultivars x salinity levels effects:

The results revealed that the interaction among humic acid soaking studied sorghum cultivars and salinity levels insignificantly influencedthe length of shoot and root, weight offresh shoot and root, the percentages of seedling height reduction and relative dry weight besides stress tolerance index.

IV. CONCLUSION

Accordingly, sown Mecca hybrid or Giza 15 cultivar with soaking in humic acid under salinity of 6 dSm^{-1} maximized seedling parameters and could recommended to cultivated in saline new reclaimed soils.

REFERENCES

[1] Ahmed, S. 2009. Effect of soil salinity on the yield and yield components of Mungbean. Pakistan J. Bot., 4(1):263-268.http://www.pakbs.org/pjbot/PDFs/41(1)/PJB41(1)263.pdf

[2] Almodares, A., M.R. Hadi and B. Dosti 2007. Effect of Salt Stress on Germination Percentage and Seedling Growth in Sweet Sorghum Cultivars. J. of Biological Sciences, 7(8): 1492-1495. http://scialert.net/abstract/?doi=jbs.2007.1492.1495

[3] Ali, H., Y. Akbar, Dr. Abdul Razaq and D. Muhammad 2014. Effect of humic acid on root elongation and percent seed germination of wheat seeds. International Journal of Agriculture and Crop Sciences, 7(4): 196-201.http://ijagcs.com/wp-content/uploads/2014/04/196-201.pdf

[4] Asgharipour, M. R. and M. Rafiei 2011. The Effect of different Concentrations of humic Acid on seed Germination Behavior and Vigor of barley. Australian Journal of Basic and Applied Sciences, 5(12): 610-613. http://ajbasweb.com/old/ajbas/2011/December-2011/610-613.pdf

[5] Asfaw, K.G. 2011. Effects of Salinity on Seedling Biomass Production and Relative Water Content of Twenty Sorghum (Sorghum biolor L. Moench) Accessions. Asian Journal of Agricultural Sciences, 3(3): 242-249. https://www.medwelljournals.com/abstract/?doi=rjagr.2010.24.30

[6] Behzadnejad, J. and E. Tohidinejad 2014. Ameliorative Effects of Exogenous SA on Germination of Sorghum under Salinity Stress. Journal of Applied Science and Agriculture, 9(4): 1519-1524. www.aensiweb.com/jasa/index.html

[7] Çavuşoğlu, K. and H.G. Ergin 2015. Effects of humic acid pretreatment on some physiological and anatomical parameters of barley (Hordeumvulgare l.) Exposed to salt stress. Bangladesh J. Bot. 44(4): 591-598.http://www.bdbotsociety.org/journal/journal_issue/2015%20December/14.pdf

[8] Chauhan, R. R., C. Reema, S. Alka and P.K. Singh 2012. Salt Tolerance of Sorghum bicolor Cultivars during Germination and Seedling Growth. Research Journal of Recent Sciences, 1(3):2-10. http://www.isca.in/rjrs/archive/v1/i3/1.ISCA-RJRS-2012-033_Done.pdf

[9] Dadar, A., A. Asgharzade, and M. Nazari 2014. Investigation effects of different salinity levels on sorghum Bicolor seed germination characters. Indian J. Sci. Res. 7 (1): 1031-1034.https://www.ijsr.in/upload/1819321125Microsoft%20Word%20-%20Dadar%20et%20al.pdf

[10] El-Naim, A.M., K. E. Mohammed, E. A. Ibrahim and N. N. Suleiman 2012. Impact of Salinity on Seed Germination and Early Seedling Growth of Three Sorghum (Sorghum biolor L. Moench) Cultivars. Science and Technology, 2(2): 16-20. http://article.sapub.org/10.5923.j.scit.20120202.03.html

[11] Geressu, K. and M. Gezahagne, 2008. Response of some lowland growing sorghum (Sorghum bicolor L. Moench) accessions to salt stress during germination and seedling growth. African Journal Agriculture Research, 3(1): 044-048. http://www.academicjournals.org/article/article1380877252_Geressu%20and%20Gezaghegne.pdf

[12] Gomez, K.A. and A.A. Gomez 1991. Statistical Procedures in Agricultural Research, John Wiley and Sons, New York 2nd edition, pp. 680.http://pdf.usaid.gov/pdf_docs/PNAAR208.pdf

[13] Hefny, M.M., E. M. R. Metwali and A. I. Mohamed 2013. Assessment of genetic diversity of sorghum (Sorghum bicolor L. Moench) genotypes under saline irrigation water based on some selection indices. Australian Journal of Crop Science, 7(12): 1935-1945. http://www.cropj.com/henfy_7_12_2013_1935_1945.p d f

[14] http://agris.fao.org/agris-search/search.do?recordID=PK2013000980

[15] Hussain, S. H, A. Khaliq, A. Matloop, W.M. Ashfaq and I. Afzal 2013. Germination and growth response of three wheat cultivars to NaCl salinity.Soil & Environment, 32(1): 36-43.http://agris.fao.org/agris-search/search.do?recordID=PK2013000980

[16] Islam, M.M. and M.A. Karim 2010. Evaluation of Rice (*Oryza sativa* L.) genotypes at germination and early seedling stage for their tolerance to salinity. The Agriculturists, 8 (2): 57-65.http://www.banglajol.info/index.php/AGRIC/article/view/7578

[17] ISTA Rules. (2013). Germination Sec. Chapter 5: pp. 5 – 44.

[18] Kandil A.A., A.E. Sharief, W.A.E. Abido and M.M. Ibrahim 2012. Effect of Salinity on Seed Germination and Seedling Characters of Some Forage Sorghum Cultivars. International Journal of Agriculture

Sciences, 4(7): 306-311.http://oaji.net/articles/2014/30-1394266790.pdf

[19] Kausar, A., M. Y. Ashraf, I. Ali, M. Niaz and Q. Abbass 2012. Evaluation of sorghum varieties/lines for salt tolerance using Physiological indices as screening tool. Pak. J. Bot., 44(1): 47-52.https://inis.iaea.org/search/search.aspx?orig_q=RN:43028062

[20] Khan, A., M. Ibrar and I. Ahmad 2014. A preliminary approach to halo sensitivity of Sorghum cultivars. African Journal of Plant Science, 8(1): 76-83. http://www.academicjournals.org/journal/AJPS/article-full-text-pdf/761A51342821

[21] Movafegh, S., R. R. Jadid and S. Kiabi 2012. Effect of salinity stress on chlorophyll content, proline, water soluble carbohydrate, germination, growth and dry weight of three seedling barley (Hordeumvulgare L.) cultivars. Journal of Stress Physiology & Biochemistry, 8 (4):157-168. http://cyberleninka.ru/article/n/effect-of-salinity-stress-on-chlorophyll-content-proline-water-soluble-carbohydrate-germination-growth-and-dry-weight-of-three-seedling

[22] Nazi, F., H. Reza and R. Rahman 2014. Effect of Humic Fertilizer on Germination of Wheat Seeds under Drought Stress. Advances in Bioresearch 5(4): 98-102. http://soeagra.com/abr/abrdec2014/18.pdf

[23] Netondo, G.W., J.C.Onyango and E. Beck 2004. Sorghum and salinity:II.Gas exchange and chlorophyll fluorescence of sorghum under salt stress. Crop Sci. 44: 806-811.https://dl.sciencesocieties.org/publications/cs/abstracts/44/3/806?access=0&view=pdf

[24] Noreen, S. and M. Ashraf 2008. Alleviation of adverse effects of salt stress on sunflower (Helianthus annuus L.) by exogenous application of salicylic acid: Growth and photo-synthesis. Pakistan Journal Botany, 40(4): 1657-1663. http://sa.indiaenvironmentportal.org.in/files/Pakistan%20Journal.pdf

[25] Patil, R. 2010. Effect of Potassium Humate and Deproteinised Juice (DPJ) on Seed Germination and Seedling Growth of Wheat and Jowar. Annals of Biological Research, 1 (4): 148-151. http://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=1238755

[26] Russell, D.F. 1986. MSTAT-C computer based data analysis software Crop and Soil Science Department, Michigan State University USA. https://msu.edu/~freed/disks.htm

[27] Sam, A., A. Y. Edris and M.S.A. Abo 2014. Effect of Salinity on Seed Germination and Seedling Growth of Pearl millet (Pennisetumglaucum L.) and Sorghum (Sorghum bicolor L.), Journal of Plant and Pest Science, 1 (1): 01-08. http://journals.sfu.ca/jpps/index.php/jpps/article/viewFile/4/10

[28] Saroj, M. and D. Soumana, 2014. Salt stress induced changes in growth of germinating seeds of Vigna mungo(L.) Hepper and Vigna aconitifolia (Jacq.) Marechal. IOSR Journal of Agriculture and Veterinary Science, 7. (4):44-48. http://www.recentscientific.com/sites/default/files/4336.pdf

[29] Siddig, A.A.M. and A.A.Y. Idris 2015. Response of Sorghum (Sorghum bicolor L.) Cultivars to Salinity Levels at Early Growth Stages. J. of Agricultural Science and Engineering, 1(1): 11-16. http://www.publicscienceframework.org/journal/jase

[30] Snedecor GW and WG. Cochran 1980. Statistical Methods. 7ThEd. Iowa State University Press, Iowa, USA, ISBN-10: 0-81381560-6, Pp: 507. https://www.amazon.com/Statistical-Methods-Seventh-isbn-0813815606/dp/B0012S4NIE

[31] Sun, Y., G. Niu1, P. Osuna, L. Zhao, G. Ganjegunte, G. Peterson, J. R. Peralta-Videa, and J. L. Gardea-Torresdey 2014. Variability in Salt Tolerance of Sorghum bicolor L. Agricultural Science, 2(1): 9-21.www.iosrjournals.orgwww.iosrjournals.org

[32] Tabatabaei, S. A., and A. Anagholi 2012. Effects of salinity on some characteristics of forage sorghum genotypes at germination stage. International Journal of Agriculture and Crop Sciences, 4(14): 979-983.http://ijagcs.com/wp-content/uploads/2012/09/979-983.pdf

[33] Tigabo, E., M. Andargie and K. Tesfaye 2013. Genotypic Variation for Salinity Tolerance in Sorghum (Sorghum bicolor (L.) Moench) Genotypes at Early Growth Stages. Journal of Stress Physiology & Biochemistry, 9 (2): 253-262. http://cyberleninka.ru/article/n/genotypic-variation-for-salinity-tolerance-in-sorghum-sorghum-bicolor-l-moench-genotypes-at-early-growth-stages

[34] Wei G. and W. Qing-Xiang 2011. Effects of seed soaking with humic acid on wheat seedlings antioxidant system under salt-alkali stress. YingyongShengtaiXuebao, 22 (10):2539. http://connection.ebscohost.com/c/articles/69910778/effects-seed-soaking-humic-acid-wheat-seedlings-antioxidant-system-under-saltalkali-stress

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Screening of sugarcane genotypes for resistance against sugarcane early shoot borer, *Chilo infuscatellus* Snellen

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***Abstract*—*In vivo experiment was conducted to screen fifty six genotypes for resistance to early shoot borer, Chilo infuscatellus Snellen at Zonal agricultural research station, V.C farm, Mandya during 2014-2015. The per cent incidence of ESB at different days after planting was varied among fifty six screened genotypes. The highest per cent incidence was recorded at 60 DAP (0.00 to 41.29) followed by at 30 DAP (0.00 to 20.15) and the per cent incidence at 90 DAP ranged from 0.00 to 14.24 and lowest per cent incidence was recorded at 120 DAP (0.00 to 4.40). Based on the cumulative incidence, 47 genotypes were categorized as less susceptible to C. infuscatellus. Nine genotypes were found moderately susceptible to C. infuscatellus. However, among less susceptible genotypes lowest cumulative incidence of 0.00 per cent was recorded in genotypes, 09-60-06. Whereas highest cumulative incidence of 29.86 per cent was recorded in moderately susceptible genotype, 10-38-06.*

Keywords*— *Chilo infuscatellus, Early shoot borer, Genotypes, Per cent incidence, Resistance.

I. INTRODUCTION

In sugarcane based on feeding habit, the insect pests are broadly classified as borers, sucking pests, subterranean pests, defoliators and non-insect pests. Nine species of lepidopteran pests regularly damage sugarcane (David, 1977) in India. Among the borers, early shoot borer (ESB), *Chilo infuscatellus* Snellen is an important pest and is widely distributed in all sugarcane growing areas in the country. It infests the crop during early stages prior to internode formation. It also infests millable cane during years of drought or scanty rainfall. Borer infestation during the germination phase kills the mother shoots resulting in drying of entire clump and creating gaps in the field. But when the attack occurs during tillering phase, the clumps do not get killed although the crop stand is affected by mortality of tillers and loss in yield due to late formed canes with reduced weight and sucrose contents (Krishnamurthy Rao, 1954).

It has been computed that the shoot borer destroys 23-65 per cent mother shoots and 6.4, 27.1 and 75 per cent primary, secondary and tertiary tillers respectively (Doss, 1956; Khan and Krishanamurthy Rao, 1956). As reported by Patil and Hapase (1981) the ESB can cause a loss to the extent of 22-33 per cent in yield, 12 per cent in sugar recovery, two per cent in commercial cane sugar and 27 per cent in jaggery.

Several control methods have been evaluated from time to time. Among the different management strategies, the use of resistant genotype is one of the important components of IPM. So different genotypes have been screened under natural conditions to identify the less susceptible genotype for early shoot borer. Plant resistance is the most economical and desirable method in the management of crop pests. The use of resistant genotypes has proven to be the most efficient way to reduce the economic damage caused by early shoot borer. Among the screened genotypes the mechanism that imparts resistance to early shoot borer were investigated. Knowledge on resistance mechanism and associated factors involved is essential for effective utilization of source of resistance which is useful in future breeding programme.

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II. MATERIAL AND METHODS

Preliminary study on field screening of 56 genotypes was done to identify the less susceptible clones against ESB, *C. Infuscatellus* during 2014 at Zonal Agricultural Research Station, V.C Farm, Mandya. Three budded setts of 56 genotypes with known check CoVC 99463 and Co 86032 were obtained from Plant Breeding Department, AICRP on Sugarcane, Mandya.

The experiment was laid out in a randomised block design with fiftysix genotypes and was replicated twice. All agronomic practices were carried out as per the package of practices recommended for sugarcane cultivation by UAS, Bangalore (Anon., 2011).

Based on the per cent cumulative incidence of ESB, genotypes were graded according to Rao and Krinshamoorthy (1973).

Dead heart counts

Number of dead hearts caused by early shoot borer out of the total number of tillers observed in all the entries at 30, 60, 90 and 120 days after planting (DAP) was recorded. After each count, the dead hearts were pulled out to avoid counting them later on.

The per cent incidence of ESB, *Chilo infuscatellus* was calculated by using the formula

$$\text{Per cent incidence } = \frac{Number\ of\ dead\ hearts}{\text{Total number of tillers}} X\ 100$$

Cumulative per cent incidence of ESB, *Chilo infuscatellus*

The cumulative per cent incidence was worked out by relating the progressive total of infested tillers (deadhearts) in proportion to the total number of tillers (Sithananthan, 1973) at 120 DAP.

Based on the cumulative per cent incidence, the sugarcane varieties were grouped in to three categories (Rao and Krinshamoorthy, 1973).

Grade/Category	**Cumulative per cent incidence**
Less susceptible (LS)	0-15 per cent
Moderately susceptible (MS)	15-30 per cent
Highly susceptible (HS)	>30 per cent

Table.1: Cumulative incidence of ESB, C. infuscatellus in different Sugarcane genotypes during 2014-15 at Zonal Agricultural Research Station, V.C. Farm, Mandya

Sl. No	**Genotypes**	**% Incidence of ESB**				**Cumulative Incidence**
		30 DAP	**60 DAP**	**90 DAP**	**120 DAP**	
1	09-60-06	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
2	Co 0323	0.00(0.00)	0.96(3.66)	0.36(2.24)	0.42(2.40)	0.87(6.68)
3	09-65-02	0.00(0.00)	0.00(0.00)	1.72(6.90)	0.85(4.73)	1.29(8.38)
4	11-02-09	0.00(0.00)	1.96(7.36)	0.50(2.64)	0.24(1.82)	1.35(8.62)
5	7-62-01	0.00(0.00)	1.72(6.73)	1.01(5.23)	0.84(4.79)	1.78(9.79)
6	10-28-16	2.18(5.53)	1.13(5.59)	0.00(0.00)	0.39(2.31)	1.84(9.78)
7	09-60-28	0.00(0.00)	0.00(0.00)	2.70(8.52)	1.10(5.39)	1.90(10.06)
8	10-28-08	2.38(5.79)	0.81(3.35)	0.80(4.68)	0.74(4.50)	2.36(10.87)
9	11-11-06	0.00(0.00)	4.11(10.70)	0.60(2.89)	0.48(2.58)	2.59(11.84)
10	Co62175	0.00(0.00)	2.33(8.04)	1.59(6.61)	1.37(6.15)	2.64(12.08)
11	09-61-05	1.09(3.89)	1.29(5.97)	3.06(8.84)	0.76(4.58)	3.10(12.84)
12	10-12-14	1.47(4.54)	2.09(7.62)	1.50(4.58)	1.19(4.08)	3.12(13.15)
13	009-64	0.00(0.00)	0.00(0.00)	4.86(10.65)	1.62(6.72)	3.24(12.82)
14	12-41-25	0.00(0.00)	4.09(10.42)	2.27(7.76)	0.50(2.62)	3.43(13.81)
15	10-33-16	2.68(8.60)	0.41(2.39)	3.40(9.60)	1.17(5.71)	3.83(14.55)
16	VCF 0517	1.11(3.94)	2.85(8.93)	3.04(9.19)	1.22(5.80)	4.11(15.09)
17	10-17-08	4.05(7.60)	0.50(2.64)	4.58(11.23)	0.80(4.62)	4.97(16.53)
18	09-10-03	4.80(11.47)	1.25(5.85)	2.97(9.05)	1.13(5.60)	5.08(16.82)
19	10-38-07	2.38(5.79)	1.25(4.18)	4.20(10.85)	2.59(8.50)	5.21(16.79)
20	7-82-10	1.57(4.68)	6.93(13.66)	1.20(5.76)	1.09(5.49)	5.39(17.01)
21	11-23-05	0.00(0.00)	9.11(16.00)	1.13(5.55)	0.71(3.13)	5.47(17.27)
22	10-65-01	5.32(11.93)	0.60(2.89)	4.51(10.93)	0.75(4.56)	5.59(17.68)

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Sl. No	Genotypes	% Incidence of ESB				Cumulative Incidence
		30 DAP	60 DAP	90 DAP	120 DAP	
23	10-20-06	8.55(15.61)	1.96(7.37)	0.75(3.22)	0.47(2.56)	5.86(18.07)
24	10-43-06	3.04(9.17)	3.34(9.61)	4.83(11.57)	2.18(7.66)	6.69(19.32)
25	10-14-17	0.00(0.00)	4.91(11.66)	6.59(13.61)	1.96(7.39)	6.73(19.41)
26	08-04-01	3.45(6.99)	3.84(10.36)	1.82(5.05)	4.40(11.11)	6.75(19.42)
27	10-35-04	0.98(3.70)	9.08(16.11)	3.27(9.39)	1.58(6.59)	7.45(20.45)
28	09-65-04	4.43(10.66)	4.29(10.98)	4.38(11.09)	2.37(7.29)	7.74(20.83)
29	11-11-02	6.95(13.97)	3.75(10.19)	3.62(9.82)	1.45(6.22)	7.88(20.83)
30	09-63-01	7.79(14.76)	1.60(6.63)	4.28(10.40)	2.13(7.35)	7.90(20.64)
31	10-38-15	0.00(0.00)	7.83(14.60)	5.48(12.26)	2.50(8.36)	7.91(21.04)
32	09-29-04	6.20(12.99)	5.67(12.65)	3.24(9.23)	0.82(3.38)	7.96(21.06)
33	09-61-07	0.00(0.00)	6.12(13.05)	8.54(15.58)	1.43(6.26)	8.05(21.20)
34	09-30-01	6.67(13.64)	7.59(14.60)	1.81(6.94)	0.87(4.79)	8.46(21.76)
35	10-14-16	4.51(10.99)	2.25(7.91)	9.62(16.15)	2.49(7.34)	9.44(22.40)
36	07-21-04	8.71(15.57)	2.34(7.85)	4.89(11.57)	3.24(9.52)	9.59(22.97)
37	07-10-02	2.04(7.55)	10.69(17.48)	5.53(12.37)	2.19(7.78)	10.57(24.41)
38	10-28-02	1.47(4.54)	12.47(18.66)	2.87(8.95)	3.26(9.51)	10.03(23.72)
39	09-60-10	11.23(17.94)	5.98(12.88)	2.24(7.83)	1.07(5.40)	10.26(24.04)
40	09-60-08	11.31(17.91)	3.03(8.65)	6.70(13.77)	0.00(0.00)	10.52(24.15)
41	09-60-04	14.69(20.25)	3.81(10.19)	2.02(7.25)	0.93(5.03)	10.72(24.50)
42	10-14-15	11.30(17.97)	5.76(12.74)	4.82(11.64)	1.12(3.95)	11.50(25.42)
43	10-20-08	13.07(19.46)	8.30(15.33)	1.22(4.13)	1.48(4.55)	12.03(26.09)
44	10-38-08	0.00(0.00)	7.13(14.20)	14.24(20.36)	3.73(10.13)	12.55(26.61)
45	10-57-07	7.68(14.41)	11.52(17.93)	4.36(9.77)	1.74(4.94)	12.65(25.83)
46	09-61-02	3.70(7.25)	20.76(24.89)	3.62(10.03)	1.61(6.66)	14.85(28.99)
47	10-58-05	9.96(16.76)	15.54(20.73)	3.24(9.18)	2.04(7.46)	15.38(29.55)
48	10-17-05	11.90(18.46)	10.01(16.88)	5.79(12.74)	3.07(9.14)	15.39(29.57)
49	07-06-05	4.89(11.28)	15.00(20.36)	11.78(17.75)	1.30(5.68)	16.48(30.30)
50	10-33-33	7.11(14.15)	23.28(26.33)	4.31(11.00)	0.81(3.36)	17.75(31.76)
51	10-20-11	19.76(23.94)	13.44(18.70)	3.19(9.34)	1.35(5.94)	18.87(32.34)
52	08-15-06	16.30(21.66)	26.43(28.14)	7.97(14.62)	1.06(3.85)	25.88(38.73)
53	06-09-03	20.85(24.78)	25.47(27.64)	8.01(14.98)	0.57(2.81)	27.45(39.77)
54	10-38-06	2.78(6.26)	41.29(36.52)	12.84(18.89)	2.81(8.78)	29.86(41.04)
55	Co99463	5.16(12.04)	0.49(2.60)	2.86(8.93)	1.15(5.66)	4.83(16.41)
56	Co 86032	10.57(15.86)	27.92(28.97)	4.55(11.00)	1.74(6.79)	22.39(35.09)
SEm±		2.5	3.7	1.9	0.7	1.5
CD @ P=0.05		7.2	10.5	5.4	2.1	4.2

III. RESULTS AND DISCUSSION

Studies on the field screening of 56 genotypes were carried out to identify the less susceptible genotypes against ESB, *Chilo infuscatellus*. Genotypes were graded as less susceptible (LS), moderately susceptible (MS) and highly susceptible (HS) based on the per cent incidence of ESB at 30, 60, 90, 120 DAP and based on cumulative per cent incidence of ESB upto 120 days after planting.

The overall per cent incidence of ESB at 30 DAP in all the screened genotype ranged from 0.00 to 20.85. Based on the per cent incidence of ESB at 30 days after planting, fifty three genotypes were categorized into less susceptible category including two standard checks *viz.*, Co 99463 (5.16%) and Co 86032 (10.57%). Among these 53 less susceptible genotypes *viz.*, 09-61-07, 009-64, 10-14-17, 09-60-06, 09-60-28, 10-38-08, 11-02-09, 11-23-05, 7-62-01, 12-41-25, 11-11-06, 10-38-15, Co323, 09-65-02 and

Co62175 recorded zero per cent incidence at 30 days after planting. Whereas the genotypes *viz*., 08-15-06 (16.30%), 10-20-11 (19.76%) and 06-09-03 (20.85%) were classified into moderately susceptible group. None of the genotypes fell under highly susceptible category (Table 1). It was found that most of the genotypes were found free from the incidence of ESB. This might be due to fast growth of the genotypes which might have helped the genotypes to escape from the ESB incidence. Similar observations were also made by Gupta and Avasthy (1954c); Kalra and Chaudhary (1964).

Incidences of ESB at 60 DAP in all the screened genotypes ranged from 0.00 to 41.29%. Among the fifty six genotypes, 48 genotypes including the standard check Co 99463 (0.49%) were graded as least susceptible (LS) and the genotypes 009-64, 09-60-06, 09-60-28 and 09-65-02 found to be highly resistant to ESB with the 0.00 per cent incidence. Six genotypes 10-58-05 (15.54%), 09-61-02 (20.76%), 10-33-33 (23.28 %), 06-09-03 (25.47%) and 08-15-06 (26.43%) including standard check Co 86032 (27.92%) fell under moderately susceptible category. The genotype 10-38-06 with 41.29% of ESB incidence was classified as highly susceptible group at 60 days after planting. The genotypes 08-15-06 and 06-09-03 which were moderately susceptible at 30 days after planting remained as MS group even at 60 days after planting (Table 1). The per cent incidence of ESB had increased at sixty days after planting. The per cent incidence steadily increased from 30th to 90th day and thereafter it declined. This is in confirmation with the findings of Sithanatham *et al*., 1975 wherein the young crop of 30 to 60 days age is reported to be susceptible to this pest. Rao and Siva (1962) also reported *Chilo infuscatellus* Snellen, preference to 45 days old plants for ovipostion. This is in line with the findings of present study.

At 90 days after planting, the per cent incidence of ESB in all the genotypes decreased. The incidence ranged from 0.00 to 14.24%. All the genotypes including both the checks CoVC 99463 (2.86%) and Co 86032 (4.55%) were found less susceptible (LS). The incidence of ESB was maximum in the genotype 10-38-08 (14.24%). The genotypes10-28-16 and 09-60-06 were found to be less susceptible to ESB at 90 days after planting by registering 0.00 per cent incidence (Table 1).

Similarly at 120 days after planting the per cent incidence of ESB decreased further. The per cent incidence of ESB at 120 DAP in all the screened genotype ranged from 0.00 to 4.40%. All the 56 genotypes fell under less susceptible categories including two standard checks with incidence ranging from 0.00 to 4.44 per cent. Highest incidence was recorded in genotype 08-04-01 (4.40%). The genotypes 09-60-06 and 10-28-16 registered 0.00 per cent incidence at 120 days after planting (Table 1). At 90 and 120 days after planting, the per cent incidence of ESB in all the genotypes decreased. All the genotypes including both standard checks Co 99463 (2.86%) and Co 86032 (4.55%) were found least susceptible (LS). It is due to the fact that all genotypes have inherent capacity to produce more number of tillers, thereby reducing the shoot borer incidence. This is in agreement with the findings of Doss (1956), Khanna (1956) and Rao and Rao (1961).

The overall cumulative per cent incidence of ESB in all the screened genotype ranged from 0.00 to 29.86%. Among the 56 genotypes, 47 genotypes were graded as less susceptible including the standard check Co 99463 (4.83%). The genotype 09-60-06 was found to be highly resistant to ESB with cumulative ESB incidence of 0.00 per cent. The cumulative ESB incidence was less than two percent in five genotypes (09-65-02, 11-02-09, 7-62-01, 10-28-16 and 09-60-28). The nine genotypes fell under the category of moderately susceptible including the check Co 86032 (22.39%). None of the genotypes fell under highly susceptible category. The overall cumulative incidence of ESB ranged from 0.00 to 29.86 per cent. The highest cumulative incidence of ESB was recorded in genotypes 08-15-06 (25.88%), 06-09-03 (27.45%) and 10-38-06 (29.86%) (Table 1). The highest cumulative incidence of ESB was recorded in genotypes 08-15-06 (25.88%), 06-09-03 (27.45%) and 10-38-06 (29.86%). Similar results were also reported by the earlier workers Rajendran and Giridharan (2001), Kumar *et al*., 2002 and Bhavani *et al*., 2011.

IV. CONCLUSION

The results of field screening of different genotypes for resistance to ESB revealed that the genotypes *viz*., 009-64(3.44%), 10-65-01(5.59%), 10-65-01(3.83%), 10-17-08(4.97%), 10-57-07(12.65%), 07-10-02(10.57%), 10-28-02(10.03%) and 09-61-02(14.85%) which recorded less than 15 per cent of incidence were graded as least susceptible while genotypes 10-17-05(15.39%), 07-06-05(16.48%), 10-33-33(17.75%), 10-38-06(29.86%), 08-15-06(25.88) and 06-09-03(27.45) have recorded 15 to 30 per cent incidence of ESB were graded as moderately susceptible (MS), whereas check Co 99463(4.83%) and Co 86032(22.39%) have recorded per cent incidence of ESB.

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REFERENCES

[1] DAVID, H., 1977, Pests of sugarcane and their control. *Pestol*., **1**: 15-19.

[2] DOSS, S. N. J., 1956, Incidence of sugarcane borers in Nellikuppam factory zone, South Arcot, Madras state. Proc. Int. Soc. Sug. Cane Technol., 9: 880-895.

[3] KHAN, M. Q. AND RAO, B. H. K., 1956, Assessment of loss due to Chilotraea infuscatellus Snell. Proc. Int. Soc. Sug. Cane Technol., 9: 870-879.

[4] PATIL, A. S. AND HAPASE, D. G., 1981, Research on sugarcane borers in Maharashtra. Proc. National Symp. on Stalk Borer, Karnal, pp.165-1758.

[5] ANONYMOUS, 2011, Zonal Research and Extension Programme Workshop. University of Agriculture Sciences, Bangalore.

[6] RAO, S. AND KRISHNAMURTHY RAO M. M., 1973, Studies on loss in yield of sugarcane due to shoot borer incidence, Chilo infuscatellus snellen (Pyralidae : Lepiodoptera). Indian Sug., 22: 867-868.

[7] SITHANANTHAM, S., 1973, Performance of some new organic insecticides in the control of sugarcane shoot borer Chilo infuscatellus snellen. Indian Sug., 22: 933-938.

[8] GUPTHA, B. D. AND AVASTHY, P. N., 1957, An epidemic of cane borer, Chilo tumidicostalis Hmpsn. Indian J. Sug. Cane Res. Dev., 22: 3.

[9] KALRA, A. N. AND CHAUDHARY, 1964, Infructuous attack of top borer of sugarcane. Indian J. Sug. Cane Res. Dev., 8: 261-264.

[10] SITHANANTHAM, S., DURAI, D. AND MUTHUSAMY, S., 1975, Incidence of sugarcane shoot borer in relation to planting time. Indian Sug., 24: 867-870.

[11] RAO, AND SIVA, D. V., 1962, Studies on the resistance of sugarcane to the early shoot borer, Chilo infuscatellus Snell. M.Sc Thesis, Andhra University, waltair.

[12] KHANNA, K. L., 1956, On tiller mortality and compensation due to borers in sugarcane. Proc. Int. Soc. Sug. Cane Technol., 9: 936-971.

[13] RAO, N. V. S. AND RAO, C. K., 1961, Preliminary studies on varietal resistance of sugarcane to infestation by the early shoot borer, Chilotraea infuscatellus Snell., Andhra Agri. J., 8: 140-146.

[14] RAJENDRAN, B. AND GIRIDHARAN, S., 2001, Field screening of sugarcane clones against shoot borer. Indian. Sug. 51(8): 515-516.

[15] KUMAR, A., SINGH, A. K., SINGH, A. P., SINGH, S. K., AND SINGH, P. R., 2002, Response of sugarcane world germplasm against shoot borer (Chilo infuscatellus Snellen) infestation. Coop. Sug., 34(2): 131-134.

[16] BHAVANI, B., REDDY, K. D., RAO, N. V. AND LAKSHMI, M. B., 2012, Biochemical basis for antibiosis mechanism of resistance in sugarcane to early shoot borer, Chilo infuscatellus Snellen. Trop. Agr. Research., 23(2): 126 – 141.

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Effect of bioformulations of Phosphate Solubilizing Bacteria (PSB) on the Growth and Biochemical Characters of the *Gossypium Hirsutum* and *Zea Mays*

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***Abstract*— *Biofertilizers offer a new technology to Indian agriculture holding a promise to balance many of the short comings of the conventional chemical based technology. They are usually prepared as carrier based inoculants containing effective microorganisms. The present study was aimed at to study the nursery performance of different formulations of PSB in maize and cotton plants. The selected PSB was mass multiplied in the laboratory and incorporated into the nursery soil through different carrier material such as coirpith, vermicompost, organic manure, lignite and vermiculite. The effect of bioinoculants on the growth and biochemical characters were studied from the control and treated seedling of Gossypium hirsutum and Zea mays. The significant difference was observed in the growth and biochemical characters in both Gossypium hirsutum and Zea mays. The effect was differed with reference to the nature of carrier materials used for the preparation of bioformulations. The results indicated that the bioformulation prepared by composted coirpith had superior in plant growth and development.*

***Keywords*– PSB, carrier, bioinoculants, crop response, cotton, maize.**

I. INTRODUCTION

Biofertilizers are the bioinoculants of specific beneficial microorganisms that promote the growth and development of plant crops by converting the unavailable form of nutrients into available form. These biofertilizers also improve the soil fertility (Sivasakthivelan and Saranraj, 2013). They can be used as supplements of chemical fertilizers; they were relatively inexpensive and renewable sources of plant nutrient. Biofertilizers were selected strains of microorganisms which were beneficial to the growth of the plants. These microorganisms were cultured in laboratory, mixed with suitable carrier materials and then applied to the fields. They maintain soil health, minimizes pollution of the environment by lowering the use of chemicals (Tripti and Anshumali, 2012).

Phosphate solubilizing bacteria play an important role in converting low grade insoluble inorganic phosphate sources like rock phosphate, bone meal, basic slag and the chemically fixed soil phosphorus into available form. Therefore, the use of phosphate solubilizing microbes in agricultural practice would not only offset the high cost of manufacturing phosphatic fertilizers but would also mobilize insoluble phosphorus in the fertilizers and soils to which they are applied. The mechanism of solubilization of insoluble phosphate is ability to secrete organic acids and phosphatase enzyme (Mahantesh *et al.,* 2015).

The carrier based inoculants produced in India generally have a short shelf life, poor quality, high contamination and unpredictable field performance. High quality biofertilizers would be expected to have higher population of desired microorganism, sufficient viability, and remain uncontaminated for longer period of storage. Today, advances in inoculants technology were concerned with improving quality, extending useful shelf life and developing new formulations for use under less favorable conditions. Suggested that liquid inoculants and alginate based granular formulations were two important new inoculants formulations which were an alternative to peat or lignite based ones (Brahmaprakash and Sahu, 2012). A cost effective carrier materials which were nonpolluting, biodegradable, non-toxic, capable of maintaining high viable count and long shelf life amendable to nutrient supplement and high water holding capacity (Gomare *et al.*, 2013).

Phosphate solubilizing bacteria of *Pseudomonas* and *Bacillus* species are producing phytohormones *i.e*. auxins, inhibition of deleterious pathogens or nutrient mobilization and ammonification. The plant growth benefits due to the addition of PSB include increases in germination rate, root growth, yield, leaf area, chlorophyll content, tolerance to drought, shoot and root weight. Such groups of bacteria are termed as phosphate solubilizing bacteria and inoculation with PSB as biofertilizers enhances P accumulation and biomass production of plants (Abbasi *et al*., 2015). Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with maize straw and sugarcane husk increased plant height and fresh weight and protein, proline, sugar contents and antioxidant activities. Inoculation of PSB further increased plant growth, physiology and yield characters over single inoculation with different carriers. It is inferred that carrier based biofertilizer effectively increased growth, maintained osmotic balance and enhanced the activities of antioxidant enzymes and yield parameters (Hassan and Bano, 2016).

The cultivation of cotton and maize requires higher amount of phosphatic fertilizer for their growth as well as sustainable yield. But the added phosphatic fertilizers are rapidly transformed to fixed form or unavailable forms. This is mainly due the nature of soil. These fixed forms of phosphates are unavailable to plants. It is directly affected the plant growth, development and yield of cotton and maize. These problems can be easily solved by application Phosphate Solubilizing Bacteria (PSB). These beneficial microorganism can able to soluble the fixed forms of phosphate into soluble/available form and can make it available to the plants. The application/amendment of PSB with cotton and maize will increase the soil fertility and crop yield. Further, the sustainable yield can be achieved by application biofertilizers like PSB.

II. MATERIAL AND METHODS

2.1. ISOLATION AND MASS MULTIPLICATION OF BIOINOCULANTS

Isolation and enumeration of PSB was carried out by dilution plate technique using hydroxy patite medium. The selected PSB isolate was multiplied in the culture flask with nutrient broth. The broth culture was mixed with sterilized carrier materials. The viable count in the inoculum was checked before mixing the inoculums with carrier materials. Various organic materials such as composted coir pith, lignite, organic manure, vermicompost and vermiculite were used as carrier for the mass multiplication of PSB. The carrier materials were sterilized, sieved and maintained proper water content in the carrier materials. The mass multiplied liquid culture was mixed with the carrier materials and used for nursery experiments.

2.2. NURSERY EXPERIMENT

A nursery experiment was conducted to study the nursery performance of different formulations of PSB in *Gossypium hirsutum* and *Zea mays.* The seeds with uniform size, colour and weight were chosen for the experimental purpose and surface sterilized with 0.1% $HgCl_2$ for 1 minute and thoroughly washed with distilled water 3-5 times. Seeds were pre-soaked for 12 hours in distilled water and were sown in sterilized soil mixture. The soil mixture was prepared by mixing black soil, red soil and sand in the ratio of 1:1:1. The PSB formulations were applied 10g each at top soil of the pots. The treatment details were: 1.Control 2.Coir pith 3.Vermicompost 4.Organic manure 5.Lignite 6.Vermiculite. The growth characters such as seed germination percentage, germination index, seedling vigour index I and II, shoot length, root length, number of leaves, fresh weight and dry weight were analyzed in the treated and untreated plants. Biochemical characters such as total Chlorophyll (Wellburn and Lichtenthaler, 1984), Protein (Lowry *et al*., 1951), glucose (Jayaraman 1981), amino acid (Jayaraman, 1981) and NR activity (Jaworski, 1971) were estimated.

2.3. STATISTICAL ANALYSIS

The data were reported as mean ± SE and in the figure parentheses represent the per cent activity. Values are expressed as means ± standard deviation of three independent data.

III. RESULTS

3.1 ISOLATION AND MASS MULTIPLICATION OF BIOINOCULANTS

Phosphate solubilizing bacteria (PSB) were isolated based on the solubilization zone production in the hydroxy apatite medium from the rhizosphere soils of Brinjal. Totally 10 PSB strains were isolated. Based on preliminary screening, PSB strain BP1 was selected as elite strain and used for further studies. The selected BP1 strain was mass multiplied in the laboratory with different carrier materials and used for nursery experiments. The carrier materials such as composted coirpith, vermicompost, organic manure, lignite and vermiculite were used for mass multiplication. A viable count ranged from 10^9 to 10^{10} ml^{-1} preferred for the preparations of bioformulation. Three day old culture was mixed with the sterilized carrier materials and used for the nursery experiment.

3.2 EFFECT OF PSB FORMULATIONS ON THE GROWTH CHARACTERS

Effect of bioformulations on the growth characters were studied from the control and treated seedlings of

Gossypiyum hirsutum and *Zea mays*. The germination rate was higher in the plants treatment with coirpith formulation over the control plants. Among the bioformulations, the low germination rate was organic manure in *G. hirsutum* and vermiculite formulation in *Z. mays*. The germination index was varied according to the bioformulation treatment. Little difference was shown in germination index among different bioinoculant treatments but the effect was higher to the control. The values of the seedling vigour index of the *Gossypium hirsutum* and *Zea mays* varied according to the bioformulation treatment. The highest value of seedling vigour index was observed in the plants treated with coirpith formulation. In both *G. hirsutum* and *Z. mays*, seedling vigour index II was significantly increased in the treatment of coirpith formulation followed by vermicompost formulation. The result revealed that the shoot length was higher in the plants of *G. hirsutum* and *Z. mays* treated with PSB with coirpith as carrier followed by vermicompost formulation. The effect was least with vermiculite bioformulation and control. Results also indicated that the root length was higher in all treated plants over control. It was observed that the plants grown with coirpith formulation produced taller roots than other treatments in both *G. hirsutum and Z. mays*. The number of leaves per plant was significantly influenced by bioformulation treated plants than untreated control plants. The highest number of leaves was found in plants treated with coirpith formulation in both *G. hirsutum* and *Z. mays.* Among different formulation tested, coirpith formulation significantly increased the plant fresh weight followed by vermicompost and least in vermiculite formulation. Like fresh weight, same trend was observed in plant dry weight also (Table 1 and table 2).

3.3. EFFECT OF PSB FORMULATIONS ON THE BIOCHEMICAL CHARACTERS

In the nursery experiment, the inoculation of PSB with *Gossypium hirsutum* and *Zea mays* increased the biochemical characters such total chlorophyll, protein content, aminoacids content, glucose content and NR activity. The total chlorophyll content was higher in plants treated with coirpith formulation followed by vermicompost formulation and least in organic manure formulation. In both *G. hirsutum* and *Z. mays*, the protein content was significantly higher in plants treated with PSB as coirpith formulation and least in organic manure. The result revealed that there was marked difference in the glucose content among the treatments. In both *G. hirsutum* and *Z. mays* glucose content was higher in plant treated with coirpith formulation. Application of biofertilizer increased the aminoacid content in the leaves of *G. hirsutum* and *Z. mays* in all treated plants. Among them, the effect was higher in coirpith formulation over other formulations and control. The NR activity was maximum in plants treated with PSB as coirpith formulation over other treated plants in both *G. hirsutum* and *Z. mays* (Table 3 and table 4).

IV. DISCUSSION

4.1. MASS MULTIPLICATION OF PSB STRAIN

Biofertilizers are usually prepared as carrier based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Basically, the carrier based inoculant of these bacteria can be prepared by a common procedure. In the bioinoculants preparation, various carrier materials were used such as lignite, vermiculite, charcoal, agro industrial waste, compost *etc.* The efficiency of different types of bioinoculants was varied based on the nature of carrier material used for preparation of bioinoculants. Therefore, selection of carrier material was important one in the biofertilizer production as well as in the crop response (Uma Maheswari and Kalaiyarasi, 2015).

The nature of carrier material, shelf life and inoculums potential were important in the quality of bioinoculants. Quality of bioinoculants was one of the most important factors deciding their performance. A good carrier material was one which can keep up the viability of microbes for a longer period by providing organic food base to the organisms and retaining the moisture content (Yadav and Chandra, 2012). Presently lignite powder was being used as carrier material by most of the bioinoculant producing units in India. Availability of quality lignite powder was also in doubt because of adulteration by agents and improper mesh size in the pulverizing unit. Several scientists have suggested compost as carrier material for biofertilizers. But the role of good compost is maintaining microbial population. The role of vermicompost as carrier in maintaining shelf life of inoculated bacterial culture has been studied for selecting as alternative carrier material to lignite (Gandhi and Sivakumar, 2010; Murray *et al.,* 2003).

The quantity of culture required for mass multiplication was differed based on the physical properties of carrier materials. It was also determine the quality of bioinoculants. Based on the water holding capacity and particle size of the carrier materials, the quantity of requirement of culture was varied. The quantity of culture filtrate varied with the carrier material with their water holding capacity. Coirpith required higher concentration of culture than others;

because the water holding capacity was more with coirpith than other carrier materials. High level of organic matter content increased the water holding capacity and neutral pH for better survival of the microorganisms (Roychowdhury *et al.,* 2015).

4.2 CROP RESPONSE OF PSB

Ahemad and Kibret (2014) stated that mobilization of mineral nutrients like P in soil by bacteria could be the main mechanism for increased growth and development of plants which makes these nutrients in more readily plant available forms. Improvement in shoot length, root length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, yield per plant and P contents of root and shoot by inoculation of PSB strains in combination with organic amendment was more prominent as compared to sole inoculation of PSB strains PSB containing phosphatase enzymes liberate P through mineralization of organic matter which promote the growth and yield of plants (Walpola and Yoon, 2013).

A significant difference in the root, shoot length and shoot dry weight of tomato plants was observed at 60 days of plant growth due to various inoculation treatments. The treatment receiving inoculation of PSB5 recorded maximum root length and was significantly superior over all other inoculation treatments followed by PSB7. All treatments showed significant increase in root growth over absolute control (Assefa and Fenta, 2017). Phosphate solubilizing bacteria improved the plant growth, yield and phosphorus content of several crops and used as bioinoculant to enhance the sustainable production. All strains showed a positive effect on plant growth. A significant increment in plant height, shoot dry weight was determined in plants treated with *Pseudomonas tolaasii*, while *Pseudomonas koreensis* has remarkably increased P content compared to the uninoculated control. *Pseudomonas* strain was selected and evaluated under field conditions in combination with triple superphosphate as P fertilizer. The presence of *Pseudomonas* strain stimulated seedling emergence, shoot length, grain yield, grain weight, total dry biomass and P content of maize plants. In general, *P. tolaasii,* inoculation was more efficient as bioinoculant without P fertilizer (Viruel *et al*., 2014).

The application of PSB either individually or combined, effected on the growth and biomass production of several crops (Hariprasad and Niranjana, 2009). Biofertilizer produced the plant growth regulating substances, which promotes root growth found the significant effect on number of primary flowers number of secondary flowers, total number of flowers, number of primary fruit, number of secondary fruit and total number of fruit by the application of PSB in strawberry (Zargar *et al*., 2008). Biochemical parameters of chlorophyll, total carbohydrate, total protein and total fat contents found higher in biofertilizer enriched vermicompost treatments. Increased amount of chlorophyll contents seems to correlate the increased photosynthetic properties (Khomami and Moharam, 2013). Application of PSB increased the plant growth and also the dry matter content. It also increased the physiological parameters like total chlorophyll, protein, amino acids, glucose and NR activity. The response was varied based on the nature of carrier material used for preparation of bioinoculants. From these, it was clear that PSB not only solubilize the P but also increased plant growth and development. PSB was not only due to the release of plant available phosphorus but also produce the biologically active substances like indole acetic acid, gibberellins, cytokinin. The favourable effect of the inoculants on plant growth and nutrient uptake was due to the improved phosphate nutrition and production of growth promoting substances by PSB (Yadav and Chandra, 2014).

PSB application significantly increased the biochemical parameters like the Chlorophyll a, and b, carotenoid, protein and abscorbic acid (Singh *et al.*, 2014). Ferreira *et al.* (1987) reported that wheat plants inoculated with PSB showed greater activity of the nitrate reductase enzyme. Inoculation with nitrogen fixing bacteria always increased leaf NRA suggesting a greater supply of NO_3 to the plants over uninoculated control. The increased NO_3 uptake may relate to increased root development in response to production of hormones. The biochemical characters of protein contents of leaves of pot grown plants was 33-35% higher over control when plants were inoculated with *Bacillus cereus* and *Pseudomonas moraviensis*. Co-inoculation of both *Bacillus cereus* and *Pseudomonas moraviensis* significantly increased protein content. Further, sugar contents of leaves were also significantly increased (Galal, 2003).

V. CONCLUSION

The isolated PSB strains were screened *in vitro* for the selection of elite strain. The selected strain of PSB was mass multiplied and used for nursery experiment. PSB strain was incorporated into the nursery soil through different carriers. The bioinoculation of PSB was increased the growth and biochemical characters of *Gossypium hirsutum* and *Zea mays*. The response was varied with carrier materials used in bioinoculation of PSB.

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REFERENCE

[1] Abbasi, M. K., Musa, N., & Manzoor, M. (2015). Phosphorus release capacity of soluble P fertilizers and insoluble rock phosphate in response to phosphate solubilizing bacteria and poultry manure and their effect on plant growth promotion and P utilization efficiency of Chilli (*Capsicum annuum* L.). *Biogeosci. Discuss*. 12: 1839-1873.

[2] Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *J. King Saud. Univ. Sci*. 26(1): 1-20.

[3] Assefa, F., & Fenta, L. (2017). Isolation and characterization of phosphate solubilizing bacteria from Tomato (*Solanum lycopersicum* L.) rhizosphere and their effect on growth and phosphorus uptake of the host plant under green house experiment. *Int. J. Adv. Res.* 3: 2320-5407.

[4] Brahmaprakash, G. P., & Sahu, P. K. (2012). Biofertilizers for sustainability. *J. Ind. Inst. Sci.* 92(1): 37-62.

[5] Ferreira, M. C. B., Fernandes, M. S., & Dobereiner, J. (1987). Role of *Azospirillum brasilense* nitrate reductase in nitrate assimilation by wheat plants. *Biol. Fertil. Soil*. 4: 47-53.

[6] Galal, Y. G. M. (2003). Assessment of nitrogen availability to Wheat (*Triticum aestivum* L.) from inorganic and organic N sources as affected by *Azospirillum brasilense* and *Rhizobium leguminosarum* inoculation. *Egypt. J. Microbiol*. 38: 57-73.

[7] Gandhi, A., & Sivakumar, K. (2010). Impact of vermicompost carrier based bioinoculants on the growth, yield and quality of Rice (*Oryza sativa* L.) cv. nlr 145. *Ecoscan*. 4(1): 83-88.

[8] Gomare, K. S, Mese, M., & Shetkar, Y. (2013). Isolation of *Azotobacter* and cost effective production of biofertilizer. *Indian J. Appl. Res*. 3(5): 54-56.

[9] Hariprasad, P., & Niranjana, S. R. (2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. *Plant Soil*. 316: 13-24.

[10] Hassan, T. U., & Bano, A. (2016). Biofertilizer: A novel formulation for improving wheat growth, physiology and yield. *Pak. J. Bot*. 48(6): 2233-2241.

[11] Jaworski, E. G. (1971). Nitrate reductase assay intact plant tissues. *Biochem. Biophy. Res. Commun.* 43: 1274-1279.

[12] Jayaraman, J. (1981). Laboratory manual in Biochemistry, Willey-Estern Company Limited. Madras. pp. 1-65.

[13] Khomami, A. M., & Moharam, M. G. (2013). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*. 3(4): 207-265.

[14] Lowry, O. H., Rosebrough, M. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *J. Bio. Chem.* 193: 257-262.

[15] Mahantesh, S. P., Patil, C. S., & Himanshu, V. (2015). Isolation and characterization of potent phosphate solubilizing bacteria. *ISOI J. Microbiol. Biotechnol. Food Sci.* 1: 23-28.

[16] Murray, P., Baron, E., Jorgensen, J. P., Faller, M. A., & Yorken, R. H. (2003). Susceptiability testing methods yeast and filamentous fungi, manual of clinical microbiology 8th ed. Vol. 2 American Society Microbiology press Washington DC.

[17] Roychowdhury, D., Paul, M., & Kumar Banerjee, S. (2015). Isolation, identification and characterization of phosphate solubilizing bacteria from soil and the production of biofertilizer. *Int. J. Curr. Microbiol. App. Sci.* 4(11): 808-815.

[18] Singh, C. K., John, S. A., & Jaiswal, D. (2014). Effect of organics on growth, yield and biochemical parameters of Chilli (*Capsicum annum* L.). *J. Agri. Veteri. Sci*. 7: 27-32.

[19] Sivasakthivelan, P., & Saranraj, P. (2013). *Azospirillum* and its formulations: A Review. *Int. J. Microbiol. Res.* 4(3): 275-287.

[20] Tripti, K., & Anshumali, V. (2012). Phosphate solubilizing activity of some bacterial strains isolated from chemical pesticide exposed agricultural soil. *Int. J. Eng. Res. Devl*. 3(9): 01-06.

[21] Uma Maheswari, N., & Kalaiyarasi, M. (2015). Comparative study of liquid biofertilizer and carrier based biofertilizer on green leafy vegetables. *Int. J. Pharm. Sci. Rev. Res*. 33(1): 229-232.

[22] Viruel, E., Erazzu, L. E., Martinez Calsina, L., Ferrero, M. A., Lucca, M. E., & Sineriz, F. (2014). Inoculation of maize with phosphate solubilizing bacteria: effect on plant growth and yield. *J. Soil Sci. Plant Nut.* 14 (4): 819-831.

[23] Walpola, B. C., & Yoon, M. (2013). Phosphate solubilizing bacteria: Assessment of their effect on growth promotion and phosphorous uptake of Mung bean (*Vigna radiate* (L.) R. Wilczek). *Chil. J. Agric. Res.* 73: 275-281.

[24] Wellburn, A. R., & Lichtenthaler, H. (1984). In: Advances in photosynthesis research (ed. Sybesma) Martinus Nijhoff, Co. *The Hague.* pp. 9-12.

[25] Yadav, A. K., & Chandra, K. (2012). National Seminar on Organic and Biological Inputs-New Innovations and Quality Control, Published by National Centre of Organic Farming, DAC, Ghaziabad, pp 19-24.

[26] Yadav, A. K., & Chandra, K. (2014). Mass production and quality control of microbial inoculants. *Proc. Indian Natn. Sci. Acad.* 80(2): 483-489.

[27] Zargar, M. Y., Baba, Z. A., & Sofi, P. A. (2008). Effect of N, P and biofertilizers on yield and physico-chemical attributes of strawberry. *Agro. Thesis*, 6(1): 3-8.

Table.1: Effect of PSB formulations on the growth characters of Gossypium hirsutum

Treatment	**Seed Germination (%)**	**Germination Index**	**Seedling Vigour Index I**	**Seedling Vigour Index II**	**Shoot Length (cm)**	**Root Length (cm)**	**Number of leaves (per plant)**	**Fresh Weight (g)**	**Dry Weight (g)**
Control	76	0.92	18.2 ±0.49 (100)	1.12 ±0.02 (100)	24 ±0.30 (100)	9.5 ±0.32 (100)	15 ±0.15 (100)	4.25 ±0.02 (100)	1.48 ±0.13 (100)
Coirpith formulation	96	2.04	36.5 ±0.37 (200)	5.21 ±0.06 (465)	38 ±0.32 (158)	17.3 ±0.41 (182)	33 ±0.30 (220)	12.83 ±0.75 (302)	5.43 ±0.47 (367)
Vermicompost formulation	94	1.85	34.8 ±0.35 (191)	3.49 ±0.60 (312)	36 ±0.15 (150)	16.8 ±0.72 (177)	32 ±0.40 (213)	11.40 ±0.47 (268)	3.80 ±0.65 (257)
Organic manure formulation	90	1.53	30.2 ±0.49 (166)	1.98 ±0.54 (172)	28 ±0.35 (117)	13.2 ±0.40 (139)	22 ±0.47 (147)	6.67 ±0.12 (157)	2.65 ±0.18 (179)
Lignite formulation	96	2.04	32.6 ±0.15 (179)	3.42 ±0.01 (305)	34 ±0.25 (142)	15.6 ±0.35 (164)	25 ±0.37 (166)	9.98 ±0.10 (235)	3.57 ±0.13 (241)
Vermiculite formulation	91	1.59	30.4 ±0.47 (167)	2.22 ±0.02 (198)	33 ±0.49 (137)	12.8 ±0.30 (135)	27 ±0.58 (180)	8.14 ±0.05 (192)	2.42 ±0.27 (164)

Table.2: Effect of PSB formulations on the growth characters of Zea mays

Treatment	**Seed Germination (%)**	**Germination Index**	**Seedling Vigour Index I**	**Seedling Vigour Index II**	**Shoot Length (cm)**	**Root Length (cm)**	**Number of leaves (per plant)**	**Fresh Weight (g)**	**Dry Weight (g)**
Control	75	0.78	13.4 ±0.15 (100)	0.70 ±0.03 (100)	20 ±0.25 (100)	9.0 ±0.09 (100)	13 ±0.28 (100)	3.55 ±0.11 (100)	1.05 ±0.01 (100)
Coirpith formulation	93	1.97	33.5 ±0.40 (250)	2.14 ±0.01 (305)	36 ±0.15 (180)	15.7 ±0.20 (174)	25 ±0.35 (192)	8.41 ±0.34 (237)	2.31 ±0.07 (220)
Vermicompost formulation	90	1.82	26.1 ±0.25	1.68 ±0.07	30 ±0.40	14.0 ±0.15	22 ±0.40	6.92 ±0.71	1.93 ±0.03

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Treatment	Seed Germination (%)	Germination Index	Seedling Vigour Index I	Seedling Vigour Index II	Shoot Length (cm)	Root Length (cm)	Number of leaves (per plant)	Fresh Weight (g)	Dry Weight (g)
			(195)	(240)	(150)	(156)	(169)	(195)	(182)
Organic manure formulation	82	1.30	28.0 ±0.30 (209)	1.12 ±0.02 (160)	35 ±0.20 (175)	14.3 ±0.45 (159)	23 ±0.41 (177)	5.18 ±0.60 (146)	1.41 ±0.13 (134)
Lignite formulation	87	1.55	27.8 ±0.73 (207)	1.42 ±0.03 (203)	32 ±0.55 (160)	13.7 ±0.40 (152)	20 ±0.81 (154)	5.77 ±0.49 (163)	1.64 ±0.05 (156)
Vermiculite formulation	81	1.28	18.2 ±0.20 (136)	0.92 ±0.02 (131)	25 ±0.65 (125)	12.2 ±0.55 (136)	19 ±0.40 (146)	4.39 ±0.11 (123)	1.26 ±0.24 (120)

Table.3: Effect of PSB formulations on the biochemical characters of Gossypium hirsutum

Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Aminoacid Content (mg/g LFW)	NRA (µmol NO_2 formed/g LFW/h)
Control	1.07 ±0.01 (100)	2.91 ±0.03 (100)	11.13 ±0.16 (100)	1.64 ±0.10 (100)	1.68 ±0.04 (100)
Coirpith formulation	2.83 ±0.02 (264)	6.80 ±0.19 (234)	22.35 ±0.42 (201)	3.90 ±0.35 (238)	3.13 ±0.17 (186)
Vermicompost formulation	2.32 ±0.07 (217)	5.66 ±0.23 (195)	19.92 ±0.21 (179)	2.94 ±0.32 (179)	2.95 ±0.09 (175)
Organic manure formulation	1.35 ±0.04 (126)	3.85 ±0.04 (132)	14.70 ±0.32 (132)	2.35 ±0.11 (143)	1.95 ±0.14 (116)
Lignite formulation	1.94 ±0.05 (181)	4.36 ±0.07 (150)	17.43 ±0.17 (157)	2.21 ±0.17 (134)	2.32 ±0.05 (138)
Vermiculite formulation	1.45 ±0.06 (135)	4.14 ±0.09 (142)	19.86 ±0.25 (178)	3.12 ±0.11 (190)	2.72 ±0.16 (162)

Table.4: Effect of PSB formulations on the biochemical characters of Zea mays

Treatment	Total Chlorophyll (mg/g LFW)	Protein (mg/g LFW)	Glucose (mg/g LFW)	Aminoacid Content (mg/g LFW)	NRA (µmol NO_2 formed/g LFW/h)
Control	0.92 ±0.03 (100)	2.65 ±0.05 (100)	9.81 ±0.03 (100)	1.12 ±0.02 (100)	1.18 ±0.02 (100)
Coirpith formulation	2.69 ±0.05 (292)	5.43 ±0.26 (204)	21.40 ±0.72 (218)	3.14 ±0.03 (280)	2.55 ±0.17 (216)

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Treatment	**Total Chlorophyll (mg/g LFW)**	**Protein (mg/g LFW)**	**Glucose (mg/g LFW)**	**Aminoacid Content (mg/g LFW)**	**NRA (µmol NO_2 formed/g LFW/h)**
Vermicompost formulation	2.24 ±0.11 (243)	4.71 ±0.07 (178)	17.09 ±0.53 (174)	2.21 ±0.11 (197)	2.04 ±0.07 (173)
Organic manure formulation	1.12 ±0.24 (121)	3.15 ±0.09 (119)	13.62 ±0.75 (139)	1.47 ±0.04 (131)	1.48 ±0.06 (125)
Lignite formulation	1.63 ±0.05 (177)	4.32 ±0.57 (163)	15.18 ±0.15 (155)	1.85 ±0.05 (165)	2.18 ±0.09 (184)
Vermiculite formulation	1.38 ±0.06 (150)	3.22 ±0.13 (122)	16.33 ±0.42 (166)	2.51 ±0.14 (224)	1.96 ±0.05 (166)

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Biomass production and Symbiotic Nitrogen Fixation in the Legume *Sulla carnosa* in its Natural Biotope (sebkha ElKelbia)

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***Abstract*— *Wild legumes (herbs, shrubs or trees) play a critical role in natural ecosystems, agriculture, and agroforestry, where their ability to fix nitrogen makes them excellent colonizers of low-N environments, and hence an economic and environmentally friendly species. The field natural nodulation of the Tunisian Sulla crnosa, its symbiotic-efficiency and feed production potentiality in its saline biotope (sebkha d'El kelbia) were investigated in this study. A greenhouse experiment was conducted on plants transferred from sebkha with their soil in pots to explore the maximum potentialities of biomass production and nitrogen fixation of this legume when water is not a limiting factor (natural soil salinity was maintained in greenhouse). Obtained field and greenhouse study demonstrated that Sulla carnosa can be a good candidate for saline agriculture regarding its important ability to grow, produce biomass and fix nitrogen under high level of salinity (about 150 mM NaCl). This legume protects its photosynthetic and symbiotic organs against their overload with sodium by an important uptake of potassium and accumulation of Na in the roots. Sulla carnosa can play a goal role in the sustainable development in a region traditionally considered marginal.*

***Keywords*— Feed production, ionic repartition, sebkha, Sulla carnosa, Symbiotic nitrogen fixation.**

I. INTRODUCTION

Salinity leads to several physiological stresses in plants and consequently few plants can tolerate significant salinity levels in their root medium for any length of time. Of all the worlds' species, about 1% are considered halophytes [14], which are defined by Flowers and Colmer [6] as able to complete their lifecycles under saline conditions corresponding to at least 200 mM of NaCl in the root medium.

The negative effects of salt on plant growth are a considerable problem for agriculture and thus the world food production. Currently it is causing problems in many parts of the world (especially in arid and semi arid regions); and it is predicted to get worse under climate change conditions with increasing weather extremes and rising seawater levels [14]. Worldwide, up to 20% of arable land surface is salt affected. Soil salinity relates to the build-up of salts in soil and can be both natural and anthropogenic. A soil is considered saline if the electric conductivity of a saturated paste (equivalent to the available salts in the soil pore water) of that soil is over 4 dS/m (equivalent to ± 40 mM NaCl, which is roughly equivalent to 7% of seawater salinity). Notwithstanding the sensitivity of many plants to salinity, some plants can survive and grow vigorously under saline conditions [15]. The use of such plants would be extremely helpful not only to reclaim salinized areas, but also because it would allow us to use brackish or salt water for irrigation in agriculture. The use of brackish and saline water for irrigation is related to the scarcity of fresh water in the world, especially in areas that receive little annual rainfall. About 1% of the world's water is fresh (>0.05% dissolved salts), about 97% of the water is seawater (<3% of dissolved salts [14]) and the remainder is of intermediate salinity. Humans use large quantities of fresh water (and the demand increases faster than the human population grows) for a variety of activities including industry (20%), domestic use (10%) and, most notably, agriculture which accounts for around 70% of global fresh water consumption. The salinity problems and the scarcity of fresh water point to a solution via the use of salt-tolerant plants in agricultural production. Few crop plants can tolerate even moderate levels of salinity however. Attempts to improve the salinity tolerance of conventional crops have not been successful so far. This is because tolerance to salinity is a complex trait [4], involving multiple genes and having evolved multiple times independently among different lineages [7], leading to different mechanisms of salinity tolerance. A different promising strategy is to focus on the de novo domestication of halophytes. In addition to that, seawater contains many elements required for plant growth. This would reduce the need for fertilization of arable lands under saline irrigation for many nutrients. However, the element plants require in the highest quantities, nitrogen (N), is present in sea water in very small quantities, and would therefore have to be supplemented. In natural ecosystems, the largest input of nitrogen comes from nitrogen fixed by microorganisms, called diazotrophs [18]. These can be either free living or associated to plants from, mainly, the family of the Fabaceae. Nitrogen input from legumes can be a sustainable source of nitrogen in agricultural systems.

In the arid and semi- arid lands of Tunisia, the most limiting factor of livestock and animal production is the availability of feed. The feed crops did not exceed 7% of the ploughable area. For several years we have been working to improve pastures and rangelands of the central and southern regions but it turned out we were on the wrong way. In fact, improving productivity of sown pastures in salinized and drought affected soils is a faulty operation. However, most legumes (of that some spontaneous and halophytic species) are easily able to fix 100 kg N/ha/yr, and this figure fits well with findings in other studies; reported values of 200–300 kg N/ha/yr are no exceptions [13]. About 40–60% of the nitrogen fixed is available for the subsequent crop [16]. Considering a loss of around 50% of N and that 75% of total plant nitrogen comes from atmospheric N2, if we aim for the addition of 100 kg N/ha/yr available for a subsequent crop, the legume used as green manure should fix around 265 kg N/ha/yr. two legumes (*Medicago sativa* and *Vicia faba*) are capable of fixing this amount of nitrogen with the maximum rate of N fixation more than double this requirement. The use of legumes as an alternative to the use of fertilizer, i.e. as green manure, is thus a viable strategy based on these calculations. However, semi arid regions of Tunisia are known by their richness of the natural pastoral flora including some nitrogen fixing legumes. These species could be an alternative to grow food crops and vegetables, regarding the low availability of fresh water and the abundance of saline water and soils. Therefore, in this study we will focus on the importance of using the spontaneous Fabacae, *Sulla carnosa,* in saline agriculture as source of feed and nitrogen. The potentialities of biomass production and nitrogen fixation will be studied in *Sulla carnosa* in its natural biotope (Sebkha d'El Kelbia) and in the Laboratory.

II. MATERIALS AND METHODS

The Sebkha d'El Kelbia is an intermittent lake in Tunisia (Kairouan Governorate) that covers 8000 hectares, in addition to 7000 hectares of surrounding swamps, at 35°50'34" North, 10°16'18" East south of Kondar. it is classified in the lower semi-arid bioclimatic stage.

2.1. Field study: Field study was conducted in October after the beginning of the rainfall season to follow the appearance of new *sulla* shoot after the dry season. Two sites are marked to collect plants and soil samples for analysis. A general description of the landscape and flora was made, the number of *Sulla carnosa* plants and the total number of other fodder and pastoral plants in 1 m^2 were counted in 10 different sites.

2.2. Soil and plant sampling: Sebkha soil was sampled for analysis in the Lab from two different sites. The first one around *sulla* plants (S1) and the second one from the nude area (S2), five samples for each site from the superficial layer (30 Cm deep). In order to express the maximum potentialities of biomass production and nitrogen fixation of *sulla carnosa* and to guarantee nodulated plants, new appeared plantlets are transferred with their soil in 1 Kg plastic pots (1 plant by pot). Precautions are taken to not damage the root system. Twenty pots are then placed in a greenhouse in the Centre of Biotechnology of Borj Cedria and irrigated with salinized tap water. Electric conductivity of the sebkha soil was measured firstly in order to use the same concentration of NaCl for plant irrigation. Each pot is put on a plate to recover the flowing water.

2.3. Analysis: Soil analysis was made in the Laboratory of Soil Sciences in the National Institute of Agronomy (INAT, Tunisia). Samples were dried in the open air for three days, then grind to a fine powder with a mortar (diameter < 2 mm).

pH of the soil solution was measured according to Pauwels *et al.* [12] in a mixture of soil/ water (1/ 2.5). 25 ml of deionized water were added to 10 g of fine soil, shaken for 2 hour, then pH measured with a pH meter Mettler.

Electric conductivity was measured according to Pauwels *et al.* [12] in a soil and deionized water suspension (1/5). 25 ml of deionized water were added to 5 g of fine soil, shaken for 1 hour, then EC measured.

The fodder potentialities of *Sulla carnosa* were estimated by its biomass production after 45 and 90 days of cultivation. Shoot biomass (very appreciated by livestock) was quantified.

90 days old plants were harvested, and separated into leaves, roots and nodules. Nodulated roots were washed thoroughly and successively in 3 baths of ultra-pure water in order to avoid the contamination of roots and nodules with ions and elements from the soil.

For chemical analysis, Samples of fresh material were dried at 70°C for 72 h and ground to fine powder. Nodules were previously numbered at the harvest. After extraction in 0.5% HNO_3, K^+ and Na^+ ions were

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measured according to Pauwels et al., (1992), using flame emission photometry (Corning, UK). Total nitrogen was determined by the Kjeldahl method.

2.4. Statistical analysis: Analysis of variance (ANOVA), using the AV1W MSUSTAT program with orthogonal contrasts and mean comparison procedures, was performed to detect differences between treatments. Mean separation procedures were carried out using the multiple range tests with Fisher's least significant difference (LSD) (P< 0.05).

III. RESULTS AND DISCUSSION

In its natural biotope (sebkha d'El Kalbia), *sulla carnosa* showed anarchic development. It occupy particularly the sebkha border in which its distribution decreases going deep, sometimes individual and sometimes in association with other halophytes (*Salsola vermculata* and *Atriplex halimus* ...) and glycophytes (*Medicago ciliaris, malva sylvestris* ...) (picture 1).

Picture 1. *sulla carnosa in association with halophytes and glycophytes in the Sebkha d'El Kelbia*

It is very important to mention the presence of some area where flora disappears and no plant observed. In fact, electric conductivity measured in the sampled soils show two important results. The first one, concerns the soil with important flora including *sulla carnosa* (EC = 13.6 mS/ Cm). It is a clear saline soil regarding its EC exceeding the adopted norms (a soil is considered saline if the electric conductivity of a saturated paste, equivalent to the available salts in the soil pore water, of that soil is over 4 dS/m (equivalent to ± 40 mM NaCl, which is roughly equivalent to 7% of seawater salinity)). This soil is also alkaline regarding its measured pH (7.6). The second result concerns the nude soil (EC = 64.5 mS/ Cm) in which EC is very high explaining the absence of any plant. For this reason EC of the soil of plants transferred in the greenhouse was maintained at 13.5 mS/ Cm.

The study of the frequency of existence of *sulla carnosa* in association with other species of forage and pastoral interest has shown that it represents 28%. The average number of *sulla carnosa* plants observed in this study is about 30 plants / m^2. In fact, Abdelguerfi et *al.* [1] demonstrated that this species appeared on soils with low rate of organic matter, very saline, calcareous and rich in Mg and P. Lachaal et *al*. [10] reported that the biomass production of *Sulla carnosa* decreased by 60% at 300 mM NaCl. Nevertheless, this Legume of Agronomic interest with its fodder quality and ability to improve soil fertility with nitrogen can be used in valorization program of degraded soils particularly in arid and semi arid regions. This Legume offers grazing opportunities during an important period of the year regarding its high capacity of regeneration. Rozema and Schat [15] suggested that these species of halophytes help, not only to exploit saline's zones, but also give opportunities to use sea water in irrigation.

When water is not a limiting factor (plants transferred in greenhouse), *sulla carnosa* can produce 45 g of fresh biomass after 45 days, even maintained on sebkha saline soil (EC = 13.5 mS/ Cm), and 133 g of fresh biomass after 90 days. Taking in account the 30 plants/ m^2 observed in the sebkha, this mean that we can reach a production of 14 tons/ ha of fodder fresh biomass after 45 days in this marginal saline soil when water is available, or in a non saline soil but irrigated with saline water. After 90 days, this production amounts to 40 tons/ ha (fig 1).

Cultivated on its proper saline soil (sebkha), *Sulla carnosa* express high potentialities of nodulation. In fact, fig 2 shows a high number of nodules development on root system reaching 100 nodules per plant after 45 days and exceeding 200 nodules per plant after 90 days of cultivation. We are therefore before a legume species that produce feed biomass and fix nitrogen in a very saline environment (sebkha). The number and the red color of nodules when excised (presence of Leghaemoglobin) reflect an important capacity of symbiotic nitrogen fixation.

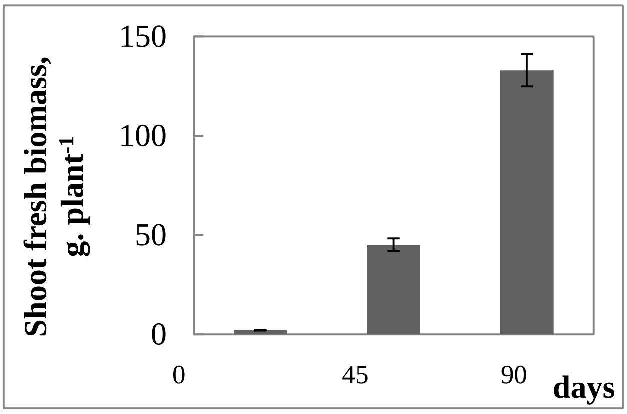

Figure 1. *Fresh biomass production of Sulla carnosa cultivated on sebkha soil (EC = 13.5 mS/ Cm) and irrigated with saline tap water. Data are means of 20 replicates (± standard error; p = 0.05).*

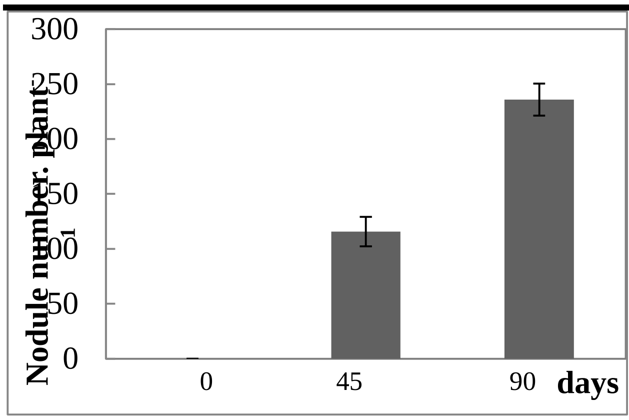

Figure 2. *Nodules number on roots of Sulla carnosa cultivated on sebkha soil (EC = 13.5 mS/ Cm) and irrigated with saline tap water. Data are means of 20 replicates (± standard error; p = 0.05).*

The analysis of nitrogen demonstrates that leaves and nodules accumulates a high levels of this nutrient (4.5 mmol N. g^{-1} DW and 3.3 mmol N. g^{-1} DW, respectively, fig 3). In roots the level of nitrogen is the lowest (1.5 mmol N. g^{-1} DW). If we consider the quantity of nitrogen measured in root and nodule organs (organs remaining in the soil, about 600 mg. $plant^{-1}$) and the number of plants founded in the sebkha (30 plant. m^{-2}), we conclude that this legume can furnish 180 Kg N/ ha within 3 months. It is generally established that the use of legumes as green manure is often equivalent to the application of 30– 80 kg N/ha/yr, with maxima of around 100 kg N/ha/yr [18]. However, for the purpose of comparison we assume 100 kg N/ha/yr as a reasonable approximation of fertilizer needs for most crops. For example, average nitrogen fertilizer use in the United States of America has been a little under 95 kg N/ha/yr (USDA ERS) [3]. Most legumes are easily able to fix 100 kg N/ha/yr, and this figure fits well with findings in other studies; reported values of 200–300 kg N/ha/yr are no exceptions [13].

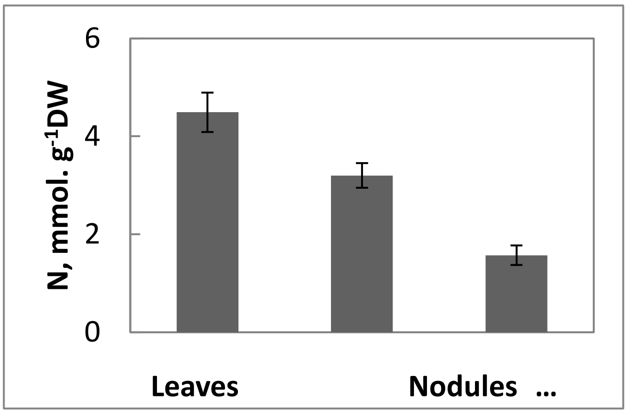

Figure 3. *Nitrogen accumulation in Sulla carnosa plants cultivated on sebkha soil (EC = 13.5 mS/ Cm) for 90 days and irrigated with saline tap water. Data are means of 10 replicates (± standard error; p = 0.05).*

However, about 40–60% of the nitrogen fixed is available for the subsequent crop [16]. This mean that by using *Sulla carnosa* in biosaline agriculture we can produce biomass and improve soil fertility in nitrogen. However, it is well documented that salt stress decreased legume productivity through the inhibition of photosynthesis, nitrogen fixation and C metabolism ([5], [8]). Velagaleti and Marsh [17] demonstrated that the decrease of nodules growth results from the inhibition of carbohydrate allocation to these organs after the reduction of leaves growth and photosynthetic capacity.

By analyzing potassium nutrition we remarks that this nutrient accumulated preferentially in leaves. Concentrations observed in nodules are also important and the roots present the less K concentration (Fig 4a). Sodium analysis repartition show very high level in leaves, then roots and nodules are the less invaded by this element (fig 4b). When calculating the ratio K/ Na (table 1), we observed that values are upper 1 in leaves and nodules and below 1 in roots. This result less think about the strategy adopted by this specie to prevent photosynthetic apparatus (leaves) and symbiotic apparatus (nodules) against the toxicity of sodium.

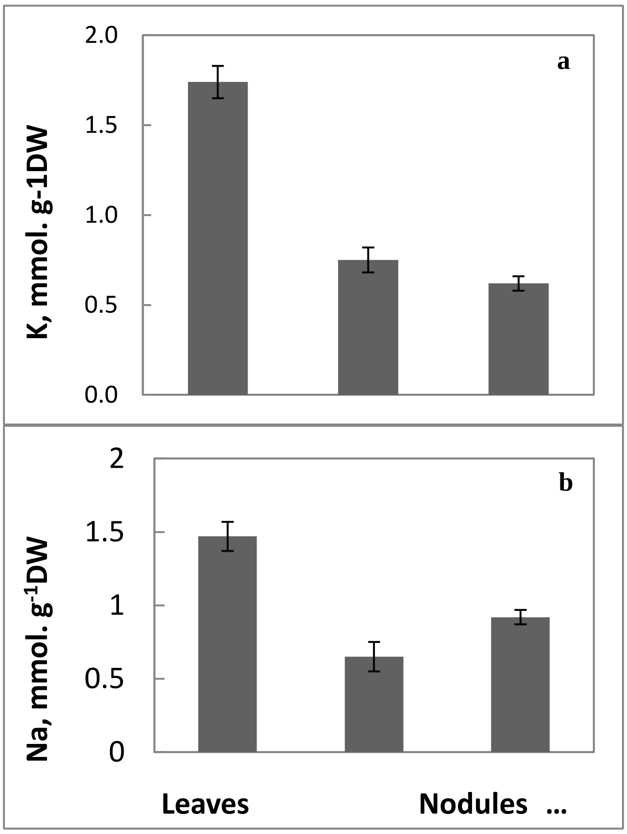

Figure 4. *Potassium (a) and sodium (b) accumulation in Sulla carnosa plants cultivated on sebkha soil (EC = 13.5 mS/ Cm) for 90 days and irrigated with saline tap water. Data are means of 10 replicates (± standard error; p = 0.05).*

Table 1. *Variation of the potassium/sodium ratio in the different organs of Sulla carnosa plants cultivated on sebkha soil (EC = 13.5 mS/ Cm) for 90 days and irrigated with saline tap water. Data are means of 10 replicates (± standard error; p = 0.05).*

Plant organ	Leaves	Roots	Nodules
K /Na	1.2± 0.11	0.7± 0.06	1.1± 0.12

Maintaining plant growth in saline conditions depends in part on the ability to keep cytoplasmic Na^+ levels low to protect the Na^+-sensitive metabolic machinery. Halophytes can utilize at least one of the three mechanisms to prevent Na^+ accumulation in the cytoplasm: reducing Na^+ entry into the cell, active Na^+ efflux from the cell, and active sequestration of Na^+ in the vacuole. In general, the vacuole is the largest sink for toxic Na^+ ions in plant cells. The plants adapt also to the ionic imbalances by osmotic adjustments of the cytoplasm. To avoid Na^+ accumulation in the leaf cytoplasm, there are a number of root-based mechanisms that minimize salt penetration to the foliar tissue ([2], [11]). It seems that our legume species, well adapted to saline environment, is able to protect its photosynthetic and symbiotic apparatus against their overload with toxic ions (Na^+ and Cl^-). In fact, mineral analysis made in this study demonstrated that *Sulla carnosa* concentrate sodium preferentially in roots and potassium preferentially in leaves and nodules. It is clear that the tolerance of *Sulla carnosa* to salinity is linked to its capacity of ions exchange. This means that our legume plant cultivated on saline soil accumulates high levels of potassium in the photosynthetic and symbiotic apparatus protecting them from toxic sodium that is trapped in roots. Bruning and Rozema [3] mention *sulla carnosa* as a xerohalophytic plant that tolerate, with some limits, drought and salinity. Kouas et *al.* [9] demonstrated that this species can tolerate 100 mM NaCl without reduction in plant growth or symbiotic nitrogen fixation. The present study demonstrated that *Sulla carnosa* can grow, produce biomass and fix nitrogen in its natural biotope in the presence of 150 Mm NaCl, but remain a native species not exploited agronomically and its symbiotic mechanism is not elucidated. The use of this Legume in the planning, development and valorization programs of areas considered for a long time marginal, like sebkha, is strategic because its low cost (no fertilizer used, no fresh water, saline abandoned area), environmental aspect (no chemical fertilizer), economic profitability (biomass and nitrogen input) and sustainable (spontaneous plants with high capacity of regeneration). Further studies should focus on the elucidation of the mechanisms of symbiotic nitrogen fixation and isolation of efficient strain of *rhizobia* for lab experiments.

IV. CONCLUSION

Saline agriculture provides a solution for at least two environmental and social problems. It allows us to return to agricultural production areas that have been lost as a consequence of salinization and it can save valuable fresh water by using brackish or salt water to irrigate arable lands. In this study, obtained results demonstrated that *Sulla carnosa* can be a good candidate for this purpose. Legume that grow in sebkha (saline, marginal and abandoned area), produce forage biomass and fix nitrogen. Further studies are in progress aiming to explore the genotypic variability of *Sulla carnosa* response to salinity using other provenance (5 regions of the arid and semi arid area of Tunisia). A special interest is granted to isolation and purification of an efficient strain of *Rhizobia* for hydroponic and La experiments.

REFERENCES

[1] Abdelguerfi-Berrekia, R., Abdelguerfi, A., Bounaga, N., Guittonneau, G.C. 1991. Répartition des espèces spontanées du genre *Hedysarum* selon certains facteurs du milieu en Algérie. *Fourrages 126:187-207.*

[2] Bieleski, R. L. 1982. Sugar alcohols. Plant Carbohydrates I, in: F.A. Loewus, W. Tanner (Eds.), Encyclopedia of plant physiology, Vol. 13A, Springer, Berlin, 1982, pp. 158– 192.

[3] Bruning, B., Rozema, J. 2013. Symbiotic nitrogen fixation in legumes: Perspectives of saline agriculture. *Environmental and Experimental Botany 92: 134– 143*.

[4] Delgado, M.J., Ligero, F., Lluch, C. 1994. Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. *Soil Biology and Biochemistry 26: 371- 376*.

[5] Ferri, A., Lluch, C., Ocana, A. 2000. Effect of salt stress on carbon metabolism and bacteroid respiration in root nodules of common bean (Phaseolus vulgaris L.). *Plant Biology 2: 396- 402*.

[6] Flowers, T.J., Colmer, T.D. 2008. Salinity tolerance in halophytes. *New Phytologyst 179: 945– 963.*

[7] Flowers, T.J., Galal, H.K., Bromham, L. 2010. Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology 37: 604– 612.*

[8] .Garg, N., Singla, R.. 2004. Growth, photosynthesis, nodule nitrogen and carbon fixation in the chickpea cultivars under salt stress. *Brazilian Journal of Plant Physiology 16(3):137- 146.*

[9] Kouas, S., Slatni, T., Salah, I.B., Abdelly, C. 2010. Eco-physiological responses and symbiotic nitrogen fixation capacity of salt-exposed *Hedysarum*

http://dx.doi.org/10.22161/ijeab/2.6.59 *ISSN: 2456-1878*

carnosum plants. African Journal of *Biotechnology, 9(44): 7462- 7469.*

[10] Lachaal, M., Abdelly, C., Soltani, A., Hajji, M., Grignon, C. 1995. Réponse physiologique de quelques légumineuses spontanées et cultivées à la contrainte saline. Colloques INRA, 77, 93- 109.

[11] Parks, G.E., Dietrich, M.A., Schumaker, K.S. 2002. Increased vacuolar Na+/H+ exchange activity in Salicornia bigelovii Torr. in response to NaCl. *Journal of Experimental Botany 53: 1055– 1065.*

[12] Peoples M.B., Herridge D.F., Ladha J.K. 1995. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? Plant and Soil 174: 3–28.

[13] Pauwels, J.M., Van Rust, E., Verloo, M., Mvoudo, Z.E. 1992. Manuel de laboratoire de pédologie: Méthodes d'analyses des sols et des plantes. Publications Agricole. 28: 1992. pp 265.

[14] Rozema, J., Flowers, T. 2008. Crops for a salinized world. *Science: 322, 1478– 1480.*

[15] Rozema, J., Schat, H. 2013. Salt tolerance of halophytes, research questions reviewed in the perspective of saline agriculture. *Environmental and Experimental Botany 92: 83- 95.*

[16] Sullivan, P. 2003. Overview of cover crops and green manures. ATTRA. Available at https://attra.ncat.org/attrapub/summaries/summary.php?pub=288

[17] Velagaleti, R.R., Marsh, S. 1989. Influence of host cultivars and *Bradyrhizobium*strains on growth and symbiotic performance of soybean under salt stress. *Plant and Soil 119: 133– 138.*

[18] Zahran, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Review 63: 968–989.*

Available nutrients and some soil properties of El-Qasr soils, El-Dakhla Oasis, Egypt

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Abstract— *Ten surface soil samples (0-30 cm) were collected from El-Qasr village, El-Dakhla Oasis, New Valley, Egypt to investigate some soil properties and nutrients availability. The results revealed that most of the soil texture varied clay loam to sandy clay loam. The mean values of bulk density (BD), soil pH, electric conductivity (EC), saturation percentage (SP), calcium carbonate ($CaCO_3$), cation exchangeable capacity (CEC), organic matter (OM), and sodium adsorption ratio (SAR) were 1.42 gcm^3, 7.89, 3.34 dSm-1, 52.10%, 7.46 %, 28.08 cmol (+) kg^{-1}, and 3.65, respectively. The mean values of N, P, and K were 99.37, 31.32, 121.93, mg/kg, respectively. Meanwhile, the mean values of Fe, Mn, Cu and Zn were 27.39, 18.38, 0.48, and 3.48 $mgkg^{-1}$, respectively. The correlations coefficient of NPK was strongly positively significant relationship with clay, OM, CEC, and SAR, but they negatively correlated with pH, EC, $CaCO_3$. Meanwhile, Fe, Mn, and Cu were positively correlated with EC, $CaCO_3$, while they negatively correlated with clay, pH, OM, CEC, and SAR. Also, Zn was negatively* ***correlated*** *with clay, pH, EC, OM, and $CaCO_3$, and it positively correlated with CEC and SAR.*

Keywords—Available nutrients, soil physico-chemical properties, El-Qasr area, El-Dakhla Oasis.

I. INTRODUCTION

El-Dakhla Oasis is belongs to the New Valley governorate that is located at the middle of the Western Desert of Egypt between longitudes 28◦ 22 00″ - 29◦ 30 00″ E and latitudes 25◦ 25 00″ – 25◦ 55 00″ N (Fig. 1). This area of El-Dakhla oasis has not conducted any studies to investigate the content of the available nutrients in their soils. El-Dakhla Oasis is the southern one in this cluster of depressions and represents an important feature in the Western Desert [11]. The general climate of El-Dakhla oasis is lies within the extremely arid belt, with long hot summer and short warm winter, since the mean monthly minimum temperature values range from 4.30 to 9.90 C° in winter and from 28.50 to 32.30 C° in summer. The groundwater is the only water resource for all life activities ([12]. Soil fertility is one of the important factors controlling the crop yield. So, soil testing provides the information about the nutrient availability of the soil upon which fertilizer recommendation for maximizing crop yield. Nutrients available depend on soil pH, organic matter content, and other physical-chemical properties [16]. Soil characterization in relation to the evaluation of fertility status of the soils of an area or region is an important aspect in the context of sustainable agriculture production. Soil variability is naturally caused by climate, topography, parent material, vegetation, time, and management practices [2]. Soil nutrients are essential for plant growth, development, and reproduction. So, if nutrients sunlight and water are present in sufficient amounts, plants can create all the compounds needed for typical development [13]. Macronutrients are commonly available to plants as exchangeable cations. These cations content an immediate source of nutrients for plants, but some insoluble sources may exist. Also, micronutrients are generally derived from parent materials, such as ferromagnesian minerals and basic igneous rocks [24].

The main objective of the current study is assessed to the availability of macro-micronutrients and some soil physical -chemical properties of El-Qasr area. These assessments will be helping us understanding the future scope of growth of plants in this region.

II. MATERIALS AND METHODS

Ten surface soil samples (0-30 cm) were collected from El-Qasr area in the western part of El-Dakhla oasis that is located between longitudes 28°42" 00 - 28°58"00 E and latitudes 25° 40" 00 - 25° 44" 00 N (**Fig.1**). The main crops grown on this area are parley, alfalfa, date balm and wheat plants. The collected soil samples were air-dried, crushed and sieved through a 2-mm sieve and kept for physic-chemical analysis. Particles-size distribution and the electrical conductivity of the soil samples were performed using the method of [19]. Soil organic matter content was determined using the dichromate oxidation method [19]. Soil calcium carbonate was estimated using the calcimeter method according to [23]. Soil reaction pH was determined in saturated soil paste suspension by a gears electrode [22]. Soil salinity (EC) was measured in 1:2.5 of soil and water extract using conductivity meter [19]. Cation exchange capacity (CEC) was determined using the sodium acetate method as proposed by [19].

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Sodium adsorption ratio (SAR) was according to [19]**.** Soluble ions were determined in the saturated soil paste extract according to [17]**.**

III. RESULT AND DISSECTION

3. 1 Some soil physical and chemical properties

Some soil physical and chemical properties investigated are shown in **Table 1** and fig. *2*.The texture class of soil samples under study was varied from loamy sand to clay texture. Sand, silt, and clay content ranged from 28 to 89, from 4 to 37 and from 7 to 47%, respectively. The bulk density and saturation percentage were ranged from 1.22 to 1.73 g/cm^3 and 16 to 76% with mean1.42 g/cm^3 to 52.10%, respectively. Soil pH values ranged from 7.56 to 8.83 with mean 7.89. The highest value of pH was found in sample No.8. The high value of pH may be attributed to the reaction of applied fertilizer material with soil colloids and increased the soluble salts and content of calcium. Also, soil tillage systems lead to an increase of soil pH, base saturation, and extractable phosphorus [25]**.** [14] he found that the levels of soil pH in some soils of Upper Egypt ranged from 7.73 to 9.45. Also, [5] found that a pH range of 6.5 to 7.5 is optimal for plant nutrient availability.

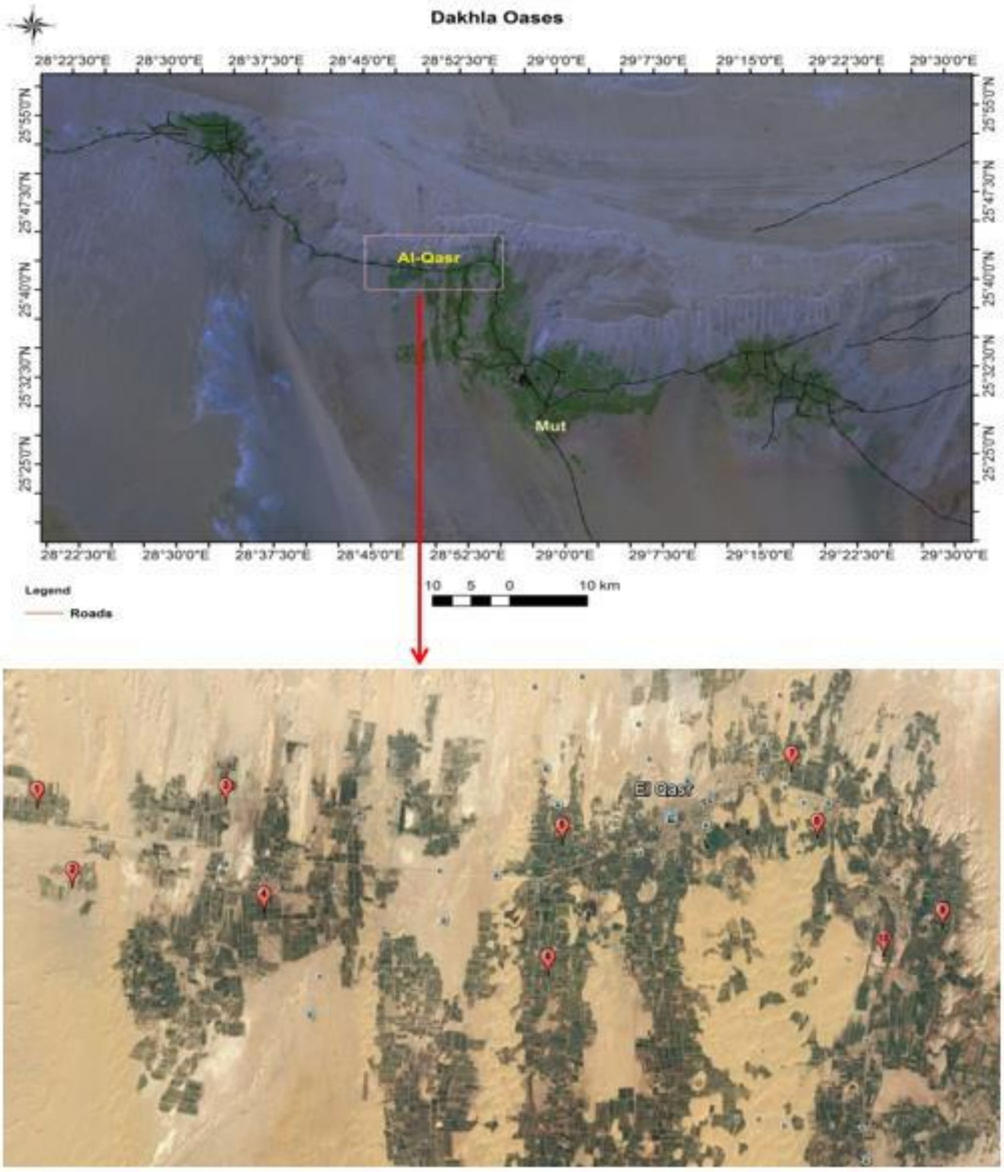

Fig.1: El-Dakhla Oasis map and location of soil sampling points in El-Qasr area.

Table.1: Some physico- chemical properties of collected soil samples in El-Qasr area.

Soil No	Particle-size distribution Sand	Silt	Clay	Texture	BD	pH (1-2.5)	EC (1-2.5)	SP	$CaCO_3$	OM	CEC	SAR
	%				gcm^{-3}		dSm^{-1}		(%)		cmol (+) kg^{-1}	
1	32.7	25	42.3	Clay	1.22	7.56	3.55	76	4.63	1.53	37.97	4.71
2	36.4	30	33.6	C L	1.27	7.55	2.64	65	2.96	1.25	29.76	1.99
3	49.8	24	26.2	S C L	1.39	7.82	1.03	50	7.65	1.07	24.32	3.66
4	36	35.9	28.1	Loam	1.43	7.75	6.58	61	2.85	1.16	32.34	5.56
5	31	35.7	33.3	C L	1.45	7.76	2.17	64	3.74	1.43	28.68	3.54
6	29.9	36.1	34	C L	1.38	8.22	1.90	70	4.12	1.11	30.10	2.87
7	28	36.8	35.2	C L	1.37	7.76	1.87	55	5.65	1.46	33.11	4.11
8	88.5	4.5	7	Sand	1.73	8.83	9.14	16	26.82	0.07	10.23	0.78
9	65.9	16	18.1	S L	1.65	8.04	2.03	23	9.30	0.12	28.21	4.96
10	50.6	23.4	26	S C L	1.33	7.63	2.44	41	6.92	1.06	26.11	4.36
Mean	**44.88**	**26.74**	**28.38**	------	**1.42**	**7.89**	**3.34**	**52.10**	**7.46**	**1.03**	**28.08**	**3.65**

CL= clay loam, SCL= sand clay loam, SL= sandy loam

In the present investigation study the $CaCO_3$content of the investigated soil samples ranged between 2.85 and 26.82%. Soil No 8 has the high amount of $CaCO_3$ (26.82%). These high amounts of $CaCO_3$ probably due to the high soil salinity or even soil texture [6]. In general, the accumulation of calcium carbonate in the soils depends on the position of the area, evaporation rates and the depth of percolating rainwater [12]. [15] they found that the values of $CaCO_3$ in some soils in Upper Egypt ranged from 0.15 to 49.00%. Also, the results in Table 2 indicate that the content of OM of the studied soil samples ranged from 0.07 to 1.53% with a mean value of 1.03%. The lower OM content is related to the coarse soil texture, while the higher one is attached to the fine and medium soil texture. This result is an agreement with those obtained by [12] whom found that soil organic matter content in El-Dakhla Oasis (GarbEl-Mawhob area) was very low and ranged from 0.05 to 1.41%. The cation exchange capacity (CEC) was varied from 10.23 to 37.97 cmol (+) kg^{-1}. The high value (37.97 cmol (+) kg^{-1}) of CEC may be due to the high of organic matter and clay content. This result agrees with those obtained by [26], [21]**)** and [7] the**y** reported that both clay content and organic matter considered as a source of nutrients by attracting cations and provide more exchange sites to get the cations adsorbed on it; so, soils that have a large amount of clay or organic matter have higher exchange capacities than sandy soils, which are usually low in clay content and organic matter. Also, the low values of CEC may be attributed to the effect of soil tillage that led to the reduction of soil organic matter [25]. The values of SAR ranged from 0.78 to 5.56 (Table 2. and figure 3), SAR values can be indicating that the majority of soils have less than 8, the non-sodic soils cover about 77% of the soils.

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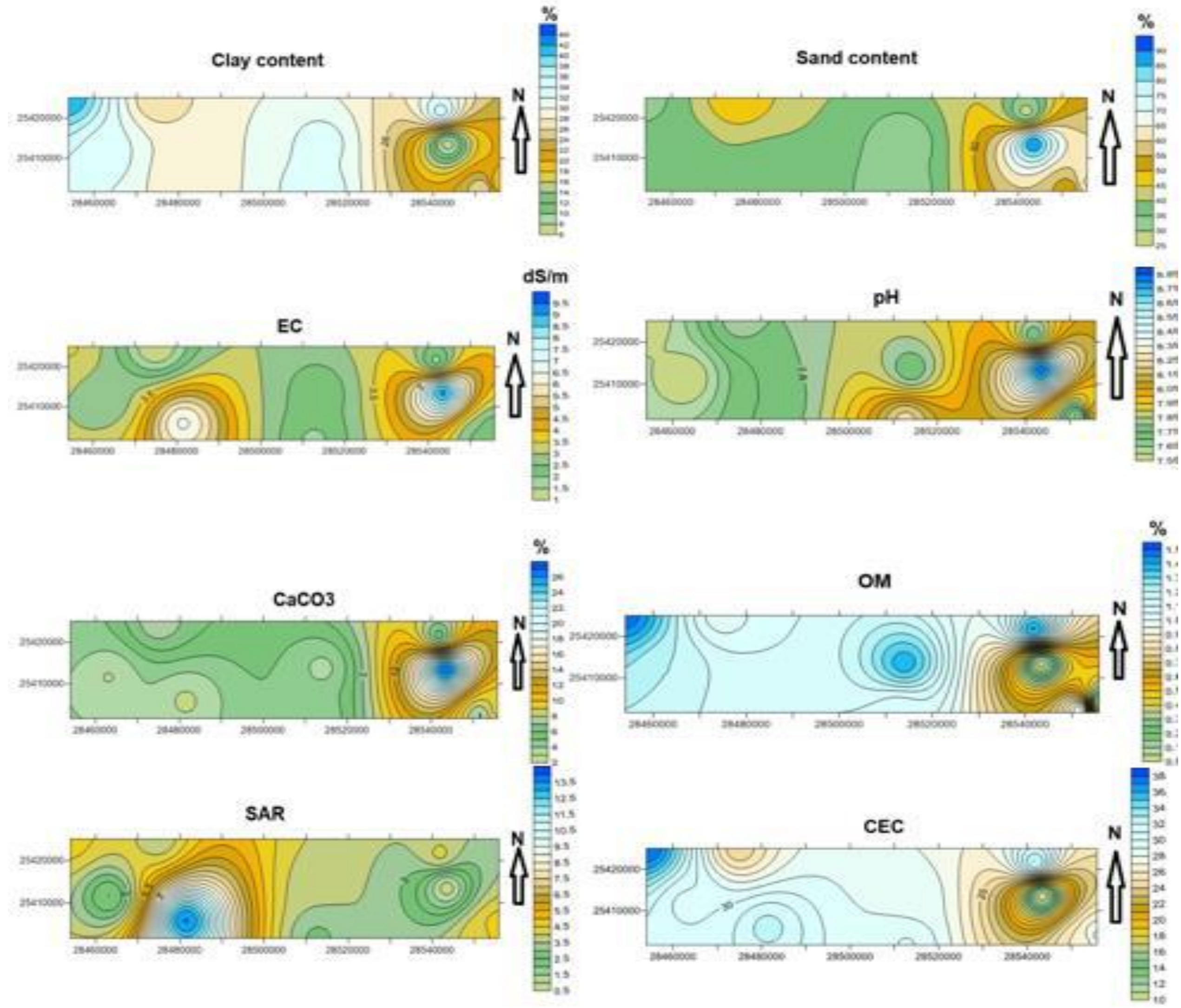

Fig.2: The distribution maps of some physical and chemical properties in El-Qasr area soils.

3. 2 Availability of macro-micronutrients

The results of the available NPK in the studied soils are shown in Table 3 and Figure 3. The values of macro elements were ranged from 70.31 to 170.22 mg/kg for N with a mean value of 99.37 mg/kg, from14.33 to 52.71mg/kg for P with a mean value of 31.32 mg/kg, and from 82.36 to 240.04 mg/kg for K with a mean value of 121.93 mg/kg. The highest level of available N, P, and K was recorded in soil sample No. 5. This may be attributed to the addition of plants residual and organic fertilizers that provide substrate for microbial growth, and subsequent microbial activity [28]. In sandy calcareous soils, the content of extractable soil P was 4.50 $mgkg^{-1}$ reported by [10]. On the other hand, [1] demonstrated that the extractable soil P ranged from 0.10-0.35 $mgkg^{-1}$in some different soils of Egypt. The mean levels of the extractable P in the loamy soils were always higher than those of sandy or clay soils. The value of available potassium ranged from 0.1 to 2.34 mg kg^{-1}.

The excess in potassium may lead to plants will exhibit typical Mg, and possibly Ca deficiency symptom due to a cation imbalance [24]. DTPA-extractable of micronutrients in the studied soils was shown in Table 3 and Fig. 3. The values of these elements ranged from 21.54 to 33.54, 14.76 to 24.87, 2.12 to 4.23, and 0.31 to 0.72 $mgkg^{-1}$with a mean value of 27.39,18.38,0.48 and 3.48 mg/kg for Fe, Mn, Cu, and Zn, respectively.

Generally, the highest levels of micronutrients were showed in soil samples No.8, respectively. In El-Dakhla oasis soils, [4] found the available Fe, Mn, Cu and Zn content range from 250 to 396, 0.21to 7.4, 0.15 to 0.43-0.77, and from 0.15 to 0.43-0.77$mgkg^{-1}$, respectively. [20] found that the Fe extracted by DTPA ranged between 1.20 to 28.60 $mgkg^{-1}$ in some soils of middle and Upper Egypt. Also, they found that the DTPA-extractable Mn ranged from 1.10 to 57.40 $mgkg^{-1}$. [18] reported that the levels of DTPA-extractable Cu varied from 0.17 to 4.88 $mgkg^{-1}$ in Sohag soils, Egypt. Generally, the availability

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of all nutrients in soil remarkably improved by the application of gypsum, which creates a more favorable environment in soil and maintain elements in a more available form due to reclamation effect [9] and [27].

Table.2: Available macro and micronutrients (mgkg^{-1}) of the collected soil samples in El-qasr area.

Soil No	**Available macronutrients**			**DTPA-extractable micronutrients**			
	N	**P**	**K**	**Fe**	**Mn**	**Cu**	**Zn**
1	140.36	44.42	133.12	29.65	14.78	3.22	0.44
2	110.32	36.31	127.11	29.87	13.43	3.22	0.48
3	70.31	28.41	116.52	13.32	14.76	2.12	0.31
4	80.16	38.35	97.34	17.43	18.32	3.65	0.52
5	170.22	52.71	243.04	31.55	12.43	4.20	0.63
6	104.21	26.34	112.54	33.54	22.54	3.97	0.39
7	99.12	24.65	105.67	29.67	21.64	3.78	0.44
8	55.71	14.33	82.36	36.75	24.42	4.23	0.72
9	55.61	21.78	92.13	21.54	20.65	4.12	0.41
10	107.64	25.91	109.44	30.54	20.87	2.23	0.43
Mean	**99.37**	**31.32**	**121.93**	**27.39**	**18.37**	**3.48**	**0.48**

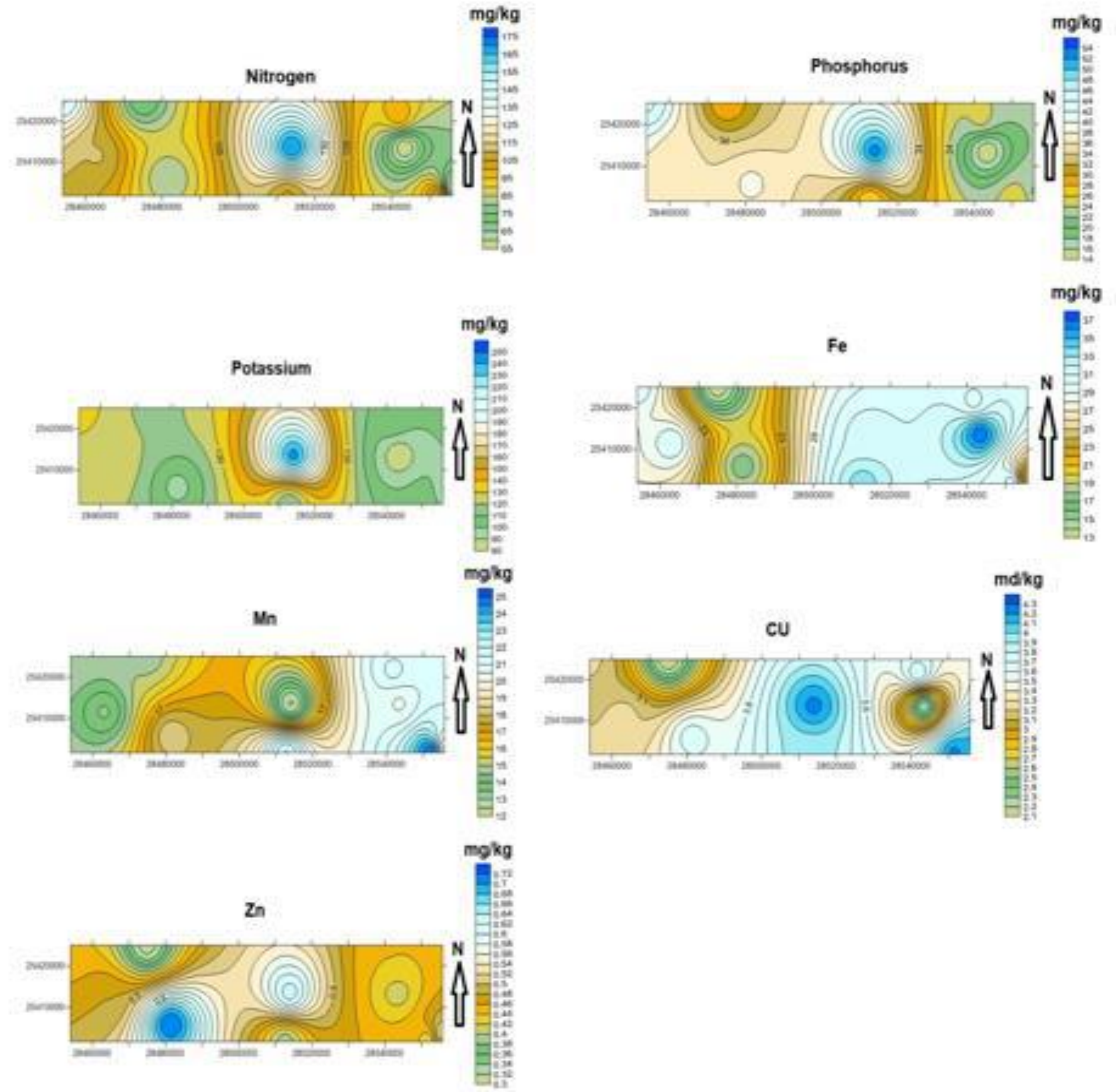

Fig.3: The distribution maps of macro and micronutrients in El-Qasr area soils.

3. 3 Linear correlation coefficients amonge macro-micronutrients and some soil physico- chemical properties in El-Qasr area.

The correlation coefficient in *Table 4* shows that NPK were related positively and significantly relationship with clay, (0.687, 0.625, 0.466 and, respectively), OM (0.728, 0.676, 0.519and, respectively), and CEC (0.415, 0.516, 0.233and, respectively), while, they negatively correlated with pH, EC, $CaCO_3$. This is evident as the proportion of clay in the soil is recognized to positively increase the amount of nutrients in the soil [26]**;** [3]**.** Meanwhile, Fe, Mn, and Cu were positively correlated with EC, $CaCO_3$, but they negatively correlated with clay, pH, OM, CEC, and SAR. Also, SAR was negatively correlated with K, Fe, Mn, and Cu, while it was positively correlated with N, P and Zn. As well as Zn was negatively correlated with clay, pH, EC, OM, and $CaCO_3$, while it was positively correlated with CEC and SAR. Generally, the high positive correlations may be attributed to imply that soil properties are influenced by similar climatic and biotic factors that are likely to influence the buildup of nutrients in the soil, while the negative associations mean that the soil properties are not influenced by similar climatic and biotic factors [8].

Table.3: The correlation coefficient among macro and micronutrients and some soil physico- chemical properties in El-Qasr area.

property	N	P	K	Fe	Mn	Cu	Zn
Clay	0.687	0.625	0.466	-0.059	-0.487	-0.157	-0.055
pH	-0.489	-0.611	-0.349	-0.402	-0.655	-0.510	-0.138
EC	-0.388	-0.264	-0.344	0.239	0.408	0.371	-0.051
OM	0.728	0.676	0.519	-0.058	-0.533	-0.285	-0.226
$CaCO_3$	-0.548	-0.666	-0.389	0.343	0.556	0.237	-0.171
CEC	0.415	0.516	0.233	-0.314	-0.349	-0.051	0.275
SAR	0.006	0.260	-0.008	-0.644	-0.102	-0.182	0.394

IV. CONCLUSION

The soil sample shows the relatively highly alkaline nature and low value of N, P, and K. The EC values were in a medium range for most soils but it's high in soil no 4 and 8. The excess amount of micro nutrient shows a medium range in most study soils. So, recommended that in location point where the soil is deficient in N, P, and K which requires addition of more fertilizer and manures to improve their physical and chemical properties and make it suitable plantation and for increasing plant growth and yield production.

REFERENCES

[1] Abd el-Regal, R.M., A.H. Abd el-Hamied and H.A. Nofal. 1995. Effect of organic and inorganic fertilizers on nutrients availability in waterlogged soils 1. Nitrogen and phosphorus. Annals of Agric. Sc. Moshtohor. 33(4):1615-1629.

[2] Arif B. T., Hikmet Günal, Kamil Sındır and Yüksel Balcı. 2001. Spatial Structure of Available Micronutrient Contents and Their Relationships with Other Soil Characteristics and Corn Yield. Fresenius Environmental Bulletin. PSP. Volume 20 – No 3a.

[3] Aweto A.O., & Enaruvbe G. O. (2010). Catenary Variation of Soil Properties under Oil Palm Plantation in South Western Nigeria. *Ethiopian Journal of Environmental Studies and Management, 3*(1), 1-7. http://dx.doi.org/10.4314/ejesm.v3i1.54389

[4] Botros, S. S.2013. Pedological studies of soils between West El-Mawhoob and Abo Monqararea Western desert-Egypt. Ph.D. Thesis: Desert Research Center, Cairo, Egypt.

[5] Brady, N.C. and Weil, R.R. 2002. The Nature and Properties of Soils, 13th Ed. Prentice- Hall Inc., New Jersey, USA., 2002, 960.

[6] Chaney, R. and Slonim, S. 1982. Determination of Calcium Carbonate Content in Soils: Geotechnical Properties Behavior and Performance of Calcareous Soils. In: "ASTM STP, Vol: 777. American Society for Testing and Materials" Demars, K. R., Chaney, R. (Eds.), Philadelphia, PP. 3-15.

[7] Chude, V. O., Malgwi, W. B., Amapu, I. Y. and Ano, A. O. (2011). *Manual on Soil Fertility Assessment*. Federal Fertilizer Department. FAO and National programme on Food security, Abuja, Nigeria. 62.

[8] Deekor, T. N.2012. Changes in Soil Properties under Different Land Use Covers in Parts of Odukpani, Cross River State, Nigeria. J. of Env. and Ecology. ISSN 2157-6092. Vol. 3, No. 1

[9] Dhanushkodi, V. and K. Subrahmaniyan, 2012. Soil Management to increase rice yield in salt affected coastal soil. Int. J. Res. Chem. Environ., 2: 1 - 5.

[10] El-Desok y, M.A. and H.M. Ragheb. 1993. Availability of P in sandy calcareous soils: II. Effects of organic matter and added P. Assiut J. Agric. Sci. 24(1): 137-153.

[11] El-Sankary, M.M. 2002. Geological, sedimentological and radioactivity studies of the Quaternary sediments, ElKharga Depression, Western Desert, Egypt.Unpublished Ph.D. Thesis, Ain Shams Univ., Cairo,Egypt, 241p.

[12] El-Sayed, M. A., Abd El-Aziz, S. H., El-Desoky, A. I. and, Selmy, S. A. H. 2016.Pedomorphic Features and Soil Classification of Gharb El-Mawhob area, El-Dakhla Oasis, Western Desert, Egypt. 1 Middle East Journal of Agriculture Research ISSN 2077-4605 Volume : 05 | Issue : 02 | April-June | 2016 Pages: 247-257.

[13] Epstein, E. and A. Bloom. 2005. Mineral nutrition of plants: Principles and perspectives.Sunderland, MA: Sinauer Associates.

[14] Faragallah, M.E.A. 1995. Relative distribution of certain nutrients in soils of the Nile valley-desert interference zone, east of Assiut city. M.Sc. Thesis, Faculty of Agric., Assiut Univ., Egypt.

[15] Ghoneim, M.F., M. Abd El-Razek, G.S. El-Gharably and K. AbdEllah. 1984. Micronutrient status in soils of upper Egypt. 1- Zinc. Assiut J. Agric. Sci. 15(3): 15-26.

[16] HaribhushanAthokpam, ShabirHussainWani, David Kame, Herojit Singh thokpam, JyotsnaNongmaithem, Deepak Kumar, YanglemKenedy Singh, Brajendra Singh, Brajendra Singh Naorem,ThokchomRenuka Devi and Lamalakshmi Devi. 2013. Soil macro- and micro- nutrient status of Senapati district, Manipur (India). African Journal of Agricultural Research. Vol. 8(39), pp. 4932-4936.

[17] Hesse, P.R. 1998. A textbook of soil chemical analysis. CBS Publishers & Distributors. Delhi, India.

[18] Ibrahim, M.S., A. Abd El-galil and M.M. Kotb. 2001. Total and availability Fe, Mn, Zn, and Cu in some soils of Sohag Governorate and their association with some soil properties. Assiut J. Agric. Sci. 32:71-85.

[19] Jackson, M. L. 1973. Soil Chemical Analysis. Prentice Hall, New Delhi.

[20] Kishk, M.A., M. AbdElrazek and G.S. El-Gharably. 1980. Status of certain micronutrients in some selected soils from Middle and Upper Egypt. Assiut J. Agric. Sci. 11: 197-206.

[21] Maji, A.K., R.G.P. Obi, S. Thayalan and N.J. Walke, 2005. Characteristics and classification of landforms and soils over basaltic terrain in sub-humid tropics of central India. J. Indian Soc. Soil Sci., 53: 154-162.

[22] Mclean, E. O. 1982. Soil pH and Lime Requirement. Part II. In: "Methods of Soil Analysis ", (Ed.): Page, A. L. 2nd Edition, ASA, Monograph, No. 9, Madison, WI, PP. 199-223.

[23] Nelson, D. W. and Sommers, L. E. 1982.Total Carbon, Organic Carbon, and Organic Matter. Part II. In: "Methods of Soil Analysis", (Ed.): Buxton, D. R. 2nd Edition, ASA, Monograph, No. 9, Madison, WI, PP. 539-579.

[24] Nigam G.K., V. K. Pandey, M. P.Tripathi, and Jitendra Sinha.2014. Assessment of Macro and Micro Nutrients of Soil in a Small Agricultural Watershed.International Journal of ChemTech Research. Vol.6, No.7, pp 3658-3664

[25] Paz-Gonzalez, A., S. R. Vieira and Ma .T. Taboada Castro, 2000.The effect of cultivation on the spatial variability of selected properties of an umbric horizon. Geoderma,97: 273-292.

[26] Reid, G. and Dirou, J. (2004). How to Interpret Your Soil Test. [Online] Available: http://www.dpi.nsw.gov.au/agriculture/resources/soils/testing/interpret.

[27] Sudan, P., R. Mathur, M.P. Chandrawat and S. Jain, 2015. Utilization of Marble slurry to Enhance Soil Fertility and to Protect Environment. Int. J. Pharm Bio Sci. 6(2): 81 – 84.

[28] Suge, J.K., M.E. Omunyin and E. N.Omami, 2011. Effect of organic and inorganic sources of fertilizer on growth, yield and fruit quality of eggplant (Solanum Melongena L). Arch. Appl. Sci. Res., 2011, 3 (6):470-479.

Assessment of Heavy Metals Level in Soil and Vegetables Grown in Peri-Urban Farms around Osun State and the Associated Human Health Risk

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Abstract—*Farming around urban centres (peri-urban farming) is a major source of fresh crop produce, notably vegetables. However, the limitation of land resources and the associated high level of soil contamination from domestic and industrial pollutants are major concerns for the safety of food materials from peri-urban farms. Thus, this study investigated heavy metals (As, Cd, Cu, Pb and Zn) concentration in soil and vegetable samples (Amaranthus hybridus and Corchorus olitorius) collected from selected peri-urban farms with a view to providing information on the human health risks associated with consumption of peri-urban vegetables.This study showed that the concentration of investigated heavy metals in the soils of peri-urban farms were within the background range for farming set by FAO/WHO (2002) and EU (2006) while appreciable level of these metals were observed in vegetable samples. Arsenic concentration was below detection limit in all samples. Amaranthus showed higher retention capacity for the assayed heavy metals except Cu. Transfer Factor values showed metal uptake by vegetables in the order Cd > Zn > Pb > Cu. The estimated daily intake showed that the highest consumption of Cd, Cu, Pb and Zn were from Amaranthus. The Health risk index showed high values for Cd and Pb but low values for Cu and Zn for both Amaranthus and Corchorus. The results obtained in this study regarding the hazard index indicate that vegetables grown in selected peri-urban farms are not safe for consumption.*

Keywords – Estimated daily intake, Heavy metals, Health risk index, Hazard index, Peri-urban farming, Transfer factor

I. INTRODUCTION

Peri-urban farming exists largely within and around boundary zones of cities all over the world (Mohammed and Folorunso, 2015). These periphery zones are characterized by off season vegetable production systems which are affected by or effecting environmental hazard (Ritcher, *et al*., 1995). The volume and diversity of demand for food stimulated the need for increasing agricultural production around vicinities of cities. Consequently, vegetable production has become intensive in peri-urban areas where there is high population and increasing demand for food (Jansen, 1992).

Irrigation is an essential component of peri-urban agriculture due to competing uses of water in urban areas (de Pascale *et al*., 2011). The burgeoning demand of water for irrigation has resulted in an increase in the reuse of waste water for agriculture. The use of waste water in peri-urban agriculture is prevalent in several localities around the world (Blumenthal *et al*., 2000; Ensink *et al*., 2002; Sharma *et al*., 2007).

The risks from peri-urban agricultural production may result from excessive agricultural inputs such as inorganic fertilizers, pesticides, sewage sludge and raw organic matter which may contain unwanted residues. Another key concern is the risk of pathogens and heavy metals contamination to consumers due to over dependence of production systems on organic waste and waste water which are readily available (Khai *et al*., 2007).

Heavy metals exposure is becoming a critical issue especially in developing regions of the world (Adriano, 2001; Jarup, 2003). Heavy metals accumulation in agricultural soil may not only result in contamination of soil but also in increased uptake by food crops which may affect its quality and safety (Muchuweti *et al*., 2006). Contamination of vegetables by heavy metals has recently received notable research attention because vegetables are consumed relatively in large amount and have the capacity to bioaccumulate heavy metals (Oluwatosin *et al*., 2010) consequently posing risk to human health.

Quite a number of researches have been carried out on contamination of soil and vegetables by heavy metals (Liu *et al.*, 2005; Mapanda *et al.*, 2005; Rattan *et al*., 2005). However, empirical data regarding heavy metals

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accumulation in soil and the resultant uptake by food crops through peri-urban farming activities are still needed. Therefore, this study was conducted to investigate heavy metals level in soil and vegetable samples collected from selected peri-urban farms, assess uptake of selected heavy metals by vegetables and to also assess the human health risk associated with consumption of peri-urban vegetables.

II. MATERIALS AND METHODS

Study Area/Sampling

The study areas are geographically located in Osun State, Southwestern part of Nigeria. The State is situated in the tropical rain forest zone. The area is characterized by rainy and dry seasons. The rainy season lasts from middle of March to late October and with peak periods in July and September. The dry season lasts from November to March. Sampling was carried out in seven cities namely; Ede, Ilesa, Ile-Ife, Ila-Orangun, Ikirun, Iwo and Osogbo. These locations were chosen because they represent the typical peri-urban off season vegetable production system in Osun State. Fifteen peri-urban farms were sampled in all from January to April (a period when irrigation was at its peak). Soil and edible vegetable samples from selected peri-urban farms were collected twice, during the first and second planting cycles. Cognizance of farming and production practises peculiar to each peri-urban farm was also taken.

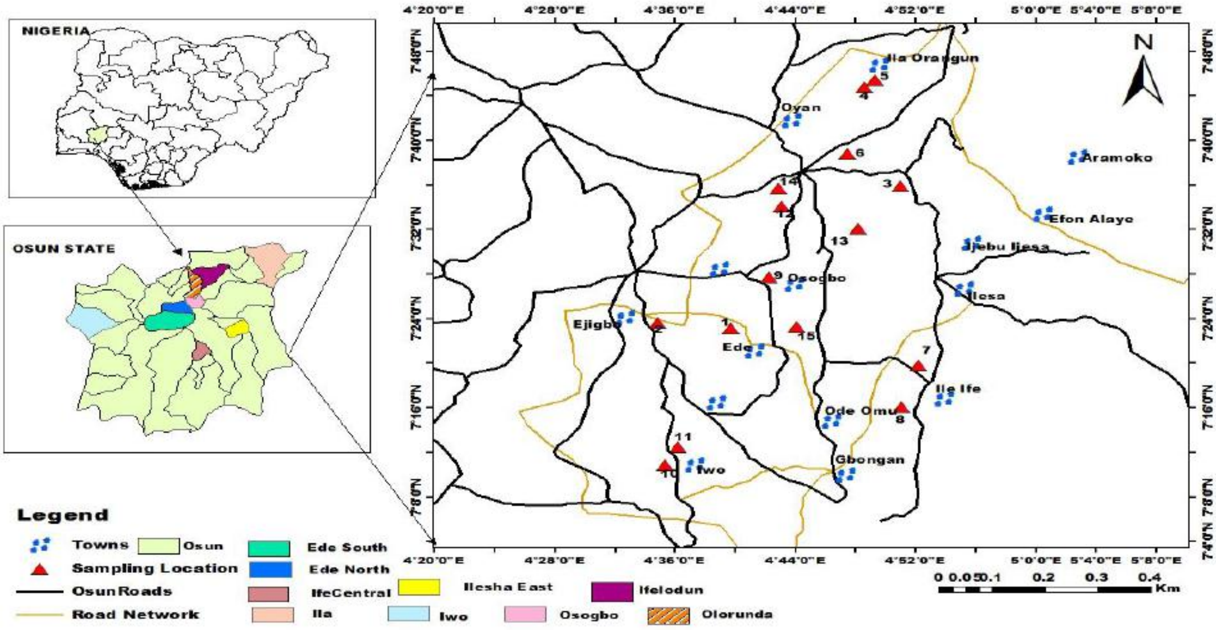

Fig.1: Map of Osun State Showing the Sampling Locations

Soil Sampling, Collection and Characterization

At each farm, soil samples were randomly collected from the upper horizon (0 -10 cm) using a soil auger and bulked together to form a composite sample. Each soil sample was placed in a labelled black polythene bag, sealed and taken to the laboratory. In the laboratory, soils were air-dried, crushed and sieved through a < 2 mm mesh, and then firmly sealed in paper envelopes until analysis. Sub-samples were used to determine the desired chemical properties. The soil pH was determined by the method of Blakemore *et al.* (1987). Organic carbon was also determined using the chromic acid determination method (Walkley and Black, 1934). Organic matter content of the soil was calculated from Organic carbon.

Plant Sampling, Collection and Preparation

Whole plant samples were collected by uprooting them from the same site where soils were collected. Two vegetable species *Amaranthus hybridus* (Amaranth) and *Corchorus olitorious* (Jute mallow) were selected for health risk assessment because they are the most widely cultivated and consumed leafy vegetables in Southwestern part of Nigeria. Vegetables sampled were between 2-3 months at harvest. After harvesting, plant samples were separated into shoot and root. The shoots were packed into brown envelope and labelled accordingly for laboratory preparation while the roots were discarded. In the laboratory, vegetable shoots were properly washed with distilled water to remove soil debris, weighed and then oven dried at 80^oC to constant weight. The oven dried samples were pulverized into fine powder using a stainless steel blender and passed through a 2 mm sieve. The resulting fine powder was stored appropriately, kept at room temperature before analysis and later digested and analyzed for As, Cd, Cu, Pb and Zn concentrations.

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Control

An experimental plot in Training and Research Farm of Obafemi Awolowo University, Ile-Ife served as the control site. Vegetable seeds were sown in soil irrigated with unpolluted water and without the application of fertilizers, manures and agrochemicals. Collection of soil and vegetable samples were made twice from January to April at about the same time sampling was being carried out in peri-urban farms.

Digestion of Samples

One gramme of both soil and vegetable samples were placed into 100 ml beaker separately to which 15 ml of trio-acid mixture (70% HNO_3, 65% $HClO_4$ and 70% H_2SO_4) was added in ratio 5:1:1. The mixture was digested at 80°C until the solution became clear indicating complete digestion. The resulting solution was then filtered and diluted to 50 ml and later analysed for metals concentration (Ogunfowokan *et al*., 2013).

Heavy Metals Analysis

The digested soil and vegetable samples were analysed for their heavy metals (As, Cd, Cu, Pb and Zn) content using Atomic Absorption Spectrophotometer PG 990 model available at the Central Science Laboratory, O.A.U., Ile-Ife. All concentrations were reported in mg/kg.

Quality Control

Measures were taken to check for background contamination and to ensure reliability of data. Blank samples were analyzed after seven samples. All analyses were replicated three times. Precision and accuracy of analysed metals were checked against standard reference material for every heavy metal.

Health Risk Assessments of Metals

Transfer Factor (TF)

Transfer factor was calculated as a ratio of heavy metals concentration in the extracts of soils and vegetables.

$$PCF = \frac{C_{plant}}{C_{soil}}$$ (Ciu *et al.*, 2005)

Where C_{plant} and C_{soil} represent heavy metal concentration in extracts of vegetables and soils on dry weight basis, respectively.

Daily Intake of Metals (DIM)

The daily intake (DIM) of heavy metals (As, Cd, Cu, Pb, Zn) was calculated as a product of heavy metals concentration in vegetables and the amount of the respective vegetable consumed. The DIM of metals was determined by the following equation.

$$\text{Daily intake of metals (DIM)} = DVC \times VMC$$

DVC = Daily vegetable consumption; VMC = Mean vegetable metal concentration (mg/kg)

Where daily vegetable consumption was considered to be 98g of vegetables per person per day for an average adult of 60 kg body weight (FAO/WHO, 1999).

Health Risk Index (HRI)

The health risk index (HRI) for the consumption of contaminated vegetables was estimated as the ratio of the daily intake of metals to the reference oral dose (RfD) for each metal. The HRI <1 means the exposed population is safe.

$$HRI = \frac{DIM}{RfD}$$

Reference oral dose are 0.003, 0.001, 0.04, 0.004 and 0.3 mg/kg/day for As, Cd, Cu, Pb and Zn respectively (FAO/WHO, 2013).

Hazard Index (HI)

The hazard index (HI) as developed by USEPA (2002) was calculated as the summation of the potential health risk index (HRI) arising from all the metals examined.

$$HI = \sum HRI_{Cd} + HRI_{Cu} + HRI_{Pb} + HRI_{Zn}$$

The value of the hazard index is proportional to the magnitude of the toxicity of the vegetables consumed.

Data Analysis

Descriptive statistics such as mean and range were used to summarize data collected from sampling sites. Statistical analysis for the cross sectional survey was carried out using Predictive Analytical software for Windows (SAS version 9.2). Analysis of variance ($p < 0.05$) and Pearson correlation coefficient were used to test for association between the different variables.

III. RESULTS AND DISCUSSION

Location of Peri-urban farms, Farming and Production Practices Peculiar to Each Peri-urban Farm

Table 1 shows the specific location of each peri-urban farm, farming and production practices peculiar to each farm. Sixty seven percent of the farmers irrigated their farms with nearby stream while 7% used shallow well and 13% each with river tributaries and waste water. About 93% of the farmers carried out weeding by hand pulling while 7% applied herbicide. Sixty percent of the farmers enhanced soil fertility by applying inorganic fertilizer, 13% applied both poultry manure and inorganic fertilizers while the remaining 27% depended on natural fertility.

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Table.1: Location of Peri-urban farms, Farming and Production Practices Peculiar to Each Peri-urban Farm

Farms	Location	Water Source	Soil Fertility Management	Agrochemical Input
1	Owode-Ede, by the roadside	Shallow well	Addition of inorganic fertilizer	-
2	Outskirt of Ede	Osun river tributaries	Addition of inorganic fertilizer and poultry manure	-
3	Ilo-Ajegunle	Nearby stream	Addition of inorganic fertilizer and poultry manure	-
4	Ila-Orangun, near an abandoned waste depot	Nearby stream channelled into the farm	Addition of inorganic fertilizer	-
5	Ila-Orangun	Nearby stream	Addition of inorganic fertilizer	-
6	Ido-Ijesa, near fish ponds	Stream chanelled into the farm	Addition of inorganic fertilizer	-
7	Outskirt of Ile-Ife	Stream chanelled in to the farm	By depending on nature fertility	-
8	By the road side, along Ede road, Ile-Ife	Waste water from O.A.U bioremediation pond	By depending on nature fertility	-
9	along Osogbo/Ilie road	Osun river tributaries	Addition of inorganic fertilizer	-
10	Outskirt of Iwo town, near a waste depot	Stream channeled into the farm	By depending on nature fertility	-
11	Between Telemu and Iwo	Waste water from a drainage basin	Addition of Inorganic fertilizer	-
12	Along Osogbo/Ikirun road	Nearby stream	By depending on nature fertility	-
13	Outskirt of Osogbo town	Nearby stream	Addition of inorganic fertilizer	Herbicide
14	Along Ikirun/Inisha road	Nearby stream	Addition of inorganic fertilizer	-
15	Outskirt of Osogbo town	Nearby stream	Addition of inorganic fertilizer	-

Heavy Metals Concentration in Peri-urban Farm Soils

In this study, soil pH ranged from 5.24 -7.87 indicating a moderately acidic to slightly alkaline pH. It was observed that where soil pH was recorded near neutral, low concentration of heavy metals was recorded in vegetables than in soil except for Cd. Total organic carbon in the peri-urban farm soils investigated ranged from 0.68-6.32%, suggesting a possibility of metals retention within the soil. Organic matter in soil samples ranged from low to high with values which varied between 1.18-10.87%. Soils of peri-urban farms hold within high amount of organic matter which could be as a result of agricultural applications. Ayolagba and Onmigbuta (2001) clearly showed that high organic matter (> 2.0%) in soil is favourable for chelation of heavy metals.

The distribution of heavy metals in the soil of peri-urban farms studied was mostly influenced by location of the peri-urban farm, prevailing farming practices and source of water for irrigation. Peri-urban farms located by the roadside, near waste depots and irrigated with waste water showed the highest level of contamination.

Accumulation of Cd in agricultural soils over time is induced by human activities (Taylor, 1997). Such activities include, excessive application of phosphate fertilizers, domestic and industrial effluents, waste water and pesticides (Kara *et al*., 2004), from traffic emission and tear and wear of alloyed parts of vehicles. Concentration of Cd in the soils of various peri-urban farms studied ranged from 0.18-0.63 mg/kg. These values were lower than the natural limit of 3.0- 5.0 mg/kg in soil as given by FAO/WHO (2002) and EU (2006). High Cd concentration in the soil of farm 10 may be due to metals mobility from a nearby waste depot while high level of Cd in the soils of farms 11 and 13 might come from agricultural applications (irrigation water source or the use of inorganic fertilizer as soil amender). The values obtained are similar to those observed by Asawalam and Eke (2006), Njoku and Ayoka (2007) and Oluyemi *et al*. (2008) who investigated heavy metal concentration and heavy metal pollutants from dump site and agricultural soils in Owerri, Ile-Ife and Osogbo, Nigeria.

The application of manure to agricultural soil increases soil Cu concentration (Mullins *et al*., 1982). Elevated levels of Cu may become harmful to plants, can affect organisms that feed on these plants adversely, and may enter water bodies through run- offs and leaching (Gupta and Charles, 1999). Copper binds strongly to organic matter and minerals in soils and so does not travel far after release (Alloway, 1990; Lentech, 2009). As a result of this, applied Cu has the tendency to accumulate in soil (Slooff *et al*., 1989). In this study, concentration of Cu in the investigated soil samples varied between 2.40-56.17 mg/kg which were below the permissible limit set by FAO/WHO (2002) and EU (2006). Soil samples collected from farm 6 and 10 had the highest concentration. Elevated levels of copper in Farm 6 could be traced to the use of Cu as additive in fish pellet (Bolan *et al.*, 2004) which might have leached into the farm while the elevated level of Cu observed in farm 10 could be traced to leaching from a nearby waste depot. The concentrations of Cu in this study were below those reported in soil samples of Torino (171.00 µg/g) by Biasioli *et al*. (2007) and Guang-dong (576.50 µg/g) by Zhou *et al*. (2007).

Lead is ranked as one of the most toxic heavy metals affecting man, animal and plant (Zude, 2000), which has been used by mankind for several years because of its wide variety of applications. Lead is found in large amount in many electronic devices, lead acid battery extensively used in car batteries which can end up in soil through corrosion. The concentration of lead in the investigated soil samples ranged from 0.70-36.75 mg/kg. In this study, soil samples from farms 1, 10 and 11 had the highest Pb concentration. High Pb concentration observed in farm 1 might be due to past atmospheric deposition derived from combustion of gasoline as a result of the farm's proximity to a highway. High concentration of Pb observed in Farm 10 and 11 could be from irrigation water source or as a result of metals mobility from a nearby waste depot to the farm through leaching and run –off. Lead levels obtained from this study were lower than those detected in British, England and Wales. Alloway (1995) reported that Pb content of normal British soil varied between 2 to 300 µg/g. Total Pb content in soils of peri-urban farms studied were below the critical concentration of 300 mg/kg (FAO/WHO, 2002) and 400 mg/kg (ICRCL, 1987).

Zinc is used in break lining because of its ability to conduct heat and is released during mechanical abrasion of vehicles, combustion of engine oil and wear and tear of tyres which are emitted into the environment as particles during deposition. In this study, Zn concentration ranged between 30 to 300 mg/kg with farms 10 and 13 having the highest concentrations. High concentration of Zn observed in farm 10 might be due to proximity of the farm to a waste depot from which zinc might have leached into the farm or could also come from irrigation water source. High concentration of Zn observed in farm 13 might come from herbicide application or irrigation water source. Normal concentration of Zn in soil ranges from 1 to 300 mg/kg (FAO/WHO, 2002). Mcgrath (1986) reported that concentration of Zn in the soil of England and Wales ranged between 5 to 3,648 mg/kg. In this study, Zn concentration is lower than this range. Ogundele *et al.* (2015) reported Zn concentration of between 30.8 to 219.23 mg/kg in soils collected along heavy traffic road which is similar to values obtained in this study. In this study, concentration of Arsenic was recorded below detection limit in almost all soil samples investigated.

Heavy Metals Concentration in Vegetables Produced from Peri-urban Farms

Concentration of heavy metals in vegetables collected from peri-urban farms showed significant variation. The variation in heavy metal concentrations in vegetables collected from the same farm may be ascribed to their morphological and physiological differences in uptake, exclusion, and accumulation of heavy metals (Kumar *et al.*, 2009). Concentration of heavy metals analysed in vegetables also varied from one farm to the other which might be due to differences in farming practices.

The concentration of Cd, Cu, Pb and Zn ranged between 0.19-0.83, 0.85-9.60, 0.80-11.55, 32.00-158.80 and 0.10-0.58, 2.18-10.33, 0.87-4.70, 14.12-88.50 mg/kg for Amaranthus and Corchorus respectively. The values of As were below detection limit in vegetables studied. Cadmium concentration in Amaranthus and Corchorus exceeded the permissible limits prescribed by FAO/WHO and EU (2006) for Cd concentration in leafy vegetables except in Corchorus collected from farms 14 and 15. Cadmium level measured in vegetables of peri-urban farms studied was lower than vegetables (10.37-17.79 mg/kg) from Titagarh West Bengal, India (Gupta *et al*., 2008), vegetables (25 mg/kg) from Turkey (Turkdogan *et al*., 2002).

Copper concentrations in Amaranthus and Corchorus collected from studied peri-urban farms were below the permissible limits set by FAO/WHO and EU (2006). The mean concentration of Cu in vegetables (4.63 mg/kg for

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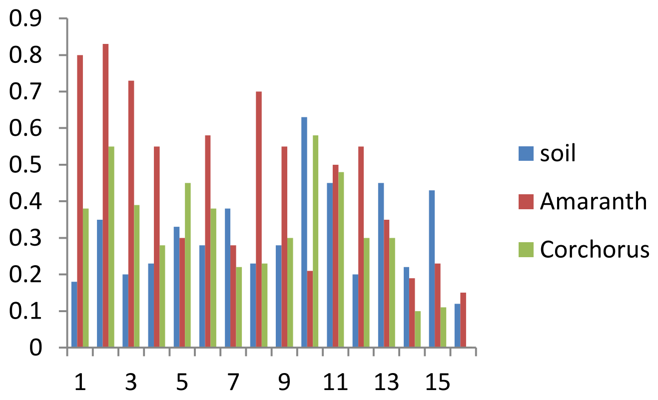
Amaranthus and 7.36 mg/kg for Corchorus) was lower than Cu content in vegetables (61.20 mg/kg) from Zhengzhou city, China (Liu *et al*., 2005) and also lower than the result (15.66-34.49 mg/kg) obtained in Titagarh West Bengal, India (Gupta *et al*., 2008). However, the variation in Cu concentration in the present study was supported by Arora *et al*. (2008) who reported Cu level of 5.21-18.2 mg/kg in vegetables. Higher Cu concentration was found in Corchorus.

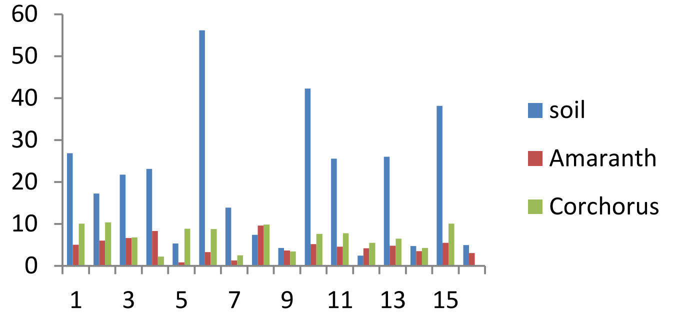
Lead concentrations in vegetables collected from studied peri-urban farms exceeded the permissible limits set by FAO/WHO and EU (2006). Lead content in vegetables was below values reported in Titagarh, West Bengal, (21.59-57.63 mg/kg) and also lower than the mean concentration of Pb (409 mg/kg) reported in vegetables from Turkey by Turkdogan *et al.* (2002) but comparable with Pb level reported (0.18-7.75 mg/kg) in China (Liu *et al.*, 2005) and in Varanasi, India (3.09-15.74 mg/kg) by Sharma *et al.*, 2008b).

Vegetables collected from peri-urban farms exceeded the permissible limits set for Zn by FAO/WHO and EU (2006) except in Amaranthus collected from farms 5 and 15 and Corchorus from farms 4, 5, 8, 14 and 15. Zinc concentration in vegetables from studied peri-urban farms was similar to vegetables (32.01-69.26 mg/kg) from Beijing, China (Liu *et al*., 2005) and also from Rajasthan, India (21.1-46.4 mg/kg) as reported by Arora *et al.* (2008) but significantly lower than Zn concentration in vegetables (1,038-1,872 mg/kg) from Harare, Zimbabwe (Thandi *et al*., 2004).

There was difference in heavy metals concentration in control soil and vegetable samples compared to heavy metals concentration in soil and vegetable samples from peri-urban farms with significant values ($p < 0.05$). Mean heavy metals (Cd, Cu, Pb and Zn) concentration in studied peri-urban farm soils, vegetables and control samples are shown in Fig. 2.



(a)



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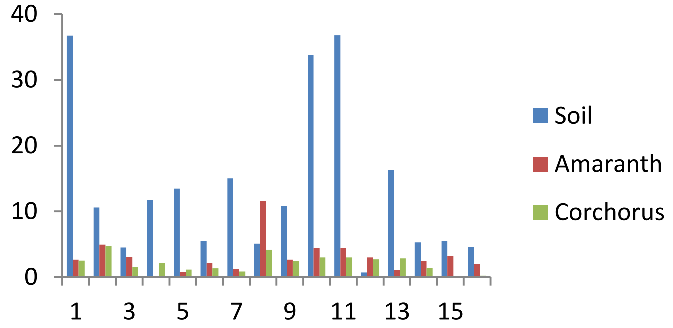

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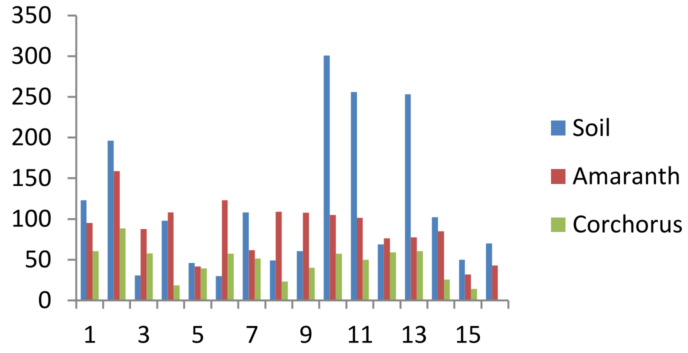

(d)

Fig. 1: Mean heavy metals distribution in peri-urban farm soils, vegetables and control samples: (a) Cd; (b) Cu; (c) Pb; and (e) Zn

Transfer Factor of Individual Metal to Vegetables (TF)

Transfer factor shows the proportion of heavy metals in the soil taken up by plants (Harrison and Chirgawi, 1989; Smith *et al.*, 1996). The soil-to-plant transfer factor is one of the pathways of human exposure to heavy metals through the food chain. The TF for Cd, Cu, Pb and Zn ranged from 0.07-4.44, 0.06-0.41, 0.07-4.28, 0.31-4.08 and 0.11-2.11, 0.06-2.27, 0.06-3.86, 0.13-2.63 mg/kg, for Amaranthus and Corchorus respectively. Amaranthus had the highest TF for all metals except Cu. Transfer factor were observed to be higher for Cd and Zn whereas lower values were found in Cu and Pb which varied with sampling site. The high transfer value of Cd and Zn indicate strong bioaccumulation of the metals by vegetables. Naser *et al*. (2011) reported similar result where they observed that Zn had the highest transfer factor among other metals. There existed strong correlation between Cd, Pb and Zn concentrations in soils and Corchorus collected from peri-urban farms including Cu and Zn concentrations in Amaranthus and Corchorus at ($p < 0.05$) which indicates similar sources of contamination. The general weak correlation between concentration of metals in soils and vegetables which has also been reported (Agbenin *et al*., 2009) indicates that other sources such as foliar absorption might have contributed to heavy metals load in vegetables. The plant transfer factor is presented in Table 2.

Table.2: Transfer Factor of Individual Metal from Soil to Amaranthus hybridus and Corchorus olitorius (mg/kg)

Farm	TFAs	TFCd	TFCu	TFPb	TFZn
1	-	4.44^a	0.19^a	0.07^a	0.77^a
	-	2.11^b	0.37^b	0.06^b	2.63^b
2	-	2.37^a	0.35^a	0.47^a	0.81^a
	-	1.57^b	0.60^b	0.44^b	0.45^b
3	-	3.63^a	0.30^a	0.68^a	2.87^a
	-	1.96^b	0.31^b	0.34^b	1.88^b
4	-	1.96^a	0.41^a	0.87^a	1.10^a
	-	1.22^b	0.45^b	0.19^b	0.19^b
5	-	0.92^a	0.15^a	0.06^a	0.91^a
	-	1.22^b	1.65^b	0.97^b	0.85^b
6	-	2.09^a	0.06^a	0.38^a	4.08^a
	-	1.36^b	0.16^b	0.08^b	1.92^b
7	-	0.73^a	0.09^a	0.08^a	0.56^a
	-	0.40^b	0.18^b	0.06^b	0.47^b
8	-	3.04^a	1.30^a	2.26^a	2.20^a
	-	1.00^b	1.33^b	0.81^b	0.47^b
9	-	2.00^a	0.85^a	0.24^a	1.78^a
	-	0.11^b	0.06^b	0.32^b	0.66^b
10	-	0.34^a	0.12^a	0.35^a	0.35^a
	-	0.92^b	0.18^b	0.15^b	0.19^b
11	-	1.05^a	0.18^a	0.12^a	0.40^a
	-	0.94^b	0.30^b	0.08^b	0.20^b
12	-	2.75^a	1.75^a	4.28^a	1.11^a
	-	1.50^b	2.27^b	3.86^b	0.85^b
13	-	0.78^a	0.18^a	0.07^a	0.31^a
	-	0.67^b	0.25^b	0.18^b	0.13^b
14	-	0.56^a	0.75^a	0.26^a	0.83^a
	-	0.45^b	0.26^b	0.29^b	0.25^b
15	-	0.50^a	0.14^a	0.01^a	0.86^a
	-	0.25^b	0.26^b	0.19^b	0.28^b

a – TF of individual metal from soil to *Amaranthus hybridus*
b – TF of individual metal from soil to *Corchorus olitorius*

Estimated Daily Intake of Metals (DIM)

The estimated daily intake of metals for adult is given in Table 3. The pathway for Cd, Cu, Pb and Zn intake was presumed to be vegetable consumption. The DIM of Cd, Cu, Pb and Zn ranged from 0.0003-0.001, 0.00021-0.016, 0.002-0.014, 0.053-0.159 and 0.0002-0.0016, 0.004-0.016, 0.0017-0.0076 and 0.023-0.144 mg/kg/day, respectively from consumption of Amaranthus and Corchorus respectively. The results for the evaluation of DIM for Cd, Cu, Pb and Zn showed that the highest intake of Cd, Cu, Pb and Zn were from consumption of Amaranthus. The estimated DIM when compared to recommended daily intake/ allowance for heavy metals (USEPA, 2009) was below the recommended daily intake/ allowance for metals studied. Zhuang *et al.* (2009) and Sharma *et al*. (2010) also reported DIM values lower than the allowable daily intake limits but Sridhara *et al*. (2007) recorded DIM values for heavy metals that were lower than tolerable daily intake limits.

Table.3: Daily Metals Intake Estimate (mg^{-1} kg^{-1} $person^{-1}$ d^{-1}) from Consumption of Amaranthus hybridus and Corchorus olitorious in Adult

Farm	As	Cd	Cu	Pb	Zn
1	-	0.0010^{a}	0.0080^{a}	0.0040^{a}	0.1550^{a}
	-	0.0006^{b}	0.0160^{b}	0.0041^{b}	0.0920^{b}
2	-	0.0010^{a}	0.0100^{a}	0.0080^{a}	0.2590^{a}
	-	0.0008^{b}	0.0168^{b}	0.0076^{b}	0.1440^{b}
3	-	0.0012^{a}	0.0110^{a}	0.0050^{a}	0.1430^{a}
	-	0.0006^{a}	0.0110^{a}	0.0025^{a}	0.0920^{a}
4	-	0.0009^{b}	0.0150^{b}	0.0140^{b}	0.1760^{b}
	-	0.0004^{a}	0.0170^{a}	0.0035^{a}	0.0290^{a}
5	-	0.0005^{b}	0.0010^{b}	0.0013^{b}	0.0680^{b}
	-	0.0007^{a}	0.0140^{a}	0.0019^{a}	0.0640^{a}
6	-	0.0009^{b}	0.0054^{b}	0.0030^{b}	0.2010^{b}
	-	0.0006^{s}	0.0140^{a}	0.0022^{a}	0.0940^{a}
7	-	0.0005^{b}	0.0021^{b}	0.0019^{b}	0.1009^{b}
	-	0.0004^{a}	0.0040^{a}	0.0014^{a}	0.0843^{a}
8	-	0.0011^{b}	0.0160^{b}	0.0190^{b}	0.1780^{b}
	-	0.0004^{a}	0.0160^{a}	0.0067^{a}	0.0380^{a}
9	-	0.0009^{b}	0.0060^{b}	0.0040^{b}	0.1760^{b}
	-	0.0004^{a}	0.0060^{a}	0.0039^{a}	0.0660^{a}
10	-	0.0003^{b}	0.0085^{b}	0.0079^{b}	0.1715^{b}
	-	0.0009^{a}	0.0120^{a}	0.0057^{a}	1.0939^{a}
11	-	0.0008^{b}	0.0070^{b}	0.0073^{b}	0.1657^{b}
	-	0.0008^{a}	0.0127^{a}	0.0049^{a}	0.0820^{a}
12	-	0.0009^{b}	0.0069^{b}	0.0050^{b}	0.1245^{b}
	-	0.0005^{a}	0.0089^{a}	0.0044^{a}	0.0960^{a}
13	-	0.0006^{b}	0.0078^{b}	0.0017^{b}	0.1260^{b}
	-	0.0005^{a}	0.0140^{a}	0.0045^{a}	0.0990^{a}
14	-	0.0003^{b}	0.0057^{b}	0.0040^{b}	0.1388^{b}
	-	0.0002^{a}	0.0068^{a}	0.0023^{a}	0.0420^{a}
15	-	0.0004^{b}	0.0070^{b}	0.0053^{b}	0.0900^{b}
	-	0.0002^{a}	0.0160^{a}	0.0017^{a}	0.0230^{a}
RDI	-	0.0640	10.000	0.2400	40.000

a – Daily metal intake estimate from consumption of *Amaranthus hybridus* b- Daily metal intake estimate from consumption of *Corchorus olitorius*

Potential Health Risk Index (HRI) and Hazard Index (HI)

Food chain is one of the most significant routes of human exposure to heavy metals. Consumption of contaminated vegetables has been pinpointed as one of the major pathways of human exposure to toxic heavy metals. The HRI for Cd, Cu, Pb and Zn ranged from 0.30-1.20, 0.03-0.38, 0.10-4.75, 0.18-0.86 and 0.20-0.90, 0.10-0.43, 0.35-1.68 and 0.08-0.48 for consumption of Amaranthus and Corchorus respectively. The result showed high values for Cd and Pb but low values for Cu and Zn for both Amaranthus and Corchorus. Ikeda *et al.* (2000) and Zhuang *et al*. (2009) also observed HRI values for Cd and Pb which were above permissible limits in vegetables and cereals. Considering individual heavy metal, the health risk index is in the order Pb > Cd > Zn > Cu but when considering vegetables type, the health risk index was Amaranthus > Corchorus. The calculated HRI for Cd and Pb from consumption of Amaranthus was greater than 1 in farms 1, 2, 3, 8 and farms 1, 2, 3, 4, 8, 9, 10, 11, 12, respectively. Health risk index for Pb from consumption of Corchorus was greater than 1 in farms 1, 2, 8, 10, 11, 12 and 13 which means that inhabitants around farms 1, 2, 3, and 8 are at significant risk of Cd toxicity from consumption of Amaranthus while inhabitants around farms 1, 2, 3, 4, 8, 9, 10, 11, 12, 13 are exposed to risk of Pb toxicity from consumption of either Amaranthus or Corchorus. The estimated hazard index for all the assayed heavy metals in Amaranthus and Corchorus of all the peri-urban farms studied was greater than 1. The result of this study regarding the HI revealed that vegetables grown in selected peri-urban farms are not safe for consumption. The HRI and HI of heavy metals through consumption of vegetables are presented in Table 4.

Table.4: Potential Health Risk and Hazard Index of Heavy Metals through Intake of Amaranthus hybridus and Corchorus olitorius in Adult

Farm	As	Cd	Cu	Pb	Zn	HI
1	-	1.00[a]	0.21[a]	1.00[a]	0.52 [a]	2.73[a]
	-	0.60[b]	0.40[b]	1.03[b]	0.31[b]	2.30[b]
2	-	1.00[a]	0.24[a]	2.03[a]	0.86[a]	4.13[a]
	-	0.80[b]	0.42[b]	1.90[b]	0.48[b]	3.60[b]
3	-	1.20[a]	0.28[a]	1.25[a]	0.48[a]	3.21[a]
	-	0.64[b]	0.28[b]	0.63[b]	0.31[b]	1.86[b]
4	-	0.90[a]	0.38[a]	3.50[a]	0.59[a]	5.37[a]
	-	0.40[b]	0.43[b]	0.88[b]	0.09[b]	1.80[b]
5	-	0.49[a]	0.03[a]	0.33[a]	0.22[a]	1.07[a]
	-	0.70[b]	0.35[b]	0.48[b]	0.21[b]	1.74[b]
6	-	0.95[a]	0.14[a]	0.75[a]	0.67[a]	2.51[a]
	-	0.60[b]	0.35[b]	0.55[b]	0.31[b]	1.81[b]
7	-	0.50[a]	0.05[a]	0.48[a]	0.33[a]	1.36[a]
	-	0.40[b]	0.10[b]	0.35[b]	0.30[b]	1.15[b]
8	-	1.10[a]	0.40[a]	4.75[a]	0.59[a]	6.84[a]
	-	0.40[b]	0.40[b]	1.68[b]	0.13[b]	2.61[b]
9	-	0.90[a]	0.15[a]	1.00[a]	0.22[a]	2.27[a]
	-	0.40[b]	0.15[b]	0.98[b]	0.22[b]	1.75[b]
10	-	0.30[a]	0.21[a]	1.98[a]	0.57[a]	3.06[a]
	-	0.90[b]	0.30[b]	1.43[b]	0.31[b]	2.94[b]
11	-	0.80[a]	0.18[a]	1.83[a]	0.55[a]	3.36[b]
	-	0.80[b]	0.32[b]	1.23[b]	0.27[b]	2.62
12	-	0.90[a]	0.17[a]	1.25[a]	0.42[a]	2.74
	-	0.50[b]	0.22[b]	1.10[b]	0.32[b]	1.09
13	-	0.60[a]	0.20[a]	0.43[a]	0.42[a]	1.65
	-	0.50[b]	0.28[b]	1.13[b]	0.27[b]	2.24
14	-	0.30[a]	0.14[a]	0.10[a]	0.46[a]	1.00
	-	0.20[b]	0.17[b]	0.58[b]	0.32[b]	1.09
15	-	0.40[a]	0.18[a]	0.13[a]	0.30[a]	1.01
	-	0.20[b]	0.40[b]	0.43[b]	0.08[b]	1.11

HI = hazard index a - HRI of heavy metals from consumption of *Amaranthus hybridus*
b - HRI of heavy metals from consumption of *Corchorus olitorius*

IV. CONCLUSION

In this study, investigated heavy metals concentration in the soils of studied peri-urban farms were within the background range for farming set by FAO/WHO (2002) and EU (2006). The results obtained from vegetables analysis for Cd, Cu, Pb and Zn indicate appreciable level of these metals in all the samples. Arsenic concentration was below detection limit in soil and vegetable samples collected from peri-urban farms. Average metal concentration in vegetables was higher in Amaranthus compared to Corchorus which suggest that Amaranthus has relatively higher bioaccumulation capacity compared to Corchorus. However, Corchorus showed higher retention capacity for Cu revealing potential use of Corchorus as a plant for environmental monitoring and soil remediation of Cu. The variability of heavy metals transfer factor was shown to be inherently strong for Cd and Zn but mild for Cu and Pb. This study also revealed that vegetables under study may constitute significant health risk to consumers as they were found to contain higher than allowable level of heavy metals such as Cd and Pb which are toxic. Also, the hazard index of heavy metals in all the peri-urban farms studied was > 1 which signifies relative presence of health risks related to ingestion of contaminated vegetables.

REFERENCES

[1] Adriano, D. C. (2001): Trace elements in terrestrial Environment. Biogeochemistry, Bioavailability and Risk of Metals. Springer Verlag, New York, pp. 3-53.

[2] Agbenin, J. O., Danko, M. and Welp, G. (2009): Soil and vegetable compositional relationships of eight potentially toxic heavy metals in urban garden fields from northern Nigeria. *Journal of Food and Agriculture.*189, 49-54.

[3] Alloway, B. J. (1990): Soil Processes and the behavior of metals. In: Alloway, B. J. (ed.). *Heavy metals in soil*. Blackie and Son Ltd. Glasgow, pp. 100-121.

[4] Alloway, B. J. (1995): The origin of heavy metals in soils. In: Alloway, B. J. (ed.), *heavy metals in soils.* Blackie Academic and Professional, London, UK. pp.38-57.

[5] Arora, M., Kiran, B., Rain, S., Rani, A., Kaura, B. and Mittal, N. (2008): Heavy metals accumulation in Vegetables irrigated with water from different sources. *Food Chemistry.* 11, 811-815.

[6] Asawalam, D. O. and Eke, C. I. (2006): Trace metal concentration in soils used for waste disposal around Owerri, Nigeria. In: *Proceedings of the 40th Conference of the Agricultural Society of Nigeria, Michael Okpara University of Agriculture, Umudike, Abia State*, Nigeria. pp. 427-430.

[7] Ayolagba, G. A. and Onwugbata, G. C. (2001): Suitability comparison of waste disposal site. *27th Proceedings of the Annual Conference of the Soil Science of Nigeria*. University of Calabar, Calabar, Nigeria, pp. 23-25.

[8] Biasioli, M., Grcman, H., Kralj, T. Madrid, F., Diaz, B. and Ajmone-Marsan, F. (2007): Potentially Toxic Elements Contamination in Urban soils: A Comparison of Three European Cities. ASA, CSSA and SSSA, *Journal of Environmental Quality.* 36, 70-79. doi: 10.2134/jeq2006.0254.

[9] Blakemore, L. C., Searle, P. L. and Daly, B. K. (1987): Methods for chemical analysis of soils. New Zealand Soil Bureau Scientific Report, 80: 103 pp.

[10] Blumenthal, U., Peasey, A., Ruiz-Palacios, G., Mara, D. D. (2000): Guidelines for wastewater reuse in agriculture and aquaculture: recommended revisions based on new research evidence. Task No. 68, Part 1. Retrieved from persistent URL http://www.lboro.ac.uk/well/resources/well-studies/full-reports-pdf on 10th of Jan, 2017.

[11] Bolan, N. S., Surinder, S., Jiafa, L., Rita, B. and Jagrati S. (2004): Gaseous emissions of nitrogen from grazed pastures: Processes, measurement and modeling, environmental implication and mitigation. *Advances in Agronomy*. 84, 37-120.

[12] De Pascale S., Costa, L. D., Vallone, S., Barbieri, G. and Maggio, A. (2011): Increasing water use efficiency in vegetable crop production: from plant to irrigation systems efficiency. *Horticultural Technology*. 21(3), 301–308.

[13] Ensink, J. H., van der Hoek, W., Matsuno, Y., Munir, S. and Aslam, M. R. (2002): Use of untreated wastewater in peri urban agriculture in Pakistan: Risks and Opportunities. Res. Rep. 64, Colombo: International Water Management. Institute. (IWMI).

[14] European Union (2006): Heavy metals in wastes, European Commission on Environment (http://ec.europa.eu/environment/waste/studies/pdf/heavy metals report.pdf)

[15] FAO (1999): Urban and Peri-Urban Agriculture. Report to the FAO Commercial.

[16] FAO/WHO (2002): Codex Alimentarius Commission: Food additives and Contaminants. Joint FAO/WHO food standard programme, ALINORM 01/12A:1-289

[17] FAO/WHO, (2013): Joint FAO/WHO food standard programme codex committee on contaminants in foods, fifth session pp 64-89Agriculture. (Coag) Meeting from Jan. 25–26. FAO, Rome.

[18] Gupta, G. and Charles, S. (1999): Trace elements in soils fertilized with poultry litter. *Poultry Science.* 78, 1695-1698.

[19] Gupta, N., Khan, D. K. and Santra, S. C. (2008): An assessment of heavy metal contamination in vegetables grown in wastewater-irrigated of Titagarh, West Bengal, India. *Bulletin of Environmental Contamination and Toxicology*. 80, 115-118.

[20] Harrison, R. M. and Chirgawi, M. B. (1989): The assessment of air, soil as contributors of some trace metals to vegetable plants. Use of a filtered air growth cabinet. *Science of the Total Environment*. 83(1-2), 13-34.

[21] ICRCL, (1987): Interdepartmental Committee on the redevelopment of contaminated land. Guidance on the assessment and redevelopment of contaminated land. Guidance Note. 59/83. Department of Environment, London, pp. 388-394.

[22] Ikeda, M., Zhang, Z. W., Shimbo, S., Watanabe, T., Nakatsuka, H., Moon, C. S., Matsuda-Inoguchi, N. and Higashikawa, K. (2000): Urban population exposure to lead and cadmium in east and south-east

Asia. *Science of the Total Environment.* 249, 373-384.

[23] Jansen, H. G. P. (1992): Supply and demand of AVRDC mandate crops in Asia: implications of past trendsfor future developments. AVRDC Working Paper Series no. 4, vol. 84. Asian Vegetable Research and Development Center, Taiwan.

[24] Jarup, L. (2003): Hazards of heavy metal contamination. *British Medical Bulletin*. 68, 167–182.

[25] Kara, E. E., Pirlak, U. and Ozdilek, H. G. (2004): Evaluation of heavy metals (Cd, Cu, Ni, Pb and Zn) distribution in sowing regions of potato fields in the province of Nigde, Turkey. *Water, Air and Soil Pollut*ion. 153, 173-186.

[26] Khai, N. M., Pham Q. H. and Irigrid, O. (2007): Nutrient flow in small scale peri-urban farming system in Southeast Asia- A case study in Hanoi. *Agriculture, Ecosystem and Environment.* 122, 192-202.

[27] Kumar, B. M., Ramachandra, P. K., Vindar, D. N. (2009): Agroforesty as a strategy for carbon sequestration. *Journal of Plant Nutrition and Soil Science.* 172(1), 10-23.

[28] Lenntech, W. T. (2009): Chemical properties, Health and Environmental Effects of Cu. Lenntech Water Treatment and Purification Holding, B. V., 3 p.

[29] Liu, W. H., Zhao, J. Z., Ouyang, Z. Y., Solderland, L. and Liu, G. H. (2005): Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environment International* 32: 805–812.

[30] Mapanda, F., Mangwayana, E. N., Nyamangara, J. and Giller, K. E. (2005): The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. *Agriculture, Ecosystem and Environment.* 107, 151-165.

[31] Mcgrath S. P. (1986): The range of metal concentration in top soil of England and Wales in relation to soil protection guidelines. In: Hempill, D. D. (ed.), *Trace substances in Environment*. University of Missouri, Columbia, 36p.

[32] Mohammed, S. A. and Folorunsho, J. O. (2015): Heavy metals concentration in soil and *Amaranthus retroflexus* grown on irrigated farmlands in the Makara Area, Kaduna, Nigeria. *Journal of Geography and Regional Planning.* 8(8), 210-217.

[33] Muchuweti, M., Birkett, J. W., Chinyanga, E., Zvauya, R., Scrimshaw, M. D. and Lester, J. N. (2006): Heavy metal content of vegetables irrigated with mixture of waste water and sewage sludge in Zimbabwe: implications for human health. *Agriculture, Ecosystem and Environment.* 112, 41-48.

[34] Mullins, G. L., Martenz, D. C., Miller, W. P., Konegay, E. T. and Hallock, D. L. (1982): Copper availability, form and mobility in soils from three annual copper-enriched Hog Manure Application. *Journal of Environmental Quality.* 11, 316-320.

[35] Naser, H. M., Shil, N. C., Mahmud, N. U., Rashid, M. H. and Hossain, K.M. (2011): Lead, Cadmium and Nickel contents of vegetables grown in industrially polluted and non-polluted areas of Bangladesh. *Bangladesh Journal of Agricultural Research.* 34, 545-554.

[36] Njoku, P. C. and Ayoka, A. O. (2007): Evaluation of heavy metal pollutant from soils at municipal solid waste deposit in Owerri, Imo State, Nigeria. *Journal of Chemical Society of Nigeria.* 32(1), 52-60.

[37] Ogundele, D. T., Adio, A. A. and Oludele, A. A. (2015): Heavy metals concentration in plants and soil along heavy traffic road in North Central Nigeria. *Journal of Environmental and analytical Toxicology*. 5, 334.

[38] Ogunfowokan, O. A., Oyekunle, J. A. O., Olutona, G. O., Atoyebi, A. O. and Lawal, A. (2013): Speciation study of heavy metals in water and sediments from Asunle River of the Obafemi Awolowo University, Ile-Ife, Nigeria, *International Journal of Environmental Protection*. 3, 6-16.

[39] Oluwatosin, G. A., Adeoyolanu, A. O., Ojo, A. O., Are, K. S., Dauda, T. O. and Aduramigba-modupe, V. O. (2010): Heavy metal uptake and accumulation by edible leafy vegetable (*Amaranthus hybridus*) grown on urban valley bottom soils in Southwestern Nigeria. *Soil and Sediment Contamination.* 19, 1-20.

[40] Oluyemi, E. A., Feuyit, G., Oyekunle, J. A. O. and Ogunfowokan, A. O. (2008): Seasonal variations in heavy metals concentrations in soil and some selected crops at a landfill in Nigeria. *African Journal of Environmental Science and Technology.* 2, 89-96.

[41] Rattan, R. K., Datta, S. P., Chhonkar, P. K., Suribabu, K., Singh, A. K. (2005): Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study. *Agriculture, Ecosystem and Environment.* 109, 310-322.

[42] Richter, J., Schnitzler, W. H. and Gura, S. (1995): Vegetable production in peri-urban areas in the tropics and sub-tropics: food, income and quality of life. Gura, S. (ed.) Proceedings of an International Workshop, DSE, Feldafing, Germany. Oxford

University Press for the Asian Development Bank, Oxford.

[43] Sharma, R. K., Agrawal, M. and Marshall, F. M. (2005): Heavy metals contamination in vegetables grown in wastewater irrigated areas of Varanasi, India. *Bulletin of Environmental Contamination and Toxicology*. 77, 311–318.

[44] Sharma, R. K., Agrawal, M. and Marshall, F. M. (2008b): Atmospheric depositions of heavy metals (Cd, Pb, Zn and Cu) in Varanasi India. *Environmental Monitoring and Assessment*. 142(1-3), 269-278.

[45] Sloof, W., Clevan, R. F., Janus, J. A. and Ros, J. P. M. (1989): Integrated Criteria Document Cu. Report no. 758474009. National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands.

[46] Smit, J., Ratta, A. and Nasr, J. (1996): In: Urban Agriculture: Food, Jobs and Sustainable Cities. UNDP. Publication series for Habitat II, New York.

[47] Sridhara, N. C., Kamala, C. T., Samuel, D. and Suman, R. (2008): Assessing risk of heavy metals from consuming food grown on sewage irrigated soil and food chain transfer. *Ecotoxicology and Environmental Safety*. 69(3), 513-524.

[48] Taylor, M. D. (1997): Accumulation of Cd derived from fertilizer in New Zealand Soils. *Science of Total Environment*. 208,123-126.

[49] Thandi, N. K., Nyamangara, J. and Bangira, C. (2004): Environmental and potential health effect of growing leafy vegetables on soil irrigated using sewage sludge and effluent: A case of Zn and Cu. *Pesticides, Food Contaminants and Agricultural Wastes*. 39,461-471

[50] Turkdogan, M. K., Kilicel, F., Kara. K., Tuncer, I. and Uygan, I. (2002): Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. *Environment, Toxicology and Pharmaco*logy. 13, 175-179. Doi. 1016/S13826689(02)00156-4.

[51] USEPA, (2002): Multimedia, Multi-pathway and Multi-receptor Risk Assessment (3MRA) Modellling System. Environmental Protection Agency, Office of Research and Development, Washington DC, pp. 1-9.

[52] USEPA (United States Environmental Protection Agency) (IRIS) (2009): Integrated Risk Information System-database. Philadelphia PA; Washington, DC USEPA (2010): Exposure Factors Handbook – General Factors. EPA/600/P-95/002Fa, vol. I. Office of Research and Development. National Center for Environmental Assessment. US Environmental Protection Agency. Washington.

[53] Walkley, A. and Black, I. A. (1934): An examination of the pegiareff method for determining soil organic matter and a proposal on modification of the chromic acid titration method. *Soil Science*. 327, 29-32.

[54] (WHO) (2006): Guidelines for the safe use of wastewater, excreta and grey water: Wastewater use in agriculture (Volume II). Retrieved from persistent URL:http://www.who.int/water_sanitation_health/wastewater/gsuweg2/en/index.html

[55] Zhou, F., Guo. H. and Hao, Z. (2007): Spatial distribution of heavy metals in Hong kong's marine sediments and their human impacts: A GIS based Chemometric Approach. *Marine Pollution Bulletin*. 54(9), 1372-1384. Doi:10.1016/j.marpolbul.2007.05.017.

[56] Zhuang, P., Zou, B., Li, Z. A. (2009): Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, china: implication for human health. *Environmental Geochemistry and Health*. Doi:10.1007/s10653-009-9248-3.

[57] Zude, A. (2000): Determination of Pb in blood, M.Sc.Thesis. An-Najah National University.

Pre-sowing Treatment Enhanced Germination and Vigour of True Shallot (Allium cepa var. aggregatum) Seeds

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Abstract— *The objective of this research was to sudy the effects of pre-sowing treatments to enhance germination, growth and transplanting of true shallot seeds. The experiment was conducted in the glasshouse at Tangtu village, Badung regency from August until November 2017. Germination experiment was carried out in petridishes, while that for seedling vigour and growth experiment was done in plastic pots containing sand and top soil mixture. Both experiments were designed as complete randomized design with eight concentrations of KNO_3 (1, 1.5 and 2 M) and GA_3 (100, 150 and 200 ppm). Those treatments were replicated four times. Seeds were soaked for 24 hours in each concentration of solution prior to planting in petridishes as well as in pots. Results of the experiment showed that GA_3 significantly increased germination percentage, speed of germination (57-63% germinated seeds at 1 dap), index of seedling vigour, speed of seedling emergence (34,39% day^{-1}) and percentage of seedlings having one true leaf (52%) at 9 dap. The effects of KNO_3 on those variables were not significantly different from those of GA_3 except on speed of seedling emergence. KNO_3 2M and GA_3 (100, 150 , 200 ppm) resulted in 12 days earlier in transplanting seedlings than control.*

Keywords— Germination, pre-sowing, true shallot seeds, vigour.

I. INTRODUCTION

The True shallot seeds (TSS) are increasingly used by farmers in Indonesia. The benefits of TSS are free from pathogen, smaller number of planting materials, easier transporting and storaging, producing healthier crops and bigger bulbs (van den Brink and Basuki, 2011). The use of TSS is economically beneficial as well, due to doubling the yields compared to seed bulb crops. The problem in using TSS is that seeds have to be grown in the nursery for 20-25 days and after that have to be transplanted into the field, therefore needs longer time to produce bulbs compared to seed bulb (bulb-propagated) crops, therefore needs longer time to produce bulbs compared to seed bulb crops. To reduce the time in the nursery efforts have to be done to increase the germination of the seeds and faster estabishment of seedlings. The use of priming and pre-sowing methods have been succeeded to improve the speed of germination, increase germination percentage and reduced the amount of abnormal seedlings of onion (*Allium cepa cv. aggregatum L.*) (Caseiro *et al*., 2004, Tajbakhsh *et al*., 2004, Sevarani and Umarani, 2011, Jagosz, 2015). Seed priming treatments were also reported to be able to increase germination capacity of parsley (*Petroselinum crispum* L.) (Dursun and Ekinel, 2010), tomato (*Lycopersicum esculentum* L.) (Mirabi and Hasanabadi, 2012; Lara *et al*., 2014), carrot (*Daucus carota* L.) (Sevarani and Umarani, 2011) and is a suitable method of shortening the time for early crops (Barlow and Haigh, 1987).

II.

The role of KNO_3 in promoting germination of tomato seeds was also reported by Lara *et al*. (2014) and in increasing speed of seed germination and establishment of *Ramonda serbica* and *Ramonda nathaliae* by Gashi (2012). Gibberellin has roles in control and promote seed germination. GA_3 enhances biochemical reaction of hydrolase (particularly α amylase) enzyme synthesis in endosperm of cereal grains. Gibberellic acid stimulates seed germination through α amylase synthesis (Finch-Savage and Leubner, 2006). Effects of GA_3 at concentration of 1000 ppm combined with 0.3% KNO_3 were reported to result in the highest final germination percentage on *Ramonda serbica*, while the concentration of 500 ppm GA_3 gave higher germination percentage on *R. nathalie* (Gashi *et al*., 2012).

Seed priming by soaking the seeds in KNO_3 solution prior to planting is expected to increase germination and seedling establishment of true shallot seeds compared to those of seed bulb crops. If the seedlings are growing faster and stronger enough to be transplanted from the nursery into the field, the seedling growth could catch up with the growth of seed bulb crops. This research has an objective to study the effect pre-sowing treatments of KNO_3 and GA_3 concentrations on seed germination and seedling establishments of true seed shallots.

II. MATERIALS AND METHODS

The experiment was conducted in the glasshouse in the village of Tangtu, Badung regency from 29 August 2017 until 21 September 2017. The commercial seeds of shallot cv. Tuk-Tuk (produced by PT. East West Indonesia, Cap Panah Merah) used in this experiment The KNO_3 solutions were prepared at the concentrations of respectively 1, 1.5 and 2 M, while those of GA_3 were respectively 100, 150 and 200 ppm. Distilled water was used as control. An amount of 50 seeds were subjected to priming by soaking them in 25 ml of each solution concentration for 24 hours prior to planting. The treatments were arranged in a randomized complete design with four replicates making 28 units for each experiment.

Half amount of seeds were then cultured in petri dishes on a single sheet of Whatman No.1 filter paper, moistened with distilled water, kept on a glasshouse bench in 25±1ºC room temperature. Another 25 seeds for each treatment were planted in a plastic pot of a pot of 12 cm diameter having 600 g of mixture of sand and fertile potting soils. Pots were watered to field capacity and leave for 24 h before planting and a volume of 50 ml water was given to all pots every day.

Seed germination was monitored and counted everyday, and a seed was considered to be germinated when the radicle was 2 cm long (Soltani *et al*, 2015).

Data were recoded daily on germination percentage (GP) of normal seedlings (protrusion of radicles of 2 cm long in the petridishes for 7 days (Heydecker and Coolbear, 1977; Gashi *et al*., 2012; Jagosz, 2015), calculated using the following formulas:

$$GP\ (\%) = \frac{\text{number of germinated seeds}}{\text{number of total seeds}} \times 100\% \qquad (1)$$

where *D* is the number of days counted from the beginning of germination, and *G* is the number of seeds which germinated on day *D* (Moradi *et al*., 2008; Jagosz, 2015).

The development of seed germination (% germinated seeds day^{-1}) was also calculated in petridishes (2)

Seedling vigour index (SVI), the speed of seedling emergence (SSE), and percentage of seedlings having true leaves (at least one true leaf has been developed) (PSTL) were recorded in pot experiment. Seedling vigour index (Hossain *et al.*, 2006; Zanjan and Asli, 2012) was calculated as:

$$SVI = \frac{\text{Seedling length (cm)} \times \text{PSE (\%)}}{100} \qquad (3)$$

The criteria of PSE was similar to that of GP. Seedling length was randomly measured on five seedlings from the root to the hypocotyl and cotyledon at 9 DAP (De Souza *et al*., 2014). The speed of seedling emergence (SSE) was calculated using formula according to Islam *et al*. (2003):

$$SSE = \frac{\text{number of seedlings emerged at 1 DAP}}{\text{number of seedlings emerged at 9 DAP}} \times 100 \qquad (4)$$

Percentage of seedlings having true leaves ((at least one true leaf has been developed) (PSTL). (5)

The pot experiment was terminated 21 DAP at which seedlings were harvested and parameters were evaluated. Data collected from the experiments were analyzed statistically with ANOVA using CoStat and MstatC computer softwares and means were compared based on 5% Least Significant Different test.

III. RESULTS AND DISCUSSION

GA_3 solution significantly ($p<0.05$) affected individually all variables measured in this research as indicated by germination percentage (GP), development of seed germination (in petridish experiment), seedling vigour index (SVI), speed of seedling emergence (SSE) and percentage of seedlings having true leaves (PSTL) (pot experiment). The first true leaf on some seedlings was appeared 9 DAP.

3.1 Germination Percentage (GP), and The Development of Seed Germination

Soaking the seeds in GA_3 and KNO_3 concentrations, except KNO_3 2 M, resulted in significantly ($p<0.05$) higher germination percentage compared to the other treatments (Fig.1). The treatments of GA_3 (100 ppm, 150 ppm and 200 ppm) and KNO_3 1 M increased germination percentage of TSS 25.1% higher than control. KNO_3 concentration of 1.5 and 2.0 M did not significantly increased seed germination percentage. The use of KNO3 infact has important role in increasing germination of shallots as nitrate provided exogenously acts as a signal molecule favouring germination (Alboresi *et al*., 2005). The nitrate could be absorbed during soaking, being used in the metabolism of the embryo, through the activity of enzyme nitrate reductase (NR). This enzyme activity in the production of nitrite/nitric oxide couls act in promoting a faster germination (Lara *et al*., 2014).

Gibberellins, as growth hormones, have a role to enhance germination besides other functions such as stimulate cell elongation, flowering and fruiting. GA_3 promotes a biochemical reaction i.e. the synthesis of *hydrolase (particular α amylase)* in endosperms of cereal seeds (Finch-Savage and Leubner, 2006). The positive effects might be due to its role in influencing the permeability of the membranes which ultimately leads to activation of enzymes involved in protein synthesis and carbohydrate metabolism (Preece and Read, 1993). The effect of GA_3 1000 ppm combined with 0.3% KNO_3 was reported to result in the highest final germination percentage on *Ramonda serbica*, while the concentration of GA_3 500 ppm gave higher germination percentage on *R. nathalie*

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(Gashi *et al*., 2012). The use of KNO3 infact has important role in increasing germination of shallots as nitrate provided exogenously acts as a signal molecule favouring germination (Alboresi *et al*., 2005). Increased germination percentage of onion seeds compared to control was also reported by Duman (2002) and Yıldırım *et al* (2002).

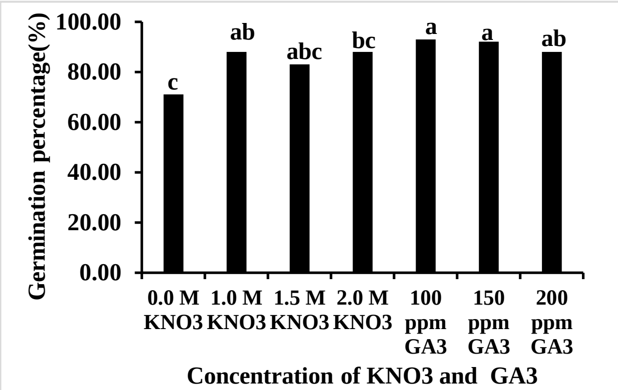

Fig. 1: Effect of KNO_3 dan GA_3 concentrations on germination percentage of true shallot seeds.

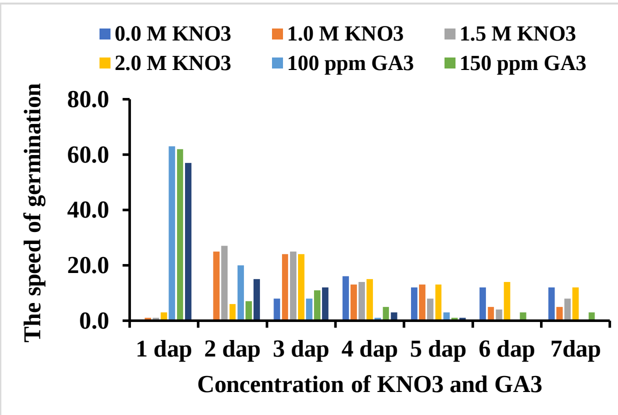

Fig.2: Effect of KNO_3 dan GA_3 concentrations on the speed of germination (% germinated seeds day^{-1}).

3.2 Seedling Vigour Index (SVI)

Vigour of seedling is an important factor to indicate the power of seedlings to grow and establish further. Such power is often reflected from seedling vigour index (SVI). The higher SVI indicates higher vigour of the seedling, guarantying they can be survived in unfavourable condition for growth. In this experiment GA_3 and KNO_3 concentrations resulted in average seedling vigour index of 2.43 higher than control (Fig. 3). The effects of GA_3 concentration were not significantly different from that of KNO_3 although this compound's ability in increasing seed germination was significantly lower. Higher average seedling vigour index indicated higher potential of seedlings to determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions. Increased seedling length due to effects of KNO_3 concentration could be resulted in vigourous seedlings, the similar effect found in tomato (Farooq *et al*., 2005). The ability of GA_3 in increasing seedling vigour was associated with the role of GA_3 as an activator of seed germination (Finkelstein *et al*., 2008) and as a promoter of seedling elongation and growth.

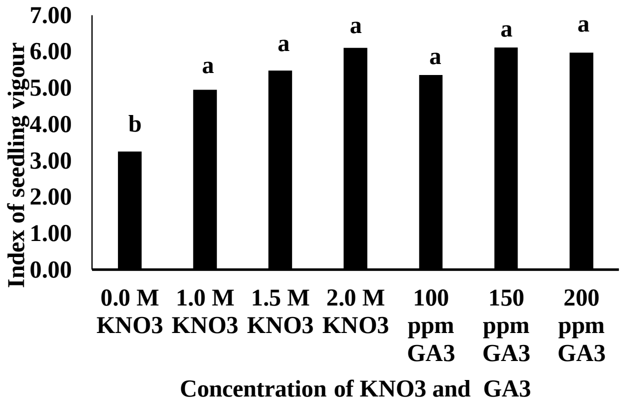

Fig. 3: Effect of KNO_3 dan GA_3 concentrations on index of seedling vigour

3.3 The Speed of Seedling Emergence (SSE)

The calculation of this variable involves number of seedlings emerge at one dap. multiply by 100 then divided by number of total seedlings emerged (Islam *et al*. (2003). GA_3 resulted in average of seedlings emergence (SSE) significantly 34.39% higher than that of KNO_3 as well as control (Fig. 4). This could be due to better permeability of the membranes, which contribute activation of enzymes involved in protein synthesis and carbohydrate metabolism (Preece and Read, 1993). Rapid emergence rate after priming or soaking may be due to increased rate of cell division in the root tips of primed seedlings as reported in wheat (Bose and Mishra, 1992) and in tomato (Farooq *et al*., 2005). Results of this experiment was not in line with that of Frett *et al*. (1991) on tomato, Demir and Mavi (2004) on watermelon and Govinden-Soulange and Levantard (2008) on other plant species, who reported that priming with KNO_3 increased emergence of seedlings.

3.4 Percentage of seedlings having true leaves (PSTL)

The effect of concentration of KNO3 2 M, which was not significantly different from that of GA_3 (100, 150 and 200 ppm), resulted in 52% higher seedlings having at least one true leaf at the of 9 dap. The lower concentration of KNO_3 (1 and 1.5 M) produced significantly lower percentage of seedlings having at least one true leaf, although they were still higher than that in control (Fig. 5). Nitrate (NO_3) that was absorbed during soaking, then used in the metabolism of the embryo, and as a nitrogen source may contribute to seedling growth and early vegetative development. KNO_3 as nitrogen-source fertilizer has a role in promoting vegetative growth of seedlings. GA_3, due to its role in stimulating cell elongation, also contributes to seedling growth. The treatment of GA_3 resulted in 34.39% more seedlings emerged in comparison to the average effect of KNO3 as well as control (Fig. 4). Seeds with high speed of emergence are indicated by their high emergence index (SVI).

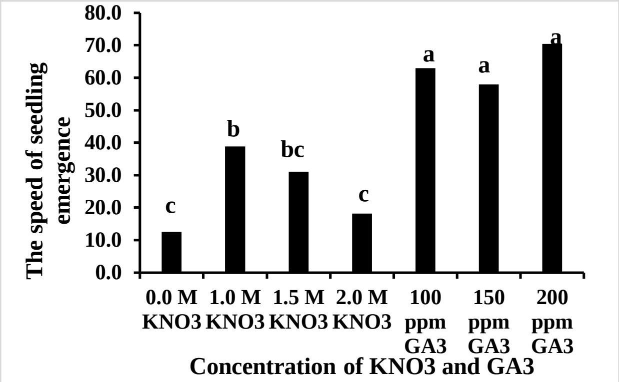

Fig. 4: Effect of KNO_3 dan GA_3 concentrations on the speed of seedling emergence (% emerged seedlings day^{-1})

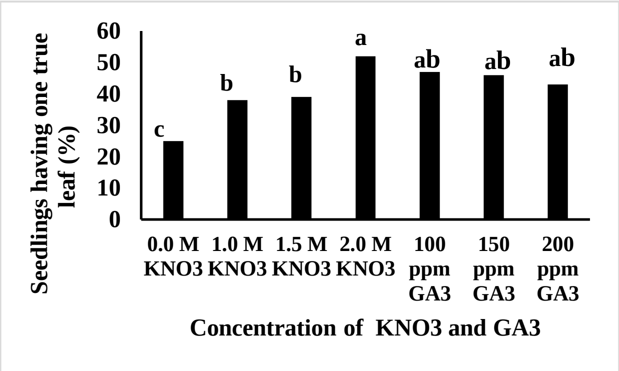

Fig. 5: Effect of KNO_3 dan GA_3 concentrations on the percentage of seedlings having one true leaf.

IV. CONCLUSION

GA_3 significantly enhanced: a) seed germination percentage (25,1% higher than control), b) The speed of seed germination (57-63% germinated at 1 dap, compared to other treatments), c) index of seedling vigour (2,43 compared to control), d) the speed of seedling emergence (34,39% seedlings day^{-1} higher than KNO_3), e) percentage of seedlings having at least one first leaf (true leaf) (52% at 9 dap). KNO_3 also had similar significant effects on the same variables, except on the speed of seed germination, the speed of seedling emergence and percentage of seedlings having one true leaf. Pre-sowing treatment with GA_3 dan KNO_3 2 M enhanced time of seedling transplanting to the field (12 days earlier than with no pre-sowing treatment).

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REFERENCES

[1] Afzal, I., Basra, S.M.A. and Iqbal, A. (2005). The effects of seed soaking with plant growth regulators on seedling vigour of wheat under salinity stress. *Journal of Stress Physiology and Biochemistry* 1 (1): 6-14.

[2] Alboresi, A., Gestin, C., Leydecker, M.T., Bedu, M., Meye, C., Truong, H.N. (2005). Nitrate, a signal relieving seed dormancy in arabidopsis. *Plant Cell Environment* 28 (4): 500-512.

[3] Amjad, M. K., Ziap, K., Iqbal, Q., Ahmad, I., Riaz, M.A. and Z.A. Saqib. Effect of seed priming on seed vigour and salt tolerance in Hot pepper. (2007). *Pakistan Journal of Agricultural Sciences* 44 (3): 408-416.

[4] Argerich, C.A. and Bradford, K.J. (1989). The effects of priming and ageing on seed vigor in tomato. *Journal of Experimental Botany* 40 (5): 599-607.

[5] Barlow, E.W.R. and Haigh, A.M. (1987). Effect of seed priming on the emrgence, growth and yield of UC 82B tomatoes in the field. ISHS *Acta Horticulturae* 200:II. International Symposium on Processing tomatoes, XXV IHC, Davis, 1 March 1987, 22(1).

[6] Bose, B. and Mishra, T. (1992). Response of wheat seed to presowing seed treatment with Mg $(NO_3)_2$. *Annual Agriculture Research* 5:11-16.

[7] Bray, C.M., Davidson, P.A., Ashraf, M. and Taylor,R.M. (1989). Biochemical changes during osmopriming of leek seeds. *Annals of Botany* 63:185-193.

[8] Caseiro, R., Bennett, M.A. and Marcos-Filho, J. (2004). Comparison of three priming techniques for onion seed lots differing in initial seed quality. *Seed Science and Technology* 32: 365-375.

[9] Cheib, A.L. and Garcia, Q.S. (2012). Longevity and germination ecology of seeds of endemic Cactaceae species from high-altitude sites in south-eastern Brazil. *Seed Science Research* 21:45–53.

[10] De Souza, A., Garcia, D. Sueiro, L. and Gilart. F. (2014). Improvement of seed germination, growth and yield of onion plants by extremely low frequency non- uniform magnetic fields. *Scientia Horticulturae* 176: 63-69.

[11] Demir, I and Mavi, K. (2004). The effect of priming on seedling emergence of differentially matured watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) seeds. *Scientia Horticulturae* 102 (4): 467-47.

[12] Duman, İ. (2002) Soğan (*Allium cepa* L.) tohumların ınçimlenmesini iyileştirici farkl ı osmotik uygulama yöntemlerinin karş ılaşt ırılmas ı. *Ege Üniversitesi Ziraat Fakültesi Dergisi* 39 (2**)**: 1-8.

[13] Dursun, A. and Ekinsi, M. (2010). Effects of different priming treatments and priming durations on germination percentage of parsley (*Petroselinum crispum* L) seeds. *Agricultural Sciences* 1 :17-23.

[14] Farooq, M., Basra, S.M.A., Saleem, B.A., Nafees, M. and Chrishti, S.A. (2005). Enhancement of tomato seed germination and seedling vigour by osmopriming. *Pakistan Journal of Agricultural Sciences* 42 (3-4): 36-41.

[15] Farooq, M., Basra, S.M.A., Saleem, B.A., Nafees, M., Gashi, B., Abdullai, K., Mafa, V. and Kongjika, E. (2012). Effect of gibberellic acid and potassium nitrate on seed germination of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae.* African *Journal of Biotechnology* 11(20): 4537-4542.

[16] Govinden-Soulange, J. and Levantard, M. (2008). Comparative studies of seed priming and pelleting on percentage and mean time to germination of seeds of tomato (*Lycopersicon esculentum* Mill.). *African Journal of Agricultural Research* 3 (10): 725-731.

[17] Preece, J.E. and Read, P.E. (1993). Mineral Nutrition In: The Biology of Horticulture Crop 2nd ed., Jhon Wiley and Sons Publisher. P.257-259.

[18] Heydecker, W. and Coolbear, P. (1977). Seed treatments for improved-performan.ce-survey and attempted prognosis. *Seed Science and Technology*. 5: 353-425.

[19] Hossain, M. A., Arefin, M.K., Khan, B.M. and Rahman, M. A. (2006). Effects of seed treatments on germination and seedling growth attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. *Research Journal of Agriculture and Biological Sciences* 1(2): 135-141.

[20] Islam, A.K., Anuar, N. and Yaakob, Z. Effect of genotypes and pre-sowing treatments on seed germination behavior of jatropha. (2003). *Asian Journal of Plant Science* 8 (6), 433-439**.**

[21] Jagosz, B. (2015). Improving onion seed germination using priming treatments. *Infrastructure and Ecology of Rural Areas* **IV** (4): 147-1447.

[22] Khayeh-Hossaeni, M., Lomholt, A. and Mattews, S. (2009). Mean germination time in the laboratory estimates the relative vigour and field performance of commercial seed lots of maize (*Zea mays* L.). *Seed Science and Technology* 37 (1): 446-456.

[23] Maguire, J. D. (1962). Speed of germination – aid in selection and evaluation for seedling emergence and vigour. *Crop Science* 2**:** 176-177.

[24] Mattews, S. and Khayeh-Hossaeni, M. (2007). Length of the lag period of germination and metabolic repair explain vigour differences in seed lots of maize (*Zea mays*). *Seed Science and Technology* 35 (1): 200-212.

[25] Mauromicale, G. and Cavallaro, V. (1995). Effects of seed osmopriming on germination of tomato at different water potential. *Seed Science and Technology* 23: 393-40.

[26] Mirabi, E. and Hasanabadi, M. (2012). Effect of seed priming on some characteristic of seedling and seed vigour of tomato (*Lycopercicum esculentum*). *Journal of Advance Laboratory Research in Biology* III (III): 37-240.

[27] Mock, J. J. and Skrdla, W.H. (1978). Evaluation of maize plant introductions for cold tolerance. *Euphytica* 27: 27-32.

[28] Moradi, D.P., Sharif-zadeh, F., Janmohammadi, M. (2008). Influence of priming techniques on seed germination behaviour of maize inbred lines (*Zea mays* L.). *Journal of Agriculture and Biological Sciences* 3(3): 22-25.

[29] Oliveira, P.G. and Garcia, Q.S. (2001). Germination characteristics of Syngonanthus seeds (Eriocaulaceae) in campos rupestres vegetation in south-eastern Brazil. *Seed Science Research* 21(01): 39-45.

[30] Don, R. (2003). ISTA Handbook on seedling evaluation. 3rd ed. International Seed Testing Association, Zùrich, Switzerland.

[31] Ramzan, A., Hafiz, L.A., Ahmad, T. And Abbasi, N.A. (2010). Effect of priming with potassium nitrate and dehusking on seed germination of gladiolus (*Gladiolus alatus*). *Pakistan Journal of Botany* 42 (1): 247-258.

[32] Selvarani, K. and Umarani, R. (201). Evaluation of seed priming methods to improve seed vigour of onion (*Allium cepa cv.aggregatum*) and carrot (*Daucus carota*). *Journal of Agricultural Technology 7*(3): 857-867.

[33] Soltani, E., Ghaderi-Far, F., Baskin, C.C. and Baskin, J.M. (2015). Problems with using mean germina tion time to calculate rate of seed germination. *Australian Journal of Botany* 63: 631-635.

[34] Tajbakhsh, M., Brown, P.H., Gracie, A.J., Spurr, C.J., Donovan, N. and Clark, R.J. (2004).

Mitigation of stunted root abnormality in onion (*Allium cepa* L.) using seed priming treatments. *Seed Science and Technology* 32: 683-692.

[35] van den Brink, L. and Basuki, R.S. (2011). Production of true seed shallots in Indonesia. ISHS Acta Horticulturae 958: I International Symposium on Sustainable Vegetable Production in Southeast Asia. DOI:10.1.7660/*Acta.Hortic*.2012.958.12.Sustainable Vegetable Production in Southeast Asia. DOI: 10.1.7660/*Acta.Hortic*.2012.958.12.

[36] Yıldırım, E., Dursun, A., Güvenç, İ. and Kumlay, A.M. (2002). Effects of different seed germination of some vegetable species. *Acta Agrobotanica* 55 (2), 75-80.

[37] Zanjan, M.G. and Asli, D.E. (2012). A study of seed germination and early seeding growth of wheat genotypes affected by different seed pyridoxine-priming duration. *Annals of Biological Research* 3 (12): 5687-5691.

Germination Characters as Affected by Salinity Stress and Soaking Grain Sorghum Genotypesin Humic acid

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Abstract— *In order to investigate salinity stress on sorghum germination indices, an experiment conducted in the Faculty of Agriculture, Mansoura University from June and July 2017 in Agronomy Department, Seed Science Laboratory. The goals of the investigation aimed to screening for five (Sorghum bicolor L.(Moench) cultivarsviz. Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305 under salinity stress, sodium chloride (Na Cl) at the levels of 0 (as control), 3, 6, 9, 12 and 15 dS/m^{-1} and soaking in humic acid. The results showed that soaking seed in humic acid exceeded percentage of germination, germination rate, germination index, energy of germination and chlorophyll content by 5.2, 7.7, 17.1, 65.8 and 17.8 %, respectively. The highest germination percentage (91.9 %), germination rate (3.08), germination energy (59.7), seedling vigor index (1483.7) and chlorophyll content (2.88) were obtained from sown Mecca hybrid. The maximum germination index (117.22) was obtainedfrom sown Giza 15 cultivar.The results point out that cumulative salinity level from 3 to 15 dSm^{-1}condensedall studied germination characters. Accumulative salinity levels to 15 dSm^{-1}condensedpercentage of germination, germination rate, index of germination, germination energy and seedling vigor index by 15.9, 15.0, 30.0, 35.9 and 37.6 %, respectively compared without salinity application. It could recommended that soaking sorghum seed of Meeca hybrid with humic acid for 12 h under salinity of concentration of 6 dSm^{-1} enhanced germination characters compared with other cultivars and salinity concentrations, it mean cultivated it under reclaimed saline soil in Egypt.*

Keywords— Salinity Stress, Genotypesin Humic acid, Mansoura University.

I. INTRODUCTION

Sorghum is one of the most important staple food crops especially in the drier and smaller areas of the semi-arid Tropics of Africa **(Ali et al. 2011).**Salinity is adversely affecting crop yield of crops to various extents of Egypt and the World. To overcome the shortage of cereal productivity. It could have achieved by growing grain sorghum cultivars tolerant of salinity to get economical yields from saline reclaimed soils, particularly in early seedling stages is very important. Soil salinization is a great determinate factor of crop productivity, especially in dried area **(Ahmed, 2009).**Many physiological variations are induced germination percentage and affecting seedling growth and development(**Saroj and Soumana, 2014).**

Seed germination positively affected by humic acid soaking and resulted more root length. Germination testes using various levels of humic extract, the doses higher than 20% v/v repressed seed germination **(Asenjo et al., 2000).**Extraof 90% from seeds germinated after five days with soaking of 100-ppm potassium humate, for 24 hours **(Ali and Elbordiny, 2009).**Wheat seed primed with humic enhanced the germination and produced higher germination% as compared to that soakedin water **(Ali et al., 2014).** The highest percentage of germination gone to Sepideh cultivar and the less percentages of germination fitted to Payam cultivar under stress conditions (**Mohammadi and Mojaddam, 2014).**The inhibiting effect of salt on the seed germination improved in variable degrees by pretreatment of humic acid**(Çavuşoğlu and Ergin, 2015).**

The recommended cultivar for new reclaimed saline soils were Soavecultivar **(Almodares et al., 2007).** Genotypes of235461 and 69239recorded the less germination rate and the great percentages of germination **(Geressu and Gezahagne, 2008).**Delay in germination time due to salinity stress. Maximum germination percentage produced from KFS4 cultivar **(Aishah et al., 2010).** Genotypes differed from salt sensitive to germination percentage**(Asfaw, 2011).**Sorghum genotypes differed from response to salinity, the medium tolerant recorded by cultivars of Hegari and JS-263, while the less sensitive produced from Noor cultivar and the sensitive produced from FJ-115 and PSV-4 cultivars**(Kausar et al., 2012).**The STI, GMP and MP genotypes were better to cultivate salinity and unsolidity stress**(Hefny et al., 2013).**The highest germination percentage recorded from Meko, Gambella1107, ICSV-111 and Melkam varieties and were more salt tolerant cultivars. However, ESH-2 and Go bye varieties were salt sensitive. The rest sorghum varieties were middle in their salt tolerance **(Tigabo et al., 2013).** Sown Shallu, Desert Maize, and 1790E genotypes graded the most salt tolerant cultivars, while the least salt tolerance genotypes produced from Schrock and RTx430 cultivars**(Sun et al., 2014).**

Salinity at a rate of 300 mM, the time of germination of CSF 18 variety increased, but germination speed and germination test variables decreased. The CSF 20 variety was in the same trend, except for the speed germination and the time of germination, which influenced by sodium chloride at the 150 mM level. The CSF 18 variety had more tolerance to salinity than CSF 20 variety during the germination stage**(De oliveir and Gomes-Filho, 2009).**The percentage of germination increased from thelowest level of salinity (2 dS/m^{-1}), while inhibited the germination % atsalinity levels of 4, 8 and 16 dS/m^{-1}. Wad Ahmed cultivar recorded the more salt tolerant, but Arfagadamak and Butana cultivars were the more salt sensitive **(El Naim et al., 2012).**The germination percentage decreased with salinity concentrations increased. The lowest germination percentage produced from CSV-15 and Pant-1 cultivars at highest salinity level**(Chauhan et al., 2012).**KFS2, KFS4 genotypes produced the highest rate of germination. LFS56 genotype recorded the lowest rate of germination by the 10% **(Tabatabaei and Anagholi, 2012)**. SSV84 cultivar recorded the highest salt tolerant variety and the highest percentage of germination produced from IS6973 cultivar **(Almodares et al., 2014).**A significant reduced from germination characteristics of sorghum triggered by salinity.The percentage of germination, germination rate and time of germination were decreased with salinity concentration increased **(Behzadnejad and Tohidinejad, 2014).**The rate of germination and germination percentage decreased as salinity levels increased. Germination parameters were the lowest at salinity level of 9 and 12 dS/m^{-1}**(Dadar et al., 2014).**Seed germination reduced at 1.5% NaCl treatment. Germinating percentage reduced by 35.8% in higher salt concentrations **(Sam et al., 2014).** The lessening connected to the salinity rate of 12 dS/m^{-1}betweensorghum genotypes, the greatest in terms of germination parameters were Kimia, Sepideh, and Payam genotypes. Salinity stress significantly influencedmost germination traits and reduced the germination related the same characters **(Sawamery and Mojaddam, 2014).** The highest percentage of germination produced from the control, but the lowest onesobtained from 1.5% salinity level. The sensitive cultivar to salinity produced from Barbarei cultivars compared to Tabat and Wad-Ahmed cultivars, Tab at cultivar was recorded themost- salt tolerant cultivars **(Siddig and Idris, 2015).**Therefore, the goals of this exploration intended to study the performance of seed viability of studied sorghum cultivars under salinity stresses.

II. MATERIALS AND METHODS

2.1. Treatments and Experimental Design:

A laboratory experiment carried out in the Faculty of Agriculture, Mansoura University from June 2017 to July 2017 in Agronomy Department, Seed Science Laboratory. The aimed of the investigation conducted to screening forfive (Sorghum bicolor L.(Moench) cultivars *i.e.* Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305 under salinity stress, Na Cl at the levels of 3, 6,9, 12 and 15 dS/m^{-1}beside the control, soaked or non-soaked in humic acid.The conducted experimentwas assignedto factorial experiment in RCBDin four replication. The first factorcontain two treatments with and without soaking in humic acid. The second factor includesthe five-grain sorghum cultivars *i.e.* Dorado, hybrid 306, Giza 15, Mecca hybrid and H-305 obtained from ARC Ministry of Agriculture. The third factor contained withinfive different levels of NaCl include 0, 3, 7, 11 and 15 dS/m^{-1}. The experiment comprised 240Petri dishes assigned in factorial experiment of Randomized Complete Block Design (RCBD). Then, the Petri dishes placed in a growth chamber for 14 days at 28 ± C º for germination according to **ISTA, 2013** roles.

2.2. Studied characters:

The studied of germination parameters as follows:

1-The final percentage of germination (FGP):Thepercentage of germination from total calculated after 14 days from sowing following by **Ellis and Roberts, (1981) and Ruan *et al*., (2002)**equation:

$$\mathbf{FGP} = \frac{\text{Number of germinated seeds}}{\text{Total Number of seed tested}} \times 100$$

2-The germination rate (GR) wasintendedconferring to the following equation by**(Ellis and Roberts 1981).**

$$\mathbf{GR} = \frac{\text{Number of germinated seeds}}{\text{Number of germination days}}$$

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3-The germination index (GI) was calculated according to **Karim *et al*. (1992)**equation**.**

$$\mathbf{GI} = \frac{\text{\% Germination in each treatment}}{\text{\% Germination in the control}} \times 100$$

4-The energy of germination (EG) was measured according to**Ruan *et al*., (2002).**

$$\mathbf{EC} = \frac{\text{Number of germinated seeds after four days}}{\text{Number of germination days}} \times 100$$

5-Average of seedling vigor index (SVI)was measured according to**Abdel Baki and Anderson (1970)**formula**.**

$$\mathbf{SVI} = (\text{Average shoot length} + \text{Average root length}) \times \text{Germination \%}$$

6-Total chlorophyll (SPAD): Average of total chlorophyll (SPAD) in seedling leaf samples assessed by SPAD-502 (Minolta Co. Ltd., Osaka, Japan).

2.3. Statistical Analysis:

According to the technique of variance (ANOVA) for the factorial in RCBD as published by Gomez and **Gomez (1991)**of the subjected data was statistically analyzed**.**LSDmethod was used as defined by **Snedecor and Cochran (1980) the**.The data analyzed statisticallyin RCBD design by MSTAT-C computer package as described by **Russel (1986)**.

III. RESULTS AND DISCUSSIONS

3.1. Humic acid soaking effect:

The results presented in Table (1) clearly showed that soaking in humic acid significantly affected studied germination parameters and chlorophyll content, except seedling vigor index insignificantly affected. Soaking seed in humic acid exceeded germination %, germination rate, germination index, energy of germination and chlorophyll content by 5.2, 7.7, 17.1, 65.8 and 17.8 %, respectively. Extra of 90% from seeds germinated after five days with soaking of 100-ppm potassium humate, for 24 hours **(Ali and Elbordiny, 2009).**Priming of wheat seed with humic produced higher percent seed germination as compared to priming seeds in alone water **(Ali et al., 2014).**

3.2. Cultivars performance:

The outcomes of statistical analysis was presented in Table (1) clearly revealed that studied sorghum cultivars significantly affected studied germination parameters and chlorophyll content. The highest germination percentage (91.9 %), germination rate (3.08), energy of germination (59.7), seedling vigor index (1483.7) and chlorophyll content (2.88) were obtained from sown Mecca hybrid. The highest germination index (117.22) was produced from sown Giza 15 cultivar. However, the lowest germination percentage (40.8 %), germination rate (1.35), germination index (77.97), energy of germination (15.9) and seedling vigor index (481.9). While, the lowest chlorophyll content (2.20) was produced from sown Dora cultivar. The recommended cultivar was Soave for soils with high salinity **(Almodares et al., 2007).** NM-92 cultivar was more adaptability under saline conditions **(Ahmed, 2009).** The time of germination of CSF 18 variety increased at salinity of 300 mM, but the speed of germination and germination test variables decreased. The CSF 20 variety was in the same trend, except for the speed germination and the time of germination, which influenced by NaCl at the 150 mM level. The CSF 18 variety had better tolerance to salt stress during the germination stage than CSF 20 **(De oliveir and Gomes-Filho, 2009).** Deferral in germination time related to salinity, the highest germination percentage produced from KFS4 cultivar **(Aishah et al., 2010).** Genotypes differed from salt sensitive to germination percentage **(Asfaw, 2011).** The percentage of germination increased from the lowest level of salinity (2 dS/m^{-1}), while inhibited the germination percentage of salinity levels of 4, 8 and 16 dS/m^{-1}. Wad Ahmed cultivar recorded the more salt tolerant, but Arfagadamak and Butana cultivars were the more salt sensitive **(El Naim et al., 2012).** The more tolerant of salinity stress produced from Hoars cultivar followed by Mabrouk cultivar, which will cultivate under new reclaimed saline soils **(Kandil, et al., 2012).** Germination percentage was clearly demonstrated varietal differences **(Khan et al., 2014).**

3.3. Salinity level effects:

The outcomes of statistical analysis was presented in Table (1) clearly revealed that salinity concentration significantly affected studied germination parameters, except chlorophyll content insignificantly affected. The results indicated that increasing salinity concentration from 3 to 15 dSm^{-1} significantly condensed the percentage of germination, and germination rate, germination index, energy of germination and seedling

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vigor index. The highest germination percentage (73.7 %), germination rate (2.46), germination index (122.5), energy of germination (52.0) and seedling vigor index (1166.8) were produced from the control treatment. However, the lowest percentage of germination (62.0 %), germination rate (2.09), germination index (85.26), energy of germination (33.3) and seedling vigor index (727.9) were produced from the highest salinity concentration of 15 dSm^{-1}. Increasing salinity concentration to 15 dSm^{-1} significantly decreased germination (%), germination rate, germination index, energy of germination and seedling vigor index by 15.9, 15.0, 30.0, 35.9 and 37.6 %, respectively compared with the control treatment. It exercises an adverse effect of osmotic inhibition and ionic toxicity **(Munns et al., 2006).**

Table.1: Averages of percentage of germination, and germination rate, germination index, energy of germination, seedling vigor index and chlorophyll content as affected by humic acid soaking, sorghum cultivars and salinity concentrations.

Treatment	Germination percentage	Germination rate	Germination index	Energy of germination	Seedling vigor index	Chlorophyll content
A. Humic acid:						
Without	65.7	2.17	86.80	20.9	924.7	2.40
Soaking	69.3	2.35	104.76	60.0	965.1	2.92
F-test	*	*	*	*	NS	*
B. Sorghum cultivars:						
Dora	63.1	2.12	89.05	47.6	759.0	2.20
H.306	53.2	1.75	96.58	25.1	712.2	2.51
Giza 15	89.1	3.00	117.22	53.9	1287.7	3.24
Mecca hybrid	91.9	3.08	98.09	59.7	1483.7	2.88
H-305	40.8	1.35	77.97	15.9	481.9	2.49
F-test	*	*	*	*	*	*
L.S.D 5%	4.6	0.16	25.11	4.2	97.8	0.41
C. Salinity concentrations:						
0 dsm^{-1}	73.7	2.46	122.50	52.0	1166.8	2.64
3 dsm^{-1}	69.6	2.32	96.91	42.7	1098.9	2.90
6 dsm^{-1}	68.2	2.31	94.92	40.7	986.2	2.85
9 dsm^{-1}	66.6	2.22	89.15	36.1	854.5	2.48
12 dsm^{-1}	65.2	2.17	85.97	38.1	835.2	2.63
15 dsm^{-1}	62.0	2.09	85.26	33.3	727.9	2.49
F-test	*	*	*	*	*	NS
L.S.D 5%	5.1	0.17	27.51	4.6	107.19	0.45

Percentage of germination and germination ratedecreased as salinity levels were increased **(Jamil et al., 2006).**Percentage of germination reduced and time of germination increased due salinity stress**(Jajarmi. 2007).**The germination percentage decreased with salinity concentrations increased. The lowest germination percentage produced from CSV-15 and Pant-1 cultivars at highest salinity level**(Chauhan et al., 2012).** The KFS2 and KFS4 varieties produced the maximum rate of germination.. LFS56 variety showed the lowest rate of germination by the 10% **(Tabatabaei and Anagholi, 2012)**. Salt stress unfavorablydistressesseedling at germination stage **(Hussain et al., 2013).**These results from good agreement with those reported by **Noreen and Ashraf (2008), Almodares et al. (2014), Behzadnejad and Tohidinejad (2014), Dadar et al. (2014) and Sam et al.(2014).**

3.4. Interaction effects:

3.4.1. Interaction between humic acid soaking and cultivars effects:

Regarding to the interaction between humic acid soaking and studied sorghum cultivars, the results clearly revealed that this interaction significantly affected the moststudied germination parameters and chlorophyll content, however, germination index, insignificantly affected. The results graphically illustrated in Figs. 1, 2 and 4 indicated that highest germination percentage (96.33 %) and germination rate (3.20), seedling vigor index (1646.7) were produced from sownMecca hybrid when soaked with humic acid. In addition, energy of germination (92.83) and chlorophyll content (3.81) were obtainedfrom sown Giza 15 cultivar when soaked with humic acid as shown in Fig. 3 and 5. Whereas, the lowest germination percentage (31.33 %), energy of germination (5.50), seedling vigor index (375.46) were produced from sown Giza 15 cultivar without soaking in humic acid as shown

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in Figs 1, 3 and 4.In addition, the lowest values of chlorophyll content (1.93) was produced from sown Dora cultivar without soaking in humic acid as shown in Fig. 5.

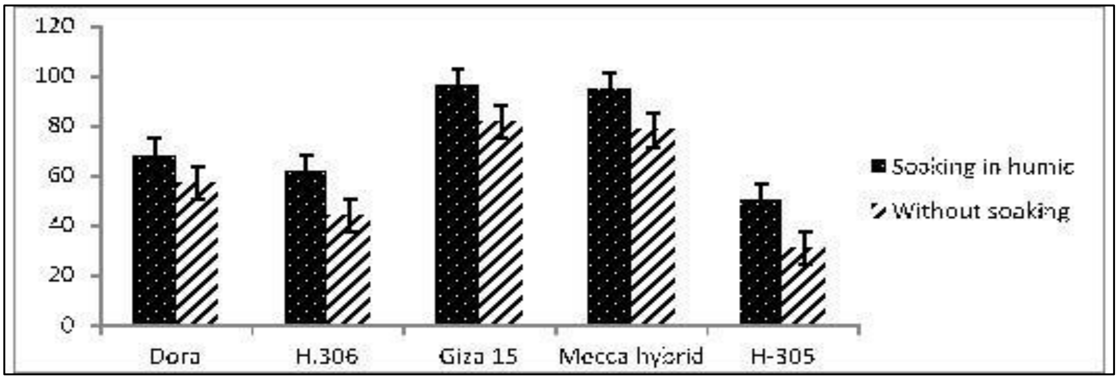

Fig.1. Average of percentage of germination as influencedby the interaction between humic acid soaking and studied sorghum cultivars.

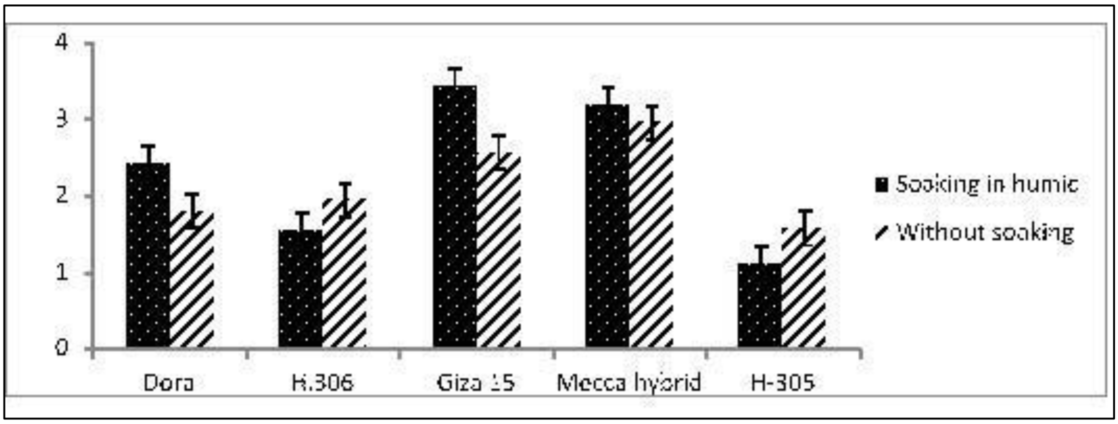

Fig.2: Average of germination rate as influenced by humic acid soaking and studied sorghum cultivars.

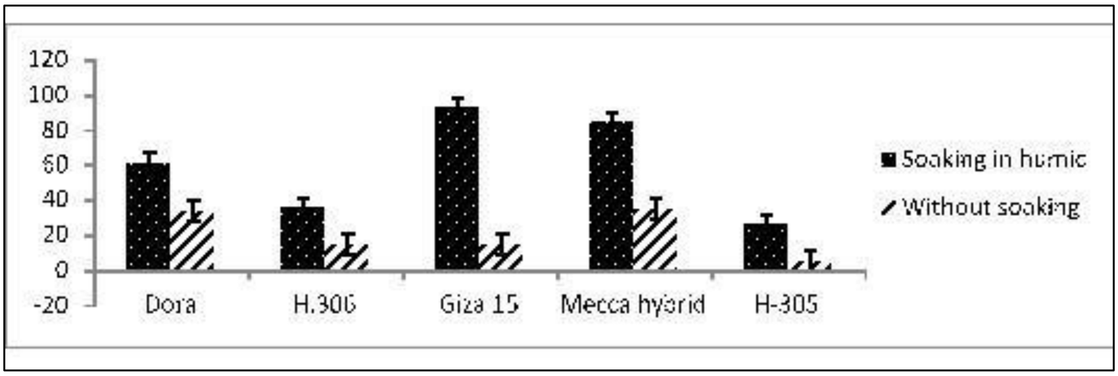

Fig.3: Averageof energy of germination as influenced by humic acid soaking and studied sorghum cultivars.

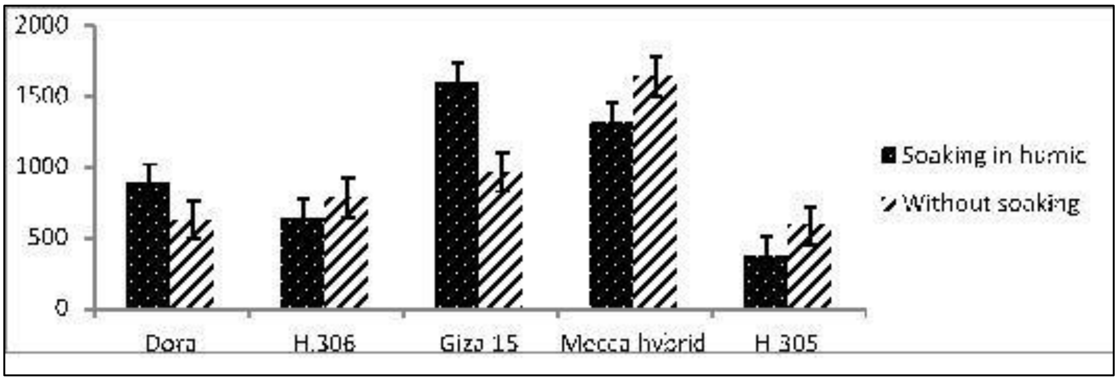

Fig.4: Average ofseedling vigor index as influenced by humic acid soaking and studied sorghum cultivars.

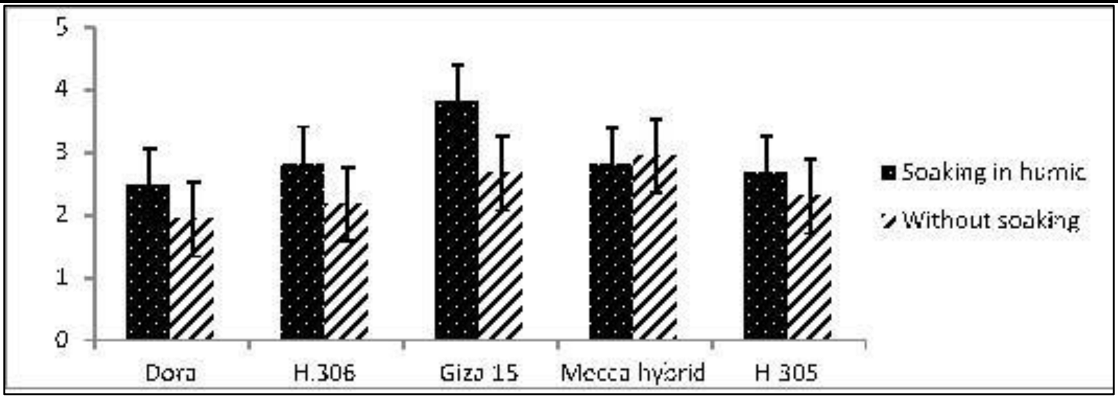

Fig.5: Average ofchlorophyll as influenced by humic acid soaking and studied sorghum cultivars.

Genotypes differed to salinity resistance level due to their different interactions with the salt ion and its concentrations **(Wei and Qing-Xiang, 2011).** The highest percentage of germination gone to Sepideh variety and the less percentage of germination went to Payam variety under stress conditions (**Mohammadi and Mojaddam, 2014).**

3.4.2. Interaction between humic acid and salinity levels effects:

Concerning to the interaction between humic acid soaking and salinity levels, the results clearly revealed that this interaction significantly affected the most studied germination parameters and chlorophyll content, however, insignificantly affected germination rate.The results graphically illustrated in Figs. 6,7, 8 and 9 indicated that highest germination percentage (79.0 %) and germination rate (2.48), energy of germination (63.6) seedling vigor index (1239.85) were produced from sown Mecca hybrid when soaked with humic acid.In addition,the highest values ofchlorophyll content (3.11) was produced from soaking seed in humic acid and salinity concentration of 3 dSm^{-1} as shown in Fig 10. However, the lowest germination percentage (58.2 %), germination rate (1.84), energy of germination (7.40), seedling vigor index (674.85) and chlorophyll content (2.20) were recorded from without soaking in humic acid at highest level of salinity of 15 dSm^{-1} as shown in Figs. 6, 7, 8, 9 and 10.

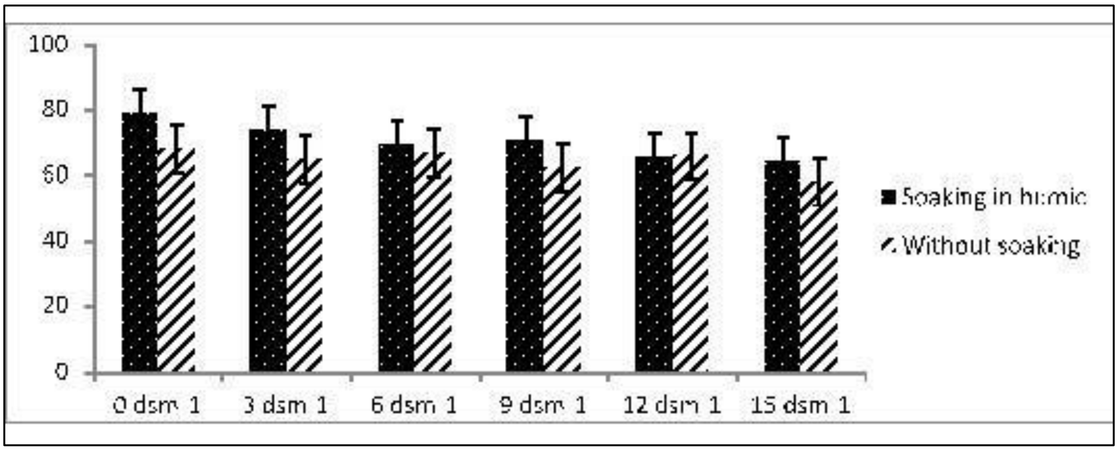

Fig.6: Average percentage of germination as influenced by humic acid soaking and salinity concentrations.

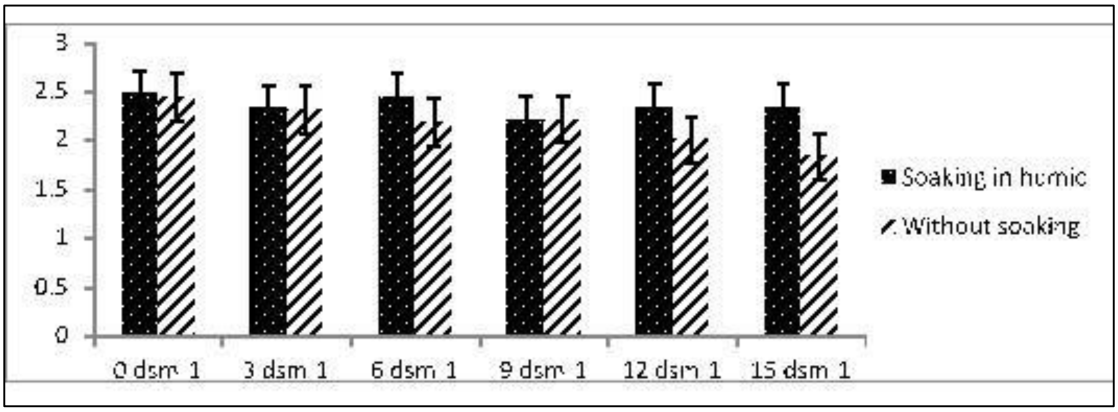

Fig.7: Average of germination rate as influenced by humic acid soaking and salinity concentrations.

Sorghum genotypes differed from response to salinity, the medium tolerant recorded by genotypes of Hegariand JS-263, while the intermediate sensitive produced from Noor cultivar and the sensitive produced from FJ-115 and PSV-4 cultivars **(Kausar et al., 2012).** During germination of Meko, Gambella1107, ICSV-111 and Melkam varieties were extra salt tolerant. However, ESH-2 and Gobye varieties were salt sensitive. The respite varieties were midway in their salt tolerance **(Tigabo et al., 2013).** SSV84 cultivar recorded the highest salt tolerant variety and the highest percentage of germination produced from IS6973 cultivar and moderate seedling characters and classified as moderate salt tolerant **(Almodares et al., 2014).** Inter cultivars genetic variation and concentration x cultivars interaction showed significant differences for all studied parameters **(Khan et al., 2014).** Similarly, many investigators such as **Dadar et al. (2014), Sam et al. (2014), Sawamery and Mojaddam (2014), Siddig and Idris (2015)** reported similar results**.**

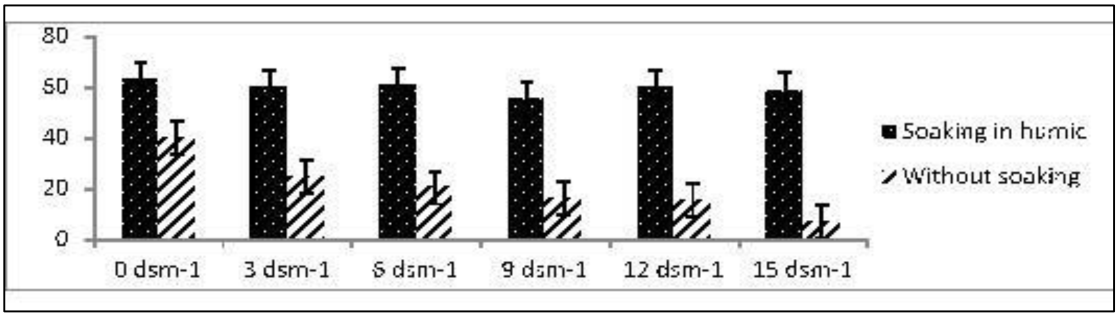

Fig.8: Average of energy of germination as influenced by humic acid soaking and salinity levels.

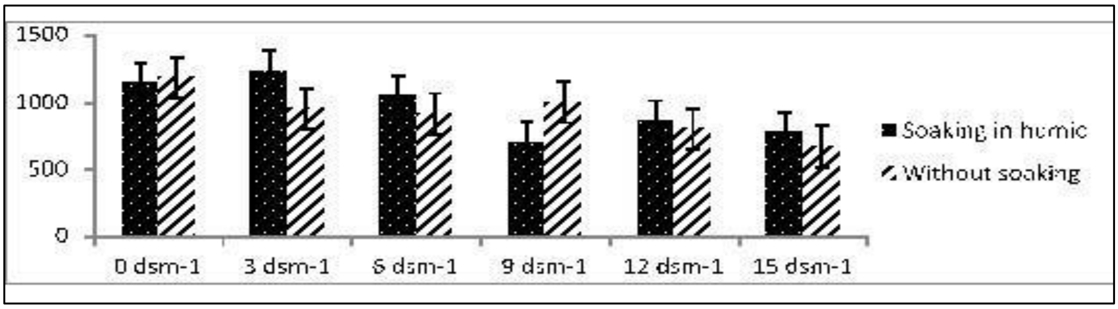

Fig.9: Average of seedling vigor index as influenced by humic acid soaking and salinity levels.

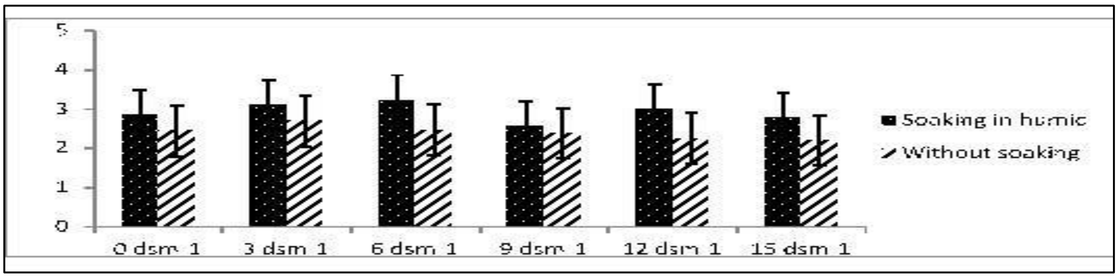

Fig.10: Average of chlorophyll as influenced by humic acid soaking and salinity levels

3.4.3. Interaction between studied sorghum and salinity levels effects:

Concerning to the interaction between studied sorghum and salinity levels, the results clearly revealed that this interaction significantly affected the most studied germination parameters and chlorophyll content insignificantly affected. The results graphically illustrated in Figs.11, 12 and 13 indicated that highest germination percentage (92.0 %**),** germination rate (3.06) and germination index (212.5) were recorded from sown Mecca hybrid at the control treatment. Where, the less percentage of germination (28.5 %), germination rate 0.96) and germination index (51.21) were produced from sown H-305 cultivar at highest salinity concentration of 15 dSm^{-1}as shown in Figs.11, 12 and 13. The highest germination (%) went to Sepideh genotype and the lowest percentage of germination fitted to Payam genotype under stress conditions (**Mohammadi and Mojaddam, 2014).**Priming wheat seeds with PEG decreased percent seed germination of wheat compared to humic matters. Seed germination enhanced with humic acid soaking under drought stress**(Nazi et al., 2014).**The inhibiting effect of salt on the seed germination improved in variable degrees by pretreatment of humic acid **(Çavuşoğlu and Ergin, 2015).**

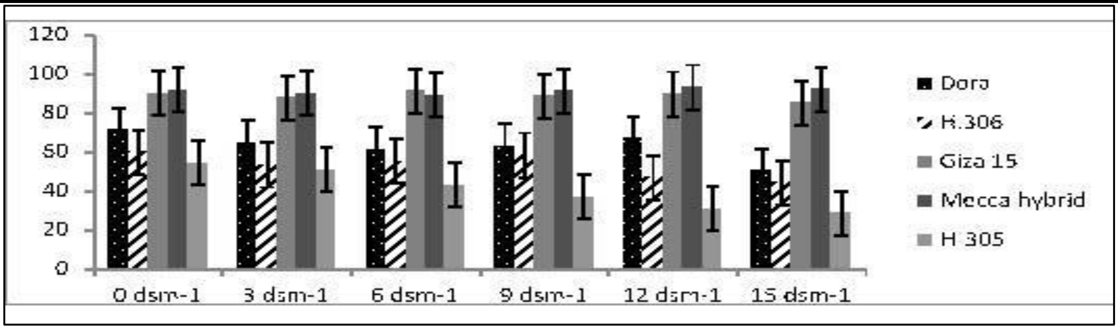

Fig.11: Average of germination percentage as influenced by studied sorghum cultivars and salinity levels.

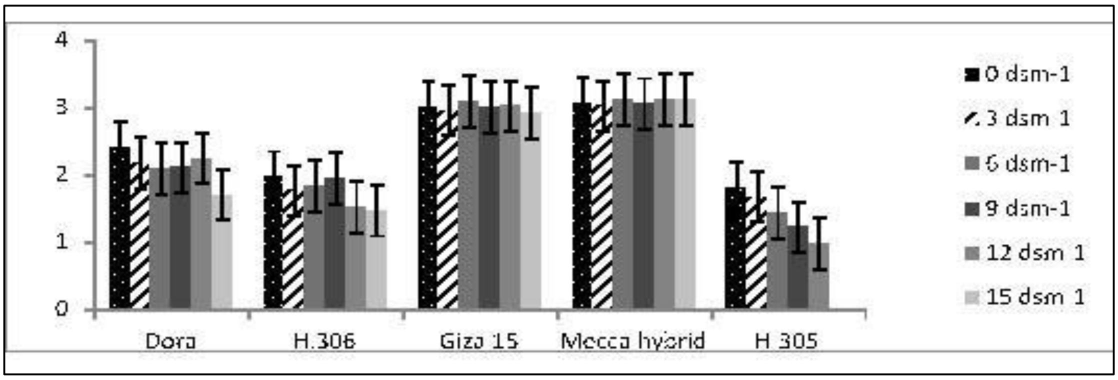

Fig.12: Average of germination rate as affected by studied sorghum cultivars and salinity levels.

3.4.4. Interaction between humic acid soaking x cultivars x salinity levels effects:

The outcomes of statistical analysis was presented indicated that the interaction between humic acid soaking x cultivars x salinity levels insignificantly influencedall studied germination parameters and chlorophyll content.

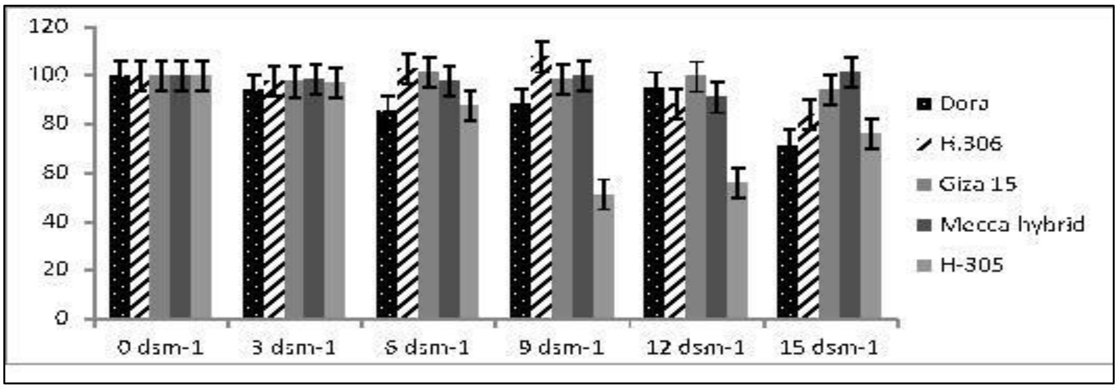

Fig.13: Average of germination index as influenced by studied sorghum cultivars and salinity levels.

IV. CONCLUSION

It could recommended that soaking sorghum seed of Meeca hybrid with humic acid for 12 h under salinity of concentration of 6 dSm^{-1} enhanced germination characters compared with other cultivars and salinity concentrations, it mean cultivated it under reclaimed saline soil in Egypt.

REFERENCES

[1] Abdel-Baki, A. A. and J.D. Anderson 1970. Viability and leaching of sugars from germinating barley. Crops Science, 10: 31 – 34.https://www.cabdirect.org/cabdirect/abstract/19701703154

[2] Ahmed, S. 2009. Effect of soil salinity on the yield and yield components of Mungbean. Pakistan J. Bot., 4(1):263-268. http://www.pakbs.org/pjbot/PDFs/41(1)/PJB41(1)263.pdf

[3] Aishah, H. S., A. R. Saberi, R. A. Halim and A. R. Zahara 2010. Salinity Effects on Germination on Forage Sorghum. Journal of Agronomy 9(4): 169-174. **DOI:**10.3923/ja.2010.169.174

[4] Almodares, A., M. R. Hadi, B. Kholdibarien, B. Samedani and Z. A. Kharazian 2014. The Response of Sweet Sorghum Cultivars to Salt Stress and Accumulation of Na^+, Cl^-, K^+ ions in relation to

Salinity. Journal of Environmental Biology, 35:733-739. https://www.ncbi.nlm.nih.gov/pubmed/25004761

[5] Almodares, A., M.R. Hadi and B. Dosti 2007. Effect of Salt Stress on Germination Percentage and Seedling Growth in Sweet Sorghum Cultivars. J. of Biological Sciences, 7(8): 1492-1495. http://scialert.net/abstract/?doi=jbs.2007.1492.1495

[6] Ali, M.A., K. Jabran, S.I. Awan, A. Abbas, M. Ehsanullah, Zulkiffal, T. Acet, J. Farooq and A. Rehman 2011: Morpho-physiological diversity and its implications for improving drought tolerance in grain sorghum at different growth stages. Australian Journal of Crop Science 5(3): 311-320.http://www.cropj.com/ali_5_3_2011_311_320.pdf

[7] Ali, H., Y. Akbar, Dr. Abdul Razaq and D. Muhammad 2014. Effect of humic acid on root elongation and percent seed germination of wheat seeds. International Journal of Agriculture and Crop Sciences, 7(4): 196-201.http://ijagcs.com/wp-content/uploads/2014/04/196-201.pdf

[8] Asenjo, M.C., J.L. Gonzales and J.M. Maldonado, 2000. Influence of humic extracts on germination and growth of ryegrass. Communications Soil Sci. Plant Anal., 31: 101-114.https://www.tib.eu/de/suchen/id/BLSE%3ARN074687957/Influence-of-Humic-Extracts-on-Germination-and/

[9] Asfaw, K.G. 2011. Effects of Salinity on Seedling Biomass Production and Relative Water Content of Twenty Sorghum (Sorghum biolor L. Moench) Accessions. Asian Journal of Agricultural Sciences, 3(3): 242-249. https://www.medwelljournals.com/abstract/?doi=rjagr.2010.24.30

[10] Behzadnejad, J. and E. Tohidinejad 2014. Ameliorative Effects of Exogenous SA on Germination of Sorghum under Salinity Stress. Journal of Applied Science and Agriculture, 9(4): 1519-1524. www.aensiweb.com/jasa/index.html

[11] Çavuşoğlu, K. and H.G. Ergin 2015. Effects of humic acid pretreatment on some physiological and anatomical parameters of barley (Hordeumvulgare l.) Exposed to salt stress. Bangladesh J. Bot. 44(4): 591-598.http://www.bdbotsociety.org/journal/journal_issue/2015%20December/14.pdf

[12] Chauhan, R. R., C. Reema, S. Alka and P.K. Singh 2012. Salt Tolerance of Sorghum bicolor Cultivars during Germination and Seedling Growth. Research Journal of Recent Sciences, 1(3):2-10. http://www.isca.in/rjrs/archive/v1/i3/1.ISCA-RJRS-2012-033_Done.pdf

[13] Dadar, A., A. Asgharzade, and M. Nazari 2014. Investigation effects of different salinity levels on sorghum Bicolor seed germination characters. Indian J. Sci. Res. 7 (1): 1031-1034.https://www.ijsr.in/upload/1819321125Microsoft%20Word%20-%20Dadar%20et%20al.pdf

[14] De oliveir, A. B. and E. Gomes-Filho 2009. Germination and Vigor of Sorghum Seeds Under Water and Salt Stress. RevistaBrasileira de Sementes, 31(3):.048-056. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-31222009000300005

[15] Ellis, R.A. and E.H. Roberts 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci. Technol., 9: 373-409.http://agris.fao.org/agris-search/search.do?recordID=XE8182678

[16] El-Naim, A.M., K. E. Mohammed, E. A. Ibrahim and N. N. Suleiman 2012. Impact of Salinity on Seed Germination and Early Seedling Growth of Three Sorghum (Sorghum biolor L. Moench) Cultivars. Science and Technology, 2(2): 16-20. http://article.sapub.org/10.5923.j.scit.20120202.03.html

[17] Geressu, K. and M. Gezahagne, 2008. Response of some lowland growing sorghum (Sorghum bicolor L. Moench) accessions to salt stress during germination and seedling growth. African Journal Agriculture Research, 3(1): 044-048. http://www.academicjournals.org/article/article1380877252_Geressu%20and%20Gezaghegne.pdf

[18] Gomez, K.A. and A.A. Gomez 1991. Statistical Procedures in Agricultural Research, John Wiley and Sons, New York 2nd edition, pp. 680.http://pdf.usaid.gov/pdf_docs/PNAAR208.pdf

[19] Hefny, M.M., E. M. R. Metwali and A. I. Mohamed 2013. Assessment of genetic diversity of sorghum (Sorghum bicolor L. Moench) genotypes under saline irrigation water based on some selection indices. Australian Journal of Crop Science, 7(12): 1935-1945.
http://www.cropj.com/henfy_7_12_2013_1935_1945.pdf
http://agris.fao.org/agris-search/search.do?recordID=PK2013000980

[20] Hussain, S. H, A. Khaliq, A. Matloop, W.M. Ashfaq and I. Afzal 2013. Germination and growth response of three wheat cultivars to NaCl salinity.Soil & Environment, 32(1): 36-43.http://agris.fao.org/agris-search/search.do?recordID=PK2013000980

[21] ISTA Rules. (2013). Germination Sec. Chapter 5: pp. 5 – 44.

[22] Jamil, M., D.B. Lee, K.Y. Jung, M. Ashraf, S.C. Lee and S.E. Rha 2006. Effect of salt (NaCl) stress on

germination and early seedling growth of four Vegetable species.Journal Central European Agriculture, 7(2): 273-282.https://jcea.agr.hr/articles/358_EFFECT_OF_SALT_(NACL)_STRESS_ON_GERMINATION_AND_EARLY_SEEDLING_GROWTH_OF_FOUR_VEGETABLES_SPECIES_en.pdf

[23] Jajarmi, V. 2007. Effects of Water Stress on Germination Indices in Ten Rapeseed Cultivars (Brassica napus L.). Abstracts, The Second Seminar of Agriculture and Environment. Islamic Azad University, Khoy Branch, Iran. https://www.anau.am/images/stories/journal/4_2008/Ag_4_2008/11-14_4_2008.pdf

[24] Kandil A.A., A.E. Sharief, W.A.E. Abido and M.M. Ibrahim 2012. Effect of Salinity on Seed Germination and Seedling Characters of Some Forage Sorghum Cultivars. International Journal of Agriculture Sciences, 4(7): 306-311.http://oaji.net/articles/2014/30-1394266790.pdf

[25] Karim, M. A.; N. Utsunomiya and S. Shigenaga 1992. Effect of sodium chloride on germination and growth of hexaploid triticale at early seedling stage. Japanese Journal of Crop Science,61: 279 – 284.https://www.jstage.jst.go.jp/article/jcs1927/61/2/61_2_279/_article

[26] Kausar, A., M. Y. Ashraf, I. Ali, M. Niaz and Q. Abbass 2012. Evaluation of sorghum varieties/lines for salt tolerance using Physiological indices as screening tool. Pak. J. Bot., 44(1): 47-52.https://inis.iaea.org/search/search.aspx?orig_q=RN:43028062

[27] Khan, A., M. Ibrar and I. Ahmad 2014. A preliminary approach to halo sensitivity of sorghum cultivars. African Journal of Plant Science, 8(1): 76-83.http://www.academicjournals.org/journal/AJPS/article-full-text-pdf/761A51342821

[28] Mohammadi, N. and M. Mojaddam 2014. The Effect of Water Deficit Stress on Germination Components of Grain Sorghum Cultivars. Indian Journal of Fundamental and Applied Life Sciences, 4(4): 284-291.http://www.cibtech.org/J-LIFE-SCIENCES/PUBLICATIONS/2014/Vol-4-No-4/JLS-043-046-MANI-EFFECT-CULTIVARS.pdf

[29] Munns, R., James, R.A. and A. Lauchli 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot., 57: 1025-1043. https://www.ncbi.nlm.nih.gov/pubmed/16510517

[30] Nazi, F., H. Reza and R. Rahman 2014. Effect of Humic Fertilizer on Germination of Wheat Seeds under Drought Stress. Advances in Bioresearch 5(4): 98-102. http://soeagra.com/abr/abrdec2014/18.pdf

[31] Noreen, S. and M. Ashraf 2008. Alleviation of adverse effects of salt stress on sunflower (Helianthus annuus L.) by exogenous application of salicylic acid: Growth and photo-synthesis. Pakistan Journal Botany, 40(4): 1657-1663. http://sa.indiaenvironmentportal.org.in/files/Pakistan%20Journal.pdf

[32] Ruan, S., Q. Xue and K. Tylkowsko 2002. The influence of priming on germination of rice (oryza sativa L.) seeds and seedling emergence and in flooded performance soils. Seed Sci. Technil., 30: 61– 67. https://www.scienceopen.com/document?vid=d0879887-4833-4f80-b5a8-3fefaac5c7c7

[33] Russell, D.F. 1986. MSTAT-C computer based data analysis software Crop and Soil Science Department, Michigan State University USA.https://msu.edu/~freed/disks.htm

[34] Sam, A., A. Y. Edris and M.S.A. Abo 2014. Effect of Salinity on Seed Germination and Seedling Growth of Pearl millet (Pennisetumglaucum L.) and Sorghum (Sorghum bicolor L.), Journal of Plant and Pest Science, 1 (1): 01-08. http://journals.sfu.ca/jpps/index.php/jpps/article/viewFile/4/10

[35] Saroj, M. and D. Soumana, 2014. Salt stress induced changes in growth of germinating seeds of Vigna mungo(L.) Hepper and Vigna aconitifolia (Jacq.) Marechal. IOSR Journal of Agriculture and Veterinary Science, 7. (4):44-48. http://www.recentscientific.com/sites/default/files/4336.pdf

[36] Sawamery, N. and M. Mojaddam 2014. The Effect of Salinity Stress on Germination Components of Grain Sorghum Cultivars. Indian Journal of Fundamental and Applied Life Sciences, 4(4): 431-437. http://www.cibtech.org/J-LIFE-SCIENCES/PUBLICATIONS/2014/Vol-4-No-4/JLS-063-66-DEC-ACCEPT-MANI-THE-CULTIVARS.pdf

[37] Siddig, A.A.M. and A.A.Y. Idris 2015. Response of Sorghum (Sorghum bicolor L.) Cultivars to Salinity Levels at Early Growth Stages. J. of Agricultural Science and Engineering, 1(1): 11-16. http://www.publicscienceframework.org/journal/jase

[38] Snedecor GW and WG. Cochran 1980. Statistical Methods. 7ThEd. Iowa State University Press, Iowa, USA, ISBN-10: 0-81381560-6, Pp: 507. https://www.amazon.com/Statistical-Methods-Seventh-isbn-0813815606/dp/B0012S4NIE

[39] Sun, Y., G. Niu1, P. Osuna, L. Zhao, G. Ganjegunte, G. Peterson, J. R. Peralta-Videa, and J. L. Gardea-Torresdey 2014. Variability in Salt Tolerance of Sorghum bicolor L. Agricultural Science, 2(1): 9-21. www.iosrjournals.orgwww.iosrjournals.org

[40] Tabatabaei, S. A., and A. Anagholi 2012. Effects of salinity on some characteristics of forage sorghum genotypes at germination stage. International Journal of Agriculture and Crop Sciences, 4(14): 979-983.http://ijagcs.com/wp-content/uploads/2012/09/979-983.pdf

[41] Tigabo, E., M. Andargie and K. Tesfaye 2013. Genotypic Variation for Salinity Tolerance in Sorghum (Sorghum bicolor (L.) Moench) Genotypes at Early Growth Stages. Journal of Stress Physiology & Biochemistry, 9 (2): 253-262. http://cyberleninka.ru/article/n/genotypic-variation-for-salinity-tolerance-in-sorghum-sorghum-bicolor-l-moench-genotypes-at-early-growth-stages

[42] Zafar, S., M. Y. Ashraf, M. Niaz1, A. Kausar and J. Hussain 2015. Evaluation of wheat genotypes for salinity tolerance using Physiological indices as screening tool. Pakistan J. Botany, 47(2): 397-405.https://www.pakbs.org/pjbot/PDFs/47(2)/02.pdf

[43] Wei G. and W. Qing-Xiang 2011. Effects of seed soaking with humic acid on wheat seedlings antioxidant system under salt-alkali stress. YingyongShengtaiXuebao, 22 (10):2539. http://connection.ebscohost.com/c/articles/69910778/effects-seed-soaking-humic-acid-wheat-seedlings-antioxidant-system-under-saltalkali-stress

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Characterization of New Bacterial Leaf Blight of Rice Caused by *Pantoea stewartii* subsp. *indologenes* in Southern Districts of Tamil Nadu

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***Abstract*— *A survey was conducted in the rice fields of Tirunelveli, Tuticorin, Kanyakumari and Madurai districts of Tamil Nadu during 2016to assess the importance of bacterial leaf blight (BLB) of rice caused by Xanthomonas oryzae pv. oryzae. Bacterial Leaf Blight affected leaf samples showing yellowing symptoms or orange to brown stripes on one or both halves of the leaf blade were collected from seventeen places and maintained as isolates. Upon isolation, symptomatic leaf pieces were surface sterilized and placed in wakimoto semi-synthetic medium. The yellow pigmented, raised and translucent colonies with smooth margin were obtained after incubation at 28°C for 2 days. The biochemical characterization revealed that the bacteria belong to gramnegativefacultative anaerobes with small rods either arranged singly or in chains. Thirteen isolates show positive results in biochemical tests viz.,Gram staining, KOH test, starch hydrolysis, anaerobic growth test, tween 80 hydrolysis test, catalase test, citrate utilizationtest and production of yellow pigment on Yeast Dextrose Chalk agar medium. In virulence test, Isolate 1, Isolate 3 and Isolate 4 were considered virulent as they have caused severe blight symptoms both in TN1 and ADT 43, the susceptible check varieties. Based on 16S rRNA sequence analysis, the causal agent was identified as Pantoea stewartii subsp. indolegenes (Accession No. SUB2733370: MF163273; MF163274; MF16327). The biochemical and molecular analysis revealed that the causal agent was not Xanthomonas oryzae pv oryzae, but a new species of bacteriumnamely Pantoea stewartii subsp. indolegenes. This is the first report of new bacterial leaf blight disease of rice caused by Pantoea stewartii subsp.indologenesin southern districts of Tamil Nadu.*

Keywords*— Bacterial leaf blight, Xanthomonas oryzae pv. oryzae, Wakimoto semi-synthetic medium, virulence test, 16S rRNA sequence analysis, Pantoea stewartii subsp.indologenes.
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I. INTRODUCTION

Rice is one of the cereal crops of great significance in India and primary staple food for huge population in Asia, Africa and Latin America. Consumption of rice accounts for over 90 per cent of the world's population in Asia and China, India and Indonesia producing 30.85 per cent, 20.12 per cent and 8.21 per cent respectively of total global rice production (USDA, 2012; Kadu, *et al.*, 2015). Global rice utilization is projected over around 501.2 million tonnes (milled basis) in 2016-17, which is just one percent more than the 2015-16 estimates. The increase would be sustained by a 5.0 million tonnes expansion in food use to 402.5 million tonnes, much of which concentrating in Asia and Africa (FAOSTAT, 2016). In India, rice is being grown in 44.10 Mha area with production of 106.5 million tonnes and productivity of 3.52 MT/ha (USDA, 2016). In Tamil Nadu, rice is grown in an area of 20.16 lakh hectares with the production of 62.53 lakh million tonnes with the average productivity of 3,102 kg/ha (INDIASTAT, 2015). The highly valuable crop is pressurized by diverse fungal and bacterial attacks (Khan, 2009). Bacterial leaf blight caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*) is the most important and oldest known bacterial disease of rice in Asia (Hasan Naqvi *et al.*, 2014) and the most serious bacterial diseases in many of the rice growing regions of the world (Xu *et al.*, 2010). The bacterial leaf blight of rice is caused by *Xanthomonas oryzae pv oryzae* and also known to be caused by *Pantoea* (Lee *et al.* 2010; Mondal *et al.* 2011). *Pantoea spp.* are opportunistic pathogens

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documented to cause different diseases in economically important crop plants including grain discoloration of rice in China (Yan *et al.* 2010), leaf blight and bulb decay of onion in the United States (Schwartz and Otto, 2000) and leaf blight of rice reported in Korea (Lee *et al.* 2010), India (Mondal *et al.,* 2011), Venezuela (Gonzalez *et al.* 2015), Benin and Togo (Kini *et al*. 2017). *Pantoea ananatis*was reported as the causal agent of the newly emerged rice leaf blight disease reported in northern India (Mondal *et al.,* 2011).

II. MATERIALS AND METHODS

2.1. ISOLATION

The diseased leaves of rice showing typical bacterial blight (BLB) symptoms were collected in brown paper bags from seventeen places of Tirunelveli, Tuticorin, Madurai and Kanyakumari districts and maintained as different isolates.

The diseased portion alongwith adjacent healthy tissues were cut into 1.5 to 2 cm pieces separately. These diseased pieces of each isolate were surface sterilized separately for 30 seconds in 0.1 per cent mercuric chloride ($HgCl_2$) solution followed by three subsequent washing with sterilized distilled water in aseptic condition to remove the traces of $HgCl_2$. Then the pieces were kept on microscopic slide and were further cut with the help of sterilized blade. Then the cut pieces were placed on wakimoto's potato semi synthetic medium. The inoculated plates were incubated at room temperature (27 ± 2°C) for 48 hours. The bacterial colonies with typical straw or yellow colored with smooth margin and raised nature were transferred to the nutrient agar slant and maintained as isolates.

To prove the Koch's postulate and to confirm the pathogenic nature of isolated bacterium, pathogenicity test was carried out. The isolates were multiplied in nutrient broth medium and the pathogenicity test was carried out on rice plants in net house by employing clip inoculation technique.

2.2. BIOCHEMICAL CHARACTERIZATION

In order to characterize the bacteria, the following biochemical tests were carried out.

2.2.1.Gram staining

Bacteria were heat fixed on a glass slide treated with (0.5%) crystal violet for 30 seconds then washed with tape water and treated with iodine solution for 1 min, washed again and decolorized with 95 per cent ethanol for 30 seconds. Then washed again and counter stained with safranin for 1 min. While observing under microscope, gram negative bacteria stained red whereas gram positive bacteria retained color of crystal violet (Jonit e*t al.,* 2016).

2.2.2.KOH test

The bacterial culture taken in test tube was vigorously stirred in drop of 3% KOH solution. The thread like-slime formation will be indicated by the presence of gram negative bacterium (Jonit e*t al.,* 2016).

2.2.3.Catalase test

One colony from pure culture was taken out and put on the slide. One drop of 3 per cent hydrogen peroxide was placed on the colony. The production of bubble gives positive result (Jonit e*t al.,* 2016).

2.2.4.Citrate utilization test

Basal medium [Sodium chloride: 5g; Sodium citrate: 2g; Agar: 15g; Ammonium dihydrogen phosphate: 1g; Dipotassium phosphate: 1g; Magnesium sulfate: 0.2g; Bromothymol blue: 0.08g] was prepared and sterilized. Bacterial inoculum was taken from the center of the wellisolated colony and placed on the medium and incubated aerobically at 35 to 37°C for 4-7 days. Color change from green to blue will indicate the nature of bacterium (Hafiz Muhammad e*t al.,* 2015).

2.2.5.Production of yellow pigment on YDC medium

YDC agar medium was prepared and poured into petridish. The bacterium was inoculated on the plate and then incubated at 28°C for 24 h. The production of yellow pigment on the plate gives positive result (Haliatur Rahma *et al.* 2014).

2.2.6.Anaerobic growth test

Basal medium [Peptone: 2g; Nacl: 5g; Agar: 0.3g; KH_2PO_4: 0.3g; bromothymol blue: (3g) in 1% aqueous solution, 5ml] was prepared. An amount 0.5 ml 10% glucose suspension was added to each tube aseptically. For each isolate two test tubes were inoculated and incubated at 28°C. Color change occurred from blue to yellow indicates the anaerobic growth of the bacteria (Jonit e*t al.,* 2016).

2.2.7.Starch hydrolysis test

Starch is an insoluble polymer of glucose, some bacteria possess the ability to produce amylase that breaks starch into maltose and the amylase is an extra cellular enzyme which is released from microorganism. Starch agar plates (soluble starch 2 g/l; peptone 5 g/l; beefs extract 3 g/l; agar20 g/l dissolve the nutrient agar powder in water by heating and dissolve the starch in 10ml distilled water and add to molten agar) were inoculated by streaking the bacterial isolates and incubated for 4 days at 27±2°C. Plates were flooded with Lugol's iodine solution and observed for appearance of clear zone of hydrolysis around the bacterial growth which indicates that the starch has been hydrolyzed (Lelliot and Stead, 1987).

2.2.8. Tween 80 hydrolysis

The hydrolytic activity of bacterial isolates were done on Tween 80 media .This media has been prepared by adding peptone, $Nacl_2.2H_2O$, agar in distilled water and pH was maintained at 7.2-7.4 then autoclaved at 121ºC for 15 minutes, Tween 80 was mixed in sterilized media. Media was poured into autoclaved Petri plates. After 24 hours these plates were inoculated with fresh bacterial culture and incubated at 28°C for 2 days. Positive reaction of milky white precipitation was formed around the colonies (Ishaq Ahmad *et al.,* 2015).

2.3. VIRULENCE TEST

Among 17 isolates, five isolates were tested to know their virulence and the isolates were multiplied on nutrient broth medium. They were inoculated to test their virulence on highly susceptible var. TN-1 and ADT 43 by employing standard clip inoculation method. The appearance of bacterial blight symptoms and their development was recorded till the end of the experiment.

2.4. MOLECULAR CHARACTERIZATION

The isolates which show higher level of virulence have been selected for characterization based on the earlier symptom expression and per cent leaf area blighted.

2.4.1. Preparation of template DNA: It is important to use a pure cultured bacterium for the identification. The colonies were picked up using a sterilized toothpick and suspended in 0.5µl of sterilized saline in a 1.5 ml centrifuge tube and which was centrifuged at 10,000 rpm for 10 min. After removal of supernatant, the pellet was suspended in 0.5 ml of InstaGene Matrix (Bio-Rad, USA). Incubated at 56°C for 30 min and then heated up to 100°C for 10 min. After heating, supernatant can be used for PCR.

2.4.2. PCR: 1 µl of template DNA was added into 20 µl of PCR reaction solution. 518F/800R primers used for bacteria and then 35 amplification cycles at 94°C for 45 sec, 55°C for 60 sec and 72°C for 60 sec was performed. DNA fragments were amplified about 1,400 bp in the case of bacteria. It includes a positive control (*E.coli* genomic DNA) and a negative control in the PCR.

2.4.3. Purification of PCR products: Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore).

2.4.4.Sequencing: The purified PCR products of approximately 1,400 bp were sequenced by using the primers (785F 5' GGA TTA GAT ACC CTG GTA 3' and 907R 5' CCG TCA ATT CCT TTR AGT TT3'). Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA).

III. RESULTS AND DISCUSSION

The diseased leaves of rice showing typical bacterial blight (BB) symptoms were visually observed and collected in brown paper bags from Tirunelveli, Tuticorin, Madurai and Kanyakumari districts. The typical symptoms of BLB such as yellowing symptoms or one to two orange or brown stripes on one or both halves of the leafblade were visually observed and critically recorded. Similiarly, Mondal *et al.,* (2011) revealed that the symptom exhibited as water soaked lesions at the tip of rice leaves and turned light brown, exhibiting a blighted appearance. Upon isolation, symptomatic leaf pieces were surface-sterilized and macerated in sterile water and maintained as 17 isolates. Upon plating on semi selective peptone-sucrose-agar (PSA) medium, yellow pigmented straw to yellow colored, raised and translucent with smooth margin colonies were obtained after incubation at 28°C for 1 or 2 days. The bacteria are gram-negative, facultative anaerobes with small rods either arranged singly or in chains.

The pathogenicity test revealed that inoculated rice leaves exhibited bacterial blight symptoms similar to those produced under natural field condition. Thus, isolated bacteria proved pathogenic to rice beyond doubt satisfying Koch’s postulate. Similiarly, Kini *et al*., (2017) also reported that the leaves inoculated with bacterial suspension showed typical BLB-like lesions and the reisolated bacteria from diseased leaves also yielded colonies.

All the isolates showed positive results in gram staining, KOH test, starchhydrolysis,anaerobic growth test,citrate utilization test and production of yellow pigment on yeast dextrose chalk agar medium except isolates numbering 5,10,11,12,14 and 17(Table 1). Similar results were obtained by Mondal *et al*.(2011) and Gonzalez *etal.*(2015). Anaerobic growth test is a key test for the identification of the bacterial genera *Erwinia* and *Pantoea*. Most of the isolates exhibit anaerobic growth which indicated clearly that the organism belongs to Enterobacteriace (i.e.facultative anaerobes) not belongs to Xanthomonadaceae family (*i.e.* True aerobes). The production of yellow pigmentation on YDC medium indicates that the isolate belongs to *Pantoea* species. Similar results were observed by Pérez-y-Terrón *et al.* (2009) and Haliatur Rahma *et al.* (2014).

The results of virulence test indicated that hundred per cent infection was caused by all the isolates in TN 1 and ADT 43, the susceptible check varieties. Maximum of 80 per cent leaf blight was observed in Isolate 1, Isolate 3 and Isolate 4 in TN1 where as it was 70, 80 and 60 per cent respectively in ADT 43. (Table 2).

The present results are in confirmation with the findings of Gopinathan *et al.* (1991) who reported that the pathogen shows variable virulence on different cultivars. Some of the biochemical tests gave overlapping results regarding the identity of the causal organism of bacterial blight. For these reasons, PCR was performed for three virulent isolates, Based on 16S rRNA sequence analysis, the causal agent was identified as *Pantoea stewartii* subsp. *indolegenes*. The sequencing data obtained has been deposited in NCBI gene bank with accession no. SUB2733370: MF163273 (ASD 16), MF163274 (TN 1), MF163275 (CO 43). The alignment showed maximum (99%) homology with the related sequence in the data bank.

These sequences were further confirmed by constructing the phylogenetic tree to correlate with the family tree of those species. The culture sequence obtained were subjected to BLAST analysis, the phylogenetically similar type strains sequence and other phylogenetic related sequence were selected from the GenBank and they were subjected to multiple sequence alignment and then align sequences were trimmed to similar length in nucleotides and were subjected to phylogenetic tree (neighbour joining) construction using MEGA 6. In the tree, the numbers at the nodes indicate the levels of the bootstrap support [high bootstrap values (close to 100%) meaning uniform support] based on a neighbor-joining analysis of 1,000 re-sampled data sets. The bootstrap values below 50% were not indicated and bar 0.005 substitutions per site which are depicted in Fig.1.

It is concluded that the pathological investigations of the BLB of rice were undertaken by recording the natural symptoms appeared in the field. The microscopic examination and repeated isolation from BLB samples revealed the presence of bacteria and the disease was thought to be caused by *Xanthomonas oryzae* pv. *oryzae*, rice bacterial blight causing pathogen. The pathogenicity test was proved to be positive on rice and satisfied Koch's postulate. The biochemical and molecular analysis revealed that the causal agent was not *Xanthomonas oryzae* pv *oryzae*, but it was *Pantoea stewartii* subsp. *indologenes*.Similar results were reported by Lee *et.al*.(2010), Mondal *et al.* (2011),Gonzalez *et.al.*(2015) and Kini *et al.* (2017).The present finding tallied with the report of Kini *et al.* (2017) who published a paper on 'New bacterial leaf blight of rice caused by *Pantoea ananatis* and *Pantoea stewartii* in Benin'. Earlier, Mondal *et al.* (2011) also submitted a first report on'New leaf blight of rice caused by *Pantoea ananatis* in India'. Likewise, 'First report of leaf blight caused by *Pantoea agglomerans*on rice was published in Korea by Lee *et.al.* (2010).The present study proved that the leaf blight of rice can also be caused by species of *Pantoea*. To our knowledge this is the first report of new bacterial leaf blight of rice caused by *Pantoea stewartii* subsp. *indologenes* in Tamil Nadu, India.

REFERENCES

[1] FAOSTAT. 2016. Agriculture data (online)- Rome, Italy. In: http://www.fao.org/and http://faostat.fao.org. (last update 2016; accessed October, 2006).

[2] Gonzalez, A.D., Franco, M.A., Galindo-Castro,I. and Graterol, E. (2015). First report on *Pantoea agglomerans* causing Rice leaf blight in Venezuela. *Plant Disease*, 99 (4): 552.

[3] Hafiz Muhammad Imran Arshad, Saima Naureen, Kamran Saleem, Safdar Ali, Tanzila Jabeen, Muhammad Masood Babar. (2015). Morphological and biochemical characterization of *Xanthomonas oxanopodis* pv. o*ryzae* isolates collected from Punjab. *Adv.life Sci*.,2(3):125-130.

[4] Haliatur Rahma, Meity Sinaga, S., Memen Surahman and Fiyanto. (2014). First report of stewart's wilt of maize by *Pantoea stewartii* subsp. *stewartii* in Bogor district, Indonesia. J. ISSAAS., 20(2):131-141

[5] Hasan Naqvi, S., Rashida Perveen, A., Ummad ud Din Umer, Owais Malik, Ateew ur Rehman, Sajid Wazeer and Taha Majid. (2014). Determination of antibacterial activity of various broad spectrum antibiotics against *Xanthomonas oryzae* pv.*oryzae*, a cause of bacterial leaf blight of rice. *International Journal of Microbiology and Mycology*., 2**:** 12- 19.

[6] INDIASTAT. (2015). Online databases. In: http:// www.indiastat.com.

[7] Ishaq Ahmad, Muhammad Zakria, Abdul Rehman and Anjum Munir. (2015). Biochemical characterization of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) populations from kallar belt of Punjab.*Pakistan. Intern J innovations and res*., 3(4) 2319-1471.

[8] Jonit et al., (2016). *Xanthomonas oryzae* pv. *oryzae*, Biochemical tests, Rice (*Oryzasativa*), Bacterial leaf blight (BLB) disease, sekinchan. *J. appl. & Environ.microbiol*., 4(3): 63- 69.

[9] Kadu, T. P., Kale, S.S., Chayan, N.R., Agarwal, T. and Verulkar, S.B. (2015). Pyramiding of three bacterial blight resistance in Dubraj rice cultivar using marker-assisted selection. *The Ecoscan.*,7**:** 07-12

[10] Khan A.S, Imran, M. and Ashfaq, M. (2009). Estimation of genetic variability and correlation for grain yield components in rice (*Oryza sativa* L.). *Journal of Agriculture and Environmental Sciences.*, pp: 6585 – 590.

[11] Kini, R., Agnimonhan, O., Afolabi, B., Milan, B., So glonou, V., Gbogbo, R. Koebnik and Silue,D. (2017). First report of a new bacterial leaf blight of Rice caused by *Pantoea ananatis* and *Pantoea stewartii* in Benin. *Plant Dis.*,101: 242.

http://dx.doi.org/10.22161/ijeab/2.6.64 ISSN: 2456-1878

[12] Lee, H.B., J.P.Hong and S.B. Kim. (2010). First report on leaf blight caused by *Pantoea agglomerans* on Rice in Korea. *Plant Dis*. 94: 1372.

[13] Lelliot, R.A. and Stead,D.E. (1987). Diagnostic procedures for bacterial plant diseases. Methods for the diagnosis of bacterial diseases of plants. pp: 37-131.

[14] Mondal, K.K., Mani, C.and Singh. J. (2011). A New leaf blight of Rice caused by *Pantoea ananatis* in India. *Plant Dis*. 95:1582

[15] Schwartz, H.F. and Otto,K. (2000). First report of a leaf blight and bulb decay of onion by *Pantoea ananatis* in Colorado. *Plant Disease.*, 84**(**7): 808-808.

[16] USDA. 2012. Rice Outlook, Economic Research Service RCS-12j/Oct. 12, 2012. pp 1-26.

[17] USDA. 2016. Rice Outlook, Economic Research Service /RCS-16J/October 14, 2016. Pp.1-25. http://www.ers.usda.gov/ media/2150132/rice-outlook-october-2016.pdf.

[18] Xu, Y.,. Zhu, X.F., Zhou, M.G., Kuang, J., Zhang, Y., Shang, Y. and Wang, J.X. (2010). J *Phytopathol.*, 158: 601-608

[19] Yan, H., Yu, S.H., Xie, G.L., Fang, Su,W.T. and Li.,B.(2010). Grain discoloration of rice caused by *Pantoea ananatis* (synonym *Erwinia uredovora*) in China. *Plant Disease*., 94(4): 482-482.

Table.1: Biochemical characterization of the isolates

Isolates	**Gram staining**	**KOH test**	**Starch hydrolysis**	**Anaerobic growth test**	**Tween 80 hydrolysis test**	**Catalase test**	**Citrate utilization test**	**Production of yellow pigment on YDC**
1	-	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+
3	-	+	+	+	+	+	+	+
4	-	+	+	+	+	+	+	+
5	+	-	-	-	-	-	-	-
6	-	+	+	+	+	+	+	+
7	-	+	+	+	+	+	+	-
8	-	+	+	+	+	+	+	-
9	-	+	+	+	+	+	-	-
10	+	-	+	-	-	-	-	-
11	+	-	-	-	-	-	-	-
12	+	-	-	-	-	-	-	-
13	-	+	+	+	+	+	-	-
14	+	-	-	-	-	-	-	-
15	-	+	+	+	+	+	-	-
16	-	+	+	+	+	+	-	-
17	+	-	-	-	-	-	-	-

Table.2: Disease severity due to virulence of the isolates

Treatment. No	**Isolates from different varieties**	**Per cent plant infected**		**Per cent leaf area blighted**	
		TN 1	ADT 43	TN 1	ADT 43
1	TN 1	100	100	80(9*)	70(9*)
2	Navarai	100	100	20(5)	20(5)
3	ASD 16	100	100	80(9)	80(9)
4	CO 43	100	100	80(9)	60(9)
5	ADT 43	100	100	40(7)	50(7)
6	Control	0	0	0(0)	0(0)

*Data in parenthesis are disease grades

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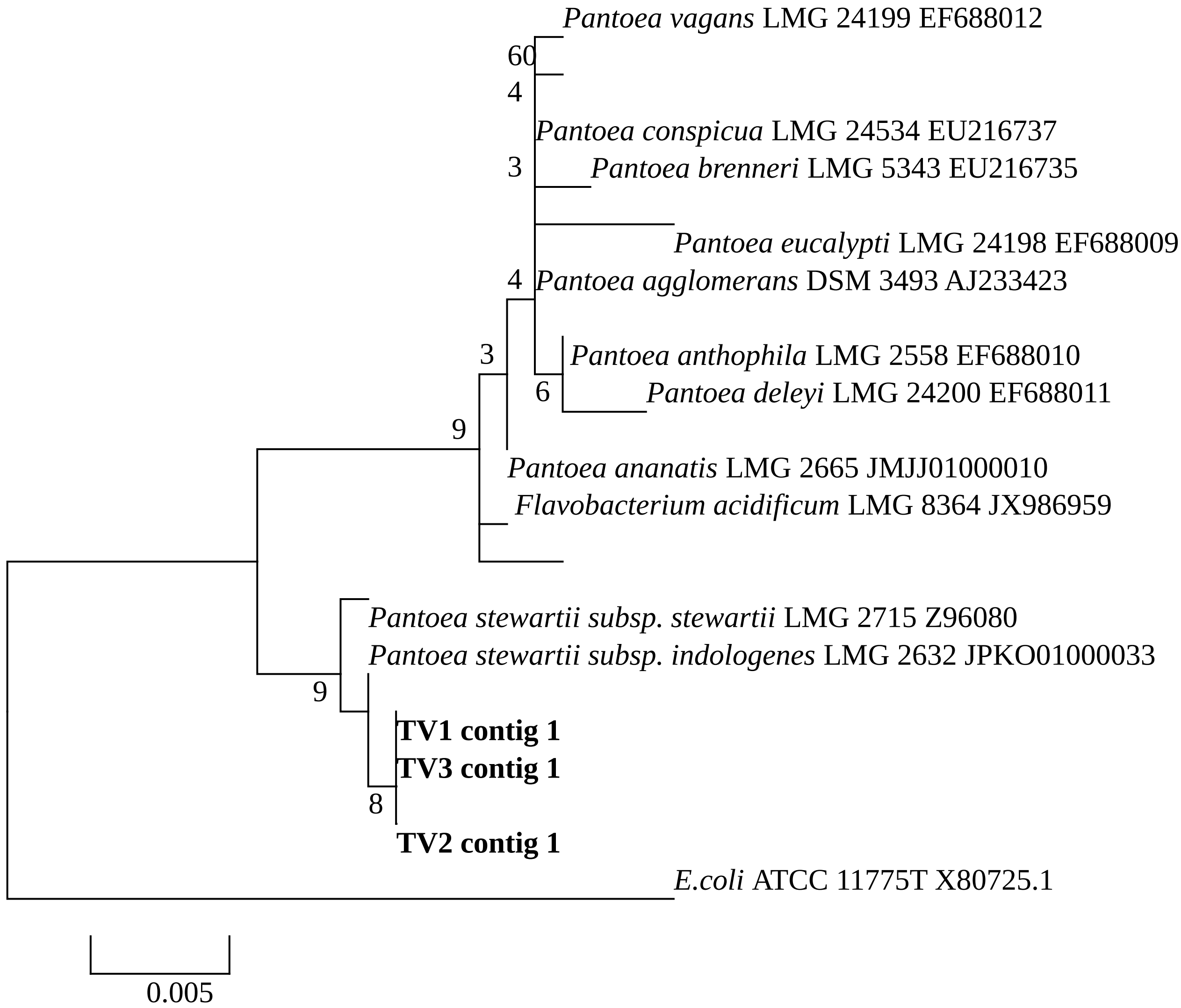

Fig.1: Phylogenetic tree of Pantoea genus alongwith new accessions

Governance for Sustainability in an Organization in Central Mexico

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Abstract *— Often, the total quality has been instrumented before being weighted. The strategies even precede a diagnosis in Mexican organizations, but in an opposite sense, the present work set out to establish the reliability and validity of an instrument to measure the perception of total quality based on three indicators related to management. , production and transfer of knowledge. A nonperimental study was carried out with a non-probabilistic selection of 124 administrative staff and employees from an organization in central Mexico. From a structural model* $\lceil X^2 = 123{,}24$ *(23df)* $p = 0{,}010$*; GFI = 0,990; CFI =,991; IFI = 0,993; RMSEA = 0,007*$\rfloor$*, it was found that management affects production (0,38) and this about the total perceived quality (0,35), although there are lines of research concerning empathy, commitment, entrepreneurship, satisfaction and happiness in relation to the implementation of continuous improvements to the quality of processes and products.*

Keywords—Client omission, Control strategy, Logistics mistake, Wrong delivery.

I. INTRODUCTION

No doubt, organizations have some mistakes in its organizational context, however, sometimes, mistakes are over-dimensioned because of clients' honest lack. It is when the organization need to have a severe control of it processes, even administrative, financial, sales, production or logistics ones. Organizations which promote the use, production or consumption of green energies, also are attached to negative factors occurrence over its processes. Present document, look forward to be a path on mistake occurrence, when it is considered the logistics' or deliveries' mistakes, in the framework of sustainability's context, due to the need of green organizations hold in the market to promote clean energy methods.

Concern about sustainability has been grown in people's mind. Debate since the release of the World Conservation Strategy in 1980, "Our Common Future" the report of the World Commission On Environment and Development in 1987 and Agenda 21 in 1992 has resulted in gradual acceptance that sustainability must integrate ecological integrity, economic efficiency and social equity (Côté& Cohen-Ronethal, 1998).

In Molina Ruiz (2013), it is mentioned that there exists an alarming situation, due to planets situation. In Mexico, it is possible to see the negative influence of population impact over environment (Molina-Ruiz, 2015). It is also possible to observe some social deterioration and economic problems. Cavagnaro& George (2017) propose a framework in which they are recognized the three main dimension of sustainability.

It is important to promote wellbeing inside the organizations. In the framework of sustainability, organizations which promote use of clean energies, sometimes are in a constant risk that threaten its stability.

It is natural for organizations to have some mistakes along its development and historical path, however, when client shows a lack of honesty and omit information sharing, the organization have a higher spend of resources to correct the mistake or repair the problem. Between organizations it is necessary to create a supporting environment in which the stakeholders share information with each other.

In order to survive on the market and achieve profitability, the companies need to meet customer requirements and perform their activities in an efficient way (Andrejić, Kilibarda & Popović, 2015). However, some clients abuse of the organizations good will, bringing extra cost in the organizational use of resources.

Sometimes, inside of the organizations, low compromised personnel have cheating attitudes that affect directly the organization performance. In Bohte& Meier (2000), it is defined organizational cheating as an attempt to manipulate performance criteria; it is also identified three major forms of

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organizational cheating:1) cutting corners (doing sloppy work); 2) lying (making up organizational results); and 3) biasing samples (reporting most conductive cases).In the organizational context it can be identified another way of organizational cheating, "client's snuggling", which means that a stakeholder inside of the organization overprotect the client, giving to it privileged information and covering bad client (or supplier) behavior that affects the organization.

Cialdini, Petrova& Goldstein (2004), proposed that organizational dishonesty can increased surveillance, (mis)matches between values of employee and organization and/or reputation degradation. It is also possible to state that organization dishonesty can make that enterprise run out of business (bankruptcy), loss of clients, loss of suppliers, loss of bank or credit-agents' support.

Enterprise in which it happened the case under study had certain particularities. It is an enterprise relatively new in the photovoltaics sector in Mexico, it was created in 2013. Due to its recent creation, there was a lack in the control and organization of different activities inside of the organization. That organization has the second place in sales in Mexican market, during 2015. During 2017, it has increased its market share to North America and Central America. In Mexico, the enterprise recovers the second position in importance by Mexican PV-market.

First detected particularity was, as here exist a cordial and close communication, delivery of final product would be required via a piece of paper written by sales manager and given to production manager.

Despite there exist four main steps to deliver a merchandize, sales manager, due to urgency of delivery, avoid the sequence of steps. The correct step by step in the enterprise would be as follows: (a) quotation price document, in which sale's agent sent the price and characteristics of the product to client, in case client accept the price and characteristics, it is generated (b) the request document, in which warehouse is notified that a product need to be packaged, it also is sent to the client so he/she can make the payment, to make (c) the invoice document, which is the official document and ensures that merchandize is now client's property, once invoice is created, it is made a (d) warehouse authorization, a list of the allowed merchandise's delivery to client, via Delivery-service outsourcing.

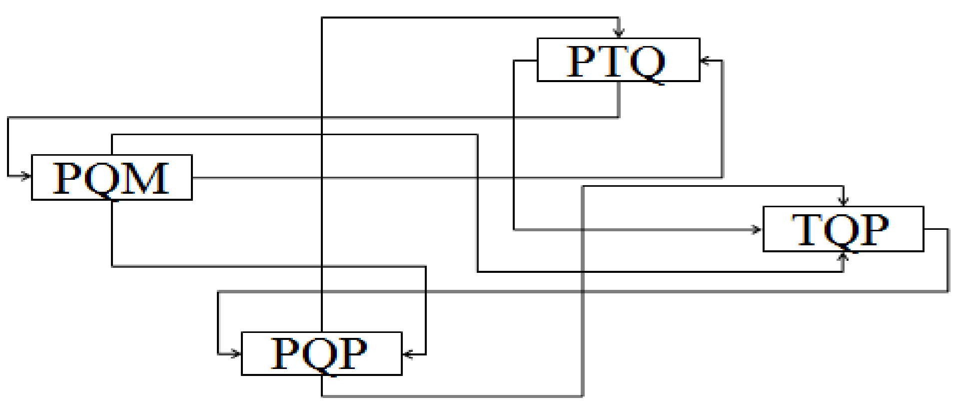
Sometimes it was authorized the delivery of merchandize, when the quotation price document was just generated, because of the request of sales manager.

There were some situations in which sales manager sent a "request document" to logistics department (warehouse), with missed information, and after, she resent mentioned document with extra information or with corrections in the information, or sales manager hold the (extra) information document (or the corrected one) for itself.

Warehouse do not have a complete folder for each delivery. Deliveries were just registered in a list with very little information, and the folder for each delivery (invoice) do not have all of the documents.

Theory of perceived quality

In the anthropocentric paradigm in which companies circumscribed their total quality control to the demands of the market and the specific demand of their clients, the function of the leader was that of an intermediary who managed and managed the risks without considering the environment or capital nor the possibilities of human or intellectual capital in face of the imbalance that the situation implied (see Figure 1).



TQP = Total Quality Perceived, PQM = Perceived quality management, PQP = Perception of quality production, PTQ = Perceived Transfer of Quality

Fig.1: Theory of Perceived Quality

Source: Prepared by the author

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In the paradigm of sustainability, the total quality lies in the evaluation, certification and accreditation of processes based on the availability of resources, policies against climate change, the effects on environmental public health and the risks inherent in the Industrial production (Acar&Acar, 2014).

While in the old anthropocentric paradigm the responsibility was centered on the leader, the manager or administrator, in the new ecocentric paradigm the responsibility is shared (Hernandez & Valencia, 2016). This implies a unilateral communication versus a bilateral communication, a unidirectional motivation versus a bidirectional motivation. It is about the confrontation of two cultures, one authoritarian and the other democratic (Anicijevic, 2013).

Even the new environmental paradigm is distinguished from the previous dominant paradigm by the continuous improvement of processes (Mendoza, Ramirez &Atriano, 2016). This supposes the entrepreneurship and the innovation of the processes that in the previous paradigm was translated in a resistance to the change. That is to say that the responsibility of participation and initiative now concerns all those who integrate the organization (Carreón et al., 2014).

The achievement of a shared responsibility precedes a shared work commitment and a climate of emotional, affective and sentimental relationships regulated and oriented to coexistence, respect, solidarity and support among those who make up the organization (Cruz, Arroyo &Marmolejo, 2016).

Therefore, there to define quality standards and criteria for its continuous improvement, the organization involves leaders and managers, managers and employees in the objectives, tasks and goals according to the availability of resources, social responsibility and organizational capabilities (Escobar, 2014).

Specification model

Formulation

Will the relationships proposed in the theory of perceived quality be adjusted to empirical observations with leaders and employees of an organization in central Mexico?

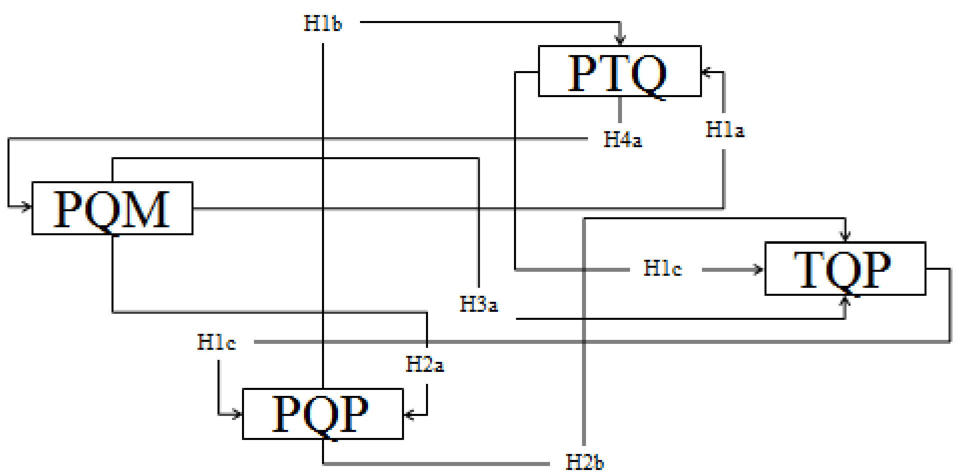
Null hypothesis

The relationships between the variables specified in the theory of perceived quality will be adjusted to the data observed in an organization in central Mexico, since it is a universal asymmetric relationship between the demands of the environment and organizational capacities, which also mark differences between leaders and employees

Alternative hypothesis

Although the theory of perceived quality anticipates scenarios of differentiation between the requirements of the environment and the capabilities of the organization, among leaders and employees, the perceptions around the total quality process, as well as control management are different in each organization reason why the relationships established in the theory will not conform to the observations of a case study

Relations on the factors



TQP = Total Quality Perceived, PQM = Perceived quality management, PQP = Perception of quality production, PTQ = Perceived Transfer of Quality

Fig.2: Specification model

Source: Prepared by the author

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In the following paragraphs, it is reported different events linked to wrong delivery made by the provider enterprise. Data have been changed or modified to protect confidential information of different enterprises and persons.

On Tuesday, July 5th, 2016 it was a wrong delivery of 8 panels of 260W and a 2.0 KW inverter, from the invoice X57X, whit tracking number AB00XX2970X, to our client, Renewable Energies Co. (SolarGroup). It because our last delivery to that client was to its address on the Southeast of Mexico.

In following figure, it is represented the invoice linked to mentioned delivery, that invoice was made by 12 PV-modules, 1 inverter of 2 KW and 1 WiFi stick for the inverter.

On Friday, July 15th, 2016, our sales manager communicates to us that the client complains because he was not received his product (delivery was sent to Southeast of Mexico). Sales department manager, request that logistics would sent 5 panels of 260W and a WiFi stick, to other address in the northwest of Mexico.

They were delivered on Saturday, July 16th, 2016. The 5 panels and 1 WiFi stick was sent to Delivery-service (center of Mexico's Office) by an outsourcing service by $ 500.00 plus taxes ($580.00), which take the merchandize from the factory to the Delivery-service's office.

It was stared the process to recover the merchandise on July 18th, 2016, with almost daily callings to Southeast's office of Delivery-service and occasional calling to Delivery-service's Call Center. Logistics department tried to establish a communication bridge. It is pointed out, that the communication with Southeast's office was very narrow and sometimes it is not possible communicate whit them.

There was also made some other calling to Southeast's Delivery-service Office. On Monday, July 18 it was made the phone call to Delivery-service's Call Center (XX XXX X10 8352), logistics department was attended by Attendant I so they were obtained the following phone numbers:

(XXX) XX3 0953
(XXX) XX3 0972
(XXX) XX3 0973

On Tuesday, July 19, 2016, it was contacted Delivery-service's Office in Southeast, so Attendant II ask to request the re-expedition of panels and inverter, by sending an e-mail to attx@deliveryserv.com.mx and attiii@deliveryserv.com.mx, to Attendant X and Attendant III

On Friday, July 22nd, 2016, it was made a call again to Delivery-service (Southeast's Office), Attendant II answered, and gave the extension number of Attendant X and Attendant III. Attendant II take the phone call to the extension of Attendant III. When Attendant III, answered said that she has already sent the quotation to send back panels and inverter to factory. It was set a price of $5634 pesos, so Logistics department request a quotation to Delivery-service Office (Center of Mexico's Office).

On Monday, July 25th, 2016, it was made another phone call to Southeast's Office, but there was no answer. On Tuesday 26th, 2016, it was called again to Southeast's Office, however in both lines the calling was stopped. It was made a phone call to Delivery-service's Call Center answering Attendant IV, she gave again the same phone numbers from Southeast's Office, and transferred the phone call to that office, that moment, answered Attendant II and logistics was hanged on the line, after a while, she asked to resend the request to Southeast's Office, so the request was resent.

On Thursday, July 28th, 2016, there was made another communication to Southeast's Office, and it was requested (Attendant X), to resend the last request, due to he has not received mail nor document.

On Friday, July 29th, 2016 and Monday, August 1st, 2016, logistics try to communicate to Southeast's Office but there was no answer. On Monday, August 8th, 2016, logistics try to communicate to Southeast's Office but there still was no answer. On Tuesday, August 16th, 2016, logistics try to communicate to Southeast's Office but again, there was no answer. On Friday, August 19th, 2016, logistics try to communicate to Southeast's Office but there was no answer. On Monday, August 22nd, 2016, logistics try to communicate to Southeast's Office but there still was no answer. On Wednesday, August 31st, 2016, logistics department try again to communicate with Southeast's Office but still no answer.

On Friday, September 2nd, 2016, logistics department make a phone call to Delivery-service's Call Center, answering Attendant V, she request the basic information of the delivery an she found out the that merchandise was already picked up by the client, the person who picked up the merchandise was named: Mauricio E. A., merchandise has been taken by that person on August 11th, due to a connection failure the calling was ended. However, logistics department call back again, attending Attendant VI, she communicate logistics with Attendant III (in Southeast's Office), and Attendant III said she was checking and she said she was calling logistics back, but Attendant III did not make any phone call.

On Monday, September the 5th, 2016, logistics try to communicate to Southeast's Office but again, there was no

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answer. On Tuesday, September 61h, 2016, there was made a phone call to Southeast's Office, that time answered Attendant III: she made the link with Attendant VII, and Attendant VII request to ask via mail for support to recover the information of the case. An e-mail was sent to request the support to recover the evidences that Southeast's Office, have in order to integrate a report or (if necessary) to start a legal motion.

On Tuesday, September 8th, 2016, there was made a phone to Southeast's Office, but there was no answer. On Friday, September 9th, 2016, there was made a phone call to Southeast's Office and answered Attendant III. It was requested to talk with Attendant VII, en the phone call was transferred to the Attendant VII's extension. Attendant II answered and she said, it was not possible to talk to Attendant VII, but Attendant II was told about the situation, so she inform that it wouldn't be possible to recover any picture or video due to Southeast's Office data base only cover 21 days of record. However, Attendant II, agree to look for the document linked to tracking number ABO0XX2970X, and send it via mail to Logistics Department, to check the person who had signed and toke the merchandise.

Some days after that communication it was received the e-mail in which a person of Renewable Energies Co.'s, required that merchandise would be given to Mauricio E. A.

On a general way, to avoid problems on merchandize delivery, it was adopted a very strong attitude over the sales manager informal requests, respecting the stablished procedure to deliver merchandize and it was stablished a delivery's binnacle in warehouse and security gate.

It has been mentioned that sales manager asked for deliveries with quotation price documents or with request document, so production and logistics department, avoid the informal authorizations to delivery products or material. The process was stablished as a four steps method: (a) quotation price document (b) request document (c) invoice document and, (d) warehouse authorization.

After the problems, it was integrated a complete folder for each delivery and added some documents to complete it. It has been mentioned that, previously, a delivery can be authorized with a quotation or request document, but with the new way of working, it was required the following documents to authorize a delivery: i) quotation, which have the price authorized to sale the merchandize; ii) request, which includes authorized price and correct data and address linked to merchandize sold; iii) payment, it is the ticket or voucher (scanned, picture taken, or PDF) in which it can be seen the linked payment for each bought material (in the case of check, it was necessary to wait three days, until the amount of money was contrasted in the enterprise's bank account); iv) invoice, generated invoice after payment check in; v) sent data ticket, which have the information to be delivered by the outsourcing delivery service; vi) warehouse binnacle, where they were registered each material (invoiced) delivered (and contains data like: date, quantity, model, client, invoice, client's Federal Taxpayer Registry, driver, license plate, sent mode); vii) tracking number, it is the obtained document linked to delivery service

II. METHOD

Design.

A descriptive, exploratory and transversal study was carried out

Sample.

124 administrative and employees of a for-profit organization in the center of Mexico.34% men and 66% women. 75% under 29 years old (M = 24,13 SD = 0,18), 15% between 29 and 65 years old (M = 41,23 SD = 10,17) and 5% over 65 years old (M = 67,32 SD = 0,16). 22% with more than 7 working years (M = 7,12 SD = 0,12), 38% with less than 7 and more than 3 working years (M = 4,35 SD = 0,84), 28% with less than 3 working years (M = 2,43 SD = 0,93).

Instrument.

The Total Perceived Quality Scale of Carreón (2016) was used, which includes four dimensions related to the management, production and the perceived transference of the quality of processes. each reagent includes five answer options that go from 0 0 it does not look like anything to my organization up to 4 = it looks a lot like my organization.

Proceeding.

The Delphi technique was used for the processing of information and the elaboration of the reagents, comparing and integrating informative information to the total quality, as well as to the opinions of different administrative and employees in an organization for profit in the center of Mexico.

Subsequently, the surveys were applied in the human resources department as part of the staff recruitment and selection protocol, as well as part of the induction, training and training courses. The confidentiality and anonymity of the respondents was guaranteed in writing, as well as the warning that the results of the study did not affect their economic or work status.

The consistency of the instrument was estimated in terms of its questions from the answers, considering the Cronbach alpha parameter, as well as the Barttlet and KMO tests for

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adequacy and sphericity as preliminary tests to the validity, which was performed with a method of extraction of main axes with promax rotation. The comparison of the model with adjustment and residual parameters for the hypothesis test.

III. RESULTS

Table 1 shows the values of internal consistency of the instrument (alpha of 0.782 for the general scale and 0.780 to 0.795 for the subscales) which suggest that in other contexts and study samples the measurement of indicators and factors will be similar in up to 70% of cases.

Table.1: Descriptives of the instrument

Code	Item	M	SD	A	F1	F2	F3	F4
PQM1	Prevention against risks	3,21	0,19	0,701				0,439
PQM2	Disasterprevention	3,25	0,28	0,702				0,329
PQM3	Preventionagainstviolence	3,45	0,38	0,731				0,431
PQM4	Conflict prevention	3,25	0,43	0,721				0,403
PQM5	Accident prevention	3,46	0,54	0,742				0,325
PQM6	Prevention against epidemics	3,67	0,83	0,721				0,345
PQM7	Preventionagainstdiseases	3,93	0,48	0,742				0,392
PQP1	Production before demands	3,02	0,91	0,743			0,431	
PQP2	Competitiveness in the face of shortages	3,01	0,29	0,741			0,423	
PQP3	Entrepreneurship before needs	3,26	0,39	0,752			0,504	
PQP4	Continuous improvement in the face of backlog	3,46	0,40	0,704			0,593	
PQP5	Continuous improvement before absences	3,41	0,53	0,725			0,502	
PQP6	Continuous improvement against rotation	3,24	0,45	0,721			0,501	
PQP7	Continuous improvement against fraud	3,25	0,41	0,793			0,504	
PTQ1	Securities against corruption	3,44	0,24	0,783		0,305		
PTQ2	Empathy in the face of absenteeism	3,12	0,32	0,702		0,416		
PTQ3	Communication in disasters	3.11	0,22	0,771		0,406		
PTQ4	Conflict support	3,02	0,33	0,772		0,493		
PTQ5	Disappearance rules	3,26	0,13	0,783		0,492		
PQT6	Incentives for absenteeism	,345	0,21	0,711		0,501		
PTQ7	Emergency response	3,46	0,34	0,705		0,403		
TQP1	Attachment to the company	3,47	0,02	0,783	0,403			
TQP2	Thanks to the company	3,41	0,38	0,783	0,302			
TQP3	Recognition to the company	3,26	0,49	0,756	0,392			
TQP4	Delivery to the company	3,27	0,93	0,736	0,491			
TQP5	Put on the company shirt	3,38	0,12	0,747	0,302			
TQP6	Respect for company values	3,04	0,21	0,746	0,321			
TQP7	Execution of company protocols	3,36	0,32	0,726	0,301			

Method of extraction of the main axes, promax rotation. Adequacy and Sphericity ⌈X^2= 452,67 (56df) p = 0,000: KMO = 0,770⌋. M = Average, DE = Standard Deviation, A = Alpha, quitting the item value. F1 = Perceived Quality Management (alpha of the 0,780 and the 24% of the variance explained), F2 = Production Perceived Quality (alpha of the 0,785 and 21% of the variance explained), 3 = Perceived Quality Transfer (alpha of the 0,790 and the 16% of the variance explained), F4 = Perception of Total Quality (alpha of the 0,795 and the 11% of the variance explained). All the items are answered with five response options: 0 = it does not look like my organization, 1 = it seems very little to my organization, 2 = it seems little to my organization, 3 = it appears in something to my organization, 4 = it looks a lot like my organization

Source: Elaborate with study data

Figure 3 shows that the perceived management of quality determines the perceived production of quality (0,38), but this last factor is determinant of the total perceived quality (0,35).

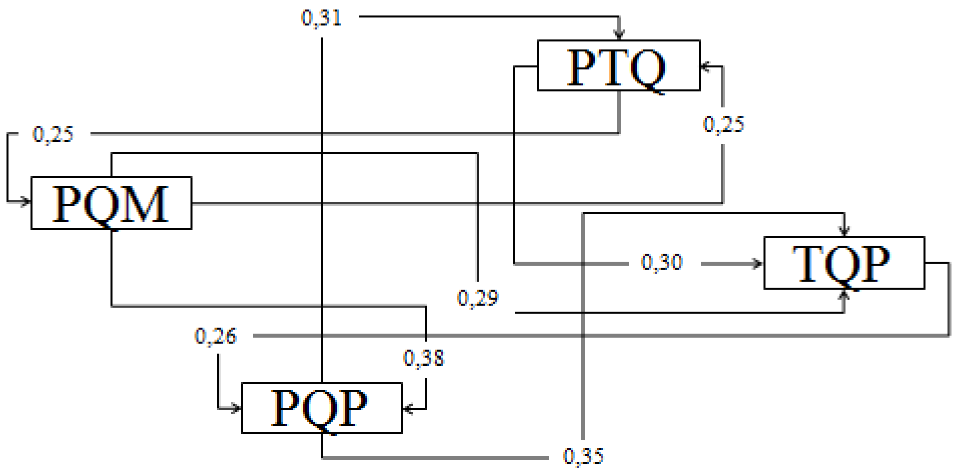

Fig.3: Structural model

Source: Elaborated with study data

The parameters of adjustment and residual ⌈X^2 = 123,24 (23df) p = 0,010; GFI = 0,990; CFI =,991; IFI = 0,993; RMSEA = 0,007⌋ suggest the acceptance of the null hypothesis, relative to the relations of dependence between the factors used in the state of the question and demonstrated in the empirical test.

Discussion

The contribution of this study to the state of the question lies in the establishment of the reliability and validity of an instrument that measures the perception of management, production, transfer and the totality of the quality of the processes, but the type of non-experimental study, the type of non-probabilistic selection and the type of exploratory factor analysis limit the results of the study to the sample and the context of the investigation.

It is recommended to extend the study to other contexts and samples, using sophisticated analysis of factors such as the least squares technique in order to confirm the structure that underlies the perception of total quality, configured by three factors related to management, production and the transfer of knowledge.

It is so important for the organization to hold a substantial list of clients. It because, the client is the stakeholder that provides organization with the financial resource to going on with its labor and remain in its market share. However, it is more important to have a selected list of clients which can be recognized a loyal to the enterprise, and in which case can be a support for the organization.

With the strict control applied on the PV-modules enterprise, apparently mistakes where reduced. In the practice, there were some mistakes on deliveries, however, all of the was due to mistakes in the information provided by sale's agents, main mistakes detected still being in the address given by sale's agents and sale's manager.

With strict control strategy application, it was also possible to determine responsibilities. Due to wrong deliveries, responsibility for each mistake was charged to logistics department or production warehouse, however, when control strategy started it application, it was recognized that mistakes and/or omissions were mainly produced by data provided thorough sales department. Very little mistakes was due to Delivery-service omissions.

IV. CONCLUSION

In the economy, the total quality is a preponderant factor in the processes and the products, although the labor climate that supposes such company is centered in the analysis of positions, worker cycle and the motivation of the worker as determining factors of a system of management, production and transfer of knowledge oriented to the continuous improvement of the scientific, technological and industrial process.

REFERENCES

[1] Acar, Z. and Acar, P. (2014). Their organizational culture types and effects on organizational performance in Turkish hospitals *Emerging Markets Journal*, 3 (3) : 1-15 [DOI: 10.5195 / emaj.2014.47].

[2] Andrejić, M., Kilibarda, M. &Popović, V. (2015). Logistics failures in distribution process, 2nd Logistics International Conference, available at: [http://logic.sf.bg.ac.rs/wp-content/uploads/Papers/LOGIC2015/ID-41.pdf].

[3] Anicijevic, N. (2013). The mutual impact of organizational culture and structure. *EconomicAnnals, 58* (198), 35-60

[4] Bohte, J. & Meier, K. J. (2000). Goal displacement: Assessing the motivation for organizational cheating. Public Administration Review, 60 (2), 173-182, available at: [http://onlinelibrary.wiley.com/doi/10.1111/0033-3352.00075/full].

[5] Carreón, J. Hernández, J., García, C. García, E., Rosas, F. Aguilar, J. (2014). Specifying a digital enterprise model for human development through intensive use of information and communication technologies. *Rural Perspectives,* 13 (25), 123-155

[6] Cavagnaro, E., & George, H. (2017). The three levels of sustainability. Routledge. ISBN-13: 978-1-906093-68-6, available at: [https://books.google.com.mx/books?hl=es&lr=&id=vqk0DwAAQBAJ&oi=fnd&pg=PT7&dq=herman+daly%27s+three+filter+economic+social+environment+1987&ots=ZWnL1VB-3E&sig=LVc-vxGU9RPT4ajTGlTbJvALQ4c&redir_esc=y#v=onepage&q&f=false].

[7] Cialdini, R.B, Petrova, P.K. & Goldstein, N.J. (2004). The hidden costs of organizational dishonesty: companies that engage in unethical practices face consequences far more harmful than is traditionally recognized. The resulting damage can easily outweigh the short-term gains, MIT Sloan Management Review. 45 (3), 67-73, available at: [http://go.galegroup.com/ps/i.do?id=GALE%7CA116484228&sid=googleScholar&v=2.1&it=r&linkaccess=fulltext&issn=15329194&p=AONE&sw=w&authCount=1&u=uaeh1&selfRedirect=true] & [http://mylearning.denverzoo.org/ets/companies/fbfdf7ad-f5a3-416e-8c31-62af837c7f0a/UserFiles/Article%20Archive/Culture%20Articles/The%20Hidden%20Cost%20of%20Organizational%20Dishonesty_Article.pdf].

[8] Côté, R.P. y Cohen-Ronethal, E. (1998). Designing eco-industrial parks: a synthesis of some experiences, Journal of cleaner production, 6 (3-4), 181-188, DOI: [https://doi.org/10.1016/S0959-6526(98)00029-8], available at: [http://www.sciencedirect.com/science/article/pii/S0959652698000298] & [https://ac.els-cdn.com/S0959652698000298/1-s2.0-S0959652698000298-main.pdf?_tid=6b56d41c-9fb0-11e7-a8e0-00000aacb35d&acdnat=1506096752_8c6e6bdac145b7fed82995e5a9dc42ff].

[9] Cruz, O., Arroyo, P. and Marmolejo, J. (2016). Technological innovations in logistics: inventory management, information systems and outsourcing operations. In M, Quintero, Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 165-178). Mexico: Miguel Angel Porrúa-UAEMEX.

[10] Escobar, R. (2014). Neural networks, cognitive processes and behavior analysis. *International Journal of behaviorism,* 2 (1), 23-43

[11] Hernandez, A. and Valencia, R. (2016). Innovation instruments: social networks in the internalization of micro, small and medium - sized Mexican companies. In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 47-66). Mexico: Miguel Angel Porrúa-UAEMEX.

[12] Mendoza, E. Ramirez, L. and Atriano, R. (2016). Use of media and technology in creating an innovation system for the common good. In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 95-114). México: Miguel Ángel Porrúa-UAEMEX.

[13] Molina Ruiz, H.D. (2013). Aproximación de cálculo de la huella de carbono en una institución de educación media superior y superior. Innovación y Desarrollo Tecnológico Revista Digital, 5 (3), ISSN: 2007-4786.

[14] Molina Ruiz, H.D. (2015). Three levels analysis of sustainability's environmental dimension in México. Innovación y Desarrollo Tecnológico Revista Digital, 7 (4), ISSN: 2007-4786.

[15] National Institute of Statistics, Geography and Informatics. . (2010) *XI National Population Census.* Mexico: INEGI

[16] Omotayo, and Adenike O., A. (2013). Impact of organizational culture on human resource practices: a study of selected Nigerian private universities. *Journal of Competitiveness,* 5 (4), 115-133 [DOI: 10.7441 / joc.2013.04.07]

[17] Quintero, M., Velázquez, E., Sales, and J. Padilla, S. (2016). A review of the state of the art on SMEs. What innovation studies? In M, Quintero, Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 31-43). Mexico: Miguel Ángel Porrúa-UAEMEX.

http://dx.doi.org/10.22161/ijeab/2.6.65 *ISSN: 2456-1878*

[18] Robles, C., Alviter, L., Ortega, A. and Martinez, E. (2016). Culture of quality and innovation in microenterprises. In M, Quintero., Sales, and J. Velázquez, E. (Coord.).*Innovation and technology challenges for practical application in companies.* (Pp. 11-30). México: Miguel Ángel Porrúa-UAEMEX.
[19] Saansongu, E. and Ngutor, D. (2012). The influence of corporate culture of employee commitment to the organization. *International Journal of Business and Management*, 7 (22) : 1-8
[20] Sales, J., Quintero, M. Velázquez, E. (2016). Adaptation versus innovation: the formation of industrial districts from rural communities. Santa Cruz Atizapan and Chiconcuac.In M, Quintero., Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies.* (Pp. 181-199). México: Miguel Ángel Porrúa-UAEMEX.
[21] Vázquez, C., Barrientos, B., Quintero, M. Velázquez, E. (2016). Government support for innovation, technology and training for small and medium enterprises in México. In M, Quintero, Sales, and J. Velázquez, E. (Coord.). *Innovation and technology challenges for practical application in companies*. (Pp. 67-78). Mexico: Miguel Angel Porrúa-UAEMEX.

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Knowledge Networks around the Strategic Alliances of Micro Coffee Producers

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***Abstract*—*The organizational process that goes from the knowledge networks to the strategic alliances supposes a management, production and transfer of information that the literature differentiates according to the degree of empathy, commitment, entrepreneurship, innovation and satisfaction of leaders and followers. In this sense, the objective of this study was to study the phenomenon in coffee growers in central Mexico. From a structural model $\lceil X^2 = 321.23\ (34gl)\ p = 0.009;\ GFI = 0.990;\ CFI = 0.975;\ RMSEA = 0.008 \rfloor$, after establishing the reliability and validity of an instrument, it was found that innovation determines satisfaction (0.51), although the type of study, sample selection and analysis limit the findings to the specific context of the investigation. Lines concerning the extension of work to different contexts and samples are noticed.*

Keywords*—*Governance, networks, alliances, entrepreneurship, innovation, satisfaction.

I. INTRODUCTION

The objective of this paper is to establish the challenges and opportunities of micro, small and medium enterprises dedicated to the management, production, administration and marketing of coffee in Xilitla, a town located in San Luis Potosi, central Mexico, facing the protectionist policies of the United States. government of Donald Trump .

Based on an economic-administrative approach focused on the production of knowledge oriented towards entrepreneurship and innovation, the present work will show that: 1) the economic, political and social crisis of developed countries negatively and positively affects emerging economies, considering the challenges of product quality and the competitiveness of services, but at the same time, encourage entrepreneurship and innovation of micro, small and medium enterprises; 2) in the case of entrepreneurship and innovation, both explain the economic relationship between the United States and Mexico, mainly the relationship established by micro, small and medium enterprises dedicated to the management, production, administration and marketing of coffee in Xilitla and that, migratory flows have exacerbated such co-dependency; 3) specifically, coffee marketing networks have generated capital - intellectual, social and administrative - from which local and regional development is explained.

However, the understanding-interpretation and measurement of the phenomenon of intellectual, social and administrative capital around entrepreneurship and innovation of coffee micro-enterprises in Xilitla has not been unveiled or linked to international markets as a factor of local and regional development. regional, but with international quality standards such as the consumer market in Germany, the main point of sale and marketing of coffee produced in Latin America and in the case of Mexico the town of Xilitla, even though the US market has historically been the client Mainly, the emergence of protectionism in the administration of President Trump has forced to diversify the client portfolio.

Therefore, the study of the challenges of the US market and the opportunities of the German market are essential in the management, production, administration and commercialization of micro, small and medium coffee companies in Xilitla.

However, the US market challenges and opportunities in the German market are confined to innovation and that, on a scale from 1 to 10 US market it ranks in third place and the German market in sixth place.

Economist Intelligence Unit (EIU) in its report for 2014 warns that there is an imbalance in the economic exchange between the EU and Germany in favor of this last Central European economy in the last 30 years .

Such commercial relationship is due to an ancestral bond of 15.7% between Americans and Germans that exceeds the relationship between Mexico and the United States, which is 10.6% .

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In other words, both economies, being linked as ancestral networks and networks of innovation, reflect the differences between both economic and organizational cultures. This is because both countries have developed from production and retail. In the case of the EU, it occupies the second place and Germany the third .

In other words, entrepreneurship and innovation in both developed economies, American and German, are focused on retail and strengthened through ancestral networks, but with a clear difference in favor of Alemania.

Even electronic commerce, buying and selling online, is focused on mobile telephony in Germany exceeded 100% and in the EU 58%, with the German market the second most dynamic and the American the last place .

Indeed, the commercial asymmetries between Germany and the EU are not only explained by their social capital or ancestral networks, not only by their retail culture, but also by their population that in Germany synchronizes technological advances and electronic devices with their lifestyle and In the US, a deceleration in consumption is evident.

However, the German population occupies the last places in terms of the birth rate -8 births per thousand inhabitants- while in the US there are 14 births per thousand inhabitants.

Although in Germany the number of births is less than the number of deaths, the economy 4.0 or digital economy is booming with the payment of online services .

That is to say that, despite the population slowdown in Germany, retail trade is essentially online and this digital retail culture is linked to the US market by its ancestral networks and its commercial exchange.

In this sense, the 81.8 million inhabitants, the German economy and its digital culture (69.5 million Internet users) and retail (37 million consumers) represent 19% of the total commercial volume of Europe, 18% in favor in the exchange with the US, 19% of central Europe.

Both US and German markets are not only linked, but also key in terms of challenges and opportunities for Mexico's products and services in general and the sale of coffee in Xilitla in particular, since both developed economies occupy the first and second place of import of products derived from coffee.

Conceptual theoretical framework

The theoretical and conceptual frameworks that explain entrepreneurial and organizational innovation and digital menudista warn determinants of growth that would be: 1) environmental, 2) sociological, 3) personal and 4) organizational.

The present work will focus on human capital, its entrepreneurship and innovation that are considered preponderant factors in developed economies.

Theory of strategic alliances

The demands of the market to envelope the resources of the organization induce the latter to establish strategic cooperation alliances in order to increase the capabilities of MSMEs with the support of transnationals, or develop competitive strategies through technology transfer and the implementation of the quality of processes and products. In this way, MSMEs survive the selection process and add to the multiplier effect of investments, transfers and procedures.

In this way, in an organizational and political sense, the strategic alliances between micro-enterprises are gestated from the confluence of objectives, tasks and goals (see Table 1).

Table.1: Taxonomy of strategic alliances

Goals	Chores	Goals
Customer satisfaction	Distribution agreements, alignment proposal, trademark cooperation	Market expansion, presence and local-global capacity, contact lines and points of sale, mutual reference, relevance of the brand
Product / service development	Joint project, technology license	Use of external technology and capacity
Creation of competitive advantages	Shared investment, reciprocal use of staff , supply risks	Economy of scale, experience and external benefits

Source: self made.

Theory of knowledge networks

From the advent of information and communication technologies (ICTs), competitiveness and the establishment of a climate relations emerges the systematization of internal and external resources in order to establish an organizational change oriented to the development of processes and products, as well as to the implementation of quality criteria and protocols. In the case

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of the adoption of technology, it involves a transfer of skills and knowledge that can be established between institutes and MSMEs with the assistance of the local or federal government, the main promoter and sponsor of investment fairs or entrepreneurial development, as well as training, practice and employment (see Table 2) .

Table.2: Taxonomy of Knowledge Networks

Dimension	Culture	Demands	Means
Translation networks	Values and norms around the construction of stability as a determinant of the informative mediation of the actors.	From the stability of the network, the exchanges of contents are reproduced.	The actors transfer the information to highlight the translation of the network.
Sociotechnicalnetworks	The values and norms that constitute the actors are preponderant to any other factor.	The actors are individuals rather than agents of change, they only select and transfer information.	The actors only use the information of their links to select it based on intuitive criteria.
Social networks	The cognition of the actors is the basis to establish the nodes that configure.	The actors are commissioned to produce information based on their identity choices.	Identity is the main currency of these networks, since from the choice of a group in relation to others establish their relationships.
Affiliation networks	The actors generate a dynamic of identities and attachments based on those that establish their productive relationships.	Attachment and identity are processes that in themselves suppose an emotional and affective demand for the actors.	The actors use their sense of attachment and affiliation strategies as the membership to establish the dynamics of the network.

Source: self made

If the theory of human capital warns that the differences between organizations are gestated from their intellectual, technological and social capital, then the theory of entrepreneurship and innovation will suggest that the asymmetries between organizations are focused on the production of knowledge indicated as market opportunities.

In the framework of ICTs , the organizations established strategic alliances and knowledge networks with the aim of enhancing their human, intellectual and innovative capital. The prevalence of the climate of relations and the climate of innovation with respect to the climate of tasks and the climate of support led to the creation of knowledge and added values to the products and organizational processes, increasing their quality, diversity and complexity. In this sense, the diffusion of innovations not only determines consumption, but also the establishment of sectors such as: innovators, followers, early, late and laggards (see Table 3).

Table.3: Academic networks taxonomy

Dimension	Social representations	Social identity	Human motivation
Translation networks	The information surrounding the networks is processed in order to establish a synthesis between the human and the technological.	The processed information of the environment is determinant of the choice of a technology and the exchange with another network.	The needs of achievement are determinants of the association between technologies and humans.
Socio technical networks	The information considered as an instrument of persuasion establishes a specific knowledge network.	An increase in the demand for information generates subsidies in the network.	The subsidy is the resource used in the face of an increase in information demands and selection in the case of a decrease in demand.
Social	The impact of the surrounding	The information that favors	Identity, as an intuitive selection tool,

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Dimension	Social representations	Social identity	Human motivation
networks	information on the prestige of the actors implies a social representation of the network and its environment.	differences between the actors with respect to the network is widely valued.	is the main currency of the network.
Affiliation networks	The information is represented as an indicator of a specific network.	The identity is assumed as a demand of the environment that determines the configuration of a network.	The sense of belonging and attachment are determinants of the network, its processes and products.

Source: self made

In the case of entrepreneurship and innovation required for the US and German markets, retailing in digital protocols involves intellectual, digital and social networks such as the commercial relationship between the EU and Germany, but as an asymmetry in favor of Germany, then the theory of entrepreneurship and innovation, in the case of Mexico, will explain the creation and emergence of scenarios in which emerging organizations will access international markets through these same digital and social knowledge networks.

Theory of human capital

From the economic theories of rational choice, the theory of human capital was established as an explanatory conceptual framework of organizational creativity, spearhead of internal and local development.

In this sense, the concepts of intellectual, technological and social capital reflected in the retail marketing of coffee in digital protocols of the US and German markets are fundamental to explain the challenges and opportunities of both international markets in local and regional MSMEs .

Intellectual capital

It alludes to capabilities; skills and knowledge related to management, production and commercialization (Pariente, 2006) . In the case of the purchase and sale of coffee, the intellectual capital would be indicated by the opportunities that MSMEs generate in the face of the challenges of the digital retail market in Germany and the retail market in the US. It deals with strategies and management styles inMSMEs in face of the challenges and opportunities of both international markets.

Technological capital

It refers to the commercialization devices and networks of products and services specialized in buying and selling retail (Caballo, R eyes and Solis , 2006). In the case of coffee, these are portals of MSMEsthat will encourage the purchase and sale of coffee between Mexico and the EU-Germany.

Social capital

It refers to the knowledge and marketing networks of products and services in developed economies and emerging countries (Domínguez and Fuentes, 2006). It deals with knowledge about the distribution of objectives, responsibilities, tasks and goals related to the purchase and sale of coffee in electronic portals and digital money transfer protocols.

II. STUDIES OF KNOWLEDGE NETWORKS AND STRATEGIC ALLIANCES

Organizational studies have focused their interest on the factors of empathy, commitment, cooperation, entrepreneurship, skills, innovation and satisfaction as structuring the process of networking with orfoundation and strategic alliances.

Ramos, (2013) established four factors related to the growth of MSMEs : production capacity (alpha of 0.912, knowledge of the market (alpha of 0.825), product differentiation (alpha of 0.742) and logistics)alpha of 0.864). However, MSMEs would have their main multiplying factor in the dissemination of their products and services in digital protocols such as Facebook (Martínez, 2012). In another study, 60% of MSMEs have not established an organizational management, production and quality control (Contreras, 2012), but customer perception that determines the purchase of products and the requisitioning of services MSMEs (Castiglioni , Castro and Galán, 2015)

2.1 Foundation of the problem

The study of entrepreneurship and innovation in micro, small and medium enterprises of online retail of coffee in Xilitla with respect to the challenges and opportunities of the US and German market, involves the study of their capital networks in terms of their capabilities intellectual, technological and social.

Therefore, the qualitative approach of the meanings around the quality of the processes and the competitiveness of the products derived from the coffee in the international mark, but especially in the American and German will show the strategies of entrepreneurship and innovation necessary to

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transfer them to the micro, small and medium enterprises of Xilitla.

In this way, the transfer of technology will be an essential management tool to strengthen the dynamics of local production and marketing with respect to the global market, mainly in the consumption of coffee by potential US and German customers.

Justification

Given that MSMEs explain 90% of global employment, 95% of national employment and 97% of local employment, it is necessary to unravel the strategies of electronic and digital entrepreneurship and innovation necessary for local and regional development with respect to international coffee market. It is an organizational process in which the retail trade of coffee explains a high percentage of the digital economy around its consumption much more frequent than water or beer in the US and Germany.

In the same sense, the policies of business and industrial development of coffee exporting countries with respect to importing countries, seem to show that the differences lie in management strategies, management, production and marketing, but these dimensions are not entirely present in micro and small companies, as well as very rare in medium-sized companies.

In this way, a strategic alliance between the mipymes of the coffee sector in Mexico, mainly in San Luis Potosí and essentially in Xilitla, seems to be a logical response to the challenge of the US and German markets.

Approach

In virtue of the fact that entrepreneurship is the result of association and economic exchange between developed and emerging countries, it consists of an exchange of production and reproduction of knowledge that would be activated by strategic alliances , the competition of capital, the skills of force labor and the evaluation of resources.

In emerging economies, entrepreneurship is the preponderant factor that will determine the learning, performance and results of companies through the links established between the networks of entrepreneurship.

Grosos modo, the entrepreneurial process starts from surrounding information about a product or service that is presumed to be indispensable for a niche market, continues with the socialization of that need or expectation, very soon, potential customers will develop an adaptive response or creative and from these two routes on the first path will experience experiences of satisfaction or dissatisfaction more likely in the trajectory of creative response.

However, this proposal lacks an explanation regarding the differences between micro, small and medium enterprises with respect to entrepreneurship and innovation around the retail marketing of coffee and its dissemination in Internet networks.

Object of study

The present work focuses its interest on entrepreneurship and innovation as factors of local development strategies from the retail and digital commerce of coffee, its derivative products and related services.

However, the generality of the process of entrepreneurship and innovation supposes its delimitation to the MSME organizations that, for the objectives of the present work will be specified to the management, production and commercialization in tune with the needs and expectations of the American and German client.

Object of investigation

The present study will observe the entrepreneurship and innovation process of MSMEs, considering the potential client. In this sense, the project is part of the creative and digital economy, since it studies essentially observable processes and phenomena in the interaction between consumer and seller online and retail.

However, the study of the coffee - growing MSMEs Xilitla is not reduced only to the present or seed capital and its emergence in the international arena, it will be crucial to observe and compare their developmental stages within the US and German retail and digital commerce.

It is an evolutionary process in which crises encourage the entrepreneurial and innovative development of organizations, observable in changes, adaptation and the adoption of revolutions. It is an evolutionary process in which organizations are circumscribed in order to thrive in developed economies.

Formulation

What will be the differences between the organizations developed with respect to emerging SMEs in terms of retail marketing of coffee through digital protocols?

What are the meanings organizations in developed economies have generated about retail marketing and electronic distribution of coffee products and derivatives?

How can transfer the meanings around entrepreneurship and innovation in MSMEs in Mexico, mainly in Xilitla meeting their particularities and localism?

What are the meanings of strategic alliances, knowledge networks and innovation climate in micro-entrepreneurs of a coffee growing area in central Mexico?

Hypothesis

The differences between organizations of the first world and the MSMEs of emerging countries will be based on the

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strategies of entrepreneurship and innovation exacerbated in developed economies with respect to emerging markets .

Because coffee marketing organizations are focused on retailing through digital protocols, then it will be possible to observe that coffee acquires a value in itself, coupled with the accessibility of Internet sales, its value will be higher.

In this regard, the marketing strategies adopted in organizations competing in developed economies, allow a transfer of focusing on entrepreneurship and innovation in order to transform the local and regional market knowledge Xilitla coffee growing MSMEs.

III. METHOD

Design

A non-experimental, cross-sectional, exploratory, qualitative and quantitative study was carried out.

Sample

A non-probabilistic sampling was carried out with an intentional selection of four informant entrepreneurs, considering their experience in strategic alliances, knowledge networks and innovation climate. Anintentional selection of 10 local coffee growers was carried out for the study in its qualitative phase and a non-probabilistic selection of 130 regional coffee merchants, considering the size of their organization, the retail approach and their accessibility or penetration in digital networks (see Table 2).

Table 4. Sample descriptive

Entrepreneur	Sex	Age	Scholarship	Status	Foundation	Alliances	Collaborations	Innovations
Candidate	Female	27	Bachelor's degree	Single	2014	1	1	0
Micro	Female	33	Bachelor's degree	Single	2010	2	3	0
Little	Male	41	Bachelor's degree	Married	2009	4	5	0
Medium	Male	52	Bachelor's degree	Married	2006	6	7	1

Source: Prepared with the study data

Instrument

An interview guide was prepared considering the categories of strategic alliances, knowledge networks and innovation climate. The questions were included: What is the starting year of your organization?What have been the objectives, tasks and goals of your organization in the last three years? What have been the strategic alliances of your organization in the last three years? How have the types of collaboration of your organization been in the last three years? What and how are processes and / or innovative products that your organization carried out in the last three years?

A scale of entrepreneurship and innovation was built in retail organizations with presence on the Internet and contacts in the US and Germany. It will include referents alluding to the dimensions of entrepreneurship and innovation such as: opportunism, utility, quality, strategy, transversality, flexibility and risk.

Process

The interviews were conducted at an entrepreneurship fair, with a guarantee of confidentiality and anonymity in writing, as well as a warning that the results of the study would not affect the economic status of the respondents. The information was processed in the qualitative data analysis package (QDA by its acronym in English version 4.0). Arrays of analysis of discourses and networks of discursive extracts were elaborated with the purpose of interpreting the meanings of the answers to the questions.

Analysis

Discursive

The meanings of the answers to the questions were analyzed from the reflective indicators of the categories of strategic alliances, knowledge networks and climate of innovation in order to select the discursive extracts. Once a list of extracts was compiled, they were sorted according to their relationship with each of the categories related to *habitus*- style of entrepreneurship, innovation and collaboration-learned, inherited, vertical or horizontal.

Reliability

The Crombachh alpha coefficient was estimated to show the internal consistency of the instrument in different contexts and samples.

Validity

Adequacy

The statistic Kay ser- Meyer- Olkin was weighted to establish the possible correlations between the indicators and the factor. A value close to 1 will show an adequate distribution, but a value lower than 0.6 suggests a reduction of the items on the scale.

Sphericity
The Bartlett test was carried out to establish the ideal factorial solution for the validity analyzes. In the same way, a value close to zero or the unit shows a distribution according to validity.

Exploratory factor analysis
An analysis of principal axes with promax rotation was carried out , following the logic of values close to the unit as evidence of construct indicators and close to zero as evidence of indicators of other constructs.

Contingency
N tables chi squared correlations to demonstrate the dependence or independence between the variables and their interpretation of differences between independent samples elaboraro. A non-significant value is interpreted as an independent relationship between the variables.

Correlation
The Pearson correlation coefficient P was estimated to establish the possible associations between the variables to be measured in the instrument. Values close to one demonstrate a strong association and probable relationship of dependence. Values close to zero will be assumed as spurious.

Regression
The dependency relations were established between the variables related to entrepreneurship and the indicators linked to innovation as factors of local and regional development. Beta values close to the unit show dependency relations and close to zero show that there are other variables not included in the relationship.

Structure

Adjustment
The goodness of fit and the fit index were weighted to show that the relations between the variables stated in the state of theoretical knowledge are adjusted to the relations observed in the empirical study, values close to unity mean the acceptance of the null hypothesis of It corresponds nce between theory and empiri a.

Residual
It was estimated the adjustment coefficients to show the probability of measurement error and the acceptance of the null hypothesis of correspondence between the matrix of expected observations and the matrix of estimated weights.

IV. RESULTS

The discourses related to knowledge networks and strategic alliances are involved in innovation and the *habitus* or instrumentation of organizational values around the management, production and sale of coffee, as well as in the insertion to the international market as an opportunity to introduce pesticide-free and organic products, friendly with the environment and occupational sustainability in terms of health, prevention of diseases and accidents.

Table 5 shows reliability values of the general scale (alpha of 0.771) and subscales (alphas of 0.755 to 0.791) sufficient to establish the internal consistency of each of them.

Table 5. Instrument descriptions

Code	Item	M	FROM	TO	F1	F2	F3	F4	F5	F6	F7
EP1	Mistrust before the elections	3.21	1.01	0,712							0.331
EP2	Post-election negotiation	3.82	1.02	0.732							0.302
EP3	common agenda before elections	3.45	1,03	0.743							0.431
CM1	Higher sales before elections	3.67	1.01	0.741						0.493	
CM2	Micro-financing before elections	3,04	1.00	0,742						0.312	
CM3	Indebtedness after elections	3,13	1.05	0.731						0.403	
CP1	Support after elections	3.82	1.08	0.754					0.319		
CP2	Competition from elections	3.05	1.08	0.721					0.401		
CP3	Solidarity before elections	3.46	1.00	0.772					0.403		
EM1	Branches before elections	3,14	1.02	0.783				0.493			
EM2	Developments after elections	3.67	1,03	0.792				0.301			
EM3	Offers before elections	3.94	1,04	0,742				0.493			
CD1	Liquidity after elections	3.05	1.02	0,715			0.399				

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Code	Item	M	FROM	TO	F1	F2	F3	F4	F5	F6	F7
EP1	Mistrust before the elections	3.21	1.01	0,712							0.331
EP2	Post-election negotiation	3.82	1.02	0.732							0.302
CD2	Contrastaciones before elections	3,12	1.05	0.725			0.413				
CD3	Expansion after elections	3.05	1.08	0.721			0.403				
IN 1	Change of rotation before elections	3.74	1,03	0.732		0.384					
IN 2	I nvesting after the election	3.56	1.02	0.743		0.302					
IN3	A sociarse before elections	3.15	1.01	0.725		0.321					
ST1	Bonanza before elections	3.52	1.01	0.731	0,315						
ST2	after election losses	3.94	1.01	0.725	0.382						
ST3	Opportunities before elections	3.05	1,11	0.731	0.324						

Extraction method: principal axes, rotation promax . Adaptation and sphericity ⌈X^2= 324.15 (234gl) p = 0.001; KMO 0,721⌋. M = mean, SD = Standard Deviation, A = Alpha Crombach removing the value of the item. F1 = empathy (alpha 0.778 and 27% of the total variance explained), F2 = Commitment (alpha 0.780 and 23% of the total variance explained), F3 = Co (alpha 0.791 and 20% of the total variance explained) , F4 = Entrepreneurship (alpha 0.775 and 17% of the total variance explained), F5 = Capacity (alpha 0.781 and 13% of the total variance explained), F6 = Innovation (alpha 0.755 and 11% of the total variance explained), F7 = Satisfaction (0.760 alpha and 9% of the total variance explained). All items are answered with one of five response options: 0 all likely, 1 = very unlikely, 2 = unlikely, 3 = somewhat likely, 4 = very likely.

Source: Elaborated with data from the study

Dependency relationships among the factors put forward in the state of the art and have been weighted case study. The innovation factor determined directly, positively and significantly satisfaction (0.51), but its effect is low, even relative to other interrelationships between factors is the highest.

Finally, the adjustment parameters and residual ⌈X^2= 321.23 (34gl) p = 0.009; GFI = 0.990; CFI = 0.975; RMSEA = 0,008⌋sugieren acceptance of the null hypothesis concerning the adjustment of the theoretical relationships regarding the data.

V. DISCUSSION

The contribution of this work lies in the interpretation of the meanings of informants entrepreneurs regarding the categories of strategic alliances, networks of knowledge and innovation climate. Regarding the study of Villegas et al., (2016) who found that knowledge of the market determines the growth of MSMEs , in this study it was observed that the informants have entrepreneurship, innovation and collaboration related to coffee growing history the location. This finding contravenes the contribution of Sixto, Aguado and Ribeiro (2017) who showed that Internet is the broadcaster par excellence of MSMEs in its growth process. Although Marquez et al., (2017) warns that the organization is essential to define the objectives, tasks and goals of MSMEs , in this study it was shown that these by establishing alliances, networks and climates feed back of organizational experience peers with which they are associated. Even the results of this study contradict the finding Hoyos and Lasso (2017) to ensure that customer perception is the determining factor in the growth of MSMEs. In the present study rather it is noted that coffee is the history of the town and the creativity of informers which had an impact for the MSMEs not only maintained, but also followed an upward trajectory.

VI. CONCLUSION

The limits of this study are: 1) the type of exploratory, transversal and qualitative research to find that coffee is the history of the locality and creativity affect entrepreneurship, innovation and collaboration of MSMEs ; 2) the interpretation of the meanings of partnership, knowledge and innovation to assume that these are the processes that guide the change of MSMEs , sidestepping their customs; 3) the differences with the state of knowledge regarding the management and administration of the three categories in comment.

It is recommended that: a) carry out a quasi-experimental study to establish the incidence of factors that control could reveal the degree of entrepreneurship, innovation and collaboration; 2) establishing paths dependency relationships between variables in comment; 3) implement the effect of other variables such as micro-financing to observe changes in a period of time.

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REFERENCES

[1] Berrou , JP and Combaunous , F. (2012). The staff networks of entrepreneurs in a casual African urban econo my. Does the strength of ties matter? *Review of Social Economy* , 1, 1-30

[2] Caballlo , A., Reyes, R. Solis, P. (2006). The competitive strategy adopted transnational company in the global market. *Accounting and Management, 50,* 89-122

[3] Carreon, J. (2016). *Human Development: Governance and Social Entrepreneurship.* Mexico: UNAM-ENTS

[4] Castiglioni , M., Castro, I. and Galan, JL (2015). The use and choice of alliances multipatner : an exploratory study. *Market Economic & Business Journal , 45 (1),* 95-123 DOI: 10.7200 / esicm.150.0461.2e

[5] Contreras, G. (2012). Asociacionismo network for integration sociodigital in Andalucia . *Andalusian Studies Journal, 29,* 105-126 DOI: 10.12795 / rea.2012.i29.05

[6] Dominguez, G. Fuentes, J. (2006). Communication and information comoo generators competitiveness in organizations. *Accounting and Management, 50,* 207-230

[7] Economist Intellig ence Unit (2010). *Women's economy opportunity* . London: EUI

[8] Economist Intelligence Unit (2015). *Evaluating the environment for public-private partnerships in Asia - Pacific* . London: EUI

[9] Manktelow , A. (2014). *Guide to Emerging Markets* . London: Economist Intelligence Unit

[10] Martinez, A. (2012). Analysis of enterprise networks and territorial impact. Technology transfer, learning and innovation. *Andalusian Studies Journal, 29,* 18-58 DOI: 10.12795 / rea.2012.i29.02

[11] Pariente, JL (2006). Training administrators in the new international environment. *Accounting and Management, 50,* 124-144

[12] Ramos, L. (2013). Alliances and networks of collaboration ng between cultural groups of ethnic arts in Andalucia . *Empriia , 15,* 26-34 DOI: 10.5944 / empiria.26.7151

[13] Robles, C. , Alviter , LE, Ortega, AO and Martinez, E. (2016). Culture of quality and innovation in microenterprises. In M. Quintero., Sales, J. and Velazquez, EB (ed.). Innovation and technology. Challenges for practical application in companies. (pp. 11-27). Mexico: Porrúa-UAEMEX

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Antifungal Activity of Petrolium and Ethanolic Extracts of *Moringa Oleifera* Leaves against *Penicillium Chrysogenum* and *Cryptococcus Neoformans*

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***Abstract*— *Moringa oleifera is well known medicinal plant. Its different parts are widely used for the treatment of different types of diseases since is have antibacterial and antifungal activity. The leaves are rich in iron, rhamnose and a unique group of compounds called glucosinolates and isothiocyanates. Other medical proprieties include antipyretic, antiepileptic, antiinflamatory, antiulcerative, antihypertensive, cholesterol lowering, antioxidant, anti diabetic. The current investigation was undertaken to evaluate the antifungal activities by petroleum ether and ethanolic extracts of Moringa oleifera leaves against Penicillium crysogenum and Cryptococcus neoformans. From our study, it is found that as concentration of Moringa leaves extract increases the diameter of zone of inhibition found increased. The results were higher and effective for ethanolic extract than petroleum extract. The result were also found more effective against Cryptococcus neoformans than Penicillium crysogenum.*

Keywords*— *Moringa oleifera, Cryptococcus neoformans, Antifungal , Antibacterial, Penicillium crysogenum.

I. INTRODUCTION

Moringa oleifera (Fig :1) is well known, widely distributed natural species belongs to monogeneric family Moringaceae and order Violes.(Table:1)[1,2]

Table.1: Classiification of Moringa oleifera

Botanical Classification	
Kingdom	Plantae
Sub-kingdom	Tracheobionta
Super-division	Spermatophyta
Division	Angiospermae/Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	Moringa
Species	Oleifera

Fig.1: Moringa oleifera tree

Moringa tree can grow well in the humid tropic or hot dry land with average height that ranges from 5 to 10 m. It can survive in harsh climatic condition including destitute soil without being much affected by drought.[3] These are true vascular plants contains xylem and phloem for conduction of water and nutrients respectively (Sub-kingdom : Trachiobionta). Its trunk is soft, white corky and branches bearing a gummy bark. Each tripinnately compound leaves bear several small leaf legs.[4,5] Moringa is flowering plant(Division: Angiospermae/Magnoliophyta).The flowers are fragrant, bisexual and pentapetalous. It contains five unequal, separate, thinly veined, yellowish-white petals.(Fig:2) Flower is syncarpous and posses united carpels(Subclass : Dilleniidae). They grow on slender, hairy stalks in spreading or drooping later flower clusters. Leaves of these plants are feathery, pale green, compound and tripinnate. They are about 30–60 cm long and contains many small leaflets.(6,7) Each leaflet is about 1 to 2 cm long and 0.5 to 1.0 cm wide. Upper surface of leaflets is smooth dark while lower is pale green. They are variable in size and shape.(Fig:3)

Fig.2: Moringa oleifera flowers

Fig.3: Moringa oleifera leaves

This plant is also known as drum stick tree or horse radish tree. It have many vernacular names such as kelor, marango, mlonge, moonga, mulangay, nebeday, saijhan, sajna or Ben oil tree(8,9) Moringa oleifera is well known medicinal plant. Its different parts are widely used for the treatment of different types of diseases since is have antibacterial and antifungal activity. The leaves are rich in iron, rhamnose and a unique group of compounds called glucosinolates and isothiocyanates. Other medical proprieties include antipyretic, antiepileptic, antiinflamatory, antiulcerative, antihypertensive, cholesterol lowering, antioxidant, anti diabetic.(10,11) The current investigation was undertaken to evaluate the antifungal activities by petroleum ether and ethanolic extracts of *Moringa oleifera* leaves against *Penicillium crysogenum and Cryptococcus neoformans.*

II. MATERIALS AND METHODS

A) Collection of leaves : Naturally dried leaves were directly plucked from Moringa plant. The collection is carried out from different plants growing in area of Solapur city and surrounding villages. Plant leaves were further dried in an oven at 40^0C for a total of three days and then finally ground to a fine powder by grinder. Extraction is carried out by using petroleum ether and ethyl alcohol. (12-16)

B) **Preparation of leaf extract :** 20 gram of finely grinded powder of Moringa leaves were soaked in two different conical flasks containing petroleum ether and ethyl alcohol respectively. The sample were shaked on rotary shaker at 200 rpm for 24 hrs. The extract were filtered using Whattman filter paper. Then solvent were evaporated to obtain dry extract using a rotary evaporator and were stored in refrigerator for antifungal analysis. Before testing five different concentration of extract were prepared for both solvents separately. This was carried by dissolving 100 mg, 200 mg, 300 mg, 400mg and 500mg of extract in 1 ml solvent respectively. (12-16)

C) **Antifungal acivity :** The antifungal activity of the Moringa oleifera leaf extracts was determined using agar well diffusion method by following the known procedure. Fungal strains were spread on the surface of agar plate aseptically by sterile cotton swab. When surface was little dried wells of 4 mm diameter were punched with the help of sterile stainless steel boarer. 20 µl of petroleum and ethanol extracts of Moringa leaves were loaded separately in wells in separate agar plates. Separate wells were used for different concentrations. Each plate further contained on well loaded with solvent act as negative control. While another well was loaded with 20 µl antibiotic Nystatin (100 mg/ml). The plates were incubated at 28°C for 72 hours and the antifungal activity was assessed by measuring the diameter of the zone of inhibition at the interval of 24 hrs. (12-16)

III. RESULT AND DISCUSSION

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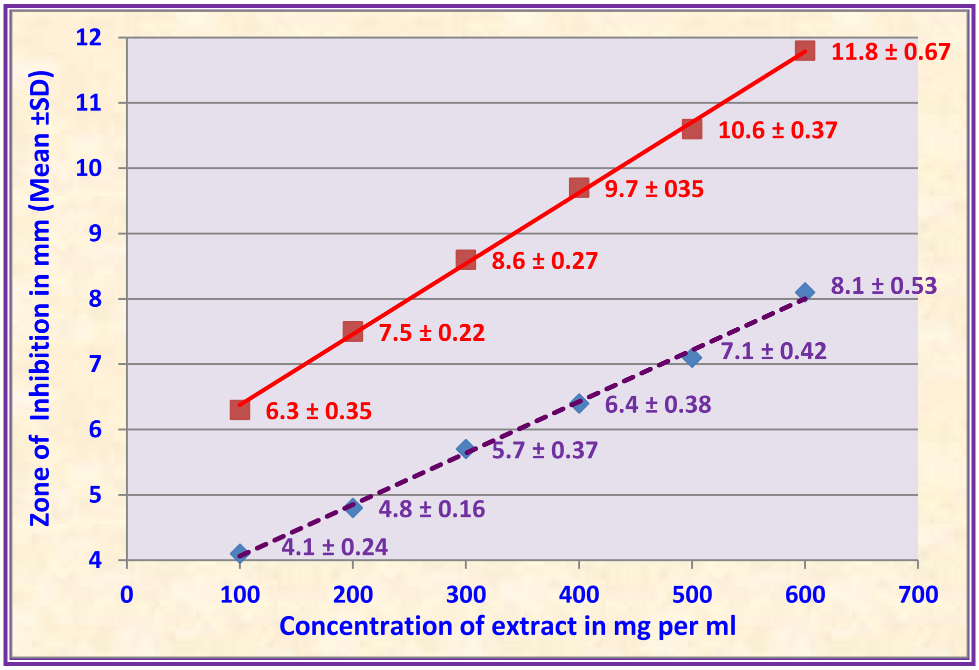
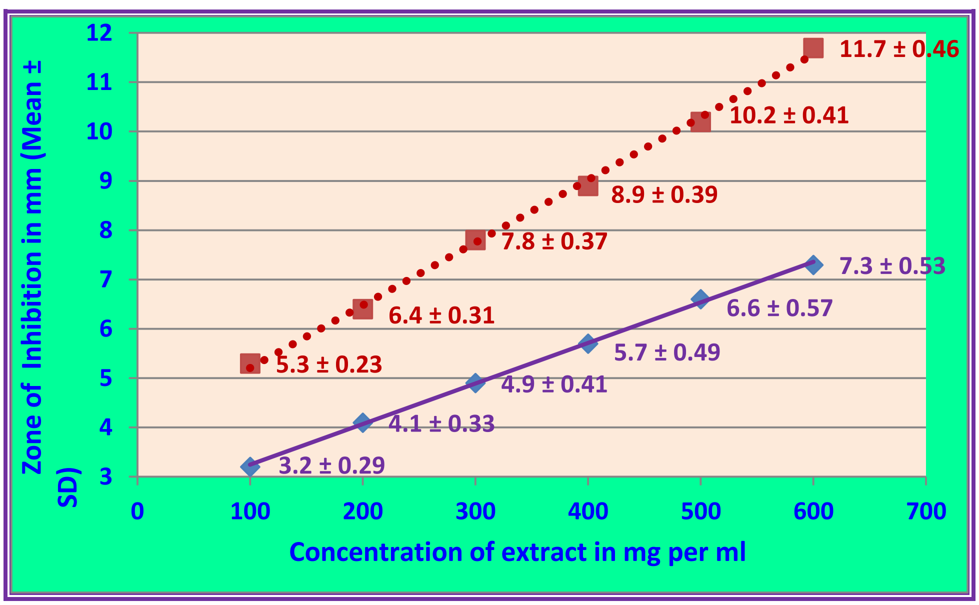
From our study, it is found that as concentration of Moringa leaves extract increases the diameter of zone of inhibition found increased. Against Cryptococcus neoformans zone of inhibition due to ethanolic extract of Moringa leaves was found increased from 6.3 mm to 11.8 mm as concentration increases from 100 to 600 mg per ml. While zone of inhibition due to petrolium extract of Moringa leaves was found increased from 4.1 mm to 8.1 mm as concentration increases from 100 to 600 mg per ml.(Graph-I) On other hand against Penicillium crysogenum zone of inhibition due to ethanolic extract of Moringa leaves was found increased from 5.3 mm to 11.7 mm as concentration increases from 100 to 600 mg per ml. While zone of inhibition due to petrolium extract of Moringa leaves was found increased from 3.2 mm to 7.3 mm as concentration increases from 100 to 600 mg per ml.(Graph-II)



Graph.1: Effect of Ethanolic extract (Red line) and Petrolium ether extract (Violet line) of Moringa oleifera leaves against Cryptococcus neoformans.



Graph:2: Effect of Ethanolic extract (Red line) and Petrolium ether extract (Violet line) of Moringa oleifera leaves against Penicillium crysogenum.

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Nystatin is a polyene antibiotic. Polyene antibiotic is a class of antimicrobial polyene compounds that target fungi. It is most commonly used for the treatment of fungal infections. It was used as a positive control against both Cryptococcus neoformans and Penicillium crysogenum.

The results were higher and effective for ethanolic extract than petroleum extract. The result were also found more effective against Cryptococcus neoformans (Graph-I) than Penicillium crysogenum.(Graph-II)

IV. CONCLUSION

From our study it comes to known that there are some alkaloids present in extract of leaves of Moringa oleifera which can be effectively used to treat infection caused by Cryptococcus neoformans and Penicillium crysogenum.

REFERENCES

[1] Nadkarni A. Indian Materia Medica. Popular Prakashan: Bombay, 1976, 810–6.

[2] Anwar F and Bhanger M. Analytical characterization of Moringa oleifera seed oil grown in temperate regions of Pakistan. J. Agric. Food Chem. 2003,51:6558-63.

[3] Anwar F et.al. Moringa oleifera: a food plant with multiple medicinal uses. Phytother. Res. 2007,21:17-25.

[4] Morton JF. The horseradish tree, Moringa pterygosperma (Moringaceae)- A boon to arid lands? Econ. Bot.1991, 45:318-333.

[5] Gupta M. et.al. CNS activities of methanolic extract of Moringa oleifera root in mice. Fitoterapia 1999(3); 70: 244-50.

[6] Shukla S et.al. Antifertility profile of the aqueous extract of Moringa oleifera roots. Journal of Ethnopharmacology 1998; 22(1): 51-62.

[7] Lurling M and Beekman W. Anti-cyanobacterial activ ity of Moringa oleifera seeds. Journal Appl Phycol 2010;22(4):503-10.

[8] Fahey J et.al. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001; 56: 5–51.

[9] Bennett R et al. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera L. (Horseradish tree) and Moringa stenopetala L. J Agric Food Chem 2003;51: 3546–53.

[10] Pal S and Saha B. Studies on the antiulcer activity of M. oleifera leaf extract on gastric ulcer models in rats. Phytother. Res. 1995; 9: 463 – 5.

[11] Bukar A and Oyeyi T. Antimicrobal profile of Moringa oleifera Lam. Extracts against some food-borne microorganisms. Bayero Journal of Pure and Applied Sciences, 2010; 3(1): 43-8.

[12] Napolean, P et.al. Isolation, analysis and identification of phytochemicals of antimicrobial activity of Moringa oleifera Lam. Current Biotica, 2009; 3(1): 33 – 7.

[13] Nwosu M and Okafor J Preliminary studies on the antifungal activities of some medicinal plants against Basidiobolus and some other pathogenic fungi. Mycoses 1995; 38(5-6): 191-5.

[14] Chuang P.et.al. In vitro antifungal activity of ethanolic extract of the leaves of Moringa oleifera against dermatophytes. Bioresources Technology 2007; 98: 232–6.

[15] Srinivasan, D et.al. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology. 2001; 94: 217 - 22.

[16] Himal Paudel Chhetri et al. Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. Kathmandu university journal of science, engineering and technology september 2008; 49-54.