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Editorial Board

FOREWORD

I am pleased to put into the hands of readers Volume-3; Issue-2: Mar-Apr 2018 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",**an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

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

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

With warm regards.

Editor-in-Chief
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A screen-printed carbon electrode modified with a chitosan-based film for *in situ* heavy metal ions measurement

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***Abstract* —** *SEM images and FTIR data of the working electrode surface showed that M^{n+} ions were adsorbed on chitosan (Chit) and crosslinked chitosan-carbon nanotube (Chit-CNT) films. XPS revealed that chelation of M^{n+} ions with the –NH_2/–OH groups from chitosan, –COOH group from carbon nanotubes, and aqua ligands represents a possible structure of the active M^{n+} species in the Chit-based film. The electrochemical behaviors of the Chit-based film modified screen-printed carbon electrode (SPCE) were characterized for individual and simultaneous detection of Cu^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, and As^{3+} ions. For individual detection, the concentration range was 0.50–3.00 ppm with a detection limit of 0.4 ppm for Cu^{2+}; 1.0–4.0 ppm with a detection limit of 0.5 ppm for Pb^{2+}; 1.0–5.0 ppm with a detection limit of 0.8 ppm for Hg^{2+}. For simultaneous detection, the lab chip sensor was successfully used to determine the concentrations of Pb^{2+}, Cu^{2+}, Hg^{2+}, and As^{3+} ions simultaneously.*

***Keywords*— Heavy metal ion, Lab chip sensor, Square-wave anodic stripping voltammetry, in situ measurement.**

I. INTRODUCTION

In recent years, environmental contamination by heavy metals has gained much attention due to the significant impact on public health. Cu, Cd, Pb, Hg, Zn, and as are used in several industrial applications and are recognized as agents that present a toxic effect to humans and other living beings. These metals are well-known water pollutants, because they are toxic (even at trace levels), not biodegradable, and have long biological half-lives; hence, they tend to bio-accumulate in higher trophic levels of the food chain. Due to increasingly rigorous environmental regulations, the limits for heavy metals in drinking water and wastewater are becoming stricter [1].

Chitosan (Chit) is a biopolymer, a feature arising from the amino and hydroxyl groups present in its structure. Chit is suitable for use to remove heavy metal ions from wastewater, as its chemical groups can act as chelation sites [2, 3]. This characteristic can be employed for the development of electroanalytical procedures for the detection of heavy metal ions, for which Chit is employed as an electrode modifier, allowing adsorption of the metals ions, and thus improving the sensitivity of the method [4-6]. Furthermore, the useful characteristics of Chit in terms of electrochemistry for the design of modified electrodes include biocompatibility, a high mechanical strength, good adhesion on traditional electrochemical surfaces, and a relatively low cost, as it is a renewable resource [7-9]. On the other hand, it is well-known that carbon nanotubes (CNTs) exhibit many excellent electric properties, which make them ideal candidates for electrode materials for heavy metal detection. Normally, they act as an adsorbent/preconcentrator agent and a transducer platform. Numerous investigations have been carried out to explore the potential applications of CNTs, due to their advantages such as good conductivity, high electron transfer rates, high surface area and providing lower detection limits [10-13]. Chit was used as an electrode modifier in the cross-linked form, with CNTs employed as the crosslinking agents. We observed that the crosslinking treatment increased the number of free hydroxyl groups in the Chit and, thus, improved the adsorption of metallic cations on the electrode surface [14-16].

Many conventional methods, such as atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectroscopy (ICP-MS), have been utilized for the measurement of heavy metals in the environment. However, these methods are limited in terms of their use for *in situ* environmental screening because of the equipment size, cost, and analysis time [17]. Electrochemical techniques, in particular stripping analysis, have been widely studied in terms of their effectiveness for *in situ* measurement of heavy metal ions [10,18]. In stripping analysis, heavy metal ions in the sample solution are identified and quantified by measuring the current generated at each reduction potential [19]. For real application, more and more heavy metal electrochemical sensors have been fabricated on screen-printed carbon electrodes (SPCEs) due to their inexpensiveness, portability and ease of mass production.

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Furthermore, a desirable combination incorporating miniaturized on-chip electrochemical sensors with microfluidic components as a micro total analysis system or lab-on-a-chip device is achievable, and provides a good platform for chemical and biological analyses in a miniaturized format [20]. Polymer substrates, such as cyclic olefin copolymers, have been used as lab-on-a-chip materials instead of the traditional silicon and glass substrates owing to their favorable properties of biocompatibility, high optical transparency, and low cost [21].

The major achievement in this study was the development of a disposable heavy metal sensor with an on-chip planar polymer film (Chit and Chit-CNT) modified SPCE (working electrode), an integrated Ag/AgCl reference electrode, and microfluidic channels using standard screen-printing technology. The proposed sensor is very low in cost and suitable for mass production, has a small analytic consumption and low waste generation, a fast sensing time, and is simple to use. This sensor is also suited to fast *in situ* environmental monitoring. Therefore, the main objective of this research was to study the selectivity of the Chit-based film modified SPCE for individual and simultaneous detection of Cu^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, and As^{3+} ions in aqueous solutions. In addition, based on the results, an adsorption mechanism was proposed. We explored the adsorption properties of the biodegradable materials of Chit and Chit-CNT, which can be utilized for the detection of heavy metal ions in wastewater and groundwater.

II. EXPERIMENTAL

2.1 Preparation of crosslinked Chit-CNT

Multi-walled carbon nanotubes (MWCNT; >95% carbon basis, 20–40 nm in diameter and 5–15 μm in length) were purchased from Aldrich. The MWCNT were submitted to an acid treatment to remove residual catalyst metal particles that remained from the synthesis process and to promote the generation of functional groups, such as carboxyl and hydroxyl groups, on the MWCNT surface [22]. Briefly, MWCNT were added to a 13 N HNO_3 solution and maintained in a stainless Teflon-lined autoclave for 30 h at 100 °C. After this time, the MWCNT were separated from the solution by centrifugation and washed thoroughly with ultrapure water until a pH of approximately 6.0 was obtained. Finally, the MWCNT were dried at 120 °C for 5 h. Chit of molecular weight 1.9–3.1 × 10^5 g/mol and an 85% deacetylation degree was purchased from Acros. 2.0 g Chit were immersed in 100 mL of 0.17 mole acetic acid aqueous solution at 40 °C and maintained under constant stirring for 12 h. Preparation of Chit-CNT was performed by mixing functionalized MWCNT (40 mg) and Chit solution (20 mL) by stirring, resulting in a homogeneous solution.

2.2 Electrode modification

Integrated SPCE sensors are based in most cases on a carbon working electrode, a carbon counter electrode, and a silver/silver chloride reference electrode. The SPCE was directly obtained by mass-production in-house via a multi-stage screen-printing process, and screens with appropriate stencil designs (100 per screen) were fabricated by a commercial firm. A photograph of the screen-printed three-electrode sensor is shown in Fig. 1. The entire chip size was 3.3 cm × 1.2 cm, and the circular reaction chamber had a 4.5-mm^2 working area and a depth of 100 μm. Then, the Chit solution or crosslinked Chit-CNT mixture solution was mixed with nafion solution (1 wt%), cast onto the SPCE surface, and dried in air. Nafion acted here as a binder to stabilize the modified species on the electrodes and as a permselective film to alleviate the interference of anions [23]. A schematic illustration of the preparation of the Chit-CNT film modified electrode is shown in Fig. 1. Furthermore, the Chit-based film modified SPCE was cleaned electrochemically by performing cyclic voltammetry for 10 cycles in the potential window -0.4 V to 1.0 V *vs.* Ag/AgCl at a potential scan rate of 50 mVs^{-1} in pH 7 phosphate buffer solution before each experiment, and served as an underlying substrate of the working electrode.

2.3 Experimental techniques

Pb^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+}, Zn^{2+}, and As^{3+} standard stock solutions (1000 ppm) were obtained from Aldrich and diluted with deionized water and a supporting electrolyte to the appropriate concentration. The supporting electrolyte used in the experiments was 0.2 M acetic acid solution, adjusted to pH 4.0 with ammonium acetate. Heavy metal-contaminated water samples were obtained from four different locations: 1,2–Groundwater was obtained from Pingtung and Taoyuan, Taiwan. 3–Factory wastewater was obtained from the industrial zone of Taoyuan, Taiwan. 4–Mine wastewater was obtained from Jinguashi Mine, New Taipei City, Taiwan. The water samples were extracted with acetic acid and the results of detection of heavy metal pollutants were compared with those obtained using an inductively-coupled plasma-mass spectrometer (ICP-MS, 7500ce). The morphology of the Chit-based film modified SPCE surface was observed using a scanning electron microscope (SEM, JSM-6330F) equipped with an energy-dispersive X-ray (EDX) microanalysis system. X-ray photoelectron spectroscopy (XPS, VG ESCALAB 250) was applied to determine the interactions between the organic functional groups in the Chit-CNT sorbent and the metals adsorbed. XPS spectra were obtained using monochromatized Al Kα radiation (1486.7 eV); the source was operated at 15 kV and 15 mA. Calibration of the binding energies (BEs) of the spectra was performed using the C_{1s} peak of the aliphatic carbons at 284.6 eV.

Anodic stripping voltammetry measurements were obtained using a conventional three-electrode cell with a CHI 660C electrochemical workstation. Square wave anodic stripping

voltammetry (SWASV) was used for the detection of metal ions at various concentrations. Studies were carried out by immersing the lab chip sensor into acetate buffers containing M^{n+} standard solutions. The pre-concentration analysis of M^{n+} proceeded in 6 ml 0.2 M pH 4.0 acetic buffer solution for 2 min while holding the electrode at -1.2 V and stirring the solution. The solutions were stirred during the pre-concentration step. After 30 s of equilibration, SWASV measurements were obtained in the potential range of -1.2 V to 1.2 V with a frequency of 50 Hz, an amplitude of 40 mV, and a potential step of 4 mV.

III. RESULTS AND DISCUSSION

3.1 Morphology and characterization

The morphologies of the fracture and surface were observed by SEM, and the EDX mapping technique was used to determine the distributions of M^{n+} ions in the Chit-based films. Fig. 2 presents SEM, Hg-mapping, and EDX images of the morphologies of the Chit-based film modified SPCE. It can be observed from Fig. 2a that the carbon layer was rough and exhibited irregular particles on the screen-printed electrode. Fig. 2b and 2c show that a smooth and dense thin film (14.3 μm) covered the surface of the SPCE coupled with nafion binder. The Chit surface had a membrane aspect and did not present porosity, which indicated that it was likely that it did not have diffusion problems, and thus the sorption process proceeded quickly. From the SEM images of the Chit-CNT film (Fig. 2d and 2e), it was clearly observed that Chit adhered uniformly to the wall of the MWCNT and fabricated a porous mesh structure. The Chit-CNT film was attached tightly to the SPCE surface and the film thickness was around 92.4 μm (Fig. 2e). Enlargement of the image revealed a highly porous structure of the material, indicating a large electroactive surface that involves fast electron transfer rates. However, the rough surface can also induce decreases in the homogeneity and reproducibility of the measurements performed between and within batches of sensors. As M^{n+} ions were introduced, the surface became rougher and exhibited a heterogeneous morphology in the matrix. Moreover, the Hg-mapping and EDX images (Fig. 2f–h) indicated the presence of a significant amount of Hg^{2+} ions in the matrix. The Hg^{2+} ions were uniformly dispersed throughout the Chit-based films, thus providing the maximum surface area for the adsorption of Hg^{2+}.

Infrared analyses were performed on the Chit film before and after being in contact with Cu^{2+}, Hg^{2+}, and As^{3+} solutions. These studies provided information regarding the functional groups and the interaction force present in the Chit film. Figure 3a shows the infrared spectrum of Chit: the bands at 3448, 1647, and 1086 cm^{-1} are closely related to the N–H and O–H stretching, N–H bending, and C–OH stretching vibrations. The differences between the IR spectra of Chit before and after Cu^{2+}, Hg^{2+}, and As^{3+} adsorption can be observed in Fig. 3b–d. It was seen that the bands at 3448, 1647, and 1086 cm^{-1} were displaced to lower wavenumbers. This occurred because the vibrations of the O–H, N–H, and C–OH bonds were modified while forming bonds between O/N (by its free pair of electrons) and the metals. The IR spectrographs suggested that coordination complexes were formed between the Chit and the metals, which reduced the vibration intensity of the O–H, N–H, and C–OH bonds due to the molecular weight increase after M^{n+} adsorption.

3.2 Adsorption mechanism study by XPS

XPS studies performed on Chit previously loaded with metal ions (M^{2+}) indicated that the main complexing sites are the amines and secondary alcohol functional groups, as the $–NH_2$ and –OH groups have a pair of electrons that can add themselves to a cation by coordinated covalent bonds. The attraction of the electron pair by the atom nucleus is stronger for oxygen, although on the other hand nitrogen has a greater tendency to donate its pair of electrons to a metal ion to form a complex through a coordinated covalent bond [24]. Similarly, CNTs, upon strong acid-based functionalization, have been reported to generate large amounts of several oxygen-rich functional groups, including carboxylic groups (–COOH), on their edge plane, and these carboxylic groups are well-known for their strong and stable functional M^{2+}-carboxylic architectures owing to their rich coordination mode [14]. Complexes between metal ions (M^{n+}) and Chit/Chit-CNT were formed according to the mechanism illustrated in Fig. 4a. In this study, we inferred that these metals chelated with the $–NH_2$/–OH groups from Chit and the –COOH group from CNTs, and a possible structure of the active M^{n+} species in the Chit/Chit-CNT systems was proposed.

Figure 4b–c show typical XPS spectra for the Chit-CNT film before and after Cu^{2+}, Pb^{2+}, and As^{3+} adsorption. Before M^{n+} adsorption, there were two peaks in the N_{1s} spectra at binding energies (BEs) of approximately 400.0 and 402.1 eV (see Fig. 4b). These peaks were attributed to the N atoms in the $R–NH_2$ and $R–NH_3^+$ groups, respectively. In an acidic solution, the following chemical reactions may be proposed to account for the adsorption of M^{n+} on Chit-CNT film:

$$R–NH_2 + H^+ \leftrightarrow R–NH_3^+ \quad (1)$$

$$R–NH_2 + M^{n+} \rightarrow R–NH_2M^{n+} \quad (2)$$

$$R–NH_3^+ + M^{n+} \rightarrow R–NH_2M^{n+} + H^+ \quad (3)$$

The reaction in eq. 1 indicates protonation and deprotonation of the amino groups in Chit. At pH 4.0, lower amino groups are protonated, thus resulting in larger amounts of $–NH_2$ (400.0 eV) than $–NH_3^+$ (402.1 eV) in the Chit-CNT film. When M^{n+} ions were added to the solution, the reaction in eq. 2 started due to sharing of the lone pair of electrons from the nitrogen atom with a M^{n+} ion, with a mechanism similar to that of the reaction shown in eq. 1. However, the binding of a M^{n+} ion to a nitrogen atom can

be expected to be stronger than the binding of a H^+ to a nitrogen atom, as the electrical attraction force between the lone pair of electrons from the nitrogen atom and the M^{n+} ion would be stronger than that of the nitrogen atom and the monovalent proton (H^+). This difference in the binding force drives the reaction in eq. 3 to take place through competitive adsorption of M^{n+} over H^+ to the nitrogen atom, which may sometimes be considered as an ion exchange mechanism [25]. The reaction in eq. 3, however, can be expected to be slower than that in eq. 2, owing to the smaller attraction force between the N in $R–NH_3^+$ and M^{n+} as compared with the force between the N in $R–NH_2$ and M^{n+}. Therefore, the peak of $–NH_2$ was of lower energy than that of $–NH_3^+$ in the Chit-CNT-M^{n+} system. After M^{n+} adsorption, moreover, the BEs of $R–NH_2$ and $R–NH_3^+$ in Chit-CNT-M^{n+} were smaller than those in Chit-CNT. This result indicated that the M^{n+} ions were chelated with the $R–NH_2$ and $R–NH_3^+$ groups ($R–NH_2\cdots M^{n+}$), and the BEs of the N atoms in $R–NH_2$ and $R–NH_3^+$ were therefore weakened.

In Fig. 4c, typical O_{1s} XPS spectra of the Chit-CNT film with and without adsorbed M^{n+} ions are presented. There was only one peak in the O_{1s} spectrum, at a BE of approximately 533.1 eV, for Chit-CNT. This was attributed to the O atoms in the R–OH and R–COOH groups. During M^{2+} ions adsorption, the O_{1s} peak shifted towards a lower energy, and its structures were close to ($[M(–NH_2)_2]^{2+}$, $2OH^–$) and ($[M(–NH_2)_2]^{2+}$, COOH, $2H_2O$). The M^{2+} ion with four coordination, during formation of a coordination compound, reacted with two $–NH_2$ amino groups and two $–OH^–$ groups or two water molecules [2]. Annamalai [14] proposed a six coordination model in which the M^{2+} ion was attached to two $–NH_2$ groups of Chit, one –COOH group of the CNTs and two water molecules. The As^{3+} ion with three or five coordination, during formation of a coordination compound, reacted with two $–NH_2$ groups and one $–OH^–$ group or one –COOH group, one $–NH_2$ group and two water molecules. For M^{n+} ion adsorption, the ΔBEs of the O_{1s} bands of the –OH and/or –COOH groups were 1.5, 1.3, and 0.5 eV before and after As^{3+}, Pb^{2+}, and Cu^{2+} adsorption, respectively. These shifts were all beyond 0.3 eV. Therefore, it could be concluded that the –OH and/or –COOH groups participated in As^{3+}, Pb^{2+}, and Cu^{2+} adsorption on the Chit-CNT adsorbent. The ΔBEs of the N_{1s} band of the $–NH_2$ groups (400.0 eV) before and after As^{3+}, Pb^{2+}, and Cu^{2+} adsorption were 1.6, 1.1, and 0.7 eV, respectively, and the ΔBEs of the N_{1s} band of the $–NH_3^+$ groups (402.1 eV) were 1.4, 1.2, and 0.6 eV, respectively. This indicated that $–NH_2$ groups may be the main functional group responsible for M^{n+} ion adsorption. Moreover, the ΔBEs of the O_{1s} and N_{1s} bands before and after M^{n+} adsorption were in the order of $As^{3+} > Pb^{2+} > Cu^{2+}$. This suggested that the interaction force of Chit-CNT with M^{n+} ions was of the order $As^{3+} > Pb^{2+} > Cu^{2+}$.

3.3 Square wave stripping voltammetric behavior of M^{n+} ions at the Chit-based film modified SPCE

3.3.1 Detection of single M^{n+} ions by the Chit-SPCE

The SWASV signals of M^{n+} ions at various concentrations measured by the Chit-SPCE sensor are shown in Fig. 5. For Cu^{2+}, dissolution peaks were observed at 0.14 V in the 0.50–3.00 ppm concentration range. The plot of peak current as a function of ion concentration was linear over the whole range studied, and the regression coefficient (R^2) was 0.987 (inset). Thus, the detection limit of Cu^{2+} ions was deduced to be 0.4 ppm, at which the stripping peak current could still be resolved. Noticeably, a larger stripping peak was found for Cu^{2+}, which showed a good electrocatalytic response to Cu^{2+}. For Pb^{2+}, dissolution peaks were observed at -0.38 V in the 1.0–4.0 ppm concentration range. The detection limit was calculated to be 0.5 ppm (R^2 = 0.981) for Pb^{2+}. Under the same experimental conditions, a small stripping peak at around -0.44 V was observed due to the strong complexing ability of Chit to Pb^{2+} on the electrode surface, and the resistivity of Chit resulted in a poor current response. For Hg^{2+}, dissolution peaks were observed at around 0.38–0.43 V in the 1.0–5.0 ppm concentration range. The detection limit was calculated to be 0.8 ppm (R^2 = 0.933) for Hg^{2+}. Moreover, there was a slight shift of the peaks towards higher values, which might be predicted by Nernst's equation, as the concentration evolved.

For the detection of individual Zn^{2+} and Cd^{2+} ions, the results showed SWASV peaks from -1.17 V to -1.24 V for Zn^{2+} ions and from -0.70 V to -0.94 V for Cd^{2+} ions, and analyses of Zn^{2+} and Cd^{2+} ions alone revealed an ill-defined stripping peak current. This was more pronounced for the lab chip sensor, for which the peaks were broader and without reproducibility. The result may be due to the existence of fissures between the Chit film and SPCE within batches of sensors, which induced an ohmic drop [26]. For As^{3+}, dissolution peaks were observed at around 0.51 V in the 30–70 ppm concentration range. The calibration curve was linear in the concentration range, and the regression coefficient (R^2) was 0.943. Thus, the detection limit of As^{3+} ions was deduced to be 1.0 ppm, at which the stripping peak current could still be resolved. Noticeably, a broader stripping peak was found and the instrumental signal was significantly different to the background signal in the range of 0.0 V to 1.0 V, which showed a poor electrocatalytic response to As^{3+}. Because elemental As is a very poor electrical conductor, the peak intensity of As^{3+} was lower than those of the other metal ions. As can be observed from Fig. 5, the sensitivity for the detection of M^{n+} ions was of the order $Hg^{2+} > Cu^{2+} > Pb^{2+} > As^{3+}$. This suggested that the interaction force of Chit with M^{n+} ions was of the order $As^{3+} > Pb^{2+} > Cu^{2+} > Hg^{2+}$; thus, the strength of the anodic redissolution signals for M^{n+} ions was $Hg^{2+} > Cu^{2+} > Pb^{2+} > As^{3+}$.

3.3.2 Multiplexing detection of M^{n+} ions at the Chit-based film modified SPCE

In order to evaluate whether the presence of several ions impacted on the stripping peaks obtained using the Chit-based film modified SPCE, we evaluated the simultaneous detection of Pb^{2+}–Cu^{2+}–Hg^{2+} and Pb^{2+}–Cu^{2+}–Hg^{2+}–As^{3+} in the same solution (see Fig. 6). In the analysis of the Pb^{2+} (5–25 ppm) and Cu^{2+} (5–25 ppm) mixture, SWASV peaks at around –0.417 V (R^2 = 0.956) and 0.212 V (R^2 = 0.983) indicated the stripping of Pb^{2+} and Cu^{2+}, respectively. In the present case, Pb had the most negative standard potential, and tended to be deposited onto the Cu metal, which presented the highest potential and was deposited first. For the reverse process, Pb was then first reoxidized, leaving a partially-covered electrode of electrodeposited Cu, but a quantity remained and was dissolved simultaneously with the Cu at a higher potential. This explained why the Pb^{2+} (from –0.425 to –0.403 V) and Cu^{2+} (from 0.157 to 0.247 V) peaks were shifted toward more positive values when the amount of dissolved Pb^{2+}–Cu^{2+} increased (from 5 to 25 ppm) in the tested solution. This could be interpreted as the formation of a Pb_xCu solid solution thin film (x being the solubility of Pb into Cu under the conditions employed) [26]. Such formation of a binary compound also explained how the Chit-SPCE sensor accelerated the enrichment of Pb^{2+} and Cu^{2+} on the electrode surface.

In Fig. 6b and 6c, for the Cu^{2+}–Hg^{2+} (1.0–4.0 ppm) and Pb^{2+}–Hg^{2+} (10–30 ppm) mixtures, SWASV peaks at potentials of −0.417 V (R^2 = 0.974), 0.106 V (R^2 = 0.998), and 0.425 V (R^2 = 0.999) represented the stripping of Pb^{2+}, Cu^{2+}, and Hg^{2+}, respectively. The results showed that the anodic peak current was different from that of the oxidation of Pb, Cu, and Hg, while the initial concentration was the same. This could be due to one or more of the following reasons. One reason for the difference could be the higher affinity between the modified electrode surface and Hg^{2+} in comparison with that of Cu^{2+} and Pb^{2+}. It could also be related to the differences between the diffusion coefficients of Cu^{2+}, Pb^{2+}, and Hg^{2+}. The kinetics of the complexation of cations with Schiff base at the electrode surface could also be responsible for the greater accumulation of Hg^{2+} as compared with Cu^{2+} and Pb^{2+} [27]. These cases could lead to a higher peak current for Hg^{2+} than for Cu^{2+} and Pb^{2+}. The SWASV responses resulting from the presence of these potentially interfering species were compared with those obtained for Cu^{2+}, Pb^{2+}, and Hg^{2+}. It was clear that no interference occurred due to these species, which implied possible successful direct application of Chit-SPCE in real samples that contain common ions or species.

SWASV responses of the Chit-SPCE sensor for the simultaneous detection of Pb^{2+}, Cu^{2+}, Hg^{2+}, and As^{3+} at successive increasing concentrations were as shown in Fig. 6d. The stripping peak potentials for Pb^{2+}, Cu^{2+}, Hg^{2+} and As^{3+} appeared at −0.417, 0.105, 0.459, and 0.625 V, respectively. The slight potential shift in comparison with the individual analyses might be caused by the complicated nature of the process of simultaneous electrode position of Cu, Hg, and As. The stripping peak currents of the three analytic ions (Pb^{2+}, Cu^{2+}, and Hg^{2+}) increased with increasing concentrations. Cu^{2+} was affected by Pb^{2+} and Hg^{2+} in the simultaneous detection, which could be the reason for which the active sites of the Chit-SPCE during deposition of Cu^{2+} were occupied by Pb^{2+} or Hg^{2+} preferentially. However, the separation between the voltammetric peaks was large enough, and simultaneous detection using the modified electrode was feasible. From the aforementioned simultaneous analysis results of the three heavy metal ions, it was observed that related parameters such as stripping peak currents and potentials changed with the presence of As^{3+} ions. Mutual interference is a common problem in the detection of several metal ions simultaneously. We considered that this result could also be explained by the intermetallic compounds formed among the four target metal ions and competitive adsorption at the limited number of active sites at the Chit-SPCE surface. Thus, the proposed electrode was successfully applied for the determination of Pb^{2+}, Cu^{2+}, Hg^{2+} and As^{3+} ions simultaneously.

The SWASV signals of M^{n+} ions at the Chit-SPCE and Chit-CNT-SPCE sensors are shown in Fig. 7. Under the same experimental conditions, a larger stripping peak at around 0.448 V was found at the Chit-CNT-SPCE (Fig. 7a), which showed a good electrocatalytic response to Hg^{2+}. The Chit-CNT component, with good conduction properties and a large surface area, was able to adsorb Hg^{2+} from the bulk solution to the electrode surface, resulting in improvement of the stripping peak current. Figure 7b shows SWASV peaks from –0.447 V to –0.436 V, from 0.07 V to 0.110 V, and from 0.439 V to 0.474 V for the mixture of Pb^{2+}, Cu^{2+}, and Hg^{2+} at the Chit-SPCE and Chit-CNT-SPCE. Analyses of the mixture of Pb^{2+}, Cu^{2+}, and Hg^{2+} ions revealed a well-defined stripping peak current, as did analyses of the three ions alone. A slight shift in peak potential and a change in the relative peak current were observed for the Chit-CNT-SPCE as compared with the Chit-SPCE. This observation can be explained by the difference in diffusivity and the intermetallic compounds formed during the accumulation process of Pb^{2+}, Cu^{2+}, and Hg^{2+} ions on the Chit and Chit-CNT film.

3.3.3 Detection of heavy metal ions in real water samples

In order to evaluate the feasibility of using the proposed sensor for *in situ* measurement of heavy metals for environmental monitoring applications, water samples taken from groundwater and wastewater of factory and pit sites were analyzed using the lab chip sensor. The water samples had been filtered and acidified in the field and stored in a fridge. The water solution was evaporated to

almost a quarter of the original volume by heating. Then, the Chit-based film modified SPCE was immersed in the mixed solution directly to determine the M^{n+} concentrations in the treated water sample. The total measurement duration for each sample was less than 3 min. 90 s was chosen as the deposition time, which was an optimized condition that balanced the detection time and the detection limit for this application. ICP-MS was used to verify the electrochemical data obtained from the real water samples using the lab chip sensor.

According to the literature, Fig. 8 shows SWASV peaks of pit wastewater at –1.059 V for the mixture of Zn^{2+} and Ni^{2+}, –0.561 V for Pb^{2+}, 0.115 V for Cu^{2+}, 0.261 V for Hg^{2+}, 0.535 V for As^{3+}, and 0.770 V for Fe^{3+}. The obtained peaks were of well-defined shapes with good reproducibility. The results obtained using the ICP-MS technique were Zn^{2+} (64.1 ppm), Ni^{2+} (30.5 ppm), Pb^{2+} (2.24 ppm), Cu^{2+} (37.0 ppm), Hg^{2+} (1.25 ppm), As^{3+} (3.81 ppm) and Fe^{3+} (49.0 ppm). Moreover, SWASV peaks of factory wastewater were observed at –0.897 V for the mixture of Zn^{2+} and Ni^{2+}, 0.105 V for Cu^{2+}, 0.491 V for As^{3+}, and 0.834 V for Fe^{3+}. The results obtained using the ICP-MS technique were Zn^{2+} (150 ppm), Ni^{2+} (8.92 ppm), Cu^{2+} (52.3 ppm), As^{3+} (13.4 ppm) and Fe^{3+} (86.3 ppm). However, no signal was obtained for the groundwater, and the concentrations measured using the ICP-MS technique were below 0.1 ppm in all samples. The experiments were repeated three times with good reproducibility, and the results indicated that the proposed method was highly accurate with good reliability, and can be used for direct analysis of relevant real samples.

IV. CONCLUSION

The use of a flexible Chit-based film to develop Chit-SPCE and Chit-CNT-SPCE lab chip sensors for individual Cu^{2+}, Pb^{2+}, Hg^{2+}, Zn^{2+}, Cd^{2+}, and As^{3+} ion detection, simultaneous detection of Pb^{2+}–Cu^{2+}, Cu^{2+}–Hg^{2+}, Pb^{2+}–Hg^{2+}, Pb^{2+}–Cu^{2+}–Hg^{2+}, and Pb^{2+}–Cu^{2+}–Hg^{2+}–As^{3+} ions, and *in situ* monitoring of heavy metals in real water samples was reported in this paper. XPS analysis revealed the occurrence of metal complexation to the carboxylic (–COOH), amino ($–NH_2$), hydroxyl (–OH), and H_2O groups in Chit-CNT, and chelation, ion exchange and electrostatic interaction formed the main adsorption mechanism. For individual detection, the detection limits were calculated to be 0.4, 0.5, 0.8, and 1.0 ppm for Cu^{2+}, Pb^{2+}, Hg^{2+}, and As^{3+}, respectively, and the detection sensitivity was of the order $Hg^{2+} > Cu^{2+} > Pb^{2+} > As^{3+}$. For simultaneous detection, the Chit-based film modified SPCE was successfully applied for the determination of Pb^{2+}, Cu^{2+}, Hg^{2+}, and As^{3+} ions simultaneously. A slight shift in the peak potential and a change in the relative peak current were observed for the Chit-CNT-SPCE sensor as compared with the Chit-SPCE sensor. This observation could be explained by the difference in diffusivity and the intermetallic compounds formed during the accumulation process of Pb^{2+}, Cu^{2+}, and Hg^{2+} ions on the Chit and Chit-CNT films. Microfabrication technology was utilized in this study to realize a new fully-integrated sensor with a planar screen-printed carbon electrode and microfluidic channels. The low cost and non-toxic electrode materials render the sensor disposable, and the simple structure and detection scheme of the proposed sensor are especially suitable for applications such as detecting trace metals *in situ* in environmental samples.

ACKNOWLEDGMENTS

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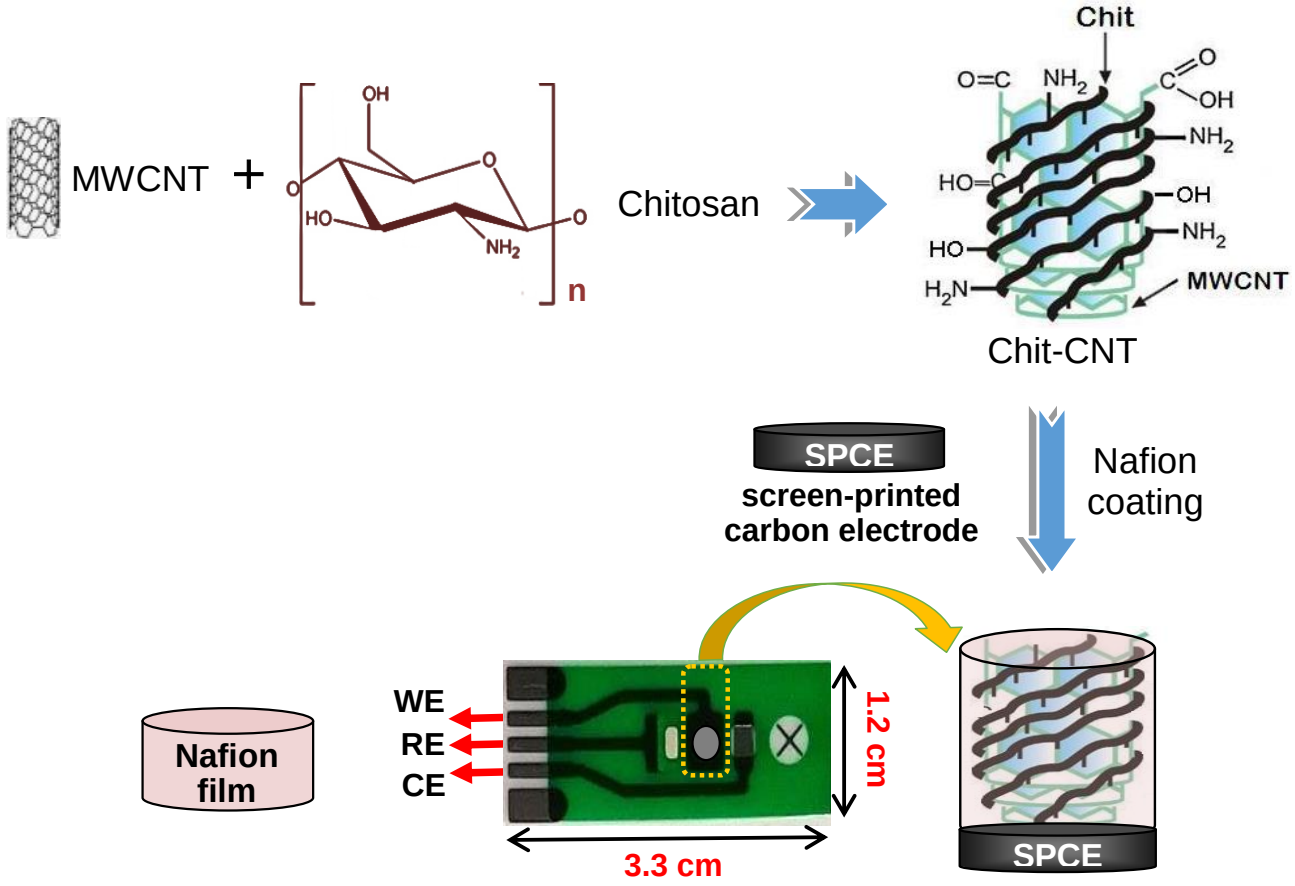

Fig. 1: Schematic illustration of the preparation of Chit-based films modified SPCE.

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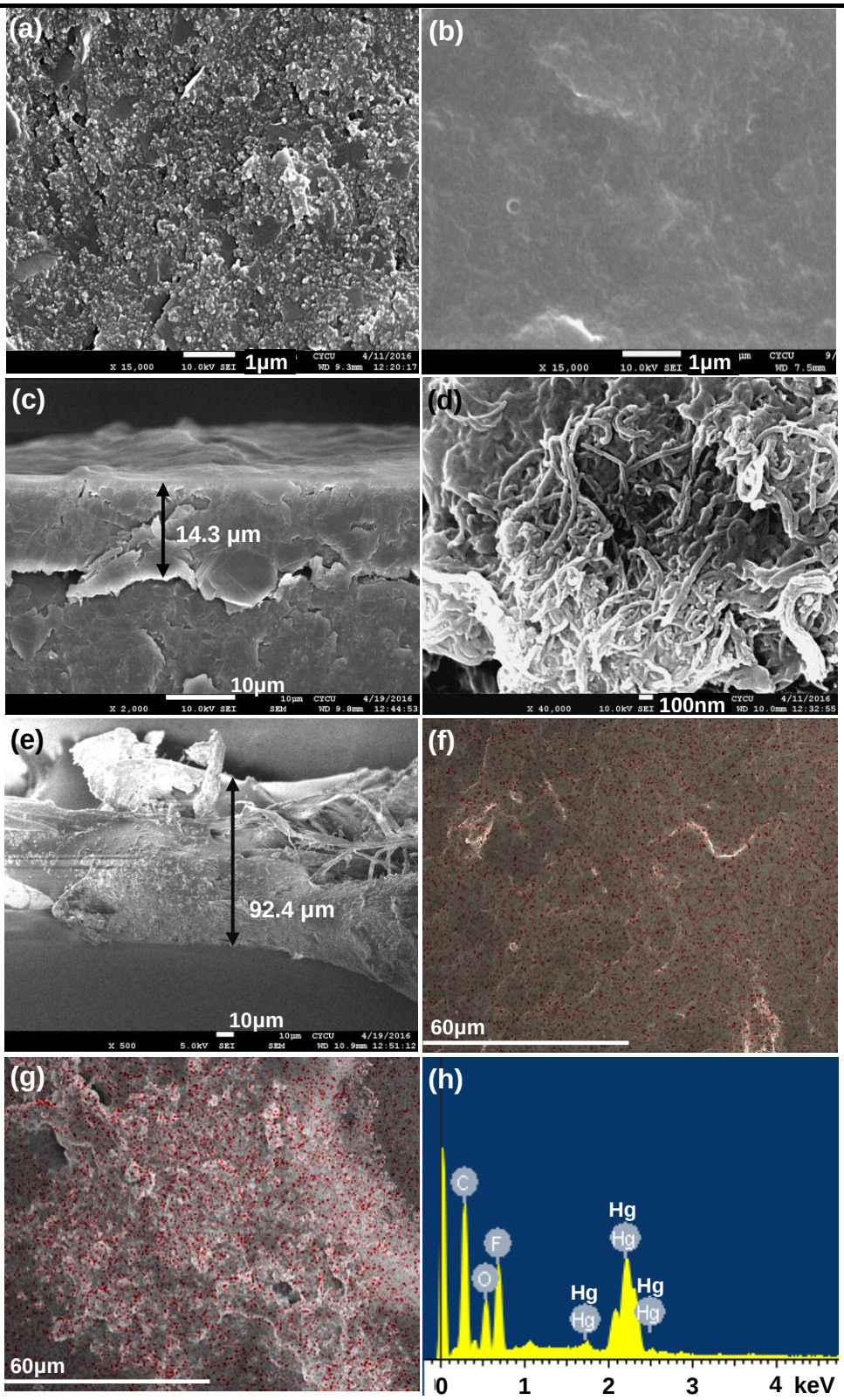

Fig.2: SEM photographs of (a) SPCE, (b) Chit-SPCE, (c) Chit-SPCE (fracture), (d) Chit-CNT-SPCE, (e) Chit-CNT-SPCE (fracture); SEM+Hg-mapping images of (f) Chit-SPCE, (g) Chit-CNT-SPCE, (h) EDX image of Chit-Hg^{2+}.

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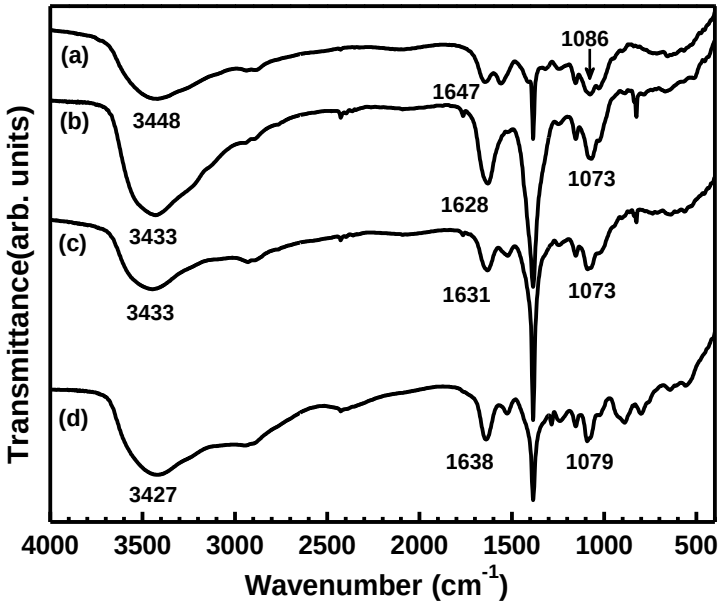

Fig. 3: FTIR spectra of (a) Chit film, (b) Chit-Cu^{2+}, (c) Chit-Hg^{2+}, (d) Chit-As^{3+}.

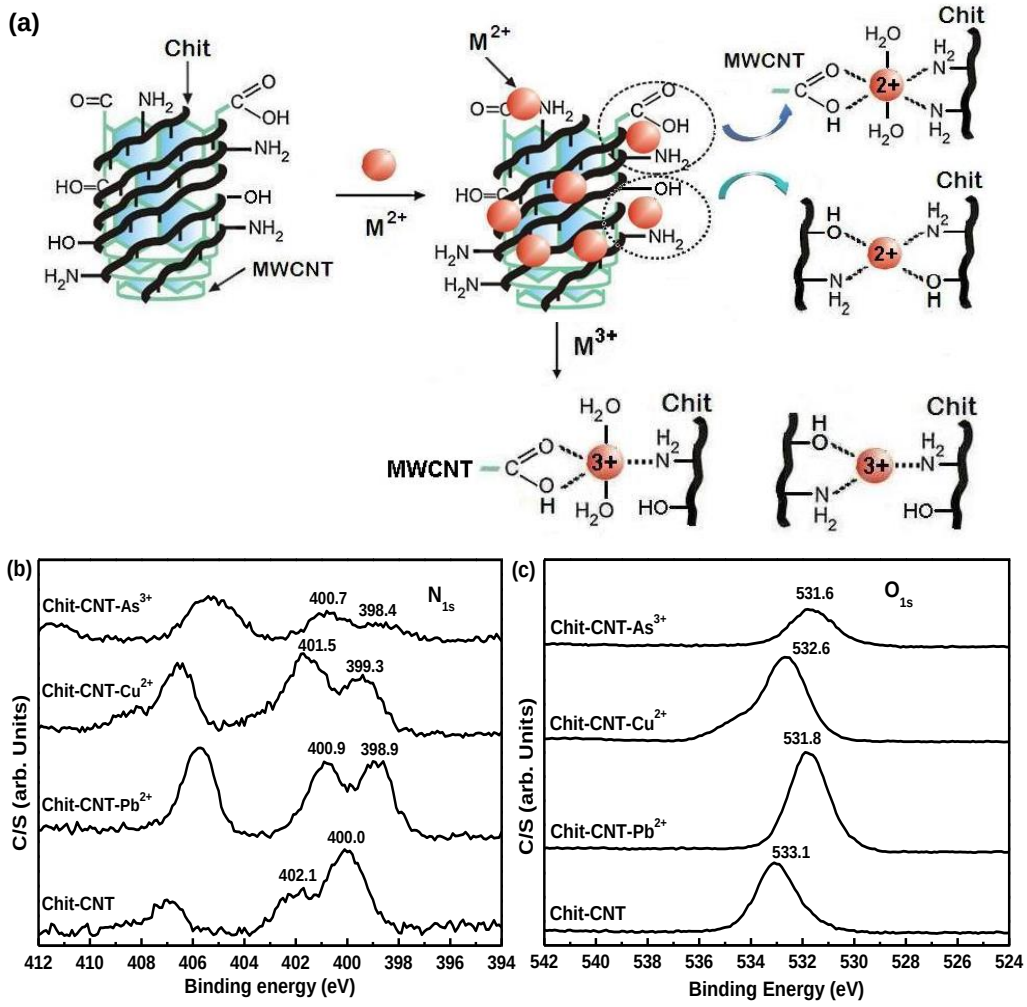

Fig.4: (a) Intermolecular complexes of Chit-CNT with metal ions in acidic solution, (b) N_{1s} and (c) O_{1s} XPS spectra of Chit-CNT before and after metal adsorption.

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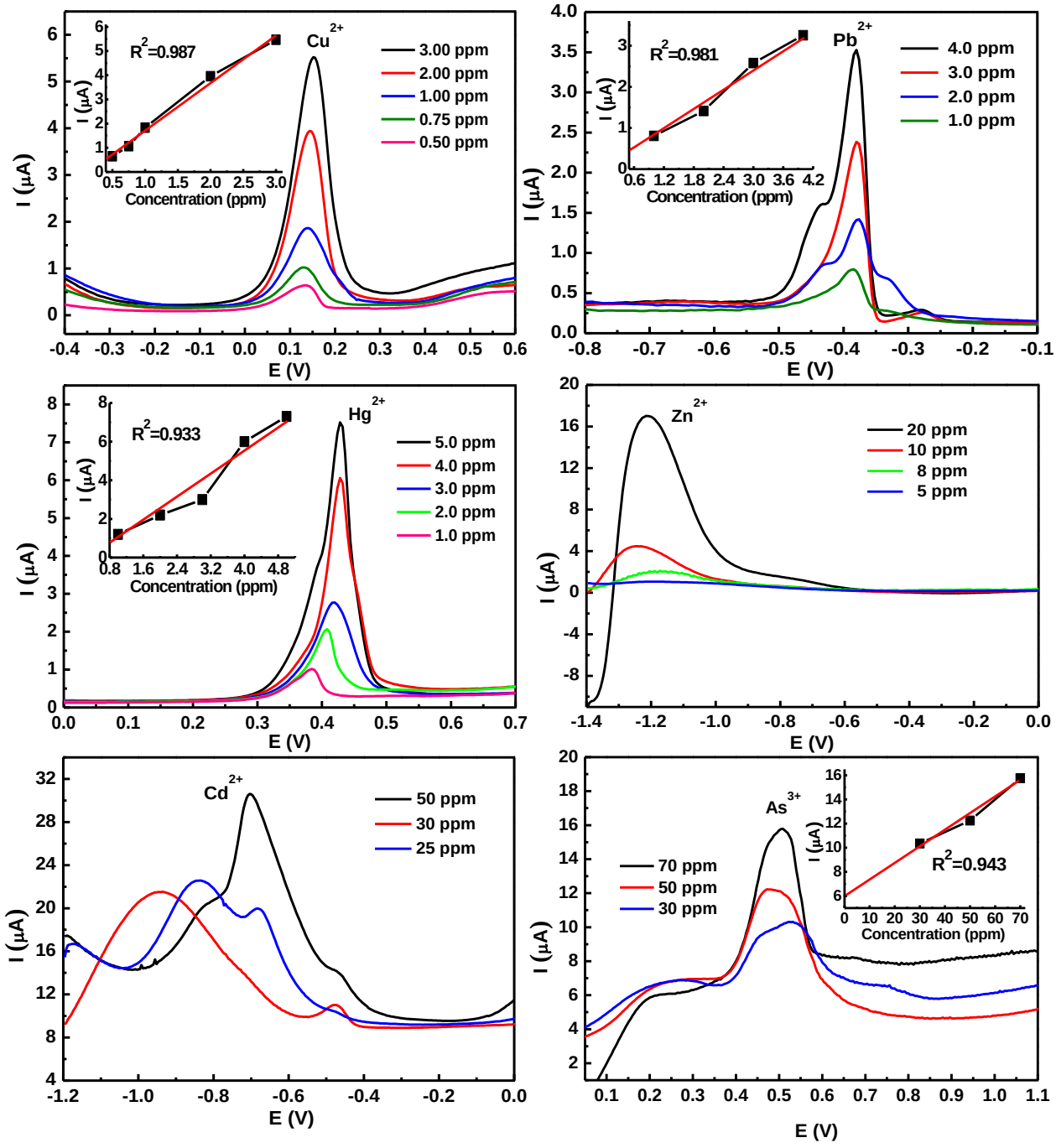

Fig. 5: SWASVs of various M^{n+} solutions at Chit-SPCE; inset: calibration plot for M^{n+} detection.

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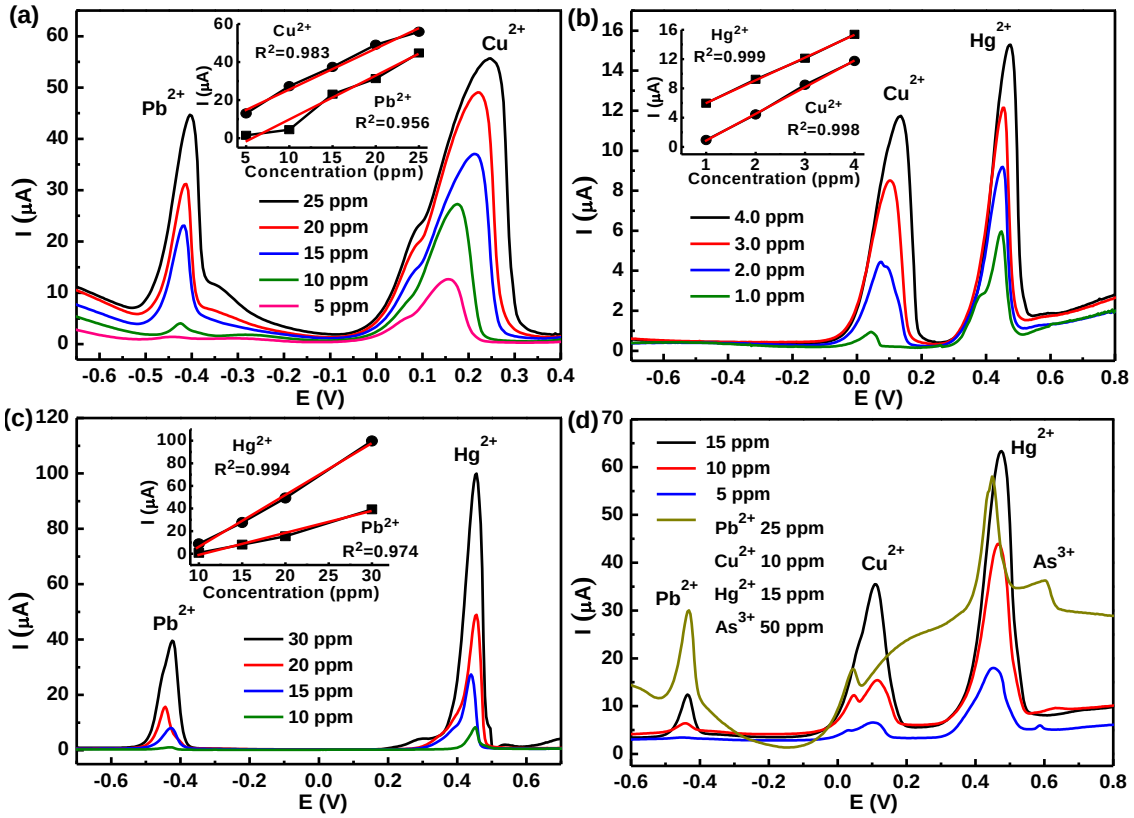

Fig. 6: SWASVs for the simultaneous standard additions of (a) Pb^{2+}-Cu^{2+} (5–25 ppm), (b) Cu^{2+}-Hg^{2+} (1.0–4.0 ppm), (c) Pb^{2+}-Hg^{2+} (10–30 ppm), (d) Pb^{2+}-Cu^{2+}-Hg^{2+} (5–15 ppm) and Pb^{2+}(25 ppm)-Cu^{2+}(10 ppm)-Hg^{2+}(15 ppm)-As^{3+}(50 ppm) at Chit-SPCE.

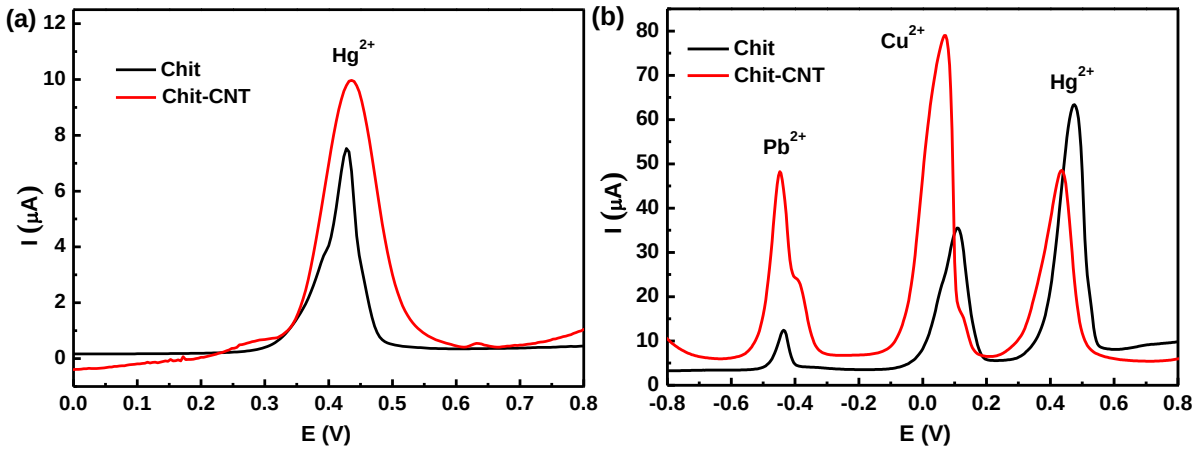

Fig. 7: Comparison of Chit-SPCE and Chit-CNT-SPCE for the determination of (a) Hg^{2+} (5ppm) and (b) Pb^{2+} (15ppm), Cu^{2+} (15ppm), Hg^{2+} (15ppm).

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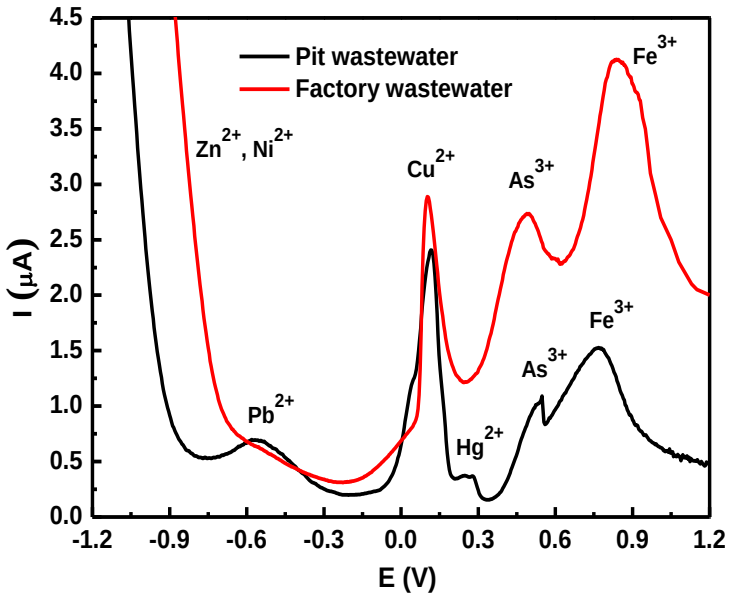

Fig. 8: SWASV determination of M^{n+} in water samples at Chit-SPCE.

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Contribution to the study of the chemical composition of Lemon Verbena: Aloysia triphylla (Hert). Britt. cultivated in Morocco

Contribution à l'étude de la composition chimique de la Verveine odorante : *Aloysia triphylla* (L'Hert.) Britt cultivée au Maroc

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Abstract**—** *By combining gas chromatography and mass spectrometry we have identified the main constituents of the essential oil of verbena tea (Alyosia triphylla) grown in Morocco. Harvests carried out at different stages of development of the plant allowed to characterize the qualitative variations of the active substances of the essential oil. The comparison of our results with the data of the literature gives some details on the different chemo types and the chemical diversity of this aromatic plant with high medicinal and economic value.*
Keywords— Lemon Verbena, Morocco , verbena tea.

Résumé*— En combinant la chromatographie en phase gazeuse et la spectrométrie de masse nous avons identifié les principaux constituants de l'huile essentielle de la verveine odorante (Alyosia triphylla) cultivée au Maroc. Les récoltes effectuées à différents stades de développement de la plante ont permis de caractériser les variations qualitatives des principes actifs de l'huile essentielle. La comparaison de nos résultats avec les données de la littérature apporte quelques précisions sur les différents chémotypes et la diversité chimique de cette plante aromatique à haute valeur médicinale et économique.*

I. INTRODUCTION

La verveine odorante, *Aloysia triphylla (l'hert.) Britt = Lippia citriodora* H.B. et K. appartient à la famille des Verbénacées. Elle est originaire du Chili et a été introduite en Europe vers 1784.

La verveine odorante est un arbuste vivace qui pousse rapidement et peut atteindre dans les meilleures conditions 2 à 3 mètres de hauteur. Ses tiges, droites ramifiées en branches étroites et minces, portent des feuilles entières et lancéolées, un peu rugueuses, caduques en hiver. Elles sont groupées par trois, d'où le qualificatif latin de *triphylla*. D'une couleur vert jaunâtre, elles dégagent un parfum citronné prononcé. Au cœur de l'été, de juillet à septembre, apparaissent à l'extrémité des tiges de longues grappes qui réunissent de nombreuses petites fleurs, blanches, violettes ou rougeâtres (Loaec, 2000).

La verveine est devenue une plante profondément ancrée dans la pharmacopée et l'art culinaire marocains. Elle est très cultivée, notamment, dans les régions de Marrakech, Agadir et Béni-Mellal, dans des jardins familiaux comme dans des plantations industrielles.

Ses cultures furent installées d'abord, vers le début des années 60, dans la région de Ghmat (environs de Marrakech), où elle prit la place du chanvre textile, puis dans la région d'Agadir. Elle occupe aujourd'hui plusieurs centaines d'hectares. Elle est surtout exportée

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séchée en vrac ou conditionnée en infusettes (Hadni, 1982).

Au cours des années 80, sa culture occupe le premier rang dans l'exploitation agricole des régions d'Ourika, Ghmat et Mesfiwa (environs de Marrakech). 80% de la production en feuilles sèches est destinée à l'exportation. Les prix ont connu des augmentations considérables (100 DH le Kg de feuilles sèches). Sachant que les rendements sont de l'ordre de 1 à 2 tonnes à l'hectare. Les recettes peuvent être estimées de 100000 à 200000 DH à l'hectare. En 2001–2002, le Maroc a exporté environ 437 tonnes de verveine séchée, ce qui représente une valeur d'environ 1.271.200 euros. Ce chiffre représente environ 4 % de la quantité des plantes exportées et 9 % de la valeur réalisée (Hadni, 1987).

Plusieurs auteurs ont rapporté les vertus thérapeutiques de la verveine odorante et de son huile essentielle. Selon Carnat *et al.* (1999), la verveine agit contre l'anxiété et l'insomnie. Elle est dotée aussi d'activités anti-oxydantes grâce à la présence dans sa composition de certains phénols (Zheng, 2001). Les feuilles ont un effet fébrifuge, antidouleurs nerveuses, antianémique et sont carminatives. La verveine est aussi tonicardiaque, hypoglycémiante et anti-migraine (Yousefzadeh et Meshkatalsadat, 2013).

Selon Roulier(1990), l'huile essentielle de verveine odorante est anti-inflammatoire articulaire et antirhumatismale. Elle est aussi calmante du système nerveux, antispasmodique, notamment dans les coliques hépatiques, antinévralgique et stimulant immunitaire en synergie avec d'autres huiles essentielles, tel que, le niaouli.

Sartoratto *et al.* (2004) ont mis en exergue le pouvoir antimicrobien de l'huile essentielle de la verveine odorante contre *Enterococcus faecium*, *Salmonella cholerasuis* et *Candida albicans*. Parodi *et al.* (2013) ont noté une inhibition modérée de l'HE de verveine odorante sur *Aeromonas* sp. Bien avant, Allegrini *et al.* (1973) et Belaich (1979) ont montré que l'HE de verveine présente une action inhibitrice sur *Staphylococcus*, *Klebsiella*, *Neisseria et Escherichia coli.*

Ali *et al.* (2011) ont signalé l'effet antibactérien intéressant de l'huile essentielle de verveine odorante contre *Bacillus subtilis* et *Staphylococcus aureus* et un effet antifongique partiel contre *Phanerochaete chysosporium* et *Trichoderma reesei*. Selon ces auteurs, ces effets antimicrobiens notables sont à corréler avec la richesse de cette huile essentielle en composés majoritaires dotés de pouvoir antimicrobien comme, entre autres, les citrals, le β-caryophyllène, le 1, 8-cinéole et le Citronellol.

Selon Belletti *et al.* (2004), l'effet inhibiteur des molécules aromatiques, comme le citral et le limonène, s'explique généralement par leur interaction avec les composants structuraux des cellules bactériennes. L'interaction avec la bicouche phospholipidique de la membrane cellulaire, entraîne une augmentation de la perméabilité et la fuite de constituants intracellulaires qui sont d'une importance vitale pour la bactérie (Singh *et al.*, 2002).

Khani *et al.* (2012) ont mis en évidence l'effet inhibiteur de l'huile essentielle de la verveine odorante contre deux insectes : *Tribolium confusum* Jacquelin du Val et *Callosobruchus maculatus* (F.). Ces auteurs ont conclu que cette huile essentielle pourrait être utilisée comme agent de contrôle potentiel contre les insectes de produits stockés.

La présente étude s'insère dans une tendance multidisciplinaire qui tente à cerner les différents aspects culturaux et phytochimiques de la verveine odorante pour une meilleure optimisation de son utilisation dans les domaines pratiques, en l'occurrence dans le domaine thérapeutique et le domaine cosmétique.

II. MATERIEL ET METHODE

Afin de mieux connaître les caractéristiques de l'huile essentielle de la verveine odorante et afin de suivre les fluctuations de sa composition chimique, cinq échantillons d'huiles essentielles ont été distillés et analysés.

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Fig.1: Verveine odorante en floraison

Ces échantillons sont issus de plantes provenant d'une parcelle qui se trouve à Tnine Ourika, à 35 km au sud-est de Marrakech (Maroc), à une altitude de 840 m. La culture a été menée selon la méthode biologique sans utilisation de produits chimiques.

Une fois les coupes réalisées, la biomasse végétale est aussitôt transportée à la distillerie de la société *Nectarome* se trouvant à 400 m de distance du lieu de la culture. Les coupes ont eu lieu selon des dates précises (Tableau 1).

Tableau.1 : Calendrier des coupes de différents lots de verveine odorante

Numéro de lots	Date de distillation	Origine
260603	26 Juin 2003	Tnine ourika(Marrakech)
050703	05 Juillet 2003	Tnine ourika(Marrakech)
120703	12 Juillet 2003	Tnine ourika(Marrakech)
160703	16 Juillet 2003	Tnine ourika(Marrakech)
200703	20 Juillet 2003	Tnine ourika(Marrakech)

Les caractéristiques de distillation des différents lots de verveine sont les suivants :

- Partie distillée : sommités fleuries à l'état frais.
- Alambic en inox : capacité 1000 litres, équipé d'un essencier, lui aussi en inox, de capacité de 70 litres.
- Technique de distillation : entraînement à la vapeur d'eau.
- Source de la vapeur : chaudière délivrant 300 kilo vapeur à l'heure et fonctionnant avec du gasoil, l'eau est issue d'une station de pompage, puis passe par un adoucisseur avant d'accéder à la chaudière.
- Durée de la distillation : 2 heures.
- Rendement en huile essentielle : 0,15 % en moyenne.

Les échantillons d'huiles essentielles obtenus ont été analysés en France au laboratoire de la société *Phytotagante* à Toulouges, en utilisant la chromatographie en phase gazeuse selon les conditions opératoires ci-dessous:

Chromatographe en phase gazeuse : HP5890
Détecteur : FID (Détecteur à ionisation de flamme)
Colonne : INNOWAX, 60 m, 32 mm, 0.5 mm.
Isotherme initial : 60°C.
Programmation : 2°C/min jusqu'à 250°C (20 min au total).
Intégrateur : HP 3380 A.
Volume injecté : 0.5 ml.
Gaz vecteur : Hélium (1 ml/min).

III. RESULTATS ET DISCUSSIONS

La distillation des cinq échantillons a permis d'avoir des rendements comparables, qui tournent autour d'une moyenne de 0,15 %. D'après Carnat e*t al.* (1999), ce pourcentage varie entre 0,2 et 1%.

L'étude des différentes huiles essentielles obtenues, a montré l'existence de quelques trente six composants appartenant à différentes familles chimiques. Le chromatogramme ci-après illustre la richesse quantitative de l'huile essentielle de verveine odorante analysée.

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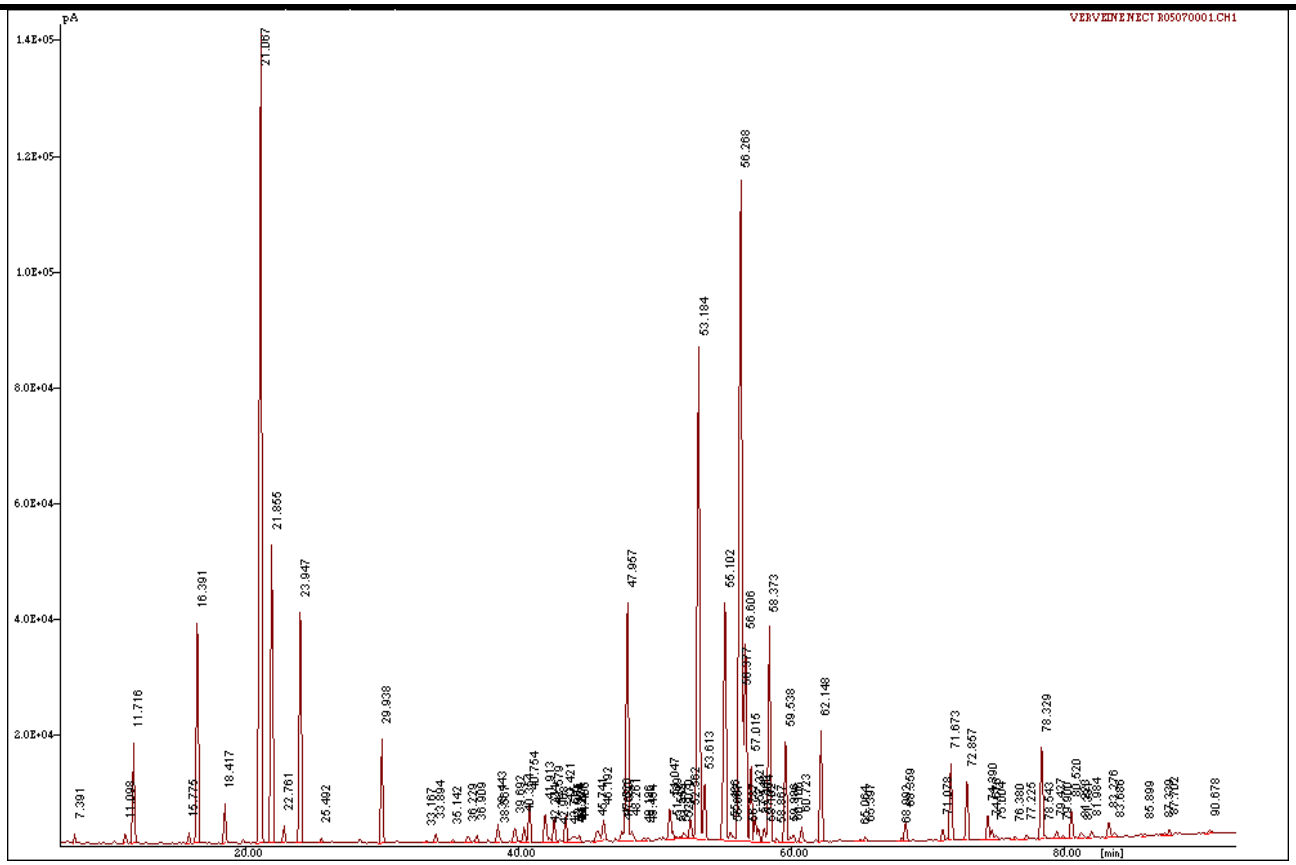

Fig.1: Chromatogramme de l'H.E de verveine, cas de l'échantillon 050703

Les composants identifiés de l'H.E de verveine odorante marocaine sont donnés dans le tableau 2:

Tableau.2: Liste des composants identifiés dans les cinq échantillons étudiés

		ECHANTILLONS D'H.E DE VERVEINE				
LOTS		**260603**	**050703**	**120703**	**160703**	**200703**
Rendement en H.E ⇨		**0,12 %**	**0,15 %**	**0,2 %**	**0,1 %**	0,17 %
Pics	**composants**	**%**	**%**	**%**	**%**	**%**
1	Alpha- pinène + alpha- thujène	1,33	1,19	1,16	1,24	1,19
2	Bêta- pinène	0,15	0,13	0,13	0,13	0,13
3	sabinène	**3,08**	**2,67**	**2,49**	**2,8**	**2,73**
4	myrcène	0,54	0,46	0,43	0,39	0,49
5	limonène	**19,54**	**16,47**	**16,6**	**16,87**	**16,9**
6	cinéole	**4,27**	**4,44**	**4,58**	**4,17**	**5,01**
7	cis bêta- oïdium	0,29	0,22	0,19	0,19	0,23
8	trans. bêta- ocimène + gamma-terpinène	**4,02**	**3,2**	**2,67**	**3,08**	**3,27**
9	para cymène	0,23	0,06	0,06	0,05	0,00
10	méthyl-6-hepten-5-one-2	1,84	1,44	1,39	1,64	1,72
11	rosefurane	0,17	0,14	0,14	0,16	0,15
12	trans-4-thujanol	0,24	0,29	0,26	0,24	0,36
13	citronnellol	0,5	0,41	0,37	0,48	0,47
14	cis cis photocitral	0,35	0,31	0,31	0,29	0,3
15	Alpha- copaène	0,74	0,59	0,68	0,76	0,61
16	Bêta- bourbonène	0,13	0,13	0,14	0,13	0,13
17	linalol	0,4	0,39	0,37	0,39	0,38

18	terpinène-4-ol	0,22	0,19	0,22	0,22	0,19
19	Bêta- caryophyllène	**6,05**	**4,81**	**5,19**	**5,4**	**4,72**
20	Bêta- cédrène	0,32	0,27	0,23	0,25	0,21
21	allo aromadendrène	0,71	0,52	0,59	0,64	0,53
22	néral	**7,41**	**11,44**	**11,34**	**9,08**	**11,58**
23	Alpha- terpinéol	0,97	1	1,01	0,96	0,99
24	zingibérine + germacrène D	**5,95**	**5,01**	**4,64**	**4,86**	**5**
25	géranial	**10,41**	**16,45**	**16,24**	**13,36**	**16,37**
26	Acétate de géranyle	**4,75**	**3,99**	**3,17**	**4,1**	**3,68**
27	bicyclogermacrène + bêta-curcumène	1,29	1,19	1,06	1,21	1,05
28	cadinène	0,32	0,22	0,3	0,28	0,22
29	ar. curcumène	**4,43**	**3,92**	**4,62**	**5,32**	**3,89**
30	nérol	**1,66**	**1,71**	**1,52**	**1,5**	**1,55**
31	géraniol	**1,77**	**1,9**	**1,77**	**1,83**	**1,8**
32	oxyde d' isocaryophyllène	0,21	0,2	0,24	0,25	0,19
33	oxyde de caryophyllène	1,47	1,43	1,85	1,98	1,32
34	trans nérolidol	0,96	0,88	0,86	1,1	0,86
35	spathulénol	1,75	1,67	1,93	2,51	1,52
36	cadinol	0,5	0,47	0,52	0,64	0,42

Parmi les 36 composants identifiés, l'intérêt a été porté sur dix éléments ayant une concentration individuelle d'au moins 1,5 %. L'ensemble des composants choisis représentent un pourcentage total moyen d'environ 68,5 % de la masse totale de l'huile essentielle. L'étude portée sur un pourcentage en concentration de 68,5 %, est significative. Le tableau 3, donne la liste des éléments sélectionnés pour le reste de l'étude.

Tableau.3: Concentrations des principaux composants de cinq échantillons de l'huile essentielle de verveine odorante cultivée au Maroc

Lots	**260603**	**050703**	**120703**	**160703**	**200703**	**MOYENNE**
Composants						
Limonène	19,54	16,47	16,6	16,87	16,9	**17,28**
1-8-cinéole	4,27	4,44	4,58	4,17	5,01	**4,49**
β -caryopyllène	6,05	4,81	5,19	5,4	4,72	**5,23**
Néral	7,41	11,44	11,34	9,08	11,58	**10,17**
Zingibérine + germacrène D	5,95	5,01	4,64	4,86	5,00	**5,09**
Géranial	10,41	16,45	16,24	13,36	16,37	**14,57**
Acétate de géranyle	4,75	3,99	3,17	4,1	3,68	**3,94**
Ar.curcumène	4,43	3,92	4,62	5,32	3,89	**4,44**
Nérol	1,66	1,71	1,52	1,5	1,55	**1,59**
Géraniol	1,77	1,9	1,77	1,83	1,8	**1,81**
TOTAL	**66,24**	**70,14**	**69,67**	**66,49**	**70,5**	**68,61**

On constate que l'huile essentielle de verveine odorante se caractérise par une présence importante de citrals: le néral et le géranial qui représentent sur les 5 échantillons analysés une moyenne d'environ 24,75%, avec 10,15 % de néral et 14,6% de géranial. Leurs alcools correspondants, le nérol et le géraniol représentent de 3,5 % environ, avec 1,6 % de nérol et 1,8 % de géraniol.

Bellakhdar *et al.* (1994) ont analysé l'huile essentielle des sommités fleuries de la verveine odorante cultivée dans la région d'Agadir (Maroc) qui s'est avérée contenir 9,9% de géranial, 6,9% de néral, 7,4% de 6-méthyl-5-hepten-2-one et 12,4% de 1,8-cinéole. Ces résultats diffèrent des nôtres en ce qui concerne les citrals qui représentent 16,8% contre 24,75% dans la présente étude. Par contre, le 1-8 cinéole est mieux représenté dans l'échantillon d'Agadir (12, 4%) que dans celui de Marrakech (Tnine Ourika) (4,49%).

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Oukerrou *et al.* (2017) ont noté chez l'HE des feuilles de verveine de Ait Imour (région de Marrakech), le trans-caryophyllène oxyde (14,22%), le β-Spathulénol (13,42%), l'Ar-curcumène (11,30%) et le néral (6,37%). A l'exception du néral qui reste, en général, comparable à nos résultats, les autres composants affichent des pourcentages trop éloignés des nôtres, c'est le cas notamment du β-Spathulénol avec 13,42% contre un pourcentage variant entre 1,52 et 2,51 dans notre étude. A signaler que la partie distillée par Oukerrou *et al.* (2017) est la feuille, tandis que dans notre étude, c'est les sommités fleuries qui ont été distillées. Ajoutons à cela, que Ait Imour se situe à une altitude de 1669 m contre seulement 840 m dans notre zone d'étude. Ces résultats mettent en évidence les variations biochimiques de l'huile essentielle en fonction de l'organe soumis à l'hydro-distillation et vraisemblablement sous l'effet de l'altitude.

Cette approche comparative pourrait avoir un grand intérêt dans la caractérisation des différentes huiles essentielles de verveine provenant de différentes zones biogéographiques du Maroc et résultant de la distillation de différents organes de la plante.

Au Portugal, Santos-Gomes *et al.* (2005), ont mis en exergue des variations de la composition chimique de l'HE de verveine en fonction de l'organe distillé. Le géranial (26,8-38,3%), le néral (20,8-29,6%) et le limonène (5,7-20,6%) sont présents dans les feuilles et dans les fleurs. Le 1-octène, le 1-octène-3-ol, le p-cymène, le (Z)-β-ocimène et le trans-carvéol, ne sont identifiés que dans l'huile essentielle extraite des fleurs. Alors que le β-citronellène, le β-pinène, l'acétate de néryle et le trans-calaménène, ne se trouvent que dans l'huile essentielle issue des feuilles.

En Turquie, Özek *et al.* (1996) ont noté que l'HE de verveine issue des feuilles contient 14,8% de limonène et 17,9% de citrals alors que celle issue de branches feuillues contient 18,6% de limonène et 27,9% de citrals.

Ibrahim *et al.* (2015) ont trouvé dans l'huile essentielle de verveine odorante cultivée en Egypte : le d-limonène (6.3 -16,2%), le 1,8 cinéole (4,7% - 7,3%) et le citral (19,9% - 28,8%).

Hudaib et al. 2013, ont décelé dans l'HE de verveine cultivée en Jordanie du limonène (17,7%), du géranial (10,1%) et du néral (9,8%). Ces derniers résultats sont proches des nôtres, notamment en ce qui concerne le limonène et les citrals.

Dans d'autres études, le taux de citrals atteint des niveaux élevés. C'est le cas notamment en Iran, avec un taux de géranial de 30,67 à 36,87% et de néral entre 21,71 et 28,33% (Shahhoseini et al. 2014).

En Argentine, Di Leo Lira *et al.* (2008), ont décelé une composition de l'HE de verveine, conforme au tableau chimique habituel : le néral (20,0%) et le géranial (29,0%). Dans d'autres échantillons analysés par ces auteurs, la présence de limonène et de citronellal à des teneurs élevés, respectivement de 40,3% et 21,6%, dote cet échantillon d'une certaine originalité. Un autre échantillon contient une proportion inhabituelle de β-thujone qui atteint 73,4%. Toujours en Argentine, Zygadlo *et al.* (1994) ont décelé une composition chimique originale à base de myrcénone (36,50%), de l'α-thujone (13,10%), de la lippifoli-1 (6) -en-5-one (8,87%) et du limonène (6,87%).

Ces spécifications sont d'un grand intérêt pratique et pourraient être utilisées pour la standardisation de la verveine odorante, en caractérisant, entre autres, les variétés riches en substances toxiques, en l'occurrence le β-thujone.

A signaler aussi que le citronellol qui est très minoritaire dans notre étude, avec un moyen de 0,47 %, atteint, dans une autre étude, le pourcentage de 8.87% (Ali et al. 2012).

La figure 2, montre la présence de trois groupes de composés, dans les huiles essentielles analysées, se distinguant nettement sur le plan quantitatif. Le premier groupe est formé de limonène, néral, et géranial ; le deuxième groupe est formé de cinéole-1-8, β-caryophyllène, zingibérine + germacrène D, acétate de géranyle et Ar-curcumène ; le troisième groupe est formé de nérol et géraniol.

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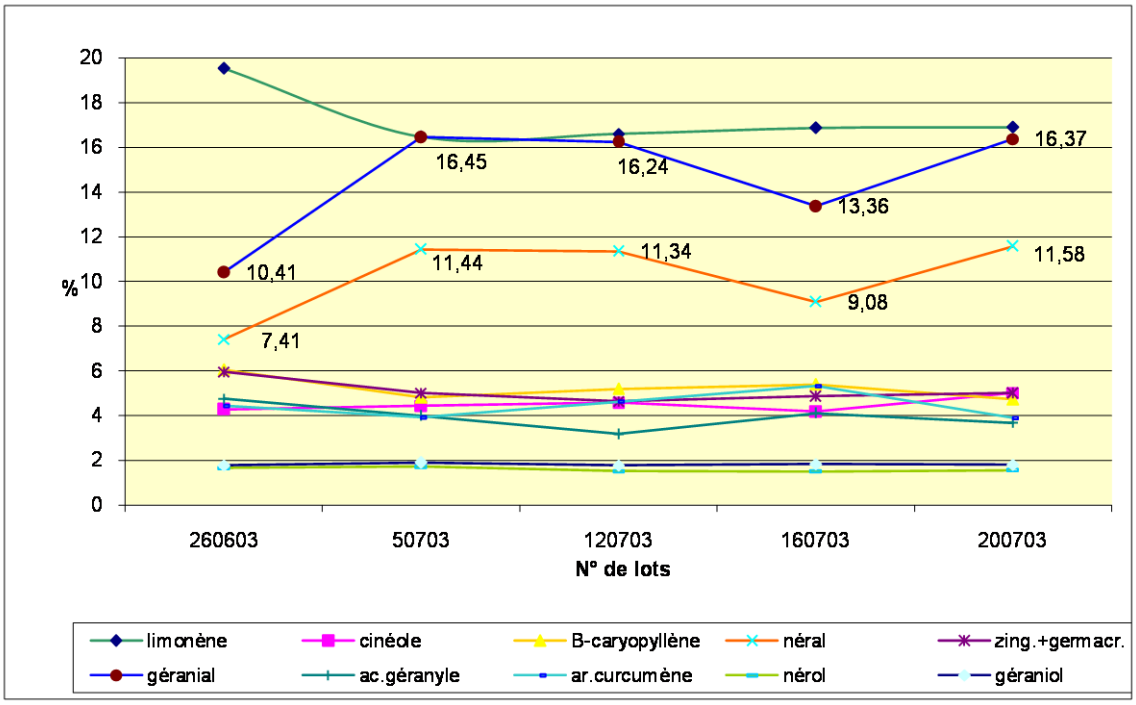

Fig.2: Evolution des concentrations de certains composants chimiques dans cinq échantillons d'H.E de verveine.

Pour le premier groupe, le limonène reste majoritaire par rapport aux autres composés étudiés et ce, pour l'ensemble des échantillons des H.E de verveine analysés. Le taux le plus élevé a été enregistré dans l'échantillon 260603 qui correspond à l'H.E distillée le 26 Juin 2003, il est de 19,5 %. Ce taux se stabilise après autour de 16 % dans les autres échantillons des H.E analysées, numérotées comme suit : 050703, 120703, 160703 et 200703

Le géranial, qui appartient à la famille chimique des aldéhydes voit sa concentration fluctuer en fonction du temps et en fonction des échantillons analysés. En effet, sa concentration passe d'un minimum de 10,4 % dans l'échantillon 260603 à un maximum de 16,45 % dans l'échantillon 050703.

Le taux le plus faible est enregistré pendant le mois de juin, il se stabilise dans les deux échantillons des H.E du 05 et 07 Juillet 2003, avec une valeur autour de 16 %. La distillation réalisée 4 jours plus tard, c'est à dire le 12 Juillet 2003, montre une concentration du géranial dans l'H.E plus faible, elle est de l'ordre de 13 %. Ce taux retrouve la valeur de concentration qui est de 16 % dans la dernière coupe réalisée le 20 Juillet 2003 et qui correspond au lot 200703. La moyenne générale de la concentration du géranial dans les 5 échantillons analysés est de 16,4 % environ.

Quant au néral, appartenant lui aussi aux aldéhydes, il a subi des variations en fonction des périodes de coupes et de distillations. A noter que l'allure correspondant à l'évolution de sa concentration suit parfaitement celle du géranial tout au long de l'étude. Toutefois, cette concentration reste plus faible sur l'ensemble des échantillons étudiés. Sa moyenne sur les 5 échantillons est de 11,6 % environ.

La concentration du néral est de 7,4 % environ au mois de Juin 2003, ce qui correspond à la concentration la plus faible enregistrée tout au long de l'étude. Cette concentration est rencontrée dans l'échantillon d'huile essentielle du lot 260603. Le pourcentage de ce composant évolue rapidement en une dizaine de jours pour atteindre environ 11,5 % dans le lot 050703, correspondant à la distillation réalisée le 05 Juillet 2003. La concentration de ce composant se stabilise à environ 11,5 % dans l'échantillon suivant qui correspond au lot 120703, il s'agit donc de la distillation qui a été effectuée une semaine plus tard.

L'analyse faite sur le lot d'H.E 160703, distillée 4 jours plus tard, montre une chute nette de la concentration de cet aldéhyde, elle atteint environ 9 %.

Dans le dernier lot analysé, référencé 200703, le taux de néral retrouve la valeur de 11,5 %.

Le deuxième groupe est formé des composants biochimiques suivants : le cinéole-1-8, le béta-caryophyllène, le zingibérine + germacrène D, l'acétate de géranyle et l'ar-curcumène (Figure 2).

Ces cinq composés, montrent un comportement similaire vis à vis de leurs concentrations dans les échantillons d'H.E analysées. En effet, aucune variation significative en fonction du temps n'a été constatée. Les différentes concentrations oscillent entre 3 % et 6 % dans les 5 échantillons. Toutefois, on peut remarquer une légère relation de proportionnalité inverse entre le cinéole-1-8 et l'acétate de géranyle, comme le montre la figure 3. On remarque, dans les 5 échantillons étudiés, que lorsque le

cinéole-1-8 augmente, l'acétate de géranyle diminue et vis versa.

C'est dans le lot 120703 où on assiste à la différence la plus importante au niveau des concentrations de ces deux éléments, le cinéole-1-8 représente 4,58 %, alors que l'acétate de géranyle représente 3,17 %, soit une différence de 1,41 %.

Il est à noter que malgré cette proportionnalité inverse, la concentration du cinéole 1-8 reste supérieure à celle de l'acétate de géranyle, sauf dans l'échantillon du 26 Juin 2003 où l'acétate de géranyle présente une concentration légèrement élevé de 4,75 % contre 4,27 % pour le 1-8-cinéole. Ces résultats nous incitent à émettre l'hypothèse d'une relation biosynthétique éventuelle entre le cinéole et l'acétate de géranyle.

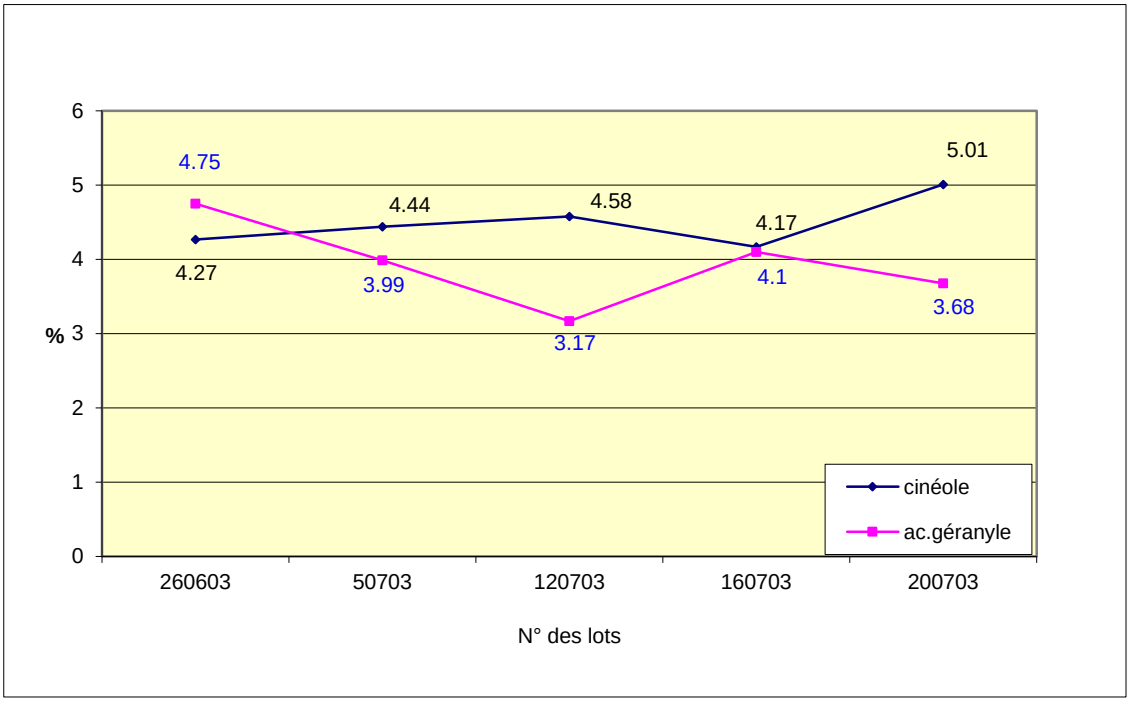

Fig.3: Comparaison et évolution des concentrations du 1-8-cinéole et de l'acétate de géranyle

Le troisième groupe est formé seulement de deux composés chimiques, à savoir le nérol et le géraniol, leur concentration reste stable autour de 1,7 % en moyenne, dans les 5 échantillons analysés. Aucune variation n'a été constatée en fonction des coupes.

Dans la littérature, les variations chimiques qualitatives, en fonction de périodes de coupe, sont fréquentes. Dans cet ordre d'idées, Argyropoulou *et al.* (2007) ont décelé des différences entre l'HE des parties aériennes de verveine récoltées en mai, en pleine croissance, et en septembre, en pleine floraison. De mai à septembre, les taux de géranial et néral ont diminué respectivement de 38,7% à 26,8% et de 24,5% à 21,8% tandis que le taux de limonène a augmenté de 5,8% à 17,7%.

Dans la même optique, Shahhoseini *et al.* (2013) ont analysé, en Iran, les parties aériennes de verveine récoltées à trois stades de croissance. Le géranial, enregistrant le taux le plus élevé au stade végétatif (33,7%), a légèrement diminué en pleine la floraison (32,7%). Le néral est passé de 26,1% au stade végétatif à 25,06 % au stade fruitier.

D'autres facteurs, biotiques ou abiotiques, peuvent influencer la composition chimique de l'HE de la verveine odorante. Dans cet ordre d'idées, Schmidt *et al.* (2016) ont examiné l'effet du gel sur la composition de l'HE de verveine odorante cultivée au Brésil. Le limonène était le plus sensible au gel, représentant 14,36% des teneurs en huile avant et 10,15% après le gel.

Kassahun *et al.* (2011) ont montré que la biomasse foliaire et le rendement en HE de verveine, cultivée en Ethiopie, augmentent avec l'âge de la première à la deuxième année, au-delà de la troisième année ces paramètres commencent à décliner. Prochnov *et al.* (2017) ont noté que les citrals enregistrent les taux les plus faibles en hiver et les taux les plus élevés en automne et en été.

Les conditions culturales peuvent aussi influencer la composition chimique de l'huile essentielle de verveine odorante. Dans cette optique, Moein *et al.* (2014) ont montré que la culture sous serre favorise la synthèse des citrals. Agah et Najafian (2013) ont montré que le séchage à l'ombre augmente le rendement en HE et favorise la synthèse de limonène, néral et géranial.

L'analyse des huiles essentielles de la verveine odorante, a révélé l'existence de deux groupes particuliers de composants chimiques, ayant des comportements

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inverses, c'est à dire, quand la concentration du premier s'élève, celle du second diminue et vis versa (tableau 4 et Figure 4).

Tableau.4 : Les des deux groupes de composés à comportements inverses

Lot analysé	Lot 26-06-03	Lot 05-07-03	Lot12-07-03	Lot16-07-03	Lot20-07-03
(groupe A) %	40,79	47,97	47,97	42,64	48,2
(groupe)%	25,45	22,17	22,2	23,85	22,3

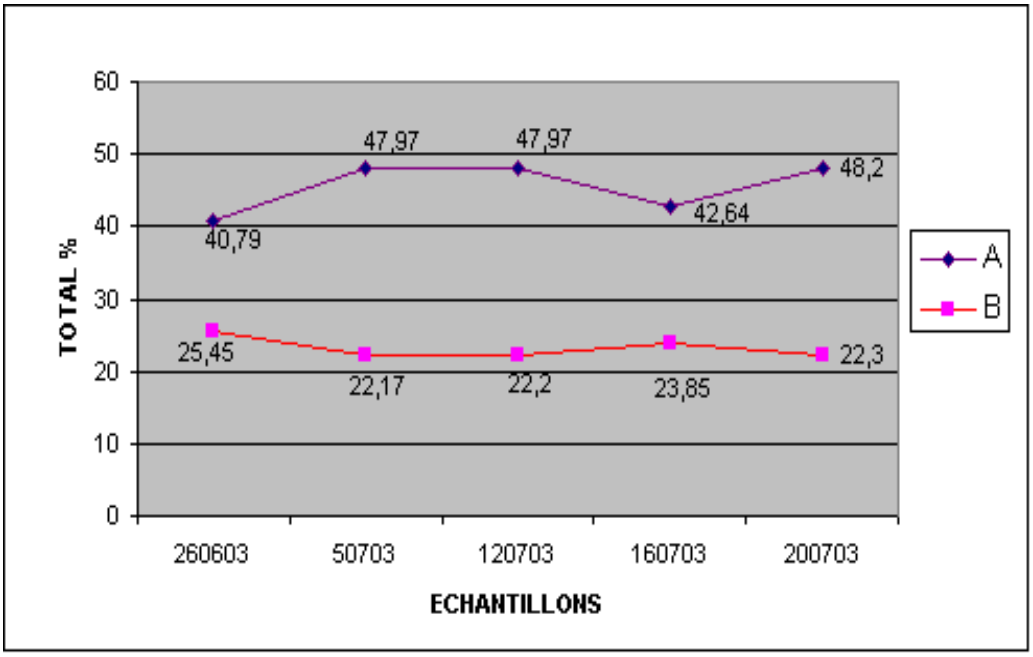

Fig.4: Evolution des concentrations de deux groupes de composés chimiques dans l'H.E de verveine odorante

La figure 4 montre que la courbe A, représente l'ensemble des composés suivants : limonène, néral, géranial, nérol et géraniol, alors que la courbe B, représente l'ensemble des composés suivants : 1-8-cinéole, béta-caryophyllène, zingibérine + germacrène D, acétate de géranyle, et ar-curcumène.

Toutefois, ces fluctuations sont beaucoup plus accentuées dans la courbe A par rapport à la courbe B. Par ailleurs, on note la présence de 3 points de concentrations maximales dans la courbe A, qui correspondent aux coupes du 05, du 12 et du 20 juillet, ces concentrations tournent autour de 48 % environ, alors que pour la courbe B, ces mêmes points correspondent plutôt à des concentrations minimales, qui tournent autour de 22 % et inversement, les deux points de concentrations minimales relevées par la courbe A et qui tournent autour d'une moyenne de 41,7 %, correspondent aux deux points de concentrations maximales repérées dans la courbe B et dont la moyenne est d'environ 24,6 %.

Cette inversion au niveau des différentes concentrations étudiées, montrent un vraisemblable phénomène biosynthétique entre les deux groupes de composés chimiques (A et B).

Dans la littérature des phénomènes similaires ont été mis en évidence. En effet, Gomez *et al.* (2006), ont montré que les niveaux de géranial qui ont évolué d'environ 40% en septembre jusqu'à 27% en novembre, ont connu une augmentation par la suite, en corrélation inverse avec les taux d'oxyde de caryophyllène et d'ar-curcumène.

Toutefois, il reste à élucider ce « phénomène » de la relation biosynthétique qui existe entre les différentes molécules de cette huile essentielle.

IV. CONCLUSION

D'après ce qui précède, on peut soupçonner l'existence d'une certaine relation biosynthétique entre les différents composants chimiques de l'huile essentielle de verveine analysée dans notre étude. L'ensemble des composants du groupe A, présente une proportionnalité inverse avec le groupe des composants du groupe B, ceci montre bien une certaine relation entre les composants et une possibilité de transformation chimiques des molécules tout au long des saisons. De même, on a bien noté le caractère inverse des concentrations entre le 1-8-cinéole et l'acétate de géranyle dans les 5 échantillons analysés.

Etant donné que les aldéhydes ont des propriétés bactéricides, il devient intéressant, principalement dans le

domaine de l'aromathérapie, de faire la coupe de la verveine odorante de la région d'étude et de pratiquer sa distillation à partir de la fin de la première semaine de juillet, ceci procure à l'huile essentielle une forte concentration en aldéhydes, en l'occurrence le groupe des citrals, composé du néral et du géranial.

Nasser Al-Deen *et al.* (2015) en analysant les huiles essentielles de verveine odorante cultivée en Syrie, ont montré que les citrals augmentent en juillet, ce qui dote l'HE extraire des feuilles récoltées pendant ce mois d'un pouvoir antibactérien plus important contre *Escherichia Coli.* C'est, d'ailleurs, à la richesse en citrals (géranial et néral) qu'est rapportée l'activité bactéricide (El Aziz, 1991).

D'autres études ont mis en exergue que le néral et le géranial pourraient induire l'apoptose dans la leucémie lymphoïde chronique (De Martino *et al.,* 2009).

A noter aussi que le taux, relativement élevé, de zingibérine et germacrène D (environ 5%) dote la verveine odorante de notre zone d'étude d'une certaine originalité et incite à pousser la recherche dans ce sens.

Il est donc fondamentale de bien connaître la meilleure période de coupe et de distillation, afin de permettre une utilisation optimale de cette huile essentielle dans les différents domaines d'applications.

Les divergences quantitatives et qualitatives enregistrées dans l'huile essentielle de verveine odorante cultivée de part le monde incitent, sur le plan pratique et appliqué, à bien caractériser les chémotypes et les conditions culturales pour bien potentialiser les utilisations de cette plante aromatique dans les domaines thérapeutiques et cosmétiques.

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Pollution and Foraging Behavior of Pied Kingfisher *Ceryle rudis* in Bujumbura Bay of Lake Tanganyika, Burundi: Conservation Implications

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Abstract-- *Lake Tanganyika is threatened by overfishing, deforestation, climate change and water pollution. Very few studies have investigated the effects of pollution on semi-aquatic communities such as aquatic birds. This study investigates the effects of pollution on the foraging behavior of pied Kingfisher Ceryle rudis in the Bujumbura bay of Lake Tanganyika. We use data on foraging behavior of this bird species collected in 2002 as background and data collected over two periods during which we assessed change in water environmental parameters. The sampling site is located in the littoral zone next to the channel mouths that collect rainfall and wastewater from Bujumbura city. The results show a change in the number of observations of foraging pied kingfishers during the course of the day with a significantly lower number of observations in the morning than in the study carried out in 2002. The most frequent foraging behavior also changed from direct dives in 2002 to repeated hovering before diving in 2016. These changes could be accounted for by pollution as the water parameters data collected during the same time periods reveal an increase in turbidity while dissolved oxygen levels dropped. Increased turbidity may have caused reduction of visibility of these visual predatory birds. Attention should be given to measures toward alleviation of pollution of Lake Tanganyika for the conservation of semi-aquatic communities which are members of this deep and ancient lake ecosystem.*

Keywords— Lake Tanganyika, pied kingfisher, piscivorous, pollution, semi-aquatic communities.

I. INTRODUCTION

As one of Africa's great lakes, Lake Tanganyika is described as an extraordinary reservoir of freshwater biodiversity (Salzburger *et al*. 2014). It has more than two thousand species of aquatic plants and animals and over 1200 faunal species (vertebrates and invertebrates) recorded within only 10% of the explored lake shore (Patterson and Makin, 1998). Among animal groups, fish show extraordinary diversity; 289 endemic species makes up 89% of fish diversity of the lake (Snoeks, 2000). The lake is also a source of income, animal proteins and drinking water for an estimated 10 million inhabitants of its catchment area (Mölsä *et al*. 1999). However, Lake Tanganyika is threatened by overfishing, deforestation, climate change and water pollution (West 2001, Odada *et al*. 2003, Nkotagu 2008, Kashaigili and Majaliwa 2010, O'reilly *et al*. 2003, Verburga and Hecky 2009).

Numerous studies have documented the response of the lake communities to pollution, mainly sedimentation (Cohen *et al*. 1993; Alin *et al*. 1999; Eggermont and Verschuren 2003; Donohue *et al*. 2003; Cohen *et al*. 2005; Donohue and Irvine 2004; McIntyre *et al*. 2005; Marijnissen *et al*. 2009). However, these studies have focused on benthic and fish communities mostly in rocky habitats and took diversity and abundance into account. On the other hand, there are very few, if any studies that investigated the effect of pollution on other aquatic and semi-aquatic communities. Semi aquatic communities such as aquatic birds can be influenced by the same lacustrine environmental features which affect fish and invertebrates (Paszkowski and Tonn 2000). They are therefore part of the lake ecosystem although they are rarely integrated in studies of Lake Tanganyika ecosystem.

Located in the northeastern and most populated part of the lake, Bujumbura, the capital of Burundi, is the largest city around the lake. It is regarded as the main source of pollution for the lake (Yu *et al*. 2017). Several rivers and water channels discharging into the Bujumbura bay of Lake Tanganyika cross the city. Their contaminating effect is presumably increasing with growing urbanization and the lack of wastewater treatment facilities (Yu *et al*. 2017). This pollution affects the lake communities including semi-aquatic communities and its effect needs to be investigated.

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The pied kingfisher, *Ceryle rudis* Linné 1758, is an aquatic bird occurring in Bujumbura bay of Lake Tanganyika (Hakizimana *et al*. 2011). It mostly feeds on fish by diving. Their distribution and foraging can be influenced by environmental parameters such as turbidity and alkalinity (Reyer *et al*. 1988). Increased pollution with higher turbidity at Bujumbura bay may have affected pied kingfisher's foraging behavior and efficiency since turbidity causes light reduction and leads to reduced sight of this piscivorous predator (Abrahams and Kattenfeld 1997; Strod *et al*. 2004). In this study we investigate the effect of pollution on the foraging behavior of pied Kingfisher in the Bujumbura bay of Lake Tanganyika. We exploited data collected by Hakizimana *et al*. (2011) for background information. Data collected during these two studies times were used to assess change in water environmental parameters.

II. MATERIAL AND METHODS

2.1 Study area

Data was collected at former "Cercle Nautique" (-3.38996°, 29.35023°), a partially protected small bay in Bujumbura, Burundi the north-eastern part of Lake Tanganyika (Fig. 1). Bujumbura has a tropical Climate of Aw type according to Köppen-Gierger classification; the mean monthly temperature ranges from 28.6°C and 31.9°C and the mean monthly precipitation is between 0 and 136.4 mm. The rain season covers the period between October and April whereas the dry season, with monthly rainfall below 50 mm, runs from May to September.

The study site is located in the littoral zone of Lake Tanganyika next to the mouths of channels that collect rainfall and domestic water from Bujumbura City and the surrounding hills (Ndikumana *et al.* 2013). The water from these channels consists mainly of rainwater runoff, household and industrial effluent and sediment discharge as well as nutrients loads from deforested areas.

2.2 Data collection

This study is based on the comparison of our results with data collected by Hakizimana *et al*. (2011). Therefore, data have been collected partially following the methodology in Hakizimana *et al*. (2011). We recorded the number of observations of pied kingfishers, time of the observations and foraging behaviors namely single hovering, repeated hovering prior to dive and direct dive without hovering. Data was collected from 8 to 11 am and 3 to 5 pm in September and October 2016.

We used data on physico-chemical parameters to compare environmental conditions in 1988 (Gasana, unpublished data), the period of Hakizimana *et al*'s (2011) study and data collected during our study period in 2016 (Nduwayezu, unpublished data). Collected parameters are pH, dissolved oxygen and turbidity.

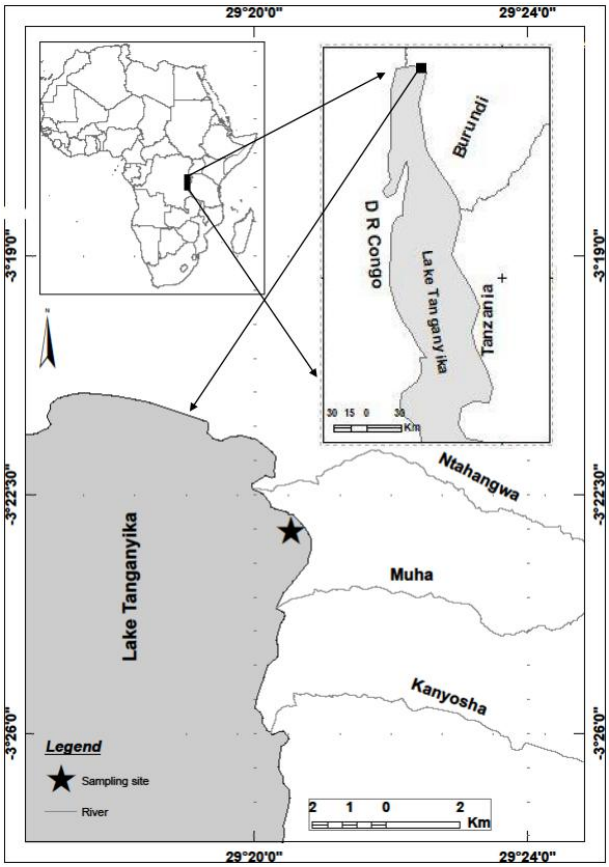

Figure 1: Study area showing the sampling site location

2.3 Data analysis

Statistical analyses were performed using ANOVA test in RStudio version 1.0.143 (R Development Core Team 2013) and graphs were generated using SigmaPlot version 11.0 (SigmaPlot, Systat Software Inc., Erkrath, Germany).

III. RESULTS

The number of observations of foraging kingfishers at the site during our study period tended to change in the course of the day with the highest values during midday and late in the afternoon. These values showed no remarkable change for the data from the 2002 study (Table 1, Fig. 2). Mean number of observations comparison from ANOVA show that there is a significant difference in all numbers of observations of foraging kingfishers at the site except for the time span from 15h-16h (Table 2). The number of observations was significantly higher for the 2002 study for the period between 8 am and 10 am in the morning.

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Table.1: Number of observations for sampled hour spans during the study period at former "Cercle Nautique", Bujumbura in 2016

Date	8h-9h	9h-10h	10h-11h	15h-16h	16h-17h	Total
01 September	1	2	8	4	7	22
03 September	0	4	9	2	5	20
04 September	1	3	8	4	6	22
15 September	2	4	7	5	8	26
17 September	0	3	6	4	12	25
18 September	1	2	5	3	6	17
06 October	0	2	6	6	8	22
08 October	0	5	9	8	10	32
09 October	0	3	6	4	7	20
20 October	1	4	7	2	5	19
22 October	0	3	6	5	2	16
23 October	1	4	7	7	5	24
Average	**0.58**	**3.25**	**7**	**4.5**	**6.75**	**22.08**
Total	**7**	**39**	**84**	**54**	**81**	**265**

However, the mean number of daily observations was not significantly different between the two studies (Table 2). Results on comparison of relative importance of fishing behavior of foraging pied kingfishers show these aquatic birds performed more direct dives in 2002 than in 2016 whereas they performed single hovering and repeated hovering more often in 2016 than in 2002. We found direct diving to be its largely dominant behavior in 2002 with a double percentage compared to other fishing behaviors (Fig. 3).

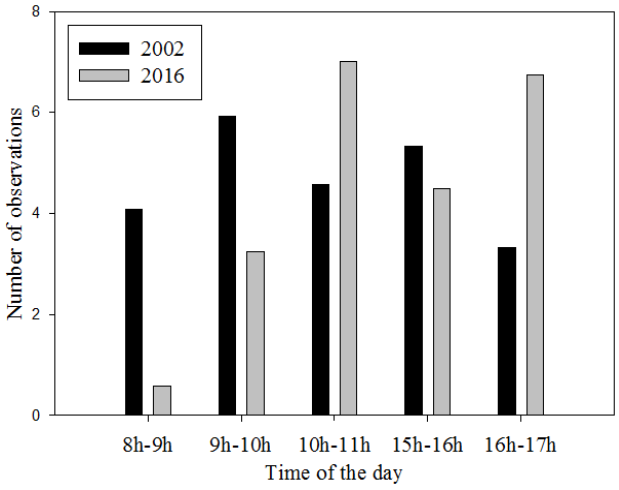

Fig.2: Comparison of observations of foraging kingfishers from 2002 and 2016 data for one-hour intervals along the day.

Tableau 2: ANOVA summary table of mean observations comparison for sampled hour span between results from 2002 and 2016.

	8h-9h	9h-10h	10-11h	15h-16h	16h-17h	Total observation
Mean sum of square	73.5	42.667	35.042	4.167	70.042	225.417
F	13.06	12.83	11.88	1.096	16.95	55.816
p-value	0.002*	0.002*	0.002*	0.307	0.0005*	0.313

*: significantly different

Comparison of the physico-chemical parameters of water in the study site between the two studies periods reveals major change in two measured water parameters. Turbidity has considerably increased while dissolved oxygen value has decreased from 1987 to 2016. On the other hand, between the same time periods pH value showed a slight decrease (Fig. 4).

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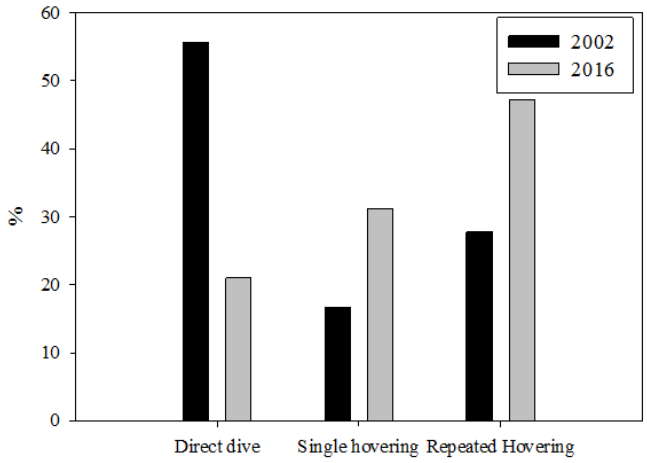
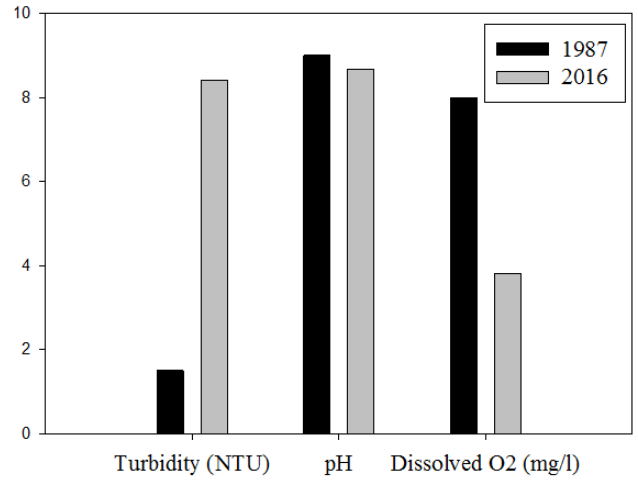


Fig.3: Comparison of fishing behavior ratio from data for observations from 2002 and 2016



Fig,4: Variation in water environmental parameters between 1987 (Gasana, unpublished data) and 2016 (Nduwayezu unpublished data).

IV. DISCUSSION

The number of observations of foraging kingfishers at the site seemed to change in the course of the day while there was no remarkable change in the number of observations for the study carried out in 2002. The number of observations was significantly lower in the morning compared to the results from the 2002 study and was significantly higher from 10 am and at the end of the afternoon. However, the difference in the number of observations between the two studies from 15 to 16 h was not significant. According to EPA (United States Environmental Protection Agency) guidance, turbidity is due to suspended matter (Kumari *et al*. 2014) and causes light attenuation leading to reduced visual range of sighted organisms (Vogel and Beauchamp 1999). Piscivorous birds are visual predators and their foraging efficiency is affected by turbidity (Abrahams and Kattenfeld 1997; Strod *et al*. 2004). The reduced number of observations of foraging pied kingfishers in our study site may have resulted from reduced visibility. This in turn could be due to increased turbidity as the number of observations of foraging kingfishers increased at midday when high sunlight contributed to more visibility. In fact, data on turbidity showed a remarkable increase from 1987 to 2016. However, the mean number of daily observations has not changed significantly between the two surveys, suggesting that pied kingfishers are still using this site for foraging but with an obvious shift in foraging timing.

Pied kingfishers fishing behavior differed from our study and the study carried out by Hakizimana *et al*. (2011) in 2002. The most frequent foraging behavior in 2002 was direct diving which is remarkably high in proportion compared to our survey where it is less frequent. The other foraging behaviors, single hovering and repeated hovering are less in proportion in 2002 but are dominant behaviors in 2016 with repeated hovering being the most frequent. Like the change in foraging timing, this change in foraging behavior could be explained by the increase in turbidity of the water at the study site. Hovering before diving is a behavior that allows the bird to hunt over large water surfaces and locate prey; thus hovering takes more time when water is more turbid. Previous studies have reported changes in the dominant foraging behavior of pied kingfishers from dives from perches to dives from hovering positions when the waters became turbid (Douthwaite 1976; Laudelout and Libois 2003).

Water parameter values measured in 1987 and 2016 have remarkably changed except pH. Turbidity has remarkably increased whereas dissolved oxygen has sensibly decreased during that period. This trend suggests that the studied littoral zone of Lake Tanganyika has undergone pollution. Our results are corroborated by a recent study that reported that rivers crossing Bujumbura city and inflowing Lake Tanganyika have become more contaminated over the past two decades (Yu *et al*. 2017). Although the nutrient loading depends on availability of nutrients in the drainage basin, Yu *et al*. (2017) argued that this change was primarily intensified by the human activity across the watershed. Another study conducted at our study site showed that there was a significant pollution by nutrient loads from water collectors discharging into the lake (Ndikumana *et al*. 2013).

Although the north-eastern part of Lake Tanganyika has been described the most polluted part of the lake, few studies have assessed the effect of this pollution on organisms. Our study shows that foraging behavior of pied kingfisher at littoral zone of Lake Tanganyika near Bujumbura city may have been affected by pollution. Foraging timing and behavior of pied kingfisher has changed in the past fourteen years but the site near

Bujumbura city is still used by this piscivorous species as a foraging site. Our study suggests that despite the detrimental effect of pollution on the ecology and behavior of pied kingfisher, the study site still offers a foraging habitat for this species. Therefore attention should be given to measures toward alleviation of pollution of Lake Tanganyika especially by anthropogenic activities from Bujumbura city not only for preservation of human health and aquatic animals but also for conservation of semi-aquatic communities which are members of Lake Tanganyika ecosystem. Since our study didn't take into account all the hour-intervals of the day due to logistic reasons, we recommend a more complete investigation and studies assessing the effects of pollution on communities of this deep and ancient lake.

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Effects of Fruit Thinning and Percentage of Retained- Fruitlets on Fruit Quantity and Quality of Siam Mandarin Cultivar at Kintamani, Bali, Indonesia

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***Abstract*—** *Fruit thinning in Siam mandarin (Citrus nobilis var microcarpa L.) has been applied in order to improve quality of the fruit involving bigger or higher fruit weght, the sweet of the fruit hence higher market price. The experiment of hand thinning was conducted in farmer's orchard in Bayung Gede village, district of Kintamani, Bangli regency, Indonesia province from 18 June until September 2015. The objective of the experiment was to investigate the effects of stage of fruit thinning and percentage of retained fruitlets on quantity and quality of fruits of siam mandarin cultivar. A randomized complete block design with three replicates was used in the experiment. Two treatment factors were the stage of fruit thinning (stage of fruitlet size of 10 mm, 30 mm and 50 mm) and percentage of retained fruitlet on a branch (100%, 75%, 50% and 25%). Results of experiment showed that thinning at the stage of 30 mm fruitlet size significantly increased fruit diameter fruit diameter, weight fruit^{-1}, and fruit weight bunch^{-1}. Thinning at the stage of 50 mm fruitlet size could only increase sugar content in the fruit of siam mandarin when retained fruitlets on the branch were 25%. Thinning at the stage of 10 mm and 30 mm fruitlet sizes resulted in higher ratio of sugar:acid content (7.34 and 7.39 respectively) than thinning at that of 50 mm. The size of fruit (diameter and fruit weight) and ratio of sugar:acid content were not significantly affected by percentage of retained fruitlets on a branch.*

Keywords*— *Fruit thinning, fruit size, ratio of sugar:acid content, Siam mandarin (Citrus nobilis var microcarpa L.).

I. INTRODUCTION

Siam mandarin cultivar (*Citrus nobilis var microcarpa* L.) is one of citrus species grown in district of Kintamani, Bangli regency, Bali province of Indonesia. As a species with many small fruits (1000-2000 fruits year^{-1}) give this species fruit quantity and quality problem. Quantity problems involve too many small fruits bunch^{-1}. Due to high competition for assimilates among fruits particularly on a branch, the size of fruits are mostly small, fruit taste more sour than sweet and sometimes suffered from pathogen which become quality problems. Those problems consequently result in lower price of this commodity. Too many fruits on a tree can cause over weight to the tree and less and uneven of sunlight penetration and oxygen circulation to the fruits.This condition could result in increasing number of fruit lost. Bigger fruits have more cells compared to smaller fruits. Fruits produced by unheavy trees will have bigger cells than those heavy ones (Ouma, 2012). One way to increase the quality of citrus fruits is by thinning. Fruit thinning is defined as reducing flowers or flower cluster or fruitlets after fruit set (Ouma, 2012). Fruit thinning is conducted to reduce number of fruits on a bunch or branch (Hardy, 2008). The ideal fruit number of siam mandarin is 6-8 fruits bunch^{-1}. Fruit thinning has been done citrus farmers (mandarin and navel orange) in Washington (Hardy, 2008) or in other citrus production center for export. Fruit thinning is usually imposed at the beginning of II stage of fruit growth (stage of cell expansion) at stage of 30-40 mm fruitlet size. Fruitlets size of <30 mm more difficult to find in the canopy (Hardy, 2008), while those of >40 mm will be too big to discard.

In Indonesia, fruits of siam mandarin cultivar has been classified based on weight fruit^{-1} into four class those are A, B, C and D. Class A has fruit diameter of 7,1 cm or 151 g fruit^{-1}, class B has fruit diameter of 6,1-7 cm or 101-150 g fruit^{-1}, class C has fruit diameter of 5.1-6.0 cm or 51-100 g fruit^{-1}, class D has fruit diameter of 4.0-5.0 cm or 50 g fruit^{-1} (Khairani and Dalapati, 2006).

Siam mandarin cultivar at Kintamani, Bali, Indonesia has fruit characteristic of fresh, relatively sour small fruis, yellowish skin colour and productivity of 40-60 kg tree^{-1}year^{-1} (Anonymous, 2015b). Siam mandarin farmers in

Bali have been reluctant to thin their fruits, therefore most fruits produced in the area are relatively small compared to orange species of keprok, hence they could not compete with the price of other imported mandarins or oranges. Besides, there is limited information available on stage of fruit thinning and percentage of retained fruitlets on siam mandarin cultivars. The objective of the present experiment was to investigate the effects of stage of fruit thinning and percentage of retained fruitlets on quantity and quality of fruits of siam mandarin cultivar in Kintamani area.

II. MATERIALS AND METHODS

The experiment was conducted at farmer's orchad in Bayung Gede village, district of Kintamani, Bangli regency, province of Bali, Indonesia. The location was at 800-900 m asl with annual amount and day of rainfall arround 142 mm and 9 days year^{-1}. Average temperature was 20-24^{o}C (Anonymous, 2015b).

Results of soil analysis of experimental land showed a neutral soil pH of 6.7, organic C of 2.66% (moderate), total N of 0.12% (low), available P of 576,05 ppm (very high), exchangeable K of 105,74 ppm (low), and soil texture of loamy sand (Anonymous, 2015c). The experiment was commenced at 18 June until September 2015. The experiment was arranged in a randomized completely block design with three replicates and two treatment factors were imposed. The first factor was stadia of fruit thinning (at fruitlet size of 10 mm, 30 mm and 50 mm), while the second factor was percentage of fruitlet retained on a branch (100%, 75%, 50% and 25% of fruitlet on a branch prior to thinning). Fruitlets at 100% did not receive any thinning treatment (control).

Thirty six healthy and almost uniform trees having flowers and young fruits on each branch were randomly selected (tagged) for the experiment samples. Hand thinning was imposed using a sterillized scissor on one selected branch at four directions (north, east, west and south). Variable measured involving fruit quantity (fruit number branch^{-1}, weight fruit^{-1}, fruit weight branch^{-1}, leaf number branch^{-1} and ratio of weight fruit^{-1}:leaf number branch^{-1}) and quality (fruit diameter, sugar content (^{o}brix), citric acid content, ratio of sugar:acids and moisture content). During the experiment there was no fertilizer applied, however cow manure and biofertilizer (finno stimulant) at 25 kg tree^{-1} and NPK fertilizer at 20 kg tree^{-1} were applied after harvest. Fungicide (scor) at 0.5 ml l^{-1} tree^{-1} and insecticide (alika) at 0.5 ml l^{-1} tree^{-1} were applied as well. Fruits were harvested when they were matured enough indicated by yellowish (25% yellow) skin colour and the fruit were not too hard when was pressed. Data were statistically analysis using ANOVA program of Costat and MstatC. Duncan's MRT and Least Significant Different at 5% level were applied to calculate mean comparisons (Gomez and Gomez, 2007).

III. RESULTS AND DISCUSION

Results of statistical analysis showed that fruit thinning had highly (P<0,01) significant effects on fruit diameter, sugar and citric acid contents, and significantly (P>0,05) affected ratio sugar:acid content, however it did not affect leaf number branch^{-1} and fruit moisture content. Percentage of retained fruitlets on a branch significantly (P<0,01) affected only number of fruits bunch^{-1}after thinning, weight of fruits harvested bunch^{-1}, leaf number branch^{-1} and ratio of weight fruit^{-1}: leaf number branch^{-1}. Interaction between stage of fruit and percentage if retained fruits on a branch only significantly (P<0,05) affected fruit sugar content (^{o}brix).

3.1 Interaction Effects Between Stage of Thinning and Percentage of Retained Fruitlets on a Branch

3.1.1 Fruit sugar content

Thinning at stage of 10 mm and 30 mm fruit size did not significantly affect fruit sugar content either at 100% or 75%, 50% and 25% (Table 1). However when thinning was imposed at stage of 50 mm fruitlet size with retained fruitlets of 25%, sugar content was increased by 23.35% (Table 1).

The sweetness of a fruit, which is indicated by sugar content in the fruit is affected by thinning. In the present experiment, high fruit sugar content of 10.67% resulted from thinning imposed at stage of 50 mm fruitlet size. That content was 23.60% higher than that at control (100% retained fruitlets). The other thinning treatments did not increase fruit sugar content (Table 1), which might be due to fruitlets had been large enough with high fruit sugar content, and competition for assimilates was relatively low among less number of fruitlets (25%). In the present experiment, sugar content of siam mandarin was higher than standard sugar content (8%) for industry in Australia (Anonymous 2009).

3.2 Individual Effect of Stage of Thinning and Percentage of Fruitlets on a Branch

3.2.1 Number of fruits harvested bunch^{-1}

Thinning at stage of 10 mm fruitlet size resulted in the highest (3.79 fruits) number of fruits bunch^{-1}which was 19.56% and 51.56% respectively higher than those thinned at stage of 30 mm dan 50 mm fruilett size (Tabel 2). Without thinning (retained fruitlets of 100%) resulted in the highest number of fruits bunch^{-1} (7.48 fruits). Thinning with retained fruitlets of 75%, 50% and 25% significantly decreased number of fruits harvested bunch^{-1} respectively 34,27%, 65,40% and 243,89% compared to those in control treatment. Decreases in number of fruits

due to thinning is one of the reason for increasing the quality of fruits and finally its marketable price (Falivene and Hardy, 2008). In addition, thinning will reduce the competition for assimilates produced in leaves (sources) then translocated to fruits (sinks) (Goldschmidts and Monselise, 1977; Guardiola 1988).

3.2.2 Weight fruit^{-1}

Thinning at stage of 30 mm significantly increased weight fruit^{-1} which made the highest value of 123.95 g, were respectively 29.76% and 8.80% compared to those done at the stage of 10 mm and 50 mm fruitlet size (Tabel 2). Percentage of retained fruitlets on a branch did not significantly affect weight fruit^{-1}. Manual (hand) thinning is an alternative way to increase citrus fruit size (Falivene and Hardy, 2008).

According to Falivene and Hardy (2008) size of fruitlet of 30 mm is the beginning stage of fruit growth, at which the assimilates produced in photosynthesis process is started to use for cell expansion. That may be due to stronger and more determinate effects of the stage thinning. Falivene and Hardy (2008) also stated that stage of fruitlet size of 30mm-40mm is the ideal time to thin citrus. Fruitlets smaller than 30 mm perhaps still small and have not received adequate assimilates, that is way has not been significantly affected by thinning. In addition the smaller fruits are not easy to see and to find.

Hardy *et al*. (2003) suggested that in order to obtain bigger fruits and better quality, first fruit thinning is better to impose at the stage of 25-35 mm through reducing fruit number up to 50%. Thinning at the stage of >40 mm fruitlet size may have been entering the stage of cell expansion, that is why its too big to thin.

Thinning results in carbohydrates more available for remaining fruits to grow and develop, reduce the number of fruit loss and increases leaf carbohydrate concentrations in comparison to control. Similar argumentation was also presented by Guardiola and Garcia-Luis (2000) that thinning is expected to increase citrus fruit size.

3.2.3 Fruit weight bunch^{-1}

Thinning at stage of 30 mm fruitlet size resulted in high fruit weight bunch^{-1} (395.64 g), particularly at 75% retained fruitlets (Table 2). Although that weight was not significantly different from that at stage of 10 mm, it was significantly 30.17% higher than that at stage of 50 mm fruitlet size. The lowest fruit weight bunch^{-1} when thinned at stage of 50 mm fruitlet size was associated with the lowest number of harvested fruits bunch^{-1} (2.50 fruits) (Table 2).

3.2.4 Fruit diameter

Thinning at stage of 30 mm fruitlet size resulted in the biggest fruit diameter (6.66 cm), which was 10.08% and 5.55% bigger respectively than those thinned at stage of 10 mm and 50 mm fruitlet size (Table 3). Percentage of retained fruitlets did not affect fruit diameter. Thinning imposed at stage of 10 mm resulted in small fruits (fruit diameter of 6.05 cm) which was 9.16% smaller than that thinned at stage of 30 mm (Table 3)..

3.2.5 Fruit acid content

Thinning at stage of 50 mm fruitlet size gave the highest (1.74 ppm) fruit acid content, which was respectively 41.46%and 18.37% higher than those thinned at stage of 10 mm and 30 mm (Table 3). Percentage of retained fruitlets did not affect fruit diameter.

3.2.6 Ratio fruit sugar:acid content

There was no significantly different ratio of fruit sugar:acid content resulted from thinning at stage of 30 mm and of 10 mm fruitlets size, however it was 33,88% higher than the ratio when thinning was imposed at stage of 50 mm fruitlet size (Table 3). The highest fruit acid content given by thinning at stage of 50 mm fruitlet size resulted in lowest ratio of sugar: acid content (5.52:1) (Table 3).Therefore, lower acid content (1.23 ppm) at thinning of stage of 30 mm fruitlet size gave higher ratio sugar;acid content (7.39:1) compared to that of stage of 50 mm fruitlet size (Table 3). Although that ratio was considered was still lower than standard for navel orange (10:1), however close enough to that for siam mandarin cultivar (8:1) (Anonymous, 2009).

3.2.7 Fruit moisture content

There was no significant effect neither of stage of thinning or percentage of retained fruitlets on a branch (Table 3). Harvested fruits contained on average of 89.03% moisture. The mandarin trees used in the present experiment were healthy (they were not suffered from serious water deficit and pest and disease), this consequently resulted in no significant difference in fruit moisture as thiining imposed.

IV. CONCLUSSIONS

Thinning at stage of 30 mm fruitlet size significantly increased the fruit quantity (weight fruit^{-1}, and fruit weight bunch^{-1}). Meanwhile thinning at stage of 10 mm and 50 mm fruitlet size did not significantly increase those variables. The quality of fruit (fruit diameter, sugar and acid content and ratio of fruit sugar: acid content) also increased when thinning was imposed at stage of 30 mm fruitlet size. .Fruit sugar content only increased when thinning was imposed at stadia of 50 mm fruitlet size with 25% retained fruitlets. Thinning at stage of 10 mm and 30 mm fruitlet size increased ratio of fruit sugar: acid content as much as 7.34 and 7.39 respectively compared to those when thinned at stadia of 50 mm fruitlet size. Fruit size (diameter and weight fruit^{-1}) and sweetness (ratio of sugar: acid content) were not significantly affected by percentage of retained fruitlets on a branch.

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Table.1: Interaction effect between stage of fruit thinning at different fruitlet size and percentage of retained fruitlets on a branch on fruit sugar content

	Percentage of retained fruitlets on a branch (%)			
Stage of thinning at fruitlet size	100	75	50	25
10 mm	9.88 ab	10.8 ab	9.67 ab	9.83 ab
30 mm	9.00 ab	8.83 ab	9.25 ab	8.63 b
50 mm	8.65 b	8.95 ab	9.93 ab	10.67 a

Notes: Means followed by the same letters in the same treatments are not significantly different at 5% DMRT.

Table.2: Individual effect of fruit thinning stage at different fruitlet size and percentage of retained fruitlets on a branch on number of harvested fruit, weight fruit^{-1}, and fruit weight bunch^{-1}

Treatments	Number of harvested fruits	Weight fruit^{-1} (g)	Fruit weight bunch^{-1} (g)
Stage of thinning at fruitlet size			
10 mm	3.79 a	95.52 c	350.87 a
30 mm	3.17 b	123.95 a	395.64 a
50 mm	2.50 c	113.04 b	276.26 b
5% LSD	0.592	10.855	69.56
Percentage of retained fruitlets on a branch (%)			
100	4.78 a	111.57 a	521.37 a
75	3.56 b	118.25 a	392.55 b
50	2.89 b	105.63 a	293.06 c
25	1.39 c	107.25 a	156.70 d
5% LSD	0.684	-	80.33

Notes: Means followed by the same letters at the same treatments and colomn are not significantly different at 5% LSD

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Table.3: Individual effect of fruit thinning stage at different fruitlet size and percentage of retained fruitlets on a branch on fruit diameter, fruit acid content, ratio fruit sugar:acid content and fruit moisture content

Treatments	Fruit diameter (cm)	Fruit acid content (ppm)	Ratio fruit sugar:acid content	Fruit moisture content (%)
Stage of thinning at fruitlet size				
10 mm	6,05 c	1,47 b	7,34 a	88,65 a
30 mm	6,66 a	1,23 b	7,39 a	89,24 a
50 mm	6,31 b	1,74 a	5,52 b	89,19 a
5% LSD	0,166	0,246	1,285	-
Percentage of retained fruitlets on a branch (%)				
100	6,36 a	1,34 a	7,15 a	88,91 a
75	6,39 a	1,49 a	6,54 a	88,83 a
50	6,24 a	1,60 a	6,49 a	89,07 a
25	6,38 a	1,49 a	6,83 a	89,32 a
5% LSD	-	-	-	-

Notes: Means followed by the same letters at the same treatments and colomn are not significantly different at 5% LSD

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Effect of stocking density on growth performance of monosex tilapia (*Oreochromis niloticus*) with Indian spinach (*Basella alba*) in a recirculating aquaponic system

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Abstract**—** *An experiment was conducted to compare effect of stocking density on growth performance of monosex tilapia (Oreochromis niloticus) with Indian spinach (Basella alba) in a recirculating aquaponic system. The experiment was set-up for 8 weeks under 4 treatments with three replications, where stocking density of tilapia were 30, 50, 70 and 90 fish/tank (300 litre) in treatments T1, T2, T3 and T4, respectively. Water from the tank was recirculated through a vegetable growing tray. Each of the tray was 0.15 m^3in size, which was planted with 12 plants (Indian spinach). The fish of all the treatments was fed two times a day. During the experimental period,the range of water temperature was 27.1 to 31.50 C, pH 7.48 to 8.28, ammonia 0.2 to 2.0 mg/l and dissolve oxygen 5.11 to 6.58 mg/l. At the end of the experiment, average weight gain, final length, specific growth rate (%/day), survival rate was significantly higher in T_1 (30 fish/tank) treatment while the net yield of fish and plant biomass was higher in T_2 (50 fish/tank) treatment.Therefore, the study suggests that stocking density of 50 fish/tank for tilapia, i.e. 167 fish/m^3, is suitable for production of both plant and fish in a recirculating aquaponic system.*

Keywords**— Aquaponic, Stocking density, Tilapia, Indian spinach, Growth.**

I. INTRODUCTION

The demand of aquaculture products is increasing to the consumers, together with the costs related with land and water and also increasing environmental constraints, have determined producers to advance their technological facilities or to implement new engineering solutions to assure the culture of high stocking densities, thus gaining enough fish supplies to cover the production costs and equally, to meet the marketed and s[1].To overcome such situation the aquaponics, an environmental friendly and sustainable food production system may be appeared as a weapon to fight against water scarcity, soil degradation, climate change and the increased population[2].

Aquaponics is a novel alternative method of fish and crop production system by combining aquaculture and hydroponics, a way of growing plants without using soil substrate. The elements which are essential for an aquaponic systemare fish rearing tank, a suspended solid removal component, a bio filter, a hydroponic component and a sump[3].In this method, plants filter waste product means ammonia which is harmful to the fish from the system and utilized them as a nutrient source[4].Very simply, the principle of aquaponic system is fish excrete contains potentially toxic nitrogen compounds, including ammonia which processed into nitrite and then nitrate by nitrifying bacteria which provided in the system. Released ammonia by the fish is not only transformed to nitrate but also removed by the plants from the water [5].The plants utilize this biologically available nutrient from the water for growth, on the other hand, fishes get suitable water quality for their health that also decrease the need to replace water for the fish tanks[3], [6].In an aquaponics system, waste input in the fish tank is reduced through a closed looped system. This symbiotic relationship between fish and plants facilitates to produce multiple crops at a time that results in increased yields while reducing costs and maintenance[7]. In the aquaponic system water, energy and fish feed are the three main physical inputs although vary in size and type of production system[8]. There also need to createa balance of the macro- and micro-nutrient amount that fish can release in the water for a given feed in the aquaponic system; this highly depends on fish species, fish density, temperature, and type of plants[9].Now, it is clear that the supplied feed and stocking density is directly related to maintain the metabolites flow into the aquaponic system

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on the other hand, in aquaculture, 'stocking density' is considered to be one of the important factors that affect fish growth, feed utilization, gross fish yield and economic returns[10], [11].

Tilapia has become the third most significant fish in aquaculture after carps and salmonids [12]. Nile tilapia is commonly cultured tilapia species all over the world that produced over 70% of the cultured tilapia[13]which is also a common choice in aquaponic system[4]. On the contrary, *Basella alba* or Indian spinach is a popular tropical leafy-green vegetable, commonly grown as backyard herb in the home gardens which is a rich source of Vitamin A and C[14].Therefore, the present investigation is carried out to identify the production of mono sex Nile tilapia and Indian spinach with varying stocking density in a recirculating aquaponic system.

II. MATERIALS AND METHODS

Site and design of the experiment

The experiment was conducted for 8 weeks in a recirculating aquaponic system in the wet-field laboratory of Faculty of Fisheries, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh from 6 May to 6 July 2015. The experiment was carried out in completely randomized design having four stocking density, (30, 50,70 and 90 fish/tank for T1, T2, T3 and T4, respectively) with triplicate.

Setting up fish rearing tank and vegetable growing bed

In this experiment, fish were reared in 12 circular plastic tanks having 300-liter capacity and vegetable were grown in rectangular trays prepared with steel sheet of (125×80×15) cm^3 or 0.15 m^3 size.All the vegetable growing trays were filled with small pieces of bricks (works as bio filter). Twelve water pumps (each of 12 watts) were used for elevating water from tank to tray. Fish rearing tanks were arranged arbitrary among the treatments. All the settings were kept under a transparent polythene shed surrounded by bamboo fencing. In this systemfish ate food and release metabolites into the water derived from the food. The metabolites containing water was pumped in the vegetable bed. These metabolites were further metabolized by bacteria (living in small pieces of bricks), and the products of this metabolism were used by plants for nourishment.

Rearing of fish, transplanting of vegetable and feeding

The tilapia juvenileswere collected from a commercial hatchery, Mymensingh, Bangladesh. After acclimatizing and nursing up to desired sized (Table 5), the fingerlings were stocked in the rearing tank. On the other hand, Indian spinach seed were collected from the local market and were grown in the earthen bed up to 15-20 cm size of the seedlings (three weeks) having at least 2–3 true leaves. After three days of stocking fish in the rearing tank, 12 vegetable seedlings/ tray were transplanted in the vegetable bed. Commercially made floating pellets for tilapia from Mega feed limited, Bangladesh were fed twice a day up to satiation. Proximate compositions of floating pellets according to manufacturer and laboratory analysis are shown in Table 1.

Table.1: Composition of experimental pellets as labeled by the manufacturer and by the laboratory analysis

Composition	**Manufacturer value**	**Laboratory analyzed value** (As fed-basis)
Moisture (%)	11	13
Crude Protein (%)	30	28
Crude lipid (%)	3	4
Ash (%)	10	9

Monitoring water quality

The water quality parameters from fish growing tanks were monitored fortnightly during the study period. Water temperature (^{0}C) was recorded using a Celsius thermometer (digi-thermo WT-2) at the experimental site. Dissolved oxygen (DO) and pH were measured using digital oxygen meter (HQ40d multi) and pH meter (sensIONTM + NH_3), respectively. Ammonia level was measured fortnightly using hach kits (Hach Co., Loveland, Colorado).

Fish sampling and growth performance

At the end of 8 weeks of rearing period, all fish from each tank were counted, measured length and weight to observe survival and growth performances. Ten fish carcasses from each tank were pooled, washed with distilled water and stored at -20^{0}C for whole body chemical composition analysis. The following formulas were used to observe the growth performances. *Weight gain (g) = Mean final weight (g) - Mean initial weight (g)*

$$\text{Specific Growth Rate (\% body weight day}^{-1}) = \frac{lnW_2 - lnW_1}{T} \times 100$$

Where,

W_1 = Average initial live body weight (g)

W_2 = Average final live body weight (g)

T = Number of days (Culture period)

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Net yield = No. of fish harvested × Mean weight gainof fish

Survival rate was calculated by the following equation.

$$Survival\ Rate\ (\%) = \frac{Total\ number\ of\ fish\ at\ harvest}{Total\ number\ of\ fish\ at\ stocking} \times 100$$

The utilization of feed was observed through Feed conversion ratio (FCR)by following equation.

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ intake\ (g)}{Live\ weight\ gain\ (g)}$$

Proximate analysis of the feed and fish carcass

The proximate composition of feed and fish was determined according to standard method given by the Association of Official Analytical Chemists (AOAC, 1995).

Moisture content (%) was determined by a thermostat oven at 105^0C for 24 hours until a constant weight obtained. Crude protein was determined indirectly by measuring total nitrogen content by standard Micro-kjeldahl method by using digestor, Model DK20 and automatic distillation by Model¬ UDK152. Crude lipid content was determined by extracting in diethyl ether in Soxhlet apparatus. Ash content was determined by igniting the sample in muffle furnace at 550°C for 6 hours. The following equations were used.

$$\%\ Nitrogen = \textbf{Error!}$$

$$\%\ Crudeprotein = 6.25 \times \%\ Nitrogen$$

$$Total\ lipid\ (\%) = \frac{Weight\ of\ lipid\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

$$Ash\ content\ (\%) = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

Harvesting ofvegetable

The first harvesting was done at 26 days after planting (DAP). Then vegetable was harvested after 10 days interval. The plants were cut manually at a length of 6 inch from the bed level by a scissor. The crop was allowed to grow, and the subsequent three harvests were done at 36 DAP, 46 DAP and 56 DAP. After harvesting weight (g) of the vegetable was recorded.

Statistical Analysis

The obtained data from the experiment were analyzed statistically by one-way ANOVA using statistical software Statistix 10. This analysis was then followed by Tukey test where significant differences in means were observed. Significance level was determined at the 5% level.

III. RESULTS

Water Quality Parameters

In aquaponics system, water quality is one of the most important factor to determinesuitable stocking density. Different physico-chemical parameters such as temperature (°C), dissolved oxygen (mg/l), hydrogen ion concentration (pH), nitrate-nitrogen (mg/l), and ammonia-nitrogen (mg/l) were recorded. Average fortnightly variations of water temperature in different treatments during the rearing period are shown in Table 2. The range of average water temperature of the treatments during the experimental period was 27.1 to 31.50C. The highest temperature was recorded on the 8th week (31.5±1.10C) in the treatment T3 where stocking density was 70 fish/tank and the lowest temperature was recorded on the 6th week (27.1±2.60C) in the treatment T1 where stocking density was 30 fish/tank. However, there was no significant difference (P> 0.05) among the treatments.

Table.2: Fortnightly variation of water temperature (^{o}C) during the experimental period in different treatments

Treatment	2nd week	4th week	6th week	8th week
T_1	28.5±2.1	30.6±1.4	27.1±2 .6	31.3±3.2
T_2	28.4±2.3	30.4±3.5	27.3±3.1	31.4±2.4
T_3	28.2±1.5	30.2±2.6	27.4±2.2	31.5±1.1
T_4	28.3±2.2	30.1±3.2	27.5±1.4	31.2±2.3

Note: Values are given as mean ± standard deviation.

Average fortnightly variations of dissolved oxygen in different treatments during the rearing period are shown in Table 3. The range of dissolved oxygen (DO) concentration was found between 5.11 to 6.58 mg/l. The highest dissolved oxygen concentration was recorded in the 8th week (6.58±0.56 mg/l) in the treatment T_1, where stocking density was 30 fish/ tank, and the lowest dissolved oxygen concentration was recorded in the 8th week (5.11±0.93 mg/l) in the treatment T_4, where stocking density was 90 fish/tank. There was no significant difference (P>0.05) of mean values of dissolved oxygen concentration among different treatments.

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Table.3: Fortnightly dissolved oxygen level (mg/l) during the experimental period in different treatments

Treatment	2nd week	4th week	6th week	8th week
T_1	6.40±0.98	6.33±0.67	6.12±0.78	6.58±0.56
T_2	6.30±0.66	6.23±0.87	6.10±0.59	6.20±0.75
T_3	5.80±0.12	5.44±0.64	5.30±0.32	5.12±0.33
T_4	5.20±0.34	5.12±0.67	5.20±0.86	5.11±0.93

Note: Values are given as mean ± standard deviation.

Hydrogen ion concentration (pH) in water body generally controls considerably the water chemistry. Any sudden fluctuation of pH causes the death of many aquatic species. Average fortnightly variations of pH in different treatments during the rearing period are shown in Table 4. The range of average values of pH were 7.48 to 8.28. The highest pH value was recorded in the 2nd week (8.28±1.11) in the treatment T_2, where stocking density was 50 fish/ tank, and the lowest dissolved oxygen concentration was recorded in the 8th week (7.48±1.34) in the treatment T_2, where stocking density was 50 fish/tank. There was no significant difference (P> 0.05) among the different treatments.

Table.4: Fortnightly water pH measured during the experiment period in different treatments

Treatment	2nd week	4th week	6th week	8th week
T_1	8.26±1.12	7.58±1.23	7.78±1.04	7.72±1.32
T_2	8.28±1.11	7.58±1.53	7.68±1.09	7.48±1.34
T_3	8.23±1.44	7.52±1.13	7.74±1.45	7.59±1.33
T_4	8.23±1.08	7.55±1.53	7.75±1.00	7.63±1.45

Unionized ammonia (NH_3) is highly toxic to fish, but ammonium ion (NH_4^{+}) is relatively nontoxic. In culture condition, the lower the value of total ammonia, the better water quality for fish. Average fortnightly variations of ammonia in tanks under different treatments are shown in Figure 1.

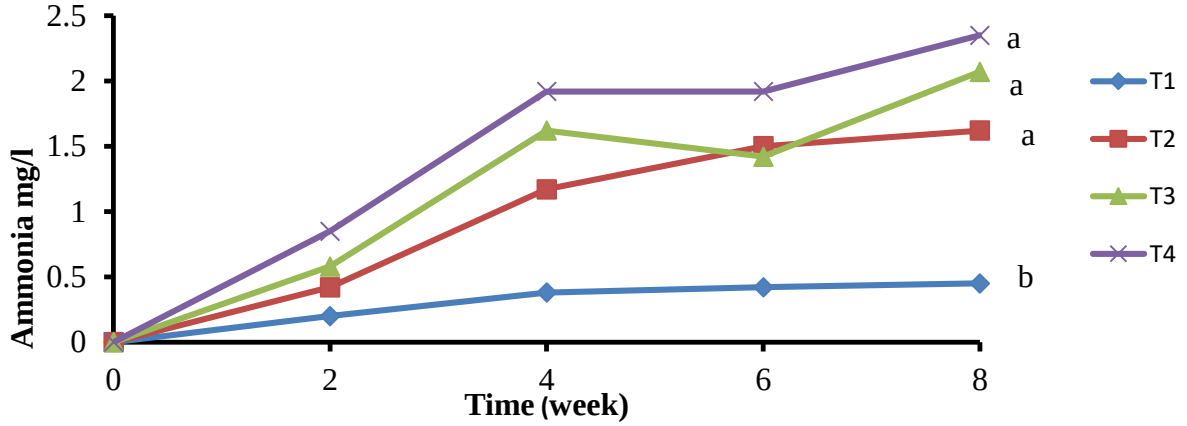

Fig.1: Fortnightly observed ammonia level (mg/l) during the experiment period in different treatments.

The maximum and minimum ammonia concentration was found in the 8th week (2 mg/l) in treatment T_4 where stocking density was 90 fish/tank and 2nd week (0.2 mg/l) in the treatment T_1 where stocking density was 30 fish/tank respectively. In all treatments ammonia level was increased with the increase in culture time. However, it was ammonia was lower in treatment T_1 compared to other treatments throughout the experimental period.

Growth performance of fish

After 8 weeks of rearing, significant difference($P<0.05$) was found in growth performances of fish (Table 5). The highest average weight gain and final length of Tilapia were observed 44.29±5.35g and 15.34±0.64 cm, respectively in the treatment T_1 where stocking density was 30 fish/tank. The lowest average weight gain and final length 19.77±0.47 g and 13.14±0.06 cm, respectively that was observed in the treatment T_4, where stocking density was 90 fish/tank. The specific growth rate (SGR, % day-1) of fish in different treatments was 3.68±0.17, 3.44±0.22, 2.86±0.11 and 2.56±0.04 in the treatment T_1, T_2, T_3 and T_4, respectively. There was significant difference in SGR ($P<0.05$) among different treatments. The highest SGR value and the lowest SGR value were found in treatment T_1 and treatment T_4, respectively. SGR value in treatment T_2, where stocking density was 50 fish/tank was statistically similar to that of treatment T_1.

Table.5: Growth performance of O. niloticus fed with floating pellets in different stocking density

Growth parameters	T_1	T_2	T_3	T_4	Level of significance
Initial weight (g)	5.23±0.04	5.25±0.02	5.24±0.00	5.25±0.20	NS
Weight gain (g)	44.29±5.35^{a}	37.72±5.64^{ab}	24.66±2.02^{bc}	19.77±0.47^{c}	*
Initial length (cm)	5.25±0.35	5.50±0.00	5.60±0.28	5.70±±0.14	NS
Final Length (cm)	15.34±0.64^{a}	14.60±0.18^{ab}	13.51±0.29^{b}	13.14±0.06^{b}	*
Survival Rate (%)	100±00^{a}	99±1.41^{ab}	95.00±3.02^{ab}	92.78±0.78^{b}	*
FCR	1.34±0.09	1.12±0.10	1.38±0.05	1.34±0.06	NS
SGR (%/day)	3.68±0.17^{a}	3.44±0.22^{ab}	2.86±0.11^{bc}	2.56±0.04^{c}	*
Net Yield (kg/m^3)	4.43±0.36	6.23±1.10	5.47±0.62	5.50±0.18	NS

Note: Values are given as mean ± standard deviation. Value in the same column bearing different letters are significantly different at 5%.

The highest survival rate was found in the treatment T_1 where stoking density was 30 fish/tank and the lowest was in T_4 treatment where stoking density was 90 fish/tank. Survival rate of Tilapiain treatment T_2, was statistically similar to that of treatment T_1. In case of FCR and net yield (kg/m^3) were found in case of T_2 though there was no significant difference among the treatments.

Proximate composition of whole body carcasses of fish

The average moisture (%) of *O. niloticus* was 72.67±3.32, 71.36±0.64, 72.75 ±3.18 and 71.15±1.20 % in the treatments T1, T2, T3 and T4, respectively. The average protein (%) of *O. niloticus* was 20.82±0.26, 20.33±0.29, 20.07±2.01 and 20.01±1.97 in the treatments T1, T2, T3 and T4, respectively. The average lipid % of *O. niloticus* was 6.72±0.24, 6.52±0.12, 6.40±0.20 and 6.22±0.11 in the treatments T1, T2, T3 and T4, respectively. The average ash percentage was1.75, 1.69, 1.73 and1.65 % in the treatments T1, T2, T3 and T4, respectively. The average protein, lipid, moisture and ash (%) of in different treatment did not differ significantly (P>0.05) (Table 6), which indicated that stocking density did not affect the proximate composition of *O. niloticus.*

Table.6: Proximate composition of whole body carcasses of O. niloticus fed with floating pellets in different stocking density

Treatments	Moisture	Protein	Lipid	Ash
T_1	72.67±3.32	20.82±0.26	6.72±0.24	1.75±0.12
T_2	71.36±0.64	20.33±0.29	6.52±0.12	1.69±0.23
T_3	72.75±3.18	20.07±2.01	6.40±0.20	1.73±0.34
T_4	71.15±1.20	20.01±1.97	6.22±0.11	1.64±0.41
Level of significance	NS	NS	NS	NS

Note: Values are given as mean ± standard deviation. Value in the same column bearing different letters are significantly different at 5%.

Yield of Indian spinach

The yield of Indian spinach per treatment varied significantly due to different stocking density of tilapia at 26, 36, 46 and 56 DAP (Table 7). At 26 DAP, the highest (3.21±0.18 kg/m^2) yield was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank, while the lowest (1.38±0.08 kg/m^2) yield was found in treatment T_1 where the stocking density of tilapia was 30 fish/tank. The highest yield (3.21±0.18, 2.75±0.44, 2.38±0.35 and 1.31±0.04 kg/m^2) was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank, at 26, 36, 46 and 56 DAP, respectively while the lowest yield (1.38±0.08, 0.82±0.24, 0.53±0.34 and 0.48±0.07 kg/m^2) was recorded in treatment T_1 where the stocking density of tilapia was 30 fish/tank.at 26, 36, 46 and 56 DAP, respectively.

Table.7: Yield (kg/m^2) of Indian spinach in different treatments

Treatment	26 DAP	36 DAP	46 DAP	56 DAP	Total Production
T_1	1.38±0.08^{c}	0.82±0.24^{c}	0.53±0.34^{c}	0.48±0.07^{b}	3.21±0.76^{d}
T_2	3.21±0.18^{a}	2.75±0.44^{a}	2.38±0.35^{a}	1.31±0.04^{a}	9.65±0.45^{a}
T_3	2.75±0.04^{b}	2.15±0.25^{a}	1.65±0.16^{b}	1.28±0.09^{a}	7.83±0.23^{b}
T_4	2.12±0.14^{b}	1.73±0.41^{b}	1.90±0.64^{b}	0.85±0.05^{b}	6.60±0.34^{c}

Note: Values are given as mean ± standard deviation. Value in the same column bearing different letters are significantly different at 5%.

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The total yield of Indian spinach per meter square was significantly different due to different stocking density of tilapia (Table 7). The highest (9.65±0.45 kg/m^2) yield was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank, while the lowest (3.21±0.76 kg/m^2) yield was found in treatment T_1 where the stocking density of tilapia was 30 fish/tank.

IV. DISCUSSION

In this aquaponic system, four different stocking densities were trialed to determine optimum stocking density of *O. niloticus*with Indian spinach. During the experiment the temperature was within suitable range for the grow out of the fish as well as for the aquaponic system. [15]exhibited that the range of water temperature of 26.06 to 31.97°C is preferable for fish culture. In aquaponics, tilapia is usually reared between 22.2 and 23.3 °C in order that the requirements of the fish, the nitrifying bacteria and the aquaponic plants are met, as plants grows better at slightly lower temperatures[16]. At a time, low concentration of dissolved oxygen can decrease water uptake by the roots and thereby decrease leaf growth of lettuce[17].In the present investigation, it is observed a decreasing trend of DO with increasing stocking density. However, the DO range 5.11±0.93 to 6.58±0.56 mg/l exhibited a suitable condition for fish culture and the system as well. pH is one of the key features in aquaponics and should be kept around 7 for smooth nitrification; converting ammonia and providing nitrate for the plants[9], [18].

It is identified that disruption may occur in the nitrification process while pH<6.5 with eventual risk of ammonia and nitrite toxicity. However, the pH was in suitable range for the system. In this present trial it was found that the level of ammonia was increased with the increasing stocking density. Accumulation of urine of fish might have caused the higher ammonia content in higher stocking density. The unionized form of ammonia (NH_3) is highly noxious to fish and other aquatic life, while the ammonium ion ($NH4^+$) is much less toxic. In the aquaponic system at pH of 7, the majority of ammonia nitrogen is in the ammonium ion form. Therefore, the ammonia level was within suitable for the system.

In terms of growth performance, lower the stocking density better the weight gains, SGR (%/day) and final length. This indicated an inverse relationship with increasing stocking density. Some researchers observed similar results, where stocking density was related to average weight gain, SGR (%/day) and length in tilapia[11]. In this trial 50 fish/tank showed the best result. The cause might be the suitable environment for this stocking density. FCR is one of the crucial parameter for the economic consideration. In this trial, the lowest FCR, 1.12±0.10 also observed in case of 50 fish/ tank, though there were no significant differences among the treatments. On the other hand, higher the stocking density lower the survival rate was observed. The cause might be the crowed condition as well as the competition though the fed was supplied up to satiation. In case of net yield highest figure 6.23±1.10 kg/m^3 was observed in T_2 where 50fish/tank were stocked. The lower production in the highest stocking density might be attributed to the fact that the growth and survival rate of fish in treatment T_3 and T_4 was the lowest and the increase in biomass was limited by available space and greater competition. The present study demonstrated that 50 fish/tank was the best stocking density in terms of production for tilapia cultured in the aquaponic system.

In aquaponic system, vegetable production is also an important factor to identify the optimum stocking density. In the present study, the highest (9.65±0.45 kg/m^2) yield was recorded in treatment T_2 where the stocking density of tilapia was 50 fish/tank. The possible reason might be theplant of treatment T_2 got suitable amount of nitrogen for their growth on the other hand in treatment T_3 and T_4 got excessive amount of nitrogen that hampered growth of plant and in treatment T_1 plant did not get enough nitrogen for their growth.

V. CONCLUSION

The effects of stocking density were determined for the *O. niloticus* growth and Indian spinach biomass in one-loop system. Though, the average weight gain, specific growth rate was higher in the stocking density of 30 fish/tank, considering the net yield of *O. niloticus* as well as biomass of Indian spinach in50 fish/tank stocking density is preferable. In aquaponic system, water quality parameters are very much important to run the system smoothly. More precisely, pH and nitrogenous substances arecrucial for the growth of the plant in the system. This nitrogen flow mostly comes from the fish. So, the optimum stocking density should be considered in aquaponic system.

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Effect of *in Ovo* injection with Nano- Selenium or Nano- Zinc on Post-Hatch Growth Performance and Physiological Traits of Broiler Chicks

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Abstract*—The current study was aimed to investigate the effect of in ovo injection with Nano-selenium or Nano-zinc on post-hatch growth performance and physiological traits of broiler chicks under heat stress. Four hundred fertile broiler eggs from cobb500™ flock were randomly divided into four treatments (100 eggs each). First was normally without injection (control), second was injected with 15 ppm Nano-Selenium (SENPs) /egg, third treatment was injected by 15 ppm Nano-Zinc (ZnNPs)/egg and fourth treatment was injected with phosphate buffered saline (PBs) 15 ppm /egg. To study the post-hatch performance, A total number of 240 one day-old chicks were randomly distributed into 4 equal experimental treatments of 60 chicks each. Every treatment was sub-divided into three replicates (20 chicks/ each), were at lasted 5weeks.*

Results obtained could be summarized as follows:

Nano-selenium explained higher chick's weight at hatch, chick's weight to egg weight ratio and hatchability % than all other treatments. At first week of age, the body weight (BW) in the nano-selenium treatment increased than the untreated (control) treatment, although the gastrointestinal tract weight was 0.44 % and the intestine weight was 0.8 %, this is explained by an augmentation in the length of both the length of the small intestine and the gastrointestinal tract by 12 % at 7 day of age. The highest live body weight and body weight gain and the best-feed conversion ratio were recorded with Nano- selenium than all other treatments at 35 day of age.

In conclusion, *under semi-arid conditions, usage the Nano-selenium are not harmful to the embryo (injected with 15 ppm) and can be used to improve the post-hatch performance of broiler under semi arid condition.*

Keywords*—*Nano-selenium, Nano- zinc, performance, In- ovo injection, immunity.

I. INTRODUCTION

Feeding the embryos in fertilized eggs by *In- ovo* injection with some nutritious solutions is beneficial after hatching. The fertilized eggs will quickly growth the gastrointestinal system to improve digestion, metabolism and growth performance of chicks (Ohtsu *et al.,* 2015). Who noted that injecting fertilized eggs into increased meat broiler production, and the trend is likely to continue in future with the advent of development in the field of genetics, nutrition, biotechnology, and developmental biology. *In ovo* nutrition could lead to improved digestive capacity, increased growth rate and feed efficiency, reduced post-hatch mortality and morbidity, improved immune response to enteric antigens, reduced incidence of developmental skeletal disorders, and increased muscle development and meat yield, Ferket (2011). However, further many advanced researches are required to explore further beneficial effects and safety of nano forms of minerals. *In -ovo* injection of minerals has also gained importance as the high-metabolism, fast-growing broiler embryos.

In tropical and semitropical regions, raising broiler out of their thermal comfort zone can cause economic loss in the poultry industry. It has been shown that, in poultry that exposed to elevated temperature showed a desperation on the immune responses, body weight and feed efficiency (Altan *et al*., 2000 and Ohtsu *et al*., 2015), while, plasma corticosterone and heterophil/lymphocyte ratio are improved (Quinteiro-Filho *et al.*, 2012). Cells exposed to elevated temperature showed an inhibition of protein synthesis through alterations in the phosphorylation state of many components of the translational process. This clarified the rise the mortality and a rapid drop in the final body weight (Syafwan *et al.*, 2011). Using fertilized egg injections can overcome such difficulties in under heat stress by in ovo feeding of minerals. Selenium is a trace mineral that is part of an antioxidant enzyme called glutathione peroxidase and is involved in the regulation of energy metabolism, thyroid hormone activation, immune response (Arthur *et al*., 2003 Ludwiczek *et al*., 2004; Lozoff *et al*., 2006 Whitnall and Richardson, 2006; Li and Zhao, 2009). Selenium is an essential micronutrient; plays

an important role in number of biological processes (essential component for the normal development of spermatozoa) and enhancing the activity of the glutathione peroxidase and seleno-enzymes which in-turn can help in protecting the body from the free radicals, which destroy the cells of the body causing autoimmune diseases. The toxicity of nano-selenium is 7 times lower than that of inorganic selenium and 3 times lower than that of organic selenium (Peng *et al.,* 2007). Trace minerals are important nutritional components for imparting immunity and in ovo enrichment can be a way for improving the immune system of the birds.

Zinc important in immune system of the broiler embryo, Kidd, *et.al.* (1992) and Kidd, (2003). Additional zinc in diet of broiler has improved enhanced antibody (Cardoso *et. al*. 2007). Zinc is function of cells mediating non-specific immunity such as neutrophils and natural killer cells (Shankar and Prasad, 1998). .

Therefore, the main objective of the present work was effect of ovo injection by nano form of selenium or zinc on post-hatch physiological and growth performance of broiler under semi-arid condition**.**

II. MATERIALS AND METHODS

First: Pre-hatch: Four hundred fertile broiler eggs from cobb500™ flock were used to investigate to effect of *In - ovo* injection by Nano forms of selenium or zinc on post-hatch physiological parameters and growth performance of broiler under semi-arid condition. The eggs were randomly divided into four treatments (100 eggs per treatment). First treatment was without injection and serves as control, second treatment was injected with 15 ppm Selenium (SENPs)/egg, third treatment was injected by 15 ppm Zinc (ZnNPs)/egg and fourth treatment was injected with phosphate buffered saline (PBs) 15 ppm /egg. The eggs were set in a hatchery in Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Nano form of selenium (SeNPs) was prepared by adopting the procedure of (Razi *et al.*, 2011). Nano form of zinc (ZnNPs) was prepared by adopting the procedure of (Patric, *et al.,* 2016). *In ovo* supplementation of nano selenium and zinc particle's (SeNPs, ZnNPs) (15ppm/egg) was carried out on 7th day of incubation, amniotic route was marked and a small pinpoint hole was made in the broad end of the egg to remove the egg shell by using Topaz Engraver as egg driller and in ovo supplementation was done according to the treatments through the amniotic route using a 24G hypodermic needle (25 mm long) and the pinpoint hole was sealed using wax (Bhanja, S.K., 2004). Eggs were candled on 7th and 17th day to remove infertile eggs. The hatchability percent was calculated using the following formulae:

Hatchability percent (%) = (Number of hatched chicks / number of eggs that were fed in ovo at 21 days) × 100.While, Chick weight is to egg weight ratio= (Chick weight (g)/ Egg weight (g)) ×100.

Second: Post- hatch: to study the post-hatch performance was carried out at South Sinai Experimental Research Station (Ras-Suder City) which fits to the Desert Research Center. A total number of 240 one-day-old chicks were randomly distributed into 4 equal experimental treatments (60 chicks each) with three replicates (20 chicks each).

The experimental treatments were as follows:

T1: Chicks produced from un-injected treatment as control

T2: Chicks produced from the injection of nano-selenium (SeNPs).

T3: Chicks produced from the injection of nano-zinc (ZnNPs).

T4: Chicks produced from the injection with phosphate buffered saline (PBs).

All chicks were kept under similar managerial, hygienic and environmental conditions. The chicks were housed in cages from hatch up to 5 weeks of age. Average of indoor ambient temperature (35.70C ±0.98) and relative humidity (24.2 RH (%) ±1.32) were recorded using electronic digital thermo-hygrometer. Feed was offered *ad libitum* that met NRC (1994) recommendations and fresh water was available all time. Weekly individual live body weight and feed intake were recorded before offering the feed. Feed conversion ratio = (g feed/g gain) were calculated. The chicks were examined against diseases and treated with antibiotics and vaccines to keep them healthy.

The end of the trial, 10 broiler from each treatment were taken randomly, blood samples were withdrawn from the wing vein into tube containing EDTA to examine immediately red blood cells count by means of hemocytometer and hemoglobin concentration according to Jaime method.

Blood samples were centrifuged at 3000 rpm for 20 minutes for the separation of serum and kept at (−20°C) until further analysis. Blood metabolites (total protein (TP), albumin (AL), total lipids (TL), Triglycerides (TG), liver enzymes (alanine transaminase (ALT), aspartic transaminase (AST)), plasma immunoglobulin IgG and IgM concentration, creatinine (Cr) and Triiodothyronine hormone (T3). While, globulin and albumin ratio (A/G ratio) were calculated. All measurements were determined calorimetrically by using kits (By BioSystems S.A. Costa Brava 30, Barcelona (Spain, Barcelona)). Thyroid hormone (Tri-iodothronine) were measured by ELISA technique using IMMUNOSPEC kits supplied by

(Immunospec Corporation, 7018 Owensmounth Ave. Suite 103 Canoga Park, CA 91303, USA).

Statistical Analysis

Statistical analysis was carried out using General Linear Model (GLM) procedures by SAS (2010) using simple one-way analysis of variance according to the model: $Y_{ij} = \mu + T_i + e_{ij}$

Where: Y_{ij}= any observation of i^{th} chicks within j^{th} treatment, μ = Overall mean, T_i= Effect of i^{th} treatment (i: 1- 4), e_{ij}= Experimental error. Significant differences among treatment means were tested by Duncan's multiple range tests, (Duncan, 1955).

III. RESULTS AND DISCUSSION

Effect of SeNPs and ZnNPs on hatchability parameters:

Effects *of In-ovo* nutrition with Nano forms of selenium or zinc on hatchability parameters are summarized in Table 1. No significant variation (P>0.01) existed in the egg weight and hatchability percent between the treatments. While, hatching weight (g) and ratio of chicks weight to egg weight % were significantly different (P<0.05) between treatments. It was noticed that the Nano- selenium recorded the highest value by 4.73, 4.31 and 4.73 %, for hatching weight (g) and ratio of chicks weight to egg weight % and hatchability %, while, Nano-zinc recorded 2.28, 2.17 and 3.30 % for hatching weight (g) and Ratio of chicks weight to egg weight % and hatchability % than control, respectively. Patric Joshua.et al., (2016) recorded that In- ovo feeding of nano minerals (at 5 ppm level of zinc, copper and selenium) did not significantly influence (P>0.01) the hatching weight, ratio of chicks weight to egg weight % and hatchability %.

Growth performance at 7 day of age.

At first week of age, (as shown in Tables 2, 3, 4, 5 and 6), the body weight (BW) in the nano-selenium treatment (T2) increased by 6.06 % than the untreated group, while, the gut weight was increased by 0.44 % and the intestine weight by 1.43 %. This is explained by an augmentation in the length of both the length of the small intestine and the gastro intestinal tract (Table 2). Thus, increased the absorption rate of the gastro intestinal tract, both mineral etc. In addition, this proves the increased of total protein, albumin and total fat by analysis of blood serum, (Table 3). It is seen from results that the chicks that got selenium Nano-particles suspension had different morphological blood indices as compared with those of the control. Which, the augmentation in the immunity of bird represented by the increase of white blood cells. White blood cells was amplified by 1.27 %, red blood cells by 1.53 % and hemoglobin by 68.1 than control. Therefore, this is explained by enhancement of hemoglobin concentration in the cell by 34.2 % and the diameter of the cell increased by 7 (Table 4). Therefore, the first week of age is that increased the protein produced because of growth hormone was directed to the production of immunity to the bird rather than to increase body weight. Immunoglobulin G (IgG), the most abundant type of antibody, is found in all body fluids and protects against bacterial and viral infections (Table 5).

Immunoglobulin M (IgM), which is found mainly in the blood and lymph fluid, is the first antibody to be made by the body to fight a new infection. The immune system of the bird is partly developed at hatch. This correlates the present study as chicks receiving in ovo injection of Se had significantly lower expression of TNF-α gene (Zhang *et al*., 2012). Selenium compound affects the expression of TLRs by modulating the TLR signaling pathway. Expression of both TLR-2 and TLR4 gene was significantly increased in Se injected chick. Chicken embryo tissues are rich in long chain polyunsaturated fatty acids and as a result are very vulnerable to lipid peroxidation. Therefore, SeNPs is a crucial factor in maintaining appropriate antioxidant defense during embryonic development (Surai, 2002). Surai also considered as an immunological enhancement agent to enhance or recover immune functions of the organism (Ru-Duan *et al.*, 1992). Se injected chicks by (Akshat, et al., 2003) has shown a positive effect by increasing the expression. They sided that it can be concluded that in ovo feeding of either SeNPs at 14^{th} day of embryonic age is beneficial for enhancing the immune response. Se has modulated the expression of adaptive or cellular immunity related genes in broiler.

Growth performance at 35 day of age.

Effects of *In -ovo* injection of broiler eggs with nano forms of selenium and zinc on growth performance of broiler during the experimental period (0-5 weeks of age) are shown in table (8). Final weight (gm) and weight gain (gm) values during the whole experimental period increased significantly (P<0.01) with the SeNPs and ZnNPs. The FI of the T2 was significantly decreased compared to other treatments. It is clear that SeNPs decreased feed intake by 9.89 % than that of the controls. Results of feed conversion ratio (FCR) (gm feed/gm gain) revealed a significant difference (P<0.01) among the experimental treatments. It was observed in this study, that SeNPs recorded the best FCR than other treatments and this may be due to the increase in feed intake and reduction of daily weight gain. It is worth to note that SeNPs improved growth performance of broiler chickens compared with other treatments and control. Ferket, P.R. (2011) recorded that *In ovo* feeding could lead to improved digestive capacity, increased growth rate and feed efficiency.

Blood analysis.

The results demonstrate that the effect of *In -ovo* injection of broiler eggs with SeNPs and ZnNPs showed increased (P<0.01) RBC counts by about (24.39 and1.63%), HGB by about (18.14 and 3.49%), MCHC by (4.59 and 2.14%), HCT by about (13.41 and 4.99 %), RDWCV by about (4.00 and 2.00%) and RDWSD by about (9.38 and 6.26%) , while it decreased (P<0.01) MCV by about by 8.78 and 9.46% , MCH by about 4.78 and 6.83% as compared to control, respectively (Table 10). The treatment of SeNPs showed a significant increase in body weight compared to the untreated treatment by about 0.28%, this is supported by increased gastrointestinal length and weight by about 0.54 % and 0.16 %, respectively and stomach weight by 0.69 %. Therefore, there was an increase in absorption and utilization of nutrients. Injected with nanoparticles at the rate of .016 %, but these few were not significant and white blood cells the lymphoma increased significantly by 0.022%, while red blood cells increased by 0.55%, increased platelets by 0.27% and increased cell diameter by 0.27%. Table (10) showed the effect of different treatment on the blood parameters of broiler at 35 day old. These results found that these results are supported by increasing the body weight by increasing the growth hormone and increase the total protein in blood and albumin, although it is an insignificant increase, followed by an increase in the immune proteins. The form of nano elemental SeNPs depends on the presence of protein and it would be interesting to investigate the relationship between protein and Se0 atoms. We found that Se0 atoms adhered easily to protein and that the coexisting system of protein and selenium could directly scavenge ROS (OH•, O•− 2 and H2O2). However, Se0 itself, after being separated from protein by centrifugation, did not show this distinctive property. Zhanga *et al.,* (2012) reported that present the first report of the preparation of a novel selenium form, nano red elemental selenium , with biological activity and effects similar to those of sodium selenite on selenium - dependent enzyme biosynthesis but with much lower acute toxicity (Zhanga, *et al.,* (2012).

It can stabilization that *In- ovo* injection with Nano particles at level 10 nm Selenium in 1 ml at 7 days increases body weight and the immune efficiency of the bird. Akshat *et al*., (2017) found that selenium supplemented chicks had higher cellular immune gene expression in vivo response to mitogen PHA-P was also higher (P<0.01) in ZnNPs or SeNPs supplemented chicks. *in ovo* supplementation of ZnNPs and SeNPs did not improve the post-hatch growth, but increased the growth related gene expression. SeNPs and ZnNPs enhanced cell-mediated immune genes expression in broiler (Akshat *et al.,* 2017).

Nano-particles can evade conventional physiological ways of nutrient distribution and transport across tissue and cell membranes, as well as protect compounds against destruction prior to reaching their targets. In ovo administration of nanoparticles, may be seen as a new method of nano-nutrition, providing embryos with an additional quantity of nutrients.

The research clearly shows that nano minerals are not harmful to the embryo and can be used to improve the post-hatch performance of broiler. However, further many advanced researches are required to explore further beneficial effects and safety of nano forms of minerals.

In conclusion, under semi-arid conditions, can be used the nano form of selenium or zinc are not harmful to the embryo (injected with 15 ppm) and can be used to improve the post-hatch performance of broiler.

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Table.1: Effect of in ovo injection by broiler eggs with Nano form (Mean ±SE) on egg weight, hatch weight of chicks, their ratio and hatchability percent.

Items	egg weight (g)	hatch weight of chicks (g)	Rat of chicks weight to egg weight %	Hatchability %
T1	60.73±0.80	47.32^{b}±0.80	77.92^{b}± 0.78	92.01± 4.11
T2	60.90±0.92	49.56^{a}± 0.75	81.28^{a}±0.90	96.36±3.08
T3	60.81±0.78	48.40^{ab}±0.94	79.61^{ab}±1.02	95.05±2.15
T4	60.8±0.79	47.43^{ab}±0.77	78.01^{ab}±0.83	94.21±3.57
Sig.	ns	*	*	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.

Table.2: Effect of In -ovo injection with broiler eggs with Nano form (Mean ±SE) on carcasses treaties of chicks at 7 day of age.

Items	BW (g)	GW (%)	SIW (%)	DTW (%)	SIL (cm)
T1	462.00± 1.05	3.96±4.02	6.56±3.01	11.32±3.50	44.50±2.40
T2	490.00±1.21	5.74±4.11	7.50±2.71	17.34±3.11	50.57±2.50
T3	448.67±1.89	4.91±4.89	7.37±2.90	13.69±3.72	49.10±2.87
T4	480.83±1.11	5.33±4.56	7.50±2.88	16.67±2.85	48.00±2.32
Sig.	ns	ns	ns	ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.
BW= body weight; GW= gut weight, SIW= small intestine weight, DTW= digestive tract weight and SIL= small intestine length.

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Table.3: Effect of In -ovo injection with broiler eggs with Nano form (Mean ±SE) on white blood cell definition treaties of chicks at 7 day of age

Items	WBC (10^9/l)	LY %	MO%	BA%
T1	53.30^{ab} ± 5.9	52.79^{ab} ±6.01	0.56^{ab} ±0.10	0.01±0.01
T2	68.11^{a} ±5.9	67.28^{a} ±6.01	0.76^{a} ±0.10	0.01±0.01
T3	42.41^{b} ±5.9	42.32^{b} ±6.01	0.16^{b} ±0.10	0.01±0.01
T4	43.63^{b} ±5.9	43.42^{b} ±6.01	0.17^{b} ±0.10	0.01±0.01
Sig.	*	*	*	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant. White blood cells (WBC), Lymphocytes (LY), Basophils (BA), Monocytes (MO), Eosinophils (EOS)

Table.5: Effect of In -ovo injection with broiler eggs with Nano form (Mean ±SE) on hematological parameters of chicks at 7 day of age

Items	RBC($x10^6$)	HGB(g/dl)	HCT (%)	MCV µm (fl)	MCH(pg)	MCHC (%)	PLT
T1	1.23±0.19	6.00±0.28	18.42±1.90	148±4.30	48.92±0.81	32.7±1.01	119±5.8
T2	1.53±0.20	7.1±0.30	20.89±1.99	135±3.85	46.58±0.98	34.2±2.33	84± 5.9
T3	1.2±0.25	5.4±0.29	16.34±0.22	135±3.05	45.58±0.75	33.5±1.88	74±6.4
T4	1.29±0.22	5.8±0.27	16.42±0.99	126±3.99	45.43±0.90	35.7±0.97	71±6.2
Sig.	ns	ns	ns	ns	ns	ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.
RBC= read blood cell; HG= hemoglobin; HCT= hematocrit; MCV= mean curricular volume; MCH= Mean corpuscular hemoglobin, pg; MCHC= Mean corpuscular hemoglobin concentration; PLT= plaited cell.

Table.6: Effect of In -ovo injection with broiler eggs with Nano form (Mean ±SE) on serum analysis of chicks at 7 day of age.

Items	Cr (mg/dL)	AST(g/dL)	ALT(g/dL)	TL (mg/dL)	TC (mg/dL)	TG (mg/dL)
T1	0.90±0.09	184±1.50	20.80±1.46	455±20.32	159±0.04	277±5.34
T2	0.88±0.09	199±1.44	18.40±1.01	567±20.32	137±0.02	169±5.34
T3	0.89±0.09	207±1.46	24.40±1.08	532±20.32	157±0.22	230±5.34
T4	1.20±0.09	198±1.48	23.60±1.44	555±20.32	176±0.03	256±5.34
Sig.	ns	ns	ns	ns	ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.

Table.7: Effect of In -ovo injection with broiler eggs with Nano form (Mean ±SE) on serum analysis of chicks at 7 day of age

Items	TP (g/dL)	Al (g/dL)	Gl (g/dL)	A/G %
T1	2.40±0.48	1.11±0.11	1.31±0.14	0.84±0.08
T2	3.85±0.55	1.49±0.11	2.36±0.14	0.63±0.08
T3	3.56±0.61	1.92±0.11	1.64±0.14	0.85±0.08
T4	2.78±0.50	1.68±0.11	1.10±0.14	0.65±0.08
Sig.	ns	ns	ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig.= Significance, * (P< 0.01), ns = not significant.

Table.8: The effect of in ovo injection of broiler on final weight, weight gain, feed intake and feed efficiency ratio at 35 day of age

Items	Chick Weight (g)	Final weight (g)	Weight gain (g period)	Feed intake (g period)	Feed conversion ratio
T1	47.41^{ab}±0.08	1499.23^{b}±22.82	1451.82^{b} ±21.89	2822.75^{a}±28.22	1.94^{a}± 0.09
T2	49.57^{a}±0.10	1890.43^{a}±24.55	1840.86^{a}±23.08	2543.45^{b}±30.01	1.38^{b}±0.17
T3	46.40^{b}±0.13	1765.27^{a}±26.78	1718.87^{a}±25.66	2886.80^{a}±32.05	1.68^{ab}±0.24
T4	47.43^{ab}±0.18	1540.90^{b}±25.91	1493.47^{b}±26.14	2676.80^{b}±27.08	1.79^{ab}±0.11
Sig.	*	*	*	*	*

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance,* (P< 0.01), ns = not significant

Table.9: The effect of In -ovo injection of broiler on carcass traits at 35 day of age

Items	LBW	HBW	CBW	DTW	DTL	DTW
T1	1514.00±23.22	1459.80±22.22	1324.14±23.63	190.80±5.5	4.08±0.8	190.80±20.5
T2	1940.00±23.22	1881.33±22.22	1798.10±23.63	221.67±5.5	6.29±0.8	221.67±20.5
T3	1811.67±23.22	1770.33±22.22	1622.61±23.63	188.67±5.5	5.66±0.8	188.67±20.5
T4	1725.67±23.22	1668.67±22.22	1349.53±23.63	200.67±5.5	4.80±0.8	200.67±20.5
Sig	ns	ns	ns	ns	ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.

Table.10: The effect of in ovo injection of broiler on white blood cell defection. at 35 day of age

Items	WBCS	LY%	MO%	BAS%
T1	144.77±20.4	60.00±5.6	31.67±6.7	5.00±1.2
T2	142.33±20.4	61.33±5.6	29.67±6.7	5.33±1.2
T3	104.23±20.4	52.00±5.6	38.00±6.7	6.00±1.2
T4	118.00±20.4	53.67±5.6	36.67±6.7	6.00±1.2
Sig.	ns	ns	Ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance,* (P< 0.01), ns = not significant. White blood cells (WBC), 10^{9}/l, Eosinophils (EOS), Monocytes (MO), Basophils (BAS), Lymphocytes (LYM),

Table.11: The effect of In -ovo injection of broiler on hematological parameters at 35 days of age.

Items	T1	T2	T3	T4
Hb (g/l)	11.57±2.51	10.27±2.51	4.00±2.51	10.77±2.51
RBCS (10^{6}/l)	2.78±0.98	2.94±0.98	9.70±0.98	3.00±0.98
HCT %	35.50±2.6	35.30±2.6	2.44±2.6	35.13±2.6
MCV µm (fl)	127.53±20.7	121.67±20.7	31.67±20.7	88.67±20.7
MCH (pg)	44.13±4.5	38.87±4.5	119.67±4.5	22.37±4.5
MCHC %	32.30±5.9	30.37±5.9	38.57±5.9	30.20±5.9
PLT	36.00±5.7	37.00±5.7	40.53±5.7	37.33±5.7
Sig	ns	ns	Ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant. Hemoglobin (Hb), Red blood cells (RBC), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV).

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Table.12: Effect of In -ovo injection with broiler eggs with Nano form (Mean± SE) on plasma fractions (g/dl) of broiler chicks at 35 day of age.

Items	Cr(mg/dl)	ALT(mg/dL)	AST(mg/dL)	TL(mg/dL)	TC(mg/dL)	TG (mg/dL)
T1	0.51±0.08	164.60±20.4	25.34±5.45	409.20±40.80	139.80±5.98	166.80±35.50
T2	0.73±0.09	194.00±20.4	10.73±5.45	431.67±40.80	134.67±5.98	234.33±35.50
T3	0.56±0.11	159.00±20.4	17.87±5.45	353.33±40.80	143.33±5.98	159.67±35.50
T4	0.62±0.07	173.67±20.4	13.63±5.45	342.00±40.80	143.67±5.98	215.67±35.50
Sig.	ns	Ns	ns	Ns	Ns	ns

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.

Table.13: Table 2: The effect of In- ovo injection of broiler on blood parameters of broiler chicks at 35 day of age.

Items	T1	T2	T3	T4	Sig.
TP (g/dL)	2.82±0.48	2.95±0.48	2.48±0.48	2.78±0.48	ns
AL (g/dL)	1.37±0.11	1.45±0.11	1.33±0.11	1.44±0.11	ns
G (g/dL)	1.46±0.14	1.51±0.14	1.15±0.14	1.34±0.14	ns
A/G ratio	1.12±0.08	1.14±0.08	1.21±0.08	1.08±0.08	ns
T3(nmol/ L)	1.38^{c}±0.85	$1.76a^{b}$±0.85	1.98^{a}±0.85	$1.58b^{c}$±0.85	ns
IGG(nmol/ L)	3.96^{d}±0.31	2.98^{e}±0.31	5.43^{b}±0.31	6.75^{a}±0.31	*
IGM(nmol/ L)	5.18^{b}±0.24	5.93^{a}±0.24	3.32^{c}±0.24	4.43^{c}±0.24	*
CK (mg/dl)	176.20^{a} ±20.51	162.00^{b}±20.51	166.00^{b}±20.51	174.03^{a}±20.51	*

a,b: Means within a column with different superscripts are significantly different (P< 0.01).
Sig. = Significance, * (P< 0.01), ns = not significant.

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Flocculation of Reactive Blue 19 (RB19) using Alum and the Effects of Catalysts Addition

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Abstract— *There are a variety of primary coagulants which can be used in a water treatment plant. One of the earliest, and still the most extensively used, is aluminum sulfate, also known as alum. Aluminum Sulfate (Alum) is one of the most commonly used flocculent in waste water treatment processes. Effectiveness of Alum in flocculation process is determined by many factors such as the effluents pH, flocculent dose as well as the use of catalyst to improve efficiency rate of flocculation. Hence special attention to these factors especially the use of catalyst has been brought about by this study. Experiments were carried out using Reactive Blue 19 Dye as the contaminant of waste water and two catalysts namely Calcium Hydroxide ($CaOH_2$) and Poly Aluminum Chloride (PACl) were evaluated. The results obtained proved that indeed after addition of catalysts, removal efficiency rates of Alum can be increased up to 25% using Calcium Hydroxide and up to 35% using Poly Aluminum Chloride compared to Alum alone. The optimum conditions for this study were at pH 5.5 ~7.5, 300 mg/L of Alum 30seconds of rapid mixing time with 300 rpm , 30rpm of mixing rate for 5 minutes and 30 minutes of settling time. Moreover, Alum showed the highest performance under these conditions and using 50 mg/L PACl as catalyst with 98.52% of COD reduction and 90.60% of color reduction. In conclusion, Alum with the support of PACl as catalyst is an effective coagulant, which can reduce the level of COD and Dye Color in Reactive Blue 19 contaminated wastewater.*

Keywords— Alum, Reactive Blue 19, Calcium Hydroxide, Poly Aluminum Chloride and Flocculation.

I. INTRODUCTION

Flocculation is the process of forming larger agglomerates of particles in suspension orof small agglomerates already formed as a result of coagulation through high molecular weight polymeric materials.[1] Flocculation is used in applications such as water purification, sewage treatment, cheese production, and brewing. It is also used in surface and physical chemistry, biology, and civil engineering. Flocculent describes a chemical or substance that promotes flocculation and usually has a positive charge.[2]Flocculation occurs when small particles in a solution lose their repelling forces and begin to attract one another. The small particles then bond together to form "flocks" or "flakes." Under most circumstances, a flocculent is necessary to begin the flocculation process. The most common flocculents are iron, aluminum, magnesium, and calcium. When flocks are fully formed, they can be removed from the solution they are in through traditional filtration methods.[3]

Example:

Aluminum:

Simple : $Al_2(SO_4)_3.\ 18H_2O$ (using for experiment)

Double: $AlK_3(SO_4)_3.18H_2O$ and $Al(NH_4)_3(SO_4)_3.18H_2O$

Iron: $FeCl_3.\ 6H_2O$ and $FeSO_4.\ 7H_2O$

Hydrolysis process of $Al_2(SO_4)_3.\ 18H_2O$

After put alum into waste water, hydrolysis reaction occurs as follows

Step 1 $Al^{3+} + H_2O = Al(OH)^{2+} + H^+$

Step 2 $Al(OH)^{2+} + H_2O = Al(OH)_2^{+} + H^+$

Step 3 $Al(OH)_2^{+} + H2O = Al(OH)_3 + H^+$

Total $Al^{3+} + 3H_2O = Al(OH)_3 + 3\ H^+$

General reaction: $Al_2(SO_4)_3 + 6H_2O = 2Al(OH)_3^{*} + 3H_2SO_4$

The attractive forces between the flocculation substance and the pollutants in wastewater are the force Vander Walls, creation of solid particles with larger size and easily settled down by the gravity.With flocculation process, no chemical reaction occurs which absorbs only physical.

Influence pH to the flocculation process in using $Al_2(SO_4)_3$.

- pH < 4.5: Flocculating process will not occur
- 5.5 < pH < 7.5: good for flocculating process
- 7.5 < pH: decreased efficiency

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Reactive Blue 19 (RB19) was chosen for this study because it is most commonly used material for dying cotton, wood, and silk with molecular weight 626.54 corresponds to 2-(3-(4-Amino-9,10-dihydro-3-sulpho-9,10-dioxoanthracen-4-yl)aminobenzenesulphonyl)vinyl)disodium sulphate. The structure of the Reactive Blue 19 is given as below.[4]

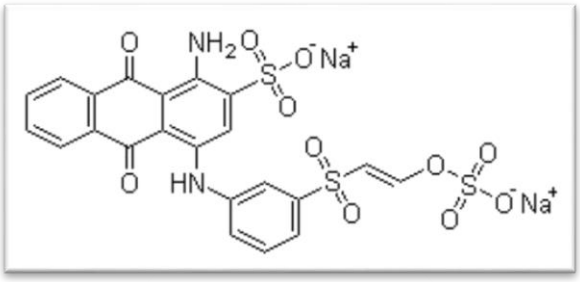

Fig.1: Molecular Structure of Reactive Blue 19 (RB19)

II. MATERIALS AND METHODS

2.1 Sample preparation and materials

A 1000ppm stock solution of RB19 was used to prepare 20 liters of 10ppm concentration waste water. After about 10 mins of homogenous mixing,1-liter sample was immediately transferred to each of the Jars of the Jar Test Equipment.

2.2 Flocculent and catalysts preparation

Stock solution of Aluminum Sulfate (Alum) should be prepared before starting the experiment. 100 g of Alum was pulverized and dissolved to 1 liter in a volumetric Flask and is well shaken to ensure that the Alum is well dissolved. Calcium Hydroxide and PolyAluminum Chloride were prepared from reagent bottles and weighted with an Analytical Balance starting from 25mg for $Ca(OH)_2$ with 25mg increments and 50mg for PACl with 10mg increments respectively.

2.3 Jar Test

A conventional jar test apparatus was used in the experiments to coagulate sample of RB19 solution by using Alum, Alum with $Ca(OH)_2$ and Alum with PACl. It was carried out as a batch test, accommodating a series of six beakers together with six-spindle steel paddles. Besides, the sample of wastewater was adjusted from the initial pH 3.6 to pH about 7.5 in the experiments due to flocculation will not occurin an acidic aqueous phases. The pH was controlled by adding either strong acid (HCl) or strong base (NaOH). Before fractionated into the beakers containing 1L of solution each, the samples of wastewater were mixed homogeneously. Then, the samples ought to be measured for Absorbance and COD for representing an initial concentration. After the desired amount of Alum was added each of the solutions, the beakers were agitated at constant mixing time and speed, which consist of rapid mixing (300 rpm) for 30 seconds and slow mixing (30 rpm) for 5 minutes. After the agitation being stopped, the suspension was allowed to settle for 30 minutes. Finally, a sample was withdrawn using a pipette from the top inch of supernatant for Absorbance and COD measurements which representing the final concentration. All tests were performed at an ambient temperature in the range of 26-30°C. In the experiment, the study was conducted by varying a few experimental parameters, which were Alum dosage (100-500 mg/L) and Catalyst, for $Ca(OH)_2$ dosage(25-150 mg/L) and PACl dosage(10-50mg/L) in order to study their effect in flocculation and obtain the optimum condition for each parameter as well as the best catalyst to be used.

2.4 Data Analysis

The COD test was performed by colorimetric method using HACH Model DR/890 Colorimeter and HACH COD Vials High Range (HR). It is used to measure the oxygen demand for the oxidation of organic matters by a strong chemical oxidant which is equivalent to the amount of organic matters in sample. Moreover, Absorbance was measured by using UV-VIS Spectrophotometer SP-300 Plus which the sample was filled into a sample cell and put into the cell holder for measurement. While the pH of wastewater was measured by using a digital Horiba pH meter F-21. The pH meter was calibrated by using buffer solutions of pH 4.0 and pH 7.0 before starting the experiments.

III. RESULTS AND DISCUSSIONS

Studies on the effects of Alum dosage and the use of Catalysts are the experiments which were conducted in order to investigate the optimum capacity of Alum in flocculation process. Since the Chemical Oxygen Demand (COD) level inRB19 contaminated wastewater is considered as the most important parameter, so it has been used as the indicator on the flocculation capacity of Alum in these experiments by supporting with other important parameter which is RB19 concentration in terms of absorbance.

3.1 Effect of Alum dosage

Dosage was one of the most important parameters that has been considered to determine the optimum condition for the performance of Alum in flocculation. Basically, insufficient dosage or overdosing would result in the poor performance in flocculation. Therefore, it was crucial to determine the optimum dosage in order to minimize the dosing cost and obtain the optimum performance in treatment.The effect of dosage was analyzed at pH 7.5, 300 rpm of mixing rate for 30 seconds and 30 rpm of mixing rate for 5 minutes and 30

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minutes of settling time for a range of Alum dosage which varied from 100 mg/l to 500 mg/l. Besides, the sample of wastewater was adjusted from the initial pH of 3.6 to pH 7.5 due to flocculation will not occur in acidic aqueous phases.[5]

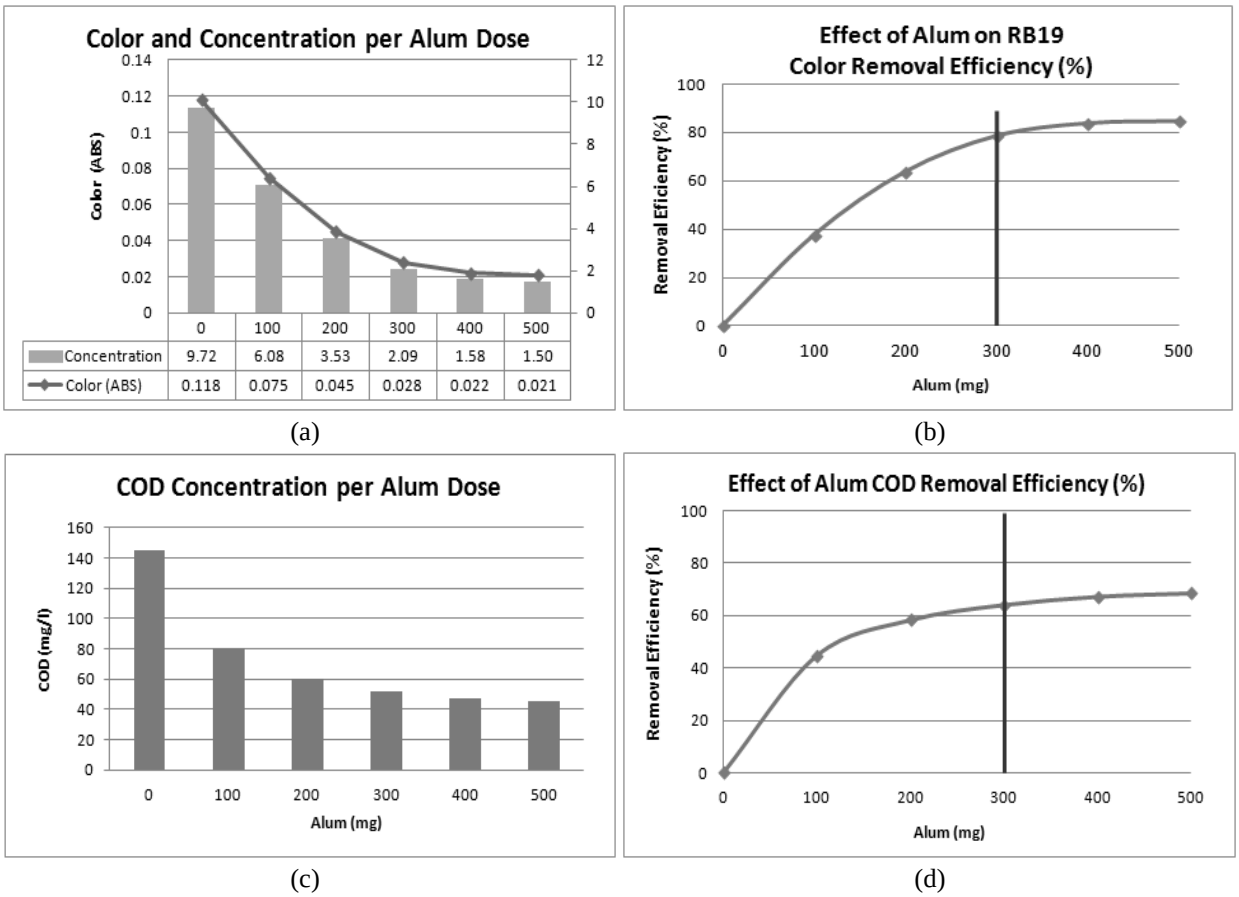

Fig.2: Effects of Alum dosage on (a) Color and Concentration (b) Color removal efficiency(c)COD Concentration (d)COD Removal Efficiency.

The results were presented in Figure 2(a) which showed the effects of Alum dosage on Color in terms of Absorbance and Concentration of RB19. While Figure 2(b) showed the effects of Alum dosage on RB19 color removal efficiency in percentage reduction. Figure 2(c) showed the effects of Alum dosage on COD concentration in mg/L. While Figure 2(d) showed the effects of Alum dosage on RB19 COD removal efficiency in percentage reduction.

From the jar test experiment, Removal efficiency for both Color and COD will be increased when amount of Alum is increased, however efficiency will increase to a point that the increase is insignificant, even if addition of Alum dosage is done.For the Alum dosage of 300 mg/L, Alum recorded the optimum reduction of parameters, which were the reduction of 84.57% and 68.45 % for Color and COD respectively. Therefore, the optimum Alum dosage in this research was 300 mg/L.

3.2 Effect of Catalysts

Addition of catalysts Calcium Hydroxide ($Ca(OH)_2$) and PolyAluminum (PACl) was evaluated using a controlled dosage of Alum which is 300mg/L. The same conditions apply for the rotational stirring speed, agitation and settling time from previous experiment. The range of dosage used for $Ca(OH)_2$ is from 25mg/L to 150 mg/L with 25mg/L increments while for PACL is from 10mg/L to 50mg/L with 10mg/L increments. pH level is also controlled by adding either strong acid (HCl) or strong base (NaOH).

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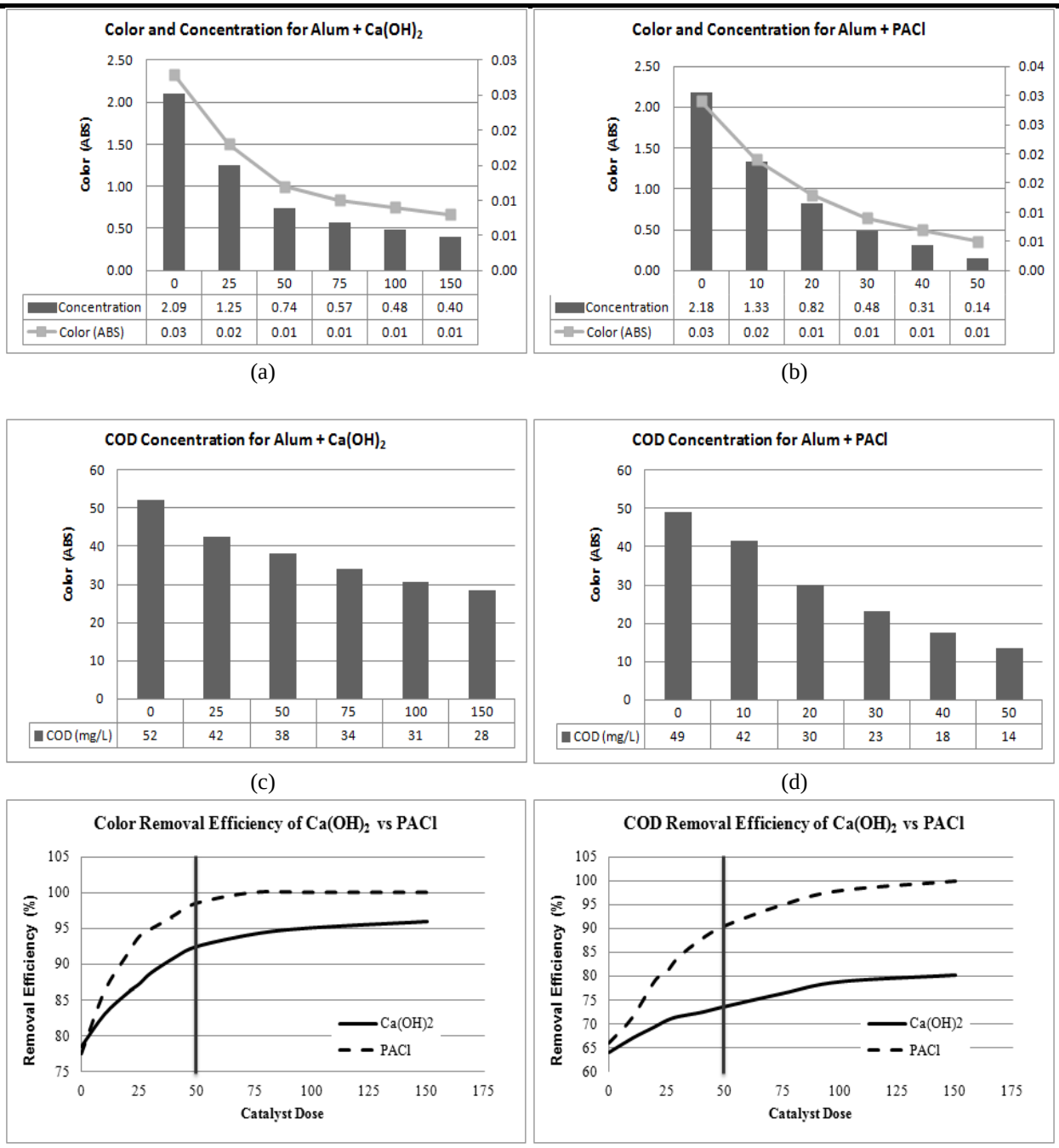

Figure 2. Effects of Catalysts on Color and Concentration for(a)$Ca(OH)_2$,(b)PACl, COD removal for (c)$Ca(OH)_2$, (d)PACl and Comparison of $Ca(OH)_2$ and PACl on (e)Color removal efficiency and (f)COD removal efficiency.

The results were presented in Figure 3(a) which showed the effects of addition of $Ca(OH)_2$ on Color in terms of Absorbance and RB19 concentration. While Figure 3(b) showed the effects of addition of PACl dosage on Color in terms of absorbance and RB19 concentration. The effects on COD removal for addition of $Ca(OH)_2$ and PACl is shown in Figure 3(c) and 3(d) respectively. Figure 3(e) and 3(f) showed a comparison between $Ca(OH)_2$ versus PACl in terms of Color and COD Removal efficiencies.

From the jar test experiment, the curves for the both Color and COD graphs were decreasing as catalyst dosage is increased. Removal efficiency for both Color and COD will be increased when amount of catalysts is increased, however efficiency will increase to a point that the increase is insignificant, even if addition of catalyst dose is done. For

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the $Ca(OH)_2$ dosage of 100 mg/L, $Ca(OH)_2$ recorded the optimum reduction of parameters, which were the reduction of 95.03% and 78.96% for Color and COD respectively. For the PACl dosage of 50mg/L, PACl recorded the highest reduction of parameters, which were the reduction of 98.52% and 90.60% for Color and COD respectively. Therefore, the best catalyst to support Alum in flocculation process is PACl with a dosage of 50mg/L.

IV. CONCLUSION

Removal efficiency both of Color and COD will be increased when amount Alum is increased however efficiency will increase to a point that the increase is insignificant, even if addition of Alum dosage is done. With the use of Alum alone as the flocculent, the speed of removed color is faster than COD. For example with 500mg Alum, color removal efficiency is 84% and COD is 68%.With the support of catalyst $Ca(OH)_2$, the treatmentability of Alum can be improved up to around 22 % for concentration and 25% for COD.$Ca(OH)_2$ produced high sludge volume, so only use $Ca(OH)_2$-like catalyst for Alum, it is not recommended to use $Ca(OH)_2$ alone in treatment by flocculation. In using large amount $Ca(OH)_2$, pH will increase and probably above 7.5, adjustment of pH should be done to promote flocculation process. With the use of PACl as catalyst, the treatment ability of Alum can be improved up to around 35 % for concentration and 37% for COD. When using PACl, use only a small amount to make the removal efficiency increase significantly for both Color and COD.PACl is able to create the larger flocks, the polluting matter can branch this flocks and aids for settling. It is advantageous to use PACl to shorten the treating time and consequently save construction costs (tank, barrel… smaller).PACl is not a chemically corrosive, so it is good for equipments in the treatment process. In conclusion, using PACl instead of other chemical in flocculation is recommended.

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Study of polyembryony and development of molecular markers for identification of zygotic and nucellar seedlings in Khasi mandarin (*Citrus reticulata* Blanco)

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Abstract*—The objective of this work was to evaluate the occurrence of polyembryonic seedlings and other morphological parameters in Khasi mandarin during three harvest years and to identify zygotic (sexual) seedlings from nucellar (asexual) ones grown under in-vitro conditions using molecular markers. Embryos from 27 polyembryonic and 7 monoembryonic seeds of Khasi mandarin were grown in-vitro. DNA from seedlings and mother parent was analyzed using 16 ISSR and 5 RAPD primers, of which 4 ISSR and a set of 3 RAPD primers were effective to identify zygotic or nucellar origin of the seedlings. In-vitro culture enables maximum embryos of each seed to grow, favouring the origin of seedlings to be identified as zygotic. Among 69 tested individuals, 37 zygotic and 32 nucellar seedlings were recognized. In polyembryonic and monoembryonic seeds, 59.6% and 42.8% of the seedlings, respectively, have the sexual origin. Morphological characteristics of seeds and the seedlings generated varied significantly and were not correlated with polyembryony except for the clutch size and the number of branches. Polyembryonic seeds in the cultivar are high, ranging from 50.0%, 55.5% to 83.3% over three harvest years with more clutch size and the possibility of obtaining zygotic plants from them is high. In polyembryonic seeds not all zygotic seedlings were produced by small embryos located at the micropyle. Identification of zygotic seedlings by ISSR and RAPD markers in Khasi mandarin cultivar is efficient and reliable at an early developmental stage.*

Keywords*— *Khasi mandarin, molecular markers, polymorphism, zygotic seedlings.

I. INTRODUCTION

Khasi mandarin (*Citrus reticulata* Blanco) is a commercially popular fruit crop of the Eastern Himalayas exhibiting the common reproductive trait polyembryony (Nakano et al., 2012). Polyembryony has serious significance in citrus breeding since nurserymen use the genetically uniform healthy offspring to revive the old clones that have lost their vigour through regular vegetative propagation (Mondal and Saha, 2014). When citrus is propagated from polyembryonic seeds, the farmers allow them to grow and produce several seedlings (including hybrid and genetically uniform ones) for a long juvenile period of 6-7 years and they normally rogue off the hybrid ones, choosing those with desirable traits and presume that this selection guarantee the origin of the seedling (Mondal et al., 2014). However, this technique is not reliable due to some unusual developments which take place during embryo maturity resulting in the formation of good proportion of twin and triplets in the population (Mondal et al., 2015). In an open-pollinated population, the morphological identification of zygotic embryos becomes even more difficult. Moreover, the orchards are suffering from decline syndrome due to genetic erosion of proper planting material. To assure the variety and good vigour, it is indispensable to identify zygotic seedlings from nucellar ones as zygotic ones are vigorous and can compete with nucellar ones (Andrade-Rodriguez et al., 2005) for establishment of new *Citrus* orchards. Thus, it is imperative to develop efficient methods for differentiating zygotic embryos from nucellar ones at an early stage, for breeding purpose in polyembryonic cultivars. Several PCR based systems are available for genomic DNA analysis, amongst which ISSR, as well as RAPD markers are simple, reproducible, and user-friendly for farmer populations (Rao et al., 2008). These markers enable early selection of the progeny and this quality is particularly useful when a mixed hybrid population has to be rapidly analyzed and differentiated (Goodwin et al., 1997). Though these markers have been proven to identify origin of seedlings in different citrus crosses such as Mandarin (*Citrus reticulata*) and Pummelo (*Citrus maxima*), Yashar [(*Citrus changsha*) × (*Citrus paradisi* × *Citrus reticulata*)], Changsha (*Citrus Changsha)* and Ponkan (*Citrus reticulata*) (Rao et al.,

2008; Golein et al., 2011), but no investigation have been reported using molecular approaches for identification of origin of seedlings in Khasi mandarin cultivar mainly from an open-population system and this finding may considerably contribute to orchard management programs for this cultivar in the Indian subcontinent. The objective of this work was to evaluate the occurrence of polyembryonic seedlings as well as other morphological parameters collected during three harvest years in Khasi mandarin and the hypothesis tested was to see if these harvest years influence on the morphological parameters in the cultivar. The study also aimed to identify zygotic (sexual) seedlings from nucellar (asexual) ones in Khasi mandarin grown under *in-vitro* conditions.

II. MATERIALS AND METHODS

2.1 Plant material

The seeds were collected from an open-pollinated Khasi mandarin (*Citrus reticulata*) trees (around fifteen years old) growing in a citrus farm located in Boko (25.97°N and 91.23°E), Assam, India. Matured and healthy fruits of Khasi mandarin were randomly harvested from six clonal mother trees located in close proximity to each other were collected during three harvest years of November in 2013, 2014 and 2015. Five fruits per plant were collected, amounting to a total of 30 fruits at each time of harvest. Five seeds per fruit were randomly selected to complete a set of 150 seeds and three replications of 150 seeds were used for each harvest year.

2.2 Study of polyembryony and morphological characteristics

To study polyembryony and morphological characteristics, the seeds were surface sterilized as described by Yun et al. (2007). The endocarp and seed coat (testa with tegmen covering all embryos) from each seed were removed and the size (length, mm) of the embryo (measured from the tip of the radicle to the opposite tip of the largest cotyledon) were recorded. The percentage of polyembryonic seeds, the number of embryos per polyembryonic seed and the average number of embryos or clutch size (Kishore et al., 2012) in polyembryonic seeds were recorded. The calculation of the average number of polymebryonic seeds were done by dividing the number of polyembryonic seeds by the total number of seeds and as a whole was calculated by dividing the total number of embryos by total number of seeds (both polyembryonic and monoembryonic) at each harvest.

For polyembryony studies, embryos from 27 polyembryonic and 7 monoembryonic seeds was grown *in-vitro* following Andrade-Rodriguez et al. (2005). *In-vitro* germination data were observed and recorded for three harvest years. The emergence of different seedlings was noted and the number of leaves/seedling, number of branches/seedling, and shoot length/seedling were recorded. Representative embryos from the open pollinated seed population were evaluated to find suitable markers for identifying the zygotic seedlings.

2.3 Plant sample for polymorphism study

Fully expanded leaves from the six mother plants (from which the fruits were collected initially), were collected in plastic bags and kept at -80 °C until DNA extraction. The leaves from *in-vitro* germinated seedlings were also collected and placed in 2 ml eppendorf tubes, labelled and placed on ice until DNA extraction. Six mother plants, 62 seedlings from polyembryonic seeds and 7 seedlings from monoembryonic seeds were used for identification of zygotic seedlings using ISSR and RAPD markers.

2.4 DNA extraction

Total genomic DNA was extracted according to the method described by Doyle and Doyle (1990). DNA concentration and purity were quantified using a Nanodrop 1000 spectrophotometer (Invitrogen, Waltham, Massachusetts, USA) at 260/280 nm absorbance.

2.5 PCR amplification

PCR amplification was carried out in a Veriti thermal cycler (Applied Biosystems, USA) in a final volume of 25 µl reactions containing 25 ng of template DNA, 0.1 mM total dNTPs, 0.3 µM primer, 2.5 µl of 1X PCR buffer with 15 mM of magnesium chloride and 0.5 unit of Taq DNA polymerase (Bangalore genei, Bangalore, India). A total of 50 ISSR primers developed by University of British Columbia, Canada and 10 RAPD primers were custom synthesized from Eurofins Genomics (Bangalore, India). Initially two representative samples each from mothers and progenies were analyzed by PCR amplification. About 10 µl of PCR-amplified product (with 2 µl of 6X loading buffer) was analyzed on a 2% agarose gel in 1X TAE buffer stained with 10 mg/ml of ethidium bromide for visualization of bands for 3 h at 80 V and examined under UV transilluminator using the UVitech gel documentation system (Bangalore genei, Bangalore, India). Molecular weights of the PCR products were estimated by comparing them with 100 bp (100 µg/ml) and *ØX174* DNA /*HaeIII* digest (500 µg/ml) DNA ladders (Bangalore genei, Bangalore, India).

2.6 Data analysis

Mean and standard error values were calculated to reveal statistical significance for the morphological parameters evaluated per harvest year. The data (means of replications) was carried out in triplicates and statistical comparison of data was performed by ANOVA. The Pearson correlations analysis was done using R software 3.2V. A probability value of $p \leq 0.05$ was adopted as the criteria for significant differences. Duncan's multiple range (DMR) test was used for comparison between the

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means of variables which was generated by the SAS program (version SAS 9.3, SAS Institute Inc., Cary, NC, USA). Scoring for polymorphism of the markers was carried out using a band-based method where bands were unambiguously scored as 1 and 0 for present and absent alleles respectively (Bonin et al., 2007). Both polymorphic and monomorphic loci were analyzed. Bands that resolved poorly on the gel were treated as missing ones.

III. RESULTS AND DISCUSSION

3.1 Polyembryony

In Khasi mandarin, polyembryony varied from 50.0% to 83.3% among three years and was recorded to be the highest in the seeds harvested in November 2013 while those harvested in November 2015 represented the lowest percentage of polyembryony. The percentage of germination was highest in the harvest year November 2015 (Table1). Factors influencing the variation in polyembryony found in our study and those reported by Andrade-Rodriguez et al., 2005 in *Citrus reshni* and Kishore et al., 2012 in *Citrus jambhiri* (90.1% and 91.4%) as well as germination traits could be attributed to genetic conditions (Kepiro and Roose, 2007), complex interrelation between genotype and surroundings such as the type of pollinators, quantity of viable pollens, fertilization, air temperature, environmental and soil humidity, plant nutrition, and wind speed (Andrade-Rodriguez et al., 2005; Mondal et al., 2014). Polyembryony varies depending on the ecological region and cultivar suggesting that it should be specific for each variety with respect to the region. The study indicated that polyembryony does not seem to be a limiting factor for germination capacity. Scalon et al. (2003) also reported that polyembryony was not a limiting factor for seed germination and plant emergence in *B. Glabra*. The number of embryos in polyembryonic seed ranged from 2 to 14 (Table 1), characterized by variation in the partitioning of embryos followed by both synchronous and asynchronous development of seedlings (Fig. 1a and 1b).

The average number of embryos (clutch size) per polyembryonic seed ranged from 2.27 ± 0.03 to 2.70 ± 0.10 and per total seed ranged from 1.13 ± 0.19 to 2.30 ± 0.12. One of the causes in the differences of clutch size during harvest years might be due to the presence of dominant trait for polyembryony, movement of auxins and ploidy level which plays prominent roles in the development of extra numerous embryo (Jaskani et al., 2005). Improper endosperm growth due to lack of nutrients and growth factors from the maternal tissue to the embryo might also lead to the early breakdown of nucellar embryos (Kishore et al., 2012). Our results corroborate the findings of Kishore et al. (2012), where the number of embryos per seed reported in citrus (including mandarin, sweet orange, rough lemon, and lime) ranged from 2 to 14 while, it was different in terms of number of embryos per polyembryonic and total seed as reported by them (3.48 and 3.26). This significant disparity in the occurrence of clutch size evidently points out the impact of location on occurrence of numerous embryos. This suggests that the difference might be due to stress-induced changes in the environment leading to change in the genetic development of cells or their hormonal behavior, thereby affecting embryo development and surrounding seed organization (Batygina and Vinogradova, 2007). The number of leaves and shoot length were maximum in November 2015 and were minimum in November 2013, respectively, while the number of branches was the highest in November 2013 and the lowest in 2015 (Table 1). The variation in the emergence of the morphological traits suggests that polyembryony was only correlated with the number of branches indicating that it can be treated as a reliable indicator of the occurrence of polyembryony in the seeds. The variation could be due to the fact that the embryos of each seed were grown *in-vitro* using conical flasks during the harvest years for each seed which enabled development of a seedling from every embryo as compared to other studies which utilized seedlings grown in greenhouse, pots, etc (Golein et al., 2011; Yun et al., 2011). Moreover, during seed formation, embryos adapt to different dynamics especially when adventitious polyembryony is considered (Batygina and Vinogradova, 2007). One way Anova analysis was carried out at 0.05% significance where the probability Pr value 0.97 is greater than 0.05 (Table 2) which resulted in the rejection of null hypothesis suggesting that there is no significant variation in time of harvest with respect to the morphological trait. This was justified by Pearson correlation test which resulted in positive correlation among total morphological traits against time of harvest. The most significant correlation was observed between November 2013 and November 2014 followed by November 2015 and November 2014 as shown in Table 3 and Fig. 2. This result suggested that there is gradual increase in morphological traits over time of harvest in three consecutive years.

3.2 ISSR and RAPD markers for identification of zygotic and nucellar seedlings

ISSR markers generated the highest number of amplified fragments with an average of 7.25 bands obtained per markers (Table 4). From the total markers, only 16 ISSR and 5 RAPD primers were selected based on the polymorphic and reproducible banding patterns. Of these, finally 4 ISSRs primers namely UBC 810, UBC 835,

UBC 840, UBC 855, and 3 RAPD primers namely OPA 18, OPAA 10 and OPA 04 were selected for further analysis based on ability to differentiate DNA amplification of mother plants and seedlings from polyembryonic seeds. **UBC 855 identified as a potential marker that recorded highest polymorphism (87.5%). In case of RAPD, primer OPA 18 amplified the highest polymorphism (71.4% of the bands) with an average of 7 bands per primer**. The sizes of amplified PCR products differed among ISSR markers i.e UBC 810-700 bp, UBC 835-280 bp, UBC 840-600 bp, and UBC 855-590 bp (Fig. 3a). The three RAPD primers generated differentiating fragments of varying sizes; OPAA 10 - 650 bp, OPA 04 -710 bp, and OPA 18 - 600 bp (Fig. 3b). In addition, a distinguishable ISSR amplification pattern, related to the mother and progeny, was obtained using the UBC855 and OPAA 10 primers. The amplified UBC 855- 590 bp fragments was not from zygotic progeny but from the mother plant (Fig. 4a) and the amplified OPAA 10- 650 bp fragment was present in the zygotic progeny but not in the mother plant as indicated by arrows (Fig. 4b). Primer UBC 855 exhibited 29.0% (18/62) zygotic identification efficiency. UBC 835 primer was the second most efficient in identifying zygotic at 27.4% (17/62) (Table 4). RAPD primer OPAA 10 exhibited 41.9% (26/62) zygotic identification efficiency, followed by OPA 18 with 24.1% (15/62). Using all 4 ISSR primers, the identification rate of zygotic seedlings was 87% (54/62), while using all the 3 RAPD primers, identification efficiency was 85.4% (53/62). Using ISSR and RAPD, the zygotic identification efficiency was increased to 59.6% (37/62) when different primers were used. However, none of these primers alone were able to categorize all zygotic seedlings. This kind of observation was also reported by reported by Rajwana et al. (2008). According to Yun et al. (2007) in Miyagawaunshiu × Ponkan mandarins, 13.4% (20/149) seedlings were zygotic. Thus, a broader selection of polymorphic primers enhances the probability of identifying zygotic individuals, as cited previously by Bastianel et al. (1998).

Of 62 polyembryonic seedlings studied, 37 seedlings showed a banding pattern different from that of the mother plant using both ISSR and RAPD primers (Table 5) indicating a zygotic origin; primer UBC 855 helped to identify 18 of these zygotic seedlings (Fig. 4a) and OPAA 10 helped to identify 26 of these zygotic seedlings (Fig. 4b). A finding reported by Vilarinhos et al. (2000) identified 12 zygotic seedlings from Volkamerian lemon × Cravo lemon using six of 20 RAPD primers in a combination. Of the 7 seedlings from monoembryonic seeds, primers UBC 835 and UBC 810 identified 14.2% (1/7) and primers OPA 04, OPAA 10 and OPA 18 identified 42.8% (3/7), 28.5% (2/7) and 14.2% (1/7), respectively to be zygotic in nature (Table 5). Additionally, among both polyembryonic and monoembryonic seedlings, 53.6% of them were zygotic in nature and 46.3% nucellar, which are in close proximity to the value of 53% and 47% of zygotic and nucellar seedlings as reported by Mondal et al. (2014) in *Citrus reticulata* using RAPD. In the present study, seedlings genetically identical to the mother plant were generated at a low incidence in polyembryonic seeds produced by the highly polyembryonic population. As reported, the percentage of nucellar seedlings declines with an increase in the proportion of polyembryonic seed generated (Soost and Roose, 1996; Andrade-Rodriguez et al., 2004). Thus, it is likely that the lower proportion of nucellar population is related to a higher range of polyembryony. In the present work, 66.6% of the Khasi mandarin seeds had 2 to 3 zygotic embryos (Table 5). This coincides with Das et al. (2007) that reports when more than one zygotic embryo per seed for citrus was observed it suggests the chances of different microgametes induced fertilization.

Our result indicated that zygotic embryos were located in the micropylar region in 40% (2 of 5) of the polyembryonic seeds; while in the remaining 60% seeds, they were located near the micropyle region but not in the micropyle (Table 5). This observations suggests that embryo distribution inside seed does not follow any specific pattern and might be genetically controlled since zygotic embryos cannot be visually distinguished based on their morphological traits such as shape, size etc (Yun et al., 2007). Similar results were also observed where it was reported that the maximum of the zygotic seedlings were obtained from embryos located close to the micropylar region (Andrade-Rodriguez et al., 2005). The reason for positioning of zygotic embryos at micropylar area could be related to their growth habit which depends on the endosperm since its presence stimulates the formation of adventitious embryos in the micropylar region but suppresses their initiation towards the chalazal part based on the distance of embryo from the micropylar end (Kishore et al., 2012).

The size of embryos in polyembryonic seeds ranged from 2-6 mm, while in monoembryonic seeds, it varied from 6-8 mm (Table 5). The study indicated that embryos of even small size (2-3 mm) were able to develop seedlings suggesting the availability of food reserve as well as hydrated condition of the mature embryos during *in-vitro* seed germination. The embryo size is an important attribute in polyembryony since smaller ones are generally found to be inviable due to insufficient food reserve (Andrade-Rodriguez et al., 2005). In citrus, as the number of embryos per seed increases, its size decreases (Soares Filho et al., 2003). In orange, it was reported that

the embryo size decreases as it approaches near the micropylar region of the seed (Villegas and Andrade, 2008).

IV. CONCLUSION

A simple outlook that emerges from the study is that the popular mandarin cultivar, Khasi mandarin is highly polyembryonic, exhibiting polyembryony in more than 80% of their seeds with more clutch size and the possibility of obtaining zygotic plants from them is high. A population of 69 individual seedlings were generated, out of which 62 individuals were tested as polyembryonic plantlets. Out of tested plantlets, 37 hybrids and 25 nucellars were recognized through molecular screening by ISSR and RAPD markers. ISSR-UBC 855 and RAPD-OPAA 10 can be used as efficient markers for identifying hybrid seedlings at an early developmental stage confirming their potential for early selection in citrus breeding. The ISSR and RAPD markers used in the present study showed 15-45% efficiency in the identification of the origin of seedlings. Observation of embryo size and position allowed us to find that zygotic seedlings were obtained from embryos located close to the micropylar region and not at the micropylar region. Since polyembryony is species and region-specific, this attempt may aid in the development of uniform population for the farmer community of the region. This work could be elaborated with a large population size or a hybrid system for overall citrus germplasm management in India.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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Table. 1: Percentage of germination (PG), percentage of polyembryony (PP) and clutch size in Khasi mandarin[(1)]

Harvest	PG (%)	PP (%)	Embryos/ PS*		Clutch size		No. of leaves	No. of branches	Shoot length
			Min	Max	Poly	Total			(cm)
2013	66.60±0.12a	83.30±1.0a	2	14	2.70±0.1a	2.30±0.12a	3.00±0.1a	3.00±0.12a	5.13±0.19 b
2014	64.23±0.09 b	55.50±1.6 b	2	13	2.50±0.0ab	1.40±0.10b	4.70±0.0 b	2.10±0.21b	6.00±0.21 a
2015	83.37±0.09c	50.00±1.6c	2	11	2.27±0.0b	1.13±0.19b	5.20±0.2c	1.33±0.12a	6.63±0.19 a

[(1)]Means followed by the same letter in each column are not significantly different based on Duncan's Multiple Range (DMR) test at 5% probability. Data are means ± standard error.

* Polyembryonic seeds

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Table. 2: Anova analysis

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Individual	2	63	31.3	0.031	0.97
Residuals	18	18376	1020.9		

Table. 3: Pearson correlation analysis

	Nov13	Nov 14	Nov 15
Nov2013	1.0000000	0.9734644	0.9041905
Nov 2014	0.9734644	1.0000000	0.9776250
Nov2015	0.9041905	0.9776250	1.0000000

Table. 4: Status of polymorphism for Khasi mandarin using four ISSR primers and three RAPD primers

Primer	Sequence (5'-3')	Total Bands amplified	Polymorphic Bands	Polymorphism (%)	Zygotic seedlings PS[(1)]	Zygotic seedlings MS[(2)]
UBC 855	(AC)8YT	8	7	87.5	18/62	0/7
UBC 835	(AG)8YC	8	5	62.5	17/62	1/7
UBC 810	(GA)9C	8	4	50.0	11/62	1/7
UBC 840	(GA)8YT	8	6	75.0	8/62	0/7
OPA 18	AGGTGACCGT	7	5	71.4	15/62	1/7
OPA 04	AATCGGGCTG	5	3	60.0	12/62	3/7
OPAA 10	TGGTCGGGTG	9	6	66.6	26/62	2/7

[(1)]polyembryonic seedling
[(2)]monoembryonic seedling

Table. 5: Zygotic (Z) or nucellar (N) origin of seedlings with respect to embryo position from polyembryonic and monoembryonic seeds analyzed using four ISSRand three RAPD primers in Khasi mandarin

Plant	Seed (no.)	Seedling (no.)	Embryo size (mm)	Position	UBC 855	UBC 835	UBC 810	UBC 840	OPA18	OPA04	OPAA10
BH1		MP			MP	MP	MP	MP	MP	MP	MP
	1	1	5	NM	N	N	N	N	N	N	N
		2	6	NM	N	Z	N	Z	N	N	N
	2	1	6	NM	N	Z	N	N	N	N	N
		2	4	NM	Z	N	N	N	N	N	N
		3	3	M	Z	N	N	Z	N	N	N
		4	4	NM	N	N	N	N	N	N	Z
	3	1	3	M	Z	Z	N	N	N	N	Z
		2	5	NM	N	N	N	Z	Z	Z	Z
		3	5	NM	N	N	Z	N	N	N	N
	4	1	4	NM	N	N	N	N	N	N	N
		2	4	NM	N	N	N	N	Z	N	N
	5	1	6	NM	N	N	N	N	Z	N	Z
		2	5	NM	N	N	N	N	N	N	Z
		3	6	NM	N	N	N	N	N	Z	N
BH2		MP			MP	MP	MP	MP	MP	MP	MP
	1	1	6	NM	Z	N	N	N	N	N	N
		2	4	NM	N	N	N	N	Z	Z	Z
	2	1	3	M	Z	Z	Z	Z	N	N	N
		2	4	NM	Z	N	N	N	N	N	N

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Plant	Seed (no.)	Seedling (no.)	Embryo size (mm)	Position	UBC 855	UBC 835	UBC 810	UBC 840	OPA18	OPA04	OPAA10
	3	1	4	NM	N	N	N	N	Z	Z	Z
		2	4	NM	N	N	N	N	N	N	N
	4	1	5	NM	Z	N	N	N	N	N	N
		2	4	NM	Z	N	N	N	N	N	N
BH3		MP			MP	MP	MP	MP	MP	MP	MP
	1	1	6	NM	N	N	N	N	N	N	N
		2	3	M	N	Z	Z	Z	N	N	N
		3	6	NM	N	N	N	N	N	N	N
		4	3	M	N	N	Z	N	N	N	N
	2	1	5	NM	N	N	N	N	N	Z	Z
		2	4	NM	N	N	N	N	N	N	Z
		3	4	NM	N	N	N	N	Z	N	Z
	3	1	6	NM	N	Z	N	N	N	N	Z
		2	5	NM	N	Z	N	N	Z	N	N
	4	1	6	NM	N	N	N	N	N	Z	Z
		2	5	NM	N	Z	N	N	N	N	N
	5	1	3	M	Z	Z	N	N	N	N	Z
		2	4	NM	Z	N	N	N	N	Z	Z
BH4		MP			MP	MP	MP	MP	MP	MP	MP
	1	1	2	NM	Z	N	N	N	N	N	Z
		2	6	NM	N	N	N	N	N	N	Z
		3	6	NM	N	N	N	N	Z	N	N
	2	1	5	NM	N	N	N	Z	N	Z	N
		2	3	M	Z	N	Z	N	N	Z	N
	3	1	6	NM	N	N	N	N	N	N	N
		2	5	NM	N	Z	N	N	N	N	Z
		3	5	NM	N	Z	N	N	N	N	Z
	4	1	6	NM	N	N	N	N	Z	N	Z
		2	3	M	Z	Z	N	Z	N	N	Z
BH5		MP			MP	MP	MP	MP	MP	MP	MP
	1	1	4	NM	Z	N	N	N	N	N	Z
		2	6	NM	N	N	N	N	N	Z	Z
	2	1	5	NM	N	N	Z	N	Z	Z	N
		2	6	NM	N	N	N	N	Z	N	N
		3	5	NM	Z	N	N	N	N	N	N
	3	1	4	NM	N	Z	N	N	N	N	N
		2	3	M	Z	Z	N	N	N	N	N
	4	1	4	NM	N	N	N	Z	Z	N	N
		2	3	M	N	N	Z	N	Z	N	N
	5	1	5	NM	N	Z	Z	N	N	N	N
		2	4	NM	Z	Z	N	N	N	N	N
BH6		MP			MP	MP	MP	MP	MP	MP	MP
	1	1	6	NM	N	N	N	N	N	N	Z
		2	5	NM	N	N	Z	N	Z	N	Z
	2	1	5	NM	N	N	N	N	N	N	Z
		2	4	NM	Z	Z	Z	N	N	N	Z
	3	1	6	NM	N	N	Z	N	N	N	N
		2	5	NM	N	N	N	N	Z	Z	N
	M1	1	7	-	N	N	N	N	N	N	N
	M2	1	8	-	N	N	N	N	N	N	N

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Plant	Seed (no.)	Seedling (no.)	Embryo size (mm)	Position	UBC 855	UBC 835	UBC 810	UBC 840	OPA18	OPA04	OPAA10
	M3	1	6	-	N	N	N	N	N	N	N
	M4	1	6	-	N	N	N	N	Z	N	Z
	M5	1	8	-	N	N	N	N	N	Z	N
	M6	1	7	-	N	Z	N	N	N	Z	N
	M7	1	8	-	N	N	Z	N	N	Z	Z

MP: mother plant; NM: non-micropylar; M: micropylar; N: nucellar; Z: zygotic; M1-M7: monoembryonic seedling; -: not applicable

Fig. 1: [a] (1, 2): Variation in partitioning of embryos formed in a seed from the polyembryonic Khasi mandarin cultivar, (3): Embryos at micropylar region, (4): Embryo from monoembryonic Khasi mandarin cultivar. Bar in [a] (1, 3, 4) is 3 cm and [a] (2) is 2 cm; [b] (1, 2, 3): Asynchronous appearance of triplet seedlings in Khasi mandarin, (4): Synchronous appearance of duplet seedlings in Khasi mandarin. Bar in [b] (1) is 0.5 cm and [b] (2, 3, 4) is 1 cm

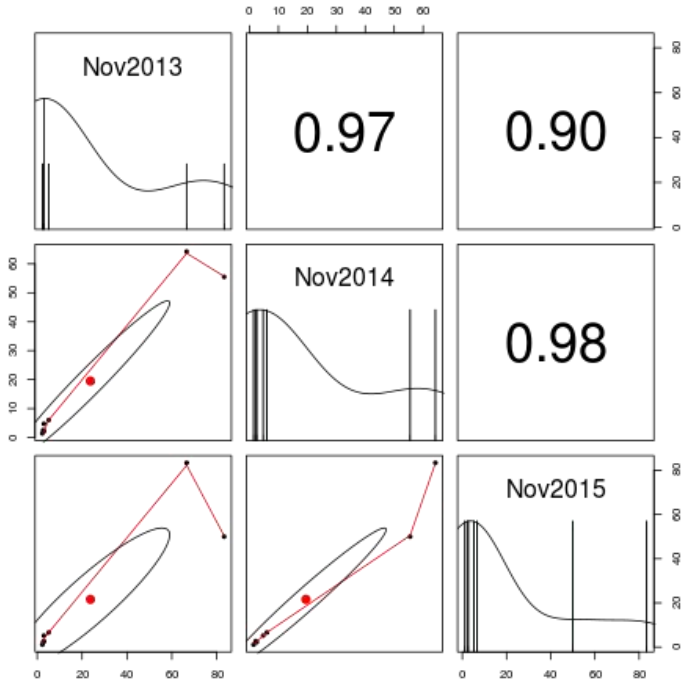

Fig. 2: Significant values among total morphological traits against time of harvest pass through positive correlation baseline as depicted through Pearson correlation test.

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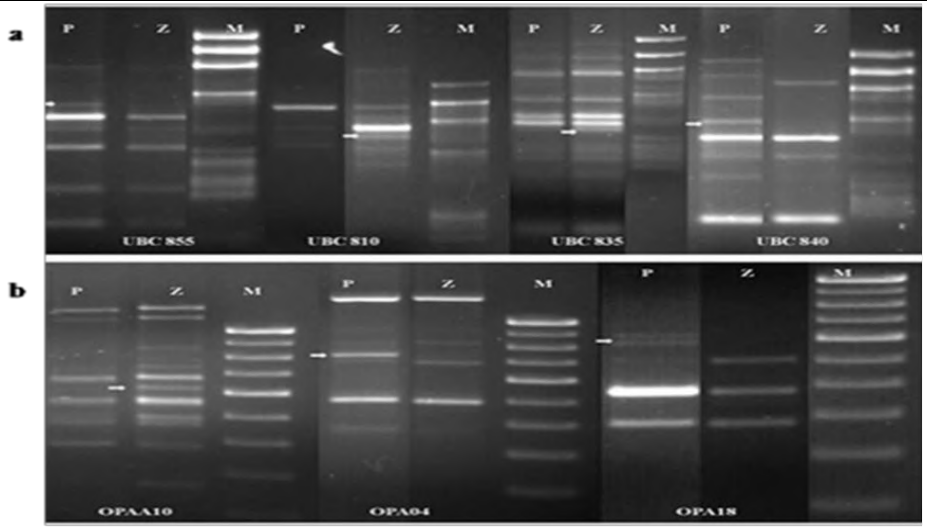

Fig. 3: (a)Mother-progeny related DNA amplification patterns showing polymorphism by using four screened ISSR primers (UBC855, UBC 810, UBC 835, and UBC 840);(b)using three RAPD primer (OPAA10, OPA04, and OPA 18). The white arrowheads indicate the above mentioned confirmed polymorphic markers that can be used for the identification of hybrid seedlings with a zygotic origin. P: mother; Z: seedling with zygotic origin; M (a) ØX/ HaeIII digest and M (b) is 1 Kb DNA ladder

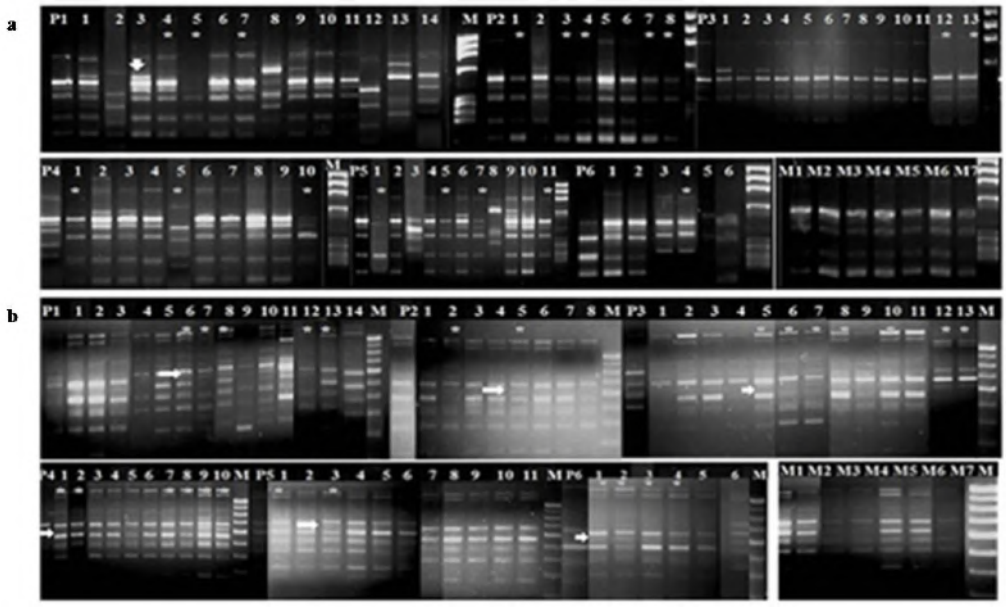

Fig. 4: (a) Putative zygotic seedlings were identified by ISSR marker UBC 855; (b) identified with RAPD marker OPAA 10. The white arrowheads indicate a 590 bp in upper one (total 18 putative zygotic seedlings) and a 650 bp in lower one (total 26 putative zygotic seedlings). M (a) ØX/ HaeIII digest and M (b) is 1 Kb DNA ladder; P1-P6: six mother trees; numerals after P1, P2, P3, P4, P5 and P6 represents polyembryonic seedling with zygotic or nucellar origin from each mother plant respectively; M1-M7: monoembryonic seedlings

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Economics of Groundnut Production among Smallholder Farmers in Michika Local Government Area of Adamawa State, Nigeria

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Abstract**—***This study assessed economics of groundnut production among smallholder farmers in Michika local government area of Adamawa State, Nigeria. Multistage sampling technique which involves purposive selection of Michika and simple random selection of farmers from eight wards was embraced in collecting primary data from 172 farmers using structured questionnaire. The analytical tools used were mainly descriptive, gross margin and regression analysis. The analysis found that groundnut production is profitable with an average gross margin of ₦97,477.80, total revenue of ₦167,160, and net farm income of ₦ 94,540.64 per hectare. The regression analysis indicated that Cobb-Douglas production function gave the best fit with R^2 value of 0.748, implying that the specified factor inputs in the regression equation explained up to 74.8% of the variation in groundnut output and only 25.2% was accounted for by the random error term. Production inputs such as farm size, labour, agrochemicals, seeds and farming experience were statistically significant at varying levels of probability. This means that any increase in such inputs would bring about increase in groundnut output. Resource use efficiency analyses indicate that the ratios of MVP and MFC in respect to seeds, labour and Agrochemicals were greater than unity and hence were under-utilized by the farmers during production period. Therefore, policies aimed at assigning more production inputs to farmers should be introduce by government in order to enhance farmers' output and profitability.*

Keywords**—Profitability, Resource use efficiency, Regression, Groundnut, Smallholders.**

I. INTRODUCTION

Groundnut (*Arachishypogaea L.*) is a leguminous oilseed crop which contains 20-50% protein, 40-50% fat and 10-20% carbohydrate. It is cultivated in the semi-arid and subtropical regions of about 114 countries of the world on nearly 31.2million hectares, with a total output of 60.5 million metric tons (mmt)at an average productivity of 1.4 metric tons per haduring2014[International Crop Research Institute for the Semi-Arid Tropics,[1,2]. Malaysia, Israel, China, Nicaragua, Nigeria, USA and Saudi Arabia are some of the leading groundnut producing countries in the world. Asia, with 25.6mmt (58.28%) and Africa, with 13.9mmt (31.62%) grasps maximum global groundnut output. Developing countries in Asia, Africa and South America account for 99.85% of the total quantity of groundnut produced in the world in 2014 [2]. Groundnut is an important crop in many developing countries where it serves as a protein source, vitamins and cooking oil. Groundnut is the 13thmost significant food crop of the world. It is the world's 4thmost vital source of edible oil and 3rdmost important source of vegetable protein[3].The haulms being a good source of feed supplement for livestock, also increases farmers income particularly during the dry season when fresh green grasses are in short supply and the silage is in high demand[1].

The meager output of groundnut in African countries may be attributed to production challenges such as rainfall variability and drought, poor soil fertility, biotic and abiotic constraints, input supply constraints, traditional smallholder farming with little or no mechanization, prevalence of pests and diseases and partial extension services. Groundnut is a major source of edible oil as well as employment generation for smallholder farmers in Northern Nigeria where 48% of the total output in West Africa was produced in 2015.It occupies about 34% of the total land area under cultivation and contributes 23% of household earnings of the country. The total output was 1.6 mmt in 1961, but fell to 4.5mmt in 1982.Since 1996, production has been increasing at an estimated rate of 2.3 mmt to about 3.4 mmt in 2015[2],as a result of both area expansion (5.52%) and increase in productivity [4].

Previous studies on groundnut production of smallholder farmers provide a variety of results. [5], in a study on the

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profit and market efficiency of modern groundnut oil extraction of RMP-12 and Ex-dakar varieties discovered that it was a profitable venture, though with a low profit margin. [6]In a study entitled economics of groundnut production in Nigeria indicated that groundnut is a profitable enterprise as farmers received a net farm income of ₦14, 355 per hectare. A similar view was shared by [7], who indicated that the total revenue, gross margin and net farm income per hectare were ₦100, 818, ₦42, 422 and ₦41, 172 respectively whereas the gross margin and net farm income per naira invested were ₦0.73 and ₦0.69 respectively, implying that groundnut production in Northern Part of Taraba State was profitable.

[8], observed that groundnut production in Adamawa State, Nigeria is profitable as farmers realized a total revenue of ₦90, 843.75, gross margin of₦31, 363.75 and net farm income of₦29, 003.75per hectare. In order to maximize profit, farmers should procure their required inputs from a competitive market and should make use of the obtainable organic manure to minimize cost of production where necessary. According to [9], pests and diseases, inadequate inputs, instability of market prices, high cost of inputs and poor storage facilities negatively affect the efficiency and marketing of groundnut product. Others as indicated by [10],include transportation cost, proximity to market location, infrastructural facilities like road network and exploitative activities of middle men that are involve in buying and selling affect profitability of groundnut production in Nigeria.

Inspite the enormous importance of this cash crop, availability of ample land and human resources in Nigeria, there seems to be inadequate supply of groundnut product to meet both local and international market demand. Therefore, there is need to explore the economics of groundnut production among smallholder farmers in Adamawa State, Nigeria. The study evaluates the costs and returns associated with groundnut production in Michika Local Government Area, determined the relationship between inputs and output as well as the resource use efficiency of groundnut production in the area.

II. METHODOLOGY

2.1 Study area

The research was conducted in Michika local government area (LGA) of Adamawa State. It is situated at the far north east of the state which lies between latitude 11^0- 8' South and longitude 15^0-13' E. It is bounded to the North by MadagaliLGA and shares an international boundary with the Republic of Cameroun in the North-East. It is bounded on the West by Borno State and by Mubi and Hong LGAsin the South. The LGA has four development areas namely Michika metropolitan, Garta, Bazza and Madzi which covers a land mass of 961km^{2}with an estimated population of 211,124[11]. The study area is heterogeneous in ethnic composition. It is agrarian in nature and has a great percentage of its populace engaged in farming as an occupation. It has a tropical type of climate marked by distinct dry and rainy seasons. The dry season commences in December and ends in April whereas the wettest months are August and September (Adebayo, 1999).

2.2 Data Collection

Multistage and simple random sampling techniques were used to gather information from the farmers. In the first stage, Michika LGA was selected based on it prominence in groundnut production. The second stage involved the selection of eight wards from a total of sixteen wards that made up the study area. This is followed by the selection of two villages each from the selected wards given rise to sixteen villages all together. Finally, a sample of 186 groundnut farmers were randomly selected from a sampling frame of 360 registered farmers in the study area. Though, only 172 questionnaires were retrieved and use for the analysis due to inappropriate response from the respondents. Data collected was based on production inputs, outputs, both inputs and output prices as well as socio-demographic characteristics of the farmers for the year 2017 cropping season.

2.3 Data analysis

This involved both descriptive and inferential analysis. Inferential analyses such as gross margin technique and regression model as used by [7], [9],[5] and[6]were adopted for the study.

2.4 Model specification

2.4.1 Gross margin

Gross margin technique was used to determine the profitability (costs and returns) of groundnut production in the study area. The model is specified as follows:

$$GM = TR - TVC \quad (1)$$

$$NFI = GI - TC \quad (2)$$

$$TC = TVC + TFC \quad (3)$$

$$RNI = \frac{NFI}{TC} \quad (4)$$

Where;

GM = Gross Margin

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TR = Total Revenue

TVC = Total Variable Cost

TFC = Total Fixed Cost

NFI = Net Farm Income

GI = Gross Income realized from groundnut production

TC = Total Cost of groundnut production

RNI = Return on every naira invested during the production process

2.4.2 Regression analysis

Regression is defined as the amount of change in the value of one variable associated with a unit change in the values of the other variables. However, this study appliedfour different functional formssuch as linear, double-log, exponential and semi-log functions to determine the inputs (independent variables) and output (dependent variable) relationship. The specifications of the functions are given as:

(i) Linear function

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + ... + b_7X_7 + \mu_i \quad (5)$$

(ii) Double-log function

$$\ln Y = \ln b_o + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + ... + b_7 \ln X_7 + \mu_i \quad (6)$$

(iii) Exponential function

$$\ln Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + ... + b_7X_7 + \mu_i \quad (7)$$

(iv) Semi-log function

$$Y = \ln b_o + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + ... + b_7 \ln X_7 + \mu_i \quad (8)$$

Where;

Y = Quantity of groundnut shell produced measured in kilogram

X_1 = Years of farming experience

X_2 = Agrochemicals used measured in liters

X_3 = Fertilizer used measured in kilogram

X_4 = Man-days of labour

X_5 = Farm size measured in hectares

X_6 = Quantity of seeds measured in kilogram

X_7 = Access to extension services (Dummy: 1 if a farmer had access to extension services, 0 = otherwise)

$b_1 - b_7$ = Estimated regression parameters

μ_i = Error term

The apriori expectation was that the coefficients of X_1-X_7 would be positive.

2.4.3 Resource use efficiency

The estimated coefficients of the linear regression model wereused to compute the marginal value product (MVP) and it ratio (r), whilemarginal factor cost (MFC)was usedto determine the economic efficiency of resource used.The model was estimated as given below:

$$r = \frac{MVP}{MFC} \quad (9)$$

Where: r represents the efficiency ratio, MVP = marginal value product of the variable inputs and MFC = marginal factor cost i.e. price per unit of input.

Based on economic theory as also reported by [13] and[14], a firm maximizes profits with respects to resource use when the ratio of the marginal return to the opportunity cost is one. However, the values are interpreted as:

(i) If r is less thanone, resource is over utilizehence reducing the quantity use of that resource willincrease farmers'profits.

(ii) If r is greater than one, resource is being underutilized during production hence increasing the quantity use of the resource will upsurge profitlevel accruing to the farmers.

(iii) If r is equal to one, it showsthe resource is being used efficiently and that is the point ofprofit maximization [15].

III. RESULTS AND DISCUSSION

3.1 Economic analysis of groundnut production

Economics of groundnut production was analyzed using 2017 prevailing market prices of inputs and output as presented in Table 1. The average output obtained per hectare was 597 kg, while the average selling price/kg was ₦280. The total variable cost incurred on production constituted the greater proportion of the total production cost which was estimated at ₦69,682.20 (95.96%), while the total fixed cost which is depreciated on land and equipment was ₦2,937.16 (4.04%) implying that variable cost is the most sensitive components in groundnut production. The gross margin and total revenue received from the sale of groundnut output in the study area on average was ₦97,477.80 and ₦167,160 respectively. The results further revealed that net farm income was estimated at ₦94,540.64, while the return per naira invested was ₦1.30 per hectare. This indicated that for every naira invested in groundnut production in the study area,a farmer

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realized a profit of₦1.30. Therefore, groundnut production is a profitable venture inMichika local government area. The results agreed with the findings of [16], [8],[17], [7], [9], who in their different studies reported that groundnut production is highly profitable.

Table.1: Average Cost and Returns in Groundnut Production/Hectare/Farmer

Variables		Amount in naira/ha	Percent
Total Variable Cost (TVC)		69,682.20	95.96
Total Fixed Cost (TFC)		2,937.16	4.04
Total cost of production (TC)		72,619.36	100.0
Returns			
Total output	247,755 kg		
Output/ha	597 kg		
Total hectare of land	415		
Price/kg		280	
Total Revenue (TR)		167,160	
Gross Margin (TR–TVC)		97,477.80	
Net Farm Income (GM–TFC)		94,540.64	
RNI		1.30	

Source: Field Survey, 2017

3.2 Regression analysis result

This section presents the results of four different functional forms which include Linear, Semi-logarithm (linear-log and log-linear) and Double-logarithm analyzed using Eviews 8 statistical package (Table 2). Based on the summary of the results, Double-logarithm function gave the best fit and was chosen as the lead equation. The selection of lead equation was based on the comparison of coefficients ofmultiple determinations (R^2), statistical significance of the F-ratios, the magnitude of standard error of the estimated parameters ($b_1 - b_7$),statistical significance of the estimated regression coefficients and the apriori expectation. Since Double-logarithm function gave the best fit, the regression coefficients are still the elasticities. Elasticity of production is defined as the measure of output response to changes in the variable input [18]. The coefficients of all the explanatory variables bore positive signs and hence reflect the apriori expectation. The predictive power of the model represented by R^2 was 0.748, meaning the specified factor inputs explained up to 74.8% of the variation in groundnut output and that only 25.2% was taken care by the random error term. The overall significance (F-statistic) of the model at 1% explains the fitness of the model.

Farm size being one of the most important variables was found to be positive (0.298) and statistically significant at 1% level of probability. This means that a 1% increase in hectare of land under cultivation would increase output by 29.8%. This also indicated that land as a factor of production is very important in groundnut production in the study area as farmers tend to derive the benefits of economies of scale. This result is in conformity with the findings of [7] and [6]who found out that farm size is one of the most important factors in groundnut production. The coefficient of labour was significant at 1% with an elasticity coefficient of 0.126 which indicated that a 1% increase in man-days of labour increased groundnut output by 12.6%. This result is similar to that obtained by[17] who observed that labour significantly influenced groundnut output in Benue State, Nigeria. Agrochemical is another significant input in groundnut production with an elasticity coefficient of 0.166 which was statistically significant at 1% probability level. This implies that a 1% increase in the use of Agrochemicals would increase output by 16.6%. In their study on groundnut production, [17] and[19]found that quantity of Agrochemicals applied is directly related to groundnut output.

As one of the factor inputs in groundnut production, seed was positive and significant with an elasticity coefficient of 0.430implying that a 5% increase in the quantity of seed would increase output by 43%. This result agrees with the findings of [20] who found that increase in seed input can increase agricultural productivity. The elasticity coefficient of farming experience was positive (0.049) and statistically significant at 1% probability level possibly depicting the impact of experience on groundnut production. This means that experience farmers were likely to achieve higher yields than the inexperience ones which could result in higher

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efficiency and net farm income as well. The result conformed to the findings of [21] who stated that experience is a significant determinant of output in agricultural production. However, the coefficients of fertilizer and extension factors were positive and in agreement with the expected sign whereas the statistical insignificance implies that the factor inputs does not determine output in groundnut production as also indicated by [13].

Table.2: Regression Results of the Various Specified Functional Forms

	Coefficient of Independent Variables										
Functions	Constant	X_1	X_2	X_3	X_4	X_5	X_6	X_7	R^2	R^{-2}	F-value
Linear	1295.541 (8.933)	7.127 (1.698)*	2.702 (3.775)***	23.582 (2.684)***	1.263 (1.697)*	-103.942 (-3.783)***	2.892 (0.483)	48.291 (0.724)	59.2	48.3	9.455***
Log-linear	7.123 (66.175)***	0.006 (1.855)*	0.002 (3.632)***	0.017 (2.583)**	0.001 (1.533)	-0.079 (-3.871)***	0.003 (0.649)	0.044 (0.891)	50.3	47.9	9.367***
Linear-log	-2.863 (-0.009)	59.875 (1.791)*	224.271 (3.957)***	92.188 (1.574)	180.217 (3.027)***	409.345 (4.123)***	34.200 (0.613)	39.110 (0.608)	61.4	58.7	10.982***
Double-log	2.170 (0.348)	0.049 (1.988)***	0.166 (3.929)***	0.059 (1.359)	0.126 (2.854)***	0.298 (4.038)***	0.430 (3.040)**	0.038 (0.790)	74.8	71.4	10.746***

Source: Field Survey, 2017
Note: ***, ** and * represents 1%, 5% and 10% significant levels
Figures in parenthesis are t-values

3.3 Efficiency of resource use analysis

The Marginal Value Product (MVP) of seeds, labour and farm size were computed and compared to their unit prices in order to determine the degree of efficiency in their use in respect to groundnut production. The results as presented in Table 3shows that the ratios of seeds, labour and Agrochemicals are greater than unity, indicating that the inputs were under-utilized hence increasing quantity of the inputs use will enhance output and profit level. However, this result is in agreement with the findings of [7], [13] and[17] who observed that resources were inefficiently utilized in groundnut production.

Table.3: Estimated Resource-Use Efficiency in Groundnut Production

Variable inputs	MVP	MFC	$\frac{MVP}{MFC}$
Seeds	909.76	763.55	1.19
Labour	453	235.83	1.92
Agrochemicals	756.56	203.74	3.71

Source: Field Survey, 2017

IV. CONCLUSION

Groundnut production in the study area has been found to be a profitable enterprise as farmers on the average, realized a net income of ₦94,540.64with a gross margin of ₦97,477.80 at an average selling price of ₦ 280/kg. The regression analysis results revealed that Cobb-Douglas function gave the best fit based on economic, econometric and statistical criteria. The elasticity coefficients of all explanatory variables bore positive signs and hence are in accordance with the apriori expectation. Factor inputs such as agrochemicals, years of farming experience, labour, seeds quantity and land hectares under cultivation

significantly affects groundnut output at 1% and 5% levels of probability. Comparison of the ratio of the MVP to MFC shows that farmer' under-utilized seeds, labour and agrochemicals inputs throughout the cropping season. Thus, increasing quantity of the inputs use will improve production outputand net income level of the farmers. The study recommends that, government should provide funds that could be used to establish research centers for the development of resistant and improved groundnut seed varieties at subsidize rate for use by the farmers. This can aid in minimizing losses arising from prolonged drought and pests as well as shortened the production period of the crop. Farmers' cooperative unions should promote and encourage farm mechanization by developing and distributing simple machines that could impede labour shortage. This can entice prospective youths in to groundnut production and lead to higher productivity and efficiency. Policies aimed at assigning more production inputs to the farmers would enhance output and profitability of the crop in the study area and the country at large.

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Sweet Potato Production for Poverty Alleviation in Nasarawa State, Nigeria

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Abstract— *The study investigated sweet potato production for poverty alleviation in Nasarawa State of Nigeria. Data were collected from 180 sweet potato farmers randomly selected from Keffi, Kokona and Karu Local Government Areas and interviewed using structured interview schedule. Results of the study show that adult males played a dominant role in sweet potato production especially in land preparation 79% and ridging 81% respectively, while women and children played major role in planting 97%, weeding 94% and harvesting 93% in the area. The study further reveals that if sweet potato is well managed, it has the potential for food security and alleviating farmers from their poverty. This suggests that sweet potato should be given adequate attention in terms of production, value addition and marketability.*

Keywords— Sweet potato, production, poverty alleviation, value addition, gender.

I. INTRODUCTION

Sweet potato (Ipomoea batatas) is a creeper of the *Convolvulaceae* family. It originated from Central America and is widely grown as important staple food in most parts of the world. Presently, Nigeria is number one producer of sweet potato in Africa with annual output of 3.46million metric tons (FAO, 2006) and globally the second largest producer after China. The crop is grown for both human and animal consumption. Sweet potato is the only crop among the root and tuber crops that has a positive per capita annual rate of increase in production in Sub-Saharan Africa (*Tewe et al, 2003*). It is the only member of the genus ipomoea whose roots are edible and is one of the world's most important foods crops due to its high yield and nutritive value (Data and Eronico, 1987). According to Chukwu (2001), within the root crop belt of Nigeria, especially the South-East agro-ecological zone, sweet potato has joined the league of life saving-crops as cassava. It blends well with rice, cowpea and plantain in most diets *(Ejechi et al., 2009)*. Fawole (2007) reported that sweet potato remains one of the three most important root crops in the world. In spite of these important aspects, less research has been done on sweet potato than on the other roots crops. The other major root crops, for instance, have had ongoing systemic studies for decades. Therefore, there is a dearth of information on the economics of sweet potato production in Nigeria.

The high agronomic potentials of sweet potato has been established at the International Institute of Tropical Agriculture (IITA) and the National Root Crops Research Institute (NRCRI), which are both located in the humid zone of Nigeria (Tewe et al, 2003). Its production provides job opportunity for the farmers, thus raising their income. Sweet potato is consumed without much processing in most parts of the Tropics. It is either eaten boiled, roasted or fried. Although sweet potato is a crop that is consumed in all parts of the country, its level of production still remains low. The crop ranks among the five most important food crops in over 50 developing countries (All about sweet potato, 2008).The roots can also be slightly fermented in water for 2-3 days to reduce the sweetness, then sun dried and milled, mixed with either yam or cassava flour for eating. The leaves and tender shoots of sweet potato are used as vegetable food. The leaves contain, on dry matter basis about 8% starch 4% sugar 27% protein and vitamins therefore are very nutritious. It also contains about 56mg carotene per 100kg dry matter. The leaves are usually eaten boiled or incorporated into soup and stews (Matthew et al, 2008).

Industrially, sweet potato flour can be used to substitute wheat bread making or maize flour in balanced feeds. Baby foods have been formulated using sweet potato while some bakeries blend 15-30% of sweet potato flour for making bread and 20-30% for pastries. It is also used in the brewing of alcoholic drinks and as sweeteners in non-alcoholics drinks. (Agbo and Ene,1992). Sweet potato starch can also have medicinal value. According to Hartwell (1971), the leafs decoction is used in folk remedies for tumor of the mouth and throat. Reported to be alternative, aphrodisiac, astringent, bactericide, demulcent, fungicide, laxative and

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tonic, industrial potentials of sweet potato have not been fully exploited due mainly to a chronic lack, of awareness of the commercial benefits derivable from sweet potato (Azgogu and Olomo, 2002). Little research is known to have been undertaken on the economics of sweet potato production compared to other roots and tubers like cassava and yam (Azogu and Olomo). Cultivation of root and tuber crops in Nigeria as in most Africa countries is threatened by the low prices of the crops and their products.

With the rising cost of labour and transportation, rural farmers can hardly sustain their farming systems considering the meager returns from their harvest. It is therefore advantageous to diversify the use of crop roots beyond those of the traditional food industry in Africa countries. Because sweet potato surpasses other root crops in terms of agronomic potentials, diversification into other food, feed and industrial uses will increase demand, ensure attractive prices and consequently encouraged farmers, to sustain and expand their root crop farming units.

In order to solve the problem of hunger in the society, there is need to increase production of crops with minimum effort, find market for the produce and improves its quality for acceptability by the public. Sweet potato is one of such crops. It requires minimum management practices such as weeding and fertilizer application. Hence the objectives of this study were: (i) to describe the socio-economic characteristics of the respondents (ii) determine the gender roles of families in the production of sweet potato (iii) to access the consumption rate of sweet potato (iv) to access marketability of sweet potato

II. METHODOLOGY

The survey was conducted in Karu, Kokona and Keffi of Nasarawa State, in 2016 to examine sweet potato production for poverty alleviation in Nasarawa State of Nigeria. The three agricultural zones in the area study were purposively selected based on strategic importance of sweet potato in the farming system of the sampled zones in the area. In each zone one Local Government Area was selected by simple random sampling technique from the list of all Local Government Areas in the State. Then in each Local Government Area, 6 communities were similarly selected by random sampling technique, and in each community, 10 sweet potato farmers were equally selected through the same sampling technique. 60 Respondents were obtained from each agricultural zone making up a sample size of 180 respondents for the entire study area, using structured questionnaires, relevant data on house hold sweet potato production were collected from the respondents. Data were analyzed with descriptive statistics.

III. RESULTS AND DISCUSSION

Socio-economic Characteristics of Respondents

Sex

Result from Table 1 showed that majority of the respondents were male farmers (62.2%)
for Nasarawa State, while the female farmers accounted for (37.8).

Age

Result from Table 1 also showed that majority of the farmers age bracket 21-30 accounted for 0% in Nasarawa State. Age bracket 31-40 accounted for 5.0% 41-50 accounted for 21.1%, 51 -60 accounted for 37.2%, greater than 60 years accounted for 36.7%. From the above, it can be seen that they have able people for farming activities. This means, if the advantage of these able men are taken, production will increase, poverty and unemployment will be reduced.

Marital Status

With reference to table 1, 90.0 % of the respondents are in Nasarawa State are married, 6.7 % and 3.3% are single and widowed in Nasarawa State, respectively. The marital status of the farmers account for marital stability, which accounted for higher productivity.

Level of Education

Majority of the respondent's table1 had formal Education; Tertiary education accounted for 7.2% in Nasarawa State, Secondary education constituted 15.6% for Nasarawa State, Primary education accounted for 32.8% for Nasarawa State. While Those farmers who had no education accounted for 44.4% for Nasarawa State. The state has fairly educated farmers, which could make adequate use of agricultural information for optimal production.

Household Size

With reference to Table 1 the average household size of 1-5 accounted for 5.0% for Nasarawa State, 6-10 constituted 35.6%, 11-15 accounted for 21.7% while 16- 20 accounted for 24.4% and above 20 accounted 13.3%, respectively. In the absence of adult males, underage males were in some places designated household heads. In all, 88% of the households were male headed and 12% female headed.

Major Occupation

The result from table 1, showed that occupation of majority of the farmers in Nasarawa State is 100%

Farm experience in Farming of Sweet potato

The result from table 1 showed that 14.9% of the respondents in Nasarawa State had between 1-5 years experience in sweet potato farming. 6-10 had 25.6%, 11-15 had 22.8%, 16-20 had 15% and above 20 had 21.7%, respectively.

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Farm Size

Majority of the respondents table1 Nasarawa State accounted for 35% between 1-2ha, 35% between 3-4ha, 15% between 5-6ha, while above 6ha accounted for 14.9%, respectively. Their hectarage is quite small because more than 62% in Nasarawa State fall within 1-2ha. Which implies that their production output is still very low, which has contributed to their poverty and unemployment.

Association Membership

The results showed that 98.9% of the farmers belong to sweet potato grower association of Nigeria (POGMAN) while 1.1% does not belong to any group in Nasarawa State while in FCT State (POGMAN) accounted for 80% membership, (ECOMC) accounted for 3.3% while (CBNCO) accounted for 0.6% and no response accounted for 6.1% Table1

Estimated Annual On-Farm Income

The distribution of respondents according to their income revealed that about 68.5% of the respondents had annual income of between N20,000 – N100,000, while 25.0% had N101,000 – N500,000 and 6.1% had N501,000– N1,000.000. The mean annual income was (N196, 226.50).Farmers with low income will not be able to purchase subsidized farm inputs provided by the government. This implies that respondents with high farm income are most likely to purchase government inputs.

Membership of Organizations

Distribution of the respondents according to their membership of organization revealed that 96.2% belonged to organizations and the remaining 3.8% did not belong to any. Being a member of any organization could be an avenue for accessing information on increased productivity.

Fertilizer use

The fertilizer used was procured from Federal and State government fertilizer programme. Because of the delay of the fertilizer getting to the farmers, the percentage usage by farmers was quite encouraging.

Estimated Annual Off-Farm Income

Distribution of respondents according to their annual off-farm income revealed that 53.9% had annual off-farm income of between N20,000 – N50,000, followed byN51,000 N100,000 (24.4%), N101,000 – N150,000 (6.7%), N151,000 – N200,000 (10.6%) and N201,000 - N250,000 (4.4%), respectively. The mean off-farm income was N38, 127.50. This is in addition to the annual on-farm income which could assist the farmer in purchasing more subsidized inputs to increase production.

Planting Materials

Distribution of respondents according to the plant materials grown by farmers' revealed that 87.2% of the respondents got their materials from International Institute for Tropical Agriculture, Ibadan and National Roots Crops Research Institute Umudike, Abia State, Nigeria. While the remaining 12.8% got their planting materials from local vendors.

Labour Use

Majority 83.3% of the respondents use family labour while the remaining 16.7% used hired labour.

Table.1: Distribution of Socio-economic Characteristics of the Respondents

Socio-economic Characteristics	**Nasarawa (n=180)**		
	Freq	**%**	**Mean**
Sex:			
Male	112	62.2	
Female	68	37.8	
Age(years)			
21 – 30	-	0	
31 – 40	9	5.0	
41 – 50	38	21.1	
51 – 60	67	37.2	
> 60	66	36.7	53.1
Marital Status			
Single	12	6.7	
Married	162	90.0	
Widowed	6	3.3	
Divorced	-	-	

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Level of Education			
No formal education	80	44.4	
Primary education	59	32.8	
Secondary education	28	15.6	
Tertiary education	13	7.2	
Mean of years spent in		8.4	
Acquiring formal education			
Household size			
(number)			
1 – 5			
6 – 10	9	5.0	
11 – 15	64	35.6	
16 – 20	39	21.7	
>20	44	24.4	
	24	13.3	
Major occupation			13
Farming			
Fishing	180	100	
Farming/Trading	-	-	
Hunting	-	-	
	-	-	
Farming experience(years)			
1 – 5			
6 – 10			
11 – 15	27	14.9	
16 – 20	46	25.6	
>20	41	22.8	
	27	15.0	
Farm size (hectares)	39	21.7	13.3
1 – 2			
3 – 4			
5 – 6	63	35.0	
>6	63	35.0	
	27	15.0	
Estimated Annual On-	27	14.9	3.3
farm Income (Naira)			
20,000 – 100,000			
101,000 – 500,000			
501,000 – 1,000,000	124	68.5	
	45	25.0	
Fertilizer use	11	6.1	
Fertilizer purchase from	196,226.5		
Government			
Not purchase from	171	95	
Government			
	9	5	
Membership of			
Organizations			
Yes			

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No		
	173	96.2
Planting materials	7	3.8
IITA/NRCRI		
Other vendors		
Labour use	157	87.2
Family labour	23	12.8
Hired Labour		
	150	83.3
	30	16.7
Estimated Annual Off-farm Income (Naira)		
20,000 – 50,000		
51,000 – 100,000		
101,000 – 150,000	97	53.9
151,000 – 200,000	44	24.4
201,000 – 250,000	12	6.7
	19	10.6
	8	4.4 38,958
Total	**180**	**100**

Source: Field Survey, 2009

Importance of sweet potato as a food security and cash crop.

Overall, sweet potato's most important role in Nigeria was as a supplementary food security crop, (Table 3), showed that Nasarawa State, still ranked 3rd in food importance and share 1st in cash with groundnut 35% each. Sorghum was the most important food security crop in Nasarawa State.

The survey reported that a woman in the household was engaged in sweet potato selling and most of the women ranked sweet potato as their most important cash crop. Sweet potato's importance as the principal cash crop varied considerably by area, with it being an extremely important source of cash for women in Nasarawa State.

Table.2: Ranking of importance of sweet potato as a food security and as a source of cash

Ranking Nasarawa State	Mz	Sg	Sp	Gn
Food security	26	39	25	10
Cash crop:	18	12	35	35

Source: Aboajah 2009 survey. Mz – maize, Sg- sorghum, Sp- sweet potato, Gn- groundnut

Sweet potato (Ipomoea batatas) is an important subsistence food security crop grown on a small-scale in the densely populated, mid- elevation areas (1,200-2,000m) of North Central Nigeria. It is a major staple food in North Central Nigeria by extension in Nigeria and a secondary food crop in the grain- based food systems of North Central Nigeria. The crop is vegetatively propagated, requires low inputs for cultivation and produces modest yields of storage roots (Ewell 1993). The storage roots have a low dry matter content (30% of the roots), with starch being the major component (Hagenimana1994). Like other root and tuber crops, fresh sweet potato does not store well because of its high moisture content. The high moisture content also make it's the crop bulky and therefore costly to transport over long distances. These attributes have made sweet potato and other root and tuber crops essentially crops for rural consumption, in setting where the chain from the producer to consumer is short. The sweet potato storage roots are usually harvested a little at a time as needed over an extended period. Harvesting this way provides a flexible source of food for households (Smit and Ocitti p' Obwoya 1994).

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Sweet Potato production, consumption and food security

Sweet Potato is the fourth most important food crop in the world (IITA, 2002). The World's annual output is greater than annual output of all other roots and tuber crops (FAOSTAT, 2008). Sweet potato is cultivated in 140 countries and more than 100 of which are located in the tropical and sub-tropical zones (Beukema et al. 1990). Annual world production currently totals 314.37 million tones and covers 19.55 million hectares (FAOSTAT, 2008). More than a million people worldwide eat sweet potato and the crop forms an important part of the diet of more than half a billion consumers in developing countries (FAOSTAT, 2008). In Africa, Nigeria occupies the seventh position in terms of total sweet potato production, 840,000 tonnes in 2007. This implies that with the average market price of ₦70, 000/tones in 2007 over ₦5 billion circulated in the Nigerian economy through sweet potato production. Aboajah (2009) revealed in a study on sweet potato consumption pattern of households, that households in North Central Nigeria spend only 10% of their food budget on sweet potato. Similarly, FAOSTAT (2008) reported that Nigeria has the lowest per capital sweet potato consumption of 3.27kg in Africa. The households in Nasarawa State of North Central Nigeria also identified sweet potato being a staple food crop, nutritional content convenient fast food, easy to cook compared with other food crops as most important factors influencing sweet potato consumption. These cardinal issues are paramount in solving food security problems. Nigerian's estimated cultivated area under sweet potato in 2007 is 266,000 hectares with an average yield of 3.27 tonnes/ha (FAOSTAT, 2008).

Labour Contribution by Gender to Farm Activities (Sweet potato) Nasarawa State

The contribution of labour by gender in farmers of activities showed that in land preparation in Nasarawa State Adult male account for (79%) adult female (3%), male children (16%) and female children 2%. Ridging, in Nasarawa State Adult male accounted for (81%), Adult female (2%), male children (16%) and female children (17%).planting activities in Nasarawa State adult male accounted for (3%) in weeding, adult female accounted for (53%), male children accounted for (8%) in harvesting adult female accounted for 21%, children male and female accounted for 72%, respectively. From the table above, showed clearly that adult male and male children are more involved in land preparation and ridging while their counterpart are pruned more in planting, weeding and harvesting. Table 3

Table.3: Contribution by Gender to Farm Activities

NASARAWA				
Activities	Adult M	Adult F	MC	FC
Land Preparation	79	3	16	2
Ridging	81	2	16	1
Planting	3	74	10	13
Weeding	8	53	21	18
Harvesting	7	21	38	34

Adult M= Adult male, Adult F = Adult female, MC= Male child and FC= female child

Income from Sales of Sweet potato

Total amount for sweet potato sales in Nasarawa State, there was a total of ₦ 286,300 of which Keffi accounted for 32.4%, Karu 34.7% and Kokona 32.0% with the average sales of ₦ 1545, ₦ 1655 and ₦1572 respectively for Keffi, Karu and Kokona.

From the above, the survey show that while Keffi in Nasarawa has the least with ₦1545. Table4**.**

Table.4: Income from Sweet Potato Sales

NASARAWA			
SWEET POTATO SALES			
	Amount (₦)	Total Sales	Average
Keffi	99,200	32.4	1545
Karu	99,300	34.7	1655
Kokona	94,300	32.9	1572
Total	286,300	100	4772

IV. CONCLUSION

The consumption patterns of sweet potato in the surveyed areas indicates that the crop is a major staple food in the diet of Nasarawa State by extension Nigeria. However, the utilization base of sweet potato is mainly limited to boiled or steamed roots for food and minimal feeding of vines and peelings to livestock. There is virtually no storage of mature fresh roots except in-ground as a crop and there is very little processing. The most important role of sweet potato is that it is a food security crop. Developing early maturity and drought tolerant varieties that are resistant/ tolerant to major pests and diseases would help fill the gap during sweet potato scarcity when demand is high. Demand of sweet potato is high immediately after drought period. There is need for research into the utilization of vines for food, feed, identifying varieties with good drying and processing properties, developing low-cost post harvesting storage technologies and processing sweet potato into food, animal feed and other products to stimulate production and increase income generation among farmers. The war against food security, which is expressed as inadequate food supply,

instability in its availability and un-affordability by consumers, could be fought by effective use and production of sweet potato. The conductive climate in Nasarawa State which allows for three cycles of sweet potato production in a year makes sweet potato highest yielding tuber crop if adequate attention is focused on research. The high nutrients content of sweet potato is an added advantage in food security. The Sweet Potato Research Programme has been playing the leading role in sweet potato research and in expanding sweet potato production to new frontiers. Sweet potato from the last decade experienced a remarkable increase because of its activities as food security crop.

For sweet potato crop to play more roles in food security and poverty alleviation in Nigeria,if well manage could boost the nation economic and reduce poverty. Sweet potato production, processing and marketing are still at subsistence level.

RECOMMENDATIONS

Varieties with good processing qualities should be scaled up through research

a. Proper funding and provision of infrastructure and other equipments necessary in research activities
b. Promotion of efforts to seek non-traditional funding for research and development of R&T.
c. Policy makers also need to be sensitive to the allocation of resources within national R & T programme in order to ensure that post-production activities is not underfunded in relation to production research
d. The problem of food insecurity in the study area can be reduced if sweet potato consumption and processing techniques are encouraged.
e. The study area need to reduce ignorance about food forms, correct faulty food habits to improve on the supply of food available.
f. Diversification of sweet potato consumption will be enhanced if sweet potato flour and sweet potato starch processing plants are provided and policies put in place to encourage adding sweet potato flour to wheat flour in the preparation of bread.

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Characteristics of hunters and the socio-economic importance of selling game on the survival of village households in the Kisangani Forest Region (R.D.C.)

Caractéristiques des chasseurs et importance socioéconomique de la vente de gibiers sur la survie des ménages villageois en région forestière de Kisangani (R.D.C)

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Abstract—*The present study focuses on the description of hunting professionals and intends to determine the contribution of hunting in the survival of village households in the former Province Orientale. It contributes to the in-depth knowledge of the profile of these key players in the commercial bushmeat market and the understanding of the socio-economic context at the first level of the game sales chain. The methods used to achieve the set objectives are summarized first of all in the questionnaire which made it possible to establish the survey form containing the identity of the interviewees and other descriptions, the interview, then the direct observation on the ground. , cost-benefit analysis and chi-square test and Anova.*

After tabulation and data processing, it appears that the hunters are all men whose age varies from 20 to 69 years. They come from different ethnic backgrounds and most of them are married and responsible for households. As a level of education, there are among them illiterates, primarians and some graduates.

The results show that bushmeat consumption occurs 1 to 3 times per week in most village households. The average monthly hunter's income estimate is USD 58.18 to meet the multiple daily needs.

Keywords—bush meat, profile, hunters, socio-economic importance, hunting income, village hunting, Rubi - Tele, Kisangani

Résumé— *La présente étude se focalise sur la description des professionnels de la chasse et compte déterminer l'apport de la chasse dans la survie des ménages villageois dans l'ancienne Province Orientale. Elle contribue à la connaissance approfondie du profil de ces acteurs clés du circuit commercial de la viande de brousse et la compréhension du contexte socioéconomique au premier niveau de la filière de vente du gibier. Les méthodes utilisées pour atteindre les objectifs fixés se résument tout d'abord au questionnaire qui a permis d'établir la fiche d'enquête contenant l'identité des enquêtés et d'autres descriptions, l'interview, ensuite l'observation directe sur terrain, l'analyse des coûts-bénéfices et le test de chi-carré et Anova.*

Après dépouillement et traitement de données, il ressort que les chasseurs sont tous des hommes dont l'âge varie de 20 à 69 ans. Ils sont issus de différentes ethnies et la plupart d'entre eux sont mariés et responsables de ménages. Comme niveau d'instruction, on compte parmi eux des analphabètes, des primairiens et quelques diplômés.

Les résultats démontrent ensuite que la consommation de la viande de brousse intervient 1 à 3 fois par semaine dans la plupart des ménages villageois. L''estimation de revenu mensuel moyen tiré par un chasseur se fixe à USD 58,18 lui permettant de faire face aux multiples besoins quotidiens.

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Mots clés —viande de brousse, profil, chasseurs, importance socioéconomique, revenus de chasse, chasse villageoise, Rubi -Télé, Kisangani.

I. INTRODUCTION

Un commerce florissant de viande de brousse s'accroit ce dernier temps en R.D.C. Plusieurs facteurs sont cités comme causes de ce phénomène. Nous retenons entre autre la demande grandissante en protéines animales (Basa *et al*., 2017),la crise économique et le sous-emploies (Bahuchet, 2000). La chasse se présente comme l'ultime alternative pour les forestiers (Wilkie *et al*., 2005). L'usage des techniques non écologiques, les armes à feu et le non-respect de la réglementation et textes légaux sur la chasse en vigueur en R.D.C sont des abus régulièrement commis par les villageois en vue de maximiser leurs gaies (Consolate Kaswera *et al.,* 2016, 2017).

De nos jours, quelques données sont disponibles par rapport au flux de viande sur les différents marchés urbains et péri urbains de Kisangani (Belembo et al.,2003 ; Nebesse, 2016). Néanmoins, les publications orientées vers les chasseurs qui du reste sont les acteurs clés du circuit de vente de cette ressource demeurent lacunaires voir inexistantes. Leurs comportements ainsi que les techniques de capture, les modes de conservation et de traitement des carcasses ne restent pas constants puisque le contexte change et ils essayent de s'adapter tant mieux que mal. Les prélèvements se font aussi bien pour l'autosubsistance que pour la vente. La durabilité des activités cynécologiques dépend donc ipso facto du comportement responsable de ces acteurs au niveau primaire du circuit de commercialisation de viande de brousse. Ces professionnels et amateurs de chasse doivent être conscientisés, éduqués, encadrés, écoutés et accompagnés. Cela n'est possible que lorsque leur profil est connu, les motivations les poussant à prélever plus d'espèces ainsi que leurs conditions sociales améliorées.

Nous nous sommes posé trois questions fondamentales à savoir :

1° Quel est le profil des chasseurs en région forestière de Kisangani?

2° Comment s'y prennent-ils actuellement pour prélever les gibiers?

3°Quel est l'apport de la chasse dans la survie des chasseurs villageois

La présente étudevise tout d'abord à décrire le portrait des chasseurs dans l'ancienne Province Orientale en République Démocratique du Congo, ensuite démontrer la tendance actuelle d'abandonner les pièges traditionnels vers l'usage d'armes à feu plus performants. Enfin, elle établit l'apport socioéconomique de la vente des gibiers sur les ménages au premier niveau de la filière viande de brousse en région forestière de Kisangani.

Comme intérêt, l'étude fournit aux scientifiques et gestionnaires de la biodiversité des données relatives aux chasseurs qui sont des collaborateurs et partenaires en matière de stratégies de gestion durable des ressources faunistiques.

Les hypothèses sont formulées comme suit :

1° Les acteurs de chasse proviennent de toutes les couches sociales présentent dans la contrée,

2° Les chasseurs font usage de plusieurs techniques pour optimiser les prélèvements.

3° L'apport de la chasse s'évalue en termes de consommation alimentaire et des revenus monétaires dans la survie des chasseurs villageois.

Présentation des zones d'études

Les investigations avaient été menées dans les zones giboyeuses situées sur deux axes routiers à savoir Kisangani-Ituri et Kisangani -Buta. Les villages visités sur la route Ituri en Province de la Tshopo sont Baegofa et Bafwaboli situés respectivement aux points kilométriques 122 et 147 de mai à juillet 2015 (figure1).

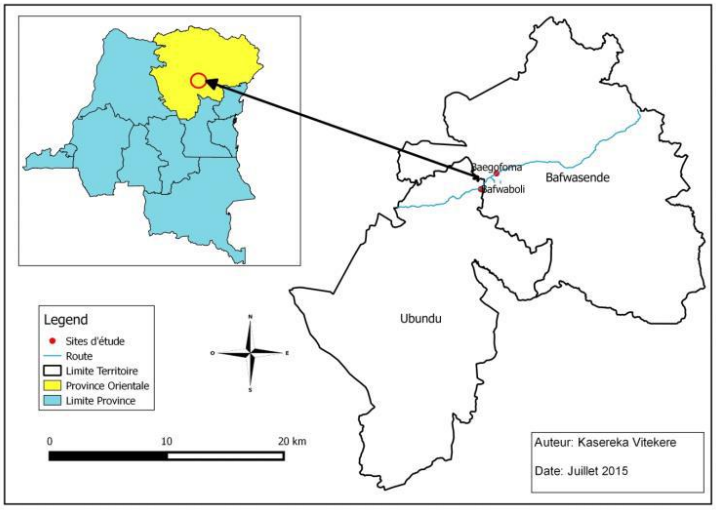

Fig.1: Localisation des sites giboyeux sur la route Ituri

Sur la route Buta, la prospection avait été menée au sein du Domaine de chasse de Rubi-Télé (DCRT). Pour cette étude, 5 localités ont été retenues. Nous sommes partis d'abord de la localité Baangba (Sukisa/centre), puis deux localités en amont dont Bondeme et Ngbete et deux autres en aval dont Bongbongo et Bobusanga. La sélection des localités a été faite sur base des critères comme l'effectivité de l'activité de chasse dans la localité; la facilité d'accéder à la localité et la distance de la localité par rapport à Baangba/Sukisa, étant donné que cette localité a constitué notre point de départ.

Le DCRT touche essentiellement cinq entités territoriales administratives dont les Territoires d'Aketi, Bambesa et Buta situés dans la Province du Bas-Uélé d'une part, et les Territoires de Banalia et Basoko qui se localisent dans la Province de la Tshopo d'autre part. Ce domaine de chasse s'étale donc sur 8 secteurs ou collectivités dont certains se trouvent dans la Province du Bas-Uélé : Bayeu-Bogbama, Mabinza, Makere II, Mongazulu et Yoko, et d'autres dans la Province de la Tshopo : Baboa de Kole, Wahanga, et Yamandundu. Ceci justifie la diversité ethnique constatée lors de l'échantillonnage.

Les cinq localités du DCRT visitées sont Baangba, Bongbongo, Bobusanga, Bondeme et Ngbete (figure 2). La collecte des données dans ce site était effectuée au cours du mois de juin 2016.

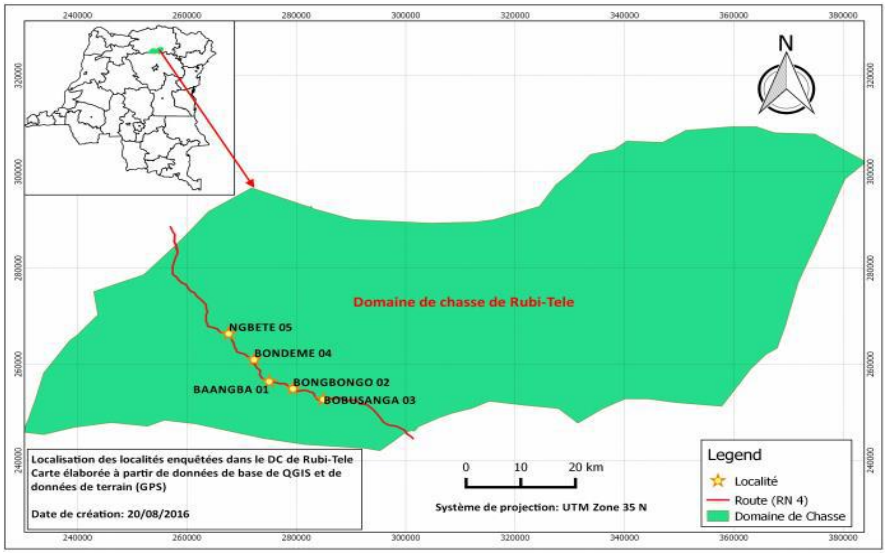

Fig.2: Domaine de chasse de Rubi -Télé (DCRT)

II. METHODOLOGIES

Nous avions capitalisé les informations données par les revendeuses de viande de brousse au marché central de Kisangani lors d'un pré enquête diligenté avant la récolte des données proprement dite.

Un guide d'interview a été mis à place et soumis aux chasseurs présents dans les villages. Les questions étaient interprétées en Kiswahili ou Lingala lors de l'administration. L'interview était complétée par des observations de terrain, un suivi des chasseurs pendant plus ou moins deux mois et des entretiens auprès de la population locale.

Compte tenu de la difficulté de disposer des listes de tous les chasseurs dans les localités retenues, nous avons procédé par la technique de boule de neige (utilisation de personnes comme source d'identification d'unités additionnelles) pour le choix de l'échantillon. Dans ce type d'échantillon, appelé aussi échantillon par réseau, les individus sont sélectionnés en fonction de leurs liens avec un « noyau» d'individus. On se base par exemple sur les réseaux sociaux, les amitiés, les relations d'affaires pour recruter de nouveaux sujets (Fortin, 2008).

Traitement des données

Les données récoltées à partir de l'enquête ont été parcourues, dépouillées, codifiées et saisies sur Microsoft Excel 2010, à partir duquel une base des données a été constituée. Partant de cette dernière, quelques calculs ont été appliqués en vue de déterminer certaines statistiques (fréquences absolues et relatives, moyennes, écart-type, valeur maximale, valeur minimale, etc). Le test statistique Anova a été appliqué pour la comparaison des moyennes. L'interprétation des résultats obtenue (p-value) au seuil de signification ($\alpha = 0,05 = 5\%$). Si p-value > α : pas de différence significative entre les moyennes ; alors que si p-value < α : la différence est significative entre les moyennes.

Le test de l'indépendance du chi-carré pour analyser la relation entre 2 variables qualitatives. Si p-value > au seuil de 5% : pas de différence significative, les deux variables sont indépendantes ; tandis que si p-value < α : il existe une différence significative, les deux variables ne sont pas indépendantes.

Détermination de l'apport de la chasse dans la survie des chasseurs

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Pour la fréquence de consommation, nous avons évalué, au cours d'une semaine le nombre de fois que le ménage consomme la viande de brousse.

Pour estimer les revenus monétaires issus de la vente des produits de chasse, nous sommes partis d'abord de l'estimation des coûts mensuels de la chasse, puis de l'estimation des recettes mensuelles issues de la vente des gibiers. Afin l'estimation de Revenu monétaire (Profit) déterminée par la différence entre les recettes et les coûts mensuels de chasse.

Les coûts retenus et estimés après entretiens avec les chasseurs sont notamment ceux liés à l'achat des munitions (cartouches), la ration mensuelle ainsi que l'achat d'autres équipements (piles de torche) par mois.

Coût Total (CT) = Achat munitions + Ration + Achat autres équipements

Les recettes étaient obtenues en multipliant le prix moyen unitaire par le nombre des gibiers estimés capturés par mois.

Recettes/Espèce = Nbre des gibiers x PMU

Recettes Totales (RT) = ∑Recettes/Espèces

Revenu monétaire mensuel issu de la vente des produits de chasse :

Revenu mensuel Total (Profit) =RT-CT

Revenu mensuel moyen=Revenu mensuel Total/n

n = taille d'échantillon (52 chasseurs enquêtés)

Rentabilité économique de la chasse (RE) : le résultat (Revenu/Profit) comparé au Chiffre d'affaires (Recettes Totales)

$$\mathbf{RE} = \frac{Revenu\ mensuel\ Total\ (Profit) * 100}{Chiffre\ d'affaires\ (Recettes\ Totales)}$$

$$\mathbf{ou}\ \frac{Revenu\ mensuel\ moyen * 100}{Recettes\ mensuelles\ moyennes}$$

III. RESULTS

1. Profil sociodémographique des chasseurs

Au total 94 chasseurs exclusivement hommes ont été questionnés dans les deux zones giboyeuses retenues. Sur la route Ituri, 21 chasseurs étaient inventoriés au village Baegofoma et 21 autres à Bafwaboli. Sur la route Buta, 52 chasseurs ont été questionnés en raison de 10 chasseurs par chaque localité: Baangba, Bondeme, Ngbete et Bongbongo, alors que 12 autres ont été enquêtés dans la localité Bobusanga.

1.1. Statut matrimonial des chasseurs et taille des ménages

Parmi les chasseurs interviewés 5,7% sont célibataires et 94,3% mariés. Les célibataires vivent dans les ménages composés de 1 à 5 individus. Par contre pour la catégorie des chasseurs mariés 36,7% ont une taille de ménage composé de 1 à 5 individus, 46,9% chasseurs ont une taille de ménage constitué de 6 à 10 individus,14,4% en ont constitué de 11 à 15 individus et enfin 2% gèrent une taille de ménage allant jusque plus de 20 individus (figure 3).

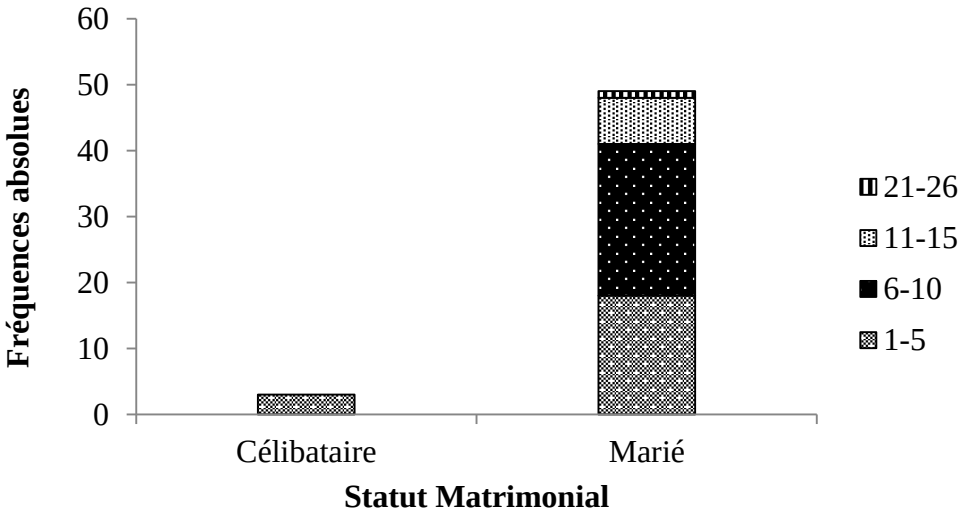

Fig.3: Répartition des chasseurs en fonction de statut matrimonial et taille de ménage

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Partant de l'âge, du statut matrimonial et de la taille des ménages, on comprend que la majorité de chasseurs exerce cette activité pour l'autosubsistance des ménages dans le cadre de la responsabilité familiale.

1.2. Activités principales des chasseurs enquêtés

L'activité principale pour la majorité des chasseurs enquêtés est l'agriculture, tandis que les restes pratiquent la chasse comme activité principale (figure 4).

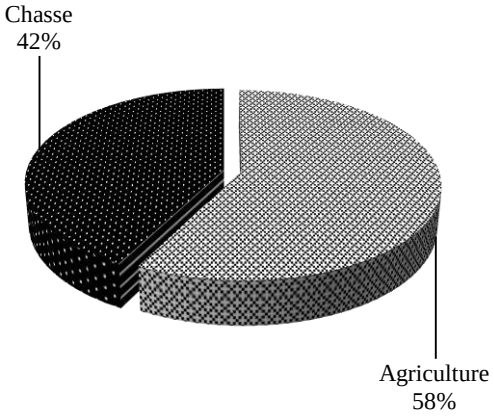

Fig.4: Répartition des chasseurs par activité principale

L'agriculture semble être l'activité principale des populations vivant en périphérie et dans les zones étudiées. Elle est cependant peu développée suite aux superficies limitées et une faible diversité des spéculations. Elle n'est pas susceptible de subvenir aux besoins de cette population en termes de revenus car les filières agricoles ne sont pas encore suffisamment développées (Pro-Routes, 2013). La collecte saisonnière des produits forestiers non ligneux tels que du miel, des chenilles, des escargots et la pêche est également pratiquée.

1.3. Age des chasseurs

L'âge des chasseurs s'étend de 20 à 69 ans d'après le tableau (1). La majorité d'enquêtés se retrouve dans la tranche d'âge comprise entre 20-49 ans caractérisée par une grande responsabilité familiale.

Tableau.1 : Effectifs par intervalle d'âge des chasseurs

Intervalle d'âge	Effectifs chasseurs Route Ituri	%	Effectifs chasseurs DCRT	%	Total chasseurs	%
20-29	9	21,4	14	26,9	23	24,4
30-39	13	30,9	19	36,5	32	34
40-49	11	26,1	13	25	24	25,5
50-59	8	19	5	9,6	13	13,8
60-69	1	2,3	1	1,9	2	2,1

Il découle de ce tableau que l'âge des chasseurs varie de 20 à 69 ans. La majorité des chasseurs actifs se retrouve entre 20 et 49 ans, avec une prédominance de la tranche d'âge 30-39 ans qui a fourni 34% de l'effectif inventorié sur les deux sites.

1.4. Niveau d'instruction des chasseurs par intervalle d'âge

Il ressort des enquêtes qu'au sein de l'intervalle d'âge 20 - 49 ans, lequel intervalle regroupe la majorité des chasseurs actifs, 41,1% ont un niveau secondaire d'instruction, 34,6% sont d'un niveau primaire et 7,6% analphabètes. Par ailleurs, pour l'intervalle d'âges 50-69 ans, 9,6% sont d'un niveau d'études primaires et 1,9% d'un niveau d'études secondaires.

Le test chi-carré appliqué pour vérifier la différence de la répartition des chasseurs par classe d'âges et par niveau d'instruction a montré une différence non significative, les deux variables sont indépendantes (X^2= 4.26, degré de liberté (ddl) = 2, p-value = 0,12 > α = 0,05, différence non significative).

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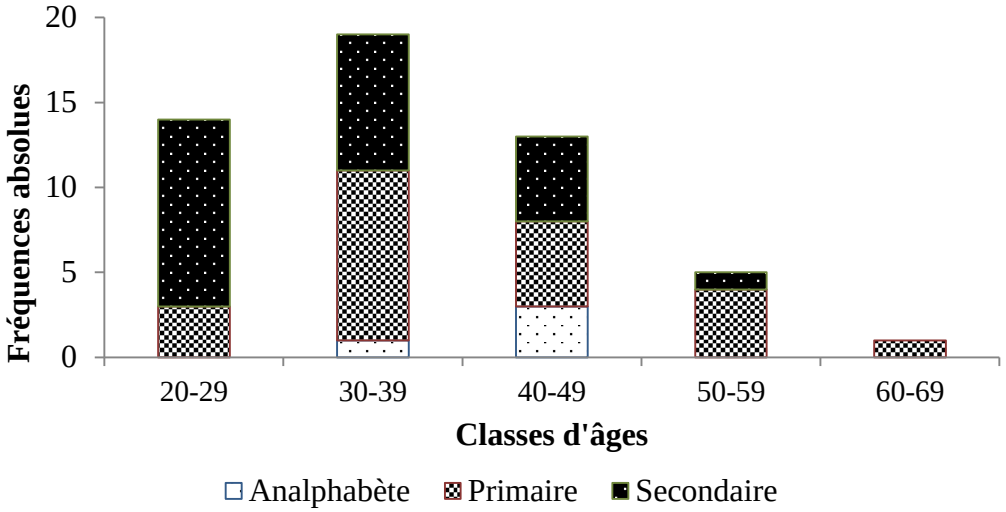

Fig.5: Niveau d'études des enquêtés par intervalle d'âge

1.5. Ages des chasseurs et techniques de chasse utilisées

Pour l'intervalle d'âges 20- 49 ans, 46,1% recourent à l'association pièges et fusil de fabrication locale (PFFL) alors que 42,3% utilisent exclusivement les fusils de fabrication locale (FFL) pour un bon rendement de l'activité. En outre, pour la catégorie des chasseurs avancés en âge (50-69 ans), 5,7% recourent aux FFL et 6 % recourent à la technique mixte PFFL. Le constat est que de plus en plus les chasseurs considèrent que les pièges seuls ne sont pas rentables et ne les font pas habituellement. C'est pour cette raison que dans le cadre de cette recherche, nous avons eu seulement à analyser les aspects socioéconomiques de la chasse au fusil. Le test d'indépendance du chi-carré appliqué démontre que la répartition des chasseurs par classe d'âges et techniques utilisées ne diffère pas significativement, c'est-à-dire que les deux variables sont indépendantes (X^2= 0, ddl = 1, p-value = 1 > α = 0,05 ; différence non significative).

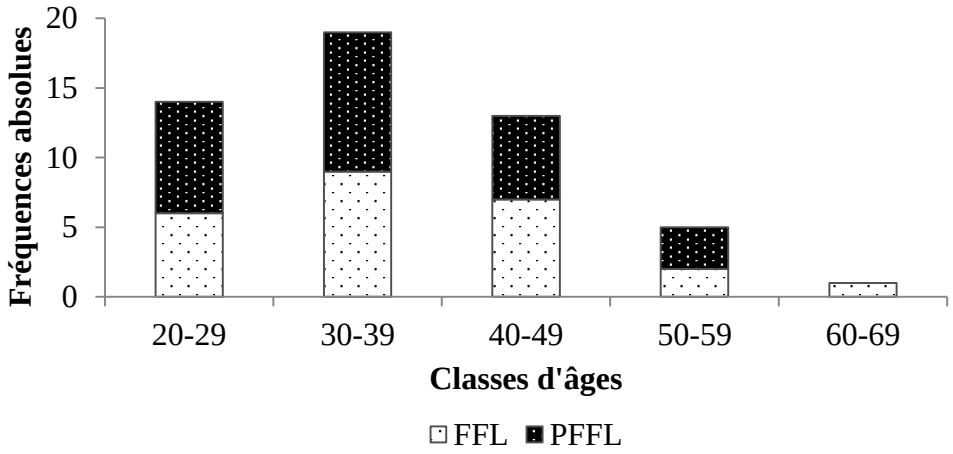

Fig.6: Répartition des chasseurs par classe d'âges et techniques utilisées

1.6. Ages des chasseurs et années d'expérience d'activité

Il ressort de la figure (figure 7) ci-dessous que les chasseurs actifs (20 à 49 ans) ont une expérience de 1 à 30 ans d'une part, et ceux avancés en âge (50 à 69 ans) en ont de 31 à 50 ans d'autre part. La répartition des chasseurs en fonction de l'âge et expérience diffère significativement, les deux variables sont dépendantes. L'expérience dépend de l'âge du chasseur(X^2 = 42.66, ddl = 1, p-value = 6.499e-11 < α = 0,05, différence significative).

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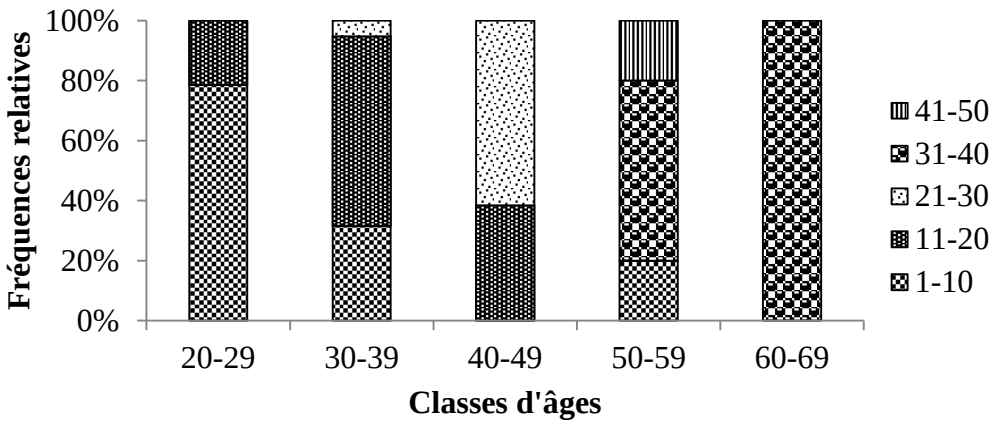

Fig.7: Répartition des chasseurs en fonction de l'âge et expérience

1.7. Appartenance ethnique

Les chasseurs recensés au sein de nos sites d'études proviennent de groupes ethniques variés. La majorité des chasseurs recensés n'est pas faite que d'autochtones, d'autres sont originaires des provinces voisines. A Baegofoma, les ethnies prédominantes sont: les Topoke (38,09%), les Mongo (19,04%) et les Bali (9,52%) tandis que les ethnies minoritaires sont constituées de Kumu, et Ngelema (1%). A Bafwaboli les chasseurs sont majoritairement Mbole (19,04%). Pour le DCRT, les Bowa représentent 51,9%, suivis des Ngelema 44,2%, des Zande 1,9% et des Ngbeto 1,9%.

2. Apport de la chasse dans la survie des chasseurs villageois du Domaine de chasse de Rubi - Télé

2.1. Espèces chassées, prix moyens unitaires (PMU) et leurs statuts

Les informations relatives aux taxa régulièrement chassés dans ce domaine, le prix moyen unitaire par espèce et le statut de protection font l'objet du tableau ci - dessous.

Tableau.2 : Fréquences des espèces chassées, PMU et leurs statuts

N°	Espèces		Fréq. relatives	PMU($)	Statuts de protection	
	Noms scientifiques	Noms vernaculaires			RDC	UICN
1	*Cephalophus monticola* Thunberg, 1789	Mboloko	98%	3,62	NP	LC
2	*Cephalophus dorsalis* Gray, 1846	Koto	83%	18,96	NP	LC
3	*Cercopithescus ascanius* Audebert, 1799	Makako	79%	4,06	NP	VU
4	*Atherurus africanus* Gray, 1842	Ndjiko	50%	2,91	NP	LC
5	*Potamocherus porcus* Linnaeus, 1758	Ngulu	35%	34,04	PP	LC
6	*Cephalophus nigrifons* Gray, 1871	Mbengele	33%	15,01	NP	LC
7	*Cricetomys emini* Waterhouse, 1840	Motomba	31%	1,40	NP	LC
8	*Cephalophus sylvicultor*	Mulimbu	8%	31,58		LC
9	*Hyemoschus aquaticus* Ogilby, 1841	Elebé	8%	10,26	PP	LC
10	*Tragelaphus spekei* Spek, 1863	Mbulimasuwa	4%	52,63	PP	LC
11	*Colobus guereza* Sclater, 1860	Magistrat	4%	6,84	TP	EN
12	*Pan troglodytes*	Sokomoto	4%	42,11	TP	EN

	Blumenbach, 1775					

Légende :
Statuts selon l'UICN
EN : Endangered (en danger)
LC : Least Concern (Préoccupation mineure)
VU : Vulnérable (Vulnérable)

TP : Totalement Protégée
PP : Partiellement Protégée
NP : Non Protégée

Statuts selon la loi Congolaise (RDC)

2.2. Consommation de la viande de brousse par les ménages des chasseurs villageois

Le tableau ci-dessous stipule que plus de la moitié des enquêtés dans les localités visitées affirment avoir consomme la viande de brousse une a trois fois par semaine alors que 5 la consomme rarement. Le chasseur prélève la faune sauvage pour deux motifs essentiels, l'autosubsistance et le lucre.

Tableau.3 : Fréquence de consommation de la viande de gibier par semaine

Fréq. de cons/sem.	Baangba	Bongbongo	Bobusanga	Bondeme	Ngbete	Total
1 fois	4	1	4	1	6	16
2 fois	1	5	5	6	3	20
3 fois	1	3	0	0	1	5
4 fois	1	0	0	1	0	2
5 fois	1	0	0	0	0	1
6 fois	1	1	0	0	0	2
Chaque jour		0	1	0	0	1
Rarement	1	0	2	2	0	5
Total	10	10	12	10	10	52

2.3. Estimation des coûts mensuels de chasse

Les principaux coûts de chasse identifiés lors de nos enquêtes dans les 5 localités du DCRT sont notamment ceux liés à l'achat des cartouches, la ration et d'autres équipements (piles et torches). Le prix moyen d'une cartouche est estimé à $ 1,58. Ainsi pour un cumul de 99 expéditions par mois, l'estimation faite sur base des entretiens avec les 52 chasseurs de 5 localités enquêtées, il se dégage des charges relatives à l'achat de cartouches de $ 915,79 pour la ration $ 159,84 alors que celles relatives à l'achat d'autres équipements sont estimées à $ 90,00.

Tableau.4 : Estimation des coûts mensuels de chasse par localités

Localités	Nbre Expédition	Cartouches	Ration	Autres équipements	Total
Baangba	18	$ 153,16	$ 24,95	$ 15,79	$ 193,89
Bongbongo	20	$ 192,63	$ 43,79	$ 23,16	$ 259,58
Bobusanga	25	$ 257,37	$ 42,74	$ 21,05	$ 321,16
Bondeme	20	$ 183,16	$ 27,32	$ 15,26	$ 225,74
Ngbete	16	$ 129,47	$ 21,05	$ 14,74	$ 165,26
Total	99	$ 915,79	$ 159,84	$ 90,00	$ 1165,63

2.4. Estimation des Recettes brutes issues de la vente des produits de chasse

Le présent tableau met en lumière l'estimation des recettes brutes mensuelles issues de la vente de viande de brousse. Il en ressort donc le prix moyen unitaire par carcasse boucanée, le nombre des gibiers prélevés par espèce débouchant à une estimation des recettes brutes par espèces puis un total général des recettes brutes par localités. En effet, dans la localité Baangba, il se dégage une estimation de 70 gibiers des espèces confondues prélevés valant une somme de $ 673.02, à Bongbongo 92 gibiers pour une valeur monétaire de $ 1,022.14, à Bobusanga 124 gibiers ont été estimés comme prélèvement mensuel générant des recettes brutes de $ 1,155.80 ; 93 gibiers à Bondeme valant des recettes brutes estimées à $ 794.49 et enfin, dans la localité de Ngbete, l'estimation des prélèvements mensuels des gibiers des espèces confondues se fixent à 63 gibiers générant des recettes brutes de $ 545.58.

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Tableau.5: Recettes brutes de vente par localités

			Baangba		Bongbongo		Bobusanga		Bondeme		Ngbete	
N°	**Espèces**	**PUM/carcasse**	**Nbre**	**Recettes**	**Nbre**	**Recettes**	**Nbre**	**Recettes**	**Nbre**	**Recettes**	**Nbre**	**Recettes**
1	Cercopithecus ascanius	$ 4,06	21	$ 85,36	34	$ 138,21	45	$ 182,92	46	$ 186,99	24	$ 97,56
2	Cephalophus monticola	$ 3,62	22	$ 79,72	26	$ 94,21	28	$ 101,46	20	$ 72,47	19	$ 68,85
3	Cephalophus dorsalis	$ 18,96	26	$ 492,92	22	$ 417,09	36	$ 682,51	26	$ 492,92	20	$ 379,17
4	Cephalophus nigrifons	$ 15,01	1	$ 15,01	0	$ -	12	$ 180,17	0	$ -	0	$ -
5	Cephalophus sylvicultor	$ 31,58	0	$ -	2	$ 63,16	0	$ -	0	$ -	0	$ -
6	Tragelaphus spekey	$ 52,63	0	$ -	2	$ 105,26	0	$ -	0	$ -	0	$ -
7	Atherurus africanus	$ 2,91	0	$ -	0	$ -	3	$ 8,74	0	$ -	0	$ -
8	Potamochoerus porcus	$ 34,04	0	$ -	6	$ 204,21	0	$ -	0	$ -	0	$ -
9	Pan troglodytes	$ 42,11	0	$ -	0	$ -	0	$ -	1	$ 42,11	0	$ -
TOTAL			70	$ 673,02	92	$ 1022,14	124	$ 155,80	93	$ 794,49	63	$ 545,58

Dans la localité Baangba, il se dégage une estimation de 70 gibiers toutes espèces confondues prélevés valant une somme de USD 673,02 ; à Bongbongo 92 gibiers pour une valeur monétaire de USD 1 022,14 ; à Bobusanga 124 gibiers ont été estimés comme prélèvement mensuel générant des recettes de USD 1 155,80 ; 93 gibiers à Bondeme valant des recettes estimées à USD 794,49 et enfin, dans la localité de Ngbete, un prélèvement estimé à 63 gibiers générant des recettes de USD 545,58.

La différence n'est pas significative entre les nombres moyens des gibiers prélevés dans les localités (Annexe 5) (p-value = 0,17 > 0,05).

2.5. Estimation des Coûts Totaux et Recettes Totales de la chasse

Les coûts totaux mensuels ont été estimés à USD 1165,63 tandis que les recettes totales mensuelles à USD 4 191,01 (pour les 52 chasseurs). Pour un nombre moyen d'expédition de 1,90 ; le coût moyen mensuel est estimé à USD 22,42 et des recettes mensuelles moyennes sont estimées à USD 80,60. Il sied de souligner que la différence entre les coûts moyens des localités n'est pas significative ($p = 0,16 > \alpha = 0,05$). C'est le cas aussi pour les recettes moyennes des localités, ($p = 0,37 > \alpha = 0,05$).

Tableau.6: Coûts Totaux et Recettes Totales de la chasse (en USD)

Coûts de chasse	
Baangba	193,89
Bongbongo	259,58
Bobusanga	321,16
Bondeme	225,74
Ngbete	165,26
Coûts totaux mensuels de chasse	**1 165,63**
Recettes brutes	
Baangba	673,02
Bongbongo	1 022,14
Bobusanga	1 155,80
Bondeme	794,49

Ngbete	545,58
Recettes totales mensuelles	**4 191,01**

2.6. Estimation de Revenu/Profit mensuel moyen

Le revenu total de l'activité de chasse pour les 52 chasseurs est estimé à **USD 3 025,38**, un chasseur tire en moyenne un revenu mensuel estimé à **USD 58,18**.

Partant des résultats de cette étude ponctuelle, la chasse villageoise dans le DCRT apparaît comme une activité financièrement rentable, ainsi la rentabilité économique est estimée à 72,19% (tableau 7). Ceci dépend de la diversification des stratégies de chasse. Il est souhaitable que des études des longues durées soient conduites, en vue de saisir les fluctuations de la rentabilité de cette activité dans le long terme.

Tableau.7: Revenu mensuel moyen issu de la vente des produits de la chasse (en USD)

	Valeur monétaire
Coûts totaux mensuels de chasse (A)	1 165,63
Recettes Totales mensuelles(B)	4 191,01
Revenu/Profit mensuel Total (C) = (B) - (A)	3 025,38
Revenu/Profit moyen mensuel (D)= (C)/52	58,18
Rentabilité économique (E) = (C)*100/(B)	72,19%

2.7. Affectation des revenus de l'activité de chasse

Partant du tableau suivant, les affectations sont classées selon l'ordre décroissant des fréquences par catégorie d'affectations. Ainsi l'achat de condiments vient en première position avec une fréquence relative de citations de 71%, ensuite vient la scolarisation des enfants avec 67%, puis les frais liés aux soins de santé avec 58%, l'habillement avec 50%. En outre l'achat des produits cosmétiques a une fréquence relative de citations de 19%, et enfin les chasseurs affectant une partie de leur revenu à l'alcool représentent une fréquence relative de 4%.

Tableau.8: Fréquences des affections de revenu de chasse

Affectations	**Fréquences absolues**	**Fréquences absolues**
Achat Condiments	37	71%
Scolarisation des enfants	35	67%
Soins de santé	30	58%
Habillement	26	50%
Produits cosmétiques	10	19%
Alcool	2	4%

IV. DISCUSSIONS

1. Du profil sociodémographique des chasseurs et moyens d'usage

De par les enquêtes menées dans les sites retenus, l'activité de chasse est masculine et pratiquée par des chasseurs actifs (20 à 49 ans), dont 52% ont un niveau secondaire d'instruction. Il a toujours été avantageux de sensibiliser et collaborer avec des partenaires qui savent lire et écrire. Les gestionnaires des ressources naturelles doivent capitaliser cet atout lors de l'élaboration de divers programmes de sensibilisation et éducation environnementale. Ces chasseurs recourent aux fusils de fabrication locale et des pièges, avec un accent prononcé de recours aux fusils de fabrication locale. L'association calibre 12 avec le chien tel que signalé par les chasseurs semble plus intéressante vu que les animaux sont d'abord dénichés par le chien puis en tentant de se sauver l'arme intervient. En fait, l'arme à calibre 12 est le fusil fabriqué localement le plus utilisé d'après les enquêtés et nos observations lors d'un suivi des chasseurs dans les zones d'études.

L'expérience d'activité varie entre 1 à 30 ans. La plupart de ces chasseurs sont mariés et combinent la chasse à l'agriculture.

Endezoumou (2012) a constaté que la chasse était une activité essentiellement masculine dans l'Unité forestière d'Aménagement de Tala-Tala, et dont l'âge des chasseurs varie entre 20 à 65 ans. Le plus grand nombre de chasseurs se trouve dans l'intervalle d'âges variant entre 30 à 45 ans et utilisant beaucoup plus les fusils. Ce sont des jeunes mariés sans emplois et cherchant des moyens de survie pour leur famille. La plupart d'entre eux se sont limités au niveau d'instruction primaire, et pratique l'agriculture comme activité principale. Les résultats de cet auteur sont similaires à ceux de la présente étude, à la seule différence au niveau d'instruction où la fréquence relative est élevée pour les chasseurs, enquêtés dans le DCRT, ayant un niveau secondaire.

Les résultats de Dufour, *et al.* (2013) démontrent que les chasseurs sont avant tout agriculteurs. Il fait remarquer

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aussi que cette activité n'est pas organisée de même que dans la sous-région Ouest-Africaine. Il en est de même pour les chasseurs de la région forestière de Kisangani.

Par contre Mpamu (2010) a constaté les mêmes tendances que nous. En effet, il a démontré que la chasse est une activité purement masculine. Cet auteur explique que la filière de viande de brousse est animée à Kinshasa ainsi que dans d'autres pays du bassin du Congo par des femmes qui pratiquent le commerce en détail (74,1%), les hommes quant à eux sont plus concentrés sur la chasse et la pratique de la vente en gros du gibier (25,8%). Il ajoute que l'usage du fusil demeure l'une de principales méthodes de chasse (29,3%) suivi du piégeage (24,1%). Nebesse (2016), a abouti aux résultats selon lesquels les chasseurs de Basukwambula à Baego utilisent souvent le calibre 12 de la fabrication artisanale que les pièges à nylon.

Les résultats de Ayaya (2012) ont mis en évidence le fait que la majorité des chasseurs sont des jeunes mariés ; ils considèrent la chasse comme le moyen le plus direct et rapide d'avoir de l'argent liquide. Pour les pygmées, la chasse est une activité principale alors qu'elle est exercée en seconde position par les bantous. Trois types de techniques de chasse ont été identifiés: les pièges, le filet avec chien, flèches (arbalète). Il signale que la population de Bandisende parait ne pas utiliser le fusil pour la chasse. Les chasseurs Bantous ont un niveau d'étude primaire ou secondaire. Nathalie V. *et al.* (2010), rapportent que la population dans le milieu rural, en Afrique Centrale, en général et les chasseurs ne sont pas instruits. Ces résultats s'apparentent aux nôtres vu que la majorité d'enquêtés savait lire et écrire mais sans diplômes. La seule différence relevée de l'étude d' Ayaya est le fait que le fusil paraît ne pas être utilisé par les chasseurs de Bandisende.

2. De l'apport de la chasse dans la survie des chasseurs villageois

2.1. De l'apport de la chasse à la consommation des ménages

L'estimation de la fréquence hebdomadaire de consommation de gibier, pour la plupart de nos enquêtés, a été de 1 à 3 fois la semaine. Les produits de chasse sont considérés comme principale source des protéines animales, d'autant plus que peu de ménages pratiquent l'élevage.

Ces résultats rencontrent ceux de Dufour, *et al.* (2013), qui estiment que plus de 70% des ménages enquêtés dans toutes les régions de leur recherche, disent consommer de la viande de brousse une à trois fois par semaine. Alors qu'Endezoumou (2012.) estime une fréquence de consommation moyenne hebdomadaire de 5 jours la semaine.

La consommation de viande par les chasseurs-cueilleurs de la forêt d'Ituri au Nord-Est de la République Démocratique du Congo est estimée à 0,16 kg/personne/jour (Wilkie et Carpenter, 1999). Les populations urbaines au Gabon, en RDC et en RCA consomment en moyenne seulement 0,013 kg/personne/jour, moins de 10% de la quantité consommée par les chasseurs-cueilleurs en forêt. Ce qui corrobore nos résultats en démontrant cette caractéristique de la viande de brousse à contribuer à l'alimentation des peuples forestiers.

Par ailleurs Fargeot (2008) démontre que la consommation de viande était plus importante dans les zones urbaines que dans les zones rurales, du fait de la plus forte densité de population. La viande de brousse est largement consommée dans les pays du bassin du Congo, les quantités consommées vont de 30g/personne/jour à 180 gr/personne/jour en RDC (Fa et *al*, 2003). Les résultats de Kambale (2015) démontrent que, à Y angambi, la consommation des animaux sauvages occupe la troisième position après les poissons et les animaux domestiques. Il poursuit en évoquant le fait que la viande de brousse est consommée à Yangambi selon les habitudes alimentaires et selon la disponibilité.

Mpamu (2010) a trouvé aussi que la viande de brousse contribue énormément à la subsistance des populations rurales les plus démunies car accessible.

Les résultats de toutes ces études soutiennent le rôle important de la viande de brousse dans la survie des ménages villageois et l'octroi des revenus telle que démontré par les résultats de cette étude. Ceci confirme l'hypothèse 3 relative à l'apport de la viande de brousse en termes de consommation alimentaire.

2.2. Du revenu monétaire issu de l'activité de chasse

Il s'est dégagé dans cette étude, une estimation de revenu mensuel moyen issu de la vente des produits de chasse se fixant à USD 58,18 permettant à un chasseur de répondre aux besoins primaires de son ménage, et celle de la rentabilité de cette activité à 72,19%. Il sied de souligner que ces valeurs peuvent fluctuer en fonction des périodes de recherche. C'est pourquoi, il est même souhaitable de mener des suivis annuels de chasse afin d'observer le comportement de ces variables pendant des longues périodes. En dépit de cela, la chasse reste une activité qui procure des moyens de subsistance considérables aux populations vivant dans les zones étudiées.

Dans la Réserve de Biosphère de Luki, Toirambe (2002) avait confirmé l'existence d'une véritable entreprise cynégétique dans cette réserve et ses environs avec 16 points de ventes de gibier comptant un effectif de 83 vendeurs (tous des hommes) dont l'âge varie de 20 à 45ans. Le revenu moyen par vendeur et par semaine était

évalué à USD 16,14 soit USD 64,56/mois/vendeur. Ces résultats se rapprochent des nôtres.

Les différentes études réalisées à Kinshasa ont montré que les bénéfices tirés par chaque intervenant dans une filière dépendent généralement de la nature du produit et de la loi de l'offre et de la demande. Les résultats de Kabongo (2005) établissent que 93% des exploitants des PFNLs estiment que leur activité est très rentable car il leur permet tous de satisfaire leurs besoins primaires.

Au Cameroun, les résultats de Lescuyer (2010) ont donné une estimation d'un revenu annuel entre 25 000 et 35 000 FCFA par chasseur dans plusieurs sites de la région du Sud, et autour de 31 000 FCFA dans la commune de Mbang à l'Est. Toujours dans la région de l'Est mais autour de la Réserve du Dja, Ekodeck (2003) estimait le revenu annuel des chasseurs à environ 80 000 FCFA par personne. Alors que dans deux sites de la région Sud-ouest du Cameroun, Willcox & Nambu (2007) parviennent à une estimation comprise entre 52 000 et 85 000 FCFA. Au Gabon (Coad *et al.*, 2010) et en Guinée Equatoriale (Kümpel *et al.*, 2010) ont observé que la majeure partie des revenus issus de la chasse est utilisée par les hommes pour des achats d'alcool et de cigarettes, permettant notamment de renforcer le lien social avec les parents et amis, au Dja (Solly, 2004) comme dans le Sud-ouest (Wright & Priston, 2010, cités par Lescuyer, 2010).

Quand bien même, cette activité ne permet pas une accumulation de richesse, mais elle permet aux chasseurs vivant dans le DCRT et ailleurs de répondre aux multiples besoins essentiels de base pour leurs familles : scolarisation des enfants, payement des frais d'hospitalisation en cas de maladie, etc.

Dans la zone d'emprise de l'UFA, les résultats d'Endezoumou (2012) démontrent que le commerce de la viande de brousse vient après l'activité forestière industrielle. En effet pour les chasseurs de cette zone, l'activité de chasse leur procure des revenus contribuant à leur bien-être. Ainsi cet auteur a estimé le revenu mensuel moyen à 24 161,94 FCFA dans la zone de Tala-Tala. Cependant, ce revenu est estimé entre 19 082,17 et 28 530,61 FCFA dans le district de Mokéko, 12 783,5 et 17 284,55 FCFA à Ngbala et 28 720 et 32 586 FCFA dans le district de Sembé. Ces résultats sont un peu différents de ceux obtenus par Noudjieu (2005) lors d'une étude similaire dans l'UTO Campo-Ma'an qui ont établi un revenu mensuel variant entre 3 092 et 12 735 FCFA. Les grandes valeurs observées sur les écarts types, démontrent la grande variabilité qui existe entre les différents revenus des répondants.

Dufour, *et al.* (op.cit.) démontrent que la chasse revêt un caractère commercial important. C'est une source d'échanges financiers tant pour les populations rurales que pour certaines populations urbaines. Mpamu (2010) confirme cette observation en élucidant le fait que la filière permet une circulation monétaire importante, dans le sens villes vers campagne, qui équilibre les besoins en numéraire des ruraux, pour financer la santé, l'éducation, les impôts ou l'achat des équipements de base (matériel agricole, matériel de construction) (Feer, 1996).

En RDC, les communautés affectent une part importante de la viande de brousse (90%) à la vente en vue de trouver des moyens financiers suffisants et le reste (10%) est réservé pour la consommation (De Merode *et al.*, 2004). Ce fait a été observé aussi par Nebesse (2016). En effet la poursuite par les chasseurs d'un but lucratif a été observée sur l'axe routier de Kisangani-Ituri. C'est d'ailleurs l'explication du recours prononcé à la technique d'arme à feu jugée plus rentable par ces derniers. C'est comme ça que Kümpel *et al.* (2010) disent que les chasseurs trouvent flou de faire une différence entre chasse de subsistance et chasse commerciale, étant donné que la viande de brousse contribue à la fois à la consommation et aux revenus.

Fa & Brown (2009) démontrent que l'activité de chasse joue un rôle essentiel durant les périodes difficiles d'un point de vue alimentaire ou financier. Sur le plan financier, elle permet de tirer des moyens financiers susceptibles de couvrir des dépenses urgentes comme la scolarité ou les soins de santé.

De par ces résultats, il ne cesse d'être mis en exergue l'importance économique de l'activité de chasse pour les riverains des forêts. En effet cette dernière est exprimée par des revenus monétaires non négligeables permettant aux chasseurs de faire face aux besoins quotidiens de leurs foyers. Ces résultats ne font que corroborer ceux démontrés par cette recherche et de ce fait, confirment l'hypothèse 3 relative à l'apport de la chasse dans la survie des chasseurs vivant dans le DCRT.

V. CONCLUSION

La viande de brousse reste une source importante des moyens de subsistance pour les populations forestières en région de Kisangani. Elle occupe une place de choix vu son apport sur le plan socioéconomique dans le quotidien des ménages de ces populations. Cependant l'exploitation de cette ressource doit se faire conformément aux textes et mesures de gestion mises en place par l' autorité. En effet, cette étude socioéconomique de la chasse villageoise a été initiée dans le souci d'appréhender toutes ces considérations.

Décrire le profil des acteurs et révéler les aspects socioéconomiques de la chasse villageoise dans l'ancienne Province Orientale tel a été l'objectif principal de cette recherche.

Dans la poursuite de cet objectif, des enquêtes par entretiens semi-structurés auprès des chasseurs répartis dans différentes localités sur les routes Ituri et Buta, des observations directes sur le terrain ont été réalisées. La

technique documentaire a été utile pour la littérature relative au thème de recherche. Le traitement des données était facilité par Microsoft Excel 2010 et l'analyse des coûts-bénéfices. Cette recherche a abouti aux résultats ci-après :

- Pour le profil des chasseurs, ils sont tous hommes, répartis dans l'intervalle d'âge allant de 20 à 69 ans. Ils sont issus de différentes ethnies de la contrée mais aussi issues des localités voisines. Comme statut matrimonial, la majorité d'entre eux est marié et responsable de ménage. Concernant le niveau d'instruction, on compte parmi eux des analphabètes, des primairiens et quelques diplômés.
- Il ressort que l'arme à calibre 12 est le moyen le plus utilisé avec 80.67%. Elle est souvent associée aux pièges, aux chiens pour améliorer le rendement.
- La consommation de la viande de brousse intervient 1 à 3 fois/semaine dans la plupart des ménages des chasseurs enquêtés, et l'estimation de revenu mensuel moyen tiré par un chasseur se fixe à USD 58,18 lui permettant ainsi de répondre aux multiples besoins de base ; la rentabilité de la chasse était estimée à 72,19%.

Ces résultats confirment toutes les hypothèses qui ont été émises pour cette recherche.

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Geochemical investigations of a Portion of Obhu Hill Marble Deposit Okpella, Edo-State, Nigeria.

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Abstract*—The Obhu marble deposit is located at latitude 7º 21′ 31.2′′ to 7º 21′ 34.9′′ and longitude 6º 25′ 11.6′′ to 6º 25′ 18.0′′. The geochemical investigations of a portion of the Obhu hill marble deposit was aimed at investigating the reserve estimate in tonnage, rock mass/overburden volume and the geochemical composition of the deposit. This study was conducted by using vertical electrical sounding (VES) geophysical method to obtain the geo-electric parameters of the deposit, and to determine the reserve estimate of the Marble deposit of study area. The result shows that the reserve tonnage is 4.6 x 10^6 Tons and rock mass reserve/overburden volume ratio is 9:1.The chemical analysis were compared with the RMRDC of Nigeria for each element suitable for production of cement, fertilizer, iron, steel and other industrial uses. The MgO values of the samples 1 and 2 does not falls within the acceptable value of 6 % of RMRDC, with the exception of sample 3 which falls within the acceptable limit. CaO, Al_2O_3 and P_2O_5 values of the samples falls within the acceptable limits of RMRDC and so suggest that the marble can be put to industrial use, while the Fe_2O_3content of sample 2 and 3 makes them probably not best suited for industrial use, except for sample 1 having a lower value of 1.85 %.The silica SiO_2 content values for the three samples exceeded the recommended standard of RMRDC of 5 %. This result reveals that the marble deposit issuitable for most industrial use.*

***Keywords*— Geochemical, Geophysical, Reserve estimate, Reserve tonnage.**

I. INTRODUCTION

The leap in the economic growth of Nigeria and its gradual evolution into the most attractive destination for foreign direct investment in Africa is placing a huge strain on the available natural resources needed to keep pace with the myriad developmental and economic needs of the country. Infrastructural deficit caused by series of factors in the past has obviously exposed the need to invest massively in relevant construction projects to really prepare the country to achieve the much talked about vision 20 – 20 – 20. These has necessitated the exploration of more mineral deposits for such rocks like Marble, limestone and granites, and quarry them to meet the ever increasing demand in the construction and other related industry. Limestone is a sedimentary rock composed largely of the mineral calcite ($CaCO_3$), formed by either organic or inorganic processes (Serra, 2006). Marble is a metamorphic rock composed essentially of calcite ($CaCO_3$), dolomite [$CaMg(CO_3)_2$], or a combination of the two, with a fine- to coarse-grained crystalline texture (Fatoye and Gideon, 2013).The basement complex terrain of Nigeria in which the site is located is endowed with a host of solid mineral deposits that can be quarried for construction and other purposes.In addition to geochemical studies, geophysical methods have also been successfully deployed in the estimation of reserves in the basement complex.Locally available calcium carbonates are relatively common in many countries of sub-Saharan Africa and are well suited for small-scale mining and processing (Van Straaten, 2002 and Nduwumuremyi et al, 2013). One key challenge is lack of accurate data on the tonnage of rock and mineral deposits, which in turn, accounts for high prevalence of illegal mining activities, (Abdulawal*et al*, 2017). Estimating reserve and tonnage of mineral resources is a highly methodical process and has been the focus of various discussions, (Downing and Giroux, 1993).

II. MATERIALS AND METHODS

2.1 Study Area Description

The study area is located at about 2km from the main town of Okpella, off the Auchi – Okene Express way in Obhu, Okpella, Edo State, at latitude 7º 21′ 31.2′′ to 7º 21′ 34.9′′ and longitude 6º 25′ 11.6′′ to 6º 25′ 18.0′′. The area is a very steep hill, with a 326m peak elevation recorded at the site.

2.2 Geology and Hydrogeology of the Study Area

The study area lies within the Precambrian basement complex of Nigeria. The site and its immediate vicinity is an extension of the crystalline rocks of the Southwestern Nigeria. According to Rahaman (1976), the basement rocks of Southwestern Nigeria are made up of Migmatite – gneiss

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– quartzite complex, Schist belt, Charnokitic, gabbroic and Dioritic rocks, Older granites, unmetamorphosed dolerite dykes and basic syenite rocks, and Volcanic and hyperbyssal rock. Specifically at the site, boulders of granite were predominantly scattered across the hill. Quartz and quartzite rubbles, boulders, cobbles and pegmantitic veins and rocks were also seen at the site. Fracturing and weathering are also evident on rocks seen at the site. Two springs that appear to be structurally controlled and likely perennial too, were noticed towards the foot of the site.

2.3 Sampling and Geophysical Survey

This study is done by collection ofsamples in the field for chemical analysis. Electrical resistivity sounding (VES) is also conducted to obtain the geo-electric parameters of the deposit, and to determine the reserve estimation of the mineral deposit in the study area, through geophysical data. Figure 1 shows a schematic diagram of the study area showing the VES points. The ABEM SAS 300C Terrameter and other field accessories were used for the geophysical data acquisition. Geographical coordinates and elevation of the VES points shown in Table 1 were taken with the aid of Garmin GPS MAP 76.

A total of four VES data were acquired across the site, using normal and modified Schlumberger array, with an electrode spread [i.e. AB/2] for each VES data was 120m. Rock samples for laboratory chemical analysis were taken from the field after pitting was done up to a depth of three meters to expose some portions of the outcrop. Samples were then cut to size before being taken to the laboratory for further analysis. A total of three samples were collected for analysis. The Atomic Adsorption Spectrometer method was employed in analyzing the rock samples.

Table.1: Geographical Coordinates and Elevations of Sampled Points

S/N	VES Point	Latitude [N]	Longitude [E]	Elevation (m)
1	VES 1	7º 21′ 33.9′′	6º 25′ 12.3′′	259
2	VES 2	7º 21′ 34.9′′	6º 25′ 17.0′′	323
3	VES 3	7º 21′ 31.4′′	6º 25′ 18.0′′	326
4	VES 4	7º 21′ 31.2′′	6º 25′ 11.6′′	261

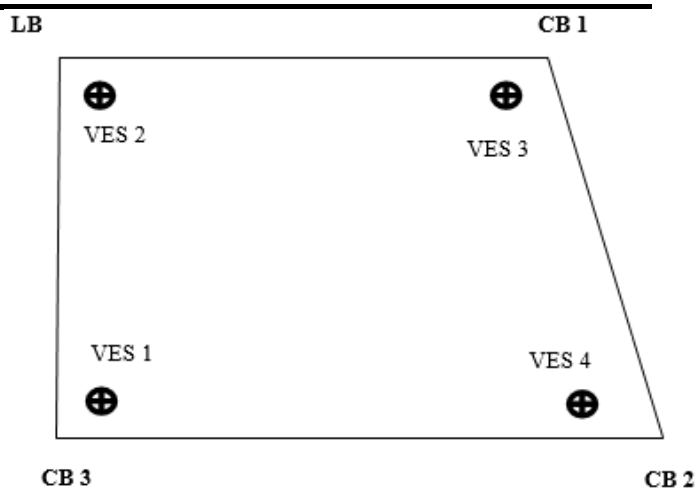

Fig.1: A schematic diagram of the study area showing the VES points

III. RESULTS

The geophysical field data from the electrical resistivity sounding is shown in Table 2. The interpreted geophysical data are presented as VES sounding curves shown in fig. 2 and the geo-electric parameters are shown in table 3. For VES 1, a total of five geo-electric layers were delineated. Layer 1 is the topsoil with a thickness of 1m and apparent resistivity of 1485Ωm. The second layer is the subsoil with a thickness of 1.11m. Layer 3 is highly resistive and is probably a boulder with a thickness of 4.74m. The fourth layer is deemed to be the weathered basement with a thickness of 6.70m. The calculated overburden thickness for VES 1 is 11.44m.

Table.2: Geophysical field data

AB/2 [m]	VES 1 App Res (Ωm)	VES 2 App Res (Ωm)	VES 3 App Res (Ωm)	VES 4 App Res [Ωm)
2	1185	598	396	59
3	1226	742	436	53
4	1301	906	515	49
6	1725	936	729	71
8	2054	812	1016	89
12	2572	513	1073	121
15	2780	427	1107	153
25	2319	553	1047	224
32	1995	650	1131	271
40	1773	798	1329	320
50	1512	951	1525	384
60	1628	1204	1827	425
80	1832	1512	2319	490
100	1965	1917	2864	462
120	2317	2230	3315	537

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*App Res – Apparent Resistivity (Ωm–ohms metre)

The data trend for the remaining VES points is not entirely similar. Calculated overburden thickness for VES 2, 3 and 4 is 6.89m, 13.64m and 45.78m respectively. The calculated average overburden thickness for the site is 19.44m.

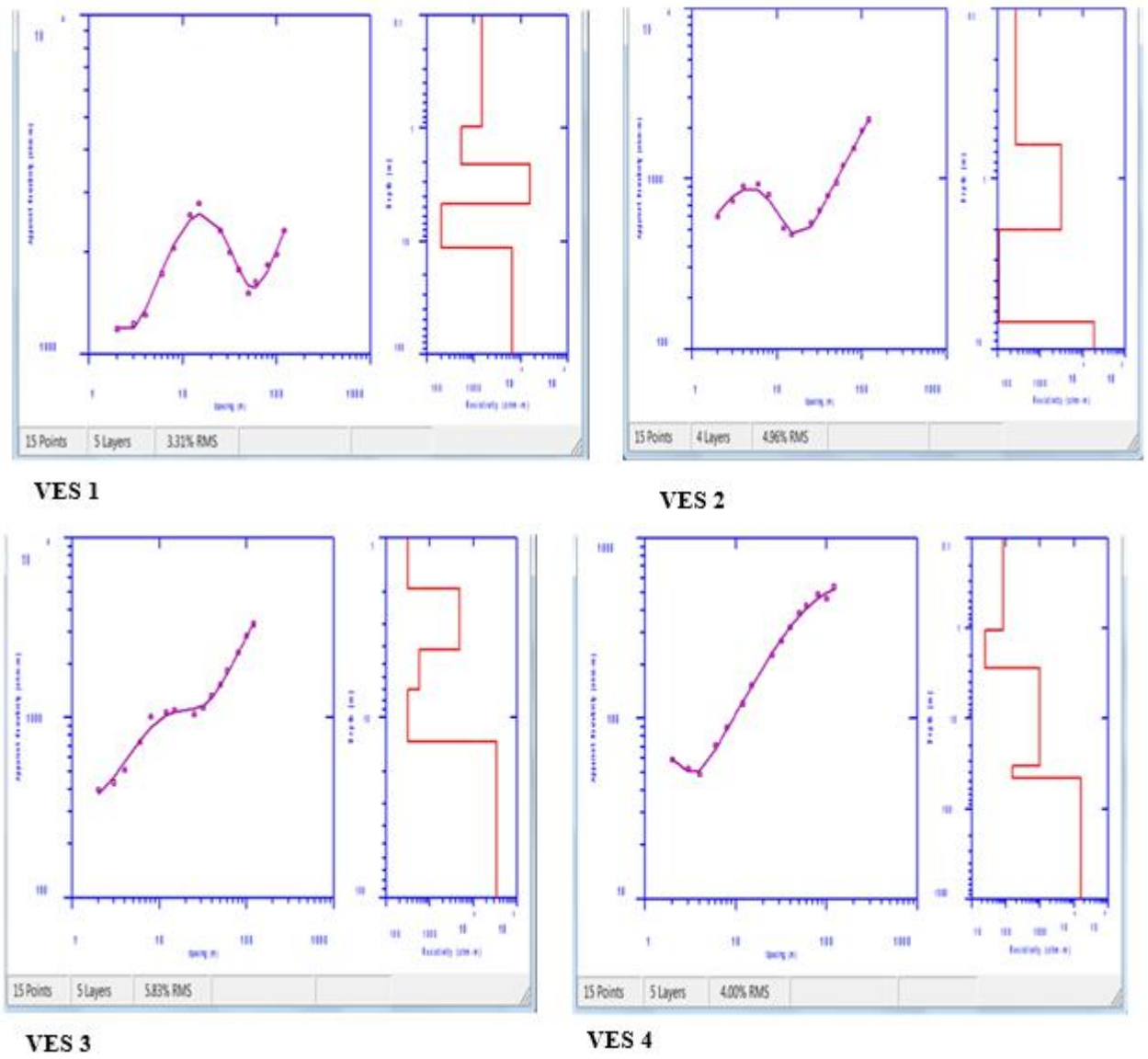

Fig.2: VES curves of the resistivity sounding in the study area

Table.3: Geoelectric Parameters and Inferred Lithology from the VES Data

Depth (m)	App Res (Ωm)	Lithology
VES 1		
1.0	1,485	Resistive/lateritic topsoil
2.11	538	Resistive/lateritic subsoil
4.74	15,825	Boulder
11.44	202	Sandy clay/clayey sand
???	6486	Fresh basement
VES 2		
0.63	264	Resistive/lateritic topsoil
2.00	3,145	Resistive/lateritic subsoil

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Depth (m)	App Res (Ωm)	Lithology
6.89	109	Weathered layer
???	18,265	Fresh basement
VES 3		
1.92	316	Resistive/lateritic topsoil
4.20	4,887	Resistive/lateritic subsoil
6.94	583	Weathered layer
13.64	314	Weathered basement
???	33,734	Fresh basement
VES 4		
1.04	82	Resistive/lateritic topsoil
2.78	24	Resistive/lateritic topsoil
33.78	998	Fresh bedrock
45.78	154	Weathered/fractured layer
???	16,135	Fresh basement

3.1 Reserve Estimation

The estimation of tonnage is derived from elevation and the overburden thickness of the area. The top surface elevation (ordinance datum) of the underlying fresh bedrock of the site is obtained by subtracting the overburden thickness from the surface elevation at each VES point.

3.2 Reserve Volume

Reserve volume = Area of the site (m^2) x thickness [trapezoidal height] of the bedrock.

Therefore, the reserve volume = 25, 484 m^2 x 71 = 1.8 x 10^6 m^3

3.3 Reserve Tonnage

This is the product of the reserve volume and the specific gravity of the bedrock. Taken the specific gravity of the bedrock at the site [Marble] to be 2.55, therefore the reserve tonnage is given thus:

Reserve tonnage = Reserve volume x specific gravity

= 1.8 x 10^6 m^3 x 2.55 = 4.6 x 10^6 Tons.

Overburden volume is the product of the average overburden thickness of the site and the trapezoidal area

Overburden volume = 19.44m x 25484m^2 = 495,408.96m^3

Therefore, rock mass reserve/overburden volume ratio = 9:1.

The chemical analysis results of the marble samples from the study area are shown in table 4. It was compared with the Raw Materials Research and Development Council of the Federal Ministry of Science and Technology standard for each element suitable for production of cement, fertilizer, iron, steel and other industrial uses.

Table.4: Marble Analysis Result from the Study Area

Parameter (%)	Sample 1	Sample 2	Sample 3	FMS (RMR&DC) Standard 2011 (%)
MgO	5.24	3.98	6.11	6
CaO	71.63	81.17	76.53	54.28
K_2O	2.75	1.64	1.22	-
Fe_2O_3	1.85	2.07	2.51	2
Al_2O_3	3.11	2.98	3.46	1.25
SiO_2	8.54	6.32	7.43	5
P_2O_5	0.71	0.69	0.74	1

Most of the values agree with the quality standard set by the Raw Materials Research and Development Council (RMR & DC) for industrial purposes. Many industrial application of marble require limits on levels of specific purities (MgO, SiO_2 and Fe_2O_3).The MgO values of the samples 1 and 2 does not falls within the acceptable value of 6 % of RMRDC, with the exception of sample 3 which falls into the acceptable limit, and it probably could be accepted for the industrial purpose it is to be put into with some strict regulation of production test control upon further studies or examination. CaO, Al_2O_3 and P_2O_5values of the samples falls within the acceptable limits of RMRDC and so suggest

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that the marble can be put to industrial use as regards its $CaOAl_2O_3$and P_2O_5content. The Fe_2O_3 content of a maximum 2 % indicates that sample 2 and 3 having values of 2.07 % and 2.51 % respectively are probably not best suited for industrial use, except for sample 1 having a lower value of 1.85 %. However, it should be noted that samples 2 and 3 values are on the bother line as well and as such the marble may well pass for use under strict test regulation regime. The silica SiO_2 content values for the three samples exceeded the recommended standard of RMRDC 5 %. These high values suggest that the marble deposit may not be very suitable for industrial purposes as it will have a high wearing effect on machineries to be used for its processing.

IV. CONCLUSION

An estimation of the limestone reserve of the deposit at Obhu, Okpella Edo state, has been evaluated based solely on geophysical data. The reserve tonnage and rock mass to overburden volume ratio depict the site as having good prospects for quarrying.From the chemical analysis done, it is noted that the samples though suitable for most industrial use, but would probably be abrasive to machines because of its high silica content. A detailed pilot test should therefore be carried out using the limestone deposit, depending on the use to which it is going to be put to. Also, a coring program should be embarked upon so as properly establish the nature and extent of overburden in the area.

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On-Street Parking Prohibition and Travel Behaviour of Motorists in Aba, Nigeria

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Abstract— *The prevalence of on-street parking in the city of Aba has continued to generate much concern to all road users and the government in Nigeria as it hinders traffic flow and increases traffic accidents. Consequently, the government initiated the policy of on-street parking prohibition at the downtown area of Aba, leading to increased travel difficulty for motorists. This study examined the policy of on-street parking prohibition and the resultant travel behaviour of motorists. The study adopted survey design whereby primary data were collected with the aid of structured questionnaire, measurement, and observation. The sample size of 400 respondents was determined from the study population of 420,665 motorists using the Standard Traffic Count (STC) approach. Cluster, purposive and simple random sampling techniques were used to proportionately select the roads and respondents used in the study. Analysis of variance (ANOVA) and Chi-square were used for data analysis. The study revealed among other thing that: there were significant differences in the geometric characteristics of roads where on-street parking was prohibited and where on-street parking was not prohibited. On-street parking prohibition was found to have impacted the travel behaviour of motorists as follows: (i) motorists travel less with single occupancy vehicles (SOVs) but more with KEKE (tricycle) during the working hours; (ii) there is reduction in the frequency of trips by motorists to the city centre whereas they divert to the outlying areas; and (iii) there is increased city bound (non-work) trips during early morning and late evening hours. It was recommended among others that government should embark on the construction of modern off-street parking facilities at designated locations within the city of Aba. Government should also recover sidewalks and road setbacks which have been totally invaded by commercial activities in Aba, and develop paid curb-parking there as alternative to on-street parking.*

Keywords— On-street, Parking, Travel behaviour, Motorists, Aba, Nigeria.

I. INTRODUCTION

Car parking especially in the city centre plays a key role in mobility, access and economic development of what appears to be an ever more car-dependent society (Rye, 2005). The city centre car parking market is a sector of the economy that has increased in importance as the market for cars has grown. Cars have become a fundamental element of journey and, in consequence, parking has as well. The car-parking sector has always been of great importance in terms of urban mobility, since it is a fundamental element in achieving a high level of accessibility in city centres. Many businesses and municipalities see an adequate supply of parking, especially for visitors, as crucial for their competitive growth. Yet, at the same time parking is, and will remain for most cities, one of the most powerful means of traffic restraint available (Andrew and Peter, 2006). The economics of car parking is also important because it is for both public and private organisations, a key source of revenue. All over the world, the demand for private-car access to cities and towns has continued to grow because of the presence of many attractions, such as public and private services, shopping and tourist centres. Since each trip has destination, therefore there is a corresponding increase in demand for parking spaces. But in most cases (especially in developing countries) developers hardly make provision for adequate off-street parking. This is because, just as Shoup (2005) observed, if everyone can easily get free parking on the street, developers have little incentive to build off-street parking and little ability to charge for the parking spaces they build. As a result, city travellers quite often do not find parking space within the property, and are forced to park on the street or engage in spill over parking on adjacent properties. The act of parking vehicles on one or both sides of the road is what is referred to as On-Street Parking.

For many years, the role of parking policy in most developed countries was to accommodate the automobile commuter by providing convenient spaces to park. As cities grew and became more complex, decision makers favoured removal of on-street parking from major arterials. They

argued that this action might increase road capacity and improve traffic flow and safety (Kennedy, 1994). On the other hand, arguments for allowing on-street parking have traditionally based on the potential benefits to the local merchants. Accessibility and parking convenience are among major factors that affect shoppers' destination choices (Andrew and Peter, 2006). Prior to the first off-street parking facilities, on-street parking had been present and naturally supplied as part of road construction, but nowhere was it regulated. With the advent of off-street parking, many larger cities like Chicago, Detroit, and Philadelphia began eliminating on-street parking to improve traffic flow (WashDOT, 1999). Others, however, took a different approach: in 1935, Oklahoma was the first city to regulate on-street parking using meters. In recent years, several communities around the world have used on-street parking prohibition and management strategies as means of achieving a wide variety of community objectives, ranging from reduction in auto trips, increased transit ridership, reduction in congestion, to improved air quality (Meyer *et. al.,* 1983). Kennedy (1994) in a study of the city of Soul found out that prohibiting or decreasing long-term on-street parking attracted additional short-term parkers, promoted transit patronage among long-term parkers, improved highway level of service, and promoted economic growth through increased retail sales.

In several other cities of the developed world there is considerable technology available for managing on-street parking, including mobile phones, ticket machines, camera enforcement, information technology for record keeping systems, et cetera, but in most cities in low income countries, manual collection systems are used (Rye, 2011). Martin (2008) has reported on the Westminster city parking system in London where a limited number of parking pricing systems have been implemented. In February 2009, Chicago city officials leased the city's metered parking spaces to private investors for a term of 75 years to attract capital to upgrade the existing parking system. In San Francisco, local agencies built a system using real-time parking data to manage congested streets and relieve a parking shortage (Battelle *et. al.,* 2011). An analysis of the Columbia District's parking management program indicated that implementation of the on-street parking enforcement program has resulted in reduced parking violation, increased on-street parking availability for short-term parkers, and significantly increased revenues from meter operations and fines (Ellis, 1987). According to Rye (2011), after an increase in on-street parking fees in Shenzhen, China, a remarkable 30% drop in demand was recorded. The benefits associated with this were short lived as the policy was reversed in 2007 and the demand soared so that the central business district was gridlocked.

In African perspective the absence of city-wide parking policies in the past has given rise to varied measures which are haphazardly applied. In some instances, placement of parking does not required action, only removal of parking does. This is because, except for roadways where parking is prohibited by statute (e.g., freeways and expressways), parking is assumed to be allowed until a specific order prohibiting it at a particular location is issued. Recently African cities appear to be waking up to the need for parking regulations. The city authorities in Cape Town have adopted the following mechanisms to address a range of parking matters: Cape Town Zoning Scheme (CTZS), Park & Ride facilities, On-street Park and pay, Parking By-law, and the Interim Policy Framework and Strategy for the Pricing of Parking (Kok, 2013). The city of Adum in Ghana established paid parking scheme in June 2006 with the objective to control on-street parking, reduce congestion through reduction of parking duration and increase in parking turnover (Charles, *et. al.,* 2014). In Egypt, the government introduced a policy in 2010 aimed at promoting modal shift from private cars to sustainable integrated public transport for Greater Cairo and its satellite cities by restricting on-street parking and introducing privately operated high quality off –street parking facilities (Mohamed, 2012). The Kampala City Council awarded a contract for the management of paid on-street parking in the central business district to the private sector in 1997. This was in response to the ever increasing congestion in the central business district. The price of parking was UGX 400 (US$ 0.17) per hour. A new ticket was purchased every hour up to 3 hours after which parking is illegal (Rye, 2011). Enforcement tactics, such as aggressive ticketing, towing, and booting illegally on-street parked vehicles have been used in many cities around Africa as in other parts of the world (Rye, 2011). They are not new, yet the use and integration of such tactics to meet broader transportation, economic, environmental and related objectives has received little attention, at least in the literature. In most communities, the police department is responsible for parking enforcement. Police priorities and sparse resources frequently become issues of prime consideration; in times of budget restrictions, parking enforcement is often one of the first targets for cuts (Kennedy, 1994). Consequently, there is an increasing trend toward using civilian personnel to enforce parking regulations. Parking characteristics of most other cities in Africa are similar, with ample supply of

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free on-street parking spaces which are often utilized to full capacity, resulting most times to increase in traffic congestion (Olorunfemi, *et. al*., 2014).

On-street parking constitutes one major problem that makes traffic situation chaotic in Nigerian cities (Osoba, 2012). Many Nigerian cities have narrow roads which lack pedestrian lanes. There are cases of double parking along these narrow roads thereby exacerbating traffic congestion. Property development in the cities is characterized by fenced compounds, and this creates exclusive interior parking spaces for residents of the compound while restricting off-street parking for visitors (Asiyanbola and Akinpelu, 2012). Majority of the cities are experiencing rapid rate of urbanization and the spatial expansion of the urban centres. This growth trend comes along with both increasing car ownership, and travel demand for shopping, leisure, education, work, and other urban activities (Osoba, 2012). As a result, most of the cities experience chaotic parking condition as well as intense traffic congestion, with cities like Lagos, Port-Harcourt, Kano, Abuja, Owerri, Aba, Uyo, Calabar, Onitsha, Kaduna, and Minna being worse off.

The parking situation in the central area of Aba has continued to generate much concern to both commuters and operators of business outfits in the area. Venues of activities such as offices, markets, shops, sports, churches and similar places often generate enormous parking demands, and the difficulty of parking vehicles at desired destinations particularly when located within the down town area of the city constitutes a major problem, becoming phenomenal in the face of increasing number of private car ownership. Huge packing demand coupled with ineffective parking management impact negatively on urban road space. The scramble for on-street parking spaces by motorists has posed serious challenge as it is arguably affecting traffic condition, volume of trips to the city, and general activities in the city centre. On-street parking is such a serious problem in the area that it erodes the available street space to the extent of causing inconvenience for pedestrians, hindering traffic flow, increasing traffic accidents, impeding access to the side-walks and degrading its function to prevent disasters. It is common experience for the shopping corridors downtown to be blocked by heavy duty trucks which are either loading or offloading goods. Shoppers are often seen driving around in search of on-street space to park, thereby exacerbating traffic congestion. Government regulation has recently prohibited on-street parking at some major roads in the downtown area of Aba. The neighbourhoods mostly affected are: Azikiwe road, Asa road, Tenant road, Ehi road, Hospital road, Kent road, Park road, and Jubilee road. Others are East road, School road, Cameroon road, Pound road, St-Michael's road, Constitution crescent, and Judges road. Consequently there has been increasing difficulty for motorists to secure parking location, and it is believed that this has affected their travel behaviour.

In Nigeria, government technical and regulatory innovations for traffic have mainly focused on mass transit issues and road rehabilitation. Previous researchers on the issue of on-street parking in Nigeria such as Olorunfemi *et. al.* (2014); Ahmed (2014); Osoba (2012); Asiyanbola and Akinpelu (2012); Aderamo *et. al.* (2011); and Obot *et. al*. (2009) studied the relationship between parking and traffic congestion, but did not address the emerging trend in parking policies and its effects on mobility at city centres. There is paucity of knowledge of the car parking market and its dynamics as there is lack of comprehensive parking policy for most cities. Parking regulations are often arbitrarily assigned just as the case in the concession of on-street parking pricing and ticketing to private firms in Abuja in 2014, and the prohibition of on-street parking in the core area of Aba in 2012. With the city of Aba as the focus of enquiry the study examined the effects of on-street parking prohibition on travel behaviour of motorists.

II. METHODOLOGY

The study is focused on the city of Aba in south-eastern Nigeria (see figure 1 for map of Abia State and Aba). Aba is located approximately between latitudes 5º 05′ N to 5º 08′ north, and longitude 7º 20′E to 7º 28′ east, and has sprawled to an approximate area of 26.7km2. The city has witnessed a high rate of urbanization which has turned it into different blocks of commercial layouts with casual and haphazard mixture of buildings, ranging from make–shift stores and shops to warehouses under large building with several floors. Within the suburb, prime agricultural lands give way to scattered ribbons of low income homes and businesses, stretching finger–like along rural road ways and streets. Urban Infrastructure is of poor quality: most of the roads lack pedestrian provisions; street lighting; parking corridors or curb parking; and bus stops. Vehicles are frequently packed at the road setbacks, especially on places that should serve for utility and sanitary purposes. Aba being a commercial town has many institutions; banks, company offices and business outlets, public and private schools and hospitals dot the entire landscape. Because of this, Aba is an important regional economic hub for Nigeria, which impacts the volume of trip generation and traffic volume in the city.

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The study adopted survey design. Both primary and secondary sources of data were used in the study. Primary data were collected with the aid of structured questionnaire, measurement, and observation. Secondary data were obtained from the Abia State Ministry of Transport handbooks. The study population consists of motorists - drivers of motor vehicles (excluding motorcycles) comprised of: commercial motorists, mainly Para-transit mode operators (mini-bus, taxi, and tricycle also known as *KEKE* in local parlance); and drivers of private cars. Some categories of motorists excluded from the study include mass transit bus drivers and lorry drivers. They were excluded because, pilot studies carried out earlier showed that these classes of motorists do not have significant contribution to on-street parking in the area as they were found to park vehicles in their off-street terminals. The population of motorists in Aba is 420,665, comprising 116,816 private motorists and 303,849 commercial motorists, and this represents average number of motorists that operate in the city daily. This data was collected from the licensing office of the Inland Revenue (Board of Internal Revenue) and the ministry of transport office, and validated through an independent traffic count by the author. The sample size of 400 respondents was determined from the study population using the Standard Traffic Count (STC) sample size determination formula. The survey recorded 92% success as 364 properly filled copies of questionnaires were returned and used for analysis. Cluster, purposive and simple random sampling techniques were used to proportionately select the roads and respondents used in the study. Cluster sampling technique was applied to divide the study area into two zones based on the areas where on-street parking was prohibited and the areas where on-street parking was permitted; and to divide each zone into four quadrants for purposes of fair representation. Purposive sampling techniques was used to select motorists at their loading bays and on-street parking positions along the roads, due to the reason that they were always on the move and were more disposed to rejecting interview requests. Then questionnaires were sampled proportionately on all the roads, at the ratio of 1:3 between private motorists and commercial motorists. The geometric variables of the roads (road carriageway width, shoulder width, parking capacity) were measured with Measuring Wheels. Reliability test of the instrument was determined using Cronbach's alpha which yielded a correlation co-efficient of 0.80. Analysis of variance and Chi-square were used to analyze the data and to test the research hypotheses at 0.05 significance level, making use of SPSS for windows version 17.

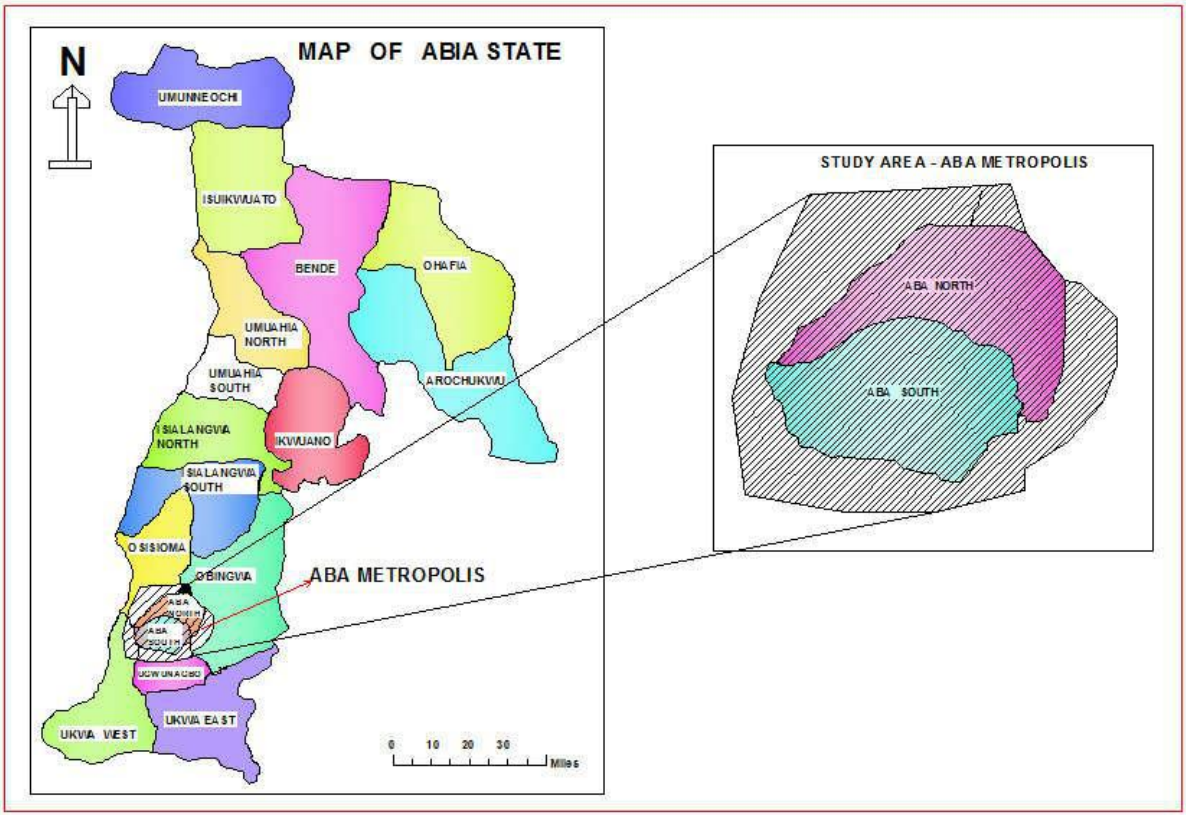

Fig.1: map of Abia State showing the metropolitan area of Aba

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III. RESULTS AND DISCUSSION

3.1 Choice of Travel Mode

Choice of travel mode in the study area is affected by period of enforcement of on-street parking prohibition. Therefore choice of travel mode for motorists in the study area was derived for three different periods of the day corresponding with strength of enforcement by traffic officers. These periods are: early morning hours (5.00am – 8.00am); Day time or working hours (8.00am – 5.00pm); and Late Evening hours (5.00pm – 10.00pm). Three different surveys were carried out to determine the choice of travel mode for the different periods of the day.

3.1.1 *Choice of Travel Mode for Early Morning Hours*

This period correspond with the time between when motorist begin to leave their houses in the morning (around 5.00am) and the time of commencement of daily working hours (8.00am). Figure 2 shows that 43 persons (11.8% of respondents) drive their cars to the downtown (CBD) during early morning hours, 41 persons (11.2%) enter mini-buses during same time, 7 persons (1.9%) enter taxi cabs, while majority of the people 272 persons or (74.5%) go to town with *KEKE* (tricycle).

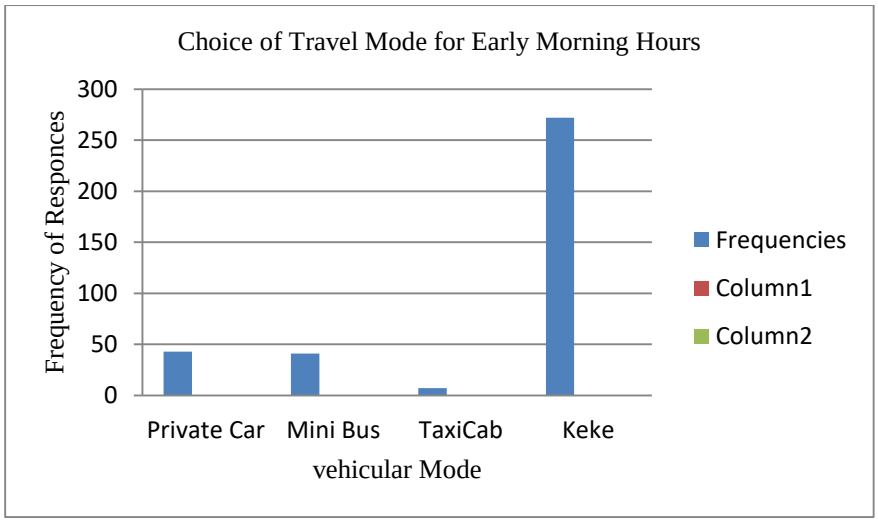

Fig.2: Choice of travel mode for early morning hours

3.1.2 *Choice of Travel Mode for Day Time (8am – 5.00pm)*

This period is the working hours of the civil service and therefore account for period of maximum enforcement because the enforcement officers are on patrol. Figure 3 indicate that motorists overwhelmingly choose to travel downtown with *KEKE* during working hours (317 respondents - 87%) while 30 persons (8%) travel with Mini-bus. 17 persons (5% of respondents) travel with their private cars while no person travel with taxi cab.

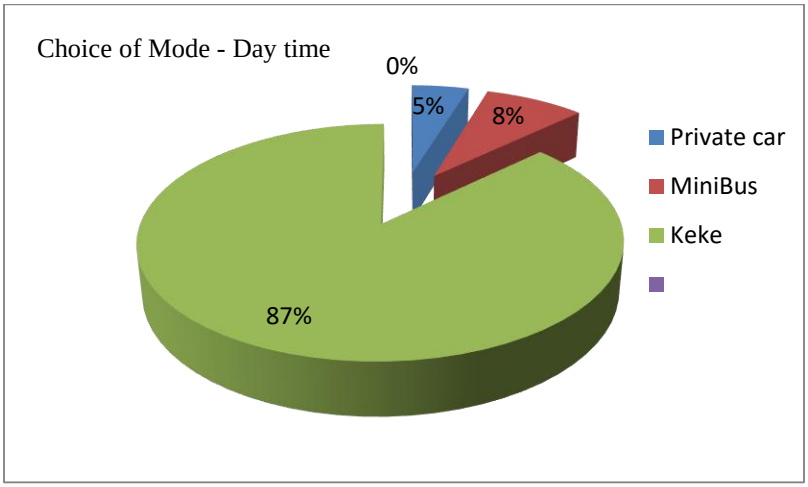

Fig.3: Choice of transport mode by day time

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3.1.3 Choice of Travel Mode for Late Evening (5.pm -10.pm)

By this period the taskforce for on-street parking prohibition must have closed work. Therefore their absence from the streets influences choice of mode of vehicular travel by motorists as shown on figure 4. This survey recorded higher choice of travel mode for private cars in the evening hours with 113 persons (31%), choice of *KEKE* mode decreased to 187 (52%), mini-bus increased to 53 (15%), while taxi is 7 (2%).

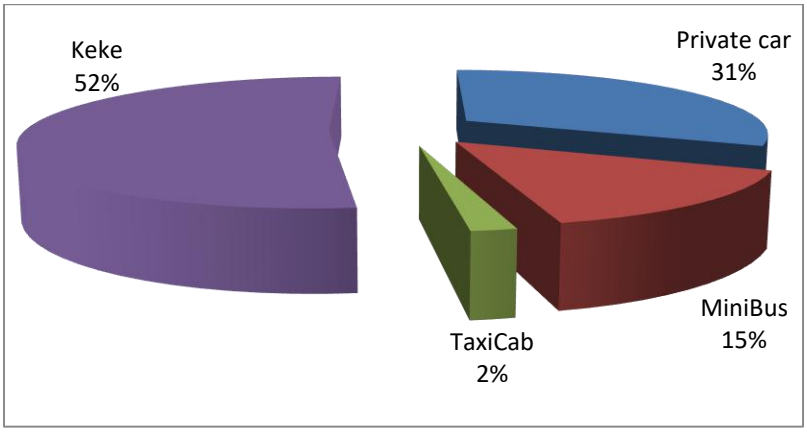

Fig.4: Choice of travel mode for during late evening

3.2 Volume of Trips to the downtown (CBD)

Like Choice of travel mode, Volume of trips to the central area of Aba is affected by period of enforcement of on-street parking prohibition. Therefore volume of trips was derived for the three primary periods identified in this study. The first of these is the early morning hours (5.00am – 8.00am).

3.2.1 *Volume of trips for early morning hours*

Table1 shows that over 62% of respondents (225 persons) make an average of 1 to 2 trips per week (for the private motorist), and 1to 2 trips per day (for commercial vehicle operators) to the CBD. Also over 36% (131persons) make between 3 - 4 trips per week (or per day) as applicable. Other frequencies here were insignificant.

Table.1: Volume of trips to the CBD during Early Morning hours

Trip Volume	Frequency	%	Valid %	Cumulative %
1 -2	225	61.6	62.5	62.5
3 -4	131	35.9	36.4	98.9
5 -6	1	.3	.3	99.2
above 6	3	.8	.8	100.0
Total	360	98.6	100.0	

3.2.2 Volume of trips to the CBD during Daytime (8am -5pm)

This period is the working hours; ordinarily every worker would travel at this time. But the survey did not measure work trips. Trips measured are non-work trips like trips for shopping, leisure, and errand. The results (table 2) show that 320 persons (88.4% of respondents) made 1 to 2 trips to the CBD per week, and per day for private motorists and commercial motorists respectively. On the other hand, 42 persons (11.6%) made 3 to 4 trips per week, or per day as applicable.

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Table.2: Volume of trips to the CBD during Daytime

Trip Volume	Frequency	Percent	Valid %	Cumulative %
1 – 2	320	87.7	88.4	88.4
3 -4	42	11.5	11.6	100.0
Total	362	99.2	100.0	

3.2.3 Volume of Trips to the CBD during Evening (5pm - 10pm)

This survey shows trip volume of motorists after working hours. The survey (see table 3) indicates that 110 persons (30.6% of respondents) made 1 to 2 trips to the CBD, 216 persons (60%) made between 3 to 4 trips to the CBD, 29 persons (8.1%) made 5 to 6 trips, while 5 persons reported making above 6 trips per week for private motorists, and per day for commercial motorists respectively.

Table.3: Volume of Trips to the CBD during Evening

Trip Volume	Frequency	%	Valid %	Cumulative %
1 – 2	110	30.1	30.6	30.6
3 -4	216	59.2	60.0	90.6
5 – 6	29	7.9	8.1	98.6
above 6	5	1.4	1.4	100.0
Total	360	98.6	100.0	

Source: Researcher's survey, (2015).

3.3 Trip Destination

It was speculated that the activities of the taskforce on on-street parking prohibition affect the choice of travel destination by motorists, with choice of destination varying between the downtown area where on-street parking is prohibited, and the outlying areas where it is presently not prohibited. Therefore this survey investigated the veracity of this assertion, considering the three periods of the day that correspond to the strengths of on-street enforcement identified in the study area.

3.3.1 *Trip destinations early Morning hours*

Figure 5 shows that 242 respondents (66.9% of motorists) travel to downtown (CBD) during early morning hours for non-work trips, whereas the rest 120 respondents (33%) travel to the outlying areas for non-work trips.

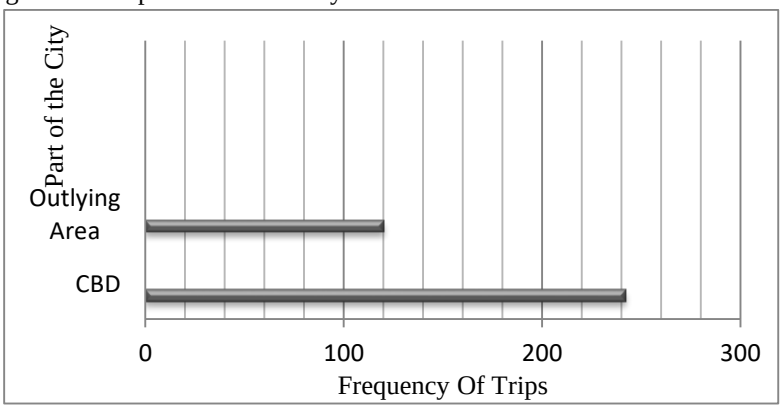

Fig.5: Trip destinations early morning hours

3.3.2 Trip Destination during Day Time

The frequency of trips by motorists during the day time (see table 4) showed that 146 motorists (40%) travelled to the CBD, but 218 (60%) preferred to travel to the outlying areas for business, leisure, errand, and other non –work activities.

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3.3.3 Trip Destination during Late Evening Period

This survey captured trip destination of motorists after the working hours, for non-work trips (Table 5). It showed an increase in the frequency of trips whose destination were at the CBD, with 240 out of 365 respondents (65.9%) visiting the CBD, and 123 (33.8%) of respondents visiting the outlying areas. This reversal of trips towards the city centre around evening time is attributed to the fact that the task force for on-street parking enforcement must have closed work at this time, and motorist were at less risk of being impounded for contravening the parking regulation hence they choose to drive downtown.

Table.4: Trip Destination during Day Time

	Frequency	%	Valid %	Cumulative %
CBD	146	40.0	40.1	40.1
Out lying area	218	59.7	59.9	100.0
Total	364	99.7	100.0	

Source: Field survey, 2016

Table.5: Trip destinations during late evening period

	Frequency	Percent	Valid %	Cumulative %
CBD	240	65.8	65.9	65.9
Out lying areas	123	33.7	33.8	99.7
11	1	.3	.3	100.0
Total	364	99.7	100.0	

Source: Field survey, 2016

3.4 Application of Traffic Management Laws in Aba

A survey was conducted to determine methods by which on-street parking prohibition is enforced in the area. Respondents identified the following measures as being applied by the taskforce on parking prohibition for enforcement (see table 6).

a) 281 persons (77.2%) of respondents reported having received all the outlined measures at one time or another, which include vehicle impoundment, payment of fines, beating–up/harassment, detention in police cell, and prosecution in court.
b) 20 persons (5.5%) reported having been prosecuted only
c) 15 motorists (4.1%) indicated vehicle impoundment and payment of fines
d) 11 persons (3.0%) said they have paid only fines
e) 10 motorists (2.7%) said they have experienced vehicle impoundment, payment of fines, beating/harassment, and detention in police cell
f) 10 respondents (2.7%) reported having been detained in police cell only
g) 9 respondents (2.5%) indicated having been beaten up only
h) 6 persons (1.6%) indicated having experienced impoundment of vehicle, payment of fines, beating and harassment.

Table 6: Enforcement of on-street parking prohibition

Enforcement Strategy	Freq.	Percent	Valid %	Cumulative %
Impound vehicle	2	.5	.5	.5
Fines	11	3.0	3.0	3.6
Beating/harassment	9	2.5	2.5	6.0
Detention	10	2.7	2.7	8.8
Prosecution	20	5.5	5.5	14.3
Impound vehicle & Fines	15	4.1	4.1	18.4
Impound vehicle & Fines & beating/harassment	6	1.6	1.6	20.1
Impound vehicle & Fines & beating/harassment &	10	2.7	2.7	22.8

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Enforcement Strategy	Freq.	Percent	Valid %	Cumulative %
detention All of the above	281	77.0	77.2	100.0
Total	364	99.7	100.0	

Source: Researcher's survey, (2016).

3.5 Hypothesis Testing

The study examined three different hypotheses which were targeted at establishing the effects which on-street parking prohibition has on travel behaviour of motorists.

3.5.1 *Hypothesis one. Ho:* There is no significant difference in the geometric characteristics of the roads where on-street parking was prohibited and roads where on-street parking was not prohibited in the study area.

The result showed that there was a significant difference in the geometric characteristics of the roads where on-street parking was prohibited against roads where on-street parking was not prohibited in the study area (F = 18.8, *P* = 0.001; *P* <0.05 significant level). The null hypothesis was rejected (see Appendix A-1 for details).

The carriageway widths of roads downtown were narrower than those in the outlying areas where on-street parking prohibition is not operational as shown in appendix A-2. This as well means that a recommendation for width expansion of some of the downtown roads in Aba is reasonable. Secondly, the on-street parking capacity on roads where policy on prohibition is operational downtown is relatively small. This has some traffic implications. Thus, the need to actually increase parking capacity on roads where prohibition policy is operational cannot be overemphasised. In addition, road shoulder widths for the downtown roads were narrower than those at the outlying areas. On-street parking ordinarily should be done on the road shoulder not on major road lane. Because road shoulder widths of the downtown roads were narrower, on-street parking significantly affects the carriageway leading to congestion. This necessitated the on-street parking prohibition in the first instance. It therefore means that expansion of the road shoulder widths of the CBD roads would accommodate marginal on-street parking without creating the usual traffic challenges.

3.5.2 *Hypothesis Two:* Three sub-hypotheses were formulated to test effects of parking prohibition as follows:

Ho: The policy of on-street parking prohibition does not significantly affect the travel behaviour of motorists in Aba, (travel behaviour as measured by choice of transport mode, frequency of trip, and choice of travel destination).

i. *Effects of on-street parking prohibition on choice of travel mode*. The analysis was conducted using SPSS for Windows; program output can be seen at Appendix – B1. The result of the 3 X 4 Chi-square showed the following:
   1. X^2 value for Private car mode = 133.225, with (*P*) value of 0.000, which is statistically significant at 0.05 significant level
   2. X^2 value for Mini-Bus mode = 40.518, with (*P*) value of 0.000, which is statistically significant at 0.05 significant level
   3. X^2 value for Taxi cab mode = 7.00, with (*P*) value of 0.321, is not statistically significant at 0.05 significant level
   4. X^2 value for *KEKE* mode = 88.399, with (*P*) value of 0.000, which is statistically significant at 0.05 significant level

ii. *Effects of on-street parking prohibition on volume of trip*. The SPSS output data for this analysis can be seen in Appendix – B2. The result is summarized as follows:
1. X^2 value for Volume of trip between 1 and 2 = 54.519, with (*P*) value of 0.000, which is statistically significant at 0.05 significant level
2. X^2 value for Volume of trip between 3 and 4 = 66.739, with (*P*) value of 0.000, which is statistically significant at 0.05 significant level
3. For Volume of trip between 5 and 6 No statistics were computed because Volume of trip during Evening hours and Volume of trips during day time were constants.
4. Also, for Volume of trips above 6, No statistics were computed because Volume of trip during Evening hours and Volume of trips during day time were constants.

iii. *Effects of on-street parking prohibition on Choice of travel destination*. The SPSS output data for this analysis has been attached as Appendix – B3. The result is summarized as follows:

1. X^2 value for Travel destination downtown (CBD) = 61.533, with (*P*) value of 0.000, which is statistically significant at 0.05 significant level
2. X^2 value for Travel Destination Outlying Areas = 88.621, with (*P*) value of 0.000, which is statistically significant at 0.05 significant level.

Inference: Results from variables in i, ii, and iii above are significant, therefore we reject the *null* hypothesis in all and accept the alternative hypothesis which states that the policy of on-street parking prohibition significantly affect the travel behaviour of motorists in Aba, (travel behaviour as measured by choice of transport mode, frequency of trip, and choice of travel destination).

3.5.3 Hypothesis Three. Ho: There is no significant difference between the travel behaviour of motorists (as measured by choice of mode and frequency of trips) when their destination is downtown (where on-street parking is prohibited), and when their destination is outlying areas (where on-street parking is permitted).

1. Result for Travel Behaviour as measured by Choice of Mode showed X^2 value of 317.425, with (*P*) value of 0.000, which is statistically significant at 0.05 significant levels, (see appendix B4)
2. For Travel behaviour as measured by frequency of Trips, the result showed X^2 value of 220.975, with (*P*) value of 0.000, and this is equally statistically significant at 0.05 significant levels (see appendixes B5).

Inference: Results from both variables are significant, therefore the *null* hypothesis was rejected, and the alternative hypothesis: there is significant difference between the travel behaviour of motorists (as measured by choice of mode and frequency of trips) when their destination is downtown (where on-street parking is prohibited), and when their destination is outlying areas (where on-street parking is permitted) is accepted.

3.6 Other Effects of On-street Parking Prohibition

The study revealed other positive and negative effects of on-street parking prohibition in the study area. The positive effects include:

i. Reduction in volume of trips to the city centre during working hours, which directly improves traffic flow on the affected roads.
ii. Reduction in the level of traffic congestion at the city centre during pick hours (7.00am – 10am; and 4.00pm to 7.00pm).
iii. Reduction in rate of accidents due to indiscriminate on-street parking
iv. Better safety to pedestrians who simply use the roadsides in the absence of pedestrian walk ways.
v. Better accessibility to emergency vehicles like ambulance and fire engines during emergencies
vi. Enforcement of on-street prohibition has encouraged private sector investment in off-street parking on commercial basis. Presently pay-as-you-go off street Motor-Parks are fast springing up in the city of Aba.
vii. On-street parking prohibition and enforcement is also a source of internally generated revenue for the State and Local Governments. Presently the traffic enforcement agency is a major revenue generating agency of government through fines, and charges.

There are however negative effects of the on-street parking prohibition policy as reported by respondents during the surveys. These include:

i. Reduction in volume of business at the city centre. The down town shopping centres reported reduced patronage mostly at major activity centres since the prohibition of on-street parking.
ii. There have been various reported cases of fighting and community uproar between the traffic officers and motorists over contravention of on-street parking regulations.
iii. The use of *KEKE* (tricycle) for commercial transport has been heightened by the prohibition of on-street parking. This is because parking problem affects buses, taxicabs and private vehicles (that occupy larger spaces, and need to park at a location either for loading and offloading or for the occupants to run their errands) more than the *KEKE*. Most times *KEKE* drivers are always on the move and may not need much space to park. The dominance of *KEKE* in urban transport system of Aba has lots of attendant difficulties: in the first place there are too many of the vehicles on any smaller section of the road space, also leading to congestion. Moreover the comparative speed of *KEKE* is very slow, and the passenger capacity also small. All these make it a major contributor to traffic congestion, though this needs to be properly investigated for conclusive evidence.

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IV. CONCLUSION AND RECOMMENDATIONS

This study investigated the effects of on-street parking prohibition on travel behaviour of motorists in the city of Aba. The study revealed that on-street parking prohibition has affected the choice of travel mode and the frequency of trips by motorists when travelling to the downtown area of the city. Besides, it has also affected motorists' choice of destination (whether to visit downtown or the outlying areas) when they embark on shopping trips, leisure or errand. The study observed that while prohibition of on-street parking has brought some relief to the city centre in terms of reduced congestion and accidents, it is gradually shifting travel and shopping pattern in favour of the outskirts, and this may be detrimental to the economic viability of the down town areas at the long run. These results also indicate that people who travel to the downtown area of Aba for activities other than work drive less and ride *KEKE* more, than when they travel to other parts of the city. The implication of this is the proliferation of *KEKE* on urban roads in Aba, as well as gradual reduction in downtown activities. This result suggests that parking policies that absolutely limit the amount of parking available to motorist without alternatives is counter productive to the goals of parking and traffic management. Therefore there is need for a review of traffic management policies in Aba especially as it applies to on-street parking. The author has therefore proposed the following recommendations.

1. The government should embark on the construction of modern off-street parking facilities at designated locations within the city of Aba. Presently only one off-street park dedicated to the public exists in the town, and this is grossly inadequate.
2. The town planning authorities should cause the government to promulgate parking minimums which must be enforced as part of commercial and residential property development in urban areas. This will guarantee adequate supply of off-street parks especially at the downtown area.
3. Government should recover sidewalks and road setbacks which have been totally invaded by commercial activities in Aba, and develop paid curb-parking by the sidewalks downtown, as alternative to on-street parking. Curb parking when properly priced would also balance both extremes of free on-street parking which encourages traffic congestion downtown, and on-street parking prohibition which negatively impacts downtown shopping. Besides, paid curb parking can be a great source of internal revenue for municipalities.
4. In parts of the city where off-street parking is not available or feasible, on-street parking must be a design consideration to ensure user convenience and economic well being of abutting properties. Addition of on-street parking is often coupled with other roadway changes such as carriage way expansion, conversion to two-way operation, widening of road shoulder widths and sidewalks, improved streetscape etc. With proper planning certain high density inner city areas of Aba with lesser traffic capacity may be developed to sustain guided on-street parking. Experience from other cities has demonstrated that a comprehensive and well-managed parking program results in significant reductions in parking violations, substantial increases in on-street parking space availability, and major increases in parking related revenues.
5. A situation where people find it extremely inconveniencing to travel to the city centre with their cars may be to the detriment of downtown activities especially shopping and service industries. Moreover the predominance of KEKE as mode of travel to the city may be counter productive to efforts at reducing traffic congestion. Therefore government should relax the stringent penalties attached to violation of on-street parking regulations. Rather than be totally prohibitive, the traffic regulations should prescribe conditions upon which motorists may be allowed to park on-street, provided such conditions would advance the goal of mitigating traffic congestion and road crash. For instance, on-street parking may be permissible within road sections at least 180meters from an intersection. Some cities have carefully marked road sections where on-street parking may be permitted along a given road lane. This method is hereby recommended to ensure guided on-street parking in the city of Aba.

V. ACKNOWLEDGEMENTS

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Appendix – A1: One Way ANOVA

geometric

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1918507	1	1918506.667	18.819	.000
Within Groups	3058371	30	101945.711		
Total	4976878	31			

Source: SPSS output

Appendix – A2:

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Geometric Data of Roads at the CBD where on-street parking prohibition is operational and outlying areas where it is permitted

Part of the city	*Road names*	*On-street parking capacity*	*Carriage way Width (m)*	*Shoulder Width (m)*
Roads in the CBD	Park road	65	10.5	0.8m
	Pound road	40	8.5	0.6
	St. Michaels' road	70	10	1.0
	Ehi road	38	9	0.6
	Tenant road	30	7.5	0.5
	Clifford road	32	7.5	0.7
	Hospital road	43	8	0.6
	Market road	31	7.7	0.4
	Georges road	15	8	0.2
	Ngwa road	89	12	1.4
	School road	68	10	1.0
	River layout road	33	11.5	0.8
	East road	21	9	0.4
	Constitution crest	0.0	7.5	0.0
	Asa road	340	24	1.5
	Jubilee road	37	8.5	0.6
	Cemetery road	0.0	7.5	0.0
	Eziukwu road	102	12	1.2
	Milverton Road	98	14	1.2
Roads Outlying Areas	Port-Harcourt road	1364	24	2.1
	Aba-Owerri road	1134	26	2.5
	Okigwe road	219	14	1.5
	Ikot –Ekpene road	1032	24	2.0
	Umoba road	41	12	0.4
	Obohia road	98	9.5	1.2
	Ohanku	174	10.5	1.4
	Ukaegbu	69	10.5	0.8
	Omuma road	604	12	1.2
	Izuogu road	591	10	1.5
	Ndiegoro road	812	12	1.5
	Umuokpu road	1013	12	1.8
	Urrata road	849	11.5	1.5

Source: Researcher's Survey, 2016

Appendix – B 1:

SPSS output for Choice of Mode

what is your choice of mode during evening hours		Value	Df	Asymp. Sig. (2-sided)
Private car	Pearson Chi-Square	133.225[a]	9	.000
	Likelihood Ratio	99.916	9	.000
	Linear-by-Linear Association	90.433	1	.000
	N of Valid Cases	113		

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Mini bus	Pearson Chi-Square	40.518[b]	6	.000
	Likelihood Ratio	47.752	6	.000
	Linear-by-Linear Association	27.193	1	.000
	N of Valid Cases	53		
Taxi	Pearson Chi-Square	7.000[c]	6	.321
	Likelihood Ratio	8.376	6	.212
	Linear-by-Linear Association	4.044	1	.044
	N of Valid Cases	7		
KEKE	Pearson Chi-Square	88.399[d]	6	.000
	Likelihood Ratio	31.548	6	.000
	Linear-by-Linear Association	42.250	1	.000
	N of Valid Cases	187		

a. 13 cells (81.3%) have expected count less than 5. The minimum expected count is .05.

b. 6 cells (50.0%) have expected count less than 5. The minimum expected count is .30.

c. 12 cells (100.0%) have expected count less than 5. The minimum expected count is .14.

d. 10 cells (83.3%) have expected count less than 5. The minimum expected count is .02.

Appendix – B 2:

The SPSS X^2 output data for volume of trips

Volume of trip during morning hours		Value	df	Asymp. Sig. (2-sided)
1 -2	Pearson Chi-Square	54.519[a]	2	.000
	Likelihood Ratio	24.045	2	.000
	Linear-by-Linear Association	19.722	1	.000
	N of Valid Cases	224		
3 -4	Pearson Chi-Square	66.739[b]	3	.000
	Likelihood Ratio	59.354	3	.000
	Linear-by-Linear Association	49.929	1	.000
	N of Valid Cases	131		
5 -6	Pearson Chi-Square	.[c]		
	N of Valid Cases	1		
above 6	Pearson Chi-Square	.[c]		
	N of Valid Cases	2		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is .29.

b. 4 cells (50.0%) have expected count less than 5. The minimum expected count is 1.03.

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Appendix – B 3:

The SPSS X^2 output data for choice of travel destination

Which part of the city do you more frequently visit day time		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
CBD	Pearson Chi-Square	61.533[a]	1	.000		
	Continuity Correction[b]	57.662	1	.000		
	Likelihood Ratio	53.187	1	.000		
	Fisher's Exact Test				.000	.000
	Linear-by-Linear Association	61.111	1	.000		
	N of Valid Cases	146				
Out lying area	Pearson Chi-Square	88.621[c]	1	.000		
	Continuity Correction[b]	86.011	1	.000		
	Likelihood Ratio	94.886	1	.000		
	Fisher's Exact Test				.000	.000
	Linear-by-Linear Association	88.211	1	.000		
	N of Valid Cases	216				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.36.

b. Computed only for a 2x2 table

c. 0 cells (.0%) have expected count less than 5. The minimum expected count is 38.30.

Appendix – B 4:

The X^2 output data for choice of Mode between CBD and Outskirts

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	317.425[a]	9	.000
Likelihood Ratio	195.539	9	.000
Linear-by-Linear Association	89.424	1	.000
N of Valid Cases	363		

a. 8 cells (50.0%) have expected count less than 5. The minimum expected count is .03.

Appendix – B 5:

Output data for Frequency of trips between CBD and Outlying areas

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	220.975[a]	9	.000
Likelihood Ratio	218.573	9	.000
Linear-by-Linear Association	143.138	1	.000
N of Valid Cases	360		

a. 6 cells (37.5%) have expected count less than 5. The minimum expected count is .13.

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Effects of different plant leaf extracts on postharvest life and quality of mango (*Mangifera indica* L.)

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***Abstract* —** *An experiment was carried out to investigate the efficacy of plant leaf extracts on elongation of shelf life and maintenance of quality of harvested mangoes. Freshly harvested mature green mangoes cv. 'Calcuttia maldah' of uniform size and weight were dipped in 50% concentration of different plant leaf extracts and stored in ambient condition (32±2ºC and 65±5 % RH). The treatments were leaf extracts from five different plants viz. neem (Azadirachta indica), chinaberry (Melia azadirach), lantana (Lantana camara), ashok (Polyalthea longifolia) and cinnamomum (Cinnamomum zeylanicum) while control was the other treatment. In addition, carbendazim (fungicide) was also kept as a benchmark treatment. Each treatment composed of 5 mangoes and replicated thrice. For each replication destructive sample was also kept. The treatment with neem leaf extract gave the most promising result as there was minimum physiological weight loss, maximum ascorbic acid content, maximum acidity and minimum pH. Similarly, shelf life, total soluble solids, freshness and firmness were highest in neem leaf extract treated fruits next to the carbendazim treated fruits. Control was the most ineffective of all the treatment regarding all the parameters.*

***Keywords*— mango, post-harvest, plant leaf extracts.**

I. INTRODUCTION

Mango (*Mangifera indica* L.), popularly known as "The king of the fruits", is one of the most popular fruit grown and consumed extensively throughout the tropical and sub-tropical region of the world. With a total production of 23.87 million, mango ranks third among the tropical fruits grown throughout the world (FAO, 2006). Owing to its unique fragrance, delicious taste and high nutritive value (Pal, 1998), mango has very high global demands. Mango contains significant amount of carbohydrates, provitamin A, vitamin C and soluble sugar (Samad et al., 1975). Being a climacteric fruit, it is generally harvested at mature green stage and ripens during post-harvest handling operations like transportation, storage, etc. Mango is a high moisture and high nutrient reserve containing commodity and as a result it is highly perishable in nature and susceptible to several post-harvest diseases (Haggag, 2010; Dodd et al., 1997). According to Islam et al. (2016), the most important problems regarding fruits production in tropical and sub-tropical regions of the world are postharvest losses and deterioration of nutritional quality of fruits. In developed countries the post-harvest losses in fresh fruit is estimated to be about 5-25% while that in developing countries it is about 20-50% (Khader, 1985 as cited in Islam et al., 2016). During post-harvest operations like natural ripening, physical handling and storage, approximately 30-50% fruits go wasted (Lashley, 1983). This high wastage of fruits is due to highly perishable nature and climacteric pattern of respiration (Islam et al., 2013). According to Gupta and Jain (2014), mango suffers 20-30% losses because of shorter storage life and faster ripening process. In addition to natural deterioration various post-harvest disease infections also play a major role in post-harvest losses of fruits. Among various diseases anthracnose, stem end rot and alternariose are the major ones that infect mango fruits (Haggag, 2010). These diseases cause rapid degradation and faster decay of fruits decreasing the quality and postharvest life of fruits. Enhancing the post-harvest life of mango fruits without losing its quality is a major challenge as it is a highly perishable commodity prone to several disease infestations.

Many post-harvest treatment methods and technologies like cold (refrigeration) storage, CA storage, MAP, treatment with ethylene inhibitors like 1-MCP, PGR treatment, wax treatment, etc. have been developed over the years for lengthening shelf life and maintaining post-harvest qualities of fruits (Pandey et al., 2017). But the accessibility and affordability of poor farmers to these advanced technologies is a matter of concern in most developing and under-

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developed countries. Most of the mango growers in those countries suffer heavy post-harvest losses of fruits as a result of natural deterioration and severity of diseases. Post-harvest diseases cause serious loss of both quality and quantity of fruits every year. Over the years, various fungicides like mancozeb, benomyl, carbendazim, thiabendazol, etc. have gained popularity among growers to control the post-harvest diseases of mango (Lee et al., 2009)and to enhance the storage life of fruits (Gupta and Jain, 2014). However, the use of these pesticides poses serious health hazards and leads to environmental contamination (Okinbo and Osuinde, 2003). In addition, due to their frequent application, there is a possibility of development of resistance in pathogen populations (Kumar et al., 2007). With growing health consciousness among people and increasing consumer demand for pesticide residue free agricultural commodities (Cutler and Cutler, 1999; Serrano et al., 2005) it is therefore important to find better alternatives that are cost effective, non-toxic and eco-friendly with low residual action to prevent disease infections and maintain post-harvest qualities of mango fruits. The necessity of developing eco-friendly post-harvest treatment methods as alternative to hazardous chemicals has become scientists' priority worldwide over the years (Phongpaichit et al., 2001).

According to Macias et al. (1997), natural plant extracts from higher plants that are non-hazardous to both human health and environment are better alternatives to chemicals for controlling post-harvest diseases of mango. In recent years numerous studies have been made on the use of natural plant extracts in controlling post-harvest diseases and there have been several reported cases of botanical extracts having antifungal activities (Das et al., 2010). Botanical extracts have attracted scientists' attention and gained popularity for their antibacterial and antifungal activity (Lee et al. 2007; Santas et al. 2010). The botanical extracts can provide an excellent opportunity to avoid or replace or reduce the use of harmful chemicals in post-harvest treatment of fruits for controlling various diseases as these extracts have been found to possess several antimicrobial properties. Moreover, plant extracts have the ability to decompose rapidly and do not cause any negative hazards to the environment unlike chemical pesticides (Fokialakis et al., 2006). Botanical extracts from different plants have been reported to have anti-fungal, anti- bacterial and other anti-microbial properties. So, the present study was carried out to evaluate the effectiveness of various plant leaf extracts for elongation of shelf life and maintenance of quality of harvested mangoes at ambient storage condition.

II. MATERIALS AND METHODS

2.1. Experimental location:

The experiment was conducted in the laboratory of Department of Horticulture, Agriculture and Forestry University, Rampur, Chitwan, Nepal in July, 2017.

2.2. Specimen collection:

The fresh and healthy leaves of neem (*Azadirachta indica)*, chinaberry *(Melia azadirach),* ashok *(Polyalthea longifolia),* cinnamomum *(Cinnamomum zeylanicum*) and lantana *(Lantana camara*) were collected from the Agriculture and Forestry University periphery. The collected leaves were washed first with tap water and finally with distilled water and shade dried at room temperature for 24 hours. Carbendazim was bought from the nearby market.

2.3. Preparation of botanical extract:

The botanical extract treatment solutions were prepared on percentage weight basis according to the method described by Gahukar (1996). Dried leaves were chopped and grinded in a laboratory mortar to fine powder. The extracts were prepared by adding 100 ml of distilled water to 100 g of leaf powder separately and kept overnight. This resulted in 100% concentration of every plant extracts (1:1 w/v). Finally the aqueous treatment solutions of different leaf extracts were prepared by diluting the extracts to 50% using distilled water. Carbendazim treatment solution was prepared by mixing carbendazim 0.1% in hot water at 55 ºC and fruits were dipped for 10-15 minutes.

2.4. Collection and preparation of fruit samples:

Freshly harvested mature green stage mango cv. 'Calcuttia Maldah' of uniform size and maturity, good quality and free from any injury or disease were bought from the mango orchard of the University. The fruits were cleaned properly with distilled water to remove all the foreign matters like dust, dirt, mud, filth, etc. Fruits were then grouped in to similar size after washing with distilled water and used for the experiment. The cleaned fruits were dipped in 50% concentration of the prepared leaf extracts for 10-15 minutes and stored at ambient room condition (32±2ºC and 65±5 % RH).

2.5. Experimental design and treatments preparation:

The experiment was conducted in Completely Randomized Design (CRD) with seven treatments and three replications. Destructive sample was kept for each replication for carrying out physico-chemical analysis. The seven treatments were; T1: Chinaberry leaf extract, T2: Neem leaf extract, T3: Lantana leaf extract, T4: Ashok leaf extract, T5: Cinnamomum leaf extract, T6: Benchmark treatment with Carbendazim and T0: Control. Each treatment was composed of 5 mangoes.

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III. OBSERVATIONS

Observations were made on the following parameters:

3.1. Physiological weight loss (%)

It was determined with the help of electronic digital balance.

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

3.2. Firmness (lbs/cm^2)

Firmness of fruits was measured using handheld penetrometer after peeling thin layer of skin.

3.3. Total Soluble Solids (^{0} Brix)

The total soluble solids (^{0} Brix) was recorded by using hand held refractometer.

3.4. Titrable Acidity

Titrable acidity was determined by the titration of diluted fruit juice with few (2-3) drops of phenolphthalein against base (0.1N NaOH) solution.

$$\text{Total Titrable Acidity (\%)} = \frac{N_B \times V_B \times \text{Milliequivalent wt. of predominant acid} \times 100 \times df}{\text{Volume of sample}}$$

Where, N_B = Normality of base (NaOH)

V_B = Volume of the base

df = Dilution factor

3.5. pH

It was measured using automatic digital pH meter.

3.6. Vitamin C (Ascorbic acid)

Vitamin C content of fruit juice was determined by volumetric method following the procedure given by Sadasivam and Manickam (1996).

Calculation of the vitamin C content was done by using the following formula.

$$\text{Amount of ascorbic acid (mg/100g sample)} = \frac{0.5\text{mg}}{V_1} \times \frac{V_2}{5\text{ml}} \times \frac{100}{\text{Wt. of sample}} \times 100$$

3.7. Shelf life (Days)

Shelf-life of fruits was measured by counting the number of days from start of storage until when more than 50% of samples per replicate have been deteriorated .

3.8. Freshness and market acceptability

Evaluation of freshness and market acceptability was done by a panel of five people based on the color and appearance of fruits. Values of freshness were given in scale of 1-5 (5 = Fresh having good market acceptability, 2.5 = critical value for market acceptability and 1 = poor in freshness having no market acceptability).

IV. STATISTICAL ANALYSIS

The collected data on various parameters were statistically analyzed using R-STAT statistical package program to find out the variation resulting from experimental treatments. Mean comparisons were made using Least Significant Difference (LSD) test at 5% probability level.

V. RESULTS AND DISCUSSIONS

5.1. Physiological weight loss

A significant difference was recorded among treatments at various days of storage with respect to the physiological loss in weight. With increasing period of storage the physiological loss in weight also increased in all the treatments. Throughout the storage, the physiological weight loss was found to be lowest (13.07%) in fruits treated with 50% neem leaf extract as compared to other treatments (Table 1). It might be because of the ability of neem leaf extract to retard the moisture loss and to delay the senescence process as reported by Gakhukar (1996). Other probable reason for this might be the ability of neem leaves to restrict the growth of micro-organisms responsible for rotting as explained by Singh et al., (2000); Chauhan et al., (2008) and Bajwa and Ahmad (2012). The reduced weight loss might also be due to the formation of thin layer of oil on surface of fruits that reduced the evapotranspiration and respiration rate in the treated fruits (Singh et al., 2000). Samanta and Prasad (1996) have also reported the positive effects of leaf extracts on minimizing the water vapor losses from fruits.

Alongside neem, lantana leaf extract gave the lowest physiological weight loss in 5th, 7th and 9th day. Except at 3rd day, there was no significant difference between neem leaf and lantana leaf extracts regarding physiological weight loss. Among the different botanical extracts treatments, polyalthea and cinnamomum leaf extract were the least effective. The maximum physiological weight loss was observed in untreated fruits (12.05%) on day 5. Neem leaf extract was more efficient than carbendazim in reducing the physiological weight loss i.e. by approximately 4 %.

Table.1: Effect of plant extracts on physiological weight loss (%) of mango during different days until the end of shelf life on ambient room storage (32±2ºC and 65±5 % RH)

Treatments	Day 3	Day 5	Day 7	Day 9
Control	6.06^{a}±0.35	12.05^{a}±0.27	Discarded	
Chinaberry leaf extract	2.72^{c}±0.34	8.23^{c}±0.43	11.34^{c}±0.8	15.68^{bc}±1.88
Neem leaf extract	1.93^{d}±0.26	7.50^{c}±0.16	9.99^{c}±0.34	13.07^{c}±0.53
Lantana leaf extract	2.53^{cd}±0.04	7.85^{c}±0.31	10.42^{c}±0.48	13.15^{c}±0.13
Polyalthea leaf extract	3.73^{b}±0.37	10.92^{ab}±0.34	16.03^{b}±0.82	19.84^{a}±0
Cinnamomum leaf extract	3.89^{b}±0.17	11.56^{a}±0.65	19.60^{a}±0.89	19.29^{a}±0
Carbendazim	4.41^{b}±0.21	9.85^{b}±0.44	14.14^{b}±0.5	17.64^{ab}±0.31
P-Value	0.00000011***	0.000000833***	0.000000736***	0.00685**
LSD	0.705	1.114	1.912	3.286
CV (%)	11.15	6.55	7.91	7.71
Grand mean	3.61	9.70	13.586	16.395

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

5.2. Firmness

Alongside physiological weight loss, firmness is very important parameter regarding postharvest storage and its value is very effective for evaluating the fruit maturity (Olmo et al., 2000). The effects of different plant extracts on the firmness of mango fruits are given in Table 2. There was a significant difference in the firmness of fruits due to the dipping of the fruits in aqueous extracts of selected plant species. Carbendazim treated fruits showed the maximum retention of firmness (0.96 lbs/cm^2) until the final days of storage followed by neem leaf and lantana leaf extract treated fruits. At the third day of storage, control and chinaberry leaf extract treatments gave the lowest firmness value and in contrast, carbendazim treatment gave the highest firmness followed by neem leaf extract. Surprisingly, polyalthea, cinnamomum and chinaberry leaf extract treated fruits all showed similar firmness value as that of untreated fruits until fifth day of storage. Carbendazim treatment had the highest firmness in all days of storage. Except Carbendazim all other treatments were similar in seventh days of storage and while in ninth day of storage, neem leaf extract and lantana leaf extract had better firmness than other remaining treatments. In case of neem leaf extract treated fruits better firmness observed might be due to the effect of azadirachtin on pectin molecules (Sandeep et al., 2010). The experiment showed that the effects of neem and lantana leaf extracts on maintaining firmness of fruits were comparable to the effects of carbendazim treatment although carbendazim gave the highest firmness value. The retardation of degradation of insoluble protopectins to the more soluble pectic acid and pectin by different plant extracts might be the possible reason for better retention of firmness of treated fruits than untreated ones (Abbasi et al., 2009). Tehrani et al. (2011) also reported that the textural changes during ripening is related the loss of pectin substances from cell wall by various degrading enzymes. Labavitch and Ahmad (1978) suggested that the gradual conversion of carbohydrate in to sugar along with change in cell wall polysaccharides and uronic acid might be the reason for decrease in firmness of fruits.

Table.2: Effect of plant extracts on firmness (lbs/cm^2) of mango during different days until the end of shelf life on ambient room storage (32±2ºC and 65±5 % RH)

Treatments	Initial	Day 3	Day 5	Day 7	Day 9
Control		8.5^{e}±0.066	4.3^{c}±0.066	Discarded	
Chinaberry leaf		8.5^{e}±0.066	4.3^{c}±0.066	2.03^{b}±0.1	0.3^{b}±0.1

extract					
Neem leaf extract		9.6^{b}±0.066	5^{b}±0.13	2.43^{b}±0.1	0.76^{ab}±0.1
Lantana leaf extract	13	9.36^{bc}±0.1	4.8^{b}±0.13	2.16^{b}±0.1	0.7^{ab}±0.1
Polyalthea leaf extract		8.96^{d}±0.1	4.03^{c}±0.1	2.16^{b}±0.1	0.4^{b}±0
Cinnamomum leaf extract		9.13^{cd}±0.13	4.4^{c}±0.13	2.16^{b}±0.1	0.4^{b}±0
Carbendazim		12.4^{a}±0.3	7.96^{a}±0.23	3.4^{a}±0.3	0.96^{a}±0.13
P-Value		0.0000000000424***	0.0000000000138***	0.000106***	0.0387*
LSD		0.380	0.354	0.415	0.459
CV (%)		2.28	4.07	9.74	25.49
Grand mean		9.49	4.97	2.39	0.586

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

5.3. Total Soluble Solids (TSS)

Statistically a highly significant variation was observed in TSS content of fruits among various treatments. TSS of treatments control, neem leaf extract and carbendazim increased throughout the storage period. TSS of other remaining treatments increased until the 7th day and then decreased (Table 3). Similar results were observed by Shinde et al., (2009). The initial increase in TSS might be due to the accumulation of sugar as a result of hydrolysis of insoluble polysaccharides (starch) into simple sugars, while the later decrease might be due to the consumption of sugar for respiration during storage (Kumar et al., 1994). TSS of carbendazim treated fruits were highest followed by neem leaf extract treated fruits at the end of storage period. There was a slow increase in TSS of neem leaf extract treated fruits in comparison to all other treatments. This might be due to the action of neem ingredients that have antifungal properties and also the thin film of neem oil on surface of fruits reduced the evapotranspiration and respiration rate and showed minimum decay thus preventing the rapid rise of TSS (Singh et al., 2000). Chauhan and Joshi (1990) also reported that botanical extracts performed better in retaining the total soluble solids in Ratna cv. of mango.

Table.3: Effect of plant extracts on TSS (^{0}Brix) of mango during different days until the end of shelf life on ambient room storage (32±2^{o}C and 65±5 % RH)

Treatments	**Initial**	**Day 3**	**Day 5**	**Day 7**	**Day 9**
Control		11.13^{b}±0.067	14.8^{a}±0.267	Discarded	
Chinaberry leaf extract		11^{b}±0.133	13.4^{b}±0.133	15.23^{b}±0.233	14.4^{cd}±0.2
Neem leaf extract		10^{c}±0.133	12.43^{c}±0.1	14.4^{c}±0.133	15.06^{b}±0.067
Lantana leaf extract	5	10^{c}±0.133	12.73^{c}±0.067	15.03^{b}±0.1	14.8^{bc}±0
Polyalthea leaf extract		10.33^{c}±0.067	14.4^{a}±0.133	16.03^{a}±0.1	14^{d}±0
Cinnamomum leaf extract		10.3^{c}±0.067	14.46^{a}±0.267	16^{a}±0.133	14^{d}±0
Carbendazim		12.06^{a}±0.2	14.73^{a}±0.067	15.13^{b}±0.067	16.26^{a}±0.067
P-Value		0.0000000185***	0.0000000271***	0.00000634***	0.0000328***
LSD		0.33	0.45	0.386	0.403
CV (%)		1.77	1.85	1.419	0.99

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Grand mean		10.68	13.85	15.30	14.75

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

5.4. Titrable Acidity (TA)

The variation of TA of fruits was found to be significant until the 7th of storage. TA on the 9th day of storage was found to be non-significant. The TA of fruit was highest at zero days of storage and then a decreasing trend of titrable acid content was observed with the advancement of storage period (Table 4). The decrease in acidity during the storage period might be due to the conversion of citric acid into sugars and their further utilization in various metabolic processes of fruit (Doreyapp and Huddar, 2001; Mizrach et al., 1997; Rathore et al., 2007; Srinivasa et al., 2002). Neem leaf extract treated fruits had the highest TA throughout the storage period. The decrease in TA of neem leaf extract treated fruits was found to be slowest followed by carbendazim treated fruits due to their effects on the utilization of organic acids in respiration which delayed the physiological ageing and restricted the starch degradation. Similar observations were also confirmed by the findings of Singh et al. (2000). Chinaberry treatment gave the lowest TA value at the end of storage period.

Table.4: Effect of plant extracts on TA of mango during different days until the end of shelf life on ambient room storage (32±2ºC and 65±5 % RH)

Treatments	Initial	Day 3	Day 5	Day 7	Day 9
Control	2.12	1.45^{b}±0.09	0.85^{b}±0.046	Discarded	
Chinaberry leaf extract		1.31^{b}±0.006	0.84^{b}±0.073	0.43^{c}±0.048	0.28^{a}±0.035
Neem leaf extract		1.75^{a}±0.026	1.09^{a}±0.08	0.76^{a}±0.053	0.44^{a}±0.046
Lantana leaf extract		1.64^{a}±0.06	0.97^{ab}±0.048	0.53^{bc}±0.031	0.37^{a}±0.05
Polyalthea leaf extract		1.33^{b}±0.06	0.86^{b}±0.07	0.50^{c}±0.051	0.30^{a}±0
Cinnamomum leaf extract		1.64^{a}±0.06	0.84^{b}±0.086	0.53^{bc}±0.053	0.30^{a}±0
Carbendazim		1.73^{a}±0.03	1.07^{a}±0.081	0.67^{ab}±0.064	0.42^{a}±0.032
P-Value		0.000105***	0.0436*	0.00277**	0.15^{NS}
LSD		0.164	0.197	0.141	0.167
CV (%)		6.05	12.04	13.82	16.47
Grand mean		1.55	0.931	0.57	0.351

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

5.5. pH

The analysis of variance between the treatments exhibited significant variation regarding the pH value of mango fruits at different days of storage except on 9th day (Table 5). The pH value was lowest at zero days of storage and it gradually increased with the advancement of storage period. This increasing trend of pH value during storage period was also observed by Shahjahan et al. (1994). This phenomenon of increasing trend of pH during storage might be possible due to the oxidation of acids resulting in higher pH (Md. Khairul Islam, M. Z. H. Khan, M. A. R. Sarkar, Nurul Absar, and S. K. Sarkar, 2013). Control treatment had the highest pH until 5th day while Cinnamomum treated fruits had the highest pH after day 5. The increase in pH value was found to be slower in carbendazim treatment on 3rd and 5th days of storage, while on 7th day both neem leaf extract and carbendazim treatment had the same pH value. But on 9th day of storage, neem leaf extract treated fruits had the lowest pH value followed by carbendazim treatment exhibiting that neem leaf extract helps in slowing down ripening process better than carbendazim. Cinnamomum treatment had the highest pH value on the 9th day of storage.

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Table.5: Effect of plant extracts on pH of mango during different days until the end of shelf life on ambient room storage (32±2ºC and 65±5 % RH)

Treatments	Initial	Day 3	Day 5	Day 7	Day 9
Control	2.2	3.26^{a}±0.1	3.90^{a}±0.067	Discarded	
Chinaberry leaf extract		3.26^{a}±0.067	3.56^{bc}±0.1	3.90^{a}±0.067	4.45^{a}±0.05
Neem leaf extract		3.03^{bc}±0.1	3.40^{cd}±0.133	3.56^{b}±0.1	4.30^{a}±0.133
Lantana leaf extract		3.10^{abc}±0.067	3.40^{cd}±0.133	3.73^{ab}±0.133	4.50^{a}±0.1
Polyalthea leaf extract		3.23^{ab}±0.033	3.53^{c}±0.033	3.90^{a}±0.067	4.40^{a}±0
Cinnamomum leaf extract		3.20^{ab}±0.067	3.80^{ab}±0.067	3.93^{a}±0.033	4.60^{a}±0
Carbendazim		2.93^{c}±0.033	3.23^{d}±0.033	3.56^{b}±0.033	4.36^{a}±0.033
P-Value		0.0149*	0.000401***	0.00699**	0.469^{NS}
LSD		0.194	0.238	0.221	0.369
CV (%)		3.535	3.841	3.311	3.094
Grand mean		3.144	3.545	3.763	4.435

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

5.6. Vitamin C (Ascorbic acid)

All the treatments had significant effects on the vitamin C content of fruits at all days of storage. In all the treatments except control, ascorbic acid content first increased for first 7 days and then was found to decrease on 9th day of storage (Table 6). Vitamin C content increased until the storage period of untreated (control) fruits. This trend of first increase in vitamin C might be attributed to the reason that the fruits are still maturing. The decrease of vitamin C during storage might be due to the rapid conversion of l-ascorbic acid in to dehydro-ascorbic acid in presence of enzyme ascorbinase (Mapson 1970) which is further consumed during metabolic process of the fruits. Some investigators have found an increase in vitamin C content with maturation (Banerjee and Romasorama, 1938 as cited in Spencer et al., 1956) while others have noted a decrease (Hawaiian Agr. Expt. Sta. Report, 1943 as cited in Spencer et al., 1956).Vitamin C was observed lowest at zero days of storage for all the treatments except Cinnamomum treatment which had lowest vitamin C at 9th day of storage. Neem leaf extract treatment had maximum retention of ascorbic acid i.e. the highest vitamin C content among all at 7th and 9th day of storage. This might be due to the influence of neem leaf extracts in retarding ripening and oxidation processes as well as slowing down the respiration rate of fruits (Singh et al., 2000).

Table.6: Effect of plant extracts on Vitamin C (mg/100g) of mango during different days until the end of shelf life on ambient room storage (32±2ºC and 65±5 % RH)

Treatments	Initial	Day 3	Day 5	Day 7	Day 9
Control	9.8	9.90^{e}±0.067	10.93^{f}±0.1	Discarded	
Chinaberry leaf extract		9.83^{e}±0.1	10.90^{f}±0.067	13^{f}±0.133	10.31^{c}±0.085
Neem leaf extract		10.43^{d}±0.02	12.39^{d}±0.133	19.34^{a}±0.267	11.45^{a}±0.16
Lantana leaf extract		10.93^{c}±0.033	12.67^{c}±0.05	17.10^{c}±0.067	10.51^{c}±0.05
Polyalthea leaf extract		10.89^{c}±0.02	12.90^{b}±0.047	14^{e}±0.073	10.98^{b}±0
Cinnamomum leaf extract		12.51^{a}±0.013	14.33^{a}±0.047	16.08^{d}±0.113	9.74^{d}±0
Carbendazim		11.75^{b}±0.033	12.04^{e}±0.073	18.54^{b}±0.053	10.54^{c}±0.0267
P-Value		0.00000000000000226***	0.0000000000000762***	0.00000000000056***	0.00047***
LSD		0.135	0.213	0.379	0.410
CV (%)		0.707	0.988	1.304	1.419
Grand mean		10.89	12.30	16.34	10.588

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

5.7. Shelf life

The variation in shelf life of fruits due to different treatments was found to be highly significant. The longest shelf life (11 days) was recorded in carbendazim treated fruits with lowest disease infestations and the shortest (5 days) was recorded in untreated (control) fruits with highest disease infestations. Neem leaf extract treated fruits had shelf life of 9 days which was the longest among all other botanical extracts although other botanical extract treatments were not statistically different (Table 7). The disease infestation was also found to be lowest in neem leaf extract among botanical extract treatments. It might be due to the antifungal properties of neem preventing the microbial growth and its thin film reducing the evapotranspiration and respiration rate (Singh et al., 2000).

Table.7: Effect of plant extracts on shelf life (days) of mango on ambient room storage (32±2ºC and 65±5 % RH)

Treatments	Shelf life
Control	5^{c}±0
Chinaberry leaf extract	8.33^{b}±0.667
Neem leaf extract	9^{b}±0
Lantana leaf extract	8.33^{b}±0.667
Polyalthea leaf extract	7.66^{b}±0.667
Cinnamomum leaf extract	7.66^{b}±0.667
Carbendazim	11^{a}±0
P-Value	0.0000592***
LSD	1.528
CV (%)	10.71
Grand mean	8.14

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

5.8. Freshness and market acceptability

There was a significant difference in the freshness or marketability of fruits as a result of treatment of the fruits with different plant extracts (Table 8). Carbendazim treatment had the highest freshness value throughout the storage period except on day 3 and discarded only on 11th day while control had the lowest value of freshness and discarded on 5th day. Carbendazim, a fungicide prevented the fruits from fungal infection (anthracnose) so the fruits had greater freshness value. Neem leaf extract treatment was next best after carbendazim on 3rd, 5th and 7th day in terms of freshness value. However both the neem leaf extract and carbendazim treatment were statistically similar. It might be due to the antifungal properties of neem preventing the microbial growth and its thin film reducing the evapotranspiration and respiration rate (Singh et al., 2000). Polyalthea and cinnamomum gave the poorest results among plant extract treatments.

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Table.8: Effect of plant extracts on freshness of mango during different days until the end of shelf life on ambient room storage (32±2ºC and 65±5 % RH)

Treatments	Initial	Day 3	Day 5	Day 7	Day 9
Control	5	4.31^{c}±0.1	2.21^{c}±0.13	Discarded	
Chinaberry leaf extract		4.81^{ab}±0.05	4.11^{b}±0.067	2.96^{ab}±0.416	2.27^{b}±0.025
Neem leaf extract		4.96^{a}±0.03	4.41^{a}±0.083	3.55^{a}±0.116	2.30^{b}±0.083
Lantana leaf extract		4.90^{ab}±0.03	4.31^{ab}±0.1	3.06^{ab}±0.366	2.37^{b}±0.075
Polyalthea leaf extract		4.80^{ab}±0.05	4.08^{b}±0.083	2.58^{b}±0.35	2.20^{b}±0
Cinnamomum leaf extract		4.78^{b}±0.067	4.08^{b}±0.083	2.55^{b}±0.283	2.20^{b}±0
Carbendazim		4.91^{ab}±0.0167	4.53^{a}±0.033	3.76^{a}±0.016	2.86^{a}±0.067
P-Value		0.00000889***	0.000000000198***	0.0356*	0.00385**
LSD		0.157	0.250	0.820	0.300
CV (%)		1.880	3.607	14.968	4.569
Grand mean		4.78	3.96	3.076	2.366

Significance codes: 0.001 '***' 0.01 '**' 0.05'*' NS= non-significant. Values are means of three replications. Within a column, values having the same letter(s) do not differ significantly as per DMRT at 5% level.

VI. CONCLUSION

Different plant leaf extracts imposed to this investigation showed significant variation in terms of post-harvest qualities and shelf life of mango. The present study revealed that postharvest dipping of mango fruits on plant extracts can improve the post-harvest quality and extend the shelf life of fruits. All the leaf extracts treatment gave decent results as all of them gave superior performance than the control. There was minimum physiological weight loss, maximum ascorbic acid content, maximum acidity and minimum pH in neem leaf extract treatment. Similarly, shelf life, total soluble solids, freshness and firmness were highest in neem leaf extract treated fruits next to the carbendazim treated fruits. The performance of neem leaf extract was superior among other leaf extract treatments and was comparable with the bench mark (carbendazim) treatment; indicating that, the use of plant extracts can be a better alternative for maintaining quality and extending post-harvest life of mango in place of hazardous chemical pesticides.

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Analysis of Channel and Structure of Cattle Marketing Intermediaries in Mubi Local Government Area of Adamawa State, Nigeria.

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Abstract *— This study determined the Marketing Channel and Structure of Cattle among Intermediaries in Mubi Local Government Area of Adamawa State, Nigeria. Objectives of this study area to examine the marketing channel for cattle; determine the marketing structure of the intermediaries and identifying the major constraints in cattle marketing in the study area. Simple random sampling technique was employed to select 123 respondents in Mubi International Cattle Market. Primary data were collected through the use of structured questionnaire from the market. Descriptive statistics and Gini-coefficient were used in analyse the data of this study. The result shows that 87% sell live cattle, 13% sell butcher pieces, while 61.8% and 27.6% sell their cattle in secondary and terminal markets respectively. About 73% had their major source of trading cattle in north-east and 26% are from other countries (Cameroon, Chad and Niger). Gini- coefficients of 0.5673, 0.6340, 0.452 and 0.5719 were obtained for wholesalers, retailers, butchers and brokers respectively, while Respondents indicates that insurgency (insecurity) (78%), inadequate market information (74%), inadequate credit facility (73.2%), cost of transportation (72.4%), double charges by market officials been the least (48.8%) were some of the major constraints. The study recommended that good roads, better and cheap means of transportation should be provided to the marketers through their cooperatives.*

Keywords— Channel, Structure, Cattle Marketing, Intermediaries, Mubi and Nigeria.

I. INTRODUCTION

Nigeria is one of the leading countries in cattle production in sub-Saharan Africa (World Health Organization, 2008, World Bank 2009). The population figure of domestic livestock in Nigeria in 2011 stood at 19.5 million cattle, consisting of 3.2 million milking cows and 16. 3 million beef cattle, where Less than 1% of these populations of cattle are managed at commercially level while the 99% of the remaining population are managed traditional level (Tibi and Aphunu ,2010). Livestock production in Nigeria had been predominately rural until recently when development in husbandry and breeding for improvement was given a prominence of place. Generally, livestock husbandry plays a very important role in the development of a nation. The limited supply of animal protein in tropical countries like Nigeria is primarily the result of low productions owing to traditional management, rather than small number of the animals (Olayide, 1980). The trend is likely to continue unless animal production efficiency through the use of improved breeds is greatly increased (Umar *et al*, 2008), and marketing systems perfected, therefore, it is believed that livestock marketing in Nigeria is traditional with a strong cultural control. It is also believed that unfavorable marketing outcome discourages production through lower output prices and consumption through high prices (Iheanacho, 2005).

Agricultural contribution to the nation's GDP is 35%, whereas livestock contributed only 5% (Bonnet *et al*, 2013). Cattle industry provides a means of livelihood for the significant proportion of the livestock rearing household and participant in the cattle value chain in Nigeria (Okunmadewa 1999). Although there are many sources of animal protein in Nigeria, recent study (Tibi and Aphumu 2010) has shown that cattle and cattle product are predominant and the most commonly consumed animal protein sources. Thus, they are highly value livestock in Nigeria where they are kept for beef, hide and milk. Cattle and beef trade provide the largest market in Nigeria with millions of Nigerians making livelihood from various beef related enterprises (Umar *et al*, (2008).

According to Bonnet *et al*, (2013), the strong demand for animal product is not only due to high rate of urbanization (60% of Nigerians are city dwellers), but above all, to consumers' greater purchasing power and emergence of new middle class. Furthermore, this trade giant accounts for nearly 60% international trade in the region. The demand for beef is up to 17,466 tones per day whereas the supply is just 3,999 tones (Oyekale 2001). Supply of cattle and

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itsproducts has witnessed a decline in the years 2010 – 2015. The per capita consumption of beef in Nigeria stood as ₦4568.4, ₦4356.2, ₦4356.2, ₦4202.4 and ₦4021.4 respectively, (National Chicken Council 2012), due partly to population growth and deficit in supply, with import at 25% (Bonnet *et al*, 2013). The high cost of marketing cattle is often the commonly cited culprit for this situation. Efficient marketing plays an important role in the attempt to achieve wider accessibility and affordability of any product to consumers (Mafimisebi *et al*, 2011). This is obvious from the long established maxim that production and marketing constitute a continuum, thus, lack of development in one will necessarily obstruct development in the other (Olayemi,S 2004; Olayemi, 1994; Seperich *et.al*, 2002).

Objectives of the Study

The main objectives of this study were to determine the marketing channel and the marketing structure of intermediaries in cattle marketing in Mubi Local Government Area of Adamawa state, Nigeria.

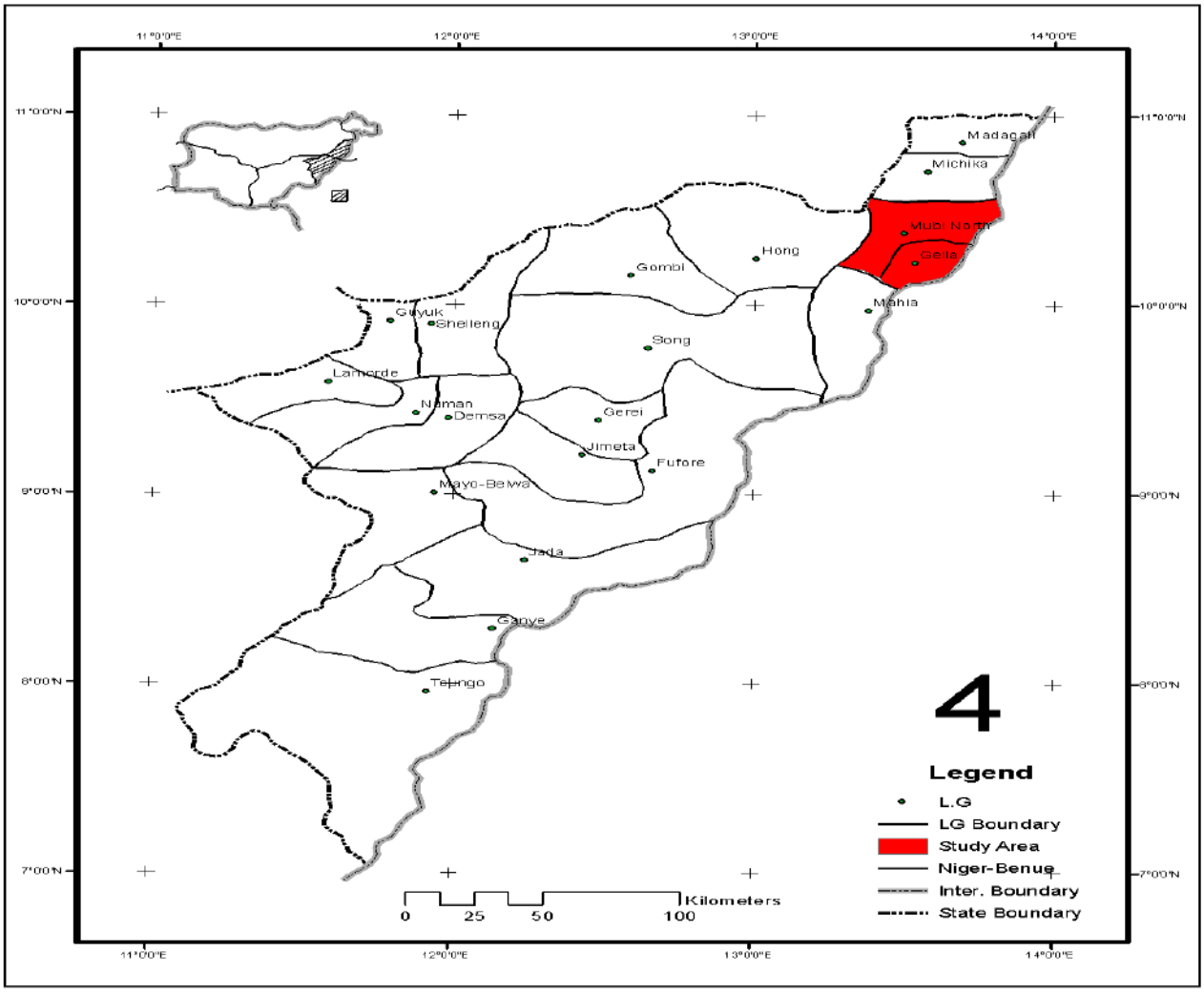
The specific objectives were to:

i. the marketing channels for cattle in the study area;
ii. determine the marketing structures of the intermediaries in cattle marketing; and
iii. examine the major constraints in cattle marketing.

II. METHODOLOGY

Study Area

The study was conducted in Mubi Area of Adamawa State, Nigeria. Mubi is located on latitudes 8^0 N and 11^0 N and longitudes 11^0 5' E and 13^0 5′ E. It is on altitude of 696 meters above sea level, with an annual mean rainfall of 700mm in North West and 1600mm in the Southern part of the State. The Maximum temperature can reach 40^OC, particularly in April, while minimum temperature can be as low as 18^oC between December and January (Mansir, 2006). It also has an international boundary with the Cameroon Republic along its eastern border (Mubi *et al*, 2013).



SOURCE: Adamawa Agricultural Development Program, (1986).

Fig.1: Map of Adamawa State showing the Study Area.

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The area is divided into two LGAs which are Mubi north and Mubi south. Mubi North has a population of 151072 people and Mubi south has a population of 128937 people (National Population Commission, 2006). They are two (2) important LGAs among the 21 LGAs of the State. Mubi International Cattle Market which is situated in Mubi south LGA of Adanawa State, forms an area of contact with cattle marketers. Figure 1 shows the Map of Adamawa State Nigeria.

Population and Sampling Procedure

The population of this study comprises all Cattle Marketers' in Mubi International Cattle Market. A sample of 123 was selected from the population of all the cattle marketers in the study area. It was selected using simple random sampling technique, from a sample frame of 50% of 246 respondents of cattle market intermediaries in Mubi International Cattle Markets.

Data Collection and Analysis

The data for the study were obtained from primary source, using well structured questionnaire administered to the cattle marketers. Data were collected on the marketing channels, sales, returns and constraints. Descriptive statistics were used to analyses the marketing channel and the major constraint affecting cattle marketing. Gini coefficient was used in analyzing the marketing structure of the intermediaries and it is expressed as fallows:

$$G.C = 1 - \sum XY$$

Where,

GC = Gini Coefficient.

X= proportion of cattle marketers

Y= cumulative proportion of cattle marketers earnings

$\sum$ = summation sign

III. RESULTS AND DISCUSSION

Marketing Channels

The marketing channels for cattle in Adamawa State are presented in Table 1 and figure 2. The result shows that 87% of the respondents sold live cattle, while 13% sold butchered pieces to the consumers in the markets and other village markets. Majority (61.8%) of the respondents sold their cattle in a secondary market. These are people who buy in the market and still sell it in the market. Also 27.6% sold their animals in a terminal or urban market. These are people who transport those cattle to other parts of the states and countries, while 10.6% of the respondents sold there cattle and its product in a primary or village markets.

Majority (62.6%) transport their cattle from place to place using truck/ lorry and few (37.4%) by trekking. Some of the respondents around the border of the country come in with their cattle by trekking to avoid some charges. Most (43.9%) of the respondents found in the markets are sellers, 36.6% buyers, while 19.5% are agents who are responsible for bringing the buyers and the sellers together. This also agrees with Okewu and Iheanacho (2015), who stated that the major market players are wholesalers, retailer and butchers who sell to one another and directly to final consumers in small quantities. Majority (82.1%) of the respondents sell their cattle to gain money so as to continue with business, while 17.9% are buyers and sellers within the market.

Most (78%) of the respondents gave resale as the primary reason for buying cattle, 11.4% for consumption, and 10.6% for breeding. Few (26%) of the marketers sourced their traded cattle from other countries around, which include Chad and Cameroon, while <1% from North West. Majorities (73.2%) of the cattle traded are from north eastern part of the country, and larger population of the cattle producers is concentrated in that region. Majority (87.8%) used their personal saving for cattle marketing and 12.2% used personal savings and loans as their sources of fund. Most (39%) of the marketers purchased Red Bororo as the breed of cattle been marketed, 26% White Fulani, 4.1% Sokoto Gudali and majority (76.4%) of those cattle are mostly sold in secondary markets.

Marketing channel for cattle shows a systematic movement of cattle from the producer to the consumers. The analysis of marketing channel for cattle in figure 2 indicates that the production is mostly done by cattle rearers (Fulani). The cattle rearer sells the cattle to the wholesaler and the local marketers, while local marketers sell to the wholesalers. The linkage between cattle rearers and butchers is a weak one. Cattle rearers only sell to butchers when in course of migration, any cattle fall sick. A butcher is then invited to purchase it. The wholesalers are responsible for selling the cattle to the retailers, butchers and brokers, whereas the retailers are responsible for selling the cattle to the butchers, brokers and consumers. The butchers and the brokers also sell their cattle to the consumers who are at the receiving end. This means that trading of cattle passes through many intermediaries before getting to the hand of the final consumers (William, *et al*, (2006). Transportation is the only value addition in the marketing channel, as there are no processors along the channel.

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Table.3: Marketing channels of cattle markets (n=123)

Variables	Frequency	Percentage
In what form do you sell your cattle.		
live cattle	107	87
butchered pieces	16	13
Where do you sale your cattle		
primary market	13	10.6
secondary market	76	61.8
terminal market	34	27.6
Mode of transporting your cattle		
trekking	46	37.4
lorry/truck	77	62.6
Major role in cattle marketing		
seller	54	43.9
buyer	45	36.6
agent	24	19.5
Reason for selling your cattle		
money	101	82.1
resale	22	17.9
Reason for buying your cattle		
consumption	14	11.4
resale	96	78
breeding	13	10.6
Major source of traded cattle		
North-east Nigeria	90	73.2
North-west Nigeria	1	0.8
others (Cameroon, Chad and Niger)	32	26
Major source of fund		
personal saving	108	87.7
personal saving/loan	15	12.3
Breed of cattle marketed		
white fulani	32	26
red bororo	48	39
bokoloji	14	11.4
sokoto gudali	5	4.1
white fulani/red bororo	17	13.8
white fulani/bokoloji	5	4.1
white fulani/red bororo/bokoloji	2	1.6
Origin of cattle sold		
primary market	29	23.6
secondary market	94	76.4

Source: Field survey data, 2017

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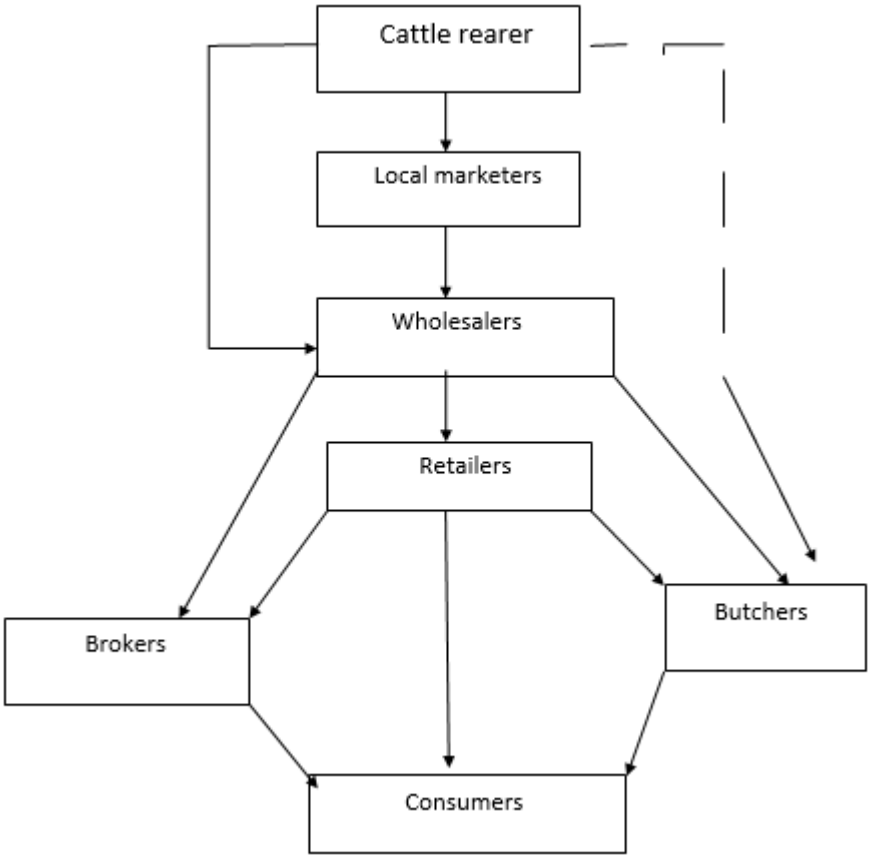

Fig.2: Marketing Channel of Cattle in Adamawa State, Nigeria

KEY:

Strong linkage

Weak linkage

Marketing Structure of the intermediaries

The cattle marketing characteristics in the study area is highly dependent upon the structure of the market. The result of Gini coefficient of market intermediaries are shown in Table 2. The results indicated that the wholesalers had total weekly sales and mean value of weekly sales of ₦9,275,000,030 and ₦15,458,333.83. The results indicate that the wholesales cattle market was concentrated, with Gini coefficient of 0.5673, which shows the possibility of non-competition in the markets. The market is controlled by fewer individuals and there is inequality distribution of wealth in the markets, for the retailers markets, it showed that they had total weekly sales and mean weekly sales of ₦44,600,014 and ₦1,651,852.37. These findings show that the retail market is more competitive with Gini coefficient of 0.6340, compared with the wholesalers (0.5673), and maximum inequality in income distribution and market concentration. (Iheanacho & Mshelia 2004), in cattle retail market, on the other hand, high capital investment makes entry easy. This makes sellers concentration moderate or less, and this is on average. It is an indication of lower profit due to presence of many buyers and sellers.

For the butchers they had total weekly sales and mean weekly sale of ₦9,000,007.5 and ₦600,000.5. These findings revealed that the butchers market was competitive with low Gini coefficient of 0.4552, which shows that people are not ready to go into business that demand more cash because they are afraid of risk. Whereas the brokers' market analysis shows that they had total weekly sales and mean weekly sales for brokers was ₦25,800,010.5 and ₦1,228,571.93. The broker's market shows market concentration with a Gini coefficient of 0.5719, showing that there is unequal distribution of wealth among them like the wholesales and brokers and non-competition.

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Table.2: Weekly Sales Distribution of Cattle Market Intermediaries in Adamawa State, Nigeria.

Market Intermediaries	Total no. 0f Intermediaries	Total Weekly Sales (₦)	Mean Value of Weekly Sales (₦)	Gini Coefficient	Market Structure
Wholesalers	60	9,275,000,030	15,458,333.83	0.5673	Concentrated
Retailers	27	44,600,014	1,651,852.37	0.6340	Concentrated
Butchers	15	9,000,007.5	600,000.5	0.4550	Non-concentrated
Brokers	21	25,800,010.5	1,228,571.93	0.5719	Concentrated

Source: Field survey data, 2017

Major Constraints in Cattle Marketing

Major constraints in cattle marketing in the study area are show in Table 3. The finding reveals that insurgency (insecurity) was indicated by 78% of the respondents as a major problem and this result from the activities of Boko haram. This was followed by inadequate market information (74%) on price and cost of production, which are not made available to the cattle marketers. Inadequate market facilities (73.2%) such as improper housing, absence of portable water, unit of measurement, lighting point and higher cost of transportation (72.4%) make it difficult for marketers to meet up with market days some times. The only means of transportation available are trekking and trucks. Other constraints include, low profitability (65%) and inadequate credit facility (52%), resulting in high interest rate, absence of collateral and improper record keeping by the marketers which are needed by lending institutions. Also double charges by market official were the least (48.8%) constraint. These include charges by Local, State and Federal Governments, and Kungiyan Miyoti Allah. These are some of the major constraints that affect cattle marketing in Adamawa State, Nigeria. This agrees with study by Okewu and Iheanacho (2015), which reveals that inadequate market information, credit, market facilities , high cost of acquisition, transportation, medication and feeding, as well as the unethical charges and levies by crook officials, especially those along the produce checking points from Local Government to Local Government are the major marketing constraints in cattle marketing.

Table.3: Major constraints in cattle marketing

Constraints	Frequency	Percentage
Inadequate market information	91	74.0
Cost of transportation	89	72.4
Cost of acquisition of Cattle	69	56.1
Cost of medication	61	49.6
Double charges	60	48.8
Inadequate credit facility	64	52.0
Low profitability	80	65.0
Inadequate market facility	90	73.2
Insurgency	96	78.0

*Multiple responses existed, hence>100%

Source: Field survey data, 2017

IV. CONCLUSION AND RECOMMENDATIONS

Marketing channel and structure of cattle among intermediaries in the study area revealed that 87% sell live cattle, 13% sell butcher pieces, while 61.8% and 27.6% sell their cattle in secondary and terminal markets respectively. Gini- coefficients of 0.5673, 0.6340, 0.452 and 0.5719 were obtained for wholesalers, retailers, butchers and brokers respectively, indicating non-competition for wholesalers, brokers and retailers but butchers having a level of competition in the markets. Insurgency/insecurity, inadequate market information, inadequate market facility, cost of transportation, double charges by markets official were some of major the constraints in cattle marketing

which shows that the market need to be improved in the area of security. The study, therefore recommended that;
extension workers should be well equipped to provide market information on cattle marketing in the study area, good roads, better and cheap means of transportation should be provide to the marketers through their cooperatives, while lending institutions should be encouraged to advance soft loans to the marketers to reduce the problems of cost of acquisition and inadequate capital among the cattle marketers in the state. Finally there should be rules and regulations on tax collection especially through the cooperatives to tackle the problems of double charges on the marketers.

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Analysis of Genetic Impurity of An Original Cultivar Duku (*Lansium parasiticum* (Osbeck.) K.C. Sahni & Bennet.), from Jambi, Indonesia Using ITS and MatK Gene

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***Abstract*—Duku Kumpeh** *is a original cultivar of Duku* (*Lansium parasiticum*) *from Kumpeh a local village in the Jambi, Indonesia. The understanding about genetic information is very important for sustainability used of this prospoctive germplasm of tropical fruit. Identification molecular is very essential to distinguish duku kumpeh with other cultivars of duku in Indonesia. Molecular characteristic of sixteen accessions of Duku Kumpeh were clarified using ITS and MatK gene. DNA from sixteen accessions duku from Jambi were extracted using Genomic KIT plant and amplified them using primer of ITS and MatK gene. The results of amplification DNA samples using both of primer ITS and MatK gene indicated that all of fifteen samples were effectively amplified. So this both of two genes are potential to use for barcoding DNA Duku. Six haplotype of ITS gene and eleven haplotype of MatK gene were identified. The accessions from kumpeh were have different haplotypes. There were genetic impurity in accessions of duku kumpeh. Genetic study and selection of duku kumpeh accessions with superior quality and similar genetic composition were needed in the future.*

***Keywords*— barcoding DNA, duku, ITS, Haplotype, Lansium parasiticum, MatK.**

I. INTRODUCTION

Duku (*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet) is a unique and potential tropical fruit belonging to the Meliaceae (Mahogany family), but it is not quite planted on a plantation scale. Most of the fruits seen in markets are being resulted from trees in village plantation. This plants have been cultivated for long period, and Ma Huan, a the Chinese traveler have been being remarked it in year 1413 [1].

The duku trees were distributed mainly in South East Asia regions particularly Indonesia in Southern part of Sumatra, Philippines, Southern part of Thailand and Peninsular Malaysia [1]. This tropical plant is not only important as a edible fruit and widely consumed fresh for dessert but also it can also be utilized in cosmetics due to its extract has antioxidant property as well as moisturizing and almost no effects with a good safety profile [2]. The people used this plants for treatment of intestinal problems, and malaria, because of its fruit, seed and bark, have specific chemical constituent such as: andirobin derivates, methyl-angolensates, exicanolides, anazadira-dione, onoceranoids and dukunolides, lansionic acid. [3].

There were eleven different synonym name of duku, thus make confuse in recognozing the taxonomic position. *Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet was the valid name for duku and accepted for scientific purposes [4]. Although it was not right scientific name, *Lansium domesticum* Corr. was often referred as the Latin name of duku. Now, duku and its related were recognizable into three main groups i.e. duku, langsat or bidjitan and kokosan. Duku has small ellipsoid and pale yellow fruits, without latex and flowers small in diameter; langsat or bidjitan possesses a ellipsoid large fruit, glabrescent, fruits with pale yellow pericarp, larger flowers and stem contain slight latex; kokosan has the biggest flowers but its fruits is smaller, globose, with orange-yellow tough pericarp, and stem produce latex and most pubescent leaves [1]. The morphological appearance of the varieties was almost similar, so the varieties were not easy to recognize.

Based on the molecular characteristic, duku was different taxonomic position with kokosan and langsat group [5]. Related to production of indigenous duku of Sumatra, duku kumpeh was been cultivated in Jambi as the source of income for fruit farmers. Hence, now Jambi area was the second largest duku producer in Indonesia.

Related to the producing of tropical fruit duku, there are five centers production of duku In Jambi that are Kumpeh, Sorolangun, Tebo, Selat and Bangko. Kumpeh

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area produce of the best quality duku that export to other areas in Indonesia, especially to South Sumatra region, i.e. Palembang. In this area, duku from Kumpeh is known as 'duku Palembang'. To produce the best quality of duku, selection of seedling resources were required. However, in the present, for propagation purpose, the seedling cannot be identified easily. So, the source of seedling and characteristics of the duku was essential to clarify.

From morphological characterization, It is difficult to know whether the duku from kumpeh or from others places such as Sorolangun, Tebo, Selat and Bangko. Molecular examination with the ISSR and RAPD markers also obtained uniform bands [6], so there is difficulties in distinguishing duku between accessions from Jambi area.

Therefore , it is crucial to use more accurate molecular markers to identify , and clarify their relationship as an effort to sustainable utilization of the prospective indigenous duku from Jambi region. DNA barcode was the new marker that uses a standardized genomic DNA sequences as a barcoding for distinguishing species more rapidly and efficiently [7].

For this reason technique barcoding DNA has been applied for analysis plant communities [8]. Application of barcode was not only the most trusty and cost efficient alternative methods for identification of species and useful for clarification the source of germplasm and similarity between the taxonomic level [9] [10], but also useful method for genotype characterization and allows a high precision to know genetic relationship between infraspesific taxa such as cultivar level [11] [12].

Based on these facts, we used ITS and MatK sequence as markers for detection genetic impurity and relationship of some accessions duku original from Kumpeh, Jambi, Indonesia.

II. MATERIAL AND METHODS

Total fifteen samples of duku were gatherred from five center localities for duku (*Lansium parasiticum*) pro-duction of in Jambi area, there were Bangko (BK04, BK 10 and BK 19), Tebo (TB 03 ,TB 11 , and TB 14), Kumpeh (KP15, KP17 and KP25), Selat (SL16, SL17 and SL18), and from Sorolangon (SR03, SR10 and SR18) represented duku accessions native in Jambi (Figure 1). The young leaves of duku trees were grinded and DNA extraction was performed using CTAB (cetyl trimethyl ammonium bromide) method [13]. The fresh young leaves about 0.1 mg were finely grinded . The resulting DNA isolation were amplified using the primers of two barcode DNA, ITS and MatK.

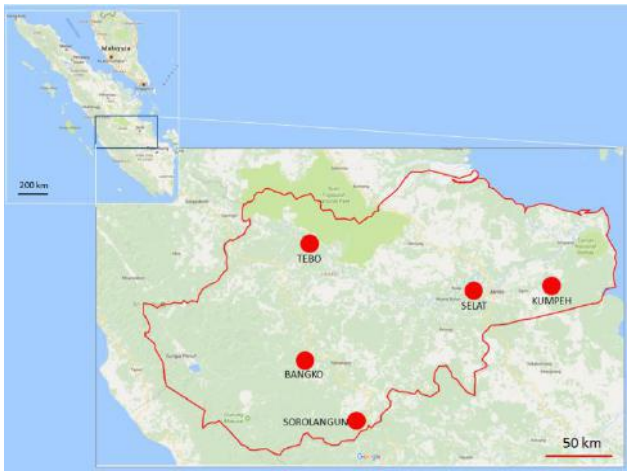

Fig. 1. Sampling sites of original cultivar from Jambi (Modification from:https://www.google.com/maps/place/Jambi,+Indonesia)

This activity was achieved using PCR (Polymerase Chain Reaction) technique andthe PCR produce was further purified using Mega Quick SpinTM PCR. Purification of DNA fragments was visualized on 1% agarose gel. Furthermore , 20 ul amplified produces were analyzed at Macrogen Inc. (Korea) to obtain the sequence of DNA. The sequences DNA were arranged using program BioEdit version 7.0. 4. [14] and the program of ClustalX was applied for alignment the homologous sequence [15]. The multiple alignment files were analyzed with MEGA version 6.0 program [16]. Concatenating sequences of the two loci (ITS and MatK) were arranged by DAMBE Version 6 .4.100 Program [17]. The evolutionary distances between fifteen accessions were analyzed by the p- distance method [8]. The phylogenetic relationship of fifteen accessions were constructed using the Kimura -2- Parameter (K-2-P) model , and Neighbor -Joining (NJ) method [21]. The bootstrapping consensus tree were inferred with 1000 replicates) to illustrate the evolutionary relationship between the accessions studied [22].

III. RESULTS AND DISCUSSION

The results of DNA extraction of fifteen accessions of duku have been done .The samples were collected from five site of producer areas of Duku (Lansium parasiticum) in Jambi area, i.e. from Bangko (BK04, BK 10 and BK 19), Tebo (TB03, TB11, and TB14), Kumpeh area (KP15, KP17 and KP25), from the Selat (SL 16, SL17 and SL18) and from Sorolangon (SR03, SR 10 and SR 18). For more details , sampling sites were shown in Figure 1.

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Table.1: Primers and their sequences of two barcodes dna (its and matk) that amplified to 15 samples dna of duku.

Primer	Sequence (5'-3')	Reference
ITS5-F	GGAAGTAAAAGTCGTAACAAGG	[19]
ITS4-R	TCCTCCGCTTATTGATATGC	
3F_KIM-F	CGTACAGTACTTTTGTGTTTACGAG	[20]
1R_KIM-R	ACCCAGTCCATCTGGAAATCTTGGTT	

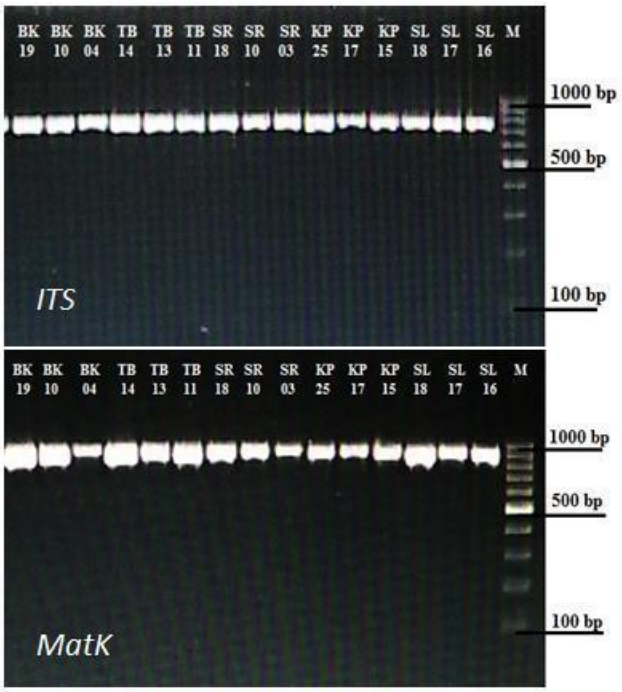

***Fig. 2**. Profile bands from electrophoresis fifteen accessions studied after amplified by ITS (upper) and Matk (lower)*

DNA of fifteen samples of duku accessions from five center production of duku i.e. Bangko (BK04, BK10 dan BK19), Kumpeh (KP15, KP17 dan KP25), Selat (SL16, SL17 dan SL18), Sorolangon (SR03, SR10 dan SR18) danTebo (TB03, TB11, and TB14) by using two primers of ITS gene (Table 1) were successfully amplified that proved by clear bands resulted from electrophoresis of samples after PCR amplification (Figure 2). The sequence length of amplification ITS gene was 800 base pair (bp) that was similar to the reported in Fig cultivars (Castro *et al*., 2015). Amplification of DNA from fifteen of duku accessions using both ITS and MatK barcode Gene produced the similar results. There were clear bands detected after electrophoresis PCR amplified samples (Figure 2). The *sequence* length of all fifteen DNA samples after amplified (± 900 bp) was similar to previous reported [11] [23].

Furthermore, analysis haplotype was performed on 16 DNA sequences of samples resulted from amplification using the *ITS* and MatK gene (Table 2). The result ofanalysis sequences based on the nucleotide single polymorphism pattern, Seventeen haplotypes were identified, seven of them were obtained from ITS gene and 10 of them were detected on sequence from MatK gene. Application of combination of two both of DNA barcoding ITS and PsbA-TrnH were successfully for discriminating the plant populations [24]. In case of application both of MatK and RbcL resulted in lower resolution for clarification of plant populations . In this study, the number haplotypes detected in accessions duku from jambi was very high (17 haplotypes) . This is very important and useful to detect the origin of the accessions. Haplotype 04 (H04) from ITS was detected over nine accessions from five central duku areas (Bangko, BK; Sorolangon, SR, Selat, SL; Kumpeh, KP; and Tebo, TB). The following haplotype H03 from MatK gene was found on four accessions from Bangko, Sorolangon, Selat, Kumpeh, but it was not detected in Tebo (TB). The haplotype diversity among all accessions studied was high (Hd = 0.74). These results was very different to the previous studied applying ISSR and RAPD marker on twenty-one accessions duku from Jambi [6], there was no different between samples analyzed. based on very high haplotype variations, not only between accession at the locality plantation, and even the unique haplotypes detected in some individual accessions from same locality. So, using these two barcodes DNA (ITS and MatK) were very potential for determination in population genetic analysis, detecting the origin of accessions the unique this tropical fruit.

For precise determination of germplasm sources from duku, it is important to provide a specific sequence that can be used to detect their phylogentic relationship. Thus DNA barcoding was found to be a practical and rapid method for identification not only at species level [25] [26] but also between varieties [27], the populations level [7], ecotypes [28] and inter individuals or accessions from one species.

Furthermore, it is well known that the production center of Duku in Jambi are Bangko, Sorolangon, Selat, Kumpeh and Tebo, with duku from Kumpeh as the best quality variety, but the source of duku accessions in kumpeh is unclear whether they were provided from Kumpeh or other locations. Based on this fact, although with the little number of accessions examined, we tried to detect the purity of duku accessions from each

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production center by using phylogenetic analysis of fifteen accessions from five locations of duku production centers in Jambi.

The Evolutionary divergence between fifteen nucleotide sequences of duku accessions was estimated and then constructed the phyllogenetic relationship. The evolutionary divergence of sequences between fifteen accessions duku from fives localities in Jambi were ranged from 0.00 (BK04 vs KP17, SL16, SR10; KP17 vs SL16, SR10) to 0.029 (SL18 vs BK19) for amplification ITS primer (Table 2, upper matrix). In case of the evolutionary divergence of sequences from amplification MatK primer was lower than by ITS with the value Evolutionary divergence was 0.00 to 0.012, respectively

Table.2: Seventeen haplotypes from two barcoding dna detected in 16 duku accessions (lansium parasisticum) from jambi.

No	Barcoding DNA	Haplotype	Acessions	Number Accessions
1	**ITS**	H01	SL18, TB11 & BK04	3
2		H02	BK20	1
3		H03	KP17	1
4		H04	BK10, SR03, SL16, KP25, SL17, TB14, TB03, KP15 & SR18	9
5		H05	BK19	1
6		H06	SR10	1
7	**MatK**	H01	BK20	1
8		H02	TB11	1
9		H03	BK04, SL16, KP17 & SR10	4
10		H04	SL17 & SR18	2
11		H05	KP15	1
12		H06	SL18	1
13		H07	KP25	1
14		H08	SR03	1
15		H09	BK19	1
16		H010	TB13	1
17		H011	TB14	1

(SR10 vs KP17) (Table 2, lower matrix). This evidence suggested that sequence DNA of duku resulted from amplification by barcode DNA ITS were more variation than those that amplification by barcode DNA MatK Joining method (Figure 4), the bootstrap consensus tree (inferred from 1000 replicates) was done to know evolutionary history of the accessions analyzed. The bootsrap values more than 50% was considered statistically significant.

There were a total of 1363 positions in the final data set. Evolutionary analysis were conducted in MEGA 6 [16]. Topology of the phyllogenetic tree indicated that the accessions of duku examined were not clustered to their collection sites. This facts showed that duku plantation in five production centers (Bangko, Sorolangon, Selat, Kumpeh and Tebo) had individual trees from various places. The individual trees of duku from kumpeh were not only origin from kumpeh but also from other locations, especially from location of centre production Duku in Jambi. For the optimal use of duku plants, the purity of the seedlings should be sought. Therefore, the clarification genetic identities (haplotype) of duku accession from kumpeh with the superior quality have to study in the future.

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Table..2: The evolutionary Divergence Using Its Gene (Lower Diagonal) And Matk Gene (Upper Diagonal) Between Fifteen Accessions Of Duku From Five Localities In Jambi

Accession	BK04	BK19	BK20	KP15	KP17	KP25	SL16	SL17	SL18	SR03	SR10	SR18	TB11	TB13
BK04	-	0.001	0.006	0.001	0.003	0.001	0.001	0.001	0.000	0.001	0.010	0.001	0.001	0.000
BK19	0.028	-	0.004	0.000	0.004	0.000	0.000	0.000	0.001	0.000	0.012	0.000	0.000	0.001
BK20	0.005	0.028	-	0.004	0.009	0.004	0.004	0.004	0.006	0.004	0.007	0.004	0.004	0.006
KP15	0.001	0.029	0.004	-	0.004	0.000	0.000	0.000	0.001	0.000	0.012	0.000	0.000	0.001
KP17	0.000	0.028	0.005	0.001	-	0.004	0.004	0.004	0.003	0.004	0.013	0.004	0.004	0.003
KP25	0.003	0.025	0.008	0.004	0.003	-	0.000	0.000	0.001	0.000	0.012	0.000	0.000	0.001
SL16	0.000	0.028	0.005	0.001	0.000	0.003	-	0.000	0.001	0.000	0.012	0.000	0.000	0.001
SL17	0.003	0.028	0.005	0.001	0.003	0.005	0.003	-	0.001	0.000	0.012	0.000	0.000	0.001
SL18	0.003	0.029	0.008	0.004	0.003	0.005	0.003	0.004	-	0.001	0.010	0.001	0.001	0.000
SR03	0.001	0.026	0.006	0.003	0.001	0.001	0.001	0.004	0.004	-	0.012	0.000	0.000	0.001
SR10	0.000	0.028	0.005	0.001	0.000	0.003	0.000	0.003	0.003	0.001	-	0.012	0.012	0.010
SR18	0.003	0.028	0.005	0.001	0.003	0.005	0.003	0.000	0.004	0.004	0.003	-	0.000	0.001
TB11	0.003	0.028	0.003	0.004	0.003	0.005	0.003	0.005	0.005	0.004	0.003	0.005	-	0.001
TB13	0.010	0.024	0.008	0.012	0.010	0.008	0.010	0.012	0.010	0.009	0.010	0.012	0.008	-
TB14	0.005	0.022	0.010	0.006	0.005	0.008	0.005	0.008	0.008	0.006	0.005	0.008	0.008	0.013

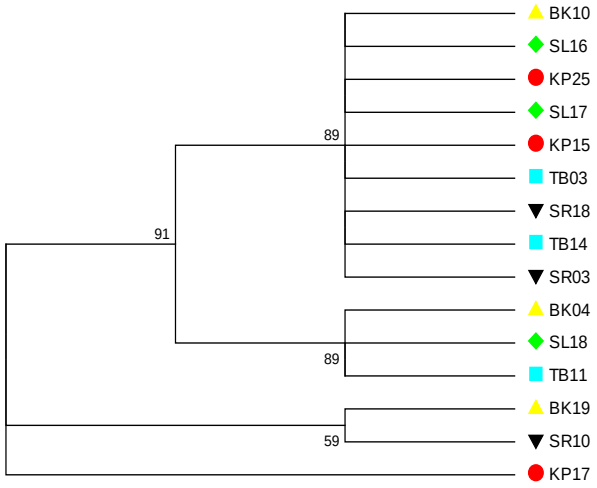

Fig. 2: The phylogenetics tree of fifteen accessions inferred using the Neighbor-Joining method based on ITS + MatK . BK = Bangko , KP = Kumpeh , SL = Selat , SR = Sorolangun and TB= Tebo

Moreover , the high variation of haplotype in other accessions or populations should be maintained as a source of germplasm for genetic conservation of local fruits typical of Jambi.

IV. CONCLUSION

Amplification of sixteen duku accessions from five locality of duku plantations in Jambi were successful using two barcode DNA ITS and MatK and very effective to detect the genetic polymorphism between accessions . Seventeen characteristics of DNA sequences (haplotypes) were detected using these two barcode DNA. The accessions from kumpeh were not originated from the same sources . the high number unique of haplotype in each accessions or populations was important genetic information for selection germplasm to produce the superior quality and yield and also potential information for genetic conservation of local fruits typical of Jambi , Indonesia.

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Evaluating the *In vivo* Efficacy of Copper-Chitosan Nanocomposition for Treating Vascular Wilt Disease in Date Palm

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***Abstract*—** *Date palm, Phoenix dactylifera, as one of the most important fruit crops in Egypt and many other countries, can be affected by many fungal diseases, among which the vascular wilt disease, caused by the fungal pathogen Fusarium oxysporum, is considered the most deteriorating one. This study aims at evaluating the efficiency of Copper-Chitosan Nanopcomposition for treating the vascular wilt disease in date palm. The study relies mainly on beleaguering the disease via the double-role functionality of copper-chitosan nanocomposition, i.e. its potential antifungal effect on the fungal pathogen, besides its capability to enhance the immune responses of the infected plant. In this regard, chitosan nanoparticles were prepared according to the ionic gelation method, whereas copper nanoparticles were prepared according to the chemical reduction method. Physicochemical characterization of both chitosan and copper nanoparticles was performed using dynamic light scattering (DLS), transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FTIR) and x-ray diffraction (XRD). Copper-chitosan nanocomposition could significantly reduce the vascular wilt disease severity; this means that the nanocomposition can be used in the future for developing new nano-fungicides to control such pathogens.*

***Keywords*—Copper nanoparticles, Chitosan nanoparticles, Date palm, Fusarium oxysporum, Vascular wilt.**

I. INTRODUCTION

Date palm, *Phoenix dactylifera*, is considered one of the most important fruit crops in many arid regions including the Middle East; This is due to its versatility, as it has a variety of uses includes eating fresh fruit and benefiting from its high nutritional value and its richness of carotenoids, citric acid, folic acid, and provitamins; this in addition to the antiviral, antibacterial, antifungal, antiulcer, antitumor and immunomodulatory properties of phenolic compounds detected in dates. Moreover, this crop has a great potential as a source of renewable energy by producing biofuel due to the high carbohydrates content in the fruits. Also, seeds are used in animal feeding, cosmetics, source of oxalic acid and charcoal, besides using them as a paste to relieve ague **(Al-Shahib and Marshall, 2003)**.

The worldwide production of dates reached **7600315** tons annually. In this context, Egypt is considered the largest producer of dates all over the world with annual production of **1465030** tons **(FAO, 2014).** This massive production of dates makes date palm a promising potential source of national income in Egypt and other large producers. But, this important crop, like all other crops, is threatened with many fungal and bacterial phytopathogens.

Generally speaking, more than 70% of the crop diseases result from fungal pathogens **(Agrios, G.N., 2005)**, this indicates to the importance of controlling such virulent phytopathogens. In this regard, surveys showed that among these fungal pathogens, the fungal pathogen *Fusarium oxysporum*, which causes vascular wilt disease (also known as fusarium wilt disease), is considered the most common and most virulent one **(Flood, 2006)**.

In this context, there are many traditional chemicals that are used to control such pathogenic fungi. But many phytopathogens have exhibited resistance against many traditional chemicals that are used to control them **(Hide *et.al*, 1992; M. J. Hajipour *et al.*, 2012; A. J. Friedman, 2013; Patel *et.al*., 2014)**. Thus, there is a dire need to develop newer and more effective controlling agents. In this regard, nanotechnology may provide more efficient alternatives for the current fungicides.

Among different types of nanomaterials, metal nanoparticles have gained a considerable attention and have a wide range of applications due to their unique catalytic, electric, magnetic and structural properties **(M. Sahu and P. Biswas, 2011; H. Tian *et al.*, 2014 and H. M. Yadav *et al.*, 2014)**. In this regard, copper nanoparticles (CuNPs) are considered a very important type of transition metals, which have many potential applications including but not limited to catalysis of some dyes such as rose Bengal dye **(S. T. H. Sherazi *et al.*, 2013)**, printing in electronics **(S. Magdassi *et al.*, 2010)**

and its antimicrobial effect **(A. D. Karthik and K. Geetha, 2013; Prachi. K. *et al.*, 2013; R. Betancourt-Galindo *et al.*, 2014 and A. M. Muthukrishnan *et al.*, 2015)**.

On the other hand, chitosan has gained much attention in many different applications, including but not limited to pharmaceutical, medical, agricultural, nutritional and industrial applications, this is due to its superior characteristics such as non-toxicity, biodegradability and biocompatibility **(Harish P. and Tharanathan, 2007)**. Also, chitosan nanoparticles (CsNPs) have a potential ability to enhance the immune responses of plants **(Chandra, S. *et al.*, 2015)**, this makes CsNPs a potential competitor in formulating the protective compositions directed toward enhancing the plant immunity.

In this work, the *In vivo* efficacy of copper-chitosan nanocomposition (CuCs) will be evaluated to treat the vascular wilt disease in date palm. The idea relies upon exploiting the dual functionality of this nanocomposition, i.e. its ability to induce and augment the innate immune responses in the plant and its potential antifungal activity against the fungal pathogen itself. This can hinder the fungal growth inside the vascular system of the infected plant and hence beleaguering the vascular wilt disease.

II. MATERIALS AND METHODS

All chemicals used were analytical grade of purity, and were used as received without any further purification.

2.1. Preparation of CsNPs and CuNPs

2.1.1. Preparation of CsNPs

CsNPs were synthesized in accordance with the ionic gelation method **(Qi, L. *et al.*, 2004)** with a simple modification, in which:

- 2 g of chitosan powder (Degree of Deactylation ≥ 90%; water content ≤ 8.0%; Carl Roth, Germany) were dissolved into 1 liter of 1% acetic acid solution.
- 0.5 g of sodium tripolyphosphate (TPP) (Sigma Aldrich) was dissolved into 1 liter of distilled water.
- TPP solution was added dropwise under stirring to the chitosan solution in 1:10 volumetric ratio.
- Temperature was kept at 25 °C and pH was adjusted at 4.

2.1.2. Preparation of CuNPs

CuNPs were prepared according to the chemical reduction method (**Mustafa B. and Ilkay S., 2010)**, in which L-ascorbic acid (0.11M) (Future Modern Co., Egypt) was added into aqueous solution of CTAB (0.09M) (Sigma-Aldrich, Egypt). NaOH solution was used to adjust pH at 6.8; temperature was kept at 85 °C. During stirring of the previous mixture of CTAB and L-ascorbic acid, Copper sulfate pentahydrate (0.03M) (Elnasr Pharmaceuticals Co., Egypt) solution was introduced. The reaction was continued until reddish brown color was developed. Then, CuNPs were collected by centrifugation for further characterization and application.

2.2. Characterization of CsNPs and CuNPs

2.2.1. Fourier Transform Infrared Spectroscopy (FTIR) of CsNPs

Fourier Transform Infrared Spectroscopy (FTIR) was performed to confirm the successful ionic gelation between chitosan and TPP. In this step, solutions of chitosan and CsNPs were firstly lyophilized, using the lyophilizer (EDWARDS Freeze Dryer), then FTIR analysis was performed on the lyophilized chitosan and CsNPs using FTIR spectrophotometer (Model Jasco 4100, Japan; 400 – 4000 cm^{-1}).

2.2.2. UV-Vis spectroscopy of CuNPs

The characteristic surface plasmon resonance of CuNPs was detected using Helios Gamma Spectrophotometer.

2.2.3. X-Ray Diffraction (XRD) of CuNPs

The characteristic X-Ray Diffractogram of CuNPs was recorded using Philips PW1840 X-Ray Diffractometer, USA (λ = 1.54056 Å; 40 kV; 25 mA). The range of recording was from 5° to 70°, with a rate of 2°/minute.

2.2.4. Determining Particle Size Distribution of CsNPs and CuNPs

Dynamic light scattering (DLS) (Zetasizer nano series (Nano ZS), Malvern, UK) was used to measure the particles size of the synthesized CsNPs and CuNPs.

2.2.5. Transmission Electron Microscopy (TEM) of CsNPs and CuNPs

Transmission Electron Microscopy (Tecnai G20, Super twin, double tilt, FEI, Netherland) was used to figure out the shape of the synthesized CsNPs and CuNPs.

2.3. The Fungus, *Fusarium oxysporum*

Fusarium oxysporum (FO1 isolate), which was isolated from infected date palm, was obtained from Department of Date Palm Pathology, Central Laboratory for Date Palm Research & Development, Agricultural Research Center, Giza, Egypt.

2.4. Preparing copper-chitosan nanocomposition (CuCs)

CuCs was prepared in four gradient concentrations (0.5, 1.0, 1.5 and 2.0 g/l) through the admixture of CuNPs and CsNPs with the respective concentration from both components.

2.5. Evaluating the Antifungal Activity of CuCs

Spores` inoculum of the fungus was prepared; then the microscopic enumeration technique was used to adjust the fungal inoculum size at 1.0×10^6 spores/ml**.** After that, 100 ul of the inoculum suspension were used to inoculate each of potato dextrose broth (control) and potato dextrose broth containing different concentrations of the nanocomposition. Both the control and treatments were kept at 30 °C for 2 days. Then, the optical densities (OD) of the cultures were detected using Uv-Vis Spectrophotometer (ORION AQUAMATE 8000) at 530 nm **(Eva Petrikkou *et al.*, 2001)**. Percentage of inhibition at each concentration was calculated according to the following formula **(Ling Yien Ing *et al.*, 2012)**.

$$Inhibition\ rate = (1 - OD_{treatment} / OD_{control}) \times 100 \quad (1)$$

2.6. Evaluating the Immune responses of Date Palm Seedlings caused by CuCs

Four groups of date palm seedlings, Sewi cultivar, (10 months) were used. Each group consists of 20 healthy seedlings. Each group was irrigated with 50 ml of CuCs with the respective concentration (0.5, 1.0, 1.5 or 2.0 g/l) per seedling. The fifth group of seedlings was used as a control, in which seedlings were irrigated with 50 ml water per seedling. Leaves from each seedling were dried, then the total phenolics and enzymes were extracted **(kâhkônen *et al.*,1999)**.

- ***The level of total phenolics*** in the extracts was quantified by the modified Folin – Ciocateu method **(Singelton and Rossi, I965)**. Gallic acid standard (5 g%) was used, and the total phenolic content was expressed as milligram Gallic Acid per gram dry weight of the original sample (mg GA/g dw).
- ***Phenoloxidase activity*** was determined according to **(Ishaaya, I971)**. The phenol oxidase activity was determined as optical density (OD_{405}) units $\times 10^3$ at an absorbance of 405 nm.
- ***Peroxidase activity*** was determined according to **(Vetter *et al.*, I958)**. The enzyme activity was expressed as the change in absorbance at 430 nm (ΔOD_{430})/minute/g fresh weight.

2.7. Evaluation of the *In vivo* Efficiency of CuCs in Treating Fusarium Wilted-Date Palm Seedlings In Comparison With Rizolex™

2.7.1. Initiation of Fusarium Wilt Disease

Firstly, the fungal culture was raised on Richard's liquid medium and incubated at 26°C for 2 weeks. Thus, the fungal mat is formed **(Riker and Riker, 1936)**. After that, the fungal inoculums were prepared through mixing of 10 g of the mycelial mat with 100 mL of distilled water in a blender **(S. Ansari *et al.*, 2012)**. Each seedling was inoculated through adding 50 mL of the fungal inoculum in its root zone.

2.7.2. *In vivo* treatment

In this experiment, three groups of the inoculated date palm seedlings, Sewi cultivar, (10 months) were used. Each group consists of 20 inoculated seedlings. 10 days after the fungal inoculation, the treatment began as follow:

The first group of inoculated seedlings was used as a treatment, in which 50 ml of CuCs with different concentrations was uniformly applied to root zone of each inoculated seedling.

The second group of inoculated seedlings was used as a positive control. In which each inoculated seedling was treated with 50 ml of 3 g/l Rizolex™ fungicide.

The third group of the inoculated seedlings was used as a negative control, in which 50 ml of water was added to the root zone of each seedling.

2.7.3. Assessment Disease Severity

Disease progression was observed through two successive weeks. Symptoms of Fusarium wilt disease on leaves (wilting and yellowing) of the date palm seedlings were used to measure the disease severity. In this regard, a standard rating **(Campbell and Madden, 1990)** was used to assess the disease severity for each group. Disease severity (DS) was calculated **(Chester *et al.*, 1959; Wheeler, 1969)** as follow:

Disease Severity (DS) = ((sum of all individual disease rating) / (total number of leaf assessed x maximum rating)) × 100. (2)

2.8. Statistical analysis

SPSS 22 software was used at $P \leq 0.05$ to distinguish between DS, antifungal efficiencies and levels of total phenolics and activity of phenoloxidase and peroxidase in different groups. The whole experiments were repeated twice **(McDonald, 2008)**.

III. RESULTS & DISCUSSION

3.1. Confirming successful preparation of CsNPs by the Ionic Gelation Method

FTIR analysis showed that chitosan has two main characteristic peaks at **3433** cm^{-1} and **1644** cm^{-1} which correspond to stretching vibrations of the primary amine group ($-NH_2$) and the amide group ($-CONH_2$), respectively; as shown in figure (1). On the other hand, FTIR analysis of chitosan nanoparticles showed that both peaks were shifted to **3428** cm^{-1} and **1580** cm^{-1}, respectively; as shown in figure (2).

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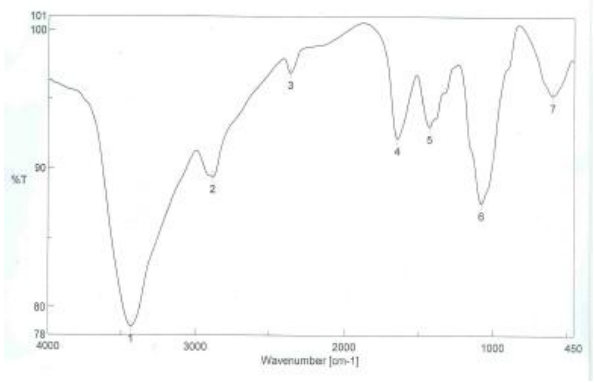

Fig.1: FTIR spectrum of chitosan

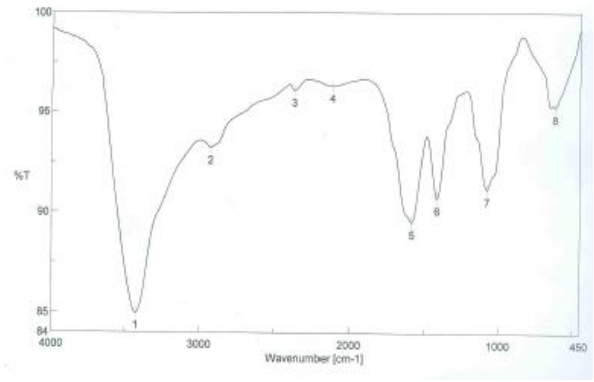

Fig.2: FTIR spectrum of chitosan nanoparticles

This reduction in stretching frequency indicates successful interaction of the polyphosphoric groups of sodium tripolyphosphate with the ammonium groups of chitosan and the more hydrogen bonding in chitosan–TPP complex.

Also, it is clear that the amine peak in chitosan nanoparticles (at **3428** cm^{-1}) is sharper than the amine peak in chitosan (at **3433** cm^{-1}), which indicates that the hydrogen bonding in chitosan nanoparticles was enhanced. **(A. Anitha *et al.*, 2009; Xu, Yongmei and Du, Yumin, 2003)**. Hence, chitosan nanoparticles were successfully synthesized by the ionic gelation method.

3.2. Characterization of CsNPs

Size distribution by number using dynamic light scattering revealed that the synthesized CsNPs have an average particle size about **50** nm as shown in figure (3). In addition, transmission electron microscopy showed spherical shape of the synthesized CsNPs, as shown in figure (4).

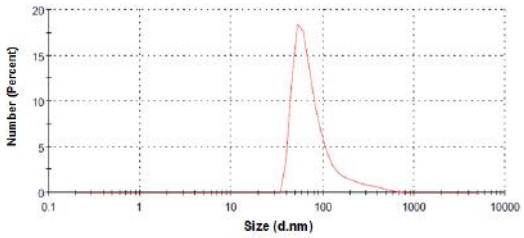

Fig.3: Particle size distribution of the synthesized CsNPs

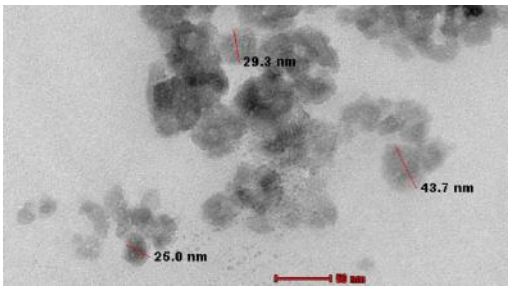

Fig.4: Transmission electron micrograph of the synthesized CsNPs, showing its spherical shape.

3.3. Confirming successful preparation of CuNPs

The Uv-vis spectroscopy of copper nanoparticles showed their characteristic resonance band at **572** nm, as shown in figure (5).

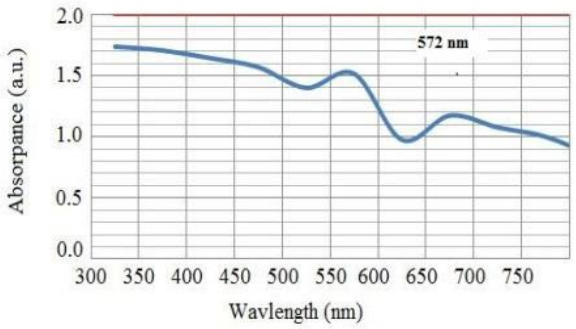

Fig.5: Uv-Vis spectrum of CuNPs showing its characteristic resonance peak at 572 nm.

Also, X-ray diffraction pattern as shown in figure (6) revealed the main characteristic diffraction peaks at 2 theta = **43.36**, **50.47** and **74.13** degrees, which correspond to the **(1 1 1)**, **(2 0 0)**, and **(2 2 0)** crystal faces of copper **(X. Zhu *et al.*, 2012)**. It is also noteworthy that there are other peaks at 2 theta = **36.45** and **61.39** degrees, that are characteristic for Cu_2O **(M. S. M. Suan *et al.*, 2011)** which indicates the formation of a Cu_2O shell covering the Cu core due to surface oxidation of CuNPs.

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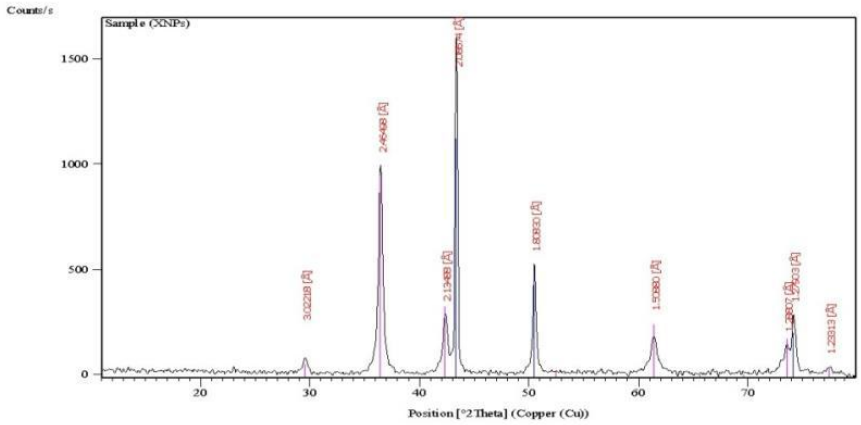

Fig.6: X-Ray Diffractogram of the synthesized CuNPs

3.4. Characterization of CuNPs

Transmission electron microscopy showed that the synthesized CuNPs have spherical shape, as shown in figure (7). Moreover, dynamic light scattering revealed that the average size of CuNPs was about **100** nm, as shown in figure **(**8).

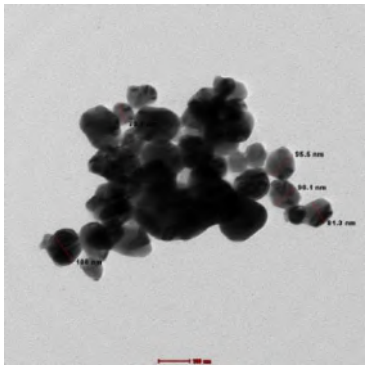

Fig.7: Transmission electron micrograph of copper nanoparticles, showing its spherical shape.

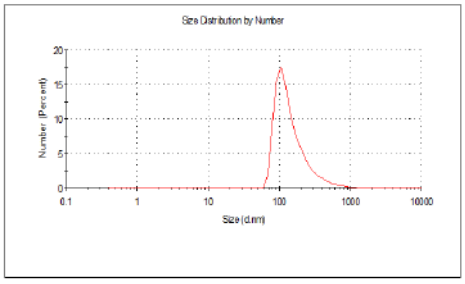

Fig.8: Size distribution by number of the synthesized CuNPs.

3.5. *In vitro* antifungal efficacy of copper-chitosan nanocomposition

All concentrations of CuCs had significant inhibition percentages, as shown in table (1); this demonstrates the potential antifungal efficacy of CuCs against the fungal pathogen, *Fusarium oxysporum*. Data express the average over triplicates.

Table.1: inhibition percentages caused by different concentrations of CuCs

Concentration (g/L)	Inhibition %
0.00 (Control)	00.00
0.50	61.94%
1.00	77.11%
1.50	89.47%
2.00	100.00%

3.6. Evaluating the Immune responses of Date Palm Seedlings caused by CuCs

Table (2) shows the positive immunomodulatory effect of CuCs with different concentrations on date palm seedlings. From the data shown, it was clear that CuCs can significantly enhance the immune responses of date palm seedlings to a great extent. Data express the average over triplicates selected randomly from each group of seedlings.

Table.2: Positive immunomodulatory effect of CuCs on date palm seedlings

Parameter	**Concentration of CuCs (g/l)**				
	0.00 Control	**0.50**	**1.00**	**1.50**	**2.00**
Total phenols *(mg/gdw)*	2.70	2.92	3.29	3.62	4.05
Phenoloxidase (OD units x10^3/min/gdw)	31.4	33.83	40.22	47.10	62.73
Peroxidase (ΔOD x10^3/min/gdw)	40.33	66.03	96.52	109.85	121.53

3.7. Evaluation of *In vivo* Efficiency of Copper-Chitosan Nanocomposition in Treating Fusarium Wilted-Date Palm Seedlings In Comparison With Rizolex^{tm}

Table (3) shows the summary of disease severity (DS) of the inoculated seedlings, which were treated with different concentrations of CuCs in comparison with those treated with water (-ve control) and with Rizolex^{TM} (+ve control).

Statistical analysis showed that the disease severity of the inoculated date palm seedlings treated with different concentrations of the nanocomposition was significantly lower than that of the inoculated date palm seedlings which were treated with **Rizolex^{TM}** (+ve Control) and water (-ve Control), except the concentration of 0.50 g/l.

Table.3: DS of the inoculated date palm seedlings treated with nanocomposition, Rizolex^{TM} and water.

	Composition concentrations (g/l)				**Rizolex^{TM}** (3.00 g/l) +ve Control	Water -ve Control
	0.50	**1.00**	**1.50**	**2.00**		
DS	70.67	58.33	46.67	34.33	69.33	84.33

IV. CONCLUSION

To this point, it was clear that copper-chitosan nanocomposition may provide a competitive candidate for treating vascular wilt disease in date palm, which clearly manifested significantly higher efficiency than the commercially available fungicide at lower concentrations. This can be interpreted by virtue of two main axes:

Firstly, the positive immunomodulatory effect of copper-chitosan nanocomposition on date palm seedlings as shown from its ability to increase peroxidase, phenoloxidase and total phenols levels, which were considered main constitutes of the plant innate immune response against the invading fungal pathogen;

Secondly, the antifungal effect of the nanocomposition on the fungal pathogen itself.

Hence, this copper – chitosan nanopcomposition with its dual complementary functionality may provide a potential approach to beleaguer the fusarium wilt disease in date palm via enhancing the plant immune responses at the same time of inhibiting the fungal growth. Thus, this nanocomposition can be used in developing new nano-fungicides to control such pathogens. But, further research should be undertaken in order to investigate its potential toxicity on plant, human and environment; Thus, determining the optimal concentration that can be used in field without considerable toxicity consequences.

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Growth Performance, Shank Pigmentation and Blood Profile of Broiler Chickens Fed Neem Leaf Meal-Based Diets

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***Abstract*—** *The many benefits of all parts of neem (Azadirachta indica) tree are well documented. Thus, this study was done to ascertain the effects of sundried neem leaf meal (NLM) on growth performance, shank pigmentation and blood profile of broiler chickens. One hundred and forty four day-old chicks were randomly distributed after 1 week pre-experimental period to four experimental diets which comprised NLM at 0% (control), 5% (5NLM), 10% (10NLM) and 15% (15NLM). A completely randomized design was adopted with 12 birds per replicate and 3 replicates per treatment. Feed and water were given ad-libitum and other management practices were carried out. Initial weight of chicks and final weights at the end of the starter and finisher phases were taken. Feed intake was measured weekly and feed conversion ratio (FCR) calculated. Shank pigmentation was assessed from 3 birds/ replicate at the end of the trial. Blood samples were collected from 3 birds/ replicate at the end of the starter and finisher phases. For the starter phase, final weight, total weight gain and FCR were significantly influenced by NLM inclusion. Chicks fed control and 5NLM diets had similar higher values than others. Blood parameters were not significant except basophil values. At the finisher phase, total weight gain and FCR were not significantly different although feed intake significantly reduced with inclusion of NLM in the diets. Blood parameters were not significant except lymphocyte values. There was a non-significant increase in shank pigmentation with increase in NLM inclusion. It is concluded that NLM inclusion in broiler diets at both phases should not exceed 5% based on growth performance. Neem leaf meal increased yellow colouration of shank and was not harmful to broiler blood parameters.*

***Keywords*—Neem leaves, Natural colourants, Broiler production, Poultry feed.**

I. INTRODUCTION

The poultry industry has great potentials that could aid the improvement of the health status of people through the provision of animal protein (Adeniji, 2005) and also help alleviate poverty (Bloom and Canning, 2000). Broiler chickens in particular are good converters of feed to meat hence their fast growth and they have wide acceptability by people all over the world.

One main constraint to poultry production mostly in developing countries is the high cost of production of which the cost of finished feed contributes significantly. According to Adebayo and Adeola (2005), high cost and shortage of feed ingredients needed by chickens is a major obstruction to the expansion of poultry production. Thus, research on the use of alternative feed ingredients which would limit the inclusion of expensive ones, which are also in stiff competition with man, is essential. High cost of finished feed would translate to high total cost of production and finally to high cost of sales of broiler chicken/ meat.

Neem leaf meal has been tested as an alternative feed ingredient in poultry production. The neem tree is abundant in Nigeria because it is used to prevent deforestation (Onyimonyi *et al.*, 2009) due to its ability to tolerate drought. Neem leaves contain about 22.37% crude protein, 14.30% crude fibre and 7.10% ash. The results reported by the various authors regarding the optimum level of neem leaf meal inclusion in broiler-chicken diets are not consistent. Onyimonyi *et al.* (2009), Obikaonu (2012) and Obun*et al.* (2013) reported 0.5, 5 and 15% respectively for broiler finishers. Thus, thisstudy was carried out to determine the optimum inclusion level of neem leaf meal in broiler-chicken diets using growth performance, shank pigmentation and blood profile as response criteria.

II. MATERIALS AND METHODS

2.1 Study site

The study was conducted at Poultry Unit of the Teaching and Research Farm and Animal Production and Health Department laboratories of the Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Test ingredient and experimental birds

Neem leaves were harvested fresh from Akoko, Ondo State and sun dried for about 3 days when it became crispy. The dried leaves were then milled and stored properly prior to diet formulation. One hundred and forty four day-old broiler chicks (Marshallbreed) were purchased from a reliable hatchery and placed on experimental diets after a one-week pre-experimental period during which they were fed commercial starter diet. Four diets were formulated to contain neem leaf meal at varying levels at both starter and finisher phases. Control diet was without neem leaf meal (0%); 5NLM, 10NLM and 15NLM contained neem leaf meal at 5, 10 and 15%, respectively. Composition of starter and finisher diets are shown in Table 1. The birds were randomly distributed to the diet groups in a completely randomized design. There were three replicates per treatment and twelve birds per replicate. The feeding trial lasted 7 weeks during which birds were managed intensively.Feed and potable water were provided *ad-libitum*.

2.3 Data collection and analysis

Initial weight was taken at the start of the experiment and final weight for each phase was taken at the end of the respective phase. Feed intake was measured weekly and feed conversion ratio calculated as ratio of feed intake to weight gain. Shank pigmentation was assessed from three birds per replicate at the end of the finisher phase by a 10-member panel using the following keys:

5- Extremely pigmented
4- Highly pigmented
3- Moderately pigmented
2- Slightly pigmented
1- Not pigmented

Blood samples were also collected from three birds per replicate at the end of starter and finisher phases respectively. Samples wereanalyzed for packed cell volume, red blood cell count, haemoglobin concentration, erythrocyte sedimentation rate, heterophil, basophil and eosinophil according to Lamb(1981). Mean cell haemoglobin concentration, mean cell haemoglobin and mean cell volume were calculated appropriately from packed cell volume, red blood cell count and haemoglobin concentration values. Data collected were subjected to one way analysis of variance using the Minitab statistical package (v 17) and means were separated using Tukey test of the same package.

III. RESULTS

3.1 Growth performance

The growth performance of broiler chicks fed neem leaf meal based diets is shown in Table 2. Final live weight (g/bird) of the chicks was significantly ($P<0.05$) different. Birds on control diet had the highest value which did not differ significantly ($P>0.05$) from that of birds fed 5NLM. Total weight gain followed same trend as final weight. Birds fed 10NLM and 15NLM had weight gain which differed significantly ($P<0.05$) from birds fed control and 5NLM diets. A progressive decrease was observed in weight gain with increasing inclusion of NLM in the diets. No significant ($P>0.05$) difference was found in total feed intake (g/bird) of the broiler chicks at all levels of inclusion. Values calculated as the feed conversion ratio were significantly ($P<0.05$) different across dietary treatments. Broiler chicks on control had the lowest feed conversion ratio (1.86) while those on 15NLM had the highest value (3.51).

As shown in Table 3, the initial live weight of the broiler-chicken finishers was a reflection of the effect of NLM at the starter phase. Final live weights of the birds (kg/bird) was significant ($P<0.05$), birds on control diet had the highest final weight, values decreased with increasing level of NLM. Birds that were fed 10NLM and 15NLM had same final weight.Total weight gain, although numerically lower in NLM diets was not significantly ($P>0.05$) different. Total feed intake was significantly ($P<0.05$) influenced by dietary NLM inclusion. Birds on control diet ate more, although the values had slight reductions. Feed conversion ratio was statistically similar ($P>0.05$) across dietary treatments.

3.2 Shank pigmentation

Shank pigmentation of the broiler-chicken finishers fed neem leaf meal based diets is presented in Table 4.The values show a non-significant ($P>0.05$) increase in pigmentation as level of NLM increased in the diets. Values increased from 1.67 in control chickens to 3.07 in those fed 15NLM diet.

3.3 Blood profile

All parameters recorded for blood profile of broiler chicks fed diets containing neem leaf meal (Table 5) viz; packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV), lymphocyte, heterophil, and eosinophil were not significantly ($P>0.05$)different except basophil, whose values did not follow any particular trend in relation to dietary treatments.

The blood profile of broiler-chicken finishers fed NLM as presented in Table 6 shows that PCV, RBC, Hb, ESR, MCHC, MCH, MCV, heterophil, basophil and eosinophil were not significantly($P>0.05$)influenced by NLM inclusion. The lymphocyte was however significantly ($P<0.05$) different, control had the lowest value.

IV. DISCUSSION

The decreasing trend observed in total weight gain of broiler chicks in this study is in agreement with Obikaonu

(2012) who reported this trend in weight gain by broiler chickens. The non-significance in feed intake is contrary to the report of Obikaonu (2012) in which a significant increase was found in feed intake of broilers as levels of NLM increased. Although feed intake was not significant in this study, chicks fed NLM diets had higher values than control and the value for chicks fed 15NLM dropped compared to other NLM groups. This decrease at 15% may be attributed to the bitter taste (Onyimonyi *et al*., 2009) of NLM or the possible reduction in aesthetic value of the diet to the birds due to its darker colour caused by the quantity of NLM included.Onyimonyi *et al*. (2009) also reported lower feed intake with the highest level of NLM used although their level of inclusion was lower than levels used in this study. Feed conversion ratio of broiler chicks increased with increasing level of NLM, this depicts that efficient utilization of feed is reduced with higher NLM inclusion. This might be due to possible interference between phytochemicals such as tannin and nutrient availability (Nworgu *et al*., 2014; Liaqat*et al*., 2016) which is characteristic to some leaf meals.

For the finisher chickens, the non-significance observed in weight gain is contrary to Obikaonu (2012) who reported a significant decrease in weight gain of broiler finishers. Although, the non-significant weight gain here was highest for control. In line with this same author, significant difference was observed in feed intake but decrease with NLM inclusion was recorded in this study as opposed to increase in feed intake with NLM inclusion there. The decrease in feed intake recorded in this study could be as deduced earlier for broiler chicks. It could also suggest that older birds have a higher perception of bitter taste. It appears that more feed was needed per kg weight gain at the starter phase than finisher phase. And it was more pronounced for birds on the control diet.

According to Blair (2008), there are variations in consumers' choice of broiler skin colour. While some like broilers to be yellow skinned, others prefer it as white. Sirri*et al*. (2010) suggested that evaluation of broiler pigmentation or yellowness could be ascertained on any part of the chicken's body, which means colour of broiler skin could be deduced from results of shank pigmentation. Onibi *et al*. (2008) reported that shanks better depicts intensity of pigmentation in broiler chickens. In this study, there was an increase in shank pigmentation with increase in NLM inclusion although it was not significant. Joseph (2016) also observed an increase in yellowish colour of cockerel shanks when NLM was included in their diets. Olabode (2015) also observed a deeper egg yolk colour with increase in NLM inclusion. So, NLM may be used as a natural colourant for chicken products.

According to Obikaonu *et al*. (2011), there were significant differences in haemoglobin (Hb), erythrocyte sedimentation rate (ESR) and packed cell volume (PCV), although the values reported did not follow any particular trend but were rather undulating with NLM inclusion. These authors also reported that eosinophils and basophils were not detected in their experiment. However, values obtained in this study for the main anaemia pointers; Hb and PCV (Turksonand Ganyo, 2015) fell within the acceptable range (Mitruka and Rownsley, 1977; Ross *et al*., 1978). At the finisher phase, there was no significance in the blood parameters except lymphocytes and it appeared that PCV, RBC and Hbhad slightly higher values with increase in NLM addition.Esonu*et al*. (2016) reported a significant increase in PCV values of laying chickens fed NLM up to 10%but Obun*et al*. (2013) reported a significant decrease in PCV values of broiler finishers and other blood parameters were not significant. Lymphocyte values, although significant fell within normal range. According to Etim*et al*. (2014), factors such as age, breed, sex and management practices could affect blood parameters of animals and thus may have caused the variability in results from these studies.

V. CONCLUSION

Based on growth performance, neem leaf meal inclusion in broiler-chicken starter and finisher diets should not exceed 5%. Neem leaf meal increased the yellow pigmentation of shanks and was not deleterious on their blood parameters.

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Table.1: Composition of experimental diets

Ingredients (kg)	**Starter Phase**				**Finisher Phase**			
	Control	**5%**	**10%**	**15%**	**Control**	**5%**	**10%**	**15%**
Maize	53.00	50.00	46.00	42.10	52.50	51.30	48.20	44.50
NLM	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Wheat offal	8.80	6.80	6.80	6.80	13.00	8.00	6.50	6.00
Soyabean meal	16.00	16.00	15.00	15.00	15.00	15.00	14.60	14.10
Groundnut cake	16.00	16.00	16.00	14.90	13.90	15.10	15.10	14.80
Fish meal (72%)	3.00	3.00	3.00	3.00	0.00	0.00	0.00	0.00
Vegetable oil	0.00	0.00	0.00	0.00	2.00	2.00	2.00	2.00
Bone meal	2.10	2.10	2.10	2.10	2.40	2.40	2.40	2.40
Limestone	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100	100	100
Calculated analysis								

Metabolizable energy (Kcal/Kg)	2923.72	2970.48	2993.33	3017.56	2989.62	3073.74	3115.62	3144.99
Crude protein (%)	22.57	23.08	23.42	23.69	19.79	20.48	20.89	21.24
Crude fibre (%)	3.68	4.16	4.73	5.32	3.83	4.15	4.65	5.20
Ether extract (%)	4.08	4.15	4.21	4.24	4.11	4.22	4.28	4.33
Calcium (%)	1.11	1.16	1.20	1.25	1.03	1.08	1.13	1.18
Phosphorus (%)	0.54	0.61	0.68	0.75	0.50	0.57	0.63	0.70

NLM= Neem Leaf Meal

Table.2: Growth performance of broiler chicks fed diets containing neem leaf meal

Diets	**Level of inclusion (%)**	**Initial weight (g/bird)**	**Final weight (g/bird)**	**Total weight gain (g/bird)**	**Total feed intake (g/bird)**	**Feed conversion ratio**
Control	0	131.94	843.40^{a}	711.40^{a}	1318.50	1.86^{a}
5NLM	5	137.50	783.40^{a}	645.90^{a}	1479.90	2.29^{ab}
10NLM	10	137.50	655.30^{b}	517.80^{b}	1425.10	2.75^{b}
15NLM	15	136.11	526.30^{c}	390.20^{c}	1354.20	3.51^{c}
Pooled standard deviation		5.77	42.24	44.45	62.78	0.23

^{abc}Means with different superscripts along the same column are significantly different (P<0.05)
NLM- Neem leaf meal

Table.3: Growth performance of broiler-chicken finishers fed diets containing neem leaf meal

Diets	**Level of inclusion (%)**	**Initial weight (kg/bird)**	**Final weight (kg/bird)**	**Total weight gain (kg/bird)**	**Total feed intake (kg/bird)**	**Feed conversion ratio**
Control	0	1.04^{a}	2.36^{a}	1.32	3.68^{a}	2.79
5NLM	5	0.93^{ab}	2.10^{ab}	1.16	3.22^{ab}	2.79
10NLM	10	0.75^{bc}	1.70^{b}	0.95	3.13^{b}	3.33
15NLM	15	0.60^{c}	1.70^{b}	1.11	3.15^{b}	3.03
Pooled standard deviation		0.08	0.21	0.23	0.20	0.42

^{abc}Means with different superscripts along the same column are significantly different (P<0.05)
NLM- Neem leaf meal

Table.4: Shank pigmentation of broiler-chicken finishers fed neem leaf meal

Diets	**Level of inclusion**	**Shank pigmentation**
Control	0	1.67
5NLM	5	2.20
10NLM	10	2.67
15NLM	15	3.07
Pooled standard deviation		0.99

NLM- Neem leaf meal

Table.5: Blood profile of broiler chicks fed diets containing neem leaf meal

Diets	Level of inclusion (%)	PCV (%)	RBC (10^6mm^3)	Hb(g/100ml)	ESR (mm/hr)	MCHC (%)	MCH (pg)	MCV (μ^3)	Lymphocyte (%)	Heterophil (%)	Basophil (%)	Eosinophil (%)
Control	0	29.63	2.72	9.89	3.50	33.37	36.61	109.70	62.00	20.75	2.38^{ab}	1.38
5NLM	5	29.22	2.67	9.72	3.67	33.27	36.73	110.39	61.44	23.11	2.00^{b}	1.78
10NLM	10	27.63	2.42	9.21	4.63	33.35	38.13	114.33	61.75	21.75	2.75^{a}	1.50
15NLM	15	28.44	2.54	9.48	4.33	33.32	37.48	112.48	62.00	21.44	2.56^{a}	1.33
Pooled standard deviation		2.32	0.35	0.77	1.91	0.09	1.82	5.41	2.28	2.03	0.51	0.50

^{ab}Means with different superscripts along the same column are significantly different (P<0.05)

PCV- Packed cell volume; RBC- Red blood cell count;Hb- Haemoglobin concentration; ESR- Erythrocyte sedimentation rate; MCHC- Mean cell haemoglobin concentration; MCH- Mean cell haemoglobin; MCV- Mean cell volume; NLM- Neem leaf meal

Table.6: Blood profile of broiler-chicken finishers fed diets containing neem leaf meal

Diets	Level of inclusion (%)	PCV (%)	RBC (10^6mm^3)	Hb(g/100ml)	ESR (mm/hr)	MCHC (%)	MCH (pg)	MCV (μ^3)	Lymphocyte (%)	Heterophil (%)	Basophil (%)	Eosinophil (%)
Control	0	27.13	2.24	9.05	5.00	33.36	41.65	124.82	59.38^{b}	23.13	2.63	1.25
5NLM	5	28.44	2.41	9.48	3.00	33.32	39.83	119.54	62.11^{ab}	21.89	2.44	1.56
10NLM	10	28.63	2.45	9.53	2.88	33.27	39.27	118.03	62.75^{a}	21.63	2.25	1.38
15NLM	15	29.22	2.56	9.73	3.22	33.31	38.91	116.82	61.89^{ab}	22.78	2.67	1.56
Pooled standard deviation		2.57	0.47	0.86	1.74	0.10	4.51	13.56	2.27	2.18	0.50	0.63

^{ab}Means with different superscripts along the same column are significantly different (P<0.05)

PCV- Packed cell volume; RBC- Red blood cell count; Hb- Haemoglobin concentration; ESR- Erythrocyte sedimentation rate; MCHC- Mean cell haemoglobin concentration; MCH- Mean cell haemoglobin; MCV- Mean cell volume; NLM- Neem leaf meal

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The Effect of Different Irrigation Systems, Discharge, and Irrigation Intervals on Green Onion Growth and Yields

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Abstract**—** *An experiment was conducted in the field of the University of Baghdad in spring season of 2015 In loamy sand soil to evaluate the effect of different irrigation systems, discharge and irrigation intervals on green onion growth and yield, using locally combine implement which used for installation subsurface irrigation pipes and surface drip irrigation. Subsurface drip irrigation system and surface drip irrigation system as main plot treatment, three levels of discharge included 2.5, 3.0 and3.5 L\hr represented subplot treatment and two levels of irrigation intervals included: 4 days and 8 days represented sub subplot treatment were used in this study. Root diameter, stem diameter, green weight, dry weight, and stem length were measured. Nested design under randomized complete block design (CRBD) with three replications was used in this experiment. Least significant differences (L.S.D) at 0.05 levels were used to compare the mean of treatments.*

The results were showed the following:

Subsurface irrigation was superior in getting higher 78.6 gm dry weight and 232.2 gm green weight and 31.8 mm stem diameter and root diameter stood 45.2 mm and stem length stood 55.3 cm comparing with other treatments. 3.5 L/h discharge was superior in getting the highest 81.8 gm dry weight, 249.4 gm green weight, stem length stood 70.8 cm, root diameter stood 45.4 mm and stem diameter stood 32.6 mm comparing with other treatments. 4 days irrigation interval was superior in getting higher 80.2 gm dry weight, 231.4 gm green weight, root diameter stood 46.3 mm, stem diameter stood 32.7 mm and stem length stood 55.3 cm comparing with other treatments. Triple interaction between subsurface irrigation system, 3.5 L / h discharge and4 days irrigation interval was superior in getting the highest dry weight stood 86.4 gm, green weight stood 252.3 gm, stem length stood 78 cm, root diameter stood 50.6 mm and stem diameter stood 35.3 mm comparing with other interactions treatments.

Keyword—root diameter, stem diameter, green weight, surface drip irrigation system, drip irrigation.

I. INTRODUCTION

Agricultural mechanization is defined as the performance of various agricultural processes such as plowing , sowing, fertilization, modification, settlement and operation of fixed irrigation pumps by agricultural machinery and implements. Tractors is the main source of the farms movement power which are used to pull various agricultural implements, (Kepner et al 1972). Mechanization is of great importance in carrying out agricultural operation, reducing manpower and production unit costs and increasing agricultural production of the land unit (Izat and Ali ,1986). Mechanization involves the identification or evaluation of various agricultural implements ,especially the combine implement , which are used to increase agricultural production and reducing the costs of agricultural operation in general (Frank, et al, 2012). Combine implement which is a group of important implements that have been published recently because they perform the work of one field by short passage of time and cost, creating good germination condition, reducing tillage and protecting the soil from compression (Camp, et al, 1988).The scarcity of irrigation water is an effective factor in reducing the cultivated areas in the world. Therefore, researchers and farmers resorted to the development and use of new systems to reduce waste in water , considering that the old irrigation systems are accompanied by large water losses with the current acute shortage of water supply in general and irrigation water especially .One of the most efficiency ways to conserve water is surface drip irrigation and subsurface drip irrigation . Kumar et al, (2007) and Enciso et al,(2009) where found that irrigation has a significant effect on onion and yield components. Drip irrigation is a slow and frequent addition of water to the soil through the picks of the picks drawn along the water delivery pipes (Al-Taie, 1999). The advantage of drip irrigation system are the efficiency of water use , as the evaporation is little ,especially in the subsurface drip irrigation system , these type of irrigation systems are also ways of reducing the growth of the bushes of the crops service operations as

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well as obtaining higher yield and quality of relatively low costs and can be add fertilizers with irrigation water (Al-taief and Alhadithi , 1988).Green onion is a winter vegetable crops of high nutritional value for its vitamins , carbohydrates and mineral salts and is used for medicinal purposes as well (Morsia and Nimet , 1973).

The purpose of this study is to evaluate the effect of subsurface drip and surface drip irrigation systems and different irrigation discharges and intervals on green onions growth and yields.

II. MATERIALS AND METHODS

The experiment was conducted to evaluate the effect of different irrigation systems, discharge and irrigation intervals on green onion growth and yield in the field of the University of Baghdad in spring season of 2015 In loamy sand soil (Tble,1). Locally combine implement (fig. 1) which used for installation subsurface irrigation pipes and surface drip irrigation was used this experiment. Subsurface drip irrigation system and surface drip irrigation system as main plot treatment, three levels of discharge included 2.5, 3.0 and3.5 L\hr represented subplot treatment and two levels of irrigation intervals included: 4 days and 8 days represented sub subplot treatment were used in this study. Root diameter, stem diameter, green weight, dry weight, and stem length were measured. Nested design under randomized complete block design (CRBD) with three replications was used in this experiment. Least significant differences (L.S.D) at 0.05 levels were used to compare the mean of treatments.

Table.1: Soil physical and chemical properties

Property	Values	Unites
Soil bulk density Soil EC PH Capron	1.3 1.1 **7.25** **619**	**Mg | m^3** **Ds| cm** **Mg | l**
Soil Texture	Loamy sand	
Soil analysis components	Sand Silt Clay	**77.6%** **12.5%** 9.9%

Fig.1: Combine implement used in instslation irrigation pipes

Studed properties

1- Root diameter (mm) -

Ten plants from the middle lines were selected and the root diameter for each plant was measured by vernier .

2- Stem daimeter (mm) -

The stem diameter of ten plants(top of the root and lower stem) was measured from the selected middle lines by vernier .

3- Stem length (cm) -

The length of the stem was measured from the previously selected plants .

4- Green weight (gm) -

The green weight of the plant was measured from the intermediate lines of the previously selected plants by the sensitive balance of each plant including the total of green onions .

5- Dry weight (gm) -

The dry weight of the plant was measured from the intermediate lines of the previously selected plants and placed in perforated bags for each plant and placed in the oven for 24 hours at a temperature of 105 CO. The measurment was taken for more than once and after the weight was established the final measurements were taken by sensitive balance for each plant

III. RESULTS AND DISCUSSION

Root diameter (mm):

Table (2) shows the effect of irrigation systems and different discharges and irrigation intervals on the root diameter of the green onion plant. Subsurface drip irrigation system was superior in getting higher root diameter stood 45.2 mm while root diameter in the surface drip irrigation system got 43.4 mm. 3.5 L / h discharge got the highest value of root diameter amounted 45.4 mm and the 4 days irrigation interval got higher root diameter amounted 46.3 mm comparing with other treatments. The triple interaction between subsurface drip

irrigation system and 3.5 L / h discharge and the 4 days Irrigation interval got the highest root diameter amounted 50.6 mm compared with the interaction between surface drip irrigation system 2.5 L / h discharge and 8 days irrigation interval reached 38.7 mm. The reason may be retention soil moisture in the subsurface irrigation system compared to the evaporation of irrigation water from the soil of the surface drip irrigation system. These results are consistent with the results reached by Khalil, (2013).

Table.2: Effect irrigation systems and different irrigation discharge and interval in root diameter (mm)

Indicators	Root Diameter (mm)			
	Interaction between Irrigation Systems and Discharge and Irrigation Intervals			
Irrigation Systems	Discharge	Irrigation Intervals		Interaction between Irrigation systems and Discharge
		4 days	8 days	
Subsurface Drip Irrigation	2.5	44.2	42.6	42.4
	3.0	48.1	42.9	45.5
	3.5	50.6	43.4	47
Surface Drip irrigation	2.5	44.3	38.7	41.5
	3.0	44.6	44.3	44.4
	3.5	46.5	41.2	43.8
Lsd = 0.05	0.86			
Irrigation Interval Medium	46.3		42.1	
Lsd =0.05	0.44			
Discharge	Interaction between Irrigation Discharge and Intervals			Discharge Medium
2.5	44.3		40.6	42.4
3.0	46.3		43.6	44.9
3.5	48.5		42.3	45.4
Lsd = 0.05	0.42			
Irrigation systems	Interaction between Irrigation Systems and Intervals			Irrigation Systems Medium
Subsurface Drip Irrigation	47.6		42.9	45.2
Surface Drip Irrigation	45.5		41.4	43.4
Lsd = 0.05	0.53			

Stem diameter (mm):

Table (3) shows the effect of irrigation systems and different discharges and irrigation intervals on the stem diameter of the green onion plant. Subsurface drip irrigation system was superior in getting higher stem diameter stood 31.8 mm while root diameter in the surface drip irrigation system got 31.2 mm. 3.5 L / h discharge got the highest value of stem diameter amounted 32.6 mm and the 4 days irrigation interval got higher stem diameter amounted 32.7 mm comparing with other treatments. The triple interaction between subsurface drip irrigation system and 3.5 L / h discharge and the 4 days Irrigation interval got the highest stem diameter amounted 35.3 mm compared with the interaction between surface drip irrigation system 3.0 L / h discharge and 8 days irrigation interval which reached 29.1 mm. The reason may be small number of days between irrigation intervals which caused increase in soil water storage. These results are consistent with the results which reached by Ati, (2014).

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Table.3: The effect of irrigation systems and different irrigation discharge and interval on stem diameter, mm.

Indicators	Stem diameter, (mm)			
Irrigation Systems	Interaction between Irrigation Systems and Irrigation Discharge and Intervals			Interaction between Irrigation Systems and Discharge
	Discharge	Irrigation Intervals		
		4 days	8 days	
Subsurface Drip irrigation	2.5	30.9	29.3	30.1
	3.0	33.4	31.1	32.2
	3.5	35.3	30.8	33.05
Surface Drip irrigation	2.5	31.7	30.5	31.1
	3.0	32.2	29.1	30.6
	3.5	32.7	31.6	32.1
Lsd = 0.05	0.094			
Medium Irrigation Intervals	32.7		30.4	
Lsd =0.05	0.73			
Discharge	Interaction between Irrigation Discharge and Intervals			Medium Discharge
2.5	31.5		29.9	30.7
3.0	32.8		30.1	31.4
3.5	34.0		31.2	32.6
Lsd = 0.05	0.78			
Irrigation Systems	Interaction between Irrigation Systems and Intervals			Medium Irrigation Systems
Subsurface Drip irrigation	33.2		30.4	31.8
Surface Drip Irrigation	32.2		30.3	31.2
Lsd = 0.05	0.26			

Stem length (cm):

Table (3) shows the effect of irrigation systems and different discharges and irrigation intervals on the stem length of the green onion plant. Subsurface drip irrigation system was superior in getting higher stem length stood 64.7 cm while stem length in the surface drip irrigation system got 60.0 cm. 3.5 L / h discharge got the highest value of stem length amounted 70.8 cm and the 4 days irrigation interval got higher stem length amounted 55.3 cm comparing with other treatments. The triple interaction between subsurface drip irrigation system and 3.5 L / h discharge and the 4 days Irrigation interval got the highest stem length amounted 78.0 cm compared with the interaction between surface drip irrigation system, 2.5 L / h discharge and 8 days irrigation interval which reached 56.0 cm. The reason may be small number of days between irrigation intervals and the retention of soil moisture in the subsurface irrigation system compared to the evaporation of irrigation water from the soil of the surface drip irrigation system. These results are consistent with the results reached by Ati, (2014).

Table.4: The effect of irrigation systems and different irrigation discharge and intervals on stem length cm.

Indicators	Stem length (cm)			
Irrigation Systems	Interaction between Irrigation Systems, and irrigation Discharge and Intervals			Interaction between Irrigation Systems and Discharge
Irrigation systems	Discharge	irrigation intervals		
		4 days	8 days	

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Subsurface Drip Irrigation	2.5	61	58	59.5
	3.0	66	60.5	63.2
	3.5	78	65	71.5
Surface Drip Irrigation	2.5	56.2	56	56.1
	3.0	59.5	57.3	58.4
	3.5	66	62.4	64.2
Lsd = 0.05		0.86		
Irrigation Intervals Medium		55.3	51.2	
Lsd =0.05		0.99		
Discharge	Interaction between Irrigation Discharge and Intervals			Medium Discharge
2.5		58.6	57	57.8
3.0		62.7	58.9	60.8
3.5		72	69.7	70.8
Lsd = 0.05	0.42			1.01
Irrigation Systems	Interaction between Irrigation systems and Intervals			Medium Irrigation Systems
Subsurface Drip Irrigation		68.3	61.1	64.7
Surface Drip Irrigation		60.5	59.5	60.0
Lsd = 0.05		1.23		0.98

Dry weight (gm) :

Table (5) shows the effect of irrigation systems and different discharges and irrigation intervals on the dry weight of the green onion plant. Subsurface drip irrigation system was superior in getting higher dry weight stood 78.6 gm/plant while dry weight in the surface drip irrigation system got 74.1 gm/plant. 3.5 L / h discharge got the highest value of dry weight amounted 81.8 gm/plant and the 4 days irrigation interval got higher dry weight amounted 80.2 gm/plant comparing with other treatments. The triple interaction between subsurface drip irrigation system and 3.5 L / h discharge and the 4 days Irrigation interval got the highest dry weight amounted 86.4 gm/plant compared with the interaction between surface drip irrigation system, 2.5 L / h discharge and 8 days irrigation interval which reached 62.0 gm/plant. The reason may be the diameter of root ,and stem diameter and length. These results are consistent with the results reached by Khalil, (2013).

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Table.5: The effect of irrigation systems and different irrigation discharge and intervals on dry weight (gm /plant)

Indicators	Dry Weight (gm/plant)		
	Interaction between Irrigation Systems and Irrigation Discharge and Intervals		
Irrigation Systems	Discharge	Irrigation Intervals	Interaction between Irrigation Systems and Discharge
		4 days	8 days
Subsurface Drip Irrigation	2.5	73.8	73.1	73.4
	3.0	85.2	76.2	80.7
	3.5	86.4	77.4	81.9
Surface Drip Irrigation	2.5	68	62	65
	3.0	82.7	68.2	75.4
	3.5	85.2	78.5	81.8
Lsd = 0.05	1.79			
Irrigation Interval Medium	80.2	72.5		
Lsd =0.05	1.22			
Discharge	Interaction between discharge and irrigation interval		Discharge Medium	
2.5	70.9	67.5	69.2	
3.0	83.9	72.7	78.3	
3.5	85.8	77.9	81.8	
Lsd = 0.05	1.86		1.99	
Irrigation Systems	Interaction between Irrigation Systems and Irrigation Intervals		Irrigation Systems Medium	
Subsurface Drip Irrigation	81.8	75.5	78.6	
Surface Drip Irrigation	78.6	69.5	74.05	
Lsd = 0.05	0.98		1.09	

Green weight (gm/plant):

Table (6) shows the effect of irrigation systems and different discharges and irrigation intervals on the dry weight of the green onion plant. Subsurface drip irrigation system was superior in getting higher green weight stood 232.3 gm/plant while green weight in the surface drip irrigation system got 206.8 gm/plant. 3.5 L / h discharge got the highest value of green weight amounted 249.4 gm/plant and the 4 days irrigation interval got higher green weight amounted 231.4 gm/plant comparing with other treatments. The triple interaction between subsurface drip irrigation system and 3.5 L / h discharge and the 4 days Irrigation interval got the highest green weight amounted 252.3 gm/plant compared with the interaction between surface drip irrigation system, 2.5 L / h discharge and 8 days irrigation interval which reached 158.1 gm/plant. The reason may be the diameter of root ,and stem diameter and length. These results are consistent with the results reached by Khalil, (2013).

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Table.6: The effect of irrigation systems and different irrigation discharge and intervals on green weight, gm/plant.

Indicators	Green weight (gm/plant)			
	Interaction between Irrigation Systems and Discharge and Irrigation Intervals			
Irrigation Systems	Discharge	Irrigation Intervals		Interaction between Irrigation Systems and Discharge
		4 days	8 days	
Subsurface Drip Irrigation	2.5	228.2	214.3	221.2
	3.0	242.0	199.9	220.9
	3.5	252.3	259.1	255.7
Surface Drip Irrigation	*2.5*	*195.9*	*158.1*	*177.0*
	3.0	227.0	174.1	200.5
	3.5	243.3	243.1	243.2
Lsd = 0.05		0.86		
Irrigation interval Medium		231.4	208.1	
Lsd =0.05	3.61			
Discharge	Interaction between discharge and irrigation interval			Discharge Medium
2.5		212.0	186.2	199.1
3.0		234.5	187.0	210.7
3.5		247.8	251.1	249.4
Lsd = 0.05		3.05		3.13
Irrigation Systems	Interaction between irrigation systems and irrigation interval			Irrigation systems Medium
Subsurface Drip Irrigation		240.0	224.4	232.2
Surface Drip Irrigation		222.0	191.7	206.8
Lsd = 0.05		2.68		2.01

IV. CONCLUSIONS AND ACOMMENDATION

From the above results, subsuarface irrigation system was superior in getting higher dry weight , green weight , root daimeter and stem daimeter and stem length . 3.5 L/h discharge was superiority in getting dry weight , green weight , root daimeter , stem detemer,stem length . 4 days irrigation intervals was superior in getting higher dry weight , green weight , root daimeter and stem daimeter and stem length. Triple interaction between subsarface drip irrigation system and discharge 3.5 L /h and first irrigation interval 4 days was superior in getting the highest dry weight , green weight , stem daimeter,root diameter , stem lenght .

Therefore, using subsarface drip irrigation system, discharge 3.5 L/h and first irrigation interval 4 days in cultivation green onion is acommend.

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Evaluation of soybean lines for resistance to rust *(phakopsorapachyrhizi)*

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Abstract*—Among the diseases that can reduce soybean production is rust disease caused by the fungus Phakopsorapachyrhizi. The aim is to evaluate the resistance of soybean genotypes to rust disease and to study there interactions between agronomic traits. The study was conducted at field and screen house in the University of Agriculture Makurdibetween julyto november2016. A total of 10 soybean genotypes were evaluated for resistance to rust disease. After inoculation in the laboratory, three lines TGX-1835-10E, TGX-1987-10F and TGX1945-4F showed a consistent moderate resistance to Phakopsorapachyrhizi. These soybeanGenotypeowered earlier and had the highest seed yield per plant (799.51kg/ha, 766.75K g/ha and 742.63 respectively). In contrast, the lines TGX-1949-10F and TGX-1485-1D which is the control , flowered at about 43 days after planting, had seed yield per plant of (404.30 and 254.23kg/ha, respectively),these lines had significantly lower yield and susceptible to rust. In the field, four lines had seed weight per plant significantly heavier than TGX-1949-10F and TGX-1485-1D, namely TGX-1835-10E, TGX-1987-10F, TGX-1904-6F and TGX-1945-4E and using a polygon view, the best performing lines were visualized as TGX-1987-10F was best in Environments one and TGX-1835-10E in two.Base on average environment coordination (AEC) procedure, TGX-1945-1F and TGX-1945-4E had yields above the grand means and stable while TGX-1945-4F and TGX-1935-3F were identified with high but unstable yield, the soybean lines with heavier seed weight per plant should potentially serve as genetic material to develop high yielding soybean varieties and resistant to rust disease.*

Keywords*— *Genotype, rust resistance, yield component, grain yield, genotype x environment Interaction (GEI).

I. INTRODUCTION

Soybean rust, caused by *Phakopsorapachyrhizi*, is a major disease limiting soybean production and has caused significant economic annual yield loss of up to 60 to 80% been reported in the eastern and southern parts of the country Levyet al.(2005). The disease originates from Japan Kitani et al. (1960) and mainly was associated with Asia and Australia. Within the last 10 years, soybean rust was reported in South America and in the continental United States. In west and central Africa, soybean rust has been reported in Nigeria, Ghana, and Democratic Republic of Congo Akinsanmi et al.(2001). The disease is now endemic in most soybean-producing areas in Nigeria. Soybean rust also has become one of the obstacles to increase soybean production in central and north East Nigeria due to climatic condition (high temperature and humidity) providing suitable conditions for disease development, especially during the raining seasonAdeleke et al. (2006).Soybean rust becomes the most destructive foliar disease of soybean worldwide due to the widespread distribution and the potential for severe yield losses Hartman et al. (2005). Soybean rust symptoms generally occur first on the leaves at the base of the plant and spread up to the canopy as the disease severity increases. Rust symptoms include presence of tan to dark brown or reddish brown lesions Hartman et al (1994). An increase in leaf density will result in leaf yellowing, early leaf senescence, and yield losses Tschanzg. (1980). The heavy defoliation due to rust disease affects pod formation and pod filling Yang et al. (2007). According to the USDA (2010), severity of losses in yield depends on the susceptibility of the soybean variety, time of the growing season in which the rust becomes established in the field and weather conditions during the growing seasons. The extent of yield loss is also dependent on crop growth stage at which the disease starts and the intensity. Time of planting also affects soybean rust severity on plant leaves Twizeyimana*et al*. (2007). The most susceptible stages are between early flowering and mid-seed development.

Resistance to soybean rust is manifested phenotypically by red-brown lesions and characterized by the plant response that have been shown to be associated with single dominant genes for soybean rust resistance, i.e. an immune response, reddish-brown lesions (or incomplete resistance), and the susceptible tan lesions Bromfield, (1984). Plant breeders routinely test genotypesin multiple locations and years to determine whether or not environment affects the magnitude of specific traits of

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genotypes, such as disease severity, as welldifferences of the values of the traits among genotypes Piepho, (1996). Several methodhave been proposed to analyze the genotype– environment (GEI) interaction such as joint regression (Finlay and Wilkinson, 1963; Perkins and Jinks, 1968), sum of squared deviations from regression (Eberhart and Russel, 1966), stability variance (Shukla, 1972), coefficient of determination (Pinthus 1973), coefficient of variability (Francis and Kanneberg 1978), and Type B genetic correlation (Burdon 1977). These methods are commonly used to analyze multi-location environment trials data to reveal patterns of GE interaction. Alternatively, the additive main effects and multiplicative interaction (AMMI) model have led to more insight in the complicated patterns of genotypic responses to the environment (Gauch and Zobel 1988,Zobel et al, 1988,Gauch 1992, 2006). Yan et al. (2000) proposed another methodology known as GGE-biplot for graphical display of GE interaction pattern of Multienvironment trial (MET) data with many advantages,among which is the graphical visualization of the interrelationshipamong environments, genotypes, and interactionsbetween genotypes and environments.The objective of the study was to evaluate soybean lines for resistance to rust disease, *Phakopsorapachyrhizi*.

II. MATERIALS AND METHODS

Experiment 1;

Evaluation of Soybean Genotypes in Wukari and Makurdi Environments.

The study was conducted at two locations Makurdi and Wukari. The experiment was carried out in the 2015 cropping season between the months of June to November at the Teaching and Research Farm of the University of Agriculture Makurdi (lat. 7.73'N, long. 8.53'E).The location falls within the southern Guinea agro-ecological zone of Nigeria, andWukari (lat. 7. 88''N, long. 9.78E).Thislocation falls within the north-east agro-ecological zone of Nigeria.Tenlines of soybean were planted out in a randomized complete block design with three replications on 30th June 2015 and 7th July, 2015 in Makurdiand Wukarirespectively. The size of each plot was $32m^2$. Each plot consisted of 4 ridges of 4m length, spaced 0.75m apart. Harvesting was carried out in November and the following parameters were measured. Days to flowering, days to maturity,plant height, number of branches per plant, number of pods per plant, 100 seed weight,seed weight per plant and yield per plot. Resistance to soybean rust, A scale of 1-5 adopted from Iqbal*et al.* (2004) was used for the disease rating where 1= highly resistant, 2= resistant, 3=moderately resistant, 4= susceptible and 5= highly susceptible.

Statistical analysis

Analysis of variance was performed initially for each of the parameters measured above in the different locations. Using the general linear model of SAS (2007) Yield data were analyzed using GGE models to determine GEI, genotype stability and winning cultivars in the locations using GENSTAT 13th Edition.

Experiment 2;

AssessingSoybean for Resistance to Bulk Isolates of Rust (*Phakopsorapachyrhizi.*

A set of three soybean plants for each of the 10 genotypes were planted in 10 litre buckets in the University of Agriculture screen house in Makurdiand arranged in a completely randomized design. Two weeks later, soybean rust isolates were collected from the field for inoculation. Soybean rust isolates were harvested using a handheld Liliput® vacuum from random soybean leaves at the R6 stage from two locations that represent the major soybean growing areas in this study (Makurdi and Wukari).These locations are described above Rust isolates, were selected from about five to twelve leaves and bulked. The bulked rust isolates were then inoculated on the 10advance soybean lines using the detached leaf technique at the second Vegetative growth stage within 48 hours of collection from the field Obua.(2012,Twizeyimana*et al.*(2010) For each isolate, freshly harvested field spores were mixed with distilled deionised water containing the surfactant Tween-20 at 0.5ml/l. Urediniospore suspensions were diluted to a concentration of 50 000 spores per millileter using a haemocytometer. Leaves at two trifoliate stages were detached from the seedlings and artificially inoculated with 1.5 ml of spore suspension on the abaxial leaf surface using a hand sprayer. Each of the inoculated detached leaves was carefully placed in 9-cm-diameter Petri dish with the adaxial side placed on the moist filter paper. After inoculation, the leaves were covered with black polythene bags for 24 hours at 22°C-24°C to maintain high relative humidity, necessary for infection. After 24 hours, the polythene bags were removed for the rest of the experimental period.

The data recorded from the study includes;

Reaction type: immune (I), Reddish-Brown (RB), Tancolored (TAN), Mixed reaction with both RB and TAN (MX), Lesion number and Frequency of lesions with uredinia. This was done using ×10 magnification lenses. Data were collected after five days of inoculation on a three day interval up to the 16th day after inoculation and subjected to analysis of variance in GENSTAT 13th Edition.

III. RESULT

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Genotype Reaction to Soybean Rust Disease

Mean rust severity scores on soybean genotypes for the locations are presented in Table 1. Genotype with lowest rust severity scores includes TGX-1835-10E (3.13) and TGX-1945-4F (3.07). On the other hand, TGX-1485-1D had the highest mean score from both location; Rust severities were significantly different across the different genotypes within locations ($p<0.01$). makurdihad the lowest mean scores of 3.17 while wukarihad higher mean score of 3.50.

Table.1: Mean Number of Rust Disease Reaction toGenotypes

Genotypes	Makurdi	Wukari
TGX-1949-10F	3.80	3.90
TGX-1987-10F	3.23	3.53
TGX-1448-2E	3.50	3.67
TGX-1485-1D	4.13	4.09
TGX-1835-10E	3.13	3.23
TGX-1904-6F	3.67	3.63
TGX-1935-3F	3.33	3.27
TGX-1945-1F	3.67	3.09
TGX-1945-4F	3.47	3.07
TGX-1951-4F	3.53	3.43
Mean	3.17	3.50
Cv%	5.46	6.32
Lsd	0.33	0.3

key; 1.0-1.9=highly resistant 2.0-2.9= resistant 3.0-3.9=moderately resistant 4.0-4.9=susceptible 5.0->=highly susceptible a scale of 1-5 *(*iqbal*et al.* 2004).

Number of Lesions per Leaf

Analysis of variance for number of lesions per leaf showed that location effect was significant ($p=0.02$) while genotypes were not significant for lesion number per leaf; this implies that isolates from different locations infected all genotypes differently. Genotypes TGX 1935-3F had a mean of 21 lesions; followed by TGX 1904-6F (15). On the other hand, TGX 1985-10F showed the lowest mean number of(12) lesions; followed by TGX 1949-10F (27), TGX 1485-1D(20) and TGX-1951-4F(25) as summarized in Table 2.

Table.2: Mean of Lesion Number per Leaf of 10 Soybean Lines after inoculation with Rust Bulk Isolates from two study locations

Genotypes	Makurdi	Wukari	Mean
TGX-1949 10F	40	14	27
TGX-1987-10F	25	13	18
TGX-1448-2E	28	20	24
TGX-1485-1D	55	15	35
TGX-1835-10E	11	12	13
TGX-1904-6F	14	6	15
TGX-1935-3F	38	14	21
TGX-1945-1F	25	21	23
TGX-1945-4E	29	11	20
TGX-1951-4F	32	16	24
Mean	30.2	14.4	20.8
LSD	1.76	3.11	3.47

Effects of Genotypes on Yield and Yield Component

The result in Table 3 show that the effects of genotypes on yield and yield component at different locations were significant ($P < 0.05$) confirming the previous studies of Lymon et al (2017) in Tanzania

In this study, the genotypes TGX 1835-10E and TGX 1987-10F outperformed the local check in all the two locations with the average mean performance of 799.51 and 766.75 kg/ha respectively, while TGX-1485-1D had the lowest (254.23kg/ha) yield in all locations. Alongside TGX 1945-4E, TGX 1904-6F and TGX 1448-2E yield performance were significantly high than the control (TGX-1485-1D) in all locations. The low yielding ability of TGX-1485-1D variety was previously reported by (Ojo et al. 2010) for the southern Guinea Savanna. The mean performance of the genotypes across the location revealed that TGX 1835-10E had the highest number of seed per plant (1.87), followed by TGX1904-6F (1.71) and TGX-1485-1D showed the lowest (1.11). TGX1835-10E and TGX 1904-6F had the largest number of pods per plant with 50.10 and 43.60 respectively, and TGX-1485-1D revealed the lower value (26.28). Similarly, the genotype TGX1835-3F and TGX 1904-6F had the highest plant height with 49.15 and 47.58cm respectively while TGX-1485-1D recorded the least (36.45cm). High yields attained by TGX 1835-10E and TGX 1935-3F genotypes could be explained by the high performance of agronomic variables such as the number of pods per plant and number of seeds per plant which featured high in these genotypes compared to others (Table 2).

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Table.3: Effect of Genotype on yield and yield component

GENOTYPES	DFF	DYSM	PLT(m)	NOB	NPPLT	SPPLT	HSW	YIELD(kg/ha)
TGX-1949-10F	43.16^{a}	88.00^{de}	37.58^{d}	1.91^{c}	30.76^{bc}	1.18^{dc}	7.65^{c}	404.30^{bc}
TGX-1987-10F	40.66^{ba}	85.50^{e}	40.67^{dc}	1.88^{c}	36.63^{c}	1.53^{bac}	9.77^{bac}	766.75^{ba}
TGX-1448-2E	41.33^{ba}	101.33^{a}	45.13^{ba}	2.63^{ba}	31.43^{bc}	1.71^{ba}	11.95^{a}	690.26^{a}
TGX-1485-1D	41.66^{ba}	94.83bc	34.45^{bc}	2.15^{bc}	26.28^{bac}	1.11^{d}	7.80^{c}	254.23^{c}
TGX-1835-10E	40.50^{b}	85.50^{e}	49.15^{bc}	2.10^{bc}	50.10^{bc}	1.87^{a}	11.03^{ba}	**799.51^{a}**
TGX-1904-6F	42.00^{bac}	99.16^{ba}	47.58^{bc}	2.06^{bc}	43.60^{bac}	1.70^{ba}	11.71^{ba}	750.68^{a}
TGX-1935-3F	41.83^{ba}	92.16^{dc}	51.46^{a}	2.93^{a}	39.43^{bac}	1.31^{bdc}	8.68^{bc}	585.40^{ba}
TGX-1945-1F	43.00^{ba}	97.83^{ba}	41.93^{bcd}	2.10^{bc}	37.46b^{a}	1.58^{ba}	10.53^{bac}	652.21^{ba}
TGX-1945-4E	41.33^{ba}	91.66^{dc}	47.36^{ba}	2.53^{ba}	33.23^{a}	1.65^{ebdac}	11.26^{ba}	742.63^{ba}
TGX-1951-4F	41.66^{bac}	94.33^{dc}	36.96^{d}	1.61^{c}	36.16^{bac}	1.60^{ba}	11.00^{ba}	559.65^{a}
Mean	41.61	93.03	43.08	2.19	35.61	10.14	1.52	775.35
SE	0.55	1.70	1.89	0.20	4.51	0.14	1.06	110.01
Cv(%)	2.60	4.48	10.71	22.73	31.33	23.26	25.62	34.71

Means with the same letter are not significantly different at the 0.05 probability level based on Tukey'sStudentized Range Test;Bolded values are highest genotype grain yield at each test environment, and highest yielding genotype across environments and the highest yielding environment;DFF: Days to flowering, DYSM: Days to maturity, PLT; Plant height, NOB; number of branches, NPPLT; number of pods per plant, SPPLT; number of seed pod per plant, HSW; hundred seed weight per plant.

Best Performing Soybean Genotypes

From The different environments best performing genotypes were visualized using a polygon view in Figure 1.This polygon view was drawn by joining five soybean genotypes at the furthest corners from the origin of the biplot. These were TGX-1987-10F, TGX-1485-1D, TGX-1935-3F, TGX-1945-4F; TGX-1835-10E from which five perpendicular lines were drawn to each of the polygon side passing through the origin of the biplot dividing the biplot into five sectors. Environments 1 (Makurdi), and Environments 2 (Wukari), lines TGX-1987-10F was the best performed genotype in Environments 1, followed by TGX-1835-10E, performed best in Environment 2, while Other vertex genotypes like TGX-1485-1D, TGX-1935-3F, TGX-1945-4E did not fall under any of the test environments. The rest of the genotypes were located within the polygon, while TGX-1945-1F was located close to the biplot origin.

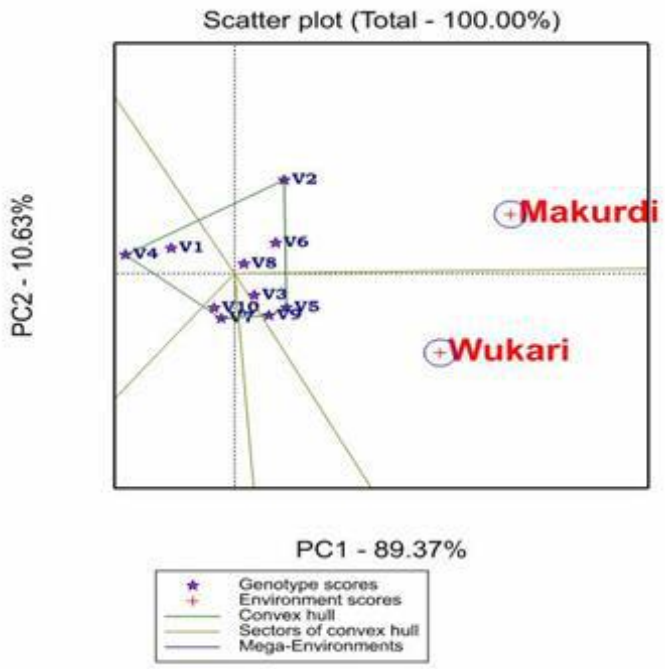

Fig.1*: Polygon view of GGE biplot based on symmetrical scaling for 10 genotypes in two environments. PC1 and PC2 are the first and second principal components, respectively.*

KEY VI= TGX-1949-10F, V2=TGX-1987-10F, V3=TGX-1448-2E, V4=TGX-1485-1D,V5=TGX1835-10E, V6=TGX-1904-6F, V7=TGX-1935-3F, V8=TGX-1945-1F, V9=TGX-1945-4F, V10=TGX1951-4F.,

Soybean Yield Performance and Stability

Below shows a GGE biplot for soybean yield performance and stability based on average environment coordination (AEC) procedure Figure 2. A straight line passing through the origin of the biplot and the average environment is represented by a small circle. A

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perpendicular line to AEC axis passing through the biplot origin separates the genotypes with more than the grand mean yield from those with less than grand mean yields. Therefore, genotypes with more than grand means and are located near the AEC line and are genetically desirable. To this regard, genotypes TGX-1448-2E (V3), and TGX-1945-4E (V9) had yields above the grand means, and the yield were stable because they were not far from the AEC line. Conversely, genotypes TGX-1835-10E (V5) and TGX-1945-1F (V10) were among the high yielding genotypes but their yields were unstable because they were located far from the AEC line. Other genotypes had yields below the grand mean but their yields were stable. These included; TGX-1904-6F (V6). On the other hand, genotypes, TGX-1485-1D (V4) and TGX-1949-10F (V1), recorded the lowest yields and were position far away from AEC line.

A comparison biplot that is genotype focused (Figure 3) showed that genotype TGX-1448-2E(V3) is the most stable genotype while TGX-1945-4E(V9) is the most ideal genotype, followed by TGX-1835-10E(V5) and TGX-1945-1F(V8) TGX-1951-4F(V10),TGX-1935-3F(V7)others were far from the AEC line which are TGX-1904-6F(V6),TGX-1949-10F(V1),TGX-1485-1D(V4) and the least is TGX-1987-10F(V2)

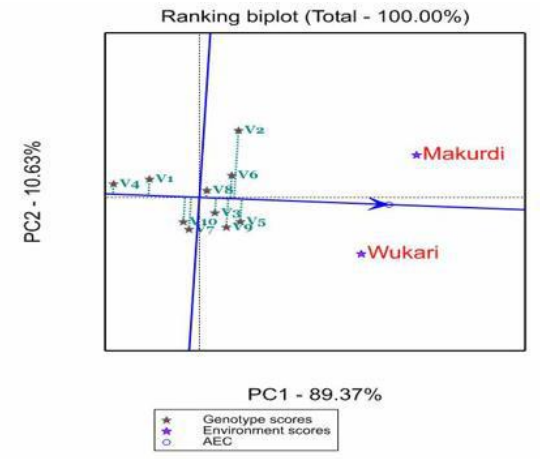

Fig.2: GGEBiplot for ranking for Yield Performance and Genotype Stability Based on Average Environment Coordination (AEC). PC1 and PC2 are the first and second principal components, respectively Where V1–V10 are codes for soybean genotypes.

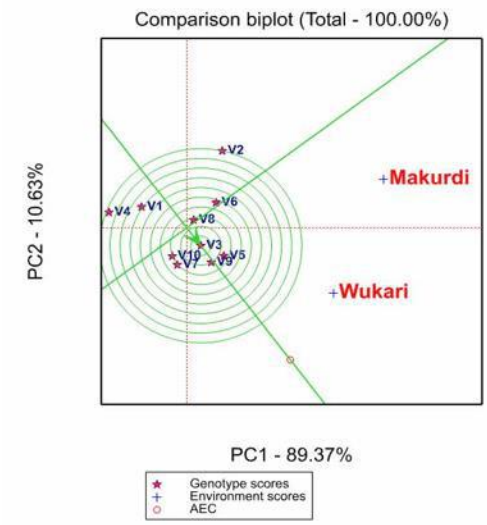

Fig.3: Genotype focused comparison biplot showing PC2 verses PC1 for10 soybean genotypes and two environments, where V1–V10 are codes for soybean genotypes.

Soybean Yield Performance and Stability

A GGE biplot based on environment-focused scaling was used to estimate the relationship of the test environments (Figure 4.) The line from the origin of the biplot to the marker of the environment is the environment vector. Environments with longer vectors (PC1 scores) and PC2 scores close to zero are desirable for discriminating genotypes and representative environments, respectively. In regard to this, Environment 1 had the longest vector (largest PC1 scores) and PC2 scores close to zero. Then Environment 2 with relatively low PC2 scores close to zero, and moderately low PC1 scores.

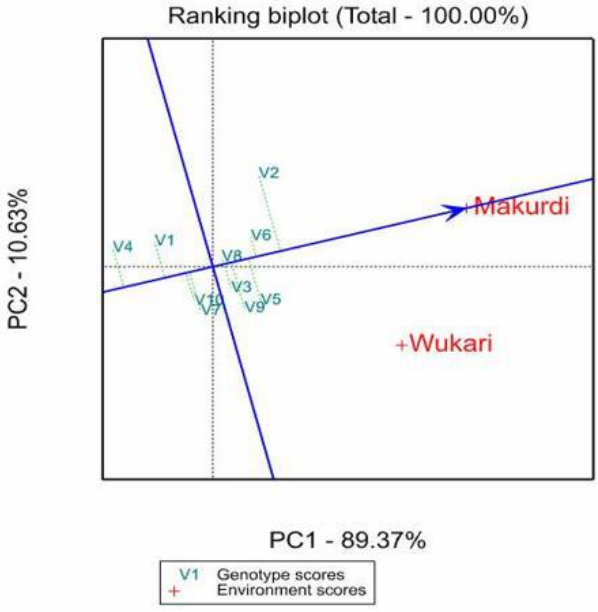

Fig.4: GGE Biplot based on environment focused scaling for 10 varieties. PC1 and PC2 are the first and second principal components, respectively. Where V1–V10 are codes for soybean genotypes.

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IV. DISCUSSION

Rust disease symptoms, in this study, started to appear since 6 to 7 days after inoculation. The incubation period in the present study was consistent when compared with the results of other researches in Africa. Twizeyimana et al. (2007) found that in Nigeria it took 5 to 7 days after inoculation to lesion of rust disease appear on the surface of leaves. Meanwhile, Maphosa et al. (2013) reported that the incubation period of rust disease in Uganda began to be seen since 4 to 5 days after inoculation. This means that the isolates of rust fungus from Nigeria are more virulent compared with isolates and or soybean genotypes from other places are more resistant than soybean genotypes from Makurdiand Wukari.

Although the incubation periods of rust disease in present study was not longerwhen compared with the results obtained from Ibadan (Twizeyimana et al 2007), the inoculation of rust disease that has been done is able to bring up the different reactions of soybean genotypes tested. The reaction differences seen in the number of lesions between one genotype to other genotype were observed. Lesions of rust disease that appears,varies between genotype and within genotype, ranging from 6 lesions cm^2 (TGX-1904-6F) to 55 lesions cm^2 (TGX-1485-1D) on observation as shown in table 2.

Differences in the reaction of genotypes tested are also found in other studies (Sulistyo et al 2016, Pham et al2010, Twizeyimana et al. 2008) stated that genotypes with non-characterized genes for resistance may be useful for host plant resistance studies and breeding soybeans for rust resistance. The reaction of soybean genotypes with resistance against rust diseases showed that all of the genotypes classified as resistant on observation were the genotypes categorized as moderately resistant. The different resistance reaction between the assessments is caused by spores of the rust disease which require time to germinate and form the new spores. According to Yang (2002), after an infection has occurred, it takes 5 to 7 days to produce uredinia by urediniospores and 10 to 20 days to produce a new generation of spores. This difference gives guidance for soybean breeders to determine the appropriate time to conduct the selection. Sulistyo and Sumartini (2015) found that there are differences in heritability of rust disease severity on observation of one, two and three weeks after inoculation.The emergence of rust diseases on the various phases of the development of soybean will determine how much yield loss will occur. Kumudini et al. (2008) found that if the rust disease began to occur at the R2 growth stage (full flowering phase), it would cause yield losses up to 66-68%, meanwhile, when it started at the R5 growth stages (seed filling phase), it will cause yield losses reach 35-39%. In this research, a soybean genotype with early flowering can avoid a large yield loss. The mechanism was shown by line TGX-1835-10E and TGX-1987-10F. Both of these soybean lines flowering at 40.50and 40.66 Days after Planting (DAP), had the highest seed yield per plant (799.51k g/ha and 766.75K g/ha, respectively) compared with other lines. In contrast, the line TGX-1949-10F and TGX-1945-1F were flowering at about 43 DAP, had a weight of seeds per plant (585.40, and 404.3kg/ha, respectively) were significantly lower than the two previous line.Plant height in this study appears to be one of the factors that will determine differences in the severity of rust disease on soybean genotypes tested. Analysis showed that there is a significant association between plant heights with the number of rust lesions in the observation. It means that the higher a plant, then the fewer rust disease lesions as with TGX 1485-1D Which have lowest height of(34.25cm) toTGX-1935-3F (51.46cm)With similar result from Abayomi et al(2009) in the southern Guinea Savannah environment. This is not surprising because *Phakopsorapachyrhizi*does not have an active mechanism for spreading the spores. According to Isard et al. (2005), wind seems to be critical factors for spreading out spores and lifting them out of the canopy. Thus, it takes quite much wind to spread the spores of rust on soybean genotypes with appearances tall plants. Rust disease in present research did not seem to affect the character of other yield components, such as the number of branches, the number of seed/plant and the number of pods. However, the three characters have an influence on seed yield per plant. According to Oz et al. (2009)number of pods per plant had significant correlations with seed yield and gave direct positive effect. Ojo et al. (2010), Valencia-Ramirez and Ligarreto-Moreno (2012) found a similar result. Malik et al. (2007) suggested that number of pods can be considered as selection criteria in improving the bean yield of soybean genotypes. The Genotype main effect and the genotype xEnvironment effect were the major sources of variation important for Genotype evaluation .The first two PCs of the biplot explained 100% of the total grain Yieldvariation which was adequate for soybean evaluation. These Findings are also supported by Yan et al. (2007), who reported that GGE Biplot analysis was effective in regard to mega environment yield. The GGE biplot aims to use the "which-won-where" pattern to facilitate Identification of the most responsive genotypes Yan et al(2000).In this study, the most responsive genotypes were five advancedlinesTGX-1945-4F,TGX-1835-10E,TGX-1935-3F,TGX-1485-1D,TGX-1987-10F. Interestingly, These genotypes demonstrated either higher (sometimes the highest) or Lower yields compared to the other genotypes in all the environments Within the sector in which they fall figure 1Other vertex genotypes includingTGX-1935-

3F, TGX-1448-2E which expressed highly Responsive behaviour but they did not fall under any of the test environments, indicating that they were not high yielding genotypes in Any of the two environments. The test environments appeared in five sectors of the polygon view figure 1, a Sign of cross-over of GEI effects, suggesting the presence of two possible mega-environments in Central and north Eastern Nigeria. According to Yan and Rajcan(2002),a mega-environment refers to Cluster of environments having the same high performing genotype(s). For instance, the first sector had one environment with TGX-1987-10F as the winning genotype. The SecondEnvironment had two sectors having TGX-1835-10E and TGX-1945-4F appearing unique and them Performing the best. Mega-environments help plant Breeders to select high yielding genotypes for a specific environment;Making better use of GEI.The other importance of mega-environments is that genotypes may be evaluated in a few Representative environments, which will provide informative data representing GEI trials to cross a much larger number of Environments. Therefore, figure 2, environments 1 may be used for evaluating soybean Genotype in Central Nigeria.Based on average environment coordination (AEC) yield performance and stability of 10 soybean genotypes were evaluated figure 2. Accordingly both yield performance (largePC1scores) and stability (PC2 close to zero) should beconsidered for effective selection of genotypes. Thus, genotype TGX-1835-10E and TGX-1945-4F were high yielding and stable. Other stable genotypes included TGX-1448-2E, TGX-1945-1F, TGX-1485-1D, TGX-1949-10F but they were low yielding. Such Genotypes would require further breeding for high yields before they are released to the farmer's figure 2. Although genotype TGX-1987-10F, TGX-1945-4F And TGX-1935-3F recorded the highest grain yields, they were unstable across the test environments. The will be recommended for specific environments or selected for their yield performance to Improve low yielding genotypes in a soybean breeding programme. In figure 3, The GGE genotype focused comparison biplot also showed that early Maturing genotypes were also low yielding and unstable. Among the locations, Makurdi had the highest seed yield. The high seed yield could have been due to optimal supply of water to the crops. In Addition to the longest period to physiological maturity, Makurdihad the Highest mean seed yield (1339kg/ha).Wukarirecorded the lowest Yield of 589kg/ha.These results seem to suggest that presence of moisture in the soil during the season delays maturity but increases seed yield of soybeans. makurdi was the most ideal environment as earlier observed by Ojoand Bello.(2012) and is therefore recommended as a primary testing centre for new soybean genotypes figure 4. According to Jandonget al.(2011) Environments with longer vectors (large PC1scores) have the ability to discriminate (informative) between Genotypes for a given trait, while short vectors identifies environmentsWith a poor ability to discriminate between genotypes figure 3 . On the other hand, small PC2 values (PC2 scores close to zero) are good representative of the target environments and vice versa. Therefore, any test environment with large PC1scores and PC2 scores close to zero are desirable. In this study, among the two environments, makurdihad the longest vector, And PC2 scores close to zero. It was, therefore, identified as the most useful environment in terms of discriminating between genotypes and was the most representative of all the test environments.

V. CONCLUSION

Four moderately resistantTGX-1835-10E,TGX-1987-10F, TGX-1904-6F and TGX-1945-4Eof the ten lines produce seeds with the seed weight per plant heavier than susceptible TGX-1949-10F and TGX-1485-1D,this Characteristics show among others the performance of plants is high with lot of number of branches and number of pods and beneficial to soybean growth and grain yield.GenotypesTGX-1448-2E and TGX-1945-1F identified as high performing genotypes and stable in test environments can be used for commercial production. While genotypes TGX-1835-10E and TGX-1987-10E thou highest yielding but highly responsive to the environments, can only be used for specific environments or be utilized to improve yields.

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Evaluation the Performance Efficiency of Manufactured, Modified and Assembled Combine Implement and Studying It's Impact on Some Soil Physical Properties and Total Costs

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Abstract**—** *The experiment was conducted to evaluate the efficiency performance of the combine implement which manufactured and assembled locally and studying it's effect on some soil physical properties and total costs in one of the Agricultural College University of Baghdad Experimental Fields in loamy soil, 2017. Brazilian Massy Ferguson Tractor (MF-650) was used with the combine implement as a machinery unit. Three machinery unit speeds included 3.15, 4.60 and 6.10 km/h and seedling treatments included manufactured combine implement, seedling and fertilizer implement and manual seedling were used in this experiment. Soil bulk density, soil moisture content, amount of added water and total costs were measured. Nested design under randomized complete block design with three replications was used in this experiment. Least significant differences (LSD = 0.05) level under 0.05 probability was used to compare treatment means.*

The results can be summarized as following:

1. *Incrased machinery unit speeds from 3.15 to 4.60 km.h^{-1} led to significant increase in soil bulk density from 1.30 to 1.36 Mg.m¯³and significant increase in soil moisture content from 0.18 to 0.20 %.*
2. *Manufactured combine implement treatment was superior in getting less soil bulk density stood 1.22 Mg.m¯³ higher soil moisture content stood 0.22%.and less amount of added water during the season stood 1103.43 mm. and less costs stood 796370 Iraqi Dinars.*
3. *The interaction between 3.15 km.h^{-1}machinery speed and manufactured combine implement got less soil bulk density stood 1.19 Mg.m¯³, while the interference between 6.1 km.h^{-1} machinery unit speed and manufactured equipment was superior in obtaining a higher moisture content* **stood** *0.229%.*
4. *Using the locally manufacturing modified combine implement for primary and secondary tillage, shallow furrow opener, seedling and fertilization in one time was successfully done in this study with high performance efficiency.*

Keywords**— performance efficiency, combine implement, shallow furrow opener, seedling, and fertilization.**

I. INTRODUCTION

Agricultural mechanization is one of the main indicators of the transition from traditional agriculture to modern agriculture. Agricultural mechanization plays a fundamental and effective role, especially if it is scientifically exploited. The international experience of developed countries and local experiences proves that agricultural mechanization is of great importance in increasing production, reducing costs and reducing working hours, where agricultural mechanization performs various agricultural operations by means of mechanical equipment, which is dependent on the mechanical or electrical driving ability using the lowest human or animal effort.

There are many combine equipment designed for the development of tillage and most of these equipment are used in primary and secondary tillage and machinery service crop and control of the bushes, seeds and fertilization, and these equipment are becoming more common because they work more than one process during the passage of one and this will reduce the number of traffic in the field, The remains of plants and their formation on the surface of the soil instead of burying them, and thus they fit very well towards the trend of reducing tillage or conservation of tillage, Frank, et, al, (2012).

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Lack of irrigation water supply due to climate change, low water and river water levels and moisture of water distribution have forced many farmers in Iraq to abandon agriculture. Some farmers have used groundwater to irrigate crops, reducing water levels and becoming poor quality due to increased salinity, and because of high pumping costs and low water table, farmers were forced to use water in a rotation manner. Therefore, alternative methods were used, one of which was to practice water deficit, which will lead to further expansion of horizontal agriculture for the same water resource, thus increasing the efficiency of water absorption that can lead to food security and reduce the risk of desertification.

The seeds of the agricultural machinery for planting the seeds, where the use of seed implements yielded positive results in the speed of completion of the seed and not waste in the quantities of seed used as the use in accordance with the design task has the ability to distribute seeds in the field on a regular basis and cover the seeds in the soil after the process Seeds and prevent their being eaten by birds. Fertilizer can also be distributed at the same time as seed when using seedling and fertilizers, (Ali, 1989).

The process of fertilization has a key role in the soil where the soil needs to replenish its fertility and compensate for the loss of elements of the addition of animal or chemical fertilizers and to reduce the persistence of the stress of erosion and decrease of nutrients, especially the basic materials such as nitrogen, phosphorus and potassium. This is done using special equipment for fertilization and varies depending on the type and nature of fertilizer, (Hassen and Ezzet, 1987).The fertilization process also has a high economic success if it is used to increase agricultural production when using fertilization equipment. Similarity is similar to that of the seed equipment, and the equipment used for sowing and fertilization is used in one,(Al-Tahan and Al-Naama, 2000).

According to the importance of choosing the best irrigation methods, irrigation interval and potato planting methods for potato planting, this experiment was done.

II. MATERIALS AND METHODS OF WORK

A field experiment was conducted in one of the fields of the Faculty of Agriculture / University of Baghdad / Al-Jadriya for the agricultural season 2016 2017. To evaluate the performanle efficiency of a combined implements used f primary and secondary tillage, opening shallow farrows and seedling, fertilization and its studying on the some soil siel properties total costs.. Then the primary tillage was carried out by the three-piece trowel plow. Then the secondary tillage was softened by the rotary plow. (18) experimental units, which are included in the comparison with the experimental units of the machine manufactured (9) experimental units, the soil samples were taken from each experimental unit in the field randomly from different locations and at a depth (0-30) cm and classified soil tissue as Soil Incubation, Table (1)

Table.1: Some physical and chemical properties of the soil

Adjective.			Unit		Value
(Electrical conductivity EC)			$ds.m^{-1}$		1.2
(pH) degree			.....		7.35
Soil particles analysis			Sand	G. Kg^{-1}	438
			Silts		394
			Clay		168
Type of soil			Silt soil		
Soil penetration resistance			Kilo Pascal	3	
(som) Organic matter of soil			G. Kg^{1-}	7.65	
Bulk Density			$^{3-}$m. Makagram		1.312
(N) Nitrogen			%		0.018
Nitrogen Ready / Ion Ammonium			Kg^{1-}.Ml gN		20.92
Moisture content under pressure	⅓field capacity	bar	%		0.335
	15welting point				0.114

The combine implement which manufacturing modified and assembling locally consists of included sweep plow, rotary plow, seedling implement, fertilization and open shallow furrows,:

1. compine implement, (Sweep plow), one of the parts of the compine implement is installed in the front part of the implement leads the pramary tillage and prepares the soil to the secondary tillage, linked to the structure of the compine implement and consists of two shares each shares is linked to a height of 70 cm and width of 10 cm and thickness of 3 cm and From the bottom V-shaped shares in English and consists of two wings have a nose for the weapon with a thickness of 4 cm convex height of 10 cm and

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two fins back the distance between 25 cm and the length of each alone 50 cm.

2. Rotary plow, used for soil pulverization (secondary tillage) soften and prepare for the soil other stage of opening the cane, the working width of the 160 cm contain 17 teeth teeth successive around the axis The tooth, which is derived from the PTO shaft, has a length of 22 cm and its thickness is pointed to the front to facilitate penetration of the soil.
3. Seedling implement, it is the third part of the combine implement and lead the process of mechanical seedling, which regulates the placement of seeds in the shrine of the seed and reduce the loss of seeds and reduce the eating of birds, and consists of the structure and seed reservoir and transmission pipes seeds and the mechanism of feeding and glass
4. Fertilization imp, the fourth part of the machine which is working on the distribution of manure mechanically and in a systematic flow and has an effective role in the removal of manure in the bottom of the plantation through pipes laid out in a regular manner and divided by equal distances, consisting of fertilizer from the structure and from the fertilization tank and fertilizer feeder and landing tubes Compost and chicken.
5. Shallow farrow opening implement, the fifth and final part of the machine which is working to open shallow nodules (grooves) and have an effective role in saving water proportion of two thirds of water and one third of land and agriculture on the rice gives ventilation of the plant and the return on the entry of light and strengthen roots, The machine to open the shallow mulch of the structure and four crumbs Each glass containing the weapon of the man of the duck works on opening a loose maze. Working machine mounted,

This combine implement is planting the corn seeds at the top two thirds of the furrow shoulder of the corn and the process of fertilization on the bottom of the furrow plant, and this implement is characterized by the ability to seed and fertilization and control the depth of the plant and any height and have the ability to horizontal movement (Figure 1) shows the work of the machine as shown in Fig. (2)

Fig.1: front view of the combine implement

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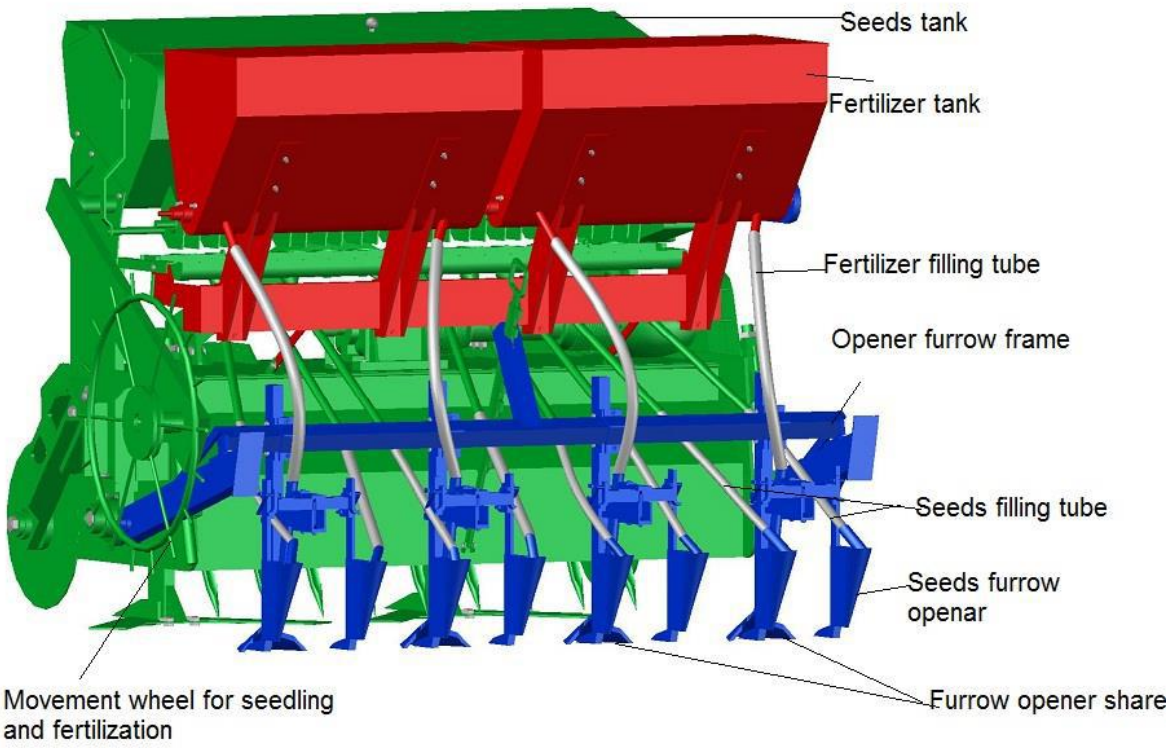

Fig.2: Rear of the machine

Studied Properties

1- :Pulk density of Soil, Mg.m⁻³

Pulk density was calculated by the method of the Core Method cylinder at the end of the agricultural season using the following equation proposed by, Black and Hartage (1986)

Pp=Ms/ Vt Mg.m^{-3}

Whereas:-

Pb = Pulk density, (1 µg.m^{-1})

Ms = mass of dry sample, (1 µg m)

Vt = sample size, (m^3)

2- Soil Moisture Content measurement (%)

The moisture content was calculated by the weight method prior to conducting the experiment and for each experimental unit as stated in, Hassan , (1990). and according to the following equation

M = (Mw / Ms) x 100

Whereas:-

(%) M = Moisture Content

Mw = Mass of Water, (grams)

) Ms = Solid block Mass, (gram

3-Amount of irrigation water added,

Shows the amount of water discharged per irrigation. The added water depth was calculated using the proposed equation proposed by, Kovda .et. al (1973).

d = (ΘFC - Θw) D

whereas

d = depth of added water, (cm)

ΘFC= Volumetric humidity at field capacity (cm-3 cm)

Θw = Volumetric humidity before irrigation 60-65% of the ready water, (cm. cm-3)

D = Depth of irrigated root zone, (cm)

The content of the prepared water is determined by the difference between the volumetric content at a water pressure of 33 kPa which shows the field capacity and the volumetric content at 1500 kPa which shows the permanent wilting point according to the following equation.

whereas:

$$A_W = \theta_{fc} - \theta_{wp}$$

Prepared water content in soil, (cm ^{3} cm^{-3}= A_W)

= θ_{fc} (Volumetric content at field capacity, (cm^3 cm^{-3})

Volumetric content at the permanent wilting point

θ_{wp} = (cm^{3}cm^{-3})

4-Total Costs, ID

The total fixed and variable costs have been calculated in accordanceto the approved economic criteria and the comparison of the transactions and the calculation of the annual revenues and profits .

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III. RESULTS AND DISCUSSION

1-Soil Bulk density, Mica gm.m^{-1}

Table (2) shows the effect of the practical velocity of the mechanic unit and seedling coefficients and their overlap in the bulk density. Table (2) shows that the increase in the operating speed from 3.15 to 4.6 and then to 6.10 cm. led to increase bulkdensity from 1.30 to 1.33 and then to 1.36 mica gm. M^{-3}. The reason is that the increase in speed helps increase the fragmentation of the soil and work on the formation of small minutes that fill the pores and over time and at the end of the agricultural season and the result of moisturizing and drying less size And increase its mass and lead to an increase in the bulk density, and with the results which proposd by of, AL-Jubouri, (2012) and Al-Sharifi, (2009).

The results indicated in Table (2) that the seed treatments had a significant effect on the soil density of the soil, where the treatment of the seedling combine implement was less apparent soil density of 1.22 µg .while while the fertilizer and fertilizer treatment recorded the highest soil bulk density of 1.41 µg.

Table (2) shows the effect of the interaction between the mechanical speed of the mechinary unit and the seedling treatments on the bulkdensity. The binary interaction between the practical speed exceeded 3.15 km.-1 and the processing of the manufactured machine obtained the lowest bulk density stood 1.19 µg.¯„ The value of the virtual density was between the speed of the operation 6.1 km.S1 - and the treatment of the fertilizer and fertilizers and amounted to 1.44 µg..¯.

Table.2: Effect of Machinary speeds and seedling treatments on soil bulk density, Micagram. m^{-3}

Soil bulk density, Mica gm .m^{-3}				
Speedkm.h^{-1}	Seed treatments			Average speed
	manufactured combine implement	seeding and fertilizer implement	manual seedling	
3.15	1.193	1.397	1.336	1.309
4.60	1.252	1.403	1.353	1.336
6.10	1.242	1.444	1.401	1.362
LSD	0.02217			0.00897
Average	1.229	1.415	1.363	
LSD	0.015			

2-Moisture content of soil(%)

Shows that the increase in process speed from 3.15 to 4.6 and then to 6.10 cm^{-1}, has had a significant effect on the soil and soil moisture content. Increase the moisture content of the soil from 0.188 to 0.202 and then to 0.208 on the relay. This is because the increase in the speed of the process helps to increase the extrusion of dust blocks and increase the degree of fragmentation of the soil, which increases the filtration or water condensation into the soil increases the moisture content of the soil , And these results correspond to the results obtained by,AL-Janabi, (2000).

The results indicated in Table (3) that the seed plant has a significant effect on the moisture content of the soil, where the treatment of the processed machine recorded the highest percentage of moisture content of 0.22%, while the treatment of the fertilizer and fertilizer was the lowest proportion of moisture content amounted to 0.182%, and may be due to the machine was used The primary tillage plow, the rotary spindle in the softening and the shallow root groove, all of which help to retain moisture in the soil with very little evaporation, which increased the moisture content of the soil compared to the two laboratories that used the plow, disc harrows and seed in mm lines Evaporation from the soil or the loss of water without the root zone increased.

Table (3) shows the significant effect of the binary interaction between the mechanical speed of the mechanic unit and the seed treatments in the moisture content of the soil. The overlap between the process speed exceeds 6.1 km .1 and the processed machine is treated with the highest soil moisture content of 0.299% Of the moisture content of the soil was between the practical speed of 3.15 km.sa^{-1}, and the treatment of alfalfa and fertilizer was 0.171%..

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Table.3: Effect of Machinary speeds and seedling treatments onsoil moisture content (%)

Soil moisture content (%)				
Speedkm.h^{-1}	Seed treatments			Average speed
	manufactured combine implement	seeding and fertilizer implement	manual seedling	
3.15	0.2127	0.1717	0.1807	0.1883
4.60	0.2197	0.1927	0.1943	0.2022
6.10	0.229	0.1837	0.214	0.2089
LSD	0.0115			0.00682
Average	0.2204	0.1827	0.1963	
LSD	0.00724			

3-Quantity of water added (water consumed), mm

Table (4) shows the results of the irrigation and the quantity of water added during the planting season of the barley barley yield. The results were obtained after sampling the moisture content at field level 0.33 and at 0.11 wilt points and with depletion of 60-65% of the prepared water before each rye and each unit.

Table (4) shows the results of the quantity of water added to the 7 irrigated wells and the rainwater recorded 4383.24 mm .Table 4 shows the results of total water added to 43177.58 (m3.hkata-1). The results show that in the treatment of the manufactured machine, the recorded water depth and the added water volume were lower than those of the powder and fertilizer and manual propagation by 0.33% The treatment of the manufactured machine was irrigated by 0.67% because it was cultivated on shallow marshes and this is the profitability in water consumption to reduce the phenomenon of water scarcity .

Table.4: Table of the quantity of water added during the planting season

Transaction number	Transactions	Quantity of water added during the season, mm	Volume of water added, M^3.ha^{-1}
1	manufactured combine implement	1103.43	11034.27
2	seeding and fertilizer implement	1595.22	15952.26
3	manual seedling	1619.1	16191.05
	Total transactions	4317.71	43177.37M^3.ha^{-1}
	Amount of rain water during the season	65.5	
	Total final amount of water added during the season	4383.24 mm	

4-Total costs of mechanized unit

The results were indicated after selecting a set of criteria that fit the production process. Comparisons were made between the two transactions. Table (5) The final value of fixed, variable and total costs after calculation of the values of each machine were compared in terms of production and fixed and variable costs

Where the efficiency of the machine manufactured in the final production exceeded the other transactions in obtaining the lowest operating cost amounted to 796370 dinars, due to the entry of the unit mechanic one-time reduced the cost of labor and reduced the rate of fuel consumption and also gave valuable results through saving water by Two thirds of water and one third without the use of shallow barley, and increased production of the crop due to the regularity of agriculture and the lack of soil soil and ventilation and increase moisture content and low density of the apparent and the multiplicity of crop branches and the introduction of solar radiation and ventilation of the plant.

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Table (5) shows the total cost of the treatment of fertilizer and fertilizers, which amounted to the highest cost of 1206350 dinars, due to the entry of the unit mechanic 4 times, which led to increased labor wages and increase in fuel consumption and the impact of the entry of machines from pressure on the soil and Dkha and reduce physical properties and increase density Of the soil and reduce the pores, which affects the productivity of the crop and the increase in costs.

Table (5) presents the results of the total costs of manual hand-handling, which amounted to KD 11.1 million. The reason for the irregularity of agriculture, the intensity of the crop and the machine's double-entry when plowing, softening, and the use of labor in machinery, prose, fertilization and non-cover during planting, Make the cost increase

Table.5: Fixed, variable and total costs, Acres^{-1}

Transactions	Fixed costs, D^{-1}	Variable costs, D^{-1}	Total costs, D^{-1}
manufactured combine implement	**517250**	**279120**	**796370**
seeding and fertilizer implement	**572750**	**633600**	**1206350**
manual seedling	**561000**	**550000**	**1110000**

IV. CONCLUSIONS

The results of the research show the following

1. The increase in the speed of the process of agriculture significantly increased the density and the moisture content.
2. The increase in the effect of seed in the treatment of the processed machine has resulted in a significant increase in moisture content, and a significant decrease in the apparent density.
3. The binary interference between the speed of the tractor exceeds 6.1 km. And the treatment of the manufactured machine to obtain the highest moisture content.
4. The interference between the speed of the machine exceeds 3.15 km.-1 and the processing of the manufactured machine in obtaining the least apparent density.
5. The results showed that the treatment of the machine manufactured the least use of water added to use the method of shallow meadows by two thirds of water and one third without water.
6. The processing of the manufactured machine has the lowest total total costs compared with the other transactions.

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The Effect of Irrigation systems and Planting Methods on Soil Porosity and Soil Electrical Conductivity and Potato Yield sunder Two Irrigation Intervals

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Abstract**—** *The experiment was conducted to evaluate the effect of irrigation systems, planting methods and irrigation intervals on soil porosity and soil electrical conductivity and potato yields for fall season of 2016 in Yousufia Area. Three irrigation systems included Sprinkler Irrigation (S), Drip irrigation (D),and Furrow Irrigation (F), two different irrigation intervals included (4 day irrigation interval (I_1) and 8 day irrigation interval (I_2)) and two methods of planting included (Mechanical planting (M) and Manual (Hand) planting (H) were used in the experiment. Soil Porosity, electrical conductivity of a saturated soil extract (Ece), average weight of potato tuber, and plant yield were measured in this study. Split split plots arrangment under Randomized Complete Block Design (RCBD) with three replicates, were used in this experiment. The means of treatments were compared by using least significant difference (LSD=0.05) under probability of 0.05.*

The results can be summarized as follows:

1- *Drip irrigation was superior in obtaining the least value of the electrical conductivity stood 2.76 ds.m^{-1}, highest potato yield stood 811 gm. plant^{-1} and highest value for the average weight of potato tuber was 150 gm. Also, the furrow irrigation treatment was superior in obtaining the highest value of soil porosity stood, 0.44 cm^3.cm^{-3}.*
2- *4 days irrigation interval got a significant higher single plant yield stood 731 gm.plant^{-1}, and potato tuber weight average stood 117.83 gm and got the least value of electrical conductivity stood 3.40 ds.m^{-1}, whereas 8 days irrigation interval was superior in getting the highest value of porosity, stood 0.40 cm^3.cm^{-3}.*
3- *Mechanical planting method resulted in obtaining the highest value of porosity, stood 0.40 cm^3.cm^{-3}, and the highest yield for a single plant value stood 703 gm.plant^{-1}, and the highest value of potato tuber average weight stood 131.33 gm.*
4- *The interaction between drip irrigation and 4 days irrigation interval was superior compared to other interactions in obtaining the least value of the electrical conductivity (Ece) stood 2.52 ds.m^{-1}, and highest value of single plant yield stood 884 gm.plant^{-1}, and highest value for the average weight of potato tuber stood 161.17 gm. On the other hand, the interaction between furrow irrigation method and the 8 days irrigation interval in obtaining the highest value for porosity which stood 0.44 cm3.cm^{-3}.*
5- *The interaction between drip irrigation method and mechanical planting method was superior compared to other interactions in obtaining the highest yield value for single plant which stood 846 gm.plant^{-1}, and the highest value for the weight average of potato tuber stood 157.50 gm. while, the interaction between furrow irrigation method and mechanical planting recorded the highest value for porosity which stood 0.46 cm^3.cm^{-3}.*
6- *The interaction between 4 days irrigation interval and mechanical planting showed a superiority in obtaining the highest value for single plant yield which stood 770 gm.plant^{-1}, and highest value for the weight average for potato tuber stood 140.44 gm, compared to other interactions, and the interaction between 8days irrigation interval and mechanical planting method was superior to obtain the highest value of porosity stood 0.42 cm^3.cm^{-3}. Also, the interaction between 4 days irrigation interval and the manual (hand) planting methods was superior to obtain the least value for electrical conductivity for soil solution stood 3.34 ds.m^{-1}.*
7- *The triple interaction between drip irrigation, 4 days irrigation interval, and mechanical planting method*

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was superior in obtaining the highest yield for a single plant which stood 936 gm.plant^{-1} and the highest value of the weight average of potato tuber which stood 169.33 gm and the lowest value for electrical conductivity of soil solution which stood 2.50 ds.m^{-1} compared to other interactions. While the interaction between furrow irrigation method, 8 days irrigation interval, and mechanical planting method was superior to obtain the highest value of soil porosity stood 0.48 cm^3.cm^{-3}.

Keywords— Sprinkler Irrigation, drip irrigation, soil porosity, mechanical planting, irrigation interval.

I. INTRODUCTION

The agricultural mechanization is one of the continuously developed requirements of agricultural production that aims to reduce the costs and increase the production, faster accomplishment of field operations, minimized manual labor and efficient use of time. Therefore, the trend had started towards the ideal use of agricultural machines and equipment from the stage of soil preparation through planting and crop service operation up to harvesting of crops and post-harvesting. Potato cultivators had an important role in developing the potato crop planting through the precise planting operation in terms planting depth and dimensions, the speed of accomplishment and the efficient use of the unit of area.

Some studies and experiments have proven that using the drip irrigation system will save large amounts of water compared to conventional irrigation methods, and as for sprinkle irrigation system which is also contributes in saving large amounts of water relatively (Altaif and Alhadithi, 1988).

The problem of water scarcity had emerged in the irrigated fields in dry and semi-dry areas in which our country is located where farmers in the mid and southern parts of Iraq are suffering from that problem. The severe reduction of the annual average of water income of Tigris and Euphrates rivers and level fluctuation from one season to another had affected and deteriorated these resources (AL- shahrabali, 2009). Therefore, there were suggestions and studies including the use of many methods to possibly confront the scarcity of water somehow. For example, the efficient management in controlling the amount of water at every single watering and the number of irrigations (irrigation scheduling) and the use of modern and proper irrigation systems with less water losses. Also, irrigation scheduling has a significant effect in potato crop production and components (Demelash, 2013).

Solanumtuberosum L Potato is an important vegetable crop that follows the solanaceae family. Its name came from solanum gender. It is one of four crops in the world in terms of the nutritious importance after wheat, corn, and rice where it comes first in terms of tuberculosis crops (Hasan, 1990). Potato tubers are important source for energy because it is rich of carbohydrates and has many proteins, vitamins, salts, minerals, and amino acids. It contains 18 out of 20 amino acidsthat are necessary to human being which gives it a high biological value (NAPCO, 2005). According to the importance of choosing the best irrigation methods, irrigation interval and potato planting methods for potato planting, this experiment was done.

II MATERIALS AND METHODS:

A field experiment has been conducted to evaluate the effect of different irrigation systems and planting methods on soil porosity and soil electrical conductivity and Solanumtuberosum L potato crop yield for fall season of 2016 in Yousufia region which is located 15 km south west of Baghdad at 75.18.44 meridian east and 84.07.33 latitude north. This land features a flat to semi-flat ground with altitude of 34.1 m above sea level.

Three irrigation systems included Sprinkler Irrigation (S), Drip irrigation (D),and Furrow Irrigation (F), two different irrigation intervals included (4 day irrigation interval (I1) and 8 day irrigation interval (I2)) and two methods of planting included (Mechanical planting (M) and Manual (Hand) planting (H) were used in the experiment. Soil Porosity, electrical conductivity of a saturated soil extract (Ece), average weight of potato tuber, and plant yield were measured in this study. Split split plots arrangement under Randomized Complete Block Design (RCBD) with three replicates, were used in this experiment. The means of treatments were compared by using least significant difference (LSD=0.05) under probability of 0.05.

Samples of field soil were taken from five different locations randomly for analysis. Chemical and physical analysis was illustrated in table (1) and the soil texture was classified as silt clay loam.

Soil was prepared by plowing using mold board plow after drenching the soil with water to get the right moisture for plowing which is (16-18) %. After primary tillage, secondary tillage was conducted using rotary harrow then leveling was conducted with leveling machine. Then, the field was divided into the experimental units.

Potato tubers type (Riviera) rank (A) were planted at 15/9/2016 on furrows with a distance of 75 cm between each line, 25 cm between each tuber, and depth of (10-18)

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cm and through the extension of planting lines. The number of the lines on a single experimental unit were 8 lines. The length of the line for one experimental unit 11 m. the number of the plants on a single line were 44. The density was 352 plants / unit. Every irrigation method had 4224 plants. The total number of plants in the field were 12672 plant.

After maturity signs appeared (vegetative growth halt, yellow leaves appearance with tuber crust hardening and colored with light brown and aerial stems hardening) the vegetative parts were cut from the contact spot with soil. After two days, i.e at 24/12/2016 the tuber was extracted manually. Then, the tuber yield was calculated from each experimental unit separately after sorting the damaged tubers.

Urea fertilizer was used (46% N) with average of 70 kg/hectare with three doses, one quarter was with planting, another quarter was with the growth of tubers, the last half was used in the stage of tuber filling. Super tri-phosphate (46% P2O5) was used by 70 kg / hectare added as a whole with soil preparation for planting. Potassium sulfate was used (52% K2O) with 80/hectare added as two doses, This procedure was according to the recommendations from Ibaa center for Agricultural research 1994 (Alzawbai, 2000).

Table.1: Chemical and physical characteristics of the studied soil

Soil characteristic	**unit**		**value**
Electrical Conductivity (ECe)	$ds.m^{-1}$		2.80
PH			7.56
Soil elements	Nitrogen	$mgm.kg^{-1}$	34.50
	Phosphor		27.13
Soil compounds	Sand	$gm.kg^{-1}$	16
	Silt		540
	Clay		300
Texture	Silt Clay Loam		
Bulk Density	$Mgm.m^{-3}$		1.40

Studied Properties Measurments:

1-Electrical conductivity for the saturated dough solution (ECe), $ds.m^{-1}$

Electrical conductivity was measured for soil solution using electrical conductivity device for soil solution (EC-meter) according to the method mentioned in (Jackson 1958).

2-Porosity, %.

Total Porosity was calculated from the value of bulk and particle densities following the equation from (Audah, 1990)

$$f = \left(1 - \frac{\rho_b}{\rho_s}\right) \times 100 \quad \ldots\ldots (9)$$

Where:

f : Soil porosity %

ρ_b : Bulk density, $Mgm.m^{-3}$

ρ_s : Particle density, $Mgm.m^{-3}$

3-Plant yield,gm. $plant^{-1}$

The total number of plants selected from each experimental unit was calculated and then divided into the number of plants selected for the same unit to obtain the plant yield.

4-Weight of the tuber,gm

10 randomly selected plants were taken from the middle lines. The weight of each plant was measured on the number of tubers per plant to extract the weight of the tuber and the weight of the tuber = the weight of the crop / number of tubers.

III RESULTS AND DISCUSSION

Electrical conductivity:

Table (2) shows the effect of irrigation methods and intervals, and planting methods on soil electrical conductivity values. Sprinkle irrigation treatment got the highest value of soil electrical conductivity stood $4.27ds.m^{-1}$. Then furrow irrigation treatment gotsoil electrical conductivity stood $3.92ds.m^{-1}$, whereas drip irrigation treatment got 2.76 $ds.m^{-1}$. These results come in agreement with the results obtained by Francois and Bernstein, 1973.

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The table also showed that irrigation intervals have significant effect on electrical conductivity for soil solution where the highest value was at 8 days irrigation interval 3.89 ds.m^{-1} compared to a less value with 4 days irrigation interval 3.40 ds.m^{-1}.

Planting methods treatments had a significant effect on the response. Mechanical planting methods had a value of 3.71 Ds.m^{-1} compared to 3.59 Ds.m^{-1} with manual planting.

The interaction between irrigation methods and irrigation intervals indicates there are significant differences. The highest value was recorded between 8 days irrigation interval and sprinkle irrigation 4.59 ds.m^{-1} compared to drip irrigation and 4 days period 2.52 ds.m^{-1}.

Results show significant differences for electrical conductivity due to the dual interaction between irrigation and planting methods. The least value of interaction was with drip irrigation and manual planting2.72 ds.m^{-1} compared to the highest value between sprinkle irrigation and manual planting 4.29 Ds.m^{-1}.

The table showed significant differences between electrical conductivity due to the interaction between irrigation methods and intervals and planting methods. The highest value was recorded with sprinkler irrigation, second period, and manual planting 4.72 ds.m^{-1} compared to drip irrigation, 4 days irrigation interval, and mechanical planting 2.50 ds.m^{-1}.

Table.2: The effect of irrigation methods and intervals and planting methods on soil electrical conductivity, ds.m^{-1}

Irrigation method	**Irrigation interval (day)**	**interaction between irrigation method and intervals and planting methods**		**Interaction between irrigation methods and irrigation intervals**
		Planting methods		
		M	H	
S	I_1	4.02	3.86	3.94
	I_2	4.46	4.72	4.59
D	I_1	2.50	2.53	2.52
	I_2	3.08	2.91	3.00
F	I_1	3.86	3.64	3.75
	I_2	4.32	3.88	4.10
L.S.D =0.05		0.14		0.09
mean		3.71	3.59	
L.S.D =0.05		0.06		
Irrigation intervals		Interaction between irrigation intervals and planting methods		mean
I_1		3.46	3.34	3.40
I_2		3.95	3.84	3.89
L.S.D =0.05		N.S		0.07
Irrigation methods		Interaction between irrigation and planting methods		mean
S		4.24	4.29	4.27
D		2.79	2.72	2.76
F		4.09	3.76	3.92
L.S.D =0.05		0.09		0.07

Total Porosity,%.

Table (3) shows the effect of irrigation methods and intervals on porosity. It can be noticed that there are significant differences in porosity values attributed by irrigation treatments where the highest value recorded with furrow irrigation stood 0.44 %. Then, drip irrigation came with a lower porosity value of 0.4 %compared with sprinkle irrigation with a value stood 0.34 %. The reason is due to the movement of soil particles with each other especially the fine ones during the irrigation and

precipitated in the big pores thus reducing the porosity from one irrigation method to another. These results come to agreement with Rose, (1961).

The table also shows significant differences between porosity values due to the effect of irrigation intervals treatments. The highest value recorded at 8 days irrigation interval 0.4 %compared to 4 days irrigation interval 0.38 %.

Also, one can notice from table (5) that there are significant differences for porosity values due to planting methods. The mechanical method gave 0.4 %whereas manual method was 0.38 %.

The table indicates a significant effect for the two-way interaction between irrigation methods and intervals on porosity. The results were 0.44 % for interaction of furrow irrigation and 8 days irrigation interval compared with 0.32 %with sprinkle irrigation and 4 days irrigation interval.

There is a significant effect for the interaction between irrigation and planting methods on porosity. Furrow irrigation and mechanical planting gave the highest values for porosity 0.46 % compared to sprinkle irrigation and manual planting where gave the least value of 0.33 %.

Results showed significant effect on porosity when using the interaction between irrigation intervals and planting methods. Porosity value was 0.42 % with 8 days period and mechanical planting compared to 0.37 % with first irrigation interval and manual planting.

Table (3) showed no significant effect for the interaction between irrigation methods and intervals, and planting methods on porosity.

Table.3): The effect of irrigation methods and intervals and planting methods on porosity,%

Irrigation method	**Irrigation interval (day)**	**Interaction between irrigation method and intervals, and planting methods**		**Interaction between irrigation methods and intervals**
		Planting methods		
		M	**H**	
S	I_1	0.32	0.32	0.32
	I_2	0.37	0.35	0.36
D	I_1	0.40	0.40	0.40
	I_2	0.42	0.39	0.41
F	I_1	0.45	0.41	0.43
	I_2	0.48	0.41	0.44
L.S.D =0.05		N.S		0.01
mean		0.40	0.38	
L.S.D =0.05		0.007		
Irrigation intervals		Interaction between irrigation intervals and planting methods		mean
I_1		0,39	0.37	0.38
I_2		0.42	0.38	0.40
L.S.D =0.05		0.008		0.005
Irrigation methods		Interaction of irrigation and planting methods		mean
S		0.34	0.33	0.34
D		0.41	0.39	0.40
F		0.46	0.41	0.44
L.S.D =0.05		0.01		0.01

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Plant yield,gm.plant^{-1}

Table 4 shows the effect of irrigation methods, and intervals, and planting methods and their interferences on the plant yield of the potato. Drip irrigation got the highest plant yield stood 811 gm.plant^{-1}followed by the sprinkler irrigation method got 642 gm.plant^{-1}and then the furrow irrigation method got the lowest yield stood 546 gm.plant^{-1}. These results are consistent with the results obtained by Nagazet.al., (2000).

The irrigation interval had a significant effect on the yield of the plant. 4 days irrigation interval was significant superior in getting higher yield stood 731gm.plant^{-1}, whereas 8 days irrigation intervals got the lowest value of the plant yield stood 601 gm.plant^{-1}.This was due to the lack of vegetation and therefore less surface area of the plant, which is the process of photosynthesis, and these results are consistent with the results obtained by Aldjoy (1999).

The table also showed significant differences in plant yield attributed by planting methods, where mechanical planting treatment got highest plant yield stood 703 gm.plant^{-1}compared with manual planting, and may The reason for the regularity of agriculture in the mechanical way in terms of the distance between the tubers and the depth of agriculture. The overlap between irrigation methods and irrigation intervals showed no significant effect.

Table 4 showed significant differences due to the double interference between the irrigation methods and planting methods. The interaction between drip irrigation and mechanical planting method got The highest value of the plant yield stood 846 gm.plant^{-1}, and also showed no significant differences in plant yield due to the bilateral interference between irrigation intervals and planting methods.

Table 4 showed significant differences in the values of the plant yield due to the triangular interference between the irrigation methods, irrigation interval and planting methods, where the highest value was recorded at the triple overlap between the drip irrigation and 4 days interval and the mechanical planting stood 936 gm.plant^{-1} while the lowest value when the overlap between irrigation furrow and 8 days interval and hand-planting method stood 470 gm.plant^{-1}**.**

Table.4: The effect of irrigation methods, irrigation intervals and planting methods on the plant yield, gm.plant^{-1}

Irrigation method	***Irrigation interval (day)***	***Interaction between irrigation method and intervals and planting methods***		***Interaction between irrigation methods and irrigation intervals***
		Planting methods		
		M	*H*	
S	I_1	*745*	*672*	*708*
	I_2	*634*	*517*	*575*
D	I_1	*936*	*833*	*884*
	I_2	*757*	*718*	*737*
F	I_1	*631*	*571*	*601*
	I_2	*515*	*470*	*492*
L.S.D =0.05		*37.27*		*N.S*
		703	*630*	
L.S.D =0.05		*13.47*		
Irrigation intervals		*Interaction between irrigation intervals and planting methods*		*mean*
I_1		*770*	*692*	*731*
I_2		*635*	*568*	*601*
L.S.D =0.05		*N.S*		*12.47*
Irrigation methods		*Interaction between irrigation and planting methods*		*mean*
S		*689*	*594*	*642*
D		*846*	*775*	*811*
F		*573*	*520*	*546*
L.S.D =0.05		*18.34*		*11.62*

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Weight of the tuber,gm

Table 5 showed the effect of irrigation methods, and intervals and planting methods on the weight of the tuber. There are significant differences in the weight of the tuber due to the irrigation methods. Sprinkler irrigation was superior in getting the highest value of the tuber weight stood121.67 gm and drip irrigation got 150 gm, and furrow irrigation got weight, of tuber stood 107.67 gm. The table also showed that there are significant differences between the weights of the tuber attributed by irrigation interval. The highest value of the weight of the tuber was recorded at the time of 4 days irrigation interval stood 135.06 gm. This is due to a relationship between water shortage and the production of potato tubers. The dryness of soil during the time of tuber formation should reduce the number and size of tubers per plant. Table 5 showed significant differences in the mean weight of the tuber due to the effect of the treatment of planting methods. Mechanical planting got the highest weight of the tuber stood 131.33 gm. may be due to the regularity of agriculture in the mechanical method in terms of distance between the tubers and the order of the depths of agriculture, which leads to consistency in germination and inequality and this increases production.

There were significant differences in the mean weight of tuber due to the double interference between the irrigation methods and irrigation interval. The interaction between drip irrigation method and 4 days irrigation interval gave the highest value of the tuber weight stood 161.17 gm. The table also showed significant differences in the mean weight of the tuber due to the interference between irrigation methods and planting methods. The highest value of the tuber weight was obtained by the drip irrigation method with mechanical planting stood 157.50 gm, furrow irrigation and manual planting got 106.33 gm. The table showed that there are no significant differences in the values of plant yield due to the bilateral interference between irrigation method and intervals and planting methods.

Table.5: The effect of irrigation methods, and intervals and planting methods on the of weight of tuber, gm.

Interaction between irrigation methods and irrigation intervals	**Interaction between irrigation method and intervals and planting methods**		**Irrigation interval (day)**	**Irrigation method**
	Planting methods			
	H	**M**		
130.67	124.67	136.67	I_1	S
112.67	107.00	118.33	I_2	
161.17	153.00	169.33	I_1	D
138.83	132.00	145.67	I_2	
113.33	111.33	115.33	I_1	F
102.00	101.33	102.67	I_2	
1.95	N.S			L.S.D =0.05
126.44	121.56	131.33		
	1.58			L.S.D =0.05
mean	Interaction between irrigation intervals and planting methods			Irrigation intervals
135.06	129.67	140.44		I_1
117.83	113.44	122.22		I_2
1.40	N.S			L.S.D =0.05
mean	Interaction between irrigation and planting methods			Irrigation methods
121.67	115.83	127.50		S
150.00	142.50	157.50		D
107.67	106.33	109.00		F
1.43	2.18			L.S.D =0.05

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Assessment of Climatic Factors on Growth and Yield of Maize Variety as Influenced by Rates of Sunshine Organic Manure and NPK 20:10:10 Fertilizer

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Abstract— *Maize is one of the most important cereal crops in West Africa. Its production in Nigeria has been hindered by inconsistency in rainfall pattern and low fertility especially in Akure, Ondo State. Two experiments were conducted at the Teaching and Research Farm, Federal University of Technology, Akure (FUTA) in 2016 growing season (wet and dry seasons) to determine the effects of Sunshine Organic Manure and NPK 20:10:10 fertilizer on the growth and yield maize variety, as well in soil fertility improvement. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications per treatment. Suwan^{-1}-SR-Ymaize varietywas used for the experiment and Sunshine Organic manure was applied at the rates of 0, 60, 90, and 120kg N ha^{-1}while NPK 20:10:10 was used as standard at the rate of 70 kg N ha^{-1}. Growth parameters (plant height and number of leaves per plant) were taken at two weeks interval for 12 weeks. At harvest, yield parameters (Seed weight/plant, Weight of 1000 seed (g), Number of seeds/cob, Yield in t ha^{-1}, cob length (cm), cob girth (cm)) were determined. The following weather data were collected; Daily rainfall, maximum & minimum temperature and solar radiation, while the soil data collected were; pH, total N, available phosphorus, potassium, organic carbon, and bulk density. Statistical Package for Social Sciences (SPSS) was used for the data analyses. Mean separation was done using Duncan's Multiple Range Test (DMRT). Results showed an increase in growth and yield parameters recorded with increasing application rates of Sunshine Organic Manure in both growing season.*

Keywords— Maize, Fertilizer, climate, Growth, Yield.

I. INTRODUCTION

Cereal production is a major component of small-scale farming in West Africa. Among cereals, maize is one of the most important as it forms the major staple food for most communities and contributes about 20% of calories to human diet (Braimoh and Vlek, 2006). However, average maize yields per unit of land have fallen over the years, partly due to loss in soil fertility as a result of unsustainable farming activities, especially in the wetter areas where the yield potential is higher (Sanchez, 2002) and partly due to low external inputs (Fosu*et al.*, 2004).

As farmers battle with low soil fertility, climate change presents an additional burden, which for them translates into production risks associated with crop yields, due to the probability of extreme events, the uncertainty of the timing of field operations, and of investments in new technologies. The concern for the present and future climate aberrations, weather trends and their implications for agriculture continue to stimulate researchers as well as public and policy-level interests regarding the analysis of climate change in relation to agricultural productivity (IPCC 2007; Cooper *et al.*, 2006). Reported projections indicate that with the trend in climate change and variability, the impacts on people's livelihoods will be greatest in Africa, where many poor smallholders largely or totally rely on rain-fed agriculture and have few alternatives (IPCC, 2001; Boko*et al.*, 2007), due to high levels of poverty, low levels of human and physical capital, and poor infrastructure (IFPRI, 2009). The specific objective was to determine the effects of organic fertilizer application rates on growth and yield of

maize as well as assessing the impacts of climate on crop performance.

II. MATERIALS AND METHODS

The research experiment was carried out during the rainy season (March - July) and dry season (September-December) at the Teaching and Research Farm of the Federal University of Technology, Akure (FUTA) ($7^0$16'N, $5^0$12'E) located in the Rain forest agro-ecological zone of Nigeria in 2016 growing season.

Each trial was laid out in a Randomized Complete Block Design (RCBD) with three replications per treatment.The size of the field was 14 m by 14 m (196 m^2) and each experimental unit was 4 m by 2 m with 1 m alley. There were 15 plots. The allocation of treatments to each experimental unit was done using the Plant Breeding Tools (PBTools) Version: 1.3.

The maize variety (Suwan^{-1}-SR-Y) was obtained from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan in Nigeria. It was an improved maize variety fortified with protein. Maize seeds were sown two seeds per hole with a spacing of 75 cm by 25 cm but were later thinned to one plant stands 2weeks after planting. Weeding was carried out manually (hoeing and hand-pulling). Growth parameters (plant height and number of leaves per plant) were taken at two weeks interval for 12 weeks. At 8 weeks after planting, fresh leaves and stem weights, oven dried leaves and stem weight were determined while the leaf area was determined using the Leaf area meter. Plant height was measured using a tape in centimeters and average leaf number was determined by counting the total number of leaves of two plant stands in each plot. At harvest, yield parameters (Seed weight/plant, Weight of 1000 seed (g), Number of seeds/cob, Yields in t ha^{-1}, Cob length (cm) and Cob girth (cm)) were determined.

The Sunshine Organic Manure (S.O.M) was applied at varying rates of 0, 60, 90, 120 kgN $ha^{-1}$and NPK 20:10:10 at 70kgN ha^{-1}as recommended rate.

The weather data required include; daily rainfall, maximum & minimum temperature and solar radiation and were collected from the West African Science Service Center on Climate Change and Adapted Land Use (WASCAL) weather observatory, Federal University of Technology, Akure.

Soil Analysis

Core samples were used for determination of soil physical properties. The auger samples were air- dried, grinded and passed through 2 mm sieve and used to determine chemical properties. The methods that were applied were: hydrometer method for soil texture (Jacob and Clark, 2002), Kjeldahl method for total nitrogen (Bremner and Mulvaney, 1982), and the modified Walkley- Black wet oxidation procedure for organic carbon content. Multiplication of the soil organic carbon by 1.72 resulted in soil organic matter (Nelson and Sommers, 1982). Titration method with EDTA solution were used for measuring calcium and magnesium (Lanyon and Heald, 1982), sampling cylinder method for Bulk density (Jacob and Clark, 2002) and the soil pH was carried out using 1:2.5 soil/water ratio and the values were read off using Beckman zeromatric pH meter (Peech, 1965). The amount of phosphorus in soil extracts of soil were determined by Spectrophotometer (Olsen and Sommers, 1982). Exchangeable K and Na after extraction were extracted using 1 N ammonium acetate (pH= 7) and read with flame photometer (Knudsen *et al.*,1982). Statistical Package for Social Sciences (SPSS) and Decision Support Systems for Agro-Technology Transfer (DSSAT) were used for data analyses. Mean separation was done using Duncan's Multiple Range Test (DMRT).

III. RESULTS

Initial Soil Physicochemical Analysis for both growing seasons

Table 1 shows the monthly means weather data (Rainfall, minimum and maximum temperature and solar radiation) for 2016 at the experimental site. The results of initial chemical and physical analyses of the soil at the experimental site in 2016 for the two growing seasons (wet and dry) are presented in Tables 2 and 3. Both experiments were carried out on the same field. The soil pH was noted to be slightly acidic across the 3 soil depths (0-15, 15-30 and 30-45 cm). The soil pH decrease down the depth (6.31, 6.18, 6.21). The total N value in the top 15 cm layer was moderately available during the first growing season, but was low in the second season (0.34 in the first growing season and 0.19 in the second season). The percentage organic matter (OM) was 0.86, 0.55 and 0.43 in top 15, 30 and 45 cm soil layer, respectively. The available phosphorus was low across the 3 layers 0-15 (10.95 mg kg^{-1}), 15-30 (6.54 mg kg^{-1}) and 30-45cm (3.43 mg kg^{-1}), exchangeable K was moderately available at 0-15 soil depth (0.33) but was low in the top 30 and 45cm layer (0.29 and 0.24 cmol kg^{-1}, respectively). The textural class of the soil is Sandy clay loam. The soil textural class increased down the soil horizon whiles % sand decreased with increasing soil depth. The bulk density (g cm^{-3}) in each layer (0-15, 15-30 and 30-45cm) were 1.52, 1.54, 1.55 respectively.

Soil available P can be rated as low and K as moderate according to Page et al. (1982). Similarly, the percent

organic carbon (0.5 and 0.55) is rated very low according to Landon (1996).

Effects of Cultivars and Organic Fertilizer Rates on Growth and Yield of Maize in the wet and dry season of 2016

Table 4 shows the effects of fertilizer application on plant height of selected maize varieties during the 2016 wet and dry season. In the wet season, significant differences ($P > 0.05$) were not observed at 4 and 10 weeks after planting (WAP) while significant differences were recorded in the other weeks. However, maize varieties planted in the control (No fertilizer) plot had the shortest plants across the weeks of the experiments while the plots treated with NPK 20:10:10 had the tallest plants. The sunshine organic manure that was applied at 120 kg N ha^{-1} performed better compared to the other rates of application (60 and 90 kg N ha^{-1}). Statistically, there was no significant difference ($P > 0.05$) in the heights of maizetreated with 60, 90 and 120 kg N ha^{-1} of Sunshine Organic Manure at 4, 6, 8 and 10WAP. In the dry season planting, significant differences ($P > 0.05$) were observed across the weeks of the experiment (4 – 12WAP). However, maize varieties planted in the control plot (No fertilizer) had the shortest plant across the other weeks of the experiments while the plot treated with NPK 20:10:10 had the tallest plant. Also from the table, sunshine organic manure that was applied at 120kg N ha^{-1} performs better compared to the other rates of application (60 and 90kg N ha^{-1}), although there were no significant differences between plant of maize treated with SOM 90 and 120kg N ha^{-1} at 6, 10 and 12WAP. Among the Sunshine Organic manure rates, the application at 60kg N ha^{-1} had the shortest plant.

The results on the effects of Sunshine Organic manure and NPK fertilizers on number of leaves and leaf area (cm^2) of selected maize varieties in the wet and dry season of 2016 were presented in (Table 5). In the wet season, significant differences were not recorded in the number of leaves across the weeks of the experiment except at 4WAP when the control was not significantly different from the others. However, maize treated with NPK 20:10:10 and sunshine organic manure applied at 90 kg N ha^{-1} had the highest number of leaves. Statistically, no significant differences were observed in the *Zea mays* number of leaves in all the Organic fertilizer plots across the weeks of the experiment. Regarding the leaf area, there were significant differences ($P > 0.05$) among the fertilizer rates, the NPK 20:10:10 fertilizer produced maizewith the largest leaf area while the control experiment had the least. Comparing the performance of different organic fertilizer rates, the Sunshine organic manure applied at the rate of 120 kg N ha^{-1} had the largest leaf area while SOM applied at 60 kg N ha^{-1}produced the smallest leaf area

In the dry season experiment, significant differences were recorded in the number of leaf across the weeks of the experiment. However, maize treated with NPK 20:10:10 and sunshine organic manure applied at 120kg N ha^{-1} had the highest number of leaf. Statistically, there were no significant differences between the number of leaf of maize treated with SOM at the rate of 90 and 120kg N ha^{-1}. Regarding the leaf area, there were significant differences ($P > 0.05$) among all the fertilizer rates, the NPK 20:10:10 fertilizer produced maize with the largest leaf area while the control experiment had the least. Comparing the performance of different organic fertilizer rates, the Sunshine organic manure applied at the rate of 120kg N ha^{-1} had the largest leaf area while SOM applied at 60kg N ha^{-1} produced maize with the smallest leaf area. All SOM rates were significantly different from one another.

Table 6 shows the effects of fertilizers on yield parameters (Seed weight/plant, Weight of 1000seed, No of seed/cob, Yield in tha^{-1}, Cob length and Cob girth) of selected maize varieties during the wet and dry season of 2016. In the wet season, significant differences were recorded in the across the aforementioned yield parameters. The NPK 20:10:10 produced the highest yield while the control experiment had the least. However, examining the performances of the maize varieties with sunshine organic manures at varying rates of application shows that the SOM 120 kg N ha^{-1} rate performed best compared to other organic manure rates. Although there was no significant difference among the organic manures rates in the following yield parameters; seed weight/plant and weight of 1000seed. The statistical analysis of the yield parameter also indicated that the control experiment had a better yield because there were no significant differences in the following yield parameters (cob girth, cob length and weight of 1000seed) when compared to the other organic fertilizer rates.

In the second growing season (dry season),significant differences were recorded in the seed weight/plant, Weight of 1000seed, No of seed/cob, Yield in tha^{-1}, Cob length and Cob girth. The NPK 20:10:10 fertilizer produced the highest yield while the control experiment had the least. However, regarding the influence of varying Sunshine Organic Manure rate on the maize varieties shows that the SOM applied at the rate of 120kg N ha^{-1} performed best compare to other organic manure rates. Although there was no significant difference between the application of SOM at 90 and 120kg N ha^{-1} in the following yield parameters; weight of 1000seed, yield in tha^{-1}, cob length and cob girth of selected maize varieties. The statistical analysis of the

yield parameter also indicated that the control experiment was significantly different from the other treatment and also had the lowest yield.

IV. DISCUSSION

This study clearly demonstrates the effects of Sunshine Organic Manure (SOM) at different rates on the growth and yield of *Zea mays*. A general assessment of the effects of SOM on growth and yield parameters recorded across the two growing seasons showed that the organic fertilizer positively influenced the performance of the crop with increasing rates of application. However, this trend was clearly observed during the dry season experiment (second growing season) compared to the wet season (first growing season). In the first growing season, the SOM applied at 0, 60, 90 and 120kg N ha^{-1}were not significantly different from one another for some parameters, such as number of leaves, plant height at 6WAP and 10WAP, weight of 1000seeds,Cob length, Cob girth and seed weight/plant. The control experiment (No fertilizer) had the lowest yield in both trials when compared to the fertilized plots. During the first growing season, significant differences were not observed between the control (no fertilizer) and some application rates of the SOM (60, 90 and 120kg N ha^{-1}) for some parameters e.g. plant height at 8WAP, cob length, cob girth and no of seeds/cob. The reason for these results may be attributed to the influence of the existing soil nutrients. From the initial soil analysis, the results revealed that the soil nutrients tested for N, P, K, Ca, Ma, OM and %OC were moderately available in the soil, according to the soil analysis guidelines for interpretation (Thiagalingam, 2000). This clearly explains the reason why maize normally planted without fertilizer application by local farmers gives a better yield.

It was also observed from the study that NPK proved to be efficacious as a good source of inorganic fertilizer that supported good vegetative growth and yield performance of maize. The NPK 20:10:10 fertilizer performance was better compared to the SOM at different rates (60, 90 and 120kg N ha^{-1}). However, this may be attributed to the percentage concentrations of Nitrogen, Phosphorus and Potassium present in the SOM (3% N: 1.5% P: 1%K). The percentage of N present in the SOM is about 6 times lower as compared to that of N content of the NPK fertilizer (20%N). The initial soil analysis that was carried out at the commencement of the second experiment revealed that the nitrogen content of the soil was low. The low amount of total soil N may be attributed to low soil organic matter because Nitrogen is one of the most essential components of organic matter. The decomposition of organic matter leads to the release of some nutrients including N.

The second experiment commenced two weeks after harvesting the first trial. Ploughing was carried out a week after harvesting and a week before planting the second trial. Therefore, the low soil organic matter may be attributed to high C: N ratio of undecomposed maize residues (straw). A study conducted by Kpongor (2007), stated that crop residues and farmyard manure increases Soil Organic Content.

Effects of environmental factors

The following environmental factors might have affected the maize performances during the two growing seasons; temperature, moisture availability, solar radiation, soil structure, and soil nutrients.

The first planting commenced on 14 May, 2016; the mean monthly weather data for the Solar Radiation, Maximum Temperature, Minimum Temperature and Rainfall were 18.0, 30.5, 22.9 and 54.8 respectively. Despite the low mean monthly rainfall recorded at the time of establishing the first trial (time of sowing), over 85% germination was recorded 6 days after sowing (visual observation). This result may be attributed to the influence of the soil physical properties on seed germination. The % Sand, Silt and Clay of the soil were 60.3%, 21.6% and 18.1% respectively. The small percentage of clay content present in the soil might have been responsible for soil water retention ability. Also, the bulk density and % soil moisture content were 1.57gcm^{-3} and 22.60% and rainfall was continuous and sufficient.

According to Walter (1967), for sowing it is important to know whether the rains are continuous and sufficient to ensure enough soil moisture during planting, and whether this level will be maintained or even increased during the growing period in order to avoid total crop failure. This is because water is essential for all plant growth and development and it is an integral part of living systems.

However, reduction in maize growth and yield parameters recorded during the 2nd growing season could not be easily linked to soil water availability because the mean average rainfall for both growing season (197mm and 139.3mm) were enough for optimum yield production. According to Oldeman and Suardi (1977), maize needs an average monthly precipitation of 100 to 140 mm. They basically takes 3 to 3.5 months for optimum growth and will need an average of 300-500 mm of precipitation during this period.

The 2nd growing season began September 1st, 2016, and ended December 8th, 2016. Comparing the performances of the maize cultivar in both growing seasons, it was observed that the yield was higher in the first growing season than the second season. Despite the fact that the same treatment

(Fertilizer types and maize varieties) and management practices were applied, there was a reduction in maize yield. In addition, the experiment was also conducted on the same site (Teaching and Research Farm of the Federal University of Technology, Akure). Also, grains harvested from the first trial were not used as seeds for the second experiment because of the likelihood of segregation, so new similar maize varieties were obtained from the Institute of Agricultural Research and Training, Moor Plantation, Ibadan in Nigeria.

After assessing all the likelihood reasons that may be responsible for low yield recorded during the second experiment, the following factors were suspected to have led to decease in yield of the selected maize cultivars; High temperature and low soil fertility.

Month	Solar Radiation	TMax	TMin	Rain
September	15.7	27.5	21.7	363.2
October	18.7	29.0	21.8	168.6
November	20.0	30.8	22.2	25.4
December	18.7	31.4	21.4	0.0

The table above is an extract from the aforementioned mean monthly weather data for 2016 (January to December). From the table above, it was observed that the solar radiation, Maximum and Minimum Temperature increases from September to December while rainfall decreases. The maize for the second trial was sown on September 1st, 2016. The mid-October marked the end of vegetative growth stage and the initiation of the tasseling, silking and milking stage. However, the implication of this on the yield of maize is that increase in temperature will accelerate physiological development i.e. hastening maturation thereby reduces yield. In addition, the soil moisture stress is critical at this development stage (grain filling) of the plant, which can have a serious effect on grain size and weight and hence on yields.

As earlier suggested by Boote and Sinclair (2006) high temperature reduces yield by accelerating physiological development (hastening maturation), not allowing the crop to progress slowly through the season so as to maximize time for the capture of resources and for assimilate partitioning to reproductive structures.

V. CONCLUSIONS

Maize grain yield was positively influenced by the rates of the organic fertilizers applied, although the inorganic fertilizer (NPK 20:10:10) had the best yields. The inorganic fertilizer led to decrease in soil pH while the sunshine organic manure helped maintain the soil pH within the optimum range. In line with the objective of this study, which was to acquire a better understanding of the yield potentiality of maize variety using the organic fertilizer rates, it shows the efficacious and productivity of the inorganic fertilizer used (NPK 20:10:10) over different organic fertilizer rates (Sunshine Organic Manure at 60, 90 and 120kgha^{-1}).

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TABLES

Table.1: Monthly Means Weather data for 2016 at the experimental site

Month	Solar Radiation (W/m^2)	Maximum Air Temperature (°C)	Minimum Air Temperature (°C)	Rainfall (mm)
January	18.8	33.8	19.6	9.4
February	19.0	35.4	22.0	0.0
March	17.1	33.1	24.0	149.8
April	19.4	32.1	23.7	15.6
May	18.0	30.5	22.9	54.8
June	15.6	28.2	21.9	321.6
July	14.4	27.4	21.6	148.4
August	13.5	27.4	21.4	263.2
September	15.7	27.5	21.7	363.2
October	18.7	29.0	21.8	168.6
November	20.0	30.8	22.2	25.4
December	18.7	31.4	21.4	0.0

Source: West African Science Service Center on Climate Change and Adapted Land Use (WASCAL) weather station

Table.2: Initial Soil Physicochemical Analysis for the first growing season

	0-15	**15-30**	**30-45**
		Depth (bottom), cm	
Particle size analysis (%)			
Clay	21.6	22	26.5
Silt	18.1	18.6	18.9
Sand	60.3	59.4	54.6
Bulk density g/cm^3	1.52	1.54	1.55
Soil pH	6.31	6.18	6.21
Nitrogen (%)	0.34	0.31	0.29
Phosphorus ($mg\ kg^{-1}$)	10.95	6.54	3.43

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Organic Matter	0.86	0.55	0.431
Exchangeable cation (cmol kg^{-1})			
Potassium	0.33	0.29	0.24
Calcium	3.10	4.01	3.20
Magnesium	2.00	1.80	1.60
ECEC	17.86	19.41	15.30

Table.3: Initial Soil Physicochemical Analysis for the second growing season

	0-15	**15-30**	**30-45**
		Depth (bottom), cm	
Particle size analysis (%)			
Clay	22.0	23.1	28.2
Silt	19.7	21.5	19.5
Sand	58.3	55.4	52.3
Bulk density g/cm^3	1.53	1.51	1.52
Soil pH	6.22	6.17	6.19
Nitrogen (%)	0.19	0.16	0.15
Phosphorus (mg/kg)	6.92	4.45	3.21
Organic Matter	0.95	0.86	0.73
Exchangeable cation (cmol kg^{-1})			
Potassium (cmol/kg)	0.25	0.23	0.20
Calcium (cmol/kg)	2.73	3.23	3.13
Magnesium (cmol/kg)	1.17	1.15	1.14
ECEC	20.12	17.54	15.26

Table.4: Effects of fertilizer treatments on plant height of maize at successive growth periods in the wet and dry season of 2016

Fertilizer	Weeks after planting			
Treatment (Kg N/ha)	4	6	8	10
		Wet season		
0	49.30a	84.87a	144.07a	190.89a
SOM 60	51.74a	91.88ab	144.67a	198.89a
SOM 90	54.60a	91.07ab	155.29a	204.11a
SOM 120	57.62a	96.20ab	158.71ab	213.67a
NPK 70	58.61a	115.06b	184.96b	218.33a
		Dry season		
0	42.44a	59.25a	95.38a	135.53a
SOM 60	46.64ab	71.11b	108.07ab	158.36b
SOM 90	50.98b	83.38c	114.67ab	163.44b
SOM 120	60.50c	88.04c	131.09b	167.64b
NPK 70	70.34d	103.40d	157.91c	191.47c

Means in the same columns not followed by same letters are significantly different at 5% level of probability by Duncan's Multiple Range Test (DMRT).

Table.5: Effects of fertilizer treatments on number of leaves and leaf area of Maizeat successive growth periods in the wet and dry season of 2016

Fertilizer	Weeks after planting				Leaf Area at 8WAP (cm^2)
Treatment (Kg N/ha)	4	6	8	10	
		Wet season			
0	7.57ab	10.09a	10.90a	9.44a	754.22a
SOM 60	6.66a	9.97a	10.73a	9.44a	867.43ab
SOM 90	8.12b	10.59a	10.92a	10.06a	979.37bc
SOM 120	8.02b	10.38a	11.43a	9.50a	1025.74cd
NPK 70	8.40b	10.83a	11.41a	10.28a	1133.12d
		Dry season			
0	6.70a	6.40a	7.91a	8.93a	747.72a
SOM 60	7.43ab	7.49ab	8.91ab	9.58ab	860.93ab
SOM 90	7.83bc	8.51bc	9.71bc	10.04bc	972.87bc
SOM 120	8.40bc	9.31bc	10.42bc	10.56bc	1019.24cd
NPK 70	9.33c	10.27c	11.04c	11.33c	1126.62d

Means in the same columns not followed by same letters are significantly different at 5% level of probability by Duncan's Multiple Range Test (DMRT).

Table.6: Effects of fertilizers on yield parameters of selected maize varietiesduring the wet and dry season of 2016

Fertilizer Treatment (Kg N/ha)	Seed weight/plant	Weight of 1000seed (g)	No of seed/cob	Yield in T ha^{-1}	Cob length (cm)	Cob girth (cm)
			Wet season			
0	171.67a	333.33a	273.56a	1147.67a	12.79a	8.28a
SOM 60	203.78ab	345.56a	328.39ab	1265.78ab	15.36ab	9.96ab
SOM 90	215.01ab	365.56a	322.94a	1392.00ab	15.08a	9.79a
SOM 120	229.00ab	360.00a	347.61ab	1511.56b	16.22ab	10.53ab
NPK 70	297.89b	377.78b	427.72b	1721.67c	19.98b	12.97b
			Dry season			
0	89.67a	237.78a	206.00a	569.44a	9.78a	6.34a
SOM 60	131.22b	277.78b	302.33bc	820.56b	14.06ab	9.10ab
SOM 90	163.11c	307.78bc	345.78bc	1007.78c	16.36bc	10.60bc
SOM 120	194.56d	330.00bc	398.89c	1199.44c	18.08bc	11.74bc
NPK 70	256.22e	363.33d	458.44d	1619.44d	21.16c	13.73c

Means in the same columns not followed by same letters are significantly different at 5% level of probability by Duncan's Multiple Range Test (DMRT).

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Effect of feeding broiler chicken on soybean oil and palm oil supplemented with some feed additives on the quality characteristics of processed chicken nuggets

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***Abstract*—** *The objective of this study was to investigate the effect of feeding broiler chicken on different vegetable oils with feed additives on the quality characteristics of chicken nuggets. A total of 216 one-day-old chicks of (Hubbard) strain were randomly assigned to six dietary treatments as (2×3) factorial designs where two sources of dietary oil contained soybean oil and palm oil with three levels of commercial multi-enzyme feed additives. Treatments were: soybean oil only (T1), soybean oil+ ZAD (T2), soybean oil+ AmPhi-BACT (T3), palm oil only (T4), palm oil + ZAD (T5) and palm oil + AmPhi- BACT (T6). Results showed that chicken nuggetsof T3 group had the higher pH value. No significant differences were found in cooking loss between (T1, T5 andT6) and nuggets of T3and T4. Nugget of T2 group had the higher T.B.A value. No significant effect on shrinkage % of nuggets samples.*

***Keywords*— Broiler feed, Vegetable oils, Feed additives, Chicken nuggets, Quality characteristics.**

I. INTRODUCTION

Chicken meat contains a high protein and low fat content and deliberated as the principal source of polyunsaturated fatty acids (PUFA) with paramount concentration of n-3 PUFA (Howe *et al.*, 2006).

Chicken has been considered an appropriate model in lipid nutrition studies, since it is highly sensitive to dietary fat modifications and many of the studies done with chickens deal with the degree of saturation or source type of the dietary replaced fat and how it influences the performance and carcass quality improvement of the animal (Rymer and Givens, 2005).

Using soybean and palm oil in poultry rations would subsequently affect human health in a positive manner by increasing 18:2 and 18:3 fatty acid contents in animal product without any negative effects on meat quality (Ayed *et al.*, 2015).Palm oil can be used as a vegetable oil in broiler chicken nutrition with positive effects on firmness of meat quality compared with soybean oil and linseed oil (Abdulla *et al.*, 2015).

Commercial enzyme preparations have been used widely to enhance nutritive value of wheat and rye-based diets because of high insoluble non-starch polysaccharides found in these feedstuffs which induce high digesta viscosity (Lázaro *et al.*, 2003). Inclusion of exogenous enzyme in animal's diet has been shown to improve broiler's performance. But the effect on meat quality has to be determined as certain feed additives have been found to affect meat quality (Wang, *et al.*, 2013; Omojola, *et al.*, 2014).

Therefore, this research aims to study the effect of using different vegetable oil sources and feed additives in finisher diets of broiler chicken, on the quality characteristics and lipid oxidation of processed chicken nuggets.

II. MATERIAL AND METHOD

2.1 Experimental Design

The experimental procedures were approved by the Poultry Production Department, Faculty of Agriculture, Ain Shams University and as followed by the Animal Breeding Department, Animal and Poultry Production Division, Desert Research Center.

The current study was conducted at Poultry Experimental Unit, Faculty of Agriculture, Ain Shams University, located in Agricultural Research Station, Shalaqan, Qalyobia Governorate, Egypt. The experiment was a 2 × 3 factorial design with two sources of vegetable oils (soybean oil and palm oil) with three levels of commercial multi-enzyme feed additives as shown in the Table (1).

Table.1: Experimental design

	Feed additives		
Type of oil	Without addition	ZAD[1] 0.5kg/ton	AmPhi-BACT[2] 0.5kg/ton
Soybean oil	Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)
Palm oil	Treatment 4 (T4)	Treatment 5 (T5)	Treatment 6 (T6)

[1] (ZAD) which contains bacteria (*Ruminococcusflavefaciens*) with concentration of (28 x 10^4). Also it contains a mixture of enzymes (Cellulase - Xylanase - α-Amylase -Protease).
[2](AmPhi-BACT), which contains bacteria (*Lactobacillus acidophilus*) and (*Lactobacillus planterum*) and (*Bifidobacteriumbifidum*) and extract ferment of both (*Bacillus subtilus*) and (*Aspergillusniger*) with concentration of 5 g / kg and also contains a mixture of enzymes that is estimated as 34.5 units / gram, that is equivalent to 2 g / kg (Cellulase - Beta-glucanase - Hemicellulase).

A total of 216 one-day-old chicks of (*Hubbard*) strain were used for this study, the chicks were randomly assigned to six treatment groups. Each group consisted of six replicates and each replicate was made up of six chicks. The basal diet was formulated to meet the nutrient requirements of broiler chicken following the National Research Council (NRC, 1994) as shown in Table (2).

Table.2: Feed ingredients and chemical analyses of experimental diets

	Starter (0-11)	Grower (12-22)	Finisher (23-35)					
Ingredients			T1	T2	T3	T4	T5	T6
Corn (grains)	52.05	55.91	56.80	56.80	56.80	56.80	56.80	56.80
Soybean Meal (44%)	31.50	30.00	28.25	28.25	28.25	28.25	28.25	28.25
Corn Gluten Meal (62%)	7.20	4.86	4.40	4.40	4.40	4.40	4.40	4.40
Soybean Oil	3.00	3.65	5.00	5.00	5.00	-	-	-
Palm Oil	-	-	-	-	-	5.00	5.00	5.00
Wheat Bran	2.00	1.50	2.00	2.00	2.00	2.00	2.00	2.00
Di-Calcium Phosphate	1.85	1.60	1.34	1.34	1.34	1.34	1.34	1.34
Calcium Carbonate	1.30	1.50	1.35	1.35	1.35	1.35	1.35	1.35
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Salt (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.29	0.28	0.21	0.21	0.21	0.21	0.21	0.21
L-Lysine HCL	0.21	0.10	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100	100	100
Nutrient content (Calculated) **								
Crude Protein %	23.00	21.00	20.00	20.00	20.00	20.00	20.00	20.00
Crude Fat %	5.69	6.39	7.76	7.76	7.76	7.76	7.76	7.76
Crude Fiber %	3.88	3.75	3.70	3.70	3.70	3.70	3.70	3.70
ME Kcal/ Kg diet	3029	3076	3171	3171	3171	3171	3171	3171
Calcium %	1.00	1.01	0.90	0.90	0.90	0.90	0.90	0.90
Available Phosphorus %	0.50	0.45	0.40	0.40	0.40	0.40	0.40	0.40
Lysine %	1.30	1.15	1.06	1.06	1.06	1.06	1.06	1.06
Methionine &Cystein %	0.97	0.93	0.84	0.84	0.84	0.84	0.84	0.84

* Each 3 Kg of premix contains: Vitamins: A: 12000000 IU; Vit. D3 2000000 IU; E: 10000 mg; K3: 2000 mg; B1:1000 mg; B2: 5000 mg; B6:1500 mg; B12: 10 mg; Biotin: 50 mg; Coline chloride: 250000 mg; Pantothenic acid: 10000 mg; Nicotinic acid: 30000 mg; Folic acid: 1000 mg; Minerals: Mn: 60000 mg; Zn: 50000 mg; Fe: 30000 mg; Cu: 10000 mg; I: 1000 mg; Se: 100 mg and Co: 100 mg.
** Nutrient content calculated based on chemical analysis data of feedstuffs provided by NRC (1994).

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• Starter: one-day-old till 11 days-of-age (basal diet – without additives - all birds).
• Grower: 12 days till 22 days (basal diet - without additives - all birds).
• Finisher: 23 days till 35 days (experimental diets specified per treatment).

Chicks were housed in galvanized cages, where nine birds were allotted to a pen cage of 100 cm long, 40 cm width and 40 cm height. The farm building was aerated naturally. Lighting program was controlled to provide 23 hours light and one hour dark daily by candescent bulb lighting system. Room temperature was maintained around 32° C for the first week and was decreased by 3° C weekly afterwards.

At the end of experiment, four chickens were randomly selected for slaughtering from each treatment to use in the processing of chicken nuggets. Slaughtered birds were scalded in hot water bath, plucked and eviscerated manually. Chicken meat from thigh and abdominal muscles were collected, packed and frozen at -18ºC until further analyses and processing of chicken burger were completed.

2.2 Preparation of chicken nuggets

Chicken meat from each experimental diet was ground through a 3mm plate grinder. Chicken nuggets samples were prepared as follows ingredients; wheat flour 3%, Condiments 3%, black pepper 2%, Salt 1.5%, and Ice flakes 8% as describe by (Nayak *et al.*, 2015). Batches of 2kg of each dietary treatment were mixed and formed by hand into circular (1 cm thicknes, 5 cm diameter and 25±2g weight). Nuggets were placed in plastic foam trayspacked in polyethylene bags and frozen at -18ºC±1until further analysis.

2.3 Physical analysis

2.3.1 pH value

pH of raw chicken nuggets was measured as described by Hood(1980). Ten grams of sample was homogenized with 100ml distilled water and measured using a digital pH-meter Jenway 3310 conductivity and pH meter. pH values were done on four replicates per treatment. Two nuggets were used for each replication.

2.3.2 Cooking measurements

Chicken nuggets samples of each treatment were dipped sequentially in plain flour and bread crumbs and fried in corn oil at 180 °C till golden brown in color. All cooking measurements were done on four replicates per treatment. For each replication three nuggets were examined for cooking loss, reduction in thickness, reduction in diameter and shrinkage.

The cooking loss was determined as reported by Naveena *et al.* (2006) as follows:

Cooking loss (%)=

$$\frac{\text{(Uncooked sample weight) - (Cooked sample weight)}}{\text{(Uncooked sample weight)}} \times 100$$

2.3.3 Shrinkage measurements

Raw and cooked samples were measured for diameter and thickness of chicken nuggets as described byBerry (1993) using the following equation: Reduction in diameter (%) =

$$\frac{\text{(Uncooked sample diameter)- (Cooked sample diameter)}}{\text{(Uncooked sample diameter)}} \times 100$$

Reduction in thickness (%)=

$$\frac{\text{(Uncooked sample thickness) - (Cooked sample thickness)}}{\text{(Uncooked sample thickness)}} \times 100$$

Shrinkage (%): Dimensional shrinkage was calculated using the following equation as reported by Murphy *et al.* (1975):

$$= \frac{\text{(Raw thickness - Cooked thickness) + (Raw diameter - Cooked diameter)}}{\text{(Raw thickness +Raw diameter)}} \times 100$$

2.4 T.B.A value

Measurement of lipid oxidation: The extent of lipid oxidation in raw chicken nuggets was assessed by measuring 2- thiobarbituric acid reactive substances (TBARS), as described by AOCS (1998).TBA values were done on three replicates per treatment. Three nuggets were used in each replication.

2.5. Color measurements

Color of raw chicken nuggets samples was measured by Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap supplied by the manufacturer (CIE, 1976). The color was expressed as L* (lightness), a* (the redness) and b* (the yellowness). The average of three spectral readings at different locations was obtained for each treatment.

2.6. Statistical analysis

Analysis of variance (ANOVA) was used to test the obtained data using the general linear modeling procedure (SAS, 2000). The used design was one way analysis. Duncan's multiple tests (1955) were applied for comparison of means.

III. RESULTS AND DISCUSSION

Table (3) showed the physiochemical properties of chicken nuggets processed from broiler chicken fed on different types of vegetable oil and feed additives. Chicken nuggets of T3 group had the higher pH value (6.11) followed by nugget of T5 (6.10). Slight differences were found between other nuggets samples.

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Table.3: Physicochemical properties of chicken nuggets

Treatments	Parameters		
	pH	Cooking loss (%)	T.B.A (mgMDA/kg)
T1	6.05±0.04^{bcd}	16.51±0.89^{c}	0.061±0.016^{c}
T2	6.02±0.03^{cd}	27.25±0.49^{a}	0.156±0.004^{a}
T3	6.11±0.02^{a}	22.97±1.55^{b}	0.048±0.008^{cd}
T4	6.00±0.03^{d}	21.08±2.71^{b}	0.059±0.005^{c}
T5	6.10±0.03^{ab}	15.85±2.29^{c}	0.088±0.001^{b}
T6	6.06±0.06^{abc}	14.20±1.02^{c}	0.035±0.006^{d}
SEM	0.01	0.97	0.004

$^{a-d}$ means within the same column with different superscripts letters are different ($p<0.05$). T1, T2 and T3: Treatments for soybean oil/ soybean oil with ZAD 0.5kg/ton and soybean oil with AmPhi-BACT 0.5kg/ton. T4, T5andT6: Treatments for palm oil/ palm oil with ZAD 0.5kg/ton and palm oil with AmPhi-BACT 0.5kg/ton. Means ± standard deviation. SEM: standard error of means.

Pekel *et al.* (2012) found that the pH of breast meat did not differ between broilers that were fed soybean oil (SO) and the neutralized sunflower soapstock (NSS) diet. Addition of commercial multi-enzyme feed additives had a significant effect on pH value of nugget processed from broiler chicken fed on soybean oil (T2 and T3), while no significant difference were found on those fed on palm oil (T5 and T6). These results are close to that obtained by Zakaria *et al.* (2010) they reported that enzymes addition had no effect on pH value of broiler chicken meat. However the effect of dietary enzyme on pH value of chicken meat was difficult to understand.

Data of cooking loss of chicken nuggets processed from broiler chicken fed on different types of vegetable oil and feed additives indicated that nugget of T2grouphad the higher cooking loss. No significant differences were found in cooking loss between(T1, T5 andT6) and nuggets of T3and T4. These results are close to that obtained by Pekel *et al.* (2012) they indicated that dietary fat source did not affect cooking loss of chicken meat.

As can be seen, addition of commercial multi-enzyme feed additives with palm oil had a significant effect on cooking loss of T2and T3 nuggets, while addition of feed additives with palm oil had no significant effects on cooking loss of T5andT6 nuggets. Omojola *et al.* (2014) found that chicken fed diets containing sesame and soybean diet supplemented with enzymes had higher cooking loss than those on sesame and soybean diet without enzymes. While, Zakaria *et al.* (2010) found that dietary enzyme had no effect on cooking loss of broiler chicken meat.

Data of T.B.A value of nuggets processed from broiler chicken fed on different types of vegetable oil and feed additives were showed in Table (3). Nugget processed from T2 group had the higher T.B.A value followed by nugget of T5, while the lowest T.B.A value found in nuggets of T6 group. No significant differences were found in T.B.A value of other nugget samples (T1, T3 and T4). These results are close to that obtained by Abdulla *et al.* (2015) they found that a significant difference in lipid oxidation was observed among the dietary oils. Breast muscles from broilers fed a diet supplemented with palm oil had a lower TBARS value compared with soybean oil. Also, Pekel *et al.* (2012) found that no significant differences were found in T.B.A value of thigh meat from broilers fed diets with different levels of fat from soybean oil or neutralized sunflower soapstock.

Data in Table (4) showed the shrinkage measurements of chicken nuggets processed from broiler fed on different types of vegetable oil and feed additives. Nugget of T2group had the higher reduction in diameter; slight significant differences were found in nugget of T1 group and nugget of T3 group. Also, no significant differences were found in nuggets samples of other dietary treatments T4, T5 and T6).

Table.4: Shrinkage measurements of chicken nuggets

Treatments	Parameters (%)		
	Reduction in diameter	Reduction in thickness	Shrinkage
T1	14.13±1.40^{b}	12.28±1.47^{c}	17.93±0.76^{a}
T2	16.99±1.25^{a}	17.16±2.13^{a}	19.44±1.39^{a}
T3	15.56±0.36^{ab}	16.88±1.02^{a}	19.24±1.28^{a}
T4	14.38±1.65^{b}	14.69±0.37^{b}	18.43±1.40^{a}
T5	13.84±0.45^{b}	13.82±0.05^{bc}	17.73±0.63^{a}
T6	13.44±1.36^{b}	12.04±0.95^{c}	17.16±1.30^{a}
SEM	0.68	0.69	0.67

$^{a-c}$ means within the same column with different superscripts letters are different ($p<0.05$).
T1, T2 and T3: Treatments for soybean oil/ soybean oil with ZAD 0.5kg/ton and soybean oil with AmPhi-BACT 0.5kg/ton. T4, T5andT6: Treatments for palm oil/ palm oil with ZAD 0.5kg/ton and palm oil with AmPhi-BACT 0.5kg/ton. Means ± standard deviation. SEM: standard error of means.

From the same Table (4), it can be found that no significant differences were found in the reduction in thickness% of nuggets of T2 and T3 groups and nuggets of T1and T6 groups. Slight significant difference was found in nuggets ofT4 andT5. Addition of vegetable oils and commercial multi enzymes feed additives had no significant effect on shrinkage % of nuggets samples. These results are consonance with that obtained by Omojola *et al.* (2014) they reported that there was no significant effect on the meat characteristics of broiler chickens fed on diets (soybean and sesame) supplemented with or without microbial phytase. Also, Dalólio *et al.* (2015) found that enzyme supplementation in diets based on corn and soybean meal did not influence the parameters of chicken meat quality. The same results were found by Pekel *et al.* (2012).
Color measurements of chicken nuggets fed on different dietary oils and commercial multi- enzyme feed additives shown in Table (5). No significant differences were found in *L** value between dietary treatments except for nugget of T1. Also, data showed no significant differences were found in *a** value between nuggets of T1, T3and T4.Slight difference was found between nuggets of T5and T6.No significant differences were found in *b* * value between nuggets of T2, T4 and T6. The differences between the other nuggets samples were not significant. These results are close to that obtained by Pekel *et al.* (2012) they found that breast meat color were not affected by the dietary fat source. Also, Zakaria *et al.* (2010) they reported that dietary enzyme had no effect on the broiler chicken meat color. Dalólio *et al.* (2015) found that enzyme supplementation in diets based on corn and soybean meal did not influence the color parameters of chicken meat.

Table.5: Color measurements of chicken nuggets

Treatments	Parameters		
	L	*a*	*b*
T1	58.97±0.89^{b}	4.05±1.33^{ab}	15.29±0.66^{c}
T2	63.35±1.15^{a}	4.62±0.87^{a}	17.27±0.62^{a}
T3	56.67±0.68$_{a}$	4.09±0.15^{ab}	15.94±0.28^{bc}
T4	62.21±2.16^{a}	4.11±0.35^{ab}	17.03±0.14^{a}
T5	63.56±2.05^{a}	3.52±0.33^{bc}	15.98±0.35^{b}
T6	63.18±1.16^{a}	2.79±0.08^{c}	16.76±0.39^{a}
SEM	0.87	0.28	0.21

$^{a-c}$Means within the same column with different superscripts letters are different ($p<0.05$). T1, T2 and T3: Treatments for soybean oil/ soybean oil with ZAD 0.5kg/ton and soybean oil with AmPhi-BACT 0.5kg/ton. T4, T5andT6: Treatments for palm oil/ palm oil with ZAD 0.5kg/ton and palm oil with AmPhi-BACT 0.5kg/ton. Means ± standard deviation. SEM: standard error of means.

IV. CONCLUSION

The purpose of the current study was to evaluate the quality characteristics of chicken nuggets processed from broiler chicken fed on different type of vegetable oils and feed additives. The addition of soybean oil and palm oil as fat sources for use in chicken diets in combination with feed additives (enzymes) had no negative effects on the quality characteristics of chicken nuggets.

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Effect of Cow Manure Dosages as Organic Fertilizer on the Productivity of Organic Rice in West Sumatra, Indonesia

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Abstract— *This research was conducted on rice paddy area at the Simarasok Village, West Sumatra Province, Indonesia, aimed at investigating the effect of dosage of cow dung as organic fertilizer on growth, yield component and production of organic rice. The experiment was arranged using a Complete Randomized Block Design with four treatments and six replications. The treatment was organic fertilizer of cow dung composted using local microbial organisms with four dosage levels, namely: a) 2 tons/ha; b) 4 tons/ha; c) 6 tons/ha; and d) 8 tons/ha. The rice variety used was the Kuriak Kusuik and the observed variables included: leaf color score, plant height, maximum number of tillers, number of productive tillers, panicle length, number of grains per panicle, percentage of empty grain, weight of 1000 grains, and grain yield. The result showed that the dosage of organic fertilizer of cow dung had significant effect on leaf color score at 56 days after planting (DAP), number of productive tillers, number of grains per panicle, and grain yield. In contrast, plant height, maximum number of tillers, panicle length, weight of 1,000 grains, and empty grain were not significantly affected. It was found that there was a positive relationship between the dosages of organic fertilizer of cow dung with the grain yield. The addition of cow dung as the organic fertilizer as much as 1 ton/ha to the soil could cause an increase in the yield of grain by 0.097 ton/ha.*

Keywords—Cow dung, Kuriak Kusuik rice, Organic fertilizer, Rice paddy, West Sumatra

I. INTRODUCTION

The organic farming system is agricultural development through a sustainable agricultural development approach, where inputs are natural and the use of chemical fertilizers, synthetic chemical pesticides, and genetic engineering for seeds, are prohibited (Jamil, *et al*., 2014). In the Province of West Sumatra, this organic farming system has been developed in the last few decades. It was recorded that, in 2007, the total area of organic rice farming was only 77.81 ha and has increased to 138.48 ha in the year period of 2013-2016. The rate of increase is relatively slow due to: a) lack of relevant institutional support, b) wide range of cross-consultation between agricultural experts and policy makers, c) low productivity of organic paddy rice, d) the un-competitiveness of the organic product price, and e) lack of farmers' interest to manage organic paddy rice (Daniel, *et al*., 2014)

The world market demand for organic agricultural products is growing rapidly about 20% per year (ISRI, 2004). In 1998, total sales of organic food products worldwide reached US$ 13 billion, increasing to US$ 26 billion in 2001 (CHO West Sumatra, 2010). Based on the growth rate of about 20% per year, it is estimated that total sales of organic food products would reach US$ 400 billion in 2020. Increasing demand for organic products may be due to the followings: a) the strengthening of environmental awareness and healthy lifestyle of the society, b) government policy support, c) support of food processing industry, d) support of modern market (supermarket absorb approximately 50% of the organic products); e) high price at consumer level; f) generic label; and g) incessant national campaign of organic farming. However, the problem is, the only realizable market share of the organic products is only 0.5-2.0%. Although the area of organic farming in Europe continues to increase, from an average of less than 1.0 percent in 1987 to 2.0-7.0% in 1997 (highest in Austria reaching 10.12%), the increase has not been able to meet the rapid demand (IAARD, 2005).

One of the factors that must be considered in producing organic rice is the management of soil fertility. To meet the nutrient needs of the plant, the effort to increase the

fertility of the soil naturally, which may be done through recycling of plant nutrients or the use of compost from animal manure. All of these efforts are targeted to the improvement of biological activity, as well as physical, and soil chemistry. The use of organic fertilizer derived from rice straw or animal manure is an alternative way to organic rice farming system in accordance with the National Standard of Indonesia (NSI) No: 01-6729-2002 (ANSI, 2002).

The organic rice field in Simarasok Village of Baso District, Agam Regency, has been developed since the planting season of January 2009. The problem was the low productivity of the rice, which was only 4.2 ton/ha (Atman and Nurnayetti, 2014). Non-organic rice productivity in West Sumatra in 2015 was 5.02 ton/ha, lower than the national productivity of 5.15 ton/ha (ISB, 2015). This low productivity may be due to the majority of the farmers (63.3%) still using local varieties, including the local organic rice variety, the Kuriak Kusuik (Nurnayetti and Atman, 2013). However, other studies have shown that the use of Integrated Crop Management technology of paddy rice with the Kuriak Kusuik variety was able to produce 7.74 ton/ha or 28.1% higher than farmer technology (6.04 ton/ha) (Winardi, 2014). On the study reported by Zen (2013), it was found that the use of improved varieties (Cisokan and IR42) were able to give rice production of 6.23 ton/ha and 6.31 ton/ha, respectively.

Another reason of the low productivity of organic paddy rice is the diversity in the use of organic fertilizers. The use of organic fertilizer on paddy rice has begun to be encouraged by local government since 2007 due to the growing issue of the damage of the quality of the rice fields (Sumarno and Kartasasmita, 2012). Organic fertilizer is a biological buffer that has the function in improving the physical, chemical, and biological properties of the soil in order to provide balanced amounts of nutrients. There is a positive correlation between organic matter content and soil productivity (Adiningsih, *et al*., 1995). According to Kartaatmadja *et al.* (2000), the amounts of nutrients transported from the soil on the rice farming system with yield rate of 8.0 ton/ha, are 269 kg N, 44 kg P2O5, 207 kg K2O, 28 kg Mg, and 24 kg S respectively. Therefore to ensure the stability of yield and sustainability of the production system, it is absolutely necessary to return nutrients in the form of organic materials or fertilizers to the soil.

The addition of organic fertilizers can increase the content of organic carbon, increase water holding capacity, and crop yields including biomass and seeds Materechera and Mehuys, 1991). According to Pratiwi and Sumarno (2014), the provision of manure of 5 ton/ha can only replace 20% of the recommended inorganic NPK fertilizer dosage on rice crops. While Kasno and Hidayat (2006), stated that the addition of manure of 2 ton/ha in paddy rice can reduce SP36 fertilizer requirement by 60%, and the use of straw as much as 5 ton/ha can reduce the requirement of KCl fertilizer by 78%.

Kasno and Rostaman (2017) found that the soil organic matter content can affect the efficiency in the use of N fertilizer, thus becoming a limiting factor for paddy rice growth. Furthermore, Sujitno *et al.* (2014), states that organic fertilizers can increase the productivity of paddy rice, ranging from 21.07 to 23.33%. In upland rice, the provision of manure can increase yield, between 19.4-27.3% (Barus, 2012).

Based on the above problems, this research was aimed to find out the effect of cow dung as organic fertilizer on growth, yield components, and yield of organic paddy rice. It is expected that the results of this study can be used as a recommendation for the optimal dosage of cow manure as organic fertilizer.

II. MATERIALS AND METHODS

This research was carried out in the area of organic farming of rice paddy in West Sumatra, in collaboration with Lurah Sepakat Farmer Group in Simarasok Village of Baso District, Agam Regency, West Sumatra Province, Indonesia. The organic rice cultivation was started in this area in early 2009 and the Organic Food Certificate (OFC) for this area was obtained in 2010 issued by the OFC Agency of West Sumatra. The nutrient contents of soil in the research location were: pH (H2O) 8.13; pH (KCl) 7.79; C-organic 1.91%; N-total 0.22%; C/N 8.68; P-Bray I 10.45 ppm; and K-can be exchanged 0.60 me/100g.

The study was arranged using a Complete Randomized Block Design, with four treatments and six replications. The treatment was organic fertilizer derived from cow dung composted using local microbial organisms (Microbial II) with four dosage levels, namely: a) 2 tons/ha; b) 4 tons/ha; c) 6 tons/ha; and d) 8 tons/ha. The nutrient contents of organic fertilizer tested were: 1.90% N; 0.80% P; 3.6% K; 10.04% C; and 5.28% C/N. Therefore, for the treatment of 2, 4, 6, and 8 ton/ha, the nutrient contents successively were 38, 76, 114, and 152 kg N/ha; 16, 32, 48, and 64 kg P2O5/ha; and 72, 144, 216, and 288 kg K2O/ha. Meanwhile, fertilizer recommendations for the study area were 69, 27, and 45 kg/ha respectively for N, P2O5, and K2O (Hasan *et al.*, 2015). According to Dobermann and Fairhurst (2000) to produce an average grain of 6 ton/ha, rice plants need 165 kg N, 19 kg P, and 112 kg K/ha or equivalent to 350 kg Urea, 120 kg SP36 and 225 kg KCl/ha.

Another nutrient source was the local liquid organic fertilizer made from local raw materials (snails, bones,

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and coconut husk) by spraying it into the plants. Dosage of liquid organic fertilizer was three tablespoons per liter of water, which was applied four times with the interval time of 15 days, starting at the plants age of 15-60 days after planting (DAP). The nutrient content of the organic liquid fertilizer materials were: snails (0.06% N; 0.07% P; 0.43% K), bone (0.01% N; 0.10% P; 0.08% K); and coconut husk (0.01% N; 0.02% P; 0.08% K).

Soil processing was conducted completely with two plows and one rake. The organic rice varieties used are the local superior variety which was propagated by farmers and continuously planted organically, namely: the Kuriak Kusuik. The seeds, with the age of less than 21 days old, were planted on a 4x5 meter plot. The spacing used was 25x25 cm with the number of seeds was 1-3 stems/clump. Weeding was done twice, i.e. at 30 and 60 DAP. Pests and diseases control carried out depending on the development of the pests and diseases, using vegetable pesticides (formulated from local plant materials). The technology of organic rice cultivation used was based on the NSI No: 01-6729-2002 (ANSI, 2002).

The observed variables included: leaf color score using leaf color chart (AIAT West Sumatra, 2012), plant height, maximum number of tillers, number of productive tillers, panicle length, number of grains per panicle, percentage of empty grain, 1,000 grains weight, and grain yield. The data obtained were analyzed statistically using analysis of variance (ANOVA) and correlation/regression analysis. If there were differences between treatments, it will be then continued by the Duncan Multiple Range Test (DMRT) at 5% level (Steel and Torri, 1960; Gomez and Gomez, 1984).

III. RESULTS AND DISCUSSION

Leaf Color Score

Observation on the leaf color score showed that there was a significant effect of organic fertilizer dosage at the plant age of 56 DAP (Table 1). From the correlation analysis it was found that the dose of organic fertilizer had a positive correlation with the leaf color score at 56 DAP, with r = 0.952*. This means that, the more organic fertilizer was applied, the leaf color score tended to be higher at 56 DAP. However, the provision of organic fertilizer up to the dose of 8 ton/ha had not been able to meet the nutrient deficiency of N in the rice crops. According to AIAT West Sumatra (2012), if the leaf color score <3.0 indicates that the plant is deficient of N, therefore additional Urea fertilizer is needed as much as of 75 kg/ha, 100 kg/ha, 125 kg/ha and 150 kg/ha respectively to obtain grain yield of 5 ton/ha, 6 ton/ha, 7 ton/ha, and 8 ton/ha.

Growth Components

The observation on the growth components showed that the treatment of organic fertilizer dosages did not have significant effect on plant height and maximum number of tillers (Table 2). However, from the correlation analysis it was found that the organic fertilizer dosages had a significant positive correlation with plant height (r = 0.974*), and the positive correlation but did not have significant with the maximum number of tillers (r = 0.546^{ns}). This means, the more the organic fertilizer was applied, will cause significant increase in plant height and the maximum number of tillers will also tended to be increased.

Yield Components and Grain Yield

It was found that the organic fertilizer dosages had significant effect on the number of productive tillers, number of grains per panicle, and grain yield (Table 3). However, it did not have significant effect on the panicle length, weight of 1,000 grains, and percentage of empty grain. From the correlation analysis it was found that the dosage of organic fertilizer had a significant positive correlation with the number of productive tillers (r = 0.965*) and the percentage of empty grain (r = 0.988*). There was no significant correlation between the dosages with the panicle length (r = 0.476^{ns}) and number of grains per panicle (r = 0.629^{ns}). In contrast, there was a negative and no significant correlation with the weight of 1,000 grains (r = -0.535^{ns}).

Good plant growth is characterized by the high yield and large number of tillers. A higher plants will produce a longer panicles (r = 0.632^{ns}), the long panicle will cause more number of grains per panicle (r = 0.670^{ns}), and more number of grains per panicle will cause more grain yield (r = 0.643^{ns}). Furthermore, more number of tillers will result in more productive tillers (r = 0.326^{ns}), so the grain yield will increase significantly (r = 0.986*). Well-grown plants will be able to utilize sunlight for the process of photosynthesis and are able to absorb nutrients optimally. According to Yoshida (1981), the availability of nutrients in the soil and the ability of plants to well utilize the sunlight could increase the plant growth and yield.

Result of the regression analysis showed that there was a real positive relationship between the organic fertilizer dosages and the organic rice yields (Fig. 1), with the equation: Y = 0.097x + 4.17 (r = 0.969). It means that, the addition of organic fertilizer as much as 1 ton/ha would increase the yield of organic paddy rice by 0.097 ton/ha. The results of research by Tufaila *et al.* (2014) on Ultisol soil in Southeast Sulawesi Province showed that dung manure compost with doses ranging from 5.0-7.5 ton/ha gave better influence to growth and production of Konawe rice variety. In the System of Rice Intensification

(SRI), the application of organic fertilizer of 4-8 ton/ha could produce rice between 6-8 ton/ha without the addition of inorganic fertilizers (Gani *et al*., 2002; Uphoff and Satyanarayana, 2006). Furthermore, research by Suhardi *et al.* (2014) found that the use of 5 ton/ha of organic fertilizer plus 20 liter/ha liquid organic fertilizer, called the biourine, on a newly open paddy field gave no significant difference in rice production with the use of inorganic fertilizers of 200 kg Urea + 300 kg NPK per ha. In this study, although the addition of organic fertilizer dosages could significantly increase the grain yields (maximum 4.96 ton/ha), the yield of these grains was much lower than its potential production which could reach up to 5.50 ton/ha (Zen et al., 2011). This is supported by Syam (2006) who stated that the superiority of SRI technique based on the supply of plant nutrients derived from organic matter is still in doubt because the rice productivity using SRI technology is lower than the conventional technology.

IV. CONCLUSION

There was a real positive relationship between the dosages of organic fertilizer with the yield of organic rice paddy. The addition of cow dung as organic fertilizer as much as 1 ton/ha would be able to increase the grain yield by 0.097 ton/ha. In order to reach the potential production of the Kuriak Kusuik rice variety of 5.50 ton/ha it is recommended to apply approximately 13.7 ton/ha of the cow dung as the organic fertilizer.

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Table.1: Leave color score of organic rice plant at different ages with various doses of organic fertilizer

Organic fertilizer dosage (ton/ha)	**Plant ages**		
	28 DAP	**42 DAP**	**56 DAP***
2	3	3	2,4 [c]
4	3	3	2,6 [b]
6	3	3	2,8 [a]
8	3	3	2,9 [a]
CV (%)			**2,72**

*) Means within a column with no common superscript differ significantly (P<0.05).

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Table.2: Growth components of organic paddy rice with various dosage of organic fertilizer

Organic fertilizer dosage (ton/ha)	Growth components*	
	Plant height (cm)	Number of tiller (per Hill)
2	105,1 [a]	19,9 [a]
4	105,8 [a]	19,1 [a]
6	106,1 [a]	20,4 [a]
8	106,4 [a]	20,3 [a]
CV (%)	**1,68**	**4,50**

*) Means within a column with no common superscript differ significantly (P<0.05).

Table.3: Yield components and grain yield of organic paddy rice with various dosage of organic fertilizer

Organic fertilizer dosage (ton/ha)	Yield components and grain yield					
	Number of productive tillers (per Hill)	Panicle length (cm)	Number of grains per panicle	Weight of 1000 grains (g)	Empty grain (%)	Grain yield (ton/ha)
2	10,5 [c]	22,8 [a]	131,9 [b]	25,14 [a]	12,91 [a]	4,33 [c]
4	11,3 [b]	23,5 [a]	142,8 [ab]	24,77 [a]	14,56 [a]	4,63 [b]
6	11,5 [b]	23,1 [a]	145,8 [a]	24,43 [a]	15,34 [a]	4,69 [b]
8	11,9 [a]	23,3 [a]	140,6 [ab]	24,85 [a]	17,34 [a]	4,96 [a]
CV (%)	**1,67**	**2,14**	**5,52**	**2,28**	**23,68**	**2,72**

*) Means within a column with no common superscript differ significantly (P<0.05).

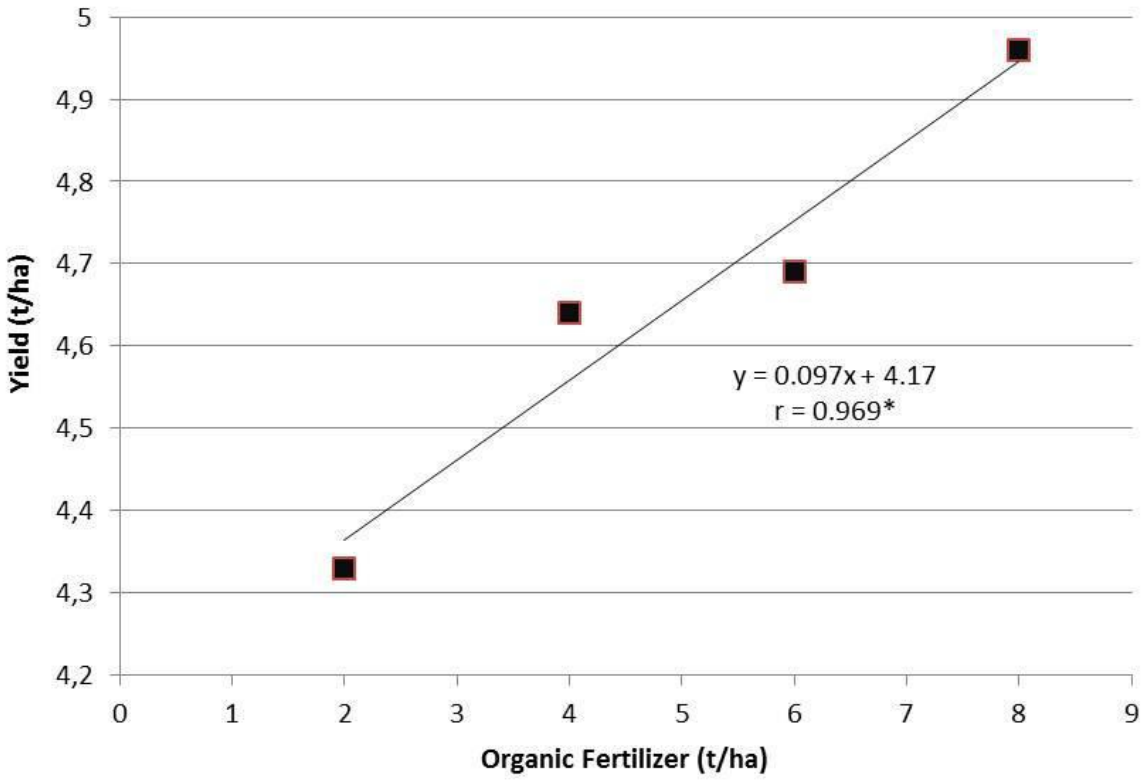

Fig.1: Relationship between the dosages of organic fertilizer of cow dung with the yield of organic rice paddy

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Analysis of Socio-Economic Factors Affecting Fish Marketing in Igbokoda Fish Market, Ondo State, Ngeria

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Abstract — *The study analyzed the socio-economic factors affecting fish marketing in Igbokoda fish market, Ondo State, Nigeria. A purposive sampling technique was used in selection of the respondents. Sample size comprised eighty fish marketing households. Questionnaire was used for data collection. Results revealed that fish marketing in the area is dominated by females (88.7%). Majority (91.3%) were married with an average household size of 7 persons. Marketers earned an average income of ₦60, 000 per month. Majority (71.3%) do not belong to cooperative societies, 46.3% of the marketers have an average of 16.5 years of experience. Findings also revealed that fish marketing is efficient and lucrative in the area. However, constraints faced by the marketers include; inadequate storage facilities, price instability, inadequate capital, lack of access to credit, distance to market among others. It was therefore recommended that effective agricultural policies and programs should address marketers' easy access to credits, infrastructural facilities such as good storage and processing facilities, electrification, good feeder roads should be provided in the area to reduce spoilage and unprofitable sales.*

Keywords— Fish Marketers, Socio-economic Characteristics, Igbokoda, Nigeria.

I. INTRODUCTION

Fish production and marketing is one of the oldest livelihood income generating activities of man since the time immemorial. Fish contains high quality protein, vitamins, minerals and other nutrients important for human health and growth. According to Ovie and Raji (2006), fish is crucial to the Nigerian economy, contributing 5.4% of the gross domestic product (GDP). Fish are significant to the nation's economy in terms of food security, income, employment, poverty alleviation, foreign exchange earnings and provision of raw materials for animal feed industries. Fish is the commonest and cheapest source of protein for the teaming Nigeria's poor, estimated to be between 65-70% of the total population. Fish is also a good source of Sulphur and essential amino acids such as lysine, leucine, valine and arginine and it is therefore suitable for supplementing diets of high carbohydrates contents (Amao *et al.*, 2006).

It has high content of Polyunsaturated (Omega III) fatty acids, which are important in lowering blood cholesterol. FAO (2012) recommended that a person takes 35 grams per caput of animal protein per day for sustainable growth and development. Fish which contributes 36.6 grams per day of net protein utilization in Nigerian homes is still below the recommended requirement by the world health organization (WHO) (Ohen and Abang, 2009) Marketing is a process of exchanging goods and services from one person to another with reference to price. A fish market is a place used for marketing of fish and fish products. However, fish marketing essentially consists of all the activities involved in delivering fish from one producer to the consumer, while distribution provides channels that link the marketing institutions and producers together. The market mechanisms have to be efficient to be able to play the role of propelling yield. An efficient market system therefore is the one that provides satisfactory and cheap services to consumers or one that maximize the ratio of input and output of marketing. In Nigeria, fish system varies depending on type of fish product and the distance between producer and source of supply of fish product and retailer and ultimately to consumer. Fish supply and marketing suffer from various sets backs, ranging from shortage of supply, price fluctuation due to drying up of the source and spoilage in transit amongst others. Despite these, the agencies involved in the marketing of the commodity appear to be on the increase as a result of increase in the population and therefore, the demand tends to be high. Also despite the nutritional and commercial values of fish and fish products,

its production and marketing remains low in Nigeria when compared to other nations of the world (FAO, 2012). Fish and fish products is consumed in all parts of the country and has a good market price. Often times, marketers are compelled if not forced to sell their product at a very low price to avoid huge wastage or total loss and this reduces their marketing margins and marketing efficiency.

Although, a number of studies have been carried out on fish and fish products in Nigeria, most of such studies dwelled on its production and consumption. Agricultural marketing is central to agricultural development and the overall growth and development of the economy. Previous studies have shown that efficient marketing system stimulates agricultural production (Awoyinka and Ikpi, 2005, Awoyinka, 2009). Efficiency in fish marketing has the potentials of stimulating fish production in the country in view of the huge deficit between local consumption and production. This will have a downward effect on the price of fish and thereby induce more consumption of fish by consumers. The importance of this development cannot be over-emphasized in country like Nigeria whose economy, life and wellbeing are immersed in agriculture, and fish alone constituted more than 40% of total protein intake in the country (Eyo, 1992). Major components of fish marketing efficiency are profitability and marketing margins of the various participants (i.e. wholesalers and retailers). Research has shown that there is continuous increase in the number of people involved in fish marketing as a result of growing population of the country (Ali et al., 2008). This is an inkling of the profitability of the enterprise as only profitable activities could be attracting increasing number of participants. Furthermore, the economy of Nigeria, just like other sub-Saharan African countries, is still developing. This has imposed a lot of challenges on marketing of goods and services in the country, especially agricultural products like fish. It has been argued that agricultural marketing is inefficient resulting in high rate of food spoilage, poverty and unaffordable food prices by consumers. However, not many studies have empirically evaluated the validity of these hypotheses in fish marketing.This study seeks to analyze economics of fish marketing and in so doing address questions affecting marketability of fish.

According to Adekanye (1988) and Abdullai (1983), marketing of food in Nigeria is characterized by multitudes of deficiencies and problems. These problems cut across processing, preservation, packaging, distribution and transportation Eze, et al (2010), identified inadequate processing skills, produce deterioration and lack of storage facilities as the major constraints perceived by women marketers. However, this may not be exhaustive bearing in mind the paucity of research in fish marketing, and also the rural nature of participants in fish marketing. According to (Adekanye, 1988), marketing is a method used to bring the interpersonal forces of demand and supply together irrespective of the location of the market. Application of various pricing criteria on sales of fish depend on efficiency with which the marketing system transmits information among the fish mongers or marketers and consequently, prices of fish changers as it passes through middlemen such that by the time it reaches consumers, it becomes expensive. Agbebi and Fagbote (2012) observed that middlemen are marketing intermediaries that do not add title to the products, but receives fee for expediting the exchange. Fish supply and marketing suffer from various setbacks ranging from shortage of supply, price fluctuation due to drying up of the source, spoilage in transit etc. (Tomek & Robinson, 1981). Despite these, the individuals involved in the marketing of the commodity appear to be on the increase as a result of increase in the population and therefore, the demand tends to be high. Tomek and Robinson (1981) indicated that increase in concentration implies more scope for the middleman to exploit either the consumers by charging high or the producer by paying them lower price. Nigeria offers the largest market for fisheries products in Africa. Fish production from capture fisheries in spite of its being expensive and risky in the coastal line regions of Nigeria has been erratic and on the decline in recent years, resulting in increase in poverty and nutritional deficiency. Fish production and marketing remains the best option to bridge the gap between the total fish demand and total domestic production in the face of high cost of production input and unstable government policy. Therefore, the socio-economic of fish marketing evaluates the structure, conduct and performance of fish marketing system as indicators of the overall efficiency of the system.

It is of essence in the determination of both consumers' living cost and producers' income and hence, the overall wellbeing and development of the country. , the study identified socio-economic characteristics of the marketers; ascertained marketing channels of fish in the area; determined the influence of the socio-economic characteristics of the fish marketers on their profits margins; determined marketing margin; determined marketing efficiency; examined the costs, return or profitability of fish marketing and identified constraints associated with fish marketing in the area.

II. OBJECTIVES OF THE STUDY

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The broad objective of this study is to determine the socio-economic factors affecting fish marketing in Igbokoda fish market, Ondo state.

The specific objectives are to:

i) To identify the economic constraints that affects the various fish Markets/settlements.
ii) Identify the nature, socio-economic structure of the market and investigate the potential for growth in the fish market and access the level of demand and consumer preference for different fish whether fresh, smoked, dried, ice or fried.
iii) Identify the factors affecting the quality and quantity of fish marketed in the study area.
iv) Make recommendation based on the findings of the study.

III. METHODOLOGY

STUDY AREA

Igbokoda fish market is located in Igbokoda, in Ilaje Local Government area of Ondo State, South-Western, Nigeria. This fish market is the largest fish market in the South-Western part of Nigeria in which fishing has been the dominant occupant of its inhabitants. The area has the Atlantic Ocean as its neighbour hence a lot of artisanal fishing is done in the area. The area also has a history of the active participation by women in fish production and also well located in relation to village downstream because it has a good road link to Akure, the capital city of Ondo State and to the other areas of Nigeria. Fishing, processing and marketing has been the dominant occupation in the area. The map of Ondo state and that of Ilaje local Government are shown in figure 1 and 2 respectively.

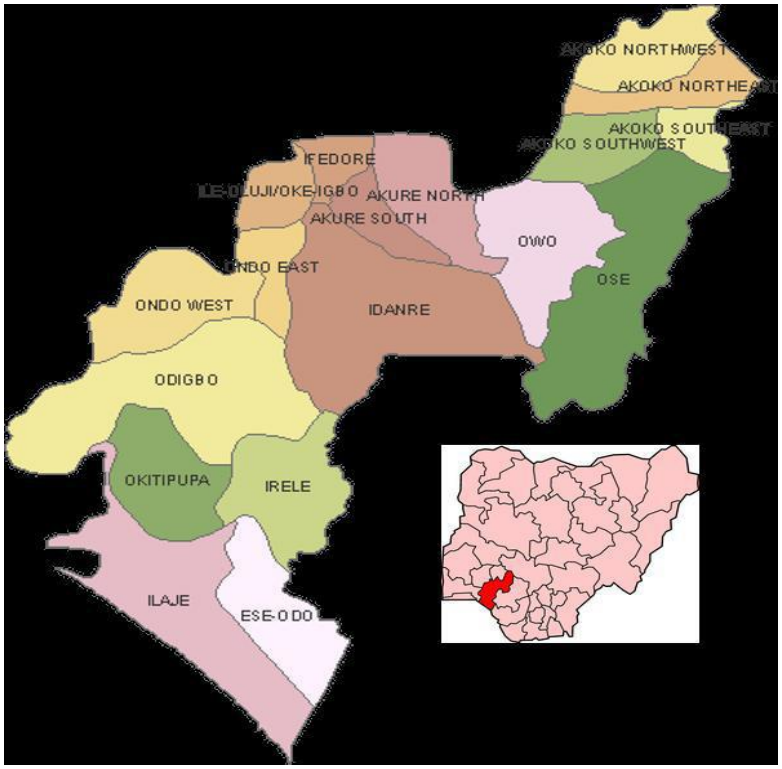

Fig.1: Map of Ondo state showing Ilaje Local Government area

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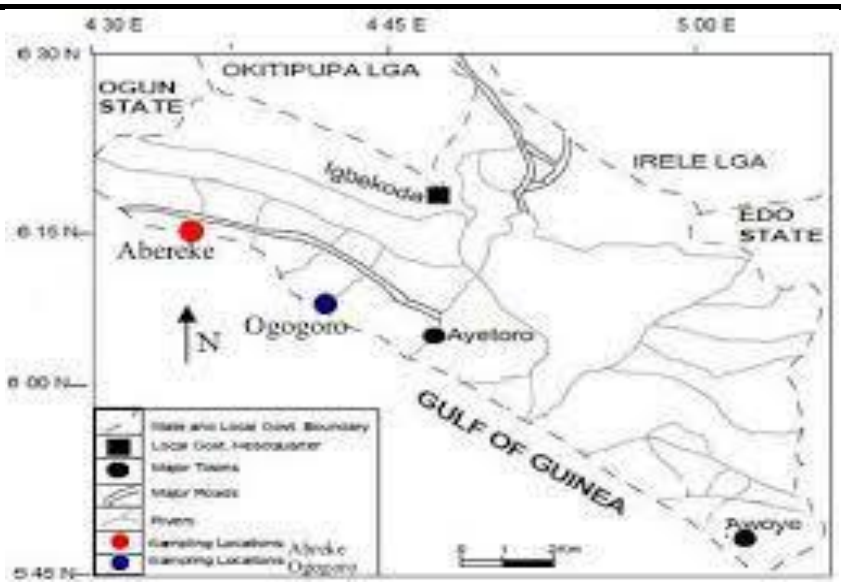

Fig.2: Map of the study area

IV. METHODOLOGY

A total number of 80 respondents were used for the study. This study entails the use of questionnaires and personal interview with men and women involved in fish marketing activities. The personal interview focused on problems and challenges faced by fish marketers from the middlemen as well as major challenges of the fish market. Purposive sampling technique was used to select respondents for this study. The main tool for data collection was a set of structured questionnaire in addition to oral interviews in places where the respondents could neither read nor write. The questionnaire sought for information on socio-economic characteristics of the fish marketers, quantities of fish purchased, quantities of fish sold, costs associated with fish marketing and the associated problems.

V. RESULTS

The results presented in Figure 1 shows the age distribution of fish marketers in the study area. 21.2% of respondents" were within the age range of 21-30 years, the average age of the marketers was 36 years while the minimum and maximum ages were 15 and 60years. Implication of these findings is that large proportions of the respondents were adults and can adequately be regarded as active, agile, and physically disposed to marketing activities. Age is very important in fish marketing activities because age has a significant influence on the decision making process of the marketers.

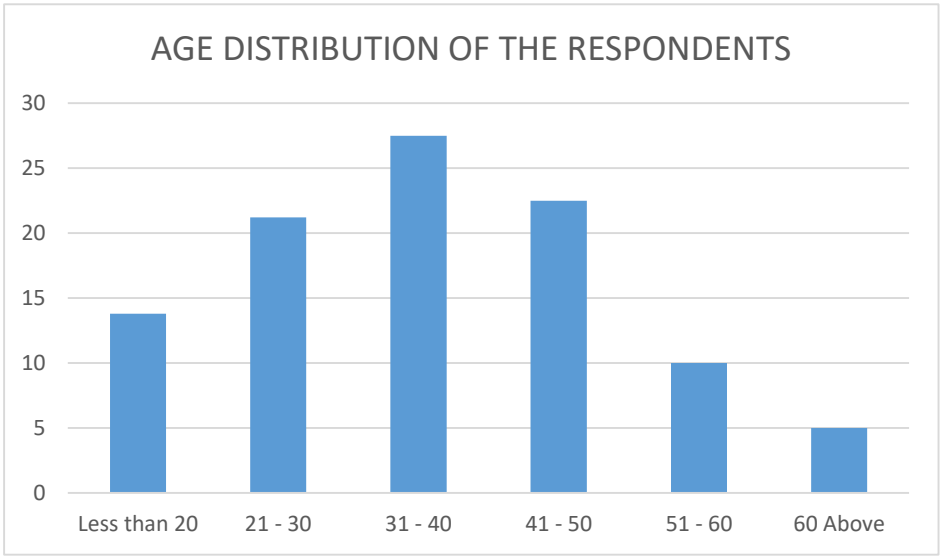

Fig.1: Age distributions of the respondents

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Gender

The result in figure 2 below shows that about 88.7% of the fish marketers were female while the (11.3%) were male. The result is in line with the findings of Agbebi and Fagbote, (2012) that fish marketers are more dominated by female gender than male and that women play a central role in fish processing and marketing and that women also have better bargaining power than men.

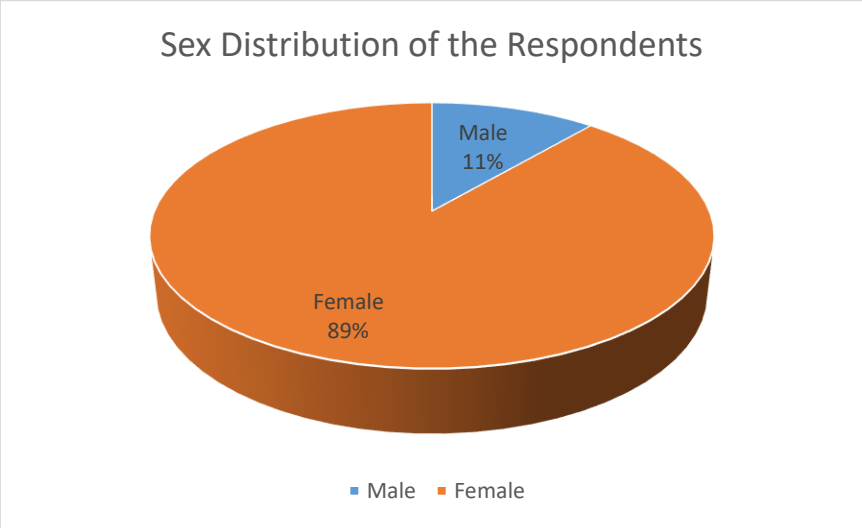

Fig.2: Distribution by gender

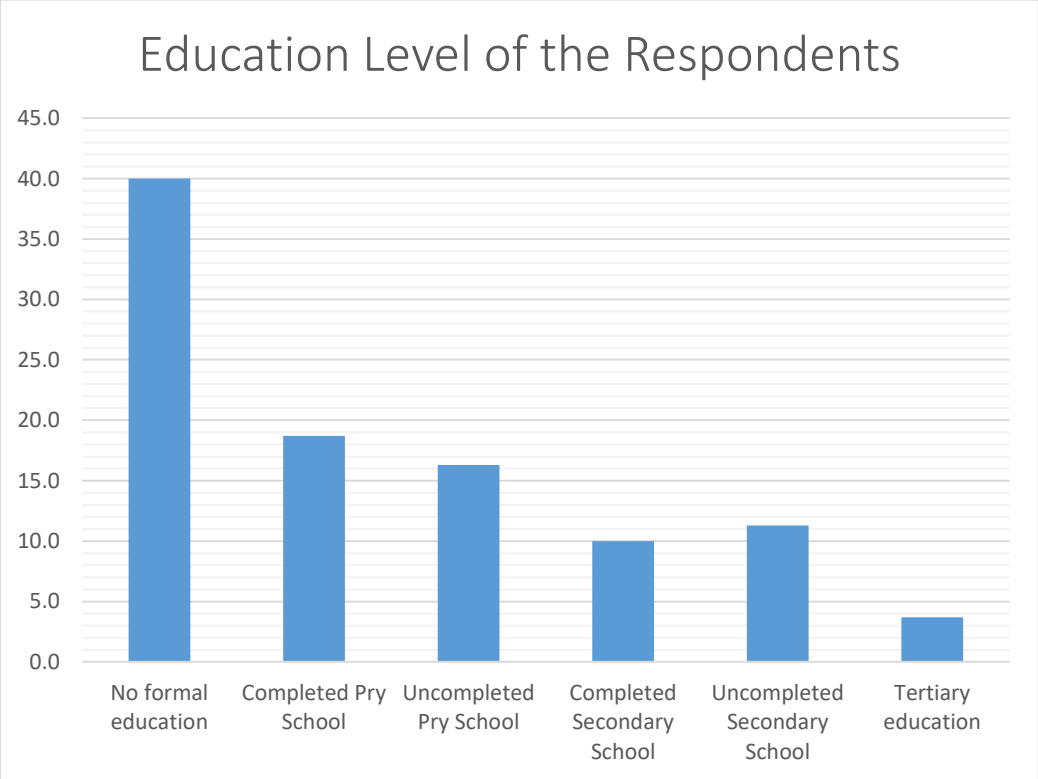

Fig.3: Educational level of Respondents

The result presented in Figure 3 shows the distribution of fish marketers by their educational level. The result indicated that about 40% of the fish marketers do not have access to formal education, which is an important factor and similar to the general believe that most marketers are illiterates or semi-illiterates most of whom dropped out of formal school system. It is also believed that a person level

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of education determines how fast and concise an individual will decode or process information.

Marital Status

The result indicated that about 5.0% are Single, 91.3% are married and 3.7% are widowed. This implies that fish marketing in the area is dominated by married individual who are responsible according to the society standard and therefore are likely to have some experience of life (Onubuogu *et al.*, 2014).

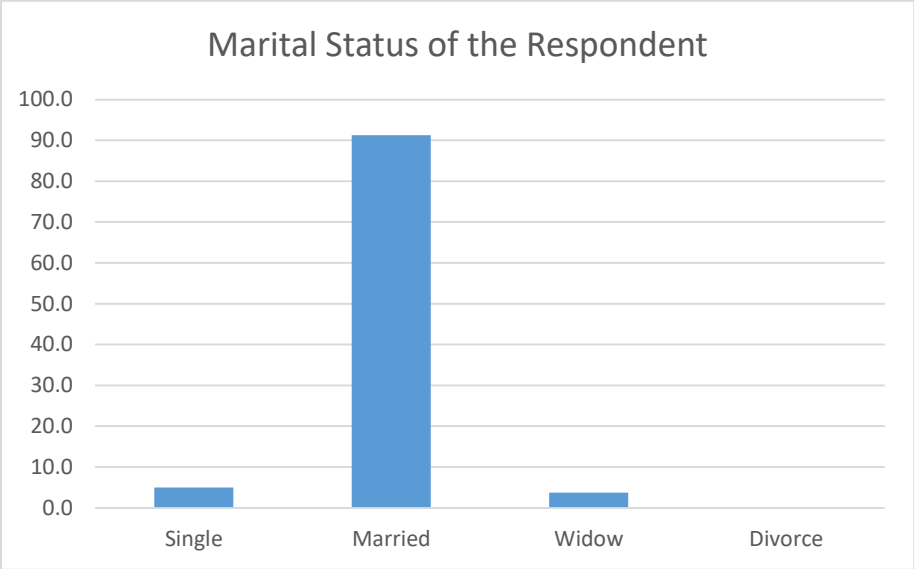

Fig.4: Marital status of respondents

High percentage of married individual is also an indication that the respondents are permanent settlers in the area and all their economic activities revolve around the area, so migration is not a case to be considered.

Marketing Experience

Greater proportion (46.3%) of the respondents had 11–20 years of marketing experience, while 11.2% constitute those with 31 years of marketing experience and above in fish marketing. This implies that marketers in the study area have sufficient experience in fish marketing. Experience in marketing is a key factor in marketing efficiency and the longer the years of marketing experience, the more exposed the marketer becomes and the more efficient and effective the marketer is expected to be.

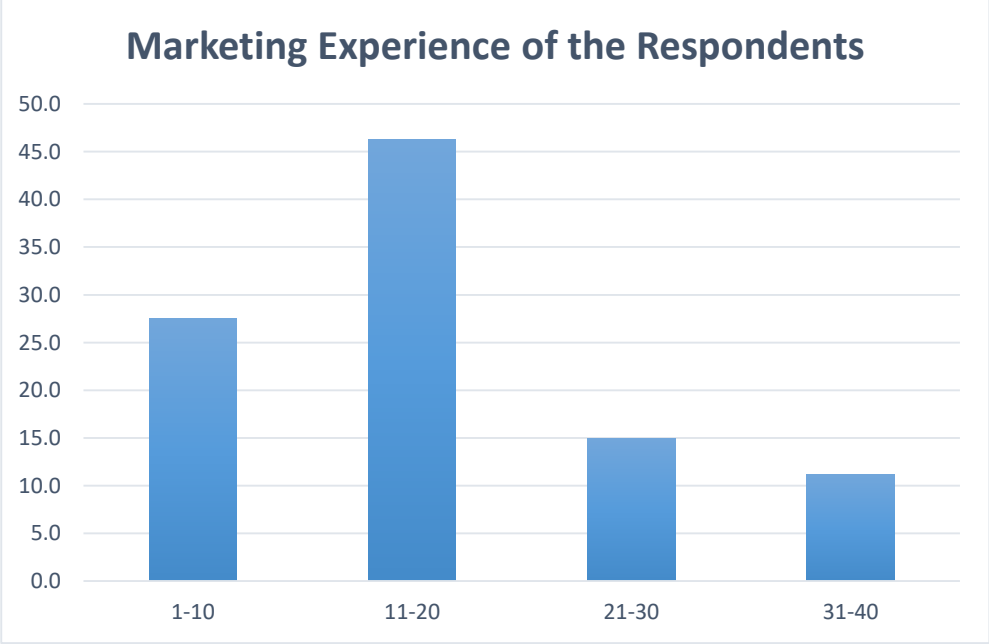

Fig..5: Marketing experience of the respondents

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House-hold size

A household comprises of all persons who generally live under the same roof and eat from the same pot (FOS, 1985; Esiobu *et al.*, 2014a and Esiobu *et al.*, 2014b). It can also be described as all people who live under one roof and who make or are subject to others making for them joint financial decision. Larger percentage (46.3%) of the fish marketers had a household size of 6-10 persons which is an indication that fish marketers in the study area have a large household size and cheap access to un-remunerated family labour. This therefore explains why the use of hired labour in small scale agribusiness enterprise is very low.

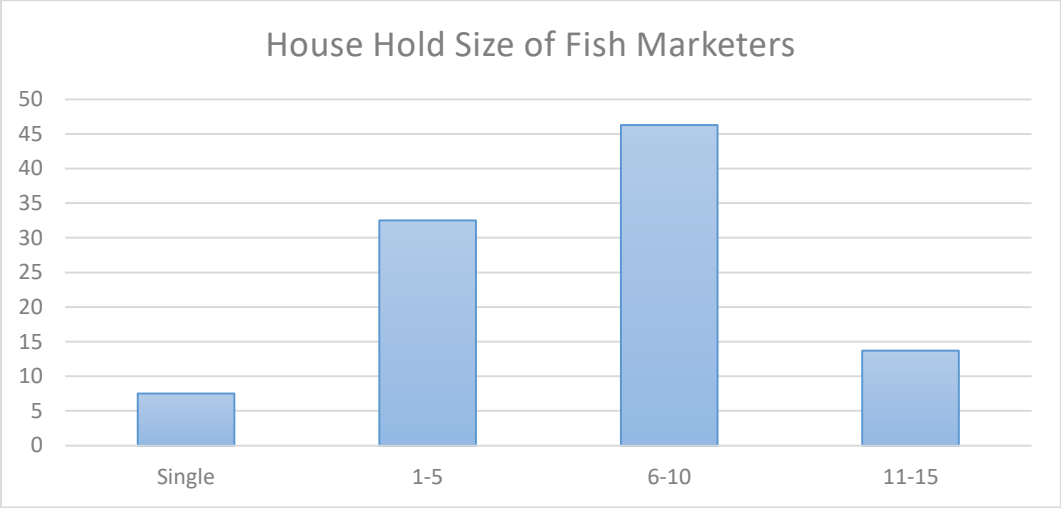

Fig.6: Household size of respondents

Table.1: Cooperative membership

Cooperative Society N=80	Frequency	Percentage
Non-members	57	71.3
Members	23	28.7
Total	**80**	**100**

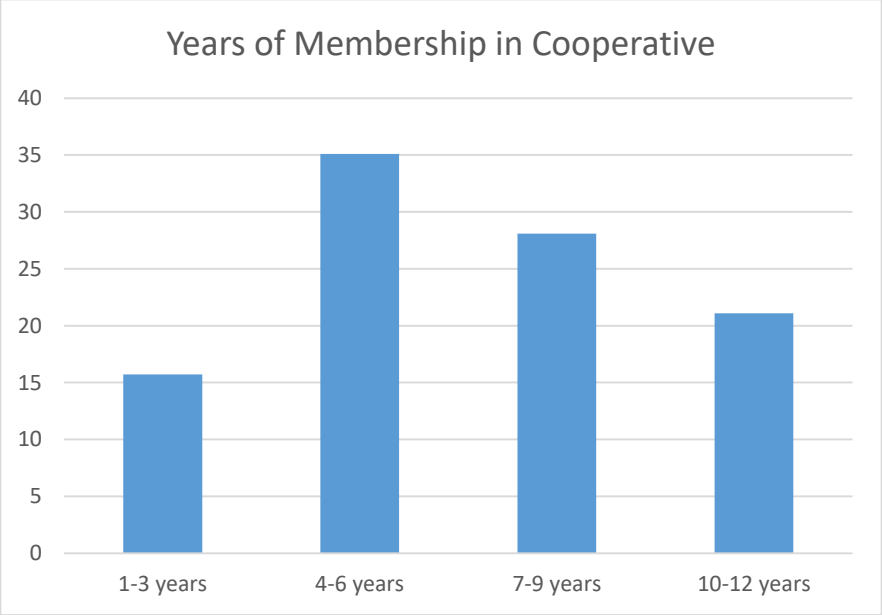

Fig..7: Years of cooperative membership

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Table 1 showed that majority 71.3% do not belong to any fish marketer's association. While only 28.7% belong to the cooperative association. Fig. 7 showed that majority (35.1%) of the members has spent 4-6 years as a member. Membership of a cooperative society enables marketers to interact with themselves, share their experiences and assist themselves. The implication of these results is that most of the marketers in the study area do not enjoy the assumed benefits accruing to cooperative societies through pooling of resources together for a better expansion and effective management of resources which in most cases could not be derive individually.

Monthly income of the Respondents

Figure 8 showed that majority (33.8%) of fish marketers make an average income between ₦41,000 - ₦60,000 while 12.5% make an average income of below ₦20, 000. Ultimately, weekly sales of fish are also presented It reveals that greater proportion (36.2%) of the fish marketers sold at least 31 - 40 fishes per week. The implication of the findings is that the demand for fish consumption is significantly high in the study area. The result confirmed the evidence of the appreciable profit margin the marketers recorded as earlier found out in the study area.

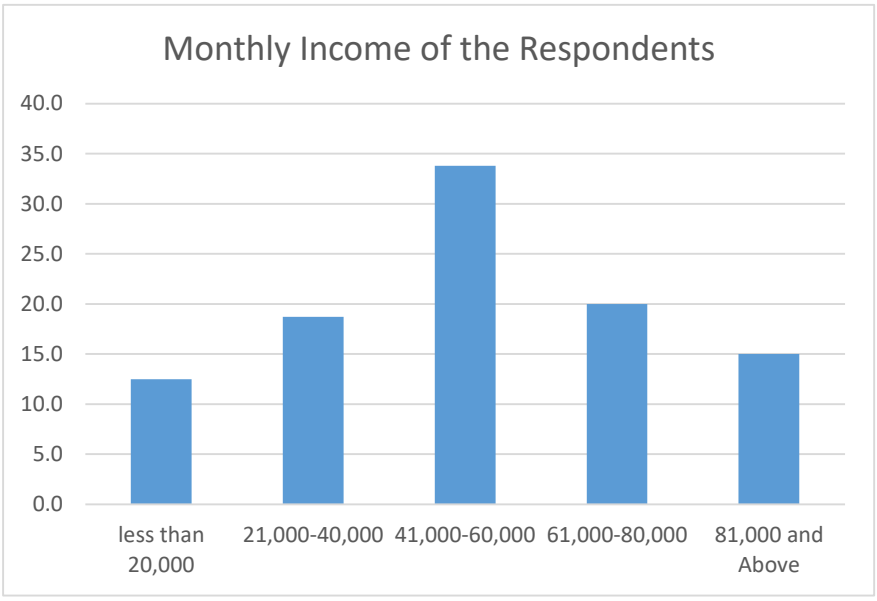

Fig.8: Monthly Income of Respondents

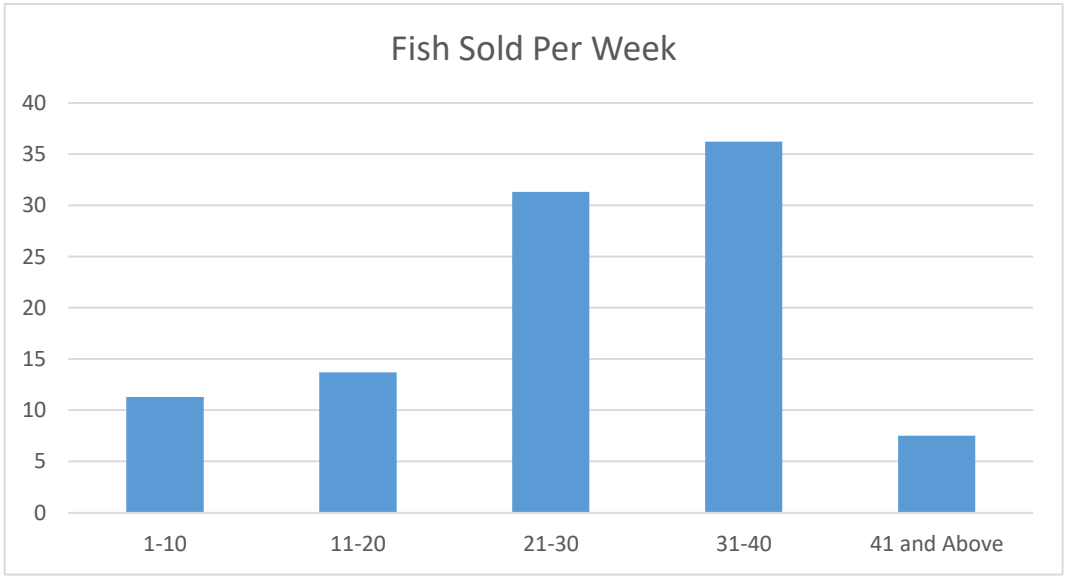

Fig.9: Fish sold per week

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Constraints Encountered by Fish Marketers in the study area

Nigeria's business sector in general as well as the fish subsector in particular has experienced some impediments which slowed the performance of the sector (Adegeye, and Dittoh, 1985). This has caused the output growth not to keep pace with its demand, thereby, resulting in declining exports and domestic supplies and a growing reliance on imports of the products. The problems faced by fish marketers in the study area were ranked according to their severity stated by the respondents. Figure 10 reveals that inadequate storage facilities were the most serious constraint faced by the fish marketers in the area as indicated by 90%. The perishable nature of the fish is a severe barrier to fish marketers this could be attributed to non-electricity in the area which is highly needed for preserving fresh fish which is in high demand in the area.

Fresh fish could only be stored for few hours in which case must be sold even when the price is not favourable, this account for the severe losses suffered by fish marketers in the study area. Fish requires large amount of capital investment for reasonable profit to be made. The study also revealed that about 72.5% of the respondents attested to the fact that Price instability and price fluctuation of fresh catfish were the first constraint faced by marketers. This finding is in line with Bureau of Statistic (2012) that says Prices for fresh fish product responded to the law of demand and supply as no price regulation mechanism exists. 63.8% identified inadequate capital. Fish marketing requires large amount of capital investment for reasonable profit to be made over time. Inadequate capital hinders the marketers from getting the necessary marketing resources and technologies which will increase their profit margin and marketing efficiency. Fresh fish marketing resources are costly. High cost of transportation as a result of bad road network as indicated by 63.8%. Lack of access to credit indicated by 58.8%, ranked fourth on marketing problems limiting operation and expansion of marketing activities in the study area. The problem in accessing credit was mainly related to absence of collateral, high interest rate of commercial bank.

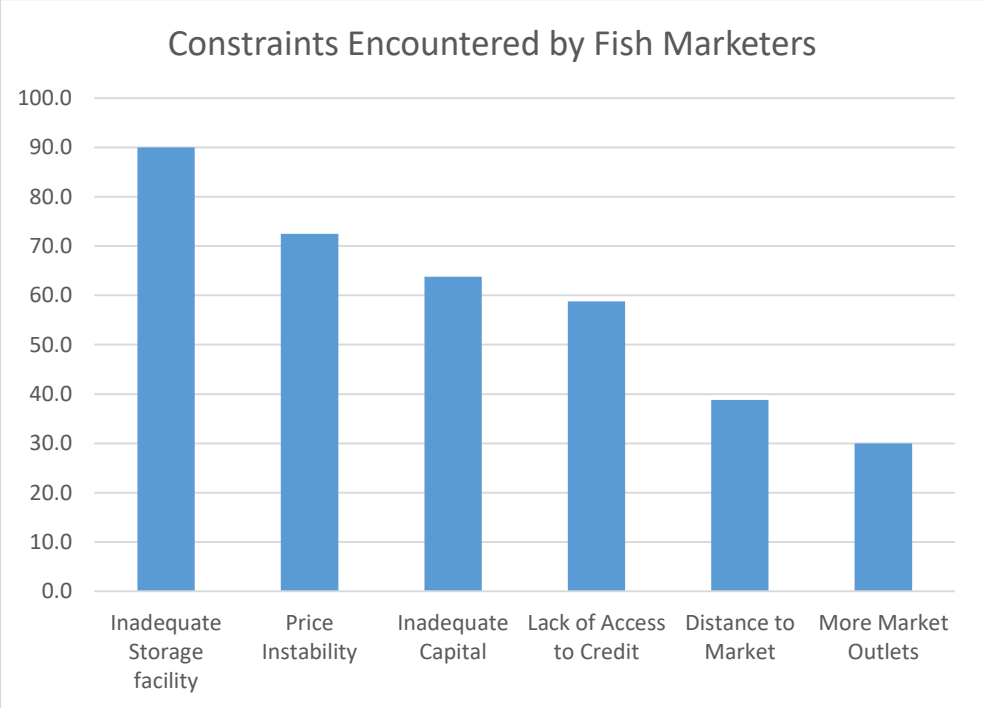

Fig..10: Constraints encontered by the respondents

VI. CONCLUSION

Fish marketing is a lucrative enterprise in the study area. Analysis revealed that age, marital status, education, marketing experience, monthly income and membership of cooperative societies were found to be the significant factors influencing profit margin and the relationships were significant. Also in the study area most of the fish marketers are females and their ages were within the economic active range which favours adoption of marketing development. Most of the fish marketers are married and highly experienced in fish marketing because of families' inheritance.

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The Impact of Crop Rotation and Nutrient Levels on Nutrition Quality, Yield and Yield Components of Maize (Zea maize L)

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Abstract*—Cropping system in crop production has many advantages and ensures better crop growth and yielding. Crop rotation methods can show optimal plants densities for maximal photosynthetic efficiencies and plant growth. This study aimed at investigating the effects of different crop rotation systems: monoculture and biculture rotations, and different nitrogen levels on yield, yield components and grain nutrient quality.*

The lowest Soil-Plant Analysis Development (SPAD), Leaf Area Index(LAI), Protein, plant height, cob length, and grain yield were found in monoculture plot signifying the influence of crop rotation on these measured variables.

Nitrogen level at 120kg/ha had a significant effect on Protein, plant height and grain yield on the monoculture field whiles Moisture content, Protein content number of rows per cob and grain yield were also significantly affected at 120kg/ha level of nitrogen application on the biculture plot

Keywords—Croprotation, maize, Nutrient quality, yield components.

I. INTRODUCTION

MAIZE (*Zea mays* L.) is a highly productive crop under optimal environmental and crop management conditions and it's a main grain crop world over, being used as a major staple food for both human consumption and as feed for animal. It has also become a major key resource for industrial applications and bioenergy production. It is a versatile crop and ranks third following wheat and rice in world production as reported by Food and Agriculture Organization [1].

Worldwide interest in long-term experiments has shown an increased in recent years, since suitable indicators of sustainable agriculture (yield trends, parameters characteristic of the quality of the ecosystem),are capable of serving as an early warning system, that can only be obtained in such experiments [2].

Crop rotation represents a way of approach in crop production research that enables the available natural resources to be preserved in a more efficient utilized ways. In crop rotation experiments, a monoculture is usually compared to various crop sequences. The fact that in most cases the yields of the cultivated crops are higher in crop rotation, as compared with a monoculture under identical conditions, is explained by the rotation effect. This rotation effect has been demonstrated irrespective of the combination of crops in the rotation system. [3] , [4] , and [5].

The benefits of crop rotation for land and water resource protection and productivity have been identified, but many of the rotation factors, processes and mechanisms responsible for increased yield and other benefits need to be better understood. Increased nitrogen supply is sometimes responsible, but improvements in soil water availability, soil nutrient availability, soil structure, soil microbial activity and weed control, decreased insect pressure and disease incidence, and the presence of phytotoxic compounds and or growth-promoting substances originating from crop residues have also been identified as contributing factors [6].

Plant nutrients especially nitrogen is an important element for crop survival and its lost from the soil or crop system may cause environmental pollution. Nitrate (NO^-_3) pollutes ground and surface waters [6], ammonia (NH_3) when deposited to land increases soil acidification and N eutrophication [8] and nitrous oxide (N2O) contributes to global warmingand breakdown of stratospheric ozone [9].

The aim of the present paper is to evaluate the effect of various crop sequences and fertilization treatments on yield and yield components of maize and grain nutrition quality of maize of fully irrigated monocultures and biculture plots of land on a longtime experimental field.

II. MATERIALS AND METHODS

The experimental site for this research is located at Latokep Research Centre of the University of Debrecen in Hungary. The experimental site is about 15km way from the city center of Debrecen with geographic coordinates of 47º33' N, 21º27' E.

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The experiment soil is flat, leveled, and has soil genetic properties belonging to the calcareous chernozem. The experiment was set up on a split-split-plot design in three replication. The research was set up based on a two-factorial parameters and these are the nutrition levels (control, 80kg of Nitrogen and 120kg of Nitrogen), and different cropping system treatment (Monoculture and Biculture) on a plot of land with plant density of (72,500/ha^{-1}). The plots were fully-irrigated in the cropping system.

On the irrigated treatment, optimal water-supply of plants shall be reached by adjusting irrigated water amount to the local temperature and precipitation values for the cropping year 2017. At the end of the cropping year of 2017, the deviation in April, July and September was positive, thus the lack of precipitation in the other months was balanced and the total precipitation of the season was higher 379.9 mm than the 30-years average of 345.1.

The issues that were considered in this research included, the morphological and physiological parameters (SPAD, LAI, NDVI) as well as yield and components thus, Cob length, cob diameter, number of rows per cob, number of kernel per row, etc. The grain moisture content and nutritional content was also measured at harvest.

The results of data for this research were processed and statistically analyzed using software Microsoft Excel and SPSS for windows. The objective of this particular study was to examine the impact of different cropping systems, monoculture and biculture, for maize and wheat rotation on the yield and yield components of maize (Zea mays l).

III. RESULTS AND DISCISSION

In this study, analysis of data indicate that cropping system has a significant effect on the Spad, LAI, Protein, plant height, cob length and crop yield. Table 1 and 2.

The maximum yield and protein of maize was achieved as a result of crop rotation system being applied as Biculture had 6178.877kh/ha as against Monoculture 4041.042kg/ha yield in this research and this study concedes with [5]. (Table2)

Crop rotation provided higher yield as compared to monoculture because of the activities of residues remaining of the previous crop on the soil. [10], especially on lands where the system is already consolidated. A general impact of the cropping system reflected only on LAI, as shown in this study. The negative impact of cropping system on monoculture with significantly lowest yield and LAI was observed. (Table1) The influence of cropping systems on LAI had been also reported by [11].

Table.I: Effects of cropping system on grain nutrient quality and photosynthetic parameters

TREATMENTS	SPAD	NDVI	LAI	PROTEIN	STARCH	MOISTURE
MONOCULTURE	50.93**	75.69	2.89**	8.52**	73.74	17.31
BICULTURE	54.09	75.87	2.47	9.07	74.04 0.41	17.76 0.54
CV (%)	1.80	1.77	0.28	0.27		

*Correlation is significant at 0.05 level, ** correlation is significant at 0.01 level

Table.II: Effect of cropping system on yield components of maize

TREATMENTS	PLANT HEIGHT	COB LENGHT	COB WEIGHT	NO. OF ROWS /COB	YIELD
MONOCULTURE	238.09**	18.36* 19.59	185.50 231.35	15.41	4041.042*
BICULTURE	247.42	1.01	22.87	15.98	6178.877
CV (%)	5.71			1.30	0.002

*Correlation is significant at 0.05 level, ** correlation is significant at 0.01 level

Different levels of Nitrogen supply to plant on both Monoculture and Biculture fields has a significant effect on maize grain nutrient quality and yield as shown in Table3 below.

High rate of nitrogen (120kg/ha) supply to crops has an influence on the protein quality of the grains and also the yield of the maize grains in both cropping systems.[12]Also reported that, nitrogen application increases plant height and protein content of maize grain.

Plant height in the monoculture was significantly affected at the rate of 120kg/ha of nitrogen supply but difference was recorded in the biculture plot.(Table 3). The number of rows per cob was also influence by nutrient levels at 120kg/ha in biculture plot. The moisture and starch content measured at harvest was significantly by nitrogen levels at 80kg/ha and 120kg/ha for monoculture and biculture respectively as shown in Table3.

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Table.III: Effects of Nitrogen levels on grain nutrient quality, yield and yield components of maize

MONO CULTURE	MOISTURE	PROTEIN	STARCH	HEIGHT	LAI	NO. ROW/COB	YIELD/KG
CONTROL	17.75*	7.92	73.90	227.2	2.66	15.11	5638
N80KG	16.95	8.08	74.47*	235.3	2.95	15.77	6788
N120KG	17.03	9.57*	73.45	251.8*	3.05	15.33	9596*
BICULTURE							
CONTROL	17.07	8.32	75.65*	248.0	2.96*	15.33	98342
N80KG	17.70	9.12	73.98	246.8	2.24	14.77	10561
N120KG	18.50*	9.78*	73.48	247.5	2.19	17.83*	12444*

*Correlation is significant at 0.05 level, ** correlation is significant at 0.01 level.

IV. CONCLUSION

From this study, I can be inferred from the obtained results that high yield and grain nutrient quality in maize is associated with cropping and high nitrogen levels. So yielding potential in maize production can be associated with relatively high nitrogen supply, crop rotation and together with best agronomical practices such weed control and proper water management supply systems

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Effect of Mulvap 100% EC. (Dichlorvos) Spray Schedules on the Control of Insect Pests and Yield of Cowpea (*Vigna Unguiculata* L. Walp) in Enugu, Southeastern Nigeria

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Abstract**—** *A field experiment to evaluate the effect of mulvap100%Ec. (Dichlorvos) spray schedules on the control of insect pests, and yield of cowpea (Vigna unguiculata L. Walp) was carried out during the 2016 cropping season at the Faculty of Agriculture and Natural Resources Management Teaching and Research Farm of Enugu State University of Science and Technology Enugu, Southeastern Nigeria, using a randomized complete block design (RCBD) with four treatments replicated five times. There was a significant (P=0.05) effect of mulvap100%Ec. Spray schedules on all the parameters assessed. Mulvap100%Ec. Sprayed every 7 days performed significantly (P=0.05) better than any other insecticide spray schedule in the control of cowpea insect pests, in addition to producing significantly higher pod yield. This was followed by the insecticide sprayed every 14 days, every 21 days and no insecticide sprayed respectively. Plants sprayed with mulvap100%Ec. every 7 days recorded mean number of 0.00 aphids per plant, 2.69% leaf damage by leaf beetles, mean number of 0.64 flower thrips, 0.11 maruca larvae per plant, 0.35% dimpled and shriveled seeds and pod yield of 0.26 tonha^{-1} , followed by plants sprayed with the insecticide every 14 days that recorded mean number of 13.38 aphids per plant, 3.89% leaf damage by leaf beetles, mean number of 1.89 flower thrips per flower, mean number of 0.57 maruca larvae per flower, 1.89% dimpled and shriveled seeds, and pod yield of 0.13 tonha^{-1} and lastly plants sprayed with no insecticide that recorded mean number of 23.39 aphids per plant, 5.49% leaf damage by leaf beetles, mean number of 4.94 flower thrips per flower, mean number of 1.41 maruca larvae per flower, 3.81% dimpled and shriveled seeds, and pod yield of 0.11 tonha^{-1} .*

Keywords**— Cowpea (Vigna unguiculata), insecticide, spray schedules, cowpea insect pests.**

I. INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is one of the most widely used legumes in the tropical world. The grain is used extensively for human nutrition. It is a major vegetable source of protein for human consumption especially in Africa (Ileke *et al*. 2013). Cowpea is a staple component of the diet in several developing countries and a major source of protein to combat malnutrition in young children in Lieu of expensive animal protein. Cowpea seed contains about 25% protein, making it extremely valuable in areas where many people cannot afford proteinous foods such as meat and fish (Lephale *et al*. 2001). It has been regarded as poor man's meat (Ileke *et al*. 2012). It is an extremely important protein source to Vegetarians and people who cannot afford animal protein (Adeyemi *et al*. 2012). Cowpea seeds are also a rich source of minerals and vitamins (Hall *et al.* 2003). The green and dry haulm are fed to livestock particularly in dry seasons when animal feed is scarce (Ababe *et al.* 2005) and also as source of income when sold to farmers who use them as livestock feed (Dugje *et al.* 2009). Cowpea is a warm weather crop that is well adapted to drier regions of the tropic like Nigeria where other food legumes do not thrive well. (Abate *et al.* 2011). Nigeria is its Largest producer and consumer, accounting for about 45 percent of its world's production (Lowenberg-Deboer and Ibro 2008), Ndong *et al.* 2012) while Africa accounts for 75%.

However, the production and the storage of this important crop have faced so many constraints. Okelede and Ariyo (2000) stated that the production of this crop in Nigeria is low and has not matched the demand of the consumers. They also noted that the shortfall in cowpea production is traceable to problem of poor yield resulting from multifarious insect pests and diseases affecting the crop at different stages of development as well as continuous use of low yielding varieties.

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Traditional farmers apply little or no insecticide on cowpea and consequently obtain low yield. Variations among environments for cowpea grain yield where greater when no insecticide was applied than where it was not used (Blade *et al.* 1992). Field insect pests can even cause colossal loss in yield of cowpea (Amatobi *et al.* 2005). They also noted that without the control of insect pests of cowpea, reasonable grain yield cannot be obtained. Several control measures are available but chemicals are more effective, giving several fold increase in grain yield. However, most small scale farmers do not adequately control insect pests and diseases because of the high cost of chemicals and labour (Opole *et al.* 2005). Many entomologists have made efforts to identify the safe and effective chemicals and also optimum number of spray for controlling the most important pests of cowpea, particularly those affecting flowers and pods (Adejumo, 2005, Opole *et al.* 2005). Therefore the general objective of this research work was to evaluate the effect of mulvap100%Ec. on the control of insect pests and yield of cowpea in Enugu, southeastern Nigeria.

II. MATERIALS AND METHODS

A field experiment to evaluate the effect of mulvap 100%Ec. spray schedules on the control of insect pests, and yield of cowpea was carried out during the 2016 cropping season at the Faculty of Agriculture and Natural Resources Management Teaching and Research Farm of Enugu State University of Science and Technology Enugu, Southeastern Nigeria.

Experimental Design.

The experiment was carried out using a randomized complete block design (RCBD) with four treatments replicated five times. The experimental area measured 14 × 11 m (154 m^2). The experimental units (plots) measured 2 m×2 m (4m^2) and were separated by 1m pathway. Three seeds were sown per hole at a spacing of 50 cm × 50 cm and later thinned down to two plants per hole at 7 days after germination.

Treatment. 1.5 liter/ha of mulvap100%Ec. at four spraying schedules viz; 0 liter/ha sprayed, 1.5 liter/ha sprayed every 7 days till harvest, 1.5 liters/ha sprayed every 14 days till harvest, 1.5 liters /ha sprayed every 21 days till harvest.

Data Collection.

Data were collected on;

- The number of cowpea aphids (*Aphis craccivora*) per plant, a total of 10 plants were sampled per experimental units. A plastic bowel was half filled with water and aphids found on each plant were dislodged into the bowel. The water that contained the aphids was filtered with a sieve of 0.15 mm or 150 micro mesh size and the aphids counted.
- Percentage leaf damage by leaf beetles (*Ootheca mutabilis* and *Luperodes lineata*).
- The number of flower thrips per flower. This was done by removal of 10 flowers every 2 days for 3 consecutive times starting from 7 days after flower initiation and counting the number of flower thrips in them.
- Number of *maruca*_larvae per flower. The same 10 flowers used for flower thrips count were used for this purpose.
- Percentage seed damage by pod sucking bugs were determined by calculating the percentage wrinkled and dimpled seeds at harvest.

Statistical Analysis.

The data collected were analyzed using the genstat release (2012) and analysis of variance outlined by Obi 2001.

III. RESULTS

Effect of Mulvap100%Ec.(Dichlorvos) spray schedules on the number of aphids per plants, percentage leaf damage by leaf beetles and number of flower thrips per flower.

The result of the experiment showed a significant (P=0.05) insecticide spray schedules on the mean number of aphids per plant, percentage leaf damage by leaf beetles and mean number of flower thrips per flower. Plants sprayed with the insecticide every 7 days has no aphids per plant indicating a hundred percent (100%) aphid control which also differed significantly from the rest of the spray schedules. Plants sprayed every 14 days had a mean number of 13.38 aphids per plant which differed significantly (P=0.05) from plants sprayed every 21 days and those sprayed with no insecticide that recorded mean number of 20.08 and 23.39 aphids per plant respectively. However, plants sprayed with insecticide every 21 days recorded mean number of aphids that did not significantly differ from those sprayed with no insecticide (Table 1).

On the mean percentage leaf damage by the leaf beetles, there was a significant (P=0.05) insecticide spray schedules effect with plants sprayed every 7 days recording the least mean percentage of 2.69 damaged leaves by leaf beetles, followed by plants sprayed every 14 days having a mean number of 3.89% damaged leaves and lastly plants sprayed with no insecticide with a mean of 5.49% damaged leaves by leaf beetles, which did not significantly differ from plants sprayed every 21 days that recorded a mean of 4.08% damaged leaves (Table 1). There was also a significant (P=0.05) insecticide spray schedules effect on the mean number of flower thrips per flower. Plants sprayed with insecticide every 7 days recorded the least mean number of 0.64 flower thrips per flower that differed significantly (P=0.05) from the rest of the spray schedule, followed by plants sprayed every 14

days that had a mean of 1.87 flower thrips per flower and lastly, plants sprayed with no insecticide having a mean of 4.49 flower thrips per flower (Table 1).

Table.1: Effect of Mulvap100%Ec.(Dichlorvos) spray schedules on the mean number of aphids per plants, percentage leaf damage by leaf beetles and mean number of flower thrips per flower.

Spray schedules (days)	mean number of Aphids per plants	mean percentage leaf damage by leaf beetles	mean number of flower thrips per flower
0	23.39	5.49	4.94
7	0.00	2.69	0.64
14	13.38	3.89	1.87
21	20.08	4.08	2.67
F-LSD $_{0.05}$	4.25	0.99	1.06

Effect of Mulvap100%Ec.(Dichlorvos) spray schedules on the number of *Maruca* larvae per flower, percentage dimpled and shriveled seeds caused by pod sucking bugs and pod yield (tonha^{-1}).

The result of the experiment showed a significant (P=0.05) effect on the mean number of *Maruca* larvae per flower with plants sprayed every 7 days recording the least mean number of 0.11 *Maruca* larvae per flower, followed by plants sprayed with the insecticide every 14 days having a mean number of 0.57 *Maruca* larvae per flower and lastly plants sprayed with no insecticide that had a greater mean number of 1.41 *Maruca* larvae per plant which differed significantly from plants sprayed every 21 days that had a mean number of 0.66. *Maruca* larvae per plant. Again, there was a significant (P=0.05) effect of Mulvap100%Ec. spray schedules on the mean percentage dimpled and shriveled seeds caused by pod sucking bugs with plants sprayed every 7 days recording the least mean percentage of 0.35 dimpled and shriveled seeds, followed by plants sprayed with the insecticide every 14 days that recorded a mean percentage of 1. 89 dimpled and shriveled seeds which differed significantly from the rest of the insecticide spray schedules. Furthermore, there was a significant (P=0.05) effect of mulvap100%Ec. spray schedules on pod yield with plants sprayed every 7 days recording the highest mean pod yield of 0.26tonha^{-1}, followed by plants sprayed every 14 days having a mean pod yield of 0.13tonha^{-1} and lastly plants sprayed with no insecticide recording 0.11tonha^{-1} that did not differ significantly (P=0.05) from the rest insecticide spray schedules, except that of every 7 days spray schedule (Table 2).

Table.2: Effect of Mulvap100%Ec.(Dichlorvos) spray schedules on the number of Maruca larvae per flower, percentage dimpled and shriveled seeds caused by pod sucking bugs, and pod yield(tonha^{-1}).

Spray schedules (days)	mean numbers of *Maruca* larvae/plant	mean number of dimpled and shriveled seed (%)	pod yield (tonha^{-1})
0	1.14	3.81	0.11
7	0.11	0.35	0.21
14	0.57	1.89	0.13
21	0.66	1.96	0.12
F-LSD $_{0.05}$	0.36	1.38	0.12

IV. DISCUSSION AND RECOMMENDATION

A hundred percent (100%) control of aphids by Mulvap100%Ec. (Dichlorvos) sprayed every 7 days showed that a regular application of this insecticide to cowpea plants is necessary for a total eradication of this cowpea insect pest. Apart from total eradication of aphids on this important leguminous crop, this insecticide sprayed every 7 days on cowpea plants that recorded lower levels of leaf beetles, flower thrips, *Maruca* larvae and pod sucking bugs infestation, also emphasized the importance of regular application of this insecticide.

Furthermore, Mulvap100%Ec.(Dichlorvos) sprayed every 7 days on cowpea plants recording a significant (P=0.05) higher mean pod yield of 2.26tonha^{-1} also showed the importance of regular application of insecticide to improve pod yield in cowpea. These findings agreed with the following researchers; Alabi *et al. (*2003) indicated that low yield is not inherent in cowpea but mainly caused by insect pests attack. They also noted that controlling flowering and podding pests resulted in highest grain yield per plot. He however recommended applying insecticide once weekly during flowering and podding stage than applying it once every week through the cowpea growing period. A similar result was reported by (Algali 1992), which suggested that insect pests of flowers and pods were most important in reducing grain

yield. Karugi *et al.* (2000) reported that regular application of insecticide generally reduce cowpea insect pests infestation and markedly increase yield. Isubikalu, (2002). Omongo *et al*. 1998 indicated that in some parts of Nigeria like the North, large scale cowpea producers, sometimes apply insecticides as many as 8-10 times during the growing season to control insect pests. They also suggested that 10 days interval insecticide application (4 times) can be as profitable as 7 days interval application (5 times) in cowpea production. Again, (Emosairue *et al.* 2004), observed that insecticide at present offer the only effective control of pests and a crop sprayed weekly from the first day after planting (DAP) can out yield an unsprayed crop by eight to nine times (784kg/ha), and less frequent application (every two weeks) gave intermediate yield of 452kg/ha, if started 21 DAP, 243kg/ha and if started 35 DAP, 187kg/ha.

As a result of this experiment, I suggest that cowpea producers in Enugu area, southeastern Nigeria should practice spraying of insecticide to growing cowpea plants weekly starting from one week after germination for the purpose of controlling cowpea insect pests attack and maximizing pod/grain yield. This is so because, this insecticide spraying interval is close enough to meet flowering and podding stages which were observed by some researchers as the critical stages of cowpea growth at which insecticide application significantly (P=0.05) minimizes pod/grain yield loss due to insect pests (Alabi *et al.* 2003).

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Determination of Physiological, Biochemical and Molecular Effects of Zinc Stress on the Growth of Sunflower Seedlings (*Helianthus annuus* L.)

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***Abstract*—** *Heavy metal contamination is an important environmental problem all over the world. High concentrations of heavy metals cause permanent damage stocells and tissues. In this study, the toxic effects of zincheavy metal in sunflower plant in population and molecular parameters were investigated. The effects of zincheavymetalon sunflower seedlings were determined using the changes in population parameters; rootlength, dry weight, and total solubleprotein content. Total protein content of sunflower plants was determined in a relationship in the opposite direction increasing the amount of metal concentrations. Genotoxic effects of heavymetal of zinc on sunflower plants were revealed by using changes in genomic template stability (GTS). According to analyses, serious changes in genomic template stability were detected and these results were compared with the growth, dry weight and total soluble protein content of the seedlings grown at various zinc concentrations. Also, it was seen that the genomic template stability significantly affected the primary root length, root dry weight and root total soluble protein content. Positive correlations were observed between physiological, biochemical and molecular parameters in sunflower seedlings under zinc stress.In conclusion, a comparison between physiological, biochemical and molecular parameters shows that zinc is a genotoxic agent for sunflower plants.*

***Keywords*— Genotoxic effect; Heavy metal; Sunflower; Zinc stress.**

I. INTRODUCTION

Sunflower (*Helianthus annuus* L.) has economic importance in terms of agriculture in the worldwide. The high level of its fatty acid (69%) easily explains the importance of the sunflower plant (Lentz et al., 2008; Blackmana, 2011). Environmental pollution took place because of the necessity to create areas for urbanized living and then it has increased since then with the improvement of industry. Especially after 1950s, the rapid growth of the population has caused our natural resources to be polluted even more and the ecosystem has got serious damages (Yarsan et al., 2000).If the environmental changes affect a plant's normal growth and development in a negative way, the reaction or the general situation of the plant is called stress. According to Levitt, stress factors have two different groups; biotic and abiotic. According to this classification, abiotic factors are the most dangerous ones which include heavy metals and threaten the ecological balance (Levitt, 1972). Heavy metals which can be heavily accumulated in soil, water and air is now a common environmental concern to take precautions immediately and it is equally dangerous for every living organism from plants to human (Yarsan et al., 2000). Several industrial activities, urban waste, exhaust gases, mining activities, some volcanic activities, disinfection and fertilisation held in agriculture, heavy use of pesticides are some of the examples of heavy metal pollution. This pollution also causes decrease in the quality of agricultural products (KirbagandMunzuroglu, 2006). Heavy metals also give damage to most of the functional biomolecules including membrane lipids and this results in increase of some reactive oxygen types such as (ROS), hydroxyl radicals, (OH·) or hydrogen peroxide, (H_2O_2) superoxide anion, (O_2^-) which cause oxidative stress (BurzynskiandKlobus, 2004;Koc et al., 2012). It is also known that heavy metals cause genotoxicity in living things. This situation occurs as a results of treating organisms with some chemical, biological and physical agents that leads to damages in their genetic materials (Steinkellner et al., 1998;Savva, 2000;Hall, 2002). Zinc is a microelement which should be taken in very less amounts by plants, animals and humans. In the deficiency of zinc, RNA levels and the cell's ribosome content decrease and this situation leads to a decrease in the protein formation mechanism. Besides, in deficiency of zinc in the plants, indexes of indol-3-acedic acid (IAA), abscisic acid and tryptophan amino acid levels also decreases. This situation gives damages to the normal growth of the plants and affects the herbal production in a serious way.The toxic effect of Zn^{2+} cause damages to the

cell division and it especially gives damages to the cell nucleus of meristematic stem cell (Koc et al., 2012). At the same time, Zn^{2+} stress results in clorosis, which is defined as a damage in the activity of chloroplast and shrinking of the plant's size. It also affects the productivity and decreases the amount of chlorophyll and resolubleproteins, the length of the root, the weight and the amount of the seed (KhuranaandChatterjee 2001;BekiarogluandKarataglis, 2002; Koc et al., 2012). In this current study, it was aimed to determine the effect of zinc heavy metal on sunflower seedlings by the use of physiological parameters such as stem/root elongation and RAPD analysis for possible genotoxicity, which is one of the PCR-based molecular indicators.

II. MATERIAL AND METHODS

Germination method, measurement of total soluble protein and length of root

Sunflower seeds' surfaces were sterilized with 70% alcohol and 30% sodium hypochlorite solution and washed three-four times with distilled water. For germinating and growing of sunflower seeds, seedling trays were filled with sterilized perlite and seeds were planted in each cell of seedling tray. The seedling trays were divided into eight groups in total, including control and seven different concentrations of zinc solution. Control group of the tray was treated with only 15mL of distilled water. The other groups of the trays were treated with 15 ml of 20, 40, 80, 160, 320, 640 and 1280 mg L^{-1} concentrations of $ZnSO_4 \cdot 6H_2O$ zinc solutions for each, respectively. These treatments were replicated twice. All these procedures were performed for 21 days. After 21 days of treatment, each plant samples' root and stem length belonging to different groups of sunflower seedlings were measured and the harvested plants were frozen in liquid nitrogen and stored at -20°C until DNA extraction. Total soluble protein of the sunflower seedlings were measured according to the Bradford method (Bradford, 1976).

DNA extraction and RAPD procedures

The piece (200mg) of roots obtained from the seedlings after 21 days of growth procedure was grounded with liquid nitrogen in eppendorf tubes, and total genomic DNA isolation was performed with the DNA isolation protocol of Lefort (Lefort,1998).The quantity and quality of DNA samples were determined by Nanodrop Spectrophotometer (ND-1000 Thermo Scientific) and also confirmed by gel electrophoresis which contains 1.5% agarose and 0.05µlml^{-1} ethidium bromide. After then, the DNA samples with suitable purity and concentration levels were selected to be used in RAPD procedure. RAPD-PCR study was performed with total 25µl of standard reaction volume for each sample. Optimum amplification conditions were obtained with 200ng genomic DNA, 1× reaction buffer, 2.5mM $MgCl_2$, 20µM dNTPs, 0.2mM primer, and 0,5U Taq DNA polymerase (Promega). 14 of 20 RAPD primers used in this study revealed polymorphic bands that are different from the control group of sunflower. Fourteen RAPD primers [5'→3'; (OPA-03) AGTCAGCCAC; (OPA-08) GTGACGTAGG; (OPB-07) GGTGACGCAG; (OPC-01) TTCGAGCCAG; (OPC-02) GTGAGGCGTC; (OPC-04) CCGCATCTAC; (OPC-05) TGGACCGGTG; (OPC-06) GAACGGACTC; (OPC-07) GTCCCGACGA; (OPC-08) TGGACCGGTG; (OPC-09) CTCACCGTCC; (OPC-10) TGTCTGGGTG; (OPC-11) AAAGCTGCGG; (OPF-05) CCGAATTCCC] were used for RAPD-PCR reactions. The thermal cycling conditions included an initial denaturation step of 95°C for 5 min, followed by 35 cycles of 94°C for 90s (denaturation), 36°C for 60s (annealing), and 72°C for 120s (extension) followed by a final extension period of 72°C for 5min. Negative control PCR, not including any DNA template was run for each samples for testing any other kinds of DNA contaminations. All PCR reactions were carried out in duplicate. PCR reaction products and DNA ladder (DNA ladder plus, Promega 100bp) were subjected to an electrophoretic separation process for 2-2,5h, under 5V cm^{-1} current in 1.5% agarose gel containing 0.05µlml^{-1} ethidium bromide. The gels were displayed with UV imaging system and photographed with using GyneSnap Software (Synoptics Co). After then, the gel photographs were analyzed for identifying the RAPD profiles.

Calculating the genomic template stability (GTS)

After analysis of the RAPD profiles, genomic template stability (%) was calculated with the following formula: $GTS_{=}$ $(1\text{-}an^{-1})$ ×100, where letter of a; refers to polymorphic band number of each sample, which was treated with the different zinc solutions and the letter of n; refers to the total band number in the control. The appearance or disappearance of bands in the treated samples' RAPD profiles in comparison to the control RAPD profiles were identified as polymorphism.

Statistical analysis

The SPSS (statistical package software v. 17.0 Multi-language for Windows) was used to analyze the changes in root length, dry weight and total soluble protein content. Data were tested by performing the paired sample t-test.

III. RESULTS AND DİSCUSSİON

The effect of zinc on physiological and biochemical parameters

One of the effects of toxins is to prevent the root and body growth. The accumulation of heavy metals in plant causes

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negative effects on roots, stems and germination of seeds; when it is exposed to the increasing concentrations of heavy metals (Zenginand Munzuroglu, 2004). Similarly, as expected in sunflower plants, the findings of this study on the length of sunflower seedlings' roots and stems are similar with the related literature. In this study, for 21 days, when the samples which were exposed to heavy metal stress with different Zn^{2+} concentrations are evaluated, the improvement of plants' roots and stems length have suggested that there is a clear decrease in parallel with the increasing concentration of Zn^{2+}, as expected. However, it was observed that there was an increase in root and stem lengths and improvement of plants which were exposed to 20mg L^{-1} and 40mg L^{-1} of zinc stress. It was found that, in the plant samples which were exposed to 20mg L^{-1} and 40mg L^{-1} of zinc stress, the root and stem lengths were more than the control group. This was also an expected finding. The reason for this is that in some levels, zinc can be used as a micronutrient (Koc et al., 2012). When it was used with 80mg L^{-1} or more concentration, the root and stem lengths were decreased. In other words, as the concentration of zinc increased, the root and stem lengths of the sunflower seedlings decreased. Decreases between 11-88% in the root lengths of the sunflower seedlings were observed compared to the control plants. Besides, when it was used with 80mg L^{-1} or more, it was observed that in the roots, there were tarnishing and it increased gradually. It is indicated that tarnishing takes place when the suberin level increases and this situation limits the water intake of the plant (BarceloandPoschenrieder, 1990) (Figure 1).

Fig.1: The views of sunflower seedlings a; control group sunflower samples, b; sunflower samples of exposed to 20 mg L^{-1} Zn stress, c; 40 mg L^{-1}, d; 80 mg L^{-1}, e; 160 mg L^{-1}, f; 320 mg L^{-1}, g; 640 mg L^{-1}, h; 1280 mg L^{-1}

Nevertheless, statistically significant differences for the effects of zinc stress on the root development was observed above 80mg L^{-1} ($P < 0.05$).A gradual decrease was determined in sunflower seedlings after 80mg L^{-1} depending on the increasing concentrations of zinc ($P < 0.01$).With 320mg L^{-1} concentration, zinc had similar negative effects on the root length of the sunflower seedlings ($P < 0.001$). However, above 160mg L^{-1}, zinc had more negative effects on the root and stem length ofsunflower seedlings compared to control group (Table 1).

Table.1: Sunflower seedlings were exposed to various zinc (Zn) concentrations (mg L^{-1}). Changes in the root length (cm seedling^{-1}), dry weight (g seedling^{-1}), total protein content (mg ml^{-1}) and GTS rate (%).

	C	Zn 20	Zn 40	Zn 80	Zn 160	Zn 320	Zn 640	Zn 1280
Root length	8.5	9.5	9.0*	7.5*	4.0**	2.3**	1.5***	1.0***
Dry weight	0.0072	0.0073	0.0072	0.0066*	0.0058**	0.0046**	0.0040**	0.0028***
Total protein content	0.0405	0.0440	0.0410	0.0328**	0.0308**	0.0249***	0.0211***	0.0187***

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GTS rate	100	92.85	91.66	90.47	88.09	85.71	83.33	83.92

*n= 14 for each group. *P<0.05, **P<0.01, ***P<0.001*

The toxic effect of some heavy metals (Pb, Cu, Ni, Zn, Mn and Cd) give damages to the cell structure and physiological activity; it especially results in clorosis, which is defined with the damage in the activity of chloroplast and decreases of the plant's size. It also affects the productivity and decreases the amount of chlorophyll and resoluble proteins. As a result, it leads to a negative effect on the production of dry weight (Khuranaand Chatterjee, 2001;BekiarogluandKarataglis, 2002; Koc et al., 2012). Compared to the control group, the exposure to zinc stress with 80mg L^{-1} and more, resulted in a decrease in sunflower seedlings' dry weight. The negative effect was observed even more in sunflower seedlings' dry weight with the losses between 8-60% when they were exposed to 80mg L^{-1} and more concentrations, respectively.Also, there was a consistent relationship between the dry weight and the length of the heavy metal treated root. R^2 value of the dry weight-root length for increasing concentrations of zinc treatment were found to be 0.9892 (Figure 2).

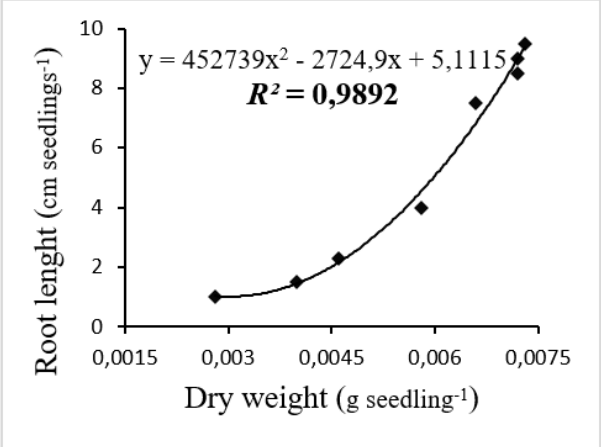

Fig.2: Influence of root dry weight to primary root length and R^2 value

Similar to the decreases in the dry weight, the length of root and total soluble protein contents of all the sunflower seedlings effected due to the increase in heavy metal concentration. Similarly, it was indicated that the increase in the concentrations of lead, cadmium and copper causes a decrease in the total protein content of sunflower cucumber and artichoke respectively (Kastori et al., 1992; Soydam et al., 2012; Batir et al., 2016). In this study, when the soluble protein indexes were evaluated in sunflower plants after the applications of zinc solution with different concentrations, the level was found as 0.0405mg L^{-1} in control group. On the other hand, in 20mg L^{-1}, the total protein level was found to be 0.0440mgL^{-1} and in 1280mg L^{-1}, it was found to be 0.0187mgL^{-1}. There was an observed increase in 20mg $L^{-1}$and 40mg L^{-1}. When it was increased to 80mg $L^{-1}$and more concentrations, the protein content was observed to decrease gradually. At the same time, positive correlations were observed between the root total soluble protein content and the root dry weight as well as between the root total soluble protein content and primary root length for zinc treatments in sunflower seedlings. It was seen that root total soluble protein content significantly affected the root dry weight (R^2 = 0.9828). In addition, primary root length was significantly affected by the root total soluble protein content in sunflower seedlings exposed to zinc stress (R^2 = 0.9365) (Figure 3).

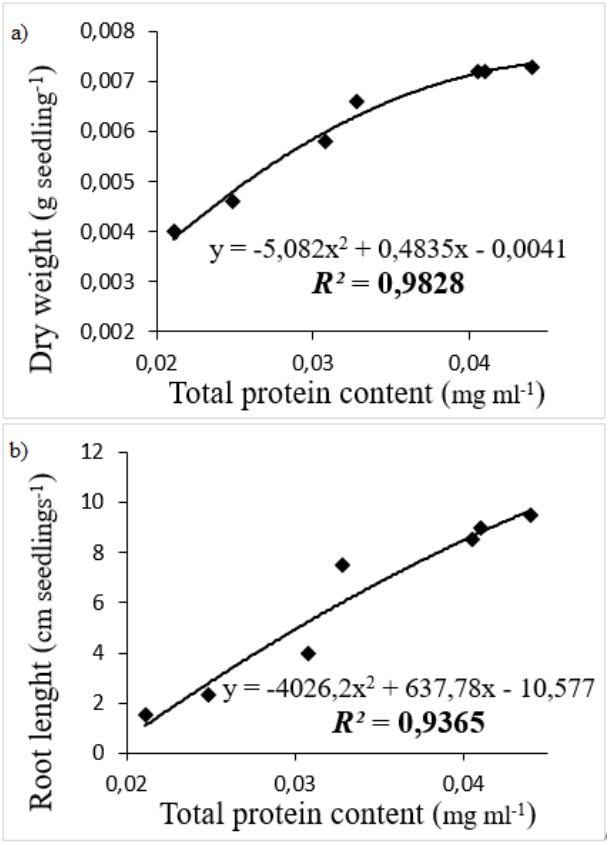

Fig.3: Influence of root total soluble protein contenttoprimaryrootlength (a) anddryweight (b) andR^2 values

The effect of zinc stress on RAPD profile

RAPD profile is used to define the genotoxicity in most of the living organisms. There are so many studies about detecting the damage of genotoxic agents on DNA by using RAPD. RAPD methods enable us to examine the relationship between the genotoxic agent and different factors such as concentration and exposure duration. From bacteria to flowery plants, it can be used for so many organisms and it helps to examine multiple samples simultaneously. (Sava, 2000;Atienzaret al.,2002; Liu et al., 2005). For example, Liu et al, (2007); applied

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cadmium (Cd) solution in different concentrations to rice (*Oryza sativa* L.) seeds and they suggested that the RAPD band patterns have changed in high concentrations. When all the other conditions were kept stable, the only change that took place occurred in the samples with high metal exposure. This situation showed that the genotoxic agent cadmium (Cd) has a mutational effect. According to RAPD analysis, the reason of observing different band profiles in the control is the mutations that occur on the genome sites where the primers are bounded on DNA structure (Liu et al., 2007). Aksoy et al. (2010); also applied copper (Cu) in different concentrations to eggplant seeds. In different copper concentrations, the grown-up seeds can be observed in terms of their genomic structure stability changes by using the RAPD profiles (Aksoyet al., 2010). Cansaran et al. (2011); clearly indicated the genotoxic effect of the air pollution and heavy metal on lichen samples by using RAPD technic. They reported DNA polymorphism induced by accumulation of heavy metal in lichens. They also expressed that RAPD is more sensitive as they give more evidence about DNA damage (Cansaran et al., 2011). In another study, Batir et al. (2016); examined the genotoxic effects of lead and copper treatments with different concentrations on artichoke seedlings. They reported that the lead and copper cause genotoxic effect on the genomes of the artichoke and generate polymorphism in the RAPD band profiles (Batir et al., 2016). In this study, according to the results of RAPD analysis, highly important polymorphism is observed in sunflower samples subjected to zinc stress. 14 of 20 RAPD primers used in this study revealed polymorphic bands that are different from the control group of sunflower. OPC09 (57.2%), OPC08 (55.5%), OPC07 (50%) and OPC11 (50%) primers showed considerable polymorphic band patterns. This showed that these primers are powerful indicator for mutagenic effect of heavy metals for sunflower plants. Genomic template stability (GTS, %) was used to compare the alteration in RAPD profiles with the morphological characters which were root length, dry weight and total protein content in sunflower seedlings. The comparison of GTS (%), root length, dry weight and total soluble protein content were calculated according to their control value which was set to 100% (Tables 1). When compared the GTS rates that were obtained from RAPD profiles, the highest rate was 92.85% at 20mg L^{-1}zinc concentration. The lowest rate was 83.33% at 640mg L^{-1}zinc. These results clearly underline the importance of the concentration applied. In addition, it was seen that the genomic template stability significantly affected the primary root length (R^2=0.9261), root dry weight (R^2=0.9358) and root total soluble protein content (R^2 = 0.9216) (Figure 4).

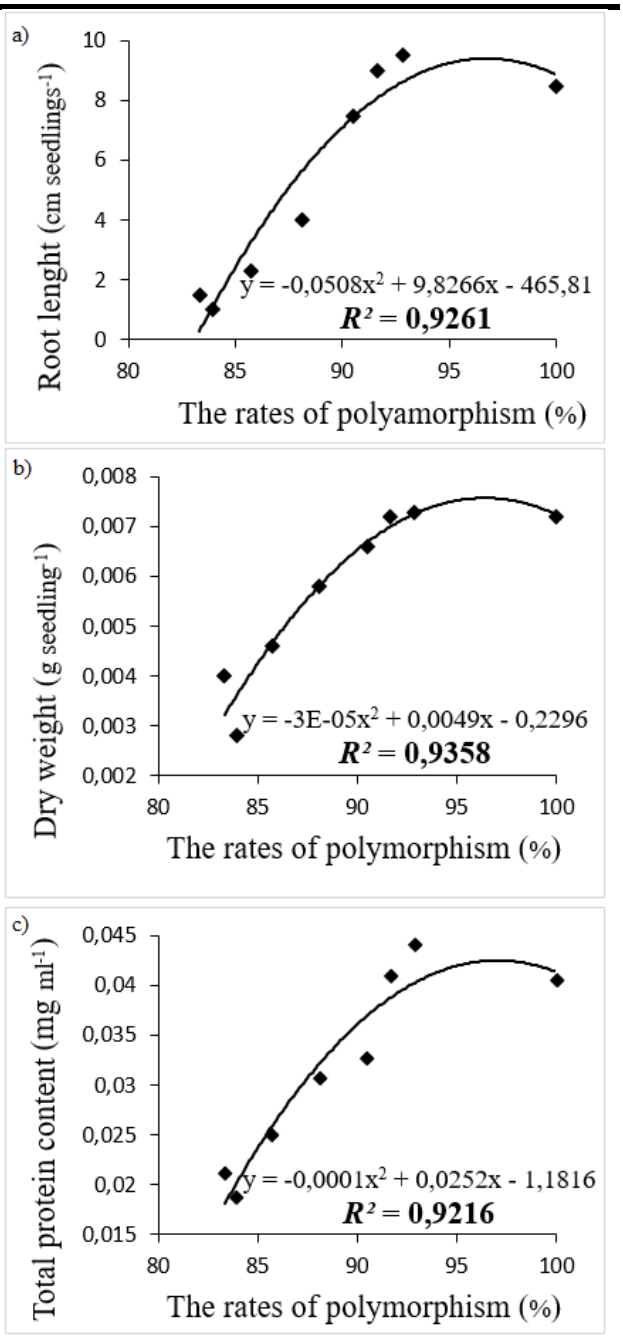

Fig.4: Influence of genomictemplate stability tother oot lenght (a), dryweight of root (b) andsoluble protein content (c) R^2 values

IV. CONCLUSIONS

In the current study, serious changes were observed in sunflower plant both in the population level and molecular level when they were exposed to zinc heavy metal. Changes in the level of DNA patterns were seen to be effective on bio-defense mechanism in sunflower plants. Our results indicate that zinc is a genotoxic agent for sunflower plant and it can be useful for restoring zinc contaminated areas with certain levels. Also, with the organism used as the bio-indicator, the biological effects of pollution were detected quantitatively in this study.

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Sensitivity of *Colletotrichum* species responsible for banana anthracnose disease to some fungicides used in postharvest treatments in Côte d'Ivoire

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***Abstract*— *Anthracnose is a major postharvest disease of banana fruit in Côte d'Ivoire. Colletotrichum musae is usually associated with banana anthracnose disease. Persistent symptoms of anthracnose despite the post-harvest treatment requires accurate characterization of pathogens for effective control. The present study was conducted to identify the species of Colletotrichum responsible for banana anthracnose and test their sensitivity to fungicides. The morphological study and molecular identification of isolated species associated with anthracnose symptoms had identified Colletotrichum gloeosporioides and Colletotrichum musae. Pathogenicity tests with representative isolates were conducted on symptomless banana fruits. All tested isolates caused anthracnose lesions on banana fruit, however C. musae was significantly more aggressive than C. gloeosporioides. Sensitivity tests including imazalil, azoxystrobin and boscalid showed a sensitivity of C. musae and C. gloeosporioides to imazalil. However, a resistance of both species to azoxystrobin and boscalid was recorded. This study highlighted the presence of resistants strains of Colletotrichum responsible for anthracnose in Côte d'Ivoire.*

Keywords*— *Banana, Anthracnose, C. musae, C. gloeosporioides, Sensitivity

I. INTRODUCTION

Banana is an economically important crop in tropical and subtropical regions. Côte d'Ivoire is one of the main African producers of banana dessert. The bulk of Ivorian production is destined for the European market with an estimated export volume of 330460 tons in 2014 (Faostat, 2016).

However, fungal diseases are responsible for large losses in the banana production chains, especially during postharvest period. Banana anthracnose is considered as one of the most important diseases of banana (Jeffries *et al*. 1990). Infection on the banana usually starts during the development of the fruit but remains quiescent until the fruit ripens; symptoms often manifest during storage. Symptoms appear as brown or black spots which enlarge depressed with ripening (Ranasinghe *et al* 2005). Anthracnose deteriorates quality of bananas and causes economic losses for producers and traders (Ara *et al*. 2012).

In Côte d'Ivoire, anthracnose was found to be recurrent on bananas despite postharvest treatments. *Colletotrichum* species are agents associated with anthracnose on many tropical and subtropical fruits (Bailey *et al* 1992). however, only *C. musae* was identified as responsible for banana anthracnose (Stover *et al*., 1987).

Chemical control methods are the most used strategies against the fungi responsible for banana postharvest diseases (Gang *et al*., 2015). Chemical control of banana anthracnose includes the use of active ingredients such as benzimidazoles, Strobulirines, and dicarboxamides (Young *et al*., 2010). However, persistence of banana anthracnose has been noted in recent decades despite antifungal treatment applied by producers in Côte d'Ivoire. Resistant strains to these fungicides have emerged. Indeed, the recurrence of banana anthracnose could be due to intensive monoculture in industrial plantations which could favor the development of new pathogens strains.

The objective of this work was to identify the species of *Colletotrichum* responsible of banana anthracnose and evaluate their sensitivity to fungicides currently used in postharvest treatment.

II. MATERIAL AND METHODS

2.1. Sampling and isolation of *Colletotrichum* spp.

Boxed banana (Cavendish Subgroup, cv. Grande Naine) treated with fungicide were randomly selected at different banana production sites in Côte d'Ivoire (Abengourou, Abgoville, Aboisso Azaguié, Dabou, Grand Bassam and Tiassalé). Banana boxes were placed in storage at the laboratory at room temperature (27 ± 1 ° C) for 21 days and observed daily. During this period, symptoms

developed on the epicarp were observed and described. Banana fruits showing anthracnose symptoms were used for fungi isolation according to the method of Davet and Rouxel (1997). Pure cultures were obtained by single spore isolation carried out using the procedure described by Choi *et al* (1999), with modifications.

2.2. Morphological and cultural characterization

Each isolate from pure cultures was plated onto PDA and incubated at room temperature (27 ± 1 ° C). After 7 days, colony size, shape, margin and colour were recorded. Colony diameter of every culture was recorded daily for 7 days. Growth rate was calculated as the 7-day average of mean daily growth (mm per day). Three cultures of each isolate were investigated and experiments were conducted twice. For examination of conidial morphology, all isolates were subcultered as mentioned above. Cultures were washed with sterile water and drops of the suspension were placed on microscope slides. Length and width were measured for 50 conidia per isolate. Conidial shape (cylindrical or falcate) was also recorded.

2.3. Pathogenicity Test

Pathogenicity tests were performed with a representative set of isolates, from all morphological groups, using non-infected matured green unripe banana fruits (Cavendish Subgroup, cv. Grande Naine). Banana fruits were disinfested by immersing them in 1 % NaOCl solution for 1 min, washed twice with sterile distilled water. The fruits were blotted dry with a sterile paper tissue and inoculated using the wound/ drop method (Lim *et al*., 2002; Kanchana-udomkan *et al*., 2004). The wound/drop method involved pin-pricking the surface of the fruit to a 1 mm depth and then placing 10 µl of conidial suspension (1×10^6 spores ml/1) over the wound. Nine fruits were tested per isolate and experiments were conducted twice. The inoculated fruit, along with appropriate controls (fruit inoculated with sterile distilled water) were placed in storage into sterile plastic containers and incubated at laboratory temperature (27 ± 1 ° C). Symptoms were recorded 5 days after inoculation (d.a.i.) and re-isolation, according to Koch's postulates, was made from all resulting lesions. The lesion diameter was measured 5 days after inoculation and the mean diameter of the lesions was calculated.

2.4. Molecular characterization

2.4.1. DNA extraction

Total genomic DNA was extracted from fungal mycelium grown on potato dextrose agar (PDA) following the protocol of Murray and Thomson. (1980).

2.4.2. PCR and sequencing

PCR amplification of the rDNA ITS region was done using the universal primers ITS1 and ITS4 (White *et al*., 1990) in a thermocycler T 100 (Bio Rad). The following amplification program was used: initial denaturation (5 min at 94 ° C), followed by 35 cycles each comprising a denaturation step (30 s at 94 ° C), a step of annealing (30 s at 55 ° C) and an elongation step (30 s at 72 ° C) and a final elongation for 10 min at 72 ° *C.* The PCR products were migrated at a voltage of 100 V for 30 min by electrophoresis in an agarose gel at 1% in a 0.5 × TBE buffer (Tris-Borate 90 mM, 1 mM EDTA) and visualized in a visual reader of EBOX-VX5 brand. The PCR products were purified observed amplicons sequenced Sporometrics, 219 Dufferin Street in Canada.

2.5. *In vitro* susceptibility of *Colletotrichum* species to fungicides

Three commercial fungicides Bankit, Cumora, Sulima (Table 1) were evaluated *in vitro* using the poison food technique (Adams and Wong, 1991). Fungicide suspension of 1 ppm, 10 ppm, 50 ppm, 100 ppm, 500 ppm, 1000 ppm and 1500 ppm were prepared by dissolving requisite quantities of each fungicide in autoclaved cooled PDA just before pouring into Petri dishes. Fifteen ml of fungicide amended media was poured into each 9 cm sterilized Petri dishes. Three replicates were performed for each concentration of each fungicide. Medium without fungicide served as a control. After solidification of the medium, each dish was inoculated with a mycelial disc (5-mm diameter) taken from the periphery of actively growing colonies on PDA. The Petri dishes were incubated in dark at 27 ± 1 °C until the control colony reached the margins of the Petri dish. The measurement of the diameter of the mycelial growth of the fungus were recorded on a daily basis, beginning with 24 hours after inoculation. Percent inhibition of growth of *Colletotrichum* species was recorded using the following formula (1):

$$I\,(\%) = \frac{dc - dt}{dc} \times 100 \quad (1)$$

where, **dc** is the average fungal colony diameter measured in control plate, with no treatment, and **dt** is the average fungal colony diameter measured in treated dishes

Table.1: Characteristics of fungicides used in banana postharvest treatment

actives ingredients	commercial name	Groups	Concentration
Azoxystrobin	Bankit	Strobulirin	250 g/l
Imazalil	Sulima	Imidazol	750 g/l
Boscalid	Cumora	Carboxamid	500 g/l

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2.6. Statistical analysis

Analysis of variance (ANOVA) to a classification criterion was performed to compare mean growth of isolates, mean length and width of conidia. The average lesion diameter and susceptibility of fungi to fungicides were also compared. The means were separated using Least Significance Different Test at $p < 0.05$. The analyzes were performed using IBM SPSS Statistics 20 software.

III. RESULTS

3.1. Morphological and cultural characterization

Common morphological characteristics of colonies allowed the grouping of the isolates into two morphological groups (Table 2). Isolates of Group 1 had colonies with reddish slight pink moderate aerial mycelia. The colonies produced by isolates of Group 2 had white colonies with moderate aerial mycelia and copious cinnamon conidial masses (Fig 1). The mean growth rate of the various groups was calculated. There was no significant difference ($P = 0.74$) in growth rate among isolates of two groups. Isolates of group 1 had growth rates ranging from 4.36 to 4.8 mm day-1. Isolates from group 2 had growth rates ranging from 4.9 to 5.2 mm day-1. Isolates of group 1 had significantly longer conidia than those of group 2. Isolates of group 1 produced hyaline, cylindrical conidia with acute apex. The average length and width of the conidia were 11.25 µm and 2.5 µm, respectively. The group 2 conidia were all cylindrical with both ends rounded. The average length and width of the conidia were 23 µm and 5.5 µm, respectively. Isolated belonging to group 1 were confirmed to be *C. gloeosporioides* and isolated of the group 2 were *C. musae* according to morphological and cultural characteristics.

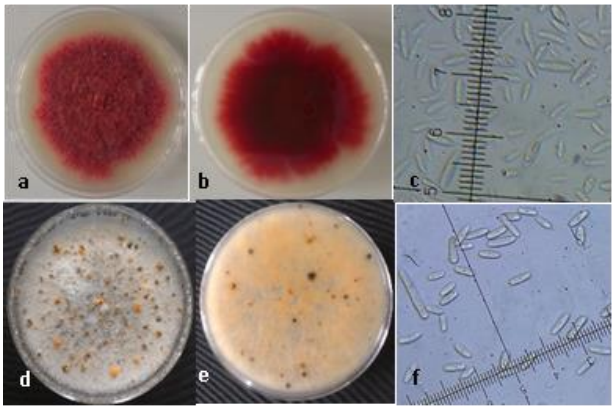

Fig.1: Morphological characteristics of Colletotrichum gloesporioides and C. musae isolated from anthracnose of banana. C. gloeosporioides (a-c): (a) upper surface, (b) lower surface, (c) conidia, C. musae (d-f): (d) upper surface, (e) lower surface, (f) conidia.

3.2. Molecular identification of *Colletotrichum* species

The ITS region, including the 5.8S gene of all isolates was successfully amplified and sequenced. The size of the amplification product obtained was estimated to be 450 bp (Fig 2). *Colletotrichum* species belonging to group 1 was identified as *Colletotrichum gloeosporioides* under accession number MG515233.1. *Colletotrichum* species belonging to the morphological group 2 were identified under the accession number MG515228.1 as *Colletotrichum musae*.

Table.2: Summary of morphological data for Colletotrichum species

species	Colony color	Conidia (mean)			Growth rate (mm/day)
		Length (µm)	Width (µm)	Shape	
C. gloeosporioides	reddish slight pink	11.25	2.5	cylindrical with acute apex.	4.36-4.8
C. musae	white	23	5.5	cylindrical with both ends rounded	4.9-5.2

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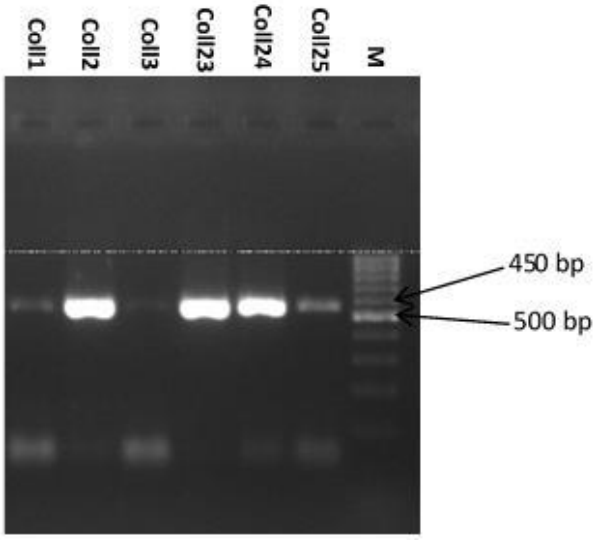

Fig.2: PCR amplification of ITS region of ribosomal DNA of Colletotrichum isolates with primer pairs ITS1 and ITS4

Fig.3: Symptoms of anthracnose disease on banana fruits after wound inoculation: (a): dry dark brown to black lesions with irregular margin and tan-to-orange-colored spores typical of Colletotrichum musae infection; (b):large black lesion with white mycelia typical of C. gloeosporioides infection ; (c): control

3.3. Pathogenicity testing

Characteristic symptoms of anthracnose were developed by all the isolates of *Colletotrichum* on banana fruits after wound inoculation. Generally, the lesions appeared 3 days post inoculation and expanded rapidly over 3–5 days. Lesions were black necrotic, circular and sunken, and these lesions showed white mycelia growth and produced orange colored conidial masses later. (Fig 3). Infections stimulate ripening of fruits, and lesions enlarge with ripening. Koch's postulates were confirmed by re-isolation of the fungi causing the lesions and identification as *C. musae* and *C. gloeosporioides.* Diameter of the anthracnose lesions varied among *Colletotrichum* isolates significantly (P< 0.05). The highest diameter of lesion was recorded with *C. musae* (4.03 cm). *C. gloeosporioides* gave the lowest lesion diameter (3.01 cm).

3.4. *In vitro* susceptibility of *Colletotrichum* species to fungicides

The susceptibility of *Colletotrichum* species to the various fungicides was presented through the mycelial growth inhibition rates in Table 3. There was a significant difference (P< 0.05) in susceptibility of *Colletotrichum* species to fungicide. For all fungicides tested, mycelial growth inhibition rate increased at the highest concentrations. Imazalil induced a complete inhibition of the mycelial growth of each species at 100 ppm. Imazalil was significantly superior to all the other fungicides followed by Azoxystrobin at all the test concentrations. *C. musae* and *C. gloeosporioides* were tolerants to Imazalil at concentrations ranging from 100 to 1500ppm. However both *Colletotrichum* species showed resistance to Azoxystrobin and Boscalid at all the test concentrations.

Table.3: Effect of different fungicides on mycelial growth (cm) of Colletotrichum species. by poisoned food technique after 7 days

species	fungicide	Percentage inhibition(%) Concentrations (ppm)						
		1	10	50	100	500	1000	1500
C. musae	Imazalil	38^{b}	58^{b}	60^{b}	100^{a}	100^{a}	100^{a}	100^{a}
	Boscalid	0	0	0	0	0	20^{c}	25^{c}
	Azoxystrobin	0	0	20^{c}	24^{b}	35^{b}	35^{b}	36^{b}
C. gloeosporioides	Imazalil	65^{a}	68^{a}	70^{a}	100^{a}	100^{a}	100^{a}	100^{a}
	Boscalid	0	0	0	0	0	25^{c}	30^{c}
	Azoxystrobin	13^{c}	22^{c}	25^{c}	28^{b}	35^{b}	37^{b}	40^{b}

Means having a common letter in the same column are not significantly different at P = 0.05 according to Least Significant Difference Test

IV. DISCUSSION

A diversity of *Colletotrichum* species, including *C. musae* and *C. gloeosporioides* were isolated on bananas showing anthracnose symptoms. These results provide evidence that, in anthracnose pathosystems, the same host is often infected by different *Colletotrichum* species. Freeman *et al* (1998) showed that anthracnose of mango was caused by several species of *Colletotrichum*, including *C.* acutatum *C. fragariae* and *C. gloeosporioides*. Than *et al* (2008) also characterized four species of *Colletotrichum* causes Capsicum spp anthracnose in Thailand. In the present study, the isolation of *C. gloeosporioides* on bananas showed anthracnose symptoms is justified by the potential of this species to infect a wide range of tropical fruits. *C. gloeosporioides* is the causative agent of anthracnose of many economically important hosts such as apple, strawberry and avocado (Afanador-Kafuri *et al*., 2003). Moreover Latiffah *et al* (2014) showed that *C. gloeosporioides* and *C. musae* were associated with anthracnose of banana, and *C. gloeosporioides* was more prevalent than *C. musae* in Malaysia.

The identification of the isolates based on morphological characteristics showed a variation among *Colletotrichum* species. *C. gloeosporioides* isolates exhibited characteristics already described by several authors (Photita *et al*., 2005; Cannon *et al*., 2008) allowing their identification. Moreover morphological characters of *C. musae* described in this study are similar to the characters observed by Photita *et al*. (2005) and Thangamani *et al*. (2011).

Pathogenicity tests with the *Colletotrichum* species isolated, showed that all were able to infect and cause symptoms in wounded banana fruit, proving that both species were causal agents of anthracnose infection on banana. This study is the first report in Côte d'Ivoire to highlight the pathogenicity of *C. gloeosporioides* on banana. The results of Latiffah *et al* (2008) also showed the involvement of *C. gloeosporioides* in banana anthracnose in Malaysia. The fact that *C. gloeosporioides* was a pathogen of banana confirmed numerous reports about the cross-infection potential among different species of *Colletotrichum* on a multitude of hosts (Freeman *et al*., 1998). A difference in virulence was found depending on the inoculated *Colletotrichum* species. The highest virulence of *C. musae* could be explained by the affinity of this species to banana. *C. musae* has been reported to be the most common causal anthracnose of many banana cultivars (Jinyoung Lim *et al*., 2002, Priyadarshanie *et al*., 2015).

Variability in fungicidal sensitivity among the *Colletotrichum* species was observed. A greater sensitivity of *C. gloeosporioides* to Imazalil was observed compared to *C. musae* which showed moderate sensitivity. The inhibition of ergesterol biosynthesis induced by Imazalil would be more pronounced on *C. gloeosporioides*. This effectiveness of Imazalil was also shown by Andrivon (1997) on *Colletotrichum coccodes*. However, with Azoxystrobin and Boscalid, marked resistance of both species was noted. The strains of *C. musae* and *C. gloeosporioides* identified in this study were not be sensitive to these actives ingredients. Resistance to azoxystrobin and boscalid fungicides by fungal pathogens of banana fruits in Côte d'Ivoire may be attributed to continuous and indiscriminate use of these fungicides without rotation or alternating with other fungicides for the control of preharvest and postharvest banana diseases. According to Hobbelen *et al*. (2013), resistance phenomen occurs after continued use of a fungicide on the target agent. A recent study in Senegal indicated that treatment with azoxystrobin showed no efficacy against post- harvest diseases of banana (Diedhiou *et al*, 2014).

V. CONCLUSION

The results of this study are relevant because, by demonstrating the diversity, virulence and sensitivity to fungicides of *Colletotrichum* species infecting banana fruits. These results will facilitate the development and implementation of disease management practices, thereby allowing producers to reduce the economic losses in banana production caused by anthracnose.

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Review of Hussain Sagar Lake Pollution, Hyderabad, India

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Abstract— *Hussain sagar lake, a historical lake which was built in 1562 by Hussain Shaw Wali is situated in the heart of Hyderabad city, India. Earlier up to 1930's, it was the major water source to meet drinking and irrigation requirements for both twin cities of Hyderabad and Secunderabad. Now this lake turned to virtual garbage bin with no adequate treatment for wastewater loads originated from point and non point sources. Therefore the lake loses the capability of self purification system which was an important factor that depends on the maximum uptake of oxygen from the atmosphere. Multiple projects were undertaken by the concerned authorities for the improvement of Hussain sagar lake and its catchment area. Recently With referring to the Lake health status, In 2006, HMDA, a governing body set up by Andhra Pradesh Government, initiated "Hussainsagar Lake and Catchment Area Improvement project" and set up STPs and wastewater interception and diversion structures. It also installed fountains to aerate the lake to improve its water quality as well as to add beauty. But these measures are lying ineffective to manage wastewater loads.*

This paper discusses that the Hussain Sagar lake water body over decades, has been polluted adversely from discharging of organic and inorganic pollutants through open drains as well as point and non point sources and also presents various measures had taken to rejuvenate Hussain Sagar Lake to its past glory.

Keywords— Hussain sagar lake, Oxygen uptake, Self purification capacity, organic pollutants.

I. INTRODUCTION

Lakes are one of the surface water sources for the human needs, and they are most useful for developmental activities in and around the areas where they existed, they serve infiltration source for ground water, for recharge of water to the water table, in addition to these, lakes can receive domestic sewage and industrial effluents from the catchment area they existed and turn into disposal sites for waste water. In contrast to the perennial flowing water bodies like streams and rivers, mostly the natural lakes are formed at mountain areas, where as the artificial lakes are also constructed in and around the cities to meet the water demand of that particular area, and also to serve wastewater basins for the catchment area they existed. Lakes can retain the waste materials without disturbing ecosystem up to certain extent, but when the discharging wastewater loads increasing, they will lose the ability of self purification process. Due to rapid industrialization and population growth, the requirement of water being increased at alarming rate in order to meet the water requirements of future generation, there is great need of protecting water bodies to promote sustainable development [1].

Many Rivers and natural water bodies across the world are being polluted by domestic and industrial sewage due to a wide range of pollutants. Some of the pollutants are persistent and stable environmental contaminants [2]. Domestic sewage poses health related issues while industrial effluents carry a wide variety of toxic elements like Cd, Cr, Pb, Hg and Zn which can cause significant toxicity even in trace amounts. The pollutants enter various parts of environment by anthropogenic activities as well as natural processes and degrade the surface and ground water quality and make them unsuitable for drinking, industrial, irrigation, recreation or other purposes [3,4].

Water bodies in flowing state normally have the capacities to purify itself from the contamination of external sources discharge into the river [5,6]. Self-purification of water is a tedious process involving physical, chemical and biological processes that occur simultaneously, allows lakes to restore its natural state over a certain period [7.8].

The process of purification is mainly depends on absorption and dissolution of atmospheric oxygen from the water body surface. This absorbed and dissolved oxygen (DO) is necessary for the growth of bacteria to break down the organic and inorganic pollutants thereby reducing its strength for a period of time [9]. Self-purification capacity of water bodies is mainly depends on natural factors. These include the water velocity, depth, discharge and temperature

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[10]. The turbulent of water bodies helps the river to very clean because of the natural capacity to absorb and digest pollutants at a very high rate. Stagnant water bodies tend to become septic because of the low rate of oxygen absorption. Furthermore, the depth of the water body also affects the rate of diffusion and mixing of the absorbed oxygen [11].

The temperature of water is high; low DO concentration because biological and chemical activity increases [12]. At a certain temperature the saturated dissolved oxygen is the maximum DO level that a river can attain. Maximum DO ranges from 14.6 mg/L of DO at 0^0c to 7.2 mg/L at 35^0c [13].

Hussain Sagar Lake :

Hussain Sagar Lake was built in 1562 during the reign of the Qutb Shahi dynasty at Golkonda fort as shown in fig.1. It is an artificial lake built on a tributary of River Musi. The lake joins the cities of Hyderabad and Secunderabad besides adding an aesthetic appeal to the twin cities. The lake water was utilized for irrigation and drinking water needs from 1884 to 1930. The total catchment area of the lake is 240 square kilometres (93 sq mi). Through four main feeder nalas named Picket Nala, Kukatpally Nala, Banjara Nala and Balkapur Nalla, wastewater from the catchment area reaches the lake. Till 1930s, the lake was the major source of water supply to the population of Hyderabad and later on it turns into wastewater basin as the lake has gradually started receiving sewage and industrial effluents through the feeder nallas. The Picket Nalla discharges mostly domestic sewage throughout the year into the lake from the north-eastern side. Similarly the Banjara Nalla (from north-western side) and Balkapur Nalla (from western side) discharge mostly domestic sewage into the lake. The Kukatpally Nalla was seen to be discharging a mix of domestic sewage and industrial effluents into the lake from the northern side. This practice of discharging municipal sewage, industry effluents and storm-water from over 240 square kilometres increased the content of organic matter, nitrogen and phosphorus. This suspended organic matter, rich in nutrients, caused eutrophication that allowed growth of algal blooms and water hyacinth[14].

Fig.1: Satellite image of Hussain Sagar Lake

The lake is mainly fed largely by the Kukatpally nala, which contributes domestic and industrial effluents from the Kukatpally industrial area while other nalas discharges domestic sewage flows only as shown in Table 1. Thus the water quality of the lake deteriorating due to the inflow of polluted water into the Hussain Sagar Lake.

Table.1: Inflows of domestic sewage and Industrial effluents

Name of the Nala	**Domestic flow, MLD**	**Industrial flow, MLD**	**Total flow, MLD**	**Remarks**
Kukatpally nala	55	15	70	Interception and diversion (I&D)
Picket nala	6	---	6	Interception and diversion after pumping, Proposed STP (30 MLD capacity)
Banjara nala	6	---	6	Interception and diversion (I&D)
Balkapur nala	13	---	13	STP (20 MLD capacity)
Total	80	15	95	50 MLD Treatment by Two STPs

II. LITERATURE REVIEW

The immersion of Ganesh idols in the lake every year has made it worse. It was reported that the water quality of Hussainsagar Lake is deteriorated with many heavy metals concentration in elevated levels than the ICMR standards [15]. It has also been reported that the kukatpally nallah carries the major quantity of pollutants which was determined by the sediment analysis at kukatpally nalla being heavily polluted by potentially toxic elements (PETs) [16]. Besides adding silt, studies indicate that these immersions have increased the pollution levels in the lake. The sediment and pore water analysis has shown elevated concentration of Cr & Pb indicating settlement of various pollutants at bottom of lake [17]. All lakes serve to recharge the water table. But because of heavy pollution of Hussain sagar Lake, many of pollutants get carried into underground

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water bodies. Though percolation filters many pollutants, open wells or bore wells receive certain pollutants causing ground water pollution. Elevated levels of certain chemicals like Hg, Cd, Pb & Ni have been reported around Hussain sagar lake[18].

Cleaning the Hussain Sagar lake programme:

To address the lake water quality, Hyderabad Metropolitan Development Authority (HMDA) has made efforts to restore a highly polluted Hussain sagar Lake to its pristine glory. The project entitled "Hussainsagar Lake and catchment area improvement project" which initiated in March 2006, aims at improving lake water quality by preventing entry of pollutants into lake from both point and non-point sources of pollution, besides removal of nutrient rich sediment. Interception and diversion of dry weather flows, improvement of nallas in catchment area [14].

As part of the project, seven fountains were installed in the lake in September 2011 to improve the dissolved oxygen content in the water and help aquatic life as well as to add the beauty to the lake. Additionally, a new 30 MLD sewage treatment plant (STP) on the Picket nalla and 20 MLD STP adjacent to Khairatabad Flyover at Balkapur nalla, construction of ring sewers around the lake and a small treatment plant of 5 MLD at Rangadhamini chervu were as a part of project implementation. Dredging and disposal of sediment was also planned for this project. It was said that dredging from Picket nalla, Balkapur nalla and Banjara nalla will result in extraction of 7 lakh cu.m of non hazardous and nutrient rich sediment. Similarly many more projects are running today with aim of cleaning the lake such as A Clean Hussain sagar campaign (launched in 2013) and Canadian technology to rejuvenate Hussain sagar lake (March 2017).

Many projects are undertaken for the improvement of Hussain sagar lake and its catchment area as shown in Table2.

Table.2: Interventions undertaken for Hussain sagar lake improvement

S.No	Name of the project	Scheme	Budget, rupees	Year of Sanctioned	Status
1.	Abatement of pollution of Hussain sagar lake project	MCH	40 Crore	1998	Completed
2.	Green Hyderabad Environment Programme	HUDA	Not Available	2002	Completed
3.	Musi River Action Plan	NRCD	344 Crore	2004	Completed
4.	Hussain sagar lake and Catchment area Improvement Project	HMDA	370 Crore	2006	Completed

Need of the Study

Hussain Sagar Lake built in 1562 by Hazrat Hussainshah Wali was the major water resource for the both hyderabad and secunderbad. Earlier up to 1930's it was used for the daily needs of people, later on due to rapid urbanization as well as industrialization it turns to sewage effluent tank with insufficient treatment due to heavy loads of pollutants. Hence the lake loses the capability of self purification system. In order to restore the capacity of self purification system of the lake as well as to promote sustainable development of Lake Ecosystem by stopping ground water contamination and managing water quality in rivers to its downstream, proper measures must be taken to bring down the pollutant loads to an acceptable range in the lake and also improvement of health status of the Lake.

Objectives of the study

- Examining health status of Hussain Sagar Lake from past to present
- Examining of Self purification capacity of the Lake

III. MATERIALS AND METHODOLOGY:

Study site:

It is estimated that nearly 350 MLD (Million Liters/ Day) of polluted water and sewage originating from the twin cities of Hyderabad and Secunderabad flow into the Musi river [19]. Out of 350 MLD, 95 – 120 MLD sewage was recieving to the lake after it was treated with the treatment plants (20 MLD STP and 30 MLD STP) which were set up upstream of lake and a very little quantity of sewage with no treatment [20].

Over a decade, Hussain sagar lake receiving huge amount of waste water through four major inflow nallahs, namely, Picket, Balkapur and Banjara channels are located at north,

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south and southwest directions of lake and receiving about 30, 17 and 15 million liters per day (MLD) of treated and untreated wastewater respectively. Kukatpally nallah located at northwest direction of the lake having four industrial estates and receiving 60 to 70 MLD of domestic & industrial effluents, and all these four stations are inflow sites to the lake [21]. Numerous efforts to clean it have failed. The Lake water is highly polluted while huge wastewater loads recieving. Therefore the water health status is necessory to make the Hussain Sagar Lake clean. The salient features of Hussain Sagar Lake are presented in the below given Table 3.

Table.3: Features of Hussain Sagar Lake

Item	Description
Inlets	4 Major nalas, namely, Banjara Nala, Picket Nala, Kukatpally Nala and Balkapur Nala
Outlets	2 surplus weirs & 4 sluice gates
Max. Depth	32 ft.
Water Spread Area	4.7 square kms
Total Catchment Area	240 km
Direct Catchment Area	67 km
Capacity	23.5million cubic meters
Circumference	14 km
Surface Elevation	1,759ft

Methodology:

Water samples were sampled from different locations in the lake at different seasons and transferred into pre cleaned polythene containers for analysis of pollutants using the standard procedures recommended by APHA [22]. Pollution indicator parameters like dissolved oxygen (DO), BOD, COD, total nitrogen and phosphates are considered for the study of reviewing the water quality status of the Hussain Sagar lake, Hyderabad.

The adopted methodology for this study is to assess the health condition of the lake with respect to the level of pollutant concentrations prevailing in it.

- Reviewing the treatment plants based on the quantity of wastewater load.
- Reviewing scientific approaches to restore self purification system of the lake.
- Recommendations to promote sustainable development by protecting aquatic ecosystem at study area.

IV. RESULTS AND DISCUSSIONS

Previous studies on Hussain sagar lake:

Table.4: VISION LABS REPORT, 27.03.2017

Parameters	**Hussain sagar lake sampling point**			**BIS (2012) STANDARDS**
	Budda sattue	Lake outlet at boats club	Lake at Necklace road	
pH	7.2	7.3	7.28	6.5-8.5
Turbidity	31.6	27.1	39.7	
EC	1616.70	1506	1582	1500
T.Akalinity	467	508	489	200
TDS	848	852	866	500
TSS	36.9	219	216	100
Cl^-	118.4	116.6	117.2	250
SO_4^{2-}				200
NO_3^-	14.16	12.76	13.84	45
T.Nitrogen	28.6	27.6	29	10
Phosphorous	4.2	3.94	3.96	5
DO	3.4	3.9	4.4	4

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BOD	41.8	50.2	53.4	30
COD	122.8	153.2	146.8	250

It was noted from the Table 4 that the values of BOD were observed at high concentration which indicates the presence of considerable pollution in the lake waters, Chlorides of water samples were observed ranging from 108-126 mg/l, Dissolved oxygen was observed in the range of 1.7-21.8 mg/l, Ammonical nitrogen was existed in the range of 4 – 29 mg/l, Nitrates were in the range of 10 -24 mg/l and total phosphates were in the range of 2.8- 9.2 mg/l, due to this there is no big evidence of eutrophication. Total suspended solids was existed in the range of 146 – 278 mg/l at the outset even though the lake was fully contaminated the consistency in the pollutant concentration remains same as there is no big differentiation of physico chemical analysis of lake water during the three months period of analysis due to the less inflow of water as the rain fall intensity during study period is minimum [23].

Table.5: Status of Hussain Sagar lake pollution

Parameters	NEERI (1996 - 97,2000)	EPTRI (2015)	2008	2010	2014	2016	2017	LIMITING STANDARDS[24]
pH	-	-	9.2	6.5-8.5	8	8.1 - 9	7.2	6.5-8.5
TDS	700-1100	940-1200	-	900	761.3	1126	848	500
TSS	-	-	52	-	-	-	36.9	100
Phosphates	1.5-5	0.4-1.5	-	-	-	-	4.2	5
Total Nitrogen	-	-	-	-	-	-	28.6	10
DO	-	-	2.3	0	-	3	3.4	4
BOD	20-48	42 - 80	55	20	-	25	41.8	30
COD	76-203	110-183	140	170	115.5	289	122.8	250

Note : All parameters have a unit of mg/l, except pH.
- Indicates no data available.

It was observed from Table 5 that different studies over years carried out by many researchers that the value of Total Dissolved Solids (TDS) holds high concentration levels, which indicates the presence of considerable pollution in the lake, while Total suspended solids concentration levels varies from 36.9 -58 mg/l were considered within the desirable limits.

The DO was observed very low value varies from 0 to 3.4 mg/L indicating fragile water quality, and the BOD was moderately high concentrations varies from 20 – 48 mg/l indicating organic pollution, the COD values are medium varies from 76 to 289 mg/L and are considered within the desirable limit. The data clearly indicates the occurrence of sewage pollution due to the convergence of untreated domestic sewage and solid waste containing oxidizable organic matter into the lake water [25]. The data indicates that the lake water was moderately alkaline and the pH values of 6.5 -9.2 were within the permissible limits. Phosphates representing eutrophication were within in the desirable, while Total nitrogen value of 28.6 mg/l was representing eutrophication conditions in the lake. The deterioration of lake water quality could be linked to nutrient loading from domestic sewage. The raw sewage is the source of nitrates and phosphates in the water [26].

V. CONCLUSIONS

Hussain sagar is a 450-year-old water body. Every day, 78 million litres of sewage and 15 million litres of industrial effluents flow into the lake through four drains. Of these, a major quanity of sewage has been treating through two STP's, while less quanity was being untreated. However the two sewage treatment plants (STPs) near the lake are insufficient to handle the wastewater load. It has reportedly found that the lake has lost its natural ability to 'self-purify' itself due to heavy load of contaminants as dissolved oxygen levels reaching the lowest and the worst pollution in lake is due to organic and inorganic substances that have seeped into the lake bed, and turned it to virtual garbage

bin. The Hussain Sagar Lake suffered continuously for decades from the sewage flowing from four nalas being dumped into it and the fear has been that one day the lake will end up as a large sewage effluent tank in the heart of Hyderabad if suitable measures have not taken up.

HMDA had made efforts to restore Hussain Sagar Lake to its past glory in all respects by appropriate technological interventions. In 2006, HMDA initiated Hussain sagar Lake and Catchment Area Improvement project and set up STPs and wastewater interception and diversion structures. It also installed fountains to aerate the lake to improve its water quality. But all these measures have been ineffective.

It was concluded that the rejuvenation and conservation of Hussain Sagar lake is utmost interest of Hyderabad city as its ecological, cultural and touristic value is immense. One of the most important steps in the rejuvenation and conservation of the lake is to restore the water quality by controlling the pollution through different remedial measures.

To restore Self purification capacity of Hussain Sagar lake, following are the alternative to be taken up, which may give best results.

- By upgrading and installing STP's at inlets
- Installing aerating devices
- Preventing Discharge of untreated domestic sewage and industrial effluents,
- Evacuate Immersed of ganesh idols contributing to the high pollution and
- Shoreline monitoring of lake acts non point source of pollution

DECLARATION OF CONFLICT OF INTEREST:

The Author declares that there is no conflict of interest.

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Socio-economic Determinants of Cassava Production in Benue State, Nigeria.

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Abstract— *The study examined the determinants of cassava production in Benue State Nigeria. A total of 180 farmers were selected across the state using multi-stage sampling technique. Data, which comprised information on the socio-economic characteristics and other quantitative variables relevant to the study, were collected using a well structured questionnaire and personal interviews. The social- economic characteristics were analyzed using descriptive statistics such as percentages and frequencies; simple multiple regression technique was used to analyze the production functions of cassava farmers. The results obtained revealed that R^2 value was 0.419 indicating that 41.9% of variation in cassava production was accounted for by the variables considered in the study. Specifically, age of the farmers, occupation, household size, farming experience, level of education of farmers, farms size and fertilizer input where all significant and are important variables that affected the production of cassava in the study area. Given the enormous potential of cassava production in the study area, it has become so imperative that youths be encourage to participate effectively in cassava production, seeing that majority of the farmers are aged and will retire from active farming. This is to ensure food security. Effective structure should be put in place for the input and credit facilities provided by the government, this will ensure that these facilities get to those who need it and will in turn help the farmers expand their production. This study, although base in Benue State, may have implications for other States with similar situation. This will help the state meet self-sufficiency in food production and so be able to feed her teeming population.*

Keywords— Socio-economic, Determinants, Cassava, Farmers, Benue State.

I. INTRODUCTION

Cassava is one of the important source of carbohydrate food in Nigerian. Nigerian is the largest producer of cassava in the word with a total output currently put at about 34 million metric tones a year (FAO, 2002). Presently, cassava is primarily product for food especially in the form of Garri, tapioca and fufu for human consumption. But the crop can be processed into several secondary products for industrial market value (World Bank survey, 1981). These products include chips, pellets, flour adhesives, alcohol and starch, which are vital raw materials in the livestock feed, alcohol/ethanol, textile, confectionary, wood, food and soft drinks industries. They are also tradable in the international market.

In view of the serious challenges of feeding world population of over 6.1billlion people, it has become imperative to pay more attention to food production issues. About 215million (43%) sub-Saharan African population is chronically undernourished and unless strong action is taken this may increase to round 315million in the year, 2010 (World Food Programs, 1995). If food production is to keep pace with rapid population growth and demand for food, a new and creative approach to agriculture development must be developed.

It is important to emphasize that despite potential benefits stemming from the expansion of the agricultural sector through various government efforts, its overall productivity remain low and the poor performance of agriculture is most clearly evidenced by the low standard of living of these small-scale rural farmers (Dogon-daji, 2006). Cassava offers a particularly significant potential for increasing food production and income in Nigeria. Like other agricultural crops, cassava has a role to play in the developing economies.

Estimates of industrial cassava use suggest that approximately 16 percent of cassava root production was utilized as an industrial raw material in 2001 in Nigeria,

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10 percent of which was used as chips in animal feed while 5 percent was processed into a syrup concentrate for soft drink and about 1 percent was processed into high quality cassava flour used in biscuits and confectionary, dextrin per-gelled starch for adhesive, starch and hydrolysates for pharmaceutical, and seasoning s (kormawa and Akoroda, 2003). This estimate leaves 84 percent or 28.9million tones of production for food consumption, a portion of this of course being lost in post harvest and waste. But, the methods used in achieving these are almost tedious which may lead to inefficiency use of resources and perhaps low quality and quantity of product. This implies that for the product from cassava to complete favorable in the international market, there is need to go beyond tedious method of producing which perhaps seem inefficiency (Ogbonna, et al, 2007). The method used by small and medium scale cassava processing in Imo state seem to be tedious, may lead to inefficiency use of technologically input and low product. An efficient processing technique in food could lead to increase in the quality and quantity of food available for consumption (Nelson and Donald, 1980, Ogbonna and Ezedinma, 2005). According to IFC, (2003) the small and medium processing technologies is enormous. Traditional cassava processing has a number of undesirable attributes. It is time consuming, provides low yields and lacks storages capacities. Many described it as drudgery. Mass production of cassava is possible because it tolerates wide range of soil and climatic conditions. Cassava is highly tolerate to acid soils and need no additional fertilizer especially phosphoric fertilizer because the roots of cassava form symbiotic association fungus in the soil helping cassava plants to absorb phosphorus and micronutrients from the soil (Howeler et al., 1990). Pander et al. (2000) on the other hand emphasized that cassava requires fertilization especially nitrogen, phosphorus and potassium; and that; cassava requires more nitrogen than phosphorus. Ezumah, and Okigbo (1980) suggested that farming systems such as intercropping grain legumes with cowpea, alley cropping, green maturing and animal dung would increase cassava yield than the mineral fertilizer.

II. METHODOLOGY

The survey was conducted in Katsina-Ala (zone A), Makurdi (zone B) and Otukpo (zone C) of Benue State, in 2016 to examine factors determining cassava production in the study area. The three agricultural zones in the area study were purposively selected based on strategic importance of cassava in the farming system of the sampled zones in the area. In each zone one Local Government Area /was selected by simple random sampling technique from the list of all Local Government Areas in the area. Then in each Local Government Area, 6 communities were similarly selected by random sampling technique, and in each community, 10 cassava farmers were equally selected through the same sampling technique. 60 Respondents were obtained from each agricultural zone making up a sample size of 180 respondents for the entire study area, Using structured questionnaires, relevant data on house hold cassava production were collected from the respondents. Data were analyzed with both descriptive and simple multiple regression statistics.

Model Specification

Categorical variables such as marital status and type of organization farmers belong was dummy coded in order to transform them into dischotomous variables. Other variables such as sex, age, level of education, farm size was entered into multiple regression equation.

Model is specified as follows;

$Y = \alpha + \beta_1 X_{1+}\beta_2 X_{2+}\beta_3 X_{3}.. + \beta_{14} X_{14+} U$

Where:

Y = Quantity of cassava produced.

A = Constant term

$\beta_1 = \beta_{14}$ regression coefficients.

X_1 = sex (male = 1, female = 2)

X_2 = age (years)

X_3 = marital status: Single, married, widowed or divorced

X_4 = level of education (level of education attainment)

X_5 = farming experience (years)

X_6 = fertilizer use (money spent in procuring fertilizer)

X_7 = farm size (in hectares)

X_8 = on-farm income (Naira)

X_9 = off-farm income (Naira)

X_{10} = household size (actual number of people living in the household)

X_{11} = Major occupation

X_{12} = membership of organization belong to (yes = 1, no =2)

X_{13} = planting materials (money spent in procuring planting materials)

X_{14} = labour input (number of persons used in cassava production)

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U = error term

Socio-economic Characteristics of the Respondents

Sex

Table 1 indicates that majority (70.6%) of participants in the scheme were male while 29.4% of the respondents were female. The predominance of male in farming operation may be attributed to the tedious nature and hard work involved in various farm enterprises. Similar finding has been reported by Abdullahi (2012) which states that 84% of the farmers in Paiko Local Government Area of Niger State were male.

Age

The study further revealed that most respondents (30.0%) belonged to the age bracket of 51-60 years, followed by 41-50 (25.6%), above 60 years (20. %), 31-40 (18.3%), 21-30 (6.1%) respectively. The mean age of the respondents was 48.3 years. This implies that most of the farmers in the study areas were very active to carry out agricultural activities. Age is considered to be an important characteristic in decision process of an innovation (Agbamu, 2006).

Marital Status

Majorities (90.6%) of the respondents were married, while (9.4%) were single (table 1). The proportion of married persons that participated in activities was high the predominance of married individuals agrees with a study carried out by Uddin (2014) which revealed that 85.8% of the Edo state farmers were married. The predominance of married persons that participated in activities implies that they are ready to improve their livelihood and that of their families, since marriage is often associated with occupational stability and responsibility (Uddin, 2014).

Level of Education

Majority (75.5%) of the respondents had formal education, whereas, 24.6% had no formal education (table 1). Among those with formal education, primary education accounted for 29.4%, 23.3% had secondary education while 22.8% had tertiary education. This implies that the respondents were literate and were more likely to utilize information on agriculture for enhanced food production. Egbule (2013) stated that education plays an important role in creating awareness in farming communities because educated people are capable of sourcing information on agricultural innovation. Benue State farmers spent M=13.1 years acquiring formal education.

Household Size

Table 1 indicates that 42.8% of the respondents had a household size of 6-10 persons, followed by 11-15 (28.6%), 16-20 (10.1%), above 20 (8.5%), 1-5 (7.8%), respectively. This implies that respondents have access to family labour which will positively increase agricultural production. This implies that the respondents had a large household size. Banmeke and Ajayi (2008) noted that large household size serves as an important source of farm labour and a strong base to adopt improved technologies so as to be able to improve productivity in order to meet up with economic needs of the family. Benue State had mean household size of (M=18 persons).

Major Occupation

Table 1 reveals that 98.9% of the respondents had farming as a major occupation while 1.1% of the respondents were involved in farming and trading. This implies that quite a number were practicing farmers.

Faming Experience

The study further revealed that 35% of the respondents had between 6 and10 years of farming experience, followed by those who were above 20 years, (21.1%), 11 and 15 years (16.7%), 16 and 20 (14.4%) and 1 and 5 (12.8%) of farming experience respectively. The mean farming experience was 13.1 years. This implies that quite a number of the respondents have been farming for a long time. According to Obinne (1991) farming experience enhances productivity and has shown to encourage rapid adoption of farming innovation.

Farm Size

A greater percentage (55.6%) had a farm size of 3.4 hectares, 35.5% had 1.2 hectares, 7.8% had 5-6 hectares and 1.1% had above 6 hectares. The mean farm size was 3.5 hectares. This implies that the respondents are small scale farmers. Benue State farmers had mean farm size of (M=3.5 ha) .

Estimated Annual On-Farm Income

The distribution of respondents according to their income revealed that about 53.9% of the respondents had annual income of between N20,000 – N100,000, while 41.1% had N101,000 – N500,000 and 5% had N501,000–N1,000.000. The mean annual income was (N154, 156.50).Farmers with low income will not be able to purchase subsidized farm inputs provided by the government. This implies that respondents with high farm income are most likely to purchase government inputs. This result agrees with Abolagba (1997) who noted that farmers with high income level are in a better position to

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afford better facilities. Benue State farmers had income of (N154, 156.50).

Membership of Organizations

Distribution of the respondents according to their membership of organization revealed that 93.3% belonged to organizations and the remaining 6.7% did not belong to any. Being a member of any organization could be an avenue for accessing information on increased productivity. Asadu (2013) reported that membership of any organization is of advantage to farmers since social organization offers an effective channel for contact with large number of farmers, as well as opportunities for interactions. This enhances farmers uptake of new practice for agricultural production, processing and storage of farm produce.

Fertilizer use

The fertilizer used was procured from Federal and State government fertilizer programme. Because of the delay of the fertilizer getting to the farmers, the percentage usage by farmers was less than 50 % in Benue State.

Estimated Annual Off-Farm Income

Distribution of respondents according to their annual off-farm income revealed that 53.5% had annual off-farm income of between N20,000 – N50,000, followed byN51,000 N100,000 (23.9%), N101,000 – N150,000 (13.9%), N151,000 – N200,000 (5.6%) and N201,000 - N250,000 (3.3%), respectively. The mean off-farm income was N38, 127.50. This is in addition to the annual on-farm income which could assist the farmer in purchasing more subsidized inputs to increase production.

Planting Materials

Distribution of respondents according to the plant materials grown by farmers' revealed that 72.2% of the respondents got their materials from International Institute for Tropical Agriculture, Ibadan and National Roots Crops Research Institute Umudike, Abia State, Nigeria. While the remaining 27.8% got their planting materials from local vendors.

Labour Use

Majority 78.9% of the respondents use family labour while the remaining 21.1% used hired labour.

Table.1: Distribution of Socio-economic Characteristics of the Respondents

Socio-economic Characteristics	**Benue (n=180)**		
	Freq	**%**	**Mean**
Sex:			
Male	127	70.6	
Female	53	29.4	
Age(years)			
21 – 30	11	6.1	
31 – 40	33	18.3	
41 – 50	46	25.6	
51 – 60	54	30.0	
> 60	36	20	48.3
Marital Status			
Single	17	9.4	
Married	163	90.6	
Widowed	-	-	
Divorced	-	-	
Level of Education			
No formal education	44	24.4	
Primary education	53	29.4	
Secondary education	42	23.3	
Tertiary education	41	22.8	
Mean of years spent in			13.1

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Acquiring formal education			
Household size (number)			
1 – 5			
6 – 10	14	7.8	
11 – 15	77	42.8	
16 – 20	54	28.6	
>20	19	10.1	
	16	8.5	
Major occupation		18	
Farming			
Fishing	178	98.9	
Farming/Trading	-	-	
Hunting	2	1.1	
	-	0	
Farming experience(years)			
1 – 5			
6 – 10			
11 – 15	23	12.8	
16 – 20	63	35.0	
>20	30	16.7	
	26	14.4	
	38	21.1	13.1
Farm size (hectares)			
1 – 2			
3 – 4			
5 – 6	64	35.5	
>6	100	55.6	
	14	7.8	
Estimated Annual On-farm Income (Naira)	2	1.1	3.5
20,000 – 100,000			
101,000 – 500,000			
501,000 – 1,000,000	97	53.9	
	74	41.1	
Fertilizer use	9	5.0	
Fertilizer purchase from Government	154,156.50		
Not purchase from Government	118	65.5	
	62	34.5	
Membership of Organizations			
Yes			
No			
	168	93.3	

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Planting materials	12	6.7	
IITA/NRCRI			
Other vendors			
	130	72.2	
Labour use	50	27.8	
Family labour			
Hired Labour			
	142	78.9	
Estimated Annual Off-farm Income (Naira)	38	21.1	
20,000 – 50,000			
51,000 – 100,000			
101,000 – 150,000	96	53.5	
151,000 – 200,000	43	23.9	
201,000 – 250,000	25	13.9	
	10	5.6	
	6	3.3	38,127.5
Total	**180**	**100**	

Source: Field Survey, 2016

Socio-economic Characteristics Influencing Rural Farmers' Production Level

Table 2 revealed that socio-economic characteristics of farmers had significant influence on farmers' production level in the activities ($F_{(9,350)} = 28.089$; $p \leq 0.05$). The result showed that the variables jointly predicted farmers' production level in activities (R = .419), and jointly accounted for 40.4% variance (adjusted $R^2 = 0.404$) in predicting farmers' production level when all the socio-economic characteristics were taken together. This implies that other characteristics not taken into consideration in this model may have accounted for the remaining 59.6% variance.

Table 2 revealed the relative contribution of socio-economic characteristics to farmers' production level in activities. The relative contributions of sex (β = -.078; t = -1.677; p>0.05), marital status (β = .049; t = 1.058; p>0.05), membership of organisations (β = .030; t = .723; p>0.05), on-farm income (β = .032; t = .743; p>0.05), off-farm income (β = .052; t = 1.220; p>0.05) planting materials (β = .007; t = .166; p>0.050) and labour input (β =0.0.80; t= 0.842; p> 0.05) were not significant at predicting respondents' production level.

The relative contributions of age (β = -.190; t = -3.939; p<.05), level of education (β = -.202; t = -3.804; p<.05), household size (β = -.108; t = -2.507; p<.05), major occupation (β = .099; t = 2.023; p<.05), farming experience (β = .117; t = 2.694; p<.05), farming size (β = -.305; t = -6.779; p<.05) and fertilizer use (β = -.305; t = -1.065; p<0.05) were significant at predicting respondents' production level .

Table 2 further revealed the extent of prediction of each of the socio-economic characteristics of farmers at different levels were ranks based on the t values. The rating is as shown: farming size (t = 6.779; p<.05) >age (t = 3.939; p<.05)>level of education (t = 3.804; p<.05)> farming experience (t = 2.694; p<.05) >household size (t = 2.507; p<.05) >major occupation (t = 2.023; p<.05)> fertilizer use (t = 1.065; p<0.05). Farm size was the socio-economic characteristics that mostly predicted farmers' production level and was followed by age, level of education, farming experience, household size, major occupation while the least predicted socio-economic characteristic was fertilizer use.

Farm size mostly predicts farmers' production level and this agrees with Giroh *et al.* (2007) who noted that 86.7% of respondents cultivated between 1-3 hectares, this implies that majority are small holder farmers. Farm sizes were found to be factors with significant influence on the farmers' production level. Indicating that farmers with larger farm holdings were more likely to be highly aware of information than those with small farm size.

Age was significant and age is considered to be an important characteristic in decision process of an innovation (Agbamu, 2006). This further revealed that

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most of the farmers were strong and very active to carry out agricultural activities.

Level of education was significant which suggest that literate farmers are more likely to source for agricultural related information for higher agricultural production than the illiterate farmers. This agrees with Egbule (2013) that literate respondents can enhance utilization of information on agricultural food production and that education plays an important role in creating awareness in farming communities because educated people are capable of sourcing information on agricultural innovations.

Farming experience was significant and this implies that quite majority of the respondents' have been into agricultural food production. This agrees with Obinne (1991), reported that increased farming experience enhances productivity and has shown to encourage rapid adoption of farming innovation.

Household size was significant and this implies that respondents have access to family labour which will positively increase agricultural production. This agrees with Banmeke and Ajayi (2008) noted that large household size serves as an important source of farm labour supply and a strong base to adopt improved technologies so as to be able to improve productivity in order to meet up economic needs of the family.

Table.2: Multiple Regression Analysis on the Determinants of cassava production in Benue State, Nigeria

	Unstandardized coefficients		Standardized coefficient	
Variables	**B**	**Std Error**	**Beta**	**T**
(Constant)	31.194	3.351		9.308
Sex	-1.194	0.712	0.-078	-1.677
Age	0.-591	0.150	0.-190	-3.939*
Marital Status	1.212	1.146	0.-049	1.058
Level of Education	-1.307	0.343	0.-202	-3.804*
Household size	0.-602	0.240	0.-108	-2.507*
Major occupation	6.789	3.357	0.099	2.023*
Farming experience	0.485	0.180	0.117	2.694*
Farm size	-2.285	0.337	0.-305	-6.779*
Membership of organization	0.759	1.050	0.030	0.723
Fertilizer use	-2.484	2.332	0.-144	-1.065*
On farm income	0.387	0.-521	0.032	0.743
Off farm income	0.334	0.273	0.052	1.220
Planting materials	0.086	0.517	0.007	0.166
Labour input	0.869	1.120	0.080	0.842

* denotes the level of significance at 0.05

A dependent variable production level of production activities, R Square = 0.419,

R square adjusted = 0.404, F value = 28.089; P≤0.05.

III. CONCLUSION AND RECOMMENDATIONS

The result of the multiple regression analysis showed that the linear functional form largely explained the variations in the quantity of cassava produced in the study area.

In the regression analysis, the age of the farmers, the occupation, farming experience, level of education of farmers, household size, farm size, fertilizer use in production were significant. By implication these variables were critical determinants of cassava production in Benue State. Given the enormous potentials cassava production in the study area, it has become so imperative that youths be encouraged to participate effectively in cassava production, seeing that majority of the farmers are aged and will retire from active farming. This is to ensure food security. Also an effective structure should be put in place to enable the farmers benefit from the subsidy on fertilizer provided by the government, this will ensure that fertilizer get to those who need it and will in turn help the farmers expand their production . Encouraging education among the farmers will also enhance the ability to adopt improved farming techniques and boast their production.

This study, although based in Benue state may have implications for other states with similar situation.

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Effects of Photomixotrophic Conditions on Plants of *Eucalyptus Urograndis* Propagated in Temporary Immersion Bioreactors

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***Abstract*—** *Eucalyptus is one of the crop, which has been investigated with commercial purposes in the world. There are more than 500 species, being the Eucalyptus urograndis one of the most important, because of its intensive use in the production of wood pulp to make papers. The multiplication by means of temporary immersion bioreactors (TIB) is among the present techniques to obtain higher productions of the pulp to satisfy the demands of the market. The effects of the photomixotrophic crop were studies during the elongation of the propagation of shoots in the TIB in order to increase the quality of them. This process consisted of the use of 30 g L^{-1} combined with two concentration of CO_2 (350 and 1200 µmol mol) and two flows of photosynthetic photon flux (PPF= 80 and 250 µmol $m^{-2}s^{-1}$). The higher percentage of suitable plants were found with the treatment of high PPF (250 µmol m^{-2} s^{-1}) and 1 200 µmol mol of CO_2. The photosynthetic capacity of propagated shoots was of 64% of the adult plants. Also was observed that photomixotrophic conditions reduced the stressed environment that is imposed by the growing in vitro. The catabolic activity in the enzymes of the metabolism of carbon was also reduced, increasing the activity of the Sucrose Phosphate Synthase.*

Keywords*— *Eucalyptus, micropropagation, photomixotrophism, vitroplants.

I. INTRODUCTION

According to predictions, the world population will increase annually in more than 80 million of people and it is estimated that in 2050 it will be about 7 000 millions of inhabitants, because of this, it is believed that the use of wood for building and for the paper industry will increase constantly. Whereas the global demand of wood products increase, the wood resources available in the world are decreasing dizzily due to the lack of knowledge and the inappropriate use of them. It has permitted the destruction and the degradation of the forests in the tropics [1].

The *Eucalyptus* specie is one of the most investigated with commercial purposes in the world. This species is characterized by its easy management in tree nursery, its fast growth, its erectile shape and the properties of its wood, which is used for numerous applications as: the production of wood pulp for making papers, charcoal and also for construction. *Eucalyptus* is another specie of great commercial in forest because of its extensive use in the production wood pulp for making paper [2]

The vegetative propagation of some species of *Eucalyptus* by means of rooting presents some limitations as the change of the rooting ability among and the gradual decrease of the potentiality of rooting associated with the ontogenetic aging of trees. A method that is exploited in several clonal propagation programs. It has also been promoted the use of the micropropagation as a method to produce clones very fast [3, 4, 5].

The techniques of tissues culture haves been used for propagating the clones with superior characteristics. More than 50 *Eucalyptus* species haves been propagated using the techniques of tissue culture, most of them using semi-solid means [6]. Micropropagation of *Eucalyptus* clones is more expensive than the techniques of macropropagation using the shoot rooting. The operations that increase cost correspond to the manual subcultivation because of the repeated labor, which are carried outs to obtain high rate of multiplications and the rooting of plantlets. These limitations can be solved with automatization of some of the phases of the organogenesis and the somatic embryogenesis to reduce labor and the cost of production [7, 8]. Starting from the first semi-automatized system of temporal immersion (e.g. RITA) some investigations about the use of this techniques in the proliferation of some crops have been carried [9, 10, 11, 12].

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The acclimatization *in vitro* is the term used when carrying out photomixotrofism and autotrofism works in plants cultivated under *in vitro* conditions [13]. These works haven't been studied in depth in the cultivation of *Eucalyptus urograndis* when temporal immersion bioreactors are utilized. That's why the objective of this investigation is to establish the photomixotrofic conditions which increase the quality of *Eucalyptus urograndis* explants propagated in TIB for the acclimatization of plants under *ex vitro* conditions.

II. MATERIAL AND METHODS

2.1 General procedures:

All the experiments carried out during this investigation were fulfilled in the specialized areas of plant cell and tissue culture laboratory in Bioplant Center of Ciego de Avila University.

In the research was used vitroplants clone of shoots that come from elite trees of *Eucalyptus urograndis (E. grandis Hill ex Maiden x E. urophylla)* which were brought from the Biotechnology Center of the Universidad Católica de Oriente, Medellín, Colombia. The donating plants, from which the explants were taken to be inserted *in vitro* conditions, were kept in greenhouses under 80% relative humidity (RH), 25.5 °C temperature and photosynthetic photon flux (PPF) of 350 µmol $m^{-2}s^{-1}$ as average under natural photoperiods during eight months. The plants were planting with a combinations (1:1, v/v) of zeolite + filter cake (derived from sugarcane bagasse) as substrate. The top of these plants were pruned in several moments. They were applied foliar fertilizer (Bayfolan Forte® produced by Bayer CropScience) each week at a rate of 2.0 ml L^{-1} in order that each plant takes 100 ml of final solution.

2.2 Establishment and micropropagation of *E. urograndis in vitro*:

The methodology proposed by Grattapaglia *et al*. [14] was the one used but with some modifications. The shoots obtained after the pruning were utilized as source of explants for the establishment phase *in vitro*. The sections taken as explants were segments of stalk from one to two centimeters of length, from 1.5 to 3.0 mm of diameter and with one or two preformed shoots after being washed with commercial detergent and rinsed several times with much fresh water, under asepsis conditions they were submerged in bichloride of mercury on 0.05% during ten minutes.

After this; the shoots were washed three times with purified sterile water. The explants were planted in test tubes in liquid medium with zeolite previously sterilized as support.

As basic means; mineral salts and MS vitamins [15], 3.0% of sucrose and 0.1 mg L^{-1} of BA were utilized. pH solution was adjusted to 5.8 with 1 N NaOH and after this it was sterilized during 20 minutes, under 121 °C and a pressure of 1.2 kg cm^2. The test tubes were placed under a photoperiod of 16 hour-light with photosynthetic photon fluxes (PPF) about µmol $m^{-2}s^{-1}$, measured by means of a sensor of photon fluxes installed in a universal cuvette matched to a portable system of measuring photosynthesis (CIRAS-2) of PP system.

After 30 days, the shoots were subcultivated and multiplied in 250 ml flask in a semisolid MS medium enriched with BA (0.5 mg L^{-1}), L-glutamine (500 mg L^{-1}), MS vitamins, saccharose (3.0%) and solidified with agar (6.5 g L^{-1}) in all the cases; a volume of 25 ml was used. Five subcultivation were carried out, each one with a frequency of three weeks.

2.3 Establishment of the methodology for the propagation in the temporary immersion bioreactors (TIB):

A design of a temporal immersion bioreactor, modified by the Bioplant Center of Ciego de Avila University, Cuba [9], was used. As recipients flasks of Nalgene Company with a capacity of one liter, which were interconnected with couples using silicone hoses, in a flask the medium of liquid cultivation was placed and in the other the *Eucalyptus* explants, additionally each one was connected to an air entrance system coming from a compressor, which was turned on by an automatic programmer to control the frequency and the length of immersion, the light and the gas fluxes. All the entrance or outgoing of air fluxes was sterilized by hydrophobic filters of 0.2 µm, in such a way that each recipient was handled independently without risk of contamination.

The number of explants (13), the time (3 minutes) and the frequency of immersion (every 12 hours) were used during the experiments. The average volume corresponded to 750 ml and 13 explants, each with five shoots, were contaminated. The environment for cultivation consisted in the mineral salt of MS from which the nitrate of ammonium was reduced to 1 237.5 mg L^{-1} also the organic (ingredients) which included: thiamine-HCI (0.4 mg L^{-1}), mio-inositol (100 mg L^{-1}), pyridoxine HCI (0.5 mg L^{-1}), nicotinic acid (0.5 mg L^{-1}), glycine (2.0 mg L^{-1}), L- glutamine (500 mg L^{-1}), saccharose (30 g L^{-1}), polyvinyl polypirrolidona (PVPP) in concentration of 250 mg L^{-1} was used as a preventive agent of oxidation, and it was also supplemented with BA (0.5 mg L^{-1}). The medium was sterilized by autoclave (40 minutes, 121 °C), previously the pH was adjusted to 5.8 with Na OH. The cultivations were incubated to 24±1 °C with a photoperiod of 16 hours with artificial light helped supported by fluorescent lamps (PHILIPS TL 40 W/54) which irradiated an intensity of 80 µmol m^{-2} s^{-1}.

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2.4 Determination of photomixotrophic effects in elongation phase of *E. Urograndis* shoots propagated in temporary immersion bioreactors:

The experiment consisted of a bifactorial under a design completely random with three repetitions for treatments, where the factors corresponded to different levels of light intensity and of CO_2 in the phase of elongation in TIB. The photomixotrophic conditions of *E. Urograndis* are shown on table 1.

Table.1: Photomixotrophic conditions in elongation phase of Eucalyptus Urograndis shoots propagated in temporary immersion bioreactors (TIB).

CO_2 Concentration (µmol mol CO_2)	**Light intensity** (µmol m^{-2} s^{-1})
350	80 250
1200	80 250

A photoperiod of 16 hours light was used during the development of this experiment, the photosynthetic photons fluxes (µmol $m^{-2}s^{-1}$) for the photosynthetic activity were measured with a sensor to measure photosynthesis (CIRAS-2), a portable PP system.

2.5 Photosynthetic, stomatic conductance and transpiration activity of *E. urograndis* plants during photomixotrophic conditions in TIB:

Net photosynthesis (A, µmol (CO_2) $m^{-2}s^{-1}$), stomatic conductance (mmol (H_2O) $m^{-2}s^{-1}$) and transpiration (µmol (H_2O) $m^{-2}s^{-1}$) was measured at the ambient relative humidity and air temperature. The analyzer and cuvette were automatically calibrated before every measurement. Light was fixed at 600 µmol $m^{-2}s^{-1}$ for the determination of the A/Ci curve, while the CO_2 was fixed at 600 µmol mol^{-1}, these values were obtained from saturation curve of light and CO_2 in *Eucalyptus* plants.

Measurement were always made on the youngest fully expanded leaves and between 9:00 am and 10:00 am. Five plants were measured with 10 repetitions each one for a total of 50 values.

2.6 Measurements of enzyme activity in *E. urogran*dis plants during photomixotrophic conditions in TIB:

Enzyme extraction and assays. Leaf samples were taken before 10:00 am and immediately frozen in liquid N_2 and stored at -80 °C until use. A 250 mg frozen tissue was ground to a very fine powder with a mortar and pestle in liquid N_2. The sucrose phosphate synthase (SPS) enzyme (EC 2.4.1.14) activity was determined to pH 7.5 with 50 µL from the unsalted extract. The mixing was incubated for 20 minutes in 30 ^{0}C. The reaction stopped with the addition of 70 µL (5.35 mol L^{-1}) of KOH. For the white the reaction stopped with the addition of 70 µL (5.35 mol L^{-1}) of KOH in 0 minutes [16]. The formation of sucrose was determined by means of the antrone method [17].

The activity of invertase (EC 3.2.1.26) was determined in a mixing of reaction (reaction mixing) of 500 µL from the total volume that contains enzymes free of salt and 50 mmol L^{-1} of saccharose, a tampon of acetate of sodium to pH 4.5 was used. The reaction started with the addition of enzyme, previously incubated during 20 minutes to 30 ^{0}C. The formation of hexosa was determined enzymatically [18].

For the case of the phosphoenolpyruvate carboxylase enzymes (PEPC; EC 4.1.1.31) and pyruvate kinase (PK; EC 2.7.1.40) were extracted in 1 ml of tampon 50 mM Hepes-KOH containing 12 mmol L^{-1} Mg Cl_2, 1 mmol L^{-1} EGTA, 1 mmol L^{-1} EDTA, 1 mmol L^{-1} DTT, 10% glycerol, 2 mmol L^{-1} benzamidine, 2 mmol L^{-1} amino-n-caproic acid to pH 7.4 [19]. The catalysis of reaction was joined to the reacting of dehydrogenated malate to 25 ^{0}C for the decreasing of NADH used to 340nm in a spectrophotometer Pharmacia. The PQ activity was joined to the dehydrogenated lactate to 25 ^{0}C by monitoring the utilization of NADH to 340 nm.

2.6 Statistical analysis:

At each sampling date, six plantlets were randomly selected to measure photosynthesis and transpiration. For enzymatic determination, randomized plantlets were used. Three extraction in each evaluation moments and three repetitions for each extraction were performed. Twenty plantlets were used for morphological measuring. Analysis of variance was conducted using SPSS Program. Duncan's multiple range tests were used for mean separation at the $p<0.05$ level.

III. RESULTS AND DISCUSSION

3.1 Effects of photomixotrophic conditions on growing variables of *E. urograndis* plants during photomixotrophic conditions in TIB.

Light intensity showed the best results with the treatment in which the plants were submitted to a greater light intensity (250 µmol m^{-2} s^{-1}), this shows statistical differences in all variables evaluated, with the exception in the dry mass. In the leaf number the plants developed five news leaves, which is in correspondence with the fresh dough found. Meanwhile CO_2 brought about significant differences just on the fresh mass variable,

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where the higher values were got in plants submitted to treatments with high concentration of CO_2 (Table 2).

Table.2: Effects of photomixotrophic conditions on growing variables of E. urograndis plants during photomixotrophic conditions in TIB.

Factors		Length (cm)	Leaf number	Mass (g)	
				fresh	Dry
I - Light	80	2.14 b	11.17 b	0.13 b	0.0189
(µmol m^{-2} s^{-1})	250	2.86 a	17.02 a	0.21 a	0.0184
	Sign.	*	*	*	ns
	SE	0.13	1.22	0.023	0.0018
II - CO_2	350	2.42	13.56	0.12 b	0.0186
(µmol mol)	1 200	2.58	15.14	0.22 a	0.0188
	Sign.	ns	ns	*	ns
	SE	0.13	1.22	0.023	0.0018
Interacction	80 + 350	2.34 bc	11.24 b	0.14 b	0.0197
I - II	80 + 1 200	1.93 c	12.16 b	0.13 b	0.0182
	250 + 350	2.49 b	19.04 a	0.11 b	0.0174
	250 + 1 200	3.23 a	14.96 ab	0.32 a	0.0194
	Sign.	*	*	*	ns
	SE	0.18	1.72	0.033	0.0025

Measures with different letters differ statistically (one-way ANOVA, Tuckey, $p<0.05$). Each data represents the mean for n=30.

Effects of the interactions of the two factors showed that plants which were submitted to a higher light intensity and enriched environment with CO_2, highest values are obtained in the studied variables of plant quality, with the exception the dry mass and the number of leafs, which shows significant differences among the other interactions. We should point out that treatments submitted to the same conditions were the ones which obtained the greater percentage of optimal size plants, motivated too for the BIT ability to aerate plant tissue and provide contact between entire explants and the liquid medium.

The shoot length, the number of new nodal segments for explant and the multiplication coefficient were significantly higher in *Eucalyptus tereticornis* under photomixotrophic conditions than in photoautotrophic condition [20]. High significant differences too were found in fresh mass and net photosynthetic rate in *E. camaldulensis* plants when they were submitted to forced ventilation [21].

Growth of plantlets *in vitro* is often greater under photomixotrophic conditions than under heterotrophic conditions, provided that the *in vitro* environment is properly controlled for promoting photosynthesis. In this case and in some others, it is speculated about the metabolic routes favored under the conditions established for cultivation, this information is too important for better interpretation of the results.

Some variables related to the photosynthetic activity, measures during the photomixotrophic treatments when finishing the phase of elongation of the *E. Urograndis* shoots propagated in temporary immersion bioreactors should be evaluated in these experiments.

3.2 Stomatic conductance, transpiration and photosynthetic activity of *E. urograndis* plants during photomixotrophic conditions in TIB:

E. urograndis plants showed a high stomatal conductance measured in terms of water flow. As the stomatal conductance is equivalent to the permeability and inverse to the resistance of water fluxes, it is inferred that transpiration in these leafs had to be proportional, however this reasoning is not always materialized (Table 3).

Table.3: Values of transpiration, stomatal conductance and net photosynthesis in E. urograndis shoots propagated under photomixotrophic conditions in TIB.

Factors	Transpiration (mmol (H_2O) $m^{-2}s^{-1}$)	Stomatic Conductance (mmol (H_2O) $m^{-2}s^{-1}$)	Photosynthesis (µmol (CO_2) $m^{-2}s^{-1}$)

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I - Light	80	6.34	4067.32 a	4.34 a
(µmol $m^{-2}s^{-1}$)	250	6.37	2633.33 b	0.00 b
	Sign.	ns	*	*
	SE	0.043	115,40	0.20
II - CO_2	350	6.78 a	4261.63 a	3.26 a
(µmol mol)	1 200	5.90 b	2439.02 b	1.12 b
	Sign.	*	*	*
	SE	0.043	115,40	0.20
Interaction	80+350	6.51 b	4960.63 a	6.52 a
I - II	80+1 200	6.17 c	3174.00 b	2.25 b
	250+350	7.05 a	3562.63 b	0.00 c
	250+1 200	5.63 d	1704.03 c	0.00 c
	Sign.	*	*	*
	SE	0.06	163,20	0.28

Means with different letters differ statistically (one–way ANOVA, Tuckey, $p<0.05$). Each data represents the means for n=30.

When analyzing the effect of light, the first variation of this postulate is found. The plants submitted to a higher PPF reduce significantly the stomatal conductance but it doesn't modify the transpiration. As it is known a general behavior of replying of the stoma to the increasing of light intensity, turns out in a greater number of open stomas and in the increasing of transpiration.

In the case of concentration of CO_2, the behavior of the *E. Urograndis* shoots cultivated in TIB was similar to the most general tendency that plants flow. When air was enriched with CO_2, plants reduced their stomatal conductance associated to a reduction in the quantity of open stomas, and the transpiration decreased significantly. It is known that the effects of CO_2 are very powerful over the opening of stomas, the increase of its concentration in occlusive cell cause a partial closure of stomas and it can inhibit the assimilation of CO_2, just partially compensated when the photosynthetic activity increases.

In the integration of the effects of both factors, the concentration of factors, the concentration of CO_2 in the environment where the shoots were cultivated, had greater influences when comparing each level of light with the two levels of CO_2 the stomatal conductance was reduced and the transpiration too. However in the comparison of each concentration of CO_2 with the two levels of PPF evaluated, it is reiterated that the increase of light reduced the stomatal conductance, but transpiration of plants cultivated with 350 µmol (CO_2) mol of air increased.

The regulation of the opening of stomas is a complex process which does not depend only on the factors previously analyzed. Others as temperature, relative humidity, cytosolic concentration of calcium, hormones, and enzymes which indicates the related metabolic routes, they also exert important influences and development. Some of these measurements should be considered in future investigations.

The effects of CO_2 over stomatal opening is adjusted in function of the demand of photosynthesis in plants. The measurement of this relative indicator demonstrated that in low levels of CO_2 and light the greater quantity of fixed CO_2 are registered.

The addition of sugar in the environment of cultivation showed a negative effect in the growing and photosynthesis in plants *in vitro* [22]. Besides the saccharose provoked the stimulations of the growth and the photosynthesis activity of tobacco plants *in vitro* [23] also in beet plants [24] and in potato plants [25]. These results indicate that the photosynthesis capacity depend mainly on the species and the environmental conditions and of cultivation that plants are submitted.

The integral analysis of the results showed on table 4 are not enough to justify the effects of treatments about the quality of plants cultivated in TIB, because the behavior of the physiological indicators is insufficient when they depend on some other factors, among them the metabolic routes.

3.23 Activity of enzymes of the carbon metabolism in the *E. urograndis* shoots propagated under photomixotrophic conditions in TIB.

In order to specify the results of the treatments under photomixotrophic conditions, in table 4 is shown the activity of important enzymes involved in the metabolism of carbon, measured in the *E. urograndis* shoots once the cultivation *in vitro* phase is finished in the TIB.

The enzymes of the catabolic routes of the metabolism of carbon were much more active than the metabolic means.

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The activity of the acid invertase and pyruvate kinase surpass several times the saccharose phosphate synthase and the phosphoenolpiruvate carboxylase (table 4).

Table.4: Activity of enzymes of the carbon metabolism, sucrose phosphate synthase (SPS) acid invertase (AI), pyruvate kinase (PK) and phosphoenolpyruvate carboxylase (PEPC) in E. urograndis shoots propagated under photomixotrophic conditions in TIB.

Factors		SPS (µmol g^{-1} FM h^{-1})	AI (µmol g^{-1} FM h^{-1})	PK (µmol g^{-1} FM h^{-1})	PEPC (µmol g^{-1} FM h^{-1})
I - Light	80	7.04	24.34 a	51.16 b	-
(µmol m^{-2} s^{-1})	250	5.95	15.54 b	223.48 a	-
	Sign.	ns	*	*	
	SE	0.77	0.84	24.38	
II - CO_2	350	5.72	10.43 b	112.90	-
(µmol mol)	1 200	7.27	29.46 a	161.74	-
	Sign.	ns	*	ns	
	SE	0.77	0.84	24.38	
Interaction	80+350	8.22 a	0.17 d	31.63 c	10.18
I - II	80+1 200	5.86 ab	48.52 a	70.68 bc	0.00
	250+350	3.21 b	20.69 b	194.16 ab	0.00
	250+1 200	8.68 a	10.40 c	252.81 a	0.00
	Sign.	*	*	*	
	SE	1.09	1.18	34.48	

Means with different letters are statistical different (one-way ANOVA, test TUCKEY, $p<0.05$). Each data represents the means for n=9.

The PK registered the greatest activity in all the treatments with significant differences marked by the light factor. As it was previously analyzed (table 3) the increasing of light reduced the stomatal conductance, apparently the resistance of the flow of water increased and although it could be one thousand superiors to the entrance of CO_2, the closing of stomas should have reduced the capture of it. Nevertheless, the entrance of oxygen, necessary for the oxidized process, specifically the respiratory ones. The relevant activity of PK is an evidence that these were the favored process.

Anyhow the activity of AI is also big, it is supposed that they degraded the saccharose from the environment of cultivation (in all cases to 30 g L^{-1}) and the resulting hexoses were degraded in the glycolytic means which marks the catalytic action of PK. Probably the high concentrations of saccharose limited the photosynthetic fixation of CO_2 as it has been shown by [26, 27], because they were sufficient to guarantee the metabolic demands which improved the quality of plants propagated under a greater flux of photosynthetic photons (PPF).

Comparing the enzyme behavior of plants propagated in the treatments with greater intensity of light, which were the ones that obtained better plants, differences between IA and SFS were observed. The treatment with best results in the production of competent plants, those which were elongated with greater levels of light and CO_2 in the air, registered lower activity of AI and higher activity of SPS. This shows that absorbed fewer saccharose from the means of cultivation and that synthesized more, favored by higher concentration of CO_2 in the atmosphere.

Activities of SPS and AI were increased much more in tomato plants cultivated in 3% of saccharose than without saccharose when they were submitted under PPF and lower concentration of CO_2, while it was observed a different behavior in the activity of SPS, when its increase was higher in conditions of high PPF and high concentration of CO_2 and in an environmental mean of cultivation without saccharose [28].

With this analysis, the interpretation of the best results in quality of plants, which were obtained under conditions of photomixotrophic cultivation, is completed. To the intense metabolic activity measured in those shoots (table 4) it is added the best management of the hydraulic related to transpiration (table 3). In *E. urograndis* plants propagated with high PPF and concentration of CO_2, these factors provoke the least stomatal conductance, the lowest loss of water and they obtained the highest values of length and fresh mass. The ones which are elongated with high PPF but with the lowest concentration of CO_2, duplicate the stomatal conductance and they registered the

highest loss of water, which showed the significant reduction of length and fresh mass.

In both treatments with higher light intensity, the null values of PEPC are corresponded with the increased activity of PK, because the two enzymes compete for the phosfoenolpiruvate and the data show that the breathing consumption prevailed because of the PK. Just with the control treatment, with the lowest levels of PPF and with CO_2, PEPC activity was detected in the *E. urograndis* shoots, the lowest activities of PK were also measured in them.

Finally, the increase of concentration of CO_2 with the lowest PPF resulted the worst combination to stimulate the elongation of the *E. urograndis* shoots in the TIB, because the percentage of plants non competent was increased to more than 20%. They were distinguished for the higher activity of the acid invertase in the catabolic process already analyzed, which together to the action of PQ, resulted determinant over the anabolic activity represented by SFS.

The interpretation of these results is complex, as it is the action of each enzyme evaluated. They go from the optimal levels enzymatic complexes to other particularities as the regulation of SPS by the CO_2 and the temperature [29] mediating other functions as the Rubisco activity and the nitrogenous metabolism, as the important relation between PEPC and the malic enzymes, the presence of isoforms in both, the regulation by light and temperature [30] as the integrated behavior for Rubisco, SPS, SS, AIC, PEPC associated to a fixation of CO_2, hormones and photoperiod [31]

Although some of the factor previously quoted were not included in this study, the results show the effects of the growth regulators in the multiplication and the photomixotrophic treatments in the elongation of *E. urograndis* shoots in TIB. The combination of high levels of PPF and of CO_2 in the air favored the quality of the propagated plants, in biochemical level approximations were obtained to reasons that justify these answers, based on the metabolism of carbon.

Nevertheless the treatments applied also influenced in the concentration of phytohormons and in its regulation [32] which are integrated to the system of signals that should have existed in this species in reply to the action of the regulators of growing, concentration of CO_2 in the air and levels of PPF, which include changes in the metabolism [33].

IV. CONCLUSION

In conclusion, the results of the present study indicated that during the photomixotrophic cultivation the light improved the quality of *E. urograndis* shoots, and that the saccharose limited the photosynthetic assimilation of CO_2 while the high PPF and concentration of CO_2 provoked less stomatal conductance, lower-loss of water and higher values of length and fresh mass. Nevertheless the catabolic activity was intense, but the combination of high levels of light and CO_2 reduced the activity of AI and increased the activity of SPS.

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Morphological Markers based Assessment of Genetic Diversity in Cultivated Tomato (*Solanum Lycopersicon* L.) Genotypes

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***Abstract*—** *Assessment of genetic diversity in any crop species provides a basis for devising future strategies for crop improvement; conservation and sustainable use. An experiment consisting of 24 genotypes of Tomato was conducted during the year 2016 at the Research Farm and Molecular Biology Laboratory of School of Biotechnology, SKUAST-J, Chatha. The experiment was conducted in Randomised Block Design (RBD) with three replications in 2 rows of 5m length with spacing of 45 x 90 cm. The extent of genetic divergence /relatedness was estimated among 24 genotypes by using 11 traits viz. plant height (cm), number of branches, number of fruits per bunch, total soluble solids, flesh thickness (mm), number of locules, fruit width (cm), fruit length (cm), yield per plant (g), average fruit weight (g), number of fruits per plant. The maximum number of fruits/bunch was recorded in "Utkal Pragyan" (3.66) and the minimum number was recorded in "Swarna Sampada" (2.03). Maximum TSS(%) was recorded in DCT-1 (8.06%) and minimum TSS was recorded in "Dhanshri" (2.83%). Maximum number of fruits and yield/plant was recorded in "DCT-1" (115.33) and "Hisar Lalit" (2507.36g), respectively. The minimum number of fruits and yield/ plant was recorded in "NDT-4" (23.20) and "DCT-1" (861.40g), respectively.Mean data revealed high range for most of studied traits. Hierarchical cluster analysis allowed the assessment of similarity and clarified some of the relationships among tomato genotypes. UPGMA produced a dendrogram with two main clusters with further sub clusters. Of all the studied 24 genotypes Anand tomato and Hisar lalit were found to be most dissimilar based on UPGMA clustering. Hisar lalit was found to be most promising variety among all the genotypes for most of the traits under study, which can be used for further breeding and crop improvement programmes.*

***Keywords*—Genetic diversity analysis, Morphological traits, cluster analysis, ANOVA, genetic advance.**

I. INTRODUCTION

Tomato is one of the significant vegetable crops of special economic importance in the horticulture industry, originating in South America and its many varieties are now commonly grown in greenhouse in cooler climates (He *et al.*, 2003). It is the most popular garden vegetable belonging to the genus *Lycopersicon*, the resemblance between leaves and flowers of potato and tomato plants seems to certify this taxonomic grouping (Wang *et al.*, 2005 and Shidfar *et al*., 2011). Systematic study and evaluation of germplasm is of great importance for current and future agronomic and genetic improvement of the crop. Furthermore, if an improvement program is to be carried out, evaluation of germplasm is imperative, in order to understand the genetic background and breeding value of the available germplasm (Singh *et al.*, 2002).

Tomato crop has wider adaptability, high yielding potential and multipurpose uses in fresh as well as processed food industries. An improvement in yield and quality in self pollinated crops like tomato is normally achieved by selecting the genotypes with desirable character combinations existing in nature or by hybridization.

Tomato fruit and its products are the main source of lycopene and other antioxidants in the human diet (Fraser *et al*., 2002) and recent epidemiological studies have shown that their consumption helps to prevent cardiovascular disease (Arab and Steck, 2000, Jarquín-Enríquez*et al*., 2013) and some types of cancer, such as prostate cancer (Barber and Barber, 2002, Shi *et al*., 2002).

The tomato plants show ample morphological variation. The plants may be in form of bushes (determinate) or vines up to six feet tall (indeterminate). The stem and leaves are pubescent having non glandular and glandular trichomes with unpleasant odour. The stem hair may develop into roots when in contact with soil. The leaves display spiral phyllotaxy i.e. one leaf at each node and are petiolate, compound, imparipinate. Tomato shoots show sympodial

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branching with apical meristems. Cultivated tomato is autogamous and style is enclosed by the staminal cone to assure self pollination.

Morphological characters have for a long time remained the means of studying genetic variations in plant species. It is a traditional approach used to quantify genetic differences, and is often used for genetic diversity analysis (Khadivi-Khub *et al.*, 2008; Nikoumanesh *et al.*, 2011). Since the quantitative characters are markedly influenced by the environment, a study under different locations and years is likely to bring out the genotype-environment interaction for precise estimation of genetic parameters and predicting the progress of selection. Moreover, knowledge about association of various characters and their relative contribution to yield is helpful for multiple trait selection. Thus, the present study was conducted with the aim to study the genetic diversity of tomato cultivars using morphological traits and development of phylogenetic tree by using bio informatics tools in order to generate a sound breeding plan for its improvement.

II. MATERIAL AND METHODS

The experimental material for the study comprised of 24 genotypes of Tomato (*Solanum lycopersicum* L.), which were grown in a Randomized Block Design (RBD) with three replications in which 21 days old seedlings were transplanted in 2 rows of 5m length with plot spacing of 45 x 90 cm. All the agronomic and plant protection practices as applicable for commercial tomato crop were adopted. In each genotype, 5 plants were selected for various observations. The materials used in this study were taken from Indian Institute of Vegetable Sciences (IIVR), Varanasi. The details of tomato genotypes are shown in Table 1.

2.1 Methodology adopted

Recommended package practices were followed for raising a good crop. Observations were recorded for the various morphological, agronomical, yield and quality traits in order to study the magnitude of variability and level of genetic divergence in the material. Five plants per plot per replication were randomly selected and tagged for recording the characters. Mean values for all the characters were worked out. Eleven characters were studied for morphological characterization of tomato viz. Plant height (cm), Number of branches per plant, Number of fruits per bunch, Fruit length (cm), Fruit width (cm), Number of fruit per plant, Number of locules per fruit, Total soluble solids (^{0}Brix), Flesh thickness(cm), Yield per plant(g) and Average fruit weight (g).

2.2 Data analysis

The morphological data recorded during the investigation was subjected to the statistical analysis which included ANOVA, Genotypic and phenotypic coefficient of variation, Heritability and Genetic advance.

III. RESULTS AND DISCUSSION

24 genotypes of tomato was evaluated for morphological characters as per the standard procedure. The significant variation in tomato genotypes with respect to yield and quality characters may be due to the genetic makeup, status of water and oxygen during the growing period of these genotypes. The oxygen deficiency restricts root respiration and negatively affects water and nutrient uptake. This eventually reduces the yield and its quality. The description of the genotypes with respect to 11 characters is described in Table 2.

3.1 Analysis of variance (ANOVA)

Analysis of variance was carried on various morpho-physiological, phenological, yield components and quality traits for studying the variation. ANOVA showed highly significant variation among the genotypes for all the characters. The analysis of variance revealed significant mean square estimates for all the characters indicating sufficient diversity among the genotypes. The variation in the genotypes would be helpful in the development of superior varieties. The results are in agreement with the observations of Golani *et al*. (2007). The analysis of variance for the data recorded on various traits viz. plant height, number of branches, number of fruits per bunch, total soluble salts, pericarp thickness, fruit length, fruit width, number of locules, average fruit weight, number of fruits per plant and yield per plant are presented in the Table 3.

3.2 Genetic parameters for various morphological, phenological, yield components and quality traits in tomato genotypes

3.2.2 Phenotypic coefficient of Variation (PCV)

The phenotypic variance ranged from 14.61 to 46.57 and the lowest variance was recorded for fruit width (14.61) and maximum was recorded for number of fruits per plant (46.57) followed by flesh thickness (33.53) and average fruit weight (33.21) (Table 5). Phenotypic coefficient of variation (PCV) was more than genotypic coefficient of variation (GCV) for all studied 11 traits. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were high for Number of fruits/plant

(45.35, 46.57%), Average fruit weight (32.71, 33.21 %), flesh thickness (29.06, 33.53 %), Yield per plant (26.55, 29.14%) and Number of locules/ Fruit (22.68, 22.97 %), which suggested greater phenotypic and genotypic variability among the accessions and sensitiveness of the attributes for making further improvement by selection. Wide difference between GCV and PCV for Number of branches and Number of fruits/bunch implied its susceptibility to environmental fluctuation, whereas narrow difference between GCV and PCV for other traits suggested their relative resistance to environmental alteration. The PCV was higher than the respective GCV for all the characters denoting environmental factors influencing their expression to some degree or other. These results are in agreement with the observations of Henareh. (2015) who showed that high PCV and GCV was observed for plant height followed by average fruit weight estimated. In present study highest estimates of GCV and PCV were recorded for number of fruits per plant (45.35 and 46.57 per cent respectively) which is an important yield component.

3.2.2 Genotypic coefficient of Variation (GCV)

The genotypic coefficient of variance (GCV) ranged from 10.07 to 45.35. High GCV was observed in number of fruits per plant (45.35) and is followed by average fruit weight (32.71) and flesh thickness (29.06). Lowest GCV was recorded in fruit width (10.07) (Table 5).

3.2.3 Heritability

The heritability for the various phenotypic traits ranged from 42.60 per cent for number of fruits per bunch to 97.00 per cent for average fruit weight (Table 5). In the present study, the broad sense heritability estimates were high for all the parameters. Such high values of heritability for Average fruit weight, Number of fruits per plant, Yield per plant, Plant height and Total soluble solids clarified that they were least affected by environmental modification and selection based on phenotypic performance would be reliable. In traits with high heritability, genotypic variance is more than environmental variance and these characters could be considered and exploited for selection in earlier generations. Whereas, in the traits with low heritability, influence of environmental factors is strong for their expression and genotype selection based on these characters should be postponed to the later generations. The results are in close conformity with Golani *et al*. (2007) who observed high heritability for average fruits weight, fruit length, number of locules/fruit and fruit yield.

3.2.4 Genetic Advance

Genetic advance ranged from 0.60(minimum) to 75.01(maximum) for all the characters under study. High genetic advance was observed for yield per plant, number of fruits per plant, average fruit weight and plant height. The results are in close conformity with Golani *et al*., (2007). High heritability (94.80%) with low genetic advance (47.73%) was reported for number of fruits per plant (Table 5). These characters also exhibited high values of GCV which portrayed that these are controlled by additive gene effect and phenotypic selection for their improvement could be achieved by simple selection.

3.3 Diversity analysis in tomato genotypes based on Ward's linkage

Distance between all pairs of 24 genotypes was calculated using Squard Euclidean Distance method and genotypes were clustered based on Ward's method (1963). All the 24 genotypes were grouped into two main clusters with sub clusters (Figure 1). The results showed that the cluster A had two sub clusters; i.e. sub cluster A_1 and sub cluster A_2. Sub cluster A_1 had 8 genotypes (BT-136, SEL-12, NDT-9, ANGHA-1, ANGHA, NDT-1, Anand Tomato-3, NDT-4) followed by cluster Sub cluster A_2 which again had 8 genotypes (ANGHA (L-E415), Dhanshri, Punjab Ratta, PANT-T-5, Hisar Anmol, AZAD-T-2, PT-11 and NDTUR-73). Cluster B had further two sub clusters; i.e. Sub cluster B_1 and B_2. Sub cluster B_1 had 4 genotypes (DCT-1, CO-3, Swarna Sampada and ANGHA (L-E415)).Sub cluster B_2 had 4 genotypes (Utkal Pragyan, Hisar Lalit, Kashi Hemant and FEB-2). Anand Tomato-3 and Hisar Lalit were found to be highly dissimilar among 24 genotypes. The results of this study are in agreement with the results of Henareh (2015) which can be exploited for breeding new tomato varieties for the development of hybrid genotypes.

IV. CONCLUSION

The study revealed considerable phenotypical (and presumably genetic) diversity among tomato genotypes. The cluster analysis grouped the genotypes into two main clusters with further sub clusters. Highest dissimilarity was found between Anand Tomato-3 and Hisar Lalit among 24 genotypes. Hisar Lalit showed large fruit size with reference to Single fruit weight, Flesh thickness, Fruit length and Fruit width and Yield per plant. The range of the mean values defines the genetic potential of different genotypes for various characters studied. The results showed that there was significant genetic distance between the genotypes for some of the characters like yield and its attributing traits. These results indicate that if the genotypes having larger value for range of variability for various characters, there will be better chance to improve the exiting cultivars by different breeding procedures. It can be used in selection or hybridization programme for the respective characters. Phenotypic coefficient of variation (PCV) was more than

genotypic coefficient of variation (GCV) for all studied 11 traits which suggested greater phenotypic and genotypic variability among genotypes and sensitiveness of the attributes for making further improvement by selection. High values of heritability for average fruit weight, fruit length, number of locules/ fruit and fruit clarified that they were least affected by environmental modification and selection based on phenotypic performance would be reliable. Considerable genetic diversity among the cultivated 24 tomato genotypes was observed at morphological levels, which is of importance for germplasm classification, management, and further utilization.

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Table.1: Genotypes of Tomato used in study

S.No	GENOTYPE	S.No	GENOTYPE
1.	UTKAL PRAGYAN	13.	HISAR ANMOL
2.	HISAR LALIT	14.	AZAD-T-2
3.	KASHI HEMANT	15.	PT-11
4.	FEB-2	16.	NDTUR-73
5.	DCT-1	17.	BT-136
6.	CO-3	18.	SEL-12
7.	ARKA ABHAY	19.	NDT-9
8.	SWARNA SAMPADA	20.	ANGHA-1
9.	ANGHA(L-E415)	21.	ANGHA
10.	DHANSHRI	22.	NDT-1
11.	PUNJAB RATTA	23.	ANAND TOMATO-3
12.	PANT –T-5	24.	NDT-4

Table.2: Mean values of morphological traits

Genotype	Plant hei (cm)	No. of branche	No. of fruits/bu	Total s salts (b	Flesh thic (mm)	No. of lo	Fruit le (cm)	Fruit wi (cm)	Average weight (g	No. of fruits/pl	Yield/pl (g)
UTKAL PRAGY	57.55	6.43	3.66	6.20	4.08	2.18	4.88	3.91	36.26	34.00	1233.40
HISAR LALIT	59.50	6.99	2.48	3.20	4.31	2.66	5.63	4.66	54.10	46.23	2507.36
KASHI HEMAN	52.19	5.44	2.40	5.40	3.00	2.50	4.13	4.42	28.66	38.33	1092.00
FEB-2	55.00	5.32	2.61	5.20	2.00	3.50	3.52	3.50	31.80	32.66	1039.20
DCT-1	52.15	6.33	3.10	8.06	1.21	2.33	2.90	2.85	7.43	115.33	861.40
CO-3	48.29	6.20	2.66	4.83	1.83	4.33	3.63	4.45	46.00	28.33	1304.33
ARKA ABHAY	60.12	7.33	2.42	5.86	3.10	3.50	3.71	4.78	44.50	46.00	2049.33
SWARNA SAMPADA	47.97	4.53	2.03	6.00	2.10	4.66	4.03	4.36	40.83	28.33	1157.66
ANGHA(L-E415	43.43	7.09	2.51	6.36	2.75	4.00	3.90	4.53	46.00	41.00	1883.00
DHANSHRI	58.23	6.99	2.10	2.83	4.31	3.00	4.48	4.98	47.33	25.00	1182.66
PUNJAB RATT	82.02	9.20	2.72	5.86	1.91	4.16	3.62	3.76	32.30	52.00	1676.80
PANT –T-5	66.72	8.44	2.55	4.66	2.66	3.33	3.69	4.03	45.56	41.00	1873.06
HISAR ANMOL	79.55	9.00	2.51	4.20	3.13	3.13	3.76	4.50	35.56	31.66	1123.63
AZAD-T-2	52.34	5.99	2.50	5.73	2.41	3.16	3.36	4.23	27.80	36.00	995.66
PT-11	74.80	8.00	3.44	5.43	3.03	3.16	3.51	4.36	33.50	65.66	2198.96
NDTUR-73	65.63	5.75	3.32	6.13	2.83	2.66	3.44	3.87	33.26	46.00	1530.10
BT-136	57.45	6.22	2.42	5.03	3.41	3.66	4.29	4.60	47.80	37.00	1764.76
SEL-12	45.00	6.66	3.00	5.80	2.46	3.83	3.51	4.11	31.86	43.00	1373.63
NDT-9	43.31	5.44	2.35	3.56	2.33	4.66	4.55	4.76	55.05	25.66	1415.40
ANGHA-1	51.40	8.33	2.95	4.76	2.76	5.50	3.35	4.48	38.36	38.66	1480.76
ANGHA	50.02	7.23	2.87	5.80	2.66	5.33	3.55	4.57	41.26	33.66	1389.30
NDT-1	55.28	7.96	2.91	6.26	2.61	3.83	3.57	4.34	36.86	48.33	1745.83
ANAND TOMA	35.10	6.66	3.00	7.00	1.50	3.33	3.83	3.33	54.00	25.66	1394.66
NDT-4	41.24	9.66	3.33	5.30	1.66	3.33	3.98	4.45	80.00	23.00	1834.33
Mean	55.59	6.96	2.74	5.39	2.67	3.57	3.86	4.24	40.67	40.94	1504.47
C.V.	8.20	15.51	14.75	7.62	16.72	16.38	10.30	10.58	5.71	10.60	11.99
S.E.	2.63	0.62	0.23	0.23	0.25	0.33	0.23	0.25	1.34	2.50	104.18
C.D. 5%	7.50	1.77	0.66	0.67	0.73	0.96	0.65	0.73	3.82	7.13	296.57
C.D. 1%	10.01	2.37	0.88	0.90	0.98	1.28	0.87	0.98	5.10	9.52	395.90

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Table.3: Analysis of Variance (ANOVA) for morphological traits in tomato genotypes

	DF	Plant Height (cm)	No. of branches	No. of fruits/ bunch	Total soluble salts (brix)	Flesh thickness (mm)	No. of locules	Fruit length (cm)	Fruit width (cm)	Average fruit weight (g)	No. of fruits/plant	Yield /plant (g)
Treatment	23	9443.27**	121.29**	12.17**	94.30**	46.23**	53.274**	22.99**	17.26**	12344.45**	24225.11**	11765066.78**
Replication	2	63.02	1.85	0.27	0.08	0.45	1.46	0.23	0.05	2.12	70.11	109133.20
Error	46	958.22	53.81	7.55	7.78	9.193	15.78	7.30	9.29	248.94	866.55	1497909.25

*P≤0.01% level of significance ** P≤0.05% level of significance

Table.4: Descriptive statistics for morphological traits in tomato genotypes

Traits	Mean±SD	Standard error	Maximum	Minimum	Range	Variance
Plant height (cm)	55.59±12.14	1.43	82.02	35.10	35.10-82.02	147.38
No. of branches	6.96±1.57	0.18	9.66	4.53	4.53-9.66	2.49
No. of fruits/bunch	2.74±0.53	0.06	3.66	2.03	2.03-3.66	0.28
Total soluble solids (brix)	5.39±1.19	0.14	8.60	2.83	2.83-8.60	1.43
Flesh thickness (mm)	2.67±0.88	0.10	4.31	1.21	1.21-4.31	0.78
No. of locules	3.57±0.99	0.11	5.5	2.18	2.18-5.5	0.99
Fruit length (cm)	3.86±0.65	0.07	5.63	2.90	2.90-5.63	0.43
Fruit width (cm)	4.24±0.61	0.07	4.98	2.85	2.85-4.98	0.37
Average fruit weight (g)	40.67±13.31	1.56	80	7.43	7.43-80	177.40
No. of fruits/plant	40.94±18.82	2.21	122	21	21-122	354.39
Yield/plant (g)	1504.47±433.98	51.14	2507.36	861.40	861.40-2507.36	188339.56

Table.5: Genetic parameters for various morphological, phenological, yield components and quality traits in tomato genotypes

Trait	GCV (%)	PCV (%)	ECV (%)	Heritability (h^2)	GA (@ 5%)	GA (@ 1%)
Plant height (cm)	20.50	22.08	8.20	86.20	21.79	27.93
No. of branches/ Plant	16.78	22.85	15.51	53.90	1.76	2.26
No. of fruits/bunch	12.70	19.47	14.75	42.60	0.46	0.60
Total soluble salts (^{0}brix)	21.20	22.53	7.62	88.60	2.21	2.84
Flesh thickness (mm)	29.06	33.53	16.72	75.10	1.38	1.77
No. of locules/Fruit	22.68	27.97	16.38	65.70	1.35	1.73
Fruit length (cm)	13.68	17.13	10.30	63.80	0.87	1.11
Fruit width (cm)	10.07	14.61	10.58	47.50	0.60	0.77
Average fruit weight (g)	32.71	33.21	5.72	97.00	27.00	34.60
No. of fruits/plant	45.35	46.57	10.60	94.80	37.24	47.73
Yield/plant (g)	26.55	29.14	11.99	83.06	75.01	96.13

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Table.6: Distribution of 24 tomato genotypes into two main clusters

Cluster	Sub clusters	Total entries	Genotypes
A	A_1	8	BT-136, SEL-12,NDT-9,ANGHA-1,ANGHA,NDT-1,ANAND TOMATO-3 NDT-4
	A_2	8	ANGHA(L-E415), DHANSHRI, PUNJAB RATTA, PANT-T-5, HISAR ANMOL, AZAD-T-2, PT-11, NDTUR-73
B	B_1	4	UTKAL PRAGYAN, HISAR LALIT, KASHI HEMANT, FEB-2
	B_2	4	DCT-1, CO-3, ARKA ABHAY, SWARNA SAMPADA

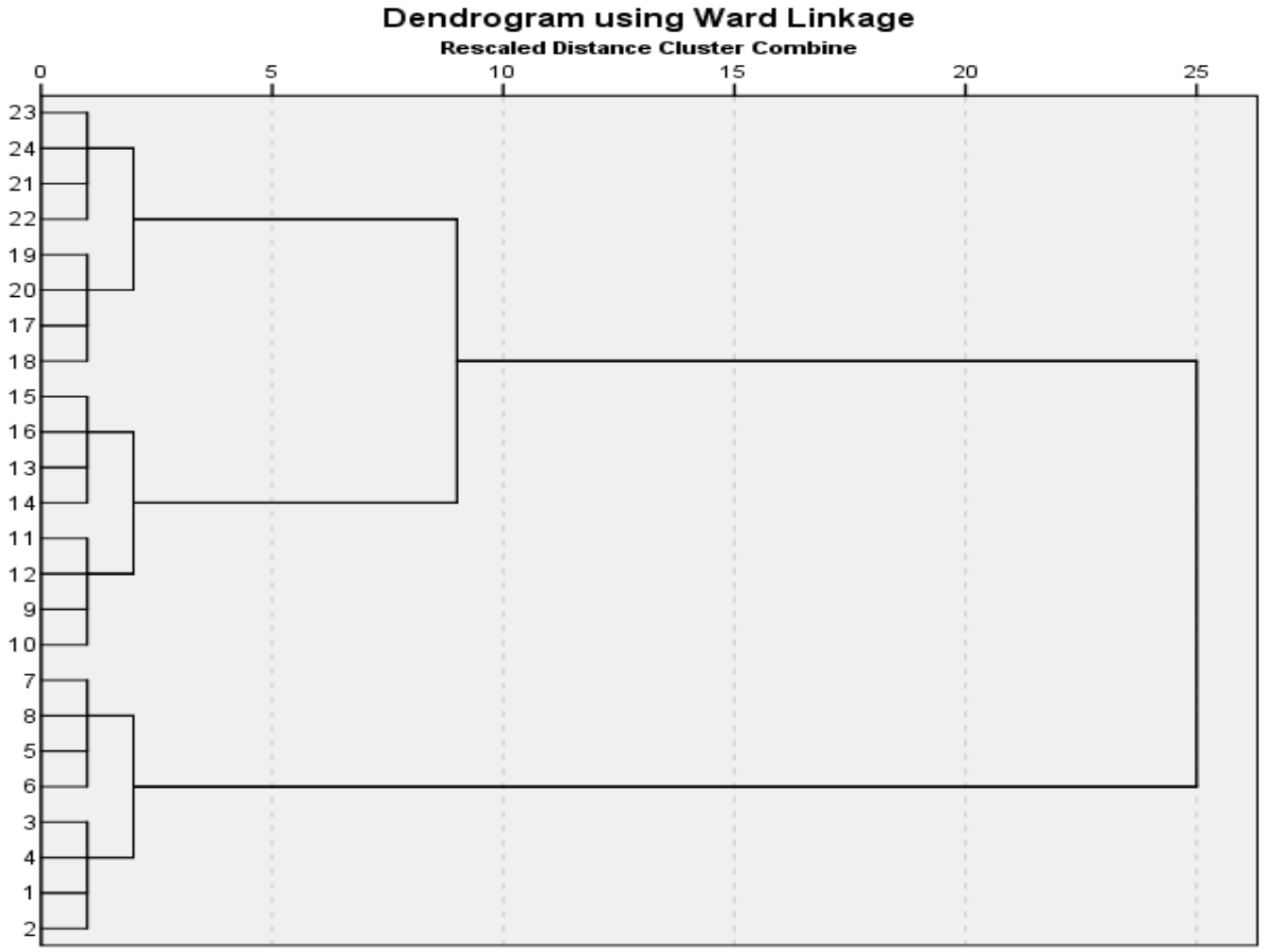

Fig.1: Dendrogram showing Genetic diversity in Tomato genotypes based on morphological markers using Ward linkage.

1-Utkal Pragyan, 2- Hisar Lalit, 3- Kashi Hemant, 4- FEB-2, 5- DCT-1, 6-CO-3, 7- Arka Abhay, 8-Swarna Sampada, 9- ANGHA (L-E415), 10- Dhanshri, 11- Punjab Ratta, 12-PANT-T-5, 13- Hisar Anmol, 14- AZAD-T-2, 15- PT-11, 16- NDTUR-73, 17- BT-136, 18- SEL-12, 19- NDT-9, 20- ANGHA-1,21- ANGHA, 22-NDT-1, 23- Anand Tomato-3, 24- NDT-4

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Distribution and Speciation of Heavy Metals in Soils around Some Selected Auto Repair Workshops in Oghara, Delta State, Nigeria.

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***Abstract*—** *Soil contamination by heavy metals is a worldwide environmental problem. Hence determining the chemical forms of a metal in soils is important to evaluate its mobility and bioavailability. This study determined the distribution and speciation of some heavy metals (Fe, Cu, Zn, Pb and Cd) in soils around some selected auto repair workshops in Oghara, Delta State, Nigeria. Soil samples were collected with the aid of soil Augar within a depth of 0 – 15 cm from the vicinity of the four selected auto repair workshops in Oghara, Delta State, Nigeria. The control samples were taken from a site free from auto repair and commercial activities. The soil samples were assessed for some physico-chemical properties, total heavy metal concentration, chemical speciation, mobility and some metal assessment indices of the heavy metals as a function of soil properties. The mean concentration of Fe, Cu, Zn, Pb and Cd in all the sites analyzed were 550.54, 31.08, 36.15, 4.21 and 1.11 mg/kg respectively. Site B and the control had the highest and lowest total concentration of the five metals analyzed respectively. The levels of Cu were above the DPR target value in sites A and B, while the levels of Cd were above the target value in all the sites except in the control site. All the metals were found to be mostly concentrated in the residual fraction except Zn which was found mostly in the carbonate fraction. The mobility factors revealed that Zn is the most mobile element with an average mobility factor of 41.54% while Cd is the least mobile element with an average mobility factor of 16.51%. Contamination factors, index of geoaccumulation and pollution load index were also calculated. This study showed that mechanic workshop is one of the major sources of anthropogenic heavy metals concentration in the environment.*

***Keywords*— Soil, Heavy Metals, Speciation, Bioavailability, Mobility.**

I. INTRODUCTION

It has been widely accepted that soil plays a key role in sustaining life in earth's ecosystems (Young and Crawford, 2004). The very survival of mankind is tied on its productivity as a medium for plants to grow (Kabata-Pendias and Mukherjee, 2007). Heavy metals emanating from anthropogenic Automobiles introduce a number of toxic metals into the environment. Also the wear of auto tires, degradation of parts, grease, peeling paint and metal in auto-catalysts are sources of heavy metal pollution (Pecheyran *et al.*, 2000). This has led to elevated levels of heavy metals in automobile mechanic workshop soils (Ipeaiyeda and Dawodu, 2008; Iwegbue, 2007). This implies that water bodies (surface and ground water) within and away from the automobile mechanic workshops may equally be polluted with these metals due to continuous interactions between soil and water and the high dispersion rate (Nwachukwu *et al.*, 2010). The fate of the various heavy metals and metalloids in the automobile mechanic workshops is of great concern because soil, water and dust in these areas may contain higher than average abundance of these elements, which may cause the formation of the more available forms of these elements (Adriano, 1992). In recent years there has been increased interest in the studies on speciation or chemical forms of heavy metals in polluted soils and sediments using sequential extraction techniques because these provide knowledge on metal affinity to soil components and the strength with which they are bound to matrix (Norvell, 1984). The use of sequential extractions, although time consuming, furnishes detailed information about the origin, mode of occurrence, biological and physicochemical availability, mobilization and transport of trace metals (Ure and Davidson, 2002). Sequential extraction procedures selectively extract metals bound by specific soil fractions with minimal effects on the soil components. In practice, sequential fractionation schemes have been suggested to identify element distribution with operationally defined soil pools (Amanda and Weindorf, 2010). As a result of ineffective law enforcement agencies to enforce existing environmental laws coupled with lack of stringency even when attempts are made to enforce, Nigerian citizens and

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indeed residents of Oghara and environs in Delta State continue to dump refuse and litter the environment indiscriminately with such toxic substances as condemned engine oil, car batteries from mechanic workshop and solid waste even on the streets. These heavy metals can become a threat to vegetation and animals and ultimately affects the quality of human life, Thus, it becomes imperative to assess the levels of physico-chemical properties, spatial distribution and chemical speciation of heavy metals in soil from auto-repair workshops in Oghara and its environs in Delta State, Nigeria in order to determine their potential hazards to humans.

II. MATERIALS AND METHODS

Study Area

Oghara is a town in Ethiope West Local Government Area of Delta State, Nigeria and is located between latitude $5^0$35′1′′N and longitude $5^0$51’16’’E. the city has road intersections connecting Sapele to Warri and Benin. This study was conducted in four popular automobile workshops in within the town Oghara, site selection was based on the distance from one another, and all samples were collected within the range of latitude $5^0$55^{l}54N to $5^0$57^{l}11N and longitude $5^0$38^{l}40E to $5^0$41^{l}19E. Global positioning system (GPS) and ground reconnaissance were used for identification of sites and geo-referencing.

Table.1: Showing Site Code, Coordinates and Site Description

Site Code	Coordinates	Site Description
Site 1	Lat- 5.95353, Long- 5.63913	This site is located along community road Ogareki-oghara
Site 2	Lat- 5.93487, Long- 5.67913	This site is located beside Ibori round about, Oghara.
Site 3	Lat- 5.94260, Long-5.68670	This site is located at the express.
Site 4	Lat -5.93819, Long- 5.65704	This site is located at Volts electrical company.

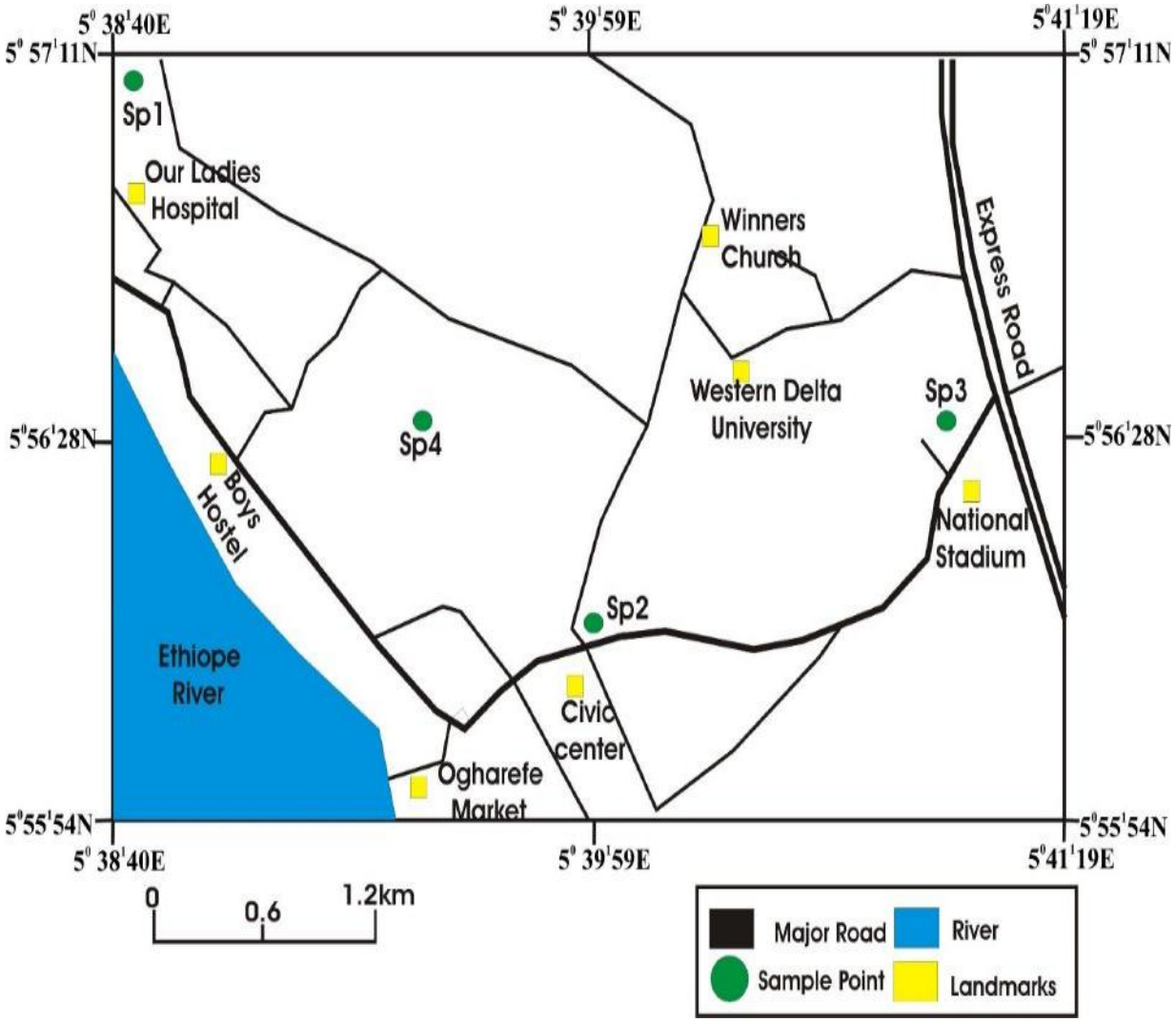

Fig.1: Map of Oghara Showing the Sampled Sites

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Collection of Soil Samples

Topsoil (0 – 15cm) samples were collected from five (5) different mechanic workshops in Oghara. At each site, three different points were chosen using cluster random sampling technique to collect the sample with the aid of soil auger, and then blended (mixed) to obtain a representative sample. Control sample was also collected from a site where there are neither car repairs nor commercial activities carried out. The collected samples were transferred into a black polythene bag, properly labelled and transported to laboratory (Tripathi and Misra, 2012). All samples were air dried and ground to pass through a 2mm sieve and used for both physico-chemical analysis and fractionation experiment (Anegbe and Okuo, 2013).

Physico-chemical Analysis of the Soil Samples

The pH and the CEC were determined as described by Anegbe and Okuo (2013). The hydrometer method described by Asagba *et al*. (2007) was used in evaluating the particle size. The concentration of phosphorus was obtained by the Oviasogie *et al.* (2006) method. The nitrogen content was determined by colorimetric method (Vogel, 2008). The method described by Anegbe *et al*. (2017) was used to determine the organic carbon content, while the total heavy metals determination was carried out according to Okuo *et al.* (2016). The chemical fractionation was carried out as described by Anegbe *et al*., (2014). All glasswares used were soaked and washed with chromic acid and rinsed with distilled water. Bulk scientific standard solution was used to calibrate the Atomic Absorption Spectrometer (Pg A500 model). Procedural blank samples were subjected to similar extraction method using the same amount of reagents.

III. RESULTS AND DISCUSSION

The physico-chemical properties of the soil samples at various sites are shown in Table 2.

Soil pH is the most widely accepted parameter which exerts a controlling influence on the availability of micro-nutrients and heavy metals in the soil to plants (Igwe *et al.*, 2005). The pH values of the soil samples from the automobile workshops were found to be in the acidic region (5.10 - 5.40) and lower than that of the control (6.40). Acidity controls availability, mobility and toxicity of heavy metal ions in the soils. Most metals tend to be less mobile in soil with high pH as they tend to form insoluble complexes (Anegbe *et al*., 2014). Electrical conductivity measures soil salinity. The electrical conductivities of the soil samples from the automobile workshops were all higher than that of the control. This indicates that movement of charge particles would be more at the workshops than that of the control because there are more soluble salts in the soil samples from the automobile workshops than the control (Karaca, 2004; Arias *et al*., 2005).

Table.2: Physico-chemical Properties of the Soil Samples from the Sites

Site	Ph	EC (µs/cm)	N (mg/kg)	P (mg/kg)	Ca (Cmol/kg)	Mg (Cmol/kg)	Na (Cmol/kg)	K (Cmol/kg)	CEC (Cmol/kg)	O.C (%)	O.M (%)	SAND (%)	SILT (%)	CLAY (%)
A	5.40	428.5	4.50	23.64	3.01	0.75	1.25	1.09	6.10	0.77	1.33	74.21	23.40	2.39
B	5.10	356.0	5.50	32.60	1.09	0.98	1.08	0.98	4.13	0.99	1.71	72.62	24.40	2.98
C	5.10	477.5	5.20	30.90	1.03	0.72	1.02	0.94	3.71	0.95	1.64	73.91	23.90	2.19
D	5.20	426.5	3.20	21.00	1.51	0.67	0.91	0.87	3.96	0.76	1.31	75.40	22.40	2.20
Control	6.40	124.0	0.26	18.09	3.20	2.50	1.42	1.12	8.33	0.38	0.66	78.60	18.20	3.20
p-value	0.000	0.004	0.017	0.001	0.014	0.032	0.000	0.000	0.004	0.002	0.002	0.000	0.000	0.000

Organic matter acts as a major adsorbent for metals through the formation of chelates and renders them immobile (Lawan *et al*., 2012). The organic matter (OM) contents at the automobile workshops (1.31-1.71 %) were higher than the control (0.66 %). This might be due to the presence of many organic matter waste residues from effluent oil and oil spills at the automobile workshops which adds more organic matter and carbon, also leading to a higher organic carbon values of the sites. The Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} of the soil samples from the automobile workshops were all lower than that of the control. the cation exchange capacity (CEC) values at the automobile

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workshops were quite low compared to the control (8.33 Cmol/kg). It was observed that at the automobile workshops, site C (3.71 Cmol/kg) and site A (6.10 Cmol/kg) had the lowest and highest CEC respectively. Soils with low CEC are more likely to develop deficiencies in potassium (K^+), magnesium (Mg^{2+}) and other cations, while high CEC soils are less susceptible to leaching of these cations (CUCE, 2007; Okiemen *et al*., 2012). The low values of the CEC were attributed to high sandy nature of the soil samples (Ugbune and Okuo, 2011). The CEC, Ca^{2+}, Mg^{2+}, Na^+ and K^+ values reported in this research were all greater than those reported by Anegbe *et al*. (2014) in a similar research carried out in Benin City. As the texture of the soil plays a very important role in the plant species establishment and development and also influences physical parameters of the soil. The soil texture class of all the soil samples (automobile workshops and control) as represented in table 2 showed that they were all sandy soils with very high percentage of sand and had very low clay contents ranging from 2.19 - 3.20 %. Similar result was obtained by (Anegbe and Okuo, 2013). Soils with high sand content exceeding 70% will have weak surface aggregation and such soils will be porous and have high rate of water infiltration and air circulation (Gbadegesin and Abua, 2011). The nitrogen and phosphorus contents of the soil samples were both higher at the automobile workshop sites compared to the control. T-test was used to indicate significant difference between variables. P-values less than 0.05 were considered statistically significant.

Table.3: Total Metal Concentrations (in mg/kg) of the Heavy Metals in the Sites

Sites	Fe	Cu	Zn	Pb	Cd	Total
A	530.80	37.44	35.81	3.67	0.87	608.59
B	714.20	44.35	39.35	6.17	1.05	805.12
C	683.90	30.55	41.38	5.28	0.99	762.1
D	560.80	28.26	48.42	3.96	2.55	643.99
Control	263.00	14.80	15.79	1.98	0.08	295.65
Average	550.54	31.08	36.15	4.21	1.11	
P-value	0.002	0.003	0.003	0.004	0.051	

Table 3 shows the heavy metal concentration and its distribution in all the sites. The soil sample showed Fe, Cu, Zn, Pb and Cd levels ranging from 263.00-714.20, 14.80-44.35, 15.79-48.42, 1.98-6.17 and 0.08-2.55 mg/kg respectively. The values of each metal at each sites are relatively higher than that of the control, the high concentration of these metals at these sites could be due to air borne sources from car exhaust fumes depositing lead and other contaminants to the environment, automobile vehicle repair process like filing and soldering of iron rods along with other metals bending processes in the workshop and industrial activities occurring close to the automobile workshops. According to the table, Fe had the highest average concentration, highest concentration of Fe compare to other metals in Nigeria soil have been reported by other researchers (Adefemi *et al.*, 2007; Emmanuel and Edward, 2010). Cd had the lowest average concentration. The total concentration of all the metals analyzed in each site varied as follows B > C > D > A > control. The highest concentration of all the metals in site B may be attributed to the large size, old age, its location within Oghara metropolis and high volume of wastes at the workshop. T-test was used to indicate significant difference between variables. P-values less than 0.05 were considered statistically significant.

Table.4: Department of Petroleum Resources (DPR, 2002) for Target and Intervention Values for Metals in Soils.

Heavy Metals	Target values (mg/kg)	Intervention values (mg/kg)
Zn	140	720
Cu	36	190
Cd	0.8	12
Pb	85	530

Comparing the concentration of each metal in each site with DPR (2002) target and intervention values, the levels of Zn and Pb found in all the sites were below the DPR target values. The levels of Cu were above the DPR target value in sites A and B, but were below the target value in sites C, D and control. The levels of Cd were above the target value in all the sites except in the control site. From table 4, it was also observed that all the individual metal analyzed in all the sites showed concentration that were

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below their DPR intervention values (DPR, 2002). There was no DPR target and intervention values for iron perhaps because of its high concentration and distribution in natural or unpolluted soils (Anietie and Labunmi, 2015).

Fractionation and Distribution of the Heavy Metals in the Soil Samples

Soil samples was fractionated for Fe, Cu, Zn, Pb and Cd using the popular Tessier *et al.* (1979) method. The amount of metal present in an extraction fraction is expressed as a percentage of the total mass of that metal in the entire extraction fraction from a given metal.

Iron: The largest portion of iron was concentrated in the residual fraction (F5) with an average percentage of 22.94 % in all the sites, similar association of iron to residual fraction was reported by Obasi *et al.* (2013) and Godwin *et al.* (2014). This was closely followed by the organic fraction (F4), Fe-Mn oxide fraction (F3) and carbonate fraction (F2) with average percentages of 21.61 %, 20.68% and 20.61% respectively. The exchangeable fraction (F1) had the lowest portion with an average percentage of 14.16 %.

Copper: Copper was found mostly in the residual fraction (F5) with a range of 28.18-42.46%, similar association of copper to residual fraction was reported by Godwin *et al.* (2014). The organic fraction (F4) is next with a range of 10.15-33.62 %, This high concentration of copper in the oxidizable fraction (F4) was due to the stability of copper organic complexes which might be attributed to the high formation constants of organic copper complexes (Obasi *et al.*, 2013). The carbonate fraction (F2), the exchangeable fraction (F1) and the Fe-Mn oxide fraction (F3) has 0.71-28.18%, 9.19-18.24% and 5.41-23.90% respectively (Figure 3).

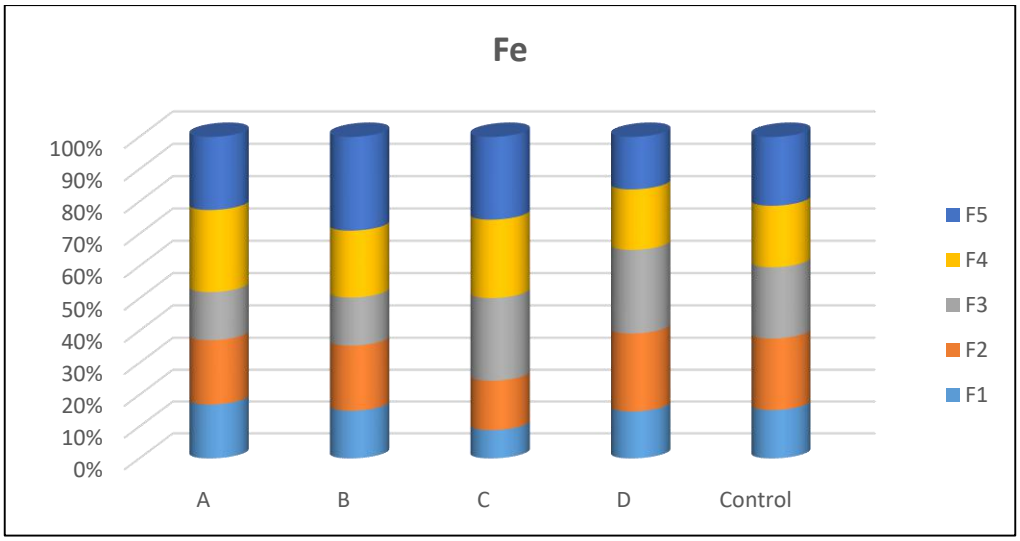

Fig.2: Percentage Concentration of Fe as a Function of Fe Content in the Soil

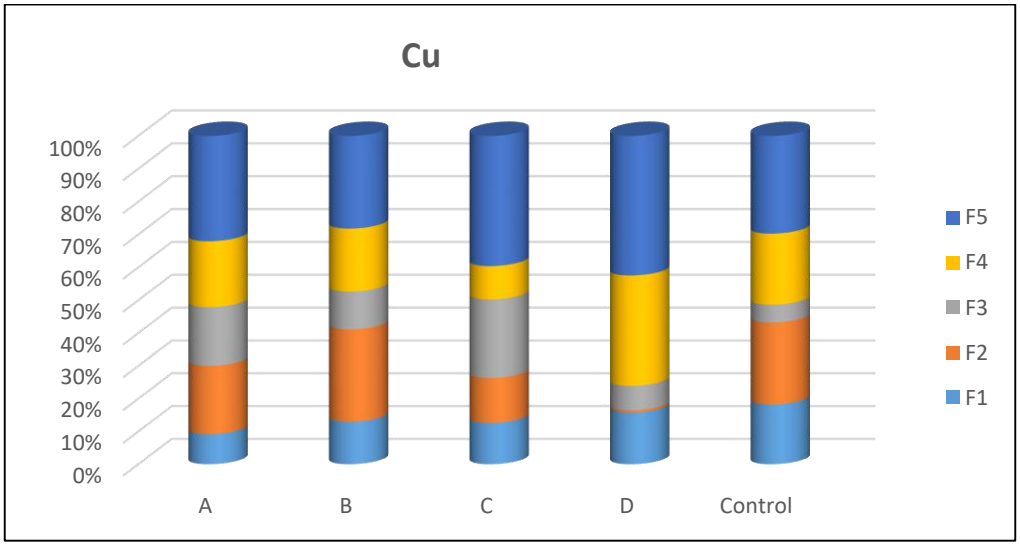

Fig.3: Percentage Concentration of Cu as a Function of Cu Content in the Soil

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Zinc: The largest portion of zinc was found in the carbonate fraction F2 with an average percentage of 27.31%. This was closely followed by the residual fraction F5 having an average of 26.82%. the remaining fractions followed the following order Fe-Mn oxide fraction > exchangeable fraction > organic fraction.

Lead: Lead was mainly found in the residual fraction F5 ranging from 27.25-95.74%, similar result was obtained by Anegbe and Okuo (2013). The metal may have co-precipitated with various silicate species as a result of their adsorption into the mineral lattice because of the sandy nature of the soil (Manceau *et al.*, 2006). This was followed by the carbonate fraction (0.00-32.10%), organic fraction (1.60-21.25%) Fe-Mn oxide bound (2.66-20.44%) and exchangeable fraction (0.00-16.85%).

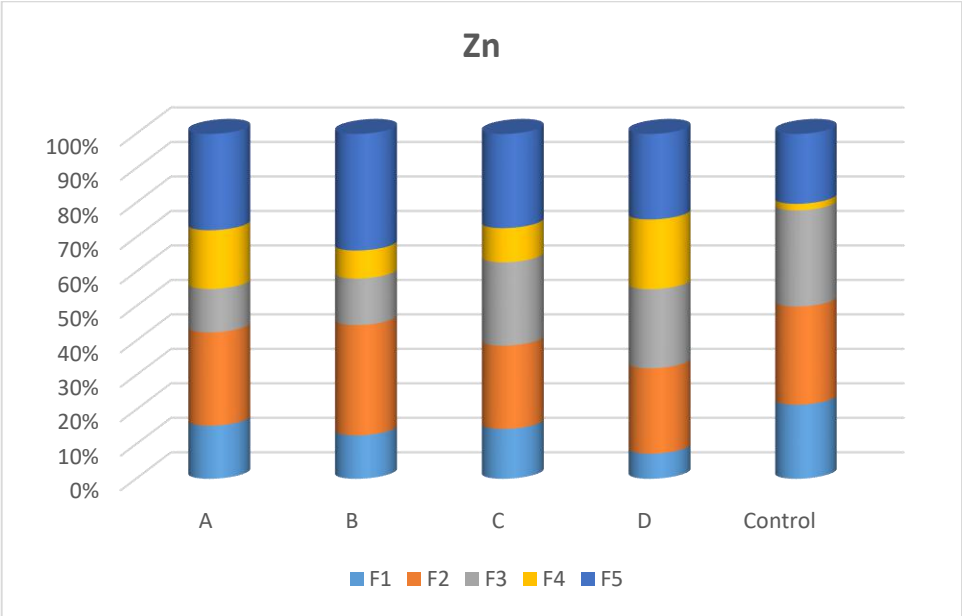

Fig.4: Percentage Concentration of Zn as a Function of Zn Content in the Soil

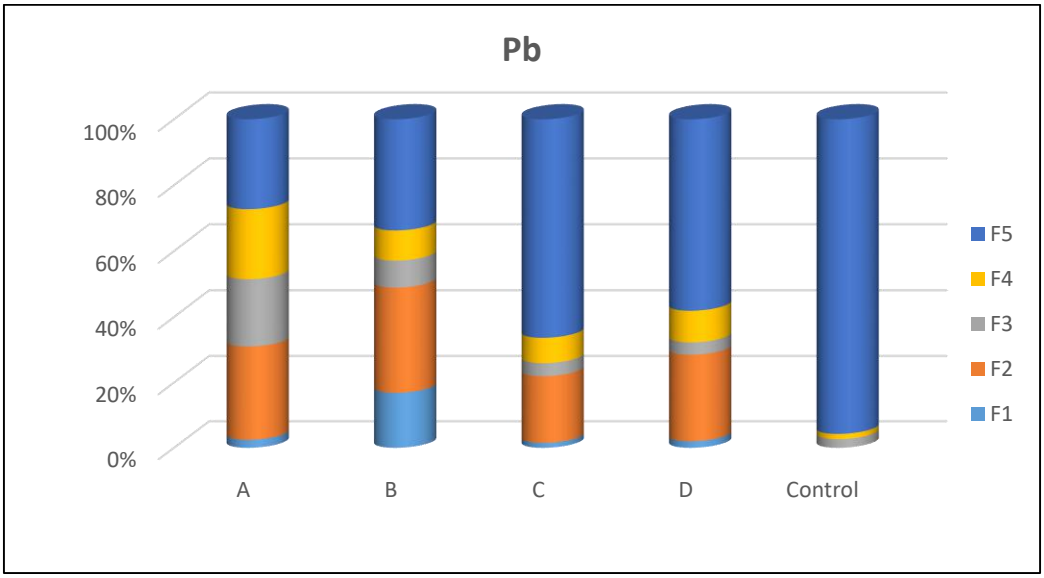

Fig.5: Percentage Concentration of Pb as a Function of Pb Content in the Soil

Cadmium: The greatest amount of cadmium was found in the residual fraction where the range is 35.27-94.24%, similar association of cadmium to residual fraction was reported by Anegbe *et al*. (2014) in a similar research carried out in Benin City. This was followed by the exchangeable fraction (F1) at a range of 0.00-54.86%. the organic fraction (F4), the carbonate fraction (F2) and the Fe-Mn oxide fraction (F3) were in the range of 1.59-12.46%, 1.19-10.57% and 1.09-6.44% respectively (Figure 6). The minor role of the organic fraction in the speciation of Cd noted in this present study is consistent with the low adsorption constant of Cd to organic matter (Yusuf, 2007).

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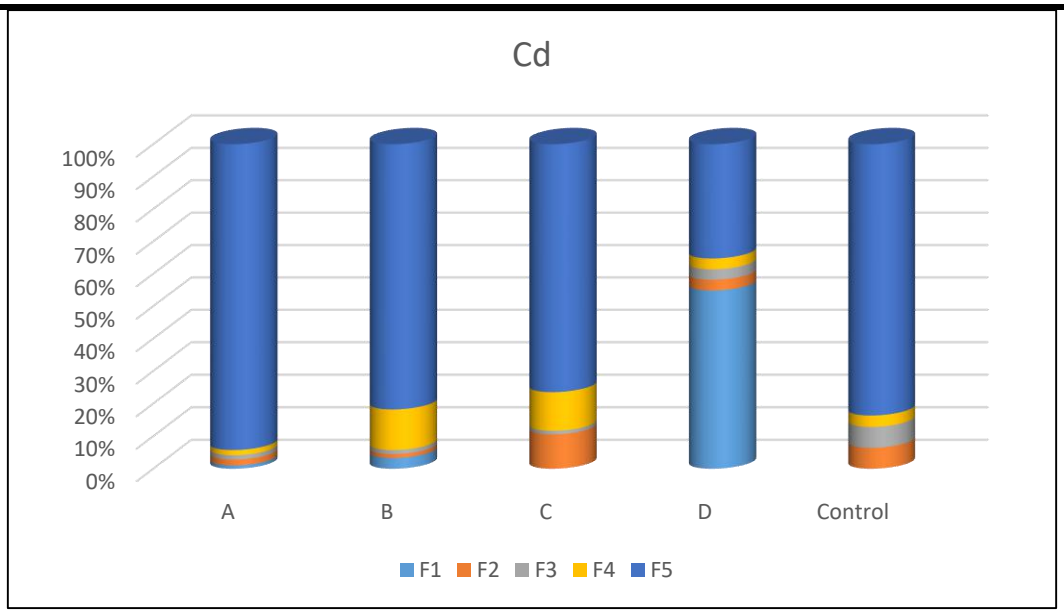

Fig.6: Percentage Concentration of Cd as a Function of Cd Content in the Soil

Mobility Factor

The operationally defined extraction sequence fractionates the heavy metals in the soil in the order of decreasing solubility. As a result, the exchangeable and carbonate (F1 + F2) fractions which are the early fractions, capture the most reactive and presumably the most mobile and bioavailable fractions (Salbu *et al.*, 1998). The relative index of metal mobility was calculated as a mobility factor (MF) on the basis of the following equation (Kabala and Singh, 2001).

$$MF = \frac{F1 + F2}{F1 + F2 + F3 + F4 + F5} \quad \text{----------(1)}$$

Where;

F1 = Exchangeable metal content fraction

F2= Metal content bound to carbonate fractions

F3= Metal content bound to Fe-Mn Oxide Fraction

F4= Metal content bound to organic matter fraction

F5= Residual metal content fraction.

The results obtained from table 5 below showed high mobility factor of the heavy metals within an average range of 16.51% - 41.54% for all the sites, which indicates a high lability and biological availability of the metals (Kabala and Singh, 2001; Anegbe and Okuo 2013). The 0.00% mobility of Pb observed in the control site indicate that the metal is not bio-available for plant uptake in that site.

According to Wong *et al.* (2007), high mobility of metals in acidic sandy loam is due to low pH, low clay and low organic matter contents. This means that soil sample with low pH, low percentage of clay and low organic matter content retains fewer metals. Thus, more metals would be released into the soil solution.

Table.5: Mobility Factors (%) of Fe, Zn, Cu, Pb and Cd in the Soil Samples

Sites	**Fe**	**Zn**	**Cu**	**Pb**	**Cd**
A	37.11	42.47	30.02	31.06	2.85
B	35.45	44.60	41.15	48.96	4.52
C	24.43	38.62	26.35	21.97	10.57
D	39.19	32.05	16.49	28.54	58.19
Control	37.64	49.97	43.24	0.00	6.44
Average	34.77	41.54	31.45	26.10	16.51

Assessment of Metal Contamination

Contamination Factor (CF)

The level of contamination of soil by metal is expressed in terms of a contamination factor (CF) calculated as:

$$CF = \frac{\text{Cm Sample}}{\text{Cm Background}} \quad \text{----------(2)}$$

Cm Sample = metal concentration in Sample

Cm Background = metal concentration in background or control Sample. (Fonge *et al.*, 2016)

Where the contamination factor CF < 1 refers to low contamination; 1 ≤ CF < 3 means moderate contamination; 3 ≤ CF ≤ 6 indicates considerable contamination and CF > 6 indicates very high contamination.

Table.6: Contamination Factors of Fe, Zn, Cu, Pb and Cd in the Soil Samples

Sites	Fe	Cu	Zn	Pb	Cd
A	2.02	2.53	2.27	1.85	10.52
B	2.72	3.00	2.49	3.12	12.72
C	2.60	2.06	2.62	2.67	12.02
D	2.13	1.91	3.07	2.00	30.86

From the results of the contamination factors shown above, the soil samples may be classified as moderately contaminated with respect to Fe, Cu, Zn, Pb, and very highly contaminated with respect to Cd in site A and site C. The soil samples may be classified as moderately contaminated with respect to Fe, Zn, considerably contaminated with respect to Cu and Pb, and very highly contaminated with respect to Cd in site B. While in site D, the soil samples may be classified as moderately contaminated with respect to Fe, Cu and Pb, considerably contaminated with respect to Zn, and very highly contaminated with respect to Cd.

Index of geoaccumulation (I_{geo})

Index of geoaccumulation (I_{geo}) was used to evaluate the heavy metal pollution by comparing current concentrations with reference (control) values as reported by Bentum *et al.* (2011).

$$\mathbf{I_{geo}} = \text{Log}_2 \frac{Cn}{1.5\ Bn} \quad \text{----------(3)}$$

Table.7: Geoaccumulation Index scale

I_{geo} Value	**I_{geo} Class**	**Designation of sediment quality**
>5	6	Very highly polluted
4-5	5	Highly polluted
>3-4	4	Moderately to highly polluted
2-3	3	Moderately polluted
>1-2	2	Moderately to unpolluted
0-1	1	Unpolluted
0 <	0	Background concentration

Source: Singh *et al.* (2003).

Where $\mathbf{I_{geo}}$ is Index of geoaccumulation of the metal, C_n is the measured concentration of the element in the sample and B_n is the geochemical background value. As reported in table 7, this index consists of seven scales (0–6) ranging from background concentration to very highly polluted. The interpretation of the results was made based on the scale above in comparison with control sample.

Table.8: Geoaccumulation Index of Fe, Zn, Cu, Pb and Cd in the Soil Samples.

Sites	Fe	Cu	Zn	Pb	Cd
A	0.43	0.75	0.60	0.30	2.81
B	0.86	1.00	0.73	1.06	3.08
C	0.79	0.46	0.80	0.83	3.00
D	0.51	0.35	1.03	0.42	4.36

From the table above, Site A is unpolluted with Fe, Cu, Zn and Pb, and moderately polluted with Cd. Site B is unpolluted with Fe, Cu and Zn, moderately to unpolluted with Pb, and moderately to highly polluted with Cd. Site C is unpolluted with Fe, Cu, Zn and Pb, and moderately polluted with Cd. Site D is unpolluted with Fe, Cu and Pb, moderately to unpolluted with Zn, and highly polluted with Cd.

The Pollution Load Index (PLI)

Generally, pollution load index (PLI) as reported by Harikumar *et al.* (2009), is as follows:

$$\text{PLI} = \sqrt[n]{Cf1\ x\ Cf2\ x\ Cf3\ x\ Cf4\ \ldots\ldots\ldots\ldots\ldots\ldots Cfn} \quad \text{----------(4)}$$

Where, CF = contamination factor, n = number of metals

The PLI value of > 1 is polluted, whereas < 1 indicates no pollution (Harikumar *et al.*,2009).

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Table.9: Pollution Load Index (PLI) for the Soil Samples in the Workshop Sites

A	B	C	D
2.96	3.81	3.39	3.78

The pollution load index values as calculated for all the workshop sites were greater than 1 (table 9). This is an indication that all sites have metal concentrations which can cause pollution to the environment. The pollution load index value was highest in site B compare to other sites. Hence, site B may cause more pollution to the environment than others.

Correlation Analysis

All data were analyzed using the SPSS statistical package 21.0. Correlation is significant at the 0.05 level (2-tailed). Correlation is significant at the 0.01 level (2-tailed).

A negative correlation exist between pH and EC (*r*=-0.920), N (*r*=-0.923), OC (*r*=-0.956), OM (*r*=-0.955), and silt (*r*=-0.958) with a positive correlation on Mg (*r*=0.952), CEC (*r*=0.954) and sand (*r*=0.915) at 0.05 level of significance. EC negatively correlates Mg (*r*=-0.979) at 0.01 level of significance and clay (*r*=-0.885) at 0.05 level of significance. N positively correlates P (*r*=0.880), OC (*r*=0.977), OM (*r*=0.977) and silt (*r*=0.991) with a negative correlation on sand at 0.05 and 0.01 level of significance respectively. P positively correlates OC (*r*=0.902) and OM (*r*=0.903) at 0.05 level of significance. Ca positively correlates CEC (*r*=0.926) at 0.05 level of significance. Mg negatively correlates silt (*r*=-0.908) at 0.05 level of significance. Na positively correlates K (*r*=0.978) and CEC (*r*=0.951) at 0.01 and 0.05 level of significance respectively. K positively correlates CEC (*r*=0.888) at 0.05 level of significance. CEC negatively correlates OC (*r*=-0.901) and OM (*r*=-0.899) at 0.01 level of significance. OC positively correlates OM (*r*=1.000) and silt (*r*=0.975) with a negative correlation on sand (*r*=-0.972) at 0.01 level of significance. OM negatively correlates sand (*r*=-0.972) with a positive correlation on silt (*r*=0.975) at 0.01 level of significance. Sand negatively correlates silt (*r*=-0.985) at 0.01 level of significance.

Table.10: Correlation Coefficient between various Physico-chemical Properties

	pH	*EC*	*N*	*P*	*Ca*	*Mg*	*Na*	*K*	*CEC*	*O.C*	*O.M*	*SAND*	*SILT*	*CLAY*
pH	1.000													
EC	-0.920*	1.000												
N	-0.923*	0.838	1.000											
P	-0.738	0.548	0.880*	1.000										
Ca	0.803	-0.602	-0.684	-0.765	1.000									
Mg	0.952*	-0.979**	-0.854	-0.545	0.614	1.000								
Na	0.861	-0.778	-0.611	-0.460	0.860	0.809	1.000							
K	0.741	-0.647	-0.452	-0.358	0.852	0.671	0.978**	1.000						
CEC	0.954*	-0.843	-0.809	-0.705	0.926*	0.863	0.951*	0.888*	1.000					
O.C	-0.956*	0.825	0.977**	0.902*	-0.822	-0.847	-0.731	-0.604	-0.901*	1.000				
O.M	-0.955*	0.823	0.977**	0.903*	-0.821	-0.845	-0.728	-0.601	-0.899*	1.000**	1.000			
SAND	0.915*	-0.782	-0.988**	-0.875	0.682	0.828	0.605	0.446	0.797	-0.972**	-0.972**	1.000		
SILT	-0.958*	0.878	0.991**	0.824	-0.692	-0.908*	-0.681	-0.524	-0.847	0.975**	0.975**	-0.985**	1.000	
CLAY	0.670	-0.885*	-0.491	-0.151	0.381	0.821	0.700	0.626	0.649	-0.487	-0.485	0.402	-0.553	1.000

Table.11: Correlation Coefficient between Heavy Metals

	Fe	Cu	Zn	Pb	Cd
Fe	1.000				
Cu	0.838	1.000			
Zn	0.821	0.615	1.000		
Pb	0.969**	0.830	0.690	1.000	
Cd	0.453	0.264	0.862	0.316	1.000

Fe positively correlates Pb (*r*=0.969) at 0.01 level of significance.

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IV. CONCLUSION

The presence of heavy metals in the environment represents one of the most important environmental hazards. The results show that the soils of the studied areas are contaminated with these metals, especially given the high total concentrations which are gradually being released into the bioavailable forms and subsequently into solution which can lead to absorption into the plants system close to these workshops and cause biomagnification along the food chain.The levels of Cd and Pb obtained from this work were found to be less than the values reported by Imasuen and Omorogieva (2013) in a similar research in Benin City. The results of geoaccumulation index revealed that all the sites are polluted with respect to Cd. By and large, mechanic workshop owners should be given stringent rules to operate with full compliance in order to minimize the level of heavy metals introduced to the environment. Furthermore, remediation of the sites should be put into consideration to reduce the amount of total metal concentration in the soil to prevent the absorption of these metals by ground water and other essential plants that are grown close to these sites.

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Antibacterial Susceptibility and Resistance Pattern of Organisms Isolated from Rectal Swab of Chicken

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Abstract— *Resistance to antibiotics is a serious matter of concern for Public and threats to the successful treatment of miscrobial disease. The prevalence of some enterobacteriaceae such as Escherichia coli and Salmonella spp resistant to antimicrobial agents is increasing. This study is intended to determine susceptibility and resistance pattern of pathogenic isolates from the rectal swabs of chicken to 8 antibiotics.*

Hundred (100) rectal swabs of chickens were collected randomly from three different farms; a farm at Montan, Awotan, Apete and Apata all in Ibadan Metropolis of Oyo State. Isolation and identification of organisms were done using standard bacteriological techniques. Antimicrobial susceptibility test was performed following standard protocol.

A total of 93 bacterial isolates were obtained from the hundred (100) rectal swaps of chicken. The isolates Escherichia Coli, Staphylococcus aureus and Salmonella spp. and their percentage of prevalence are 54, 43 and 3. Antibiotic susceptibility tests carried out on the isolates showed that most of the isolates were resistant to ofloxacin, cloxacillin and Augumentin, while almost all the isolates in this study are sensitive to gentamicin. Erythromycin did not have any effect on any of the bacterial isolates.

Bacterial isolates obtained in the study area were multi drug resistant and this suggests that the chickens are important reservoir of antimicrobial resistant organism which is a major public health concern.

Keywords—Antimicrobial, Resistance, Bacterial isolates.

I. INTRODUCTION

The control of microorganisms is critical for the prevention and treatment of disease. Microorganisms grow on and within other organisms and microbial colonization can lead to disease, disability and death. Thus, the control on destruction of microorganisms residing within the bodies of humans and other animals is of great importance.

An antibacterial agent is a compound or substance that kills or slows down the growth of bacteria. The term is often synonymously used with the term (antibiotics), today however, with increased knowledge of the causative agents of various infectious diseases, antibiotics have come to denote a broader range of antimicrobial compounds including antifungal and other compounds.

In several countries, antibiotics such as penicillin, erythromycin and tetracycline are approved for the growth promotion as well as therapeutic use in animals, many of the antibiotics that are given to animals are closely related to medically important human drugs. Thus it is possible that the indiscriminate use of antibiotics of non-human use e.g. as addictive can lead to the development of resistance which could then be passed to human pathogens.

Food animals harbor food borne pathogens and act as source of contamination, which is important in the spread of *Salmonella* and *Escherichia Coli* in human, (Acha and Szyfres, 2001; Apajalahti *et al*., 2004 and White et al., 2001.). Staphylococcal infections are frequently treated with antibiotics and consequently resistance to it and or acquired resistance develop (Normand et al., 2000). The emergence of resistance in enteric pathogens to different antimicrobial agents in farming communities will adversely affect the availability of antimicrobial therapies available for use (Wagener *et al.,* 1999, Witte *et al*., 2000).

The emergence and widespread of antimicrobial resistant *Escherichia Coli* and *Salmonella* strains in chickens and humans may be associated with the indiscrimate use of antimicrobials both in animal and human treatments (Molla *et al*., 2003).Antibiotic resistance in these bacterial is often mediated by Plasmids, some of which are self-transmissible (Dufrenne *et al*., 2001; Adesiyun and Oni, 1989; Bebora *et al*, 1994; Robab and Azadeg, 2003; Kariuki *et al*., 2005), whereas others maybe co-transferred by conjugative Plasmids (Robab and Azadeh, 2003; Kariuki *et al.,* 2005).

However, the principle behind the development of resistance is that bacteria in the guts of humans and animals are subjected to different types, concentrations and frequencies of antimicrobial agents. Overtime, selective pressure selects resistant bacteria that have specific

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fingerprints for resistance to antimicrobial agents that have been used (Prescote *et al*., 2000; Troy *et al*., 2002).

Hence this work was carried out to isolate Pathogenic bacteria from the rectal swabs of chicken and to determine the antibiotics susceptibility and resistance pattern isolates to 8 antibiotics.

II. MATERIALS AND METHODS

Sample collection

Hudnred rectal swabs of chickens were collected randomly from three different farms; the farm at Monatan, Awotan, Apete and Apata all in Ibadan metropolis in Oyo State. The rectal swabs were all transferred into sterile peptone water in McCartney bottles and were transported into the laboratory immediately for microbiological analysis.

Preparation of media

The media were prepared according to the manufacturers' instruction. These media are as follows; Eosin Methylene Blue (EMB), Nutrient Agar (NA), Salmonella Shigella Agar (SSA) and Mannitol Salt Agar (MSA).

Isolation of microorganism

The rectal swabs collected were streaked on the surface of the agar-plates.The plates were then incubated at 37ºC for 18-24hours. The isolates on each plate were sub-cultured on the different agar plates to obtain pure cultures.

Each of the rectal swab collected was streaked on the surface of each agar plate.The plates were incubated at 37ºC for 18-24hours. The isolates on each plate were sub-cultured on the different agar plates to obtain pure cultures.

Identification of Bacterial Isolates

Conventional isolation techniques such as growth on selective media, gram staining and biochemical tests were utilized for the identification for the different bacterial isolates.

The isolates were also subjected to various biochemical tests to determine their probable identity. The result of each test was recorded and the probable identity of the isolate determined using Khoos and Schlenfer (1975) and Bergey's manual of systematic bacteriology (Cheesbrough, 2000).

Antibiotic Susceptibility Test: The bacterial isolates were tested for their susceptibility to antimicrobial agents using the agar disc-diffusion method of Piddock (1990).

All the isolates were screened for their antibiotic susceptibility to routinely used antibiotics such as Gentamicin, Ceftazidime, Cloxacillin, Ofloxacin, Cefunxine, Erythromycin, Cefixime and Augmentin,obtained from Abtek biologicals.

Gram positive and negative antibiotics discs were placed and pressed on already prepared sterile solidified Muller Hinton agar with a sterile forceps on seeded agar-plates to ensure complete contact with the agar.

The plates were incubated for 24hours at 37ºC for all the isolates. Clear zone of inhibition around the antibiotic disc on the plate were measured in millimeter. The clear zone indicated the relative susceptibility of the isolate of each antibiotic.

RESULTS

A total of 144 *Escherichia coli*,104 *Staphylocccus aureus* and 24 *Salmonella spp* were isolated from the three poultry farms (Table 1). The percentage prevalence of *Escherichia coli* isolated was 54, *Staphylocccus aureus* was 43, while *Salmonella spp* had the least prevalence of 3.

Table 2 shows the result of the percentage resistance of the bacterial isolates to each of the antibiotics. Most of the bacterial isolates were resistant to Ofloxacin, Cloxacillin and Augmentin. While almost all the Isolates in this study were sensitive to Gentamicin. Erythromycin did not have effect on any of the bacterial isolates. 60% of the isolates were resistant to at least 4 antibiotic agents. 70% of *Salmonella spp* were resistant to 5 antibiotics agents while 90% of *Escherichia coli* were resistant to at least 5 antibacterial agents.

Table.1: Isolates from Selected Commercial Poultry Farms in Ibadan Metropolis.

Source (FARM)	**Organisms Isolated/ Numbers of Organisms**		
	Staphylocccus aureus	*Escherichia coli*	*Salmonella spp*
A	26	38	4
B	28	34	8
C	22	35	6
D	28	37	6

Table.2: Percentage of Organisms Resistant to each Antibiotics Escherichia coli, Staphyloccus. aureus, Salmonella spp.

Antibiotics	**Organisms Isolated/ Numbers of Organisms**		
	Staphylocccus aureus	*Escherichiacoli*	*Salmonella spp*
Ceftazidime	75	65	8
Cloxacillin	95	90	84
Gentamicin	0	10	5
Ofloxacin	90	45	70
Cefurxine	90	90	84
Erythromycin	100	90	95
Cefixime	90	84	41
Augmentin	85	70	60

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III. DISCUSSION

The microbial isolates identified in this study are *Staphylococcus aureus, Escherichia coli* and *Samonella spp. Staphylococcus aureus and Escherichia coli* appeared to be the most prevalent bacterial species isolated. *Staphylococcus aureus* is known to be easily carried in the nasopharynx, throat, skin, cuts, boils, nails and as such can easily contribute to the normal microflora (Ekhaise *et al., 2008;* Yaqub *et al., 2012).*

Salmonella is the least bacterial isolate isolated from the chickens and this trend can be attributed to the good *Salmonella* control programme practiced by most farms as examination of food to detect *Salmonella* is routinely carried out for food safety and food-borne disease surveillance.

The lower rates of *Salmonella* was similar to those reported by Robert *et al., 2002; Yaqub et al*., 2012 and was in contrast to the report of Davies *et al., 1997.*

The result of this study revealed the presence of multi-drug resistant bacteria from chickens. All isolates showed high resistance to Cloxacillin while the isolates are sensitive to Gentamicin. The result of this study clearly identifies Gentaniclin as a good choice antibiotic for treatment of infection in this study. Also all *Escherichia coli* and *Salmonella sp.*isolated in this study were found to be resistant to 4 or more antibacterial agents tested in this study which is supported by earlier report of Overdevett *et al.,* 2011, Yaqub *et al.,* 2012 that drug resistance in enterobacteriacea has increased dramatically during the past decade.

The increase being attributed to increased prevalence of extended spectrum β-lactamase producing enterobacteriaceae.

In addition, these results provided evidence that there is an increased emergence of antibiotic resistance for commensal bacterial isolates, a finding which is in agreement with the earlier reports of Chukwendu *et al.,* 2008 who found increasingly emergence of antibiotic resistance phenotypes in both clinically relevant strains and normal commensal macrobiotic. The findings in the investigation emphasize the importance of studying multiple genera of bacteria from different animals as sources of human exposure to antibiotic resistance strains.

Therefore, not only that the chickens are at risk, poultry workers and consumers are equally exposed to serious hazards due to multi-drug resistance bacteria.

This calls for urgent intervention by regulatory agencies to limit the emergence and spread of these bacteria as well as prudent use of antibacterial agents among farmers in Nigeria.

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Callus Induction and Organogenesis from *Pueraria tuberosa* (Roxb. ex Willd.) DC

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Abstract—Pueraria tuberosa *(Roxb. ex Willd.) DC. is a perennial woody climber, commonly known as Indian Kudzu in English, Vidarikand in Hindi and Vidari in Sanskrit. The tubers are used in different systems of medicine viz. Ayurveda, Folk, Homoeopathy, Siddha, Tibetan and Unani. The present study aims to develop an effective protocol for optimum callus induction and organogenesis in Pueraria tuberosa. Callus cultures were first established by inoculating tender leaf explants in Murashige and Skoog's (MS) medium supplemented with different concentrations of 6-Benzylaminopurine (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg.L-1) with 1-Naphthaleneacetic acid (0.5 mg.L-1). Maximum callus induction and shoot organogenesis was observed in MS medium containing 1.5 mg.L-1 BAP with NAA 0.5mg.L-1. Fresh weight of the organogenic greenish granular hard callus was 4.70±0.10 gm. Shoot organogenesis was observed after 65 days of culture. Maximum shoot buds developed from callus on MS media augmented with 1.5 mg.L-1 BAP with 0.5 mg.L^{-1} NAA.*

Keywords—Pueraria tuberosa, Callus, Leaf explants, Organogenesis.

I. INTRODUCTION

Pueraria tuberosa belonging to family Fabaceae is distributed throughout tropical parts of India. It is a large climbing shrub with trifoliate leaves and bluish- purple flowers. Ayurvedic physicians use the tubers for the management of general weakness, fertility disorders and also as anti-ageing, Pandey *et al*., (1998). The tuberous roots act as a galactagogue, stimulant and emollient, Warrier *et al.*, (1995). Its tubers are rich in isoflavonoids and terpenes with puetubersoanol, and tuberosin , Khan *et al*., (1996). Puerarin, diadzein, genistein and genistin are the isoflavonoids present in callus cultures of *P.tuberosa*, Kamlesh *et al*., (2006). Increased isoflavonoid production was reported by elicitation effect in cell cultures of *P.tuberosa*, Shaily and Ramawat, (2008). Callus cultures of *Pueraria candollei* var. *mirifica* was capable of producing high level of isoflavones like daidzein and genistein consistently , Sudarat and Sanha, (2006). During past decades, interest in isoflavonoids have increased considerably. Isoflavonoids have effective role in treating cancer, postmenopausal symptoms and cardiovascular diseases, Dixon and Ferreira, (2002) ; Nestel P, (2004); Duncun, *et al*., (2003); Vitrac *et al*., (2004).

There is a growing demand for plant based drugs due to the presence of biologically active compounds and it is therefore necessary to select, multiply and characterize important medicinal plants for commercial use. Further, *in vitro* protocols offer scope for multiplication and genetic enhancement of desirable genotypes and *in vitro* plant cell cultures have been considered to be an important source of secondary metabolites from the plants, Manisha *et al*., (2012).

II. MATERIALS AND METHODS

2.1. Plant sample and experiment designing

Young, tender leaves collected from three months old vegetatively propagated plants were first washed in running tap water for 10 minutes and then soaked in soap water for 3 minutes. The leaves were cut into small fragments and then immersed in cefotaxime (200 mg.L-1), tetracycline (200 mg.L-1) and bavistin (15 g.L-1) for 5 minutes. The bavistin treated explants were then washed with double distilled sterile water for five times. These explants were then sterilized by mercuric chloride solution for 3 minutes. After several distilled water wash, 4-5 explants were inoculated into MS (Murashige and Skoog, 1962) media supplemented with 3% (w/v) sucrose, 0.2 % (w/v) clerigel and1ml lactic acid taken in petridish. The pH of the media was adjusted to 5.8 after the addition of various concentrations of BAP and NAA. The culture medium was autoclaved at 121°C, 15 psi pressure for 20 minutes. The media were incubated at 25 ± 2 °C under 16 hour photoperiod at a relative humidity of 55 percent with a light intensity of 3000 lux.

Leaf explants were inoculated into media supplemented with various combinations of BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg.L-1) with NAA (0.5 mg.L-1). Percentage of callus induction, fresh weight and dry weight of callus, colour and texture of callus and the number of days for callus induction were the different parameters observed and recorded. Subcultures were routinely carried out every 10 days interval into fresh media. Growth of callus on different media compositions after 50 days of inoculation were measured in terms of fresh weight and dry weight. Dry weight of the callus was measured by drying at 60ºC in a hot air oven to a constant weight.

2.2. Statistical Analysis

Experiments were conducted with three replications, having 30 samples each. The effect of various treatments on different growth parameters was measured quantitatively and statistically tested using analysis of variance (ANOVA) using "R-statistics package" version 11.0. The significance of the mean values of various treatments was assessed by Duncan's New Multiple Range Test (DMRT) at $p < 0.05$.

III. RESULTS

The combined effect of BAP and NAA on callus induction was studied by culturing leaf explants on MS medium supplemented with various concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg.L-1) with 0.5 mg.L-1 NAA (Table 1). MS basal media without plant growth regulators were taken as control. The explants failed to establish callus on control media. Various types of callus such as compact, friable and granular were observed in different colours as green, creamy yellow and white. Decrease in percent of response (60%) was observed at lower concentration of BAP (0.5 mg.L-1) with 0.5 mg.L-1NAA. Organogenesis was not recorded from the white friable callus of this media. The fresh weight and dry weight of the callus of this particular media composition were found to be the lowest among other treatments (Table 2). Creamy yellow friable callus (Fig 1A) initiated within 12 days on media with 1 mg.L-1BAP and 0.5 mg.L-1 NAA and the fresh weight of callus harvested from this media after fifty days was 1.60±0.15 gm. Only 2 shoots were differentiated from this callus after 65 days of culture.

It was observed that the callus growth was best in MS media augmented with 1.5 mg.L-1 BAP with 0.5 mg.L-1 NAA. This particular media composition resulted in 84% callus induction within 7days of inoculation (Table 1). After 50 days of culture, the fresh weight and dry weight of callus at this optimum treatment was 4.70±0.10 and 2.43±0.15 gm respectively. The green granular hard callus (Fig 1 C) resulted after 50 days of culture showed initiation of buds. These buds developed into 5-6 shoots after 65 days of culture. At higher concentrations of 2 mg.L-1 BAP with NAA 0.5 mg.L-1, the percentage of callus induction reduced (80.3%) and the number of days for callus induction increased to14 days. The green buds which originated from the dark compact callus (Fig 1 E) of this medium developed into 3-4 shoots after 65 days of culture. During subculture after 50 days, the dark brown regions of this callus were removed to reduce contamination. The shoots elongated to a height of 3-4 cm after 85 days of culture (Fig 1F). Frequent sub culturing was done to enhance the survival rate of the callus.

IV. DISCUSSIONS

Auxin and cytokinin balance is an important factor in the control of cell division in tissue culture. In the present study, different concentrations of auxin and cytokinin influenced callus production from leaf explants. Highest percent of organogenic green granular hard callus was obtained on leaf explants grown on the medium containing 1.5 mg.L-1BAP and 0.5 mg.L-1NAA (Table 1 and 2). Our results are in line with the interaction effect of NAA and BAP on callus formation of *Alstroemeria* cv. fuego, Seyyed *et al*., (2013). The leaf explants produced callus on half strength MS media supplemented with BAP and 2,4-D, Reddy *et al.*, (2011). Maximum induction of callus was observed on a combination of 2.0 mg.L-1 2,4-D and 0.5 mg.L-1 NAA from leaf explants, Manokesh *et.al*., (2014). *Tectona grandis* produced compact and fibrous callus on MS media with 0.5 NAA and 1.5mg.L-1 BAP after two weeks of culture, Egodawatta *et al*., (2014). The colour and nature of callus from *Tinospora formanii* varied with different concentration of BAP and at higher concentration of BAP with NAA, the number of days taken for callus initiation increased and the percentage of response decreased, Sheema *et al*., (2017) which is in conformity with the results of the present study.

Callus is an important source for indirect plant organogenesis and embryogenesis, two most striking processes in plant micropropagation, Te-chato *et al*., (2006). It is evident from the results that, among the growth regulators tested, 1.5 mg.L-1BAP with 0.5 mg.L-1NAA induced maximum frequency of shoot regeneration with maximum number of leaves. These results are in conformity with those of Manokesh *et al*., (2014). Genotypes, explant source, physiological status of the donor plants, the culture medium, and the interactions between them are the factors that influence responses like callus induction and regeneration capacity, Ozgen *et al*., (1996). In the present experiment, high concentrations of cytokinin (1.5 and 2

mg.L-1) along with very low concentration of auxin promoted shoot regeneration from callus. Our findings are in line with those of Ahmad, (1996) and Ahmad and Spoor, (1999). High fresh weight, callus percentage and effective embryogenesis were observed from leaf explants of Sainfoin (*Onobrychis sativa*) on MS medium supplemented with 2.5 mg.L-1BAP and 0.5 mg.L-1 NAA, Sedegh *et al*., (2012).

Table.1: Effect of BAP and NAA on callus induction from leaf explants of P. tuberosa

Treatments	MS+PGR (mg.L^{-})		% of explants showing Callus induction	Number of days for callus induction
	BAP	NAA		
T_0	0.0	0.0	0.00^{g}	0.00^{g}
T_1	0.5	0.5	60.47 ± 1.29^{f}	16.20 ± 0.30^{c}
T_2	1.0	0.5	65.87 ± 0.61^{e}	12.50 ± 0.10^{e}
T_3	1.5	0.5	84.26 ± 0.21^{a}	07.06 ± 0.06^{f}
T_4	2.0	0.5	80.33 ± 0.15^{b}	14.41 ± 0.10^{d}
T_5	2.5	0.5	72.90 ± 0.20^{c}	18.35 ± 0.19^{b}
T_6	3.0	0.5	68.23 ± 0.25^{d}	21.30 ± 0.26^{a}

Note: Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P>0.05$).

Table.2: Weight of callus and types of callus on different BAP and NAA combinations of MS

MS+PGR (mg.L-1)		Types of callus	Fresh weight (gm)	Dry weight (gm)
BAP	NAA			
0.5	0.5	White friable	0.46±0.05^{d}	0.24±0.02^{d}
1.0	0.5	Creamy yellow friable	1.60±0.15^{c}	0.71±0.02^{c}
1.5	0.5	Green granular hard	4.70±0.10^{a}	2.43±0.15^{a}
2	0.5	Dark compact	3.33±0.21^{b}	1.33±0.15^{b}

Note: Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P>0.05$).

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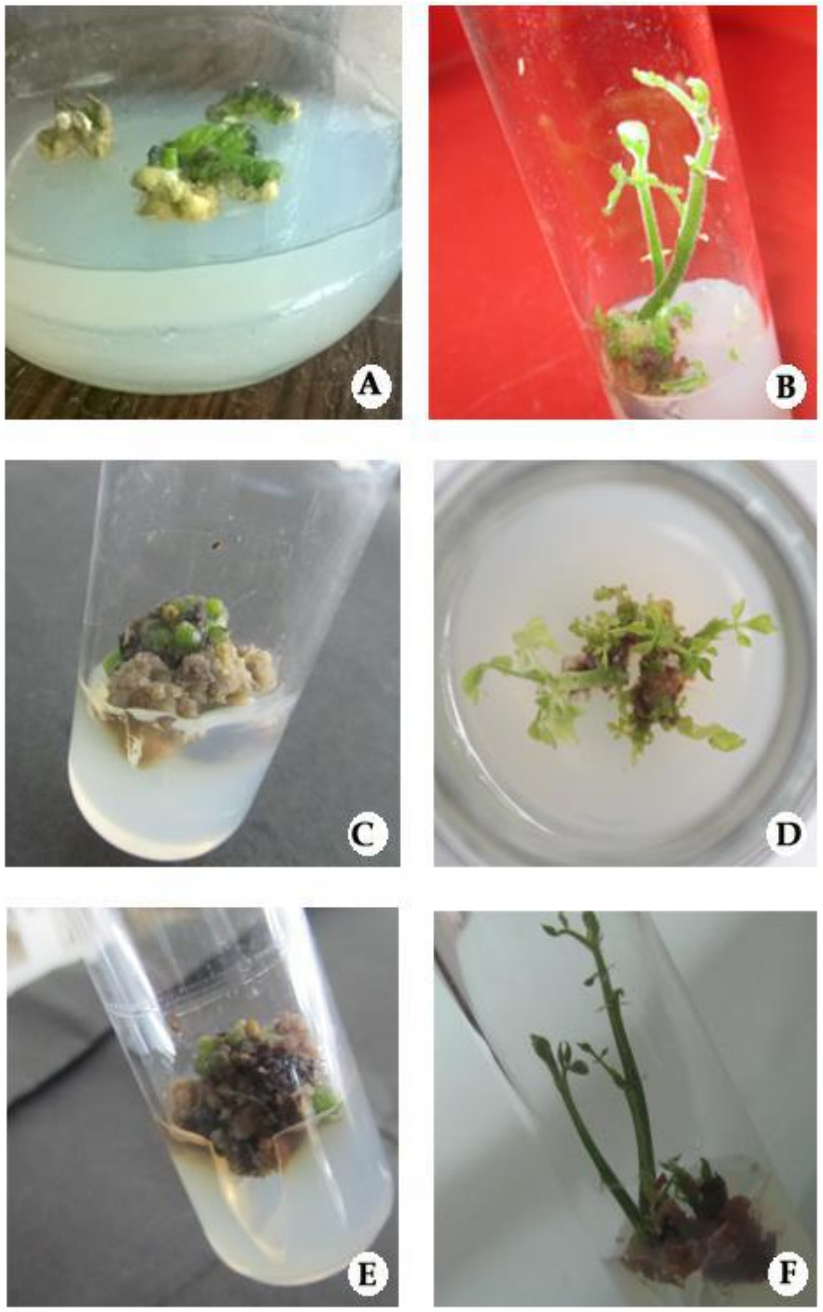

Figure 1. Callus development Organogenesis from leaf explants of *Puerarta tuberosa*.

A. Callus proliferation on MS medium with 1 mg.L- 1 BAP + 0.5 mg.L-1 NAA;

B. Shoot development from callus on MS with 1 mg.L- 1 BAP + 0.5 mg.L-1 NAA;

C. Green granular hard callus; D. Organogenesis from callus on MS with 1.5 mg.L- 1 BAP + 0.5 mg.L-1 NAA;

E. Dark compact callus; F. Organogenesis from callus on MS with 2 mg.L- 1 BAP + 0.5 mg.L-1 NAA.

V. CONCLUSION

The success of *in vitro* protocols depends on factors like type of explants and plant growth regulators. In the present study, the effect of different concentrations of BAP with NAA on callus formation and organogenesis of *P. tuberosa* was evaluated using leaf explants. We have

developed a simple and robust procedure to regenerate this important taxon with the use of BAP and NAA. The present protocol can also be applied for the mass multiplication and secondary metabolite production from the callus without harvesting the whole plant.

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Economic Analysis of Market Performance of Fresh Fish in Lagos State, Nigeria

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Abstract— *This study analysed the market performance of fresh fish marketing in Lagos state, Nigeria. It critically focused on ascertaining the market structure, determining the profitability of fish marketing and determining the marketing efficiency of fresh fish marketing in the study area. Multistage sampling procedure was used to sample 80 fresh fish marketers from Lagos state. The data collected for the study were analysed using Gini coefficient, budgetary technique and shepherd efficiency model. The study revealed that there was inequality in the income distribution among the fresh fish marketers with Gini coefficient of 0.78, it further shows that fresh fish marketing is profitable with gross margin of #27,101.36 and that fish marketing activities among fish marketers is highly efficient (517.5%). Thus, government should help in the provision of a soft loan to the marketers so as to promote fresh fish marketing being a profitable and efficient business.*

Keyword— Market Performance, Market Structure, Profitability, Market Efficiency.

I. INTRODUCTION

Fish marketing is a primordial economic activity in Nigeria (Agbebi, 2010). Its activities cover both the coastal and inland waterways and it was of tremendous economic value to the pre-colonial Nigerians (Ehinmore, 2007). Although, fresh fish were said to be marketed mostly in short distance areas owing to the perishable nature attached to it.

Fish is a major source of animal protein and an essential food item in the diet of many Nigerians, being relatively cheaper than meat. Accordingly, agricultural production and fish marketing must develop hand in hand because they are partners in a progressive system (Iliyasu, Onu, Midau and Fintan, 2011).

Assessment of how well the process of marketing is carried out, and according to Awol (2010) performance is how successfully its aims are accomplished. Is produce assembled and delivered on time and without wastage? Is it well packed and presented attractively? Is its quality reliable and are contract kept? Is the consumption of the products increasing and sales in competitive market expanding? There are such many practical indications of how well a certain marketing system is operating.

Also, the form in which markets are structure is almost assumed to rigidly determine each firm's conduct (output decisions and pricing behaviour), which yields an industry's overall performance (e.g. its efficiency and profitability) (Umoinyang, 2014).

Meanwhile, to be more profitable, fish trade requires every activity that increases sales revenue and as well decreasing the costs of marketing, thus profitability of fish is the measure of fish profit against its power to earn profit (Monica, 2014).

An efficient marketing system ensures that goods which are seasonal will be available all year round, with little variation in prices, which can be attributed to cost of marketing functions like storage, processing, transportation(Nwaru, Nwosu and Agummuo,2011).Thus, marketing efficiency increases with continued transitions and specialized functions like wholesale and retail (Enete, 2008). This supported the claim ofAdegeye and Dittoh (1985) that the general-purpose of marketing efficiency is to provide goods to consumers in the required form at the required time and place with the lowest possible marketing costs consistent with the interests of the producers.

An extensive literature survey has been carried out on economic analysis of fresh fish marketing performance with empirical evidence from many studies and special attention paid to the market structure, profitability and factors influencing it and the efficiency of fish marketing. Evidence from Adeleke and Afolabi, (2012) and Edward and Madugu, (2011)have established the profitability and marketing efficiency of fresh fish marketing. Also, Bukenya, Theodora, Twinamasiko and Molnar. (2012) and Abdal and Eglal, (2010), in their study, assert that fish marketing profitability is eminent with high market performance. However, the scholars' works on the, performance of fresh fish marketing in Nigeria are still limited. Thus, this study seeks to explore the performance of fresh fish marketing by ascertaining the market structure, determine the profitability, efficiency of fresh fish and

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estimate factors influencing the income of fresh fish marketers in the study area.

II. METHODOLOGY

The Study Area, Sampling Technique and Data Collection

This study was carried out in Lagos State, located within the southwest Nigeria. Farming is part of the notable occupation of the people most especially along the coast as well as other related activities.

Multistage sampling procedure was used for this study which involves purposive selection of Lagos State in the first stage being one of the notable fishing states in Nigeria, purposive selection of two Local Government Areas (LGA's) namely Ibeju-Lekki and Ikorodu LGA's because of the prevalence of fresh fish marketers in the area. In the third stage, two communities were selected using purposive sampling technique. The selected communities are Orimedu and Otto in Ibeju-lekki and Ijede and Ipakodo in Ikorodu local government respectively. In the last stage, ten fresh fish marketers were selected from each of the four communities using snowball sampling technique. Thus, a total of 80marketers/respondents were used for this study. Structured questionnaire were administered and responses were analyzed using descriptive statistics, Gini coefficient, budgetary techniques and shepherd index.

Analytical techniques

The data obtained from the respondents were subjected to descriptive and inferential statistics. Inferential statistics such as Gini coefficient was used to ascertain the market structure of fresh fish marketing, budgetary technique was employed to ascertain the profitability of fresh fish marketing and shepherd index was used to determine the marketing efficiency of fresh fish marketing in the study area.

Model Specification

Gini Coefficient: The Gini coefficient mathematically, it is explicitly represented by

$GC = 1 - \sum[X_{t-1} * Y_{t-1}]$

Where:

N = is the number of elements (observations)

X = Proportion of Fresh fish seller

X = Proportion of fish seller is given as $X = \frac{\text{No of fish seller in a market}}{\text{overall No of fish Marketers under study}}$

$\sigma X (X_{t-1})$ = Cumulative Proportion of fish sellers (X)

Y = Proportion of total sales by Fresh fish marketer

Y = Proportion of total sales is given as: $\frac{\text{total sales of fish in a market}}{\text{overall total sales of fish in all the Markets under study}}$

$\sigma Y (Y_{t-1})$= Cumulative Proportion of total sales (Y)

Budgetary Technique: The budgetary technique encompasses the analyses of the gross margin which involves the cost and return analysis of fish marketing in the study area. The gross margin formula is explicitly stated below:

The budgetary technique involves the cost and return analysis of fish marketing in the study area. It is explicitly stated as:

$G.M = \sum(\mathbf{P_{ij}Q_{ij} - r_{ij}X_{ij}})$

$\mathbf{P_{ij}}$= Price of fish in i^{th} for j^{th} respondent.

$\mathbf{Q_{ij}}$= Quantity of fish in i^{th} for j^{th} respondent.

$\mathbf{r_{ij}}$= Price of Variable Input in i^{th} for j^{th} respondent.

$\mathbf{X_{ij}}$= Quantity of Variable Input in i^{th} for j^{th} respondent.

The profitability and efficiency ratio was calculated as follows:

Profitability ratio is given as: $\frac{\pi}{TVC}$

Efficiency ratio is given as: $\frac{TR}{TVC}$

a. $\frac{TR}{TVC} > 0$ = It is operational efficiency
b. $\frac{TR}{TVC} < 0$ = It is operational inefficiency
c. $\frac{\pi}{TVC} > 0$ = It is profitable
d. $\frac{\pi}{TVC} < 0$ = It is not profitable

Thus, the values in the Profitability and Efficiency ratio were computed in the marketing of fish in the study area.

Where:

$\prod$ = Profit

TR = Total Revenue

TVC = Total Variable Cost

Multiple Regression Model

Multiple regression is one of the analytical tools that are used to determine the effect(s) of one or more variables on another. The marketing function postulated for fresh fish trader's annual income in the study area is implicitly presented by $Y = f(X_1, X_2, X_3, X_4, X_5, X_6, u_i)$ as shown below:

Where Y= Annual Income from Fish Marketing (₦)

X_1= Age of respondents (years)

X_2= Fish Marketing experience (years)

X_3= Number of year spent in school (year)

X_4= Cost of purchase (₦)

X_5= Cost of transportation (₦)

X_6= Membership of association (Yes =1, No = 0)

X_7= Price per kg of fish (₦)

X_8= Quantity of Fish Sold (Kg)

Shepherd efficiency models: The Shepherd efficiency models developed by Shepherd, (1965) and used by Massoud and Gowda, (2012) was used to analyze the marketing efficiency of fish marketing by estimating as follows:

Marketing cost: The total marketing cost was determined by the following formula:

$TC = C_P + \sum Mci$ (1)

Where:

i = 1

TC = Total Cost of Marketing

C_p = Producer cost of marketing

Mc_i = Marketing cost by the ith trader

Marketing margin: The absolute margins of both the processed and unprocessed fish retailers were determined as follows:

$AM = Psa – (Pba + Mc)$ (2)

AM = Absolute Margin

Psa =Selling price

Pba = Buying price

Mc = Marketing cost

Producer's share in the consumer price: The producer' share in the consumer price was calculated by the following indicator:

$$Ps = \frac{Pp}{Pr}\ x\ 100$$

Ps = Producer' share in the consumer price

Pp = Producer' price

Pr = Retail price or final consumer price

Marketing efficiency with Shepherd Index proposed to evaluate the marketing efficiency of fish marketing activities. It is given by:

$$ME = \frac{Pr}{TC+AM} \quad (4)$$

Pr = Retail price or final consumer price

TC = Total Cost of Marketing

AM = Absolute Margin

$$ME = \frac{Value\ added\ by\ Marketing}{Marketing\ cost\ or\ cost\ of\ markeying\ services}\ x\ 100$$

Pr = Retail price or final consumer price

TC = Total Cost of Marketing

AM = Absolute Margin

III. RESULTS AND DISCUSSION

Market Structure

The Gini coefficient of 0.78 was revealed (Table 1), indicated high level of inequality distribution of sales income for fresh fish market in the study area. This was in line with Dillion and Hardaker (1993) in their finding that the value of Gini coefficient greater than 0.35 is high indicating inequitable distribution of sales income/sales.

This was evidenced with the total income generated from total sales at ₦17,914,000while 82.5% and the remaining 17.5% of the total sales contributed ₦10,964,000and ₦6,950,000respectively. This deduces that *o*nly 17.5% of the respondents played an active role in the market while majority (82.5%) of the respondents have low funding for their marketing activities in the study area.

Table.1: Computation of Gini Coefficient for Fresh Fish Market Structure in the Study Area

Income	**No of sellers**	**%**	**Cum %**	**Proportion of sellers (X)**	**Cumulative proportion of sellers**	**Total sales**	**Cum Total Sales**	**Proportion of total sales (Y)**	**Cumulative proportion of total sales**	**XY**
<150,000	39	48.75	48.75	0.49	0.49	5,133,000	5,133,000	0.29	0.29	0.141375
150,001-250,000	17	21.25	70	0.21	0.7	2,761,000	7,894,000	0.15	0.44	0.031875
250,001-350,000	10	12.50	82.5	0.13	0.83	3,070,000	10,964,000	0.17	0.61	0.0215
350,001-450,000	5	6.25	88.75	0.06	0.89	2,020,000	12,984,000	0.11	0.72	0.006875
450,001-550,000	5	6.25	95	0.06	0.95	2,450,000	15,434,000	0.14	0.86	0.00875
550,001-650,000	3	3.75	98.75	0.04	0.99	1,780,000	17,214,000	0.1	0.96	0.00375
>650,000	1	1.25	**100**	0.01	1	700,000	**17,914,000**	0.04	1	0.0005
Total	**80**	**100**				**17,914,000**				0.214625

Source: Analysis of Field Survey 2017

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Using the formula, Gini-Coefficient (GC) = 1 - ∑ XY

Fresh Fish Market Structure: GC_F = 1 - 0.214625

= 0.785375

Profitability Analysis

The measure of the cost and return analysis of the marketers in the study area was carried out using the budgetary technique. The result in Table 2 showed that the cost of purchase gulped up to 91.97% of the total variable cost for the fresh fish marketers. Also, the table revealed that a marketer earned average revenue of ₦223,925.00 but incurred a total variable cost of ₦196,466.73 over the same period. This indicates that an average marketer earned ₦27,458.28 as gross margin per year suggesting that fresh fish marketing is a profitable venture in the study area. This is evident in the study of Adeleke and Afolabi, (2012) which indicates that fresh fish marketing is a profitable venture. The result of the profitability ratio or the return on investment (ROI) was 0.14 indicating that for every ₦1.00 spent on fresh fish marketing14kobo is gained by the marketers.

Table.3: Computation of cost and return analysis of the fresh fish marketers

Item	**Cost (₦)**	**% TVC**
Cost of purchase	14,455,638	91.97%
Transportation	5,76,500	3.67%
Labour	8,800	0.05%
Bowl	186,000	1.18%
Bracket	0	0.00%
Wire gauze	0	0.00%
Knife	33,000	2.09%
Salting	0	0.00%
Association fee	3,150	0.02%
Storage	154,500	0.98%
Rent (Space and others)	272,250	1.73%
Security	16,000	0.10%
Utility	11,500	0.07%
Total TVC	**15,717,338**	**100%**
Average TVC	**196,466.73**	
Total Revenue	**17,914,000**	
Gross Margin (TR-TVC)	**2,196,662**	
Average GM	**27,458.28**	
Profitability ratio	0.14	

Source: Analysis of Field Survey 2017

Income Determinants of Fresh Fish Marketing

The estimate of the factors influencing the income of the marketers in the study area was carried out using the multiple regression analysis. Three functional forms of regression analysis (Table 3) were undertaken to determine the model that best fits the data with respect to coefficient of determination, F statistics and the t-value of the marketers.

The regression results show that, linear functional form had the highest R^2 (i.e. coefficient of multiple determination) of 58.1% and was chosen as the lead equation. The regression results show that, the regressors combined are responsible for 58.1% of the variation in income due to these factors incorporated in the model. The remaining 41.9% are caused by other factors not included in the model. The entire equation measured by the F-ratio (11.981) is significant at 5% probability level. Regression result shows that, the cost of purchase (X_4) is positively significant at 1% while the number of years spent in school (X_3) and price of fish (X_7) are also significant and positive at 5% and 1% levels respectively. The implication of this is that a unit increase in cost of purchase, price of fish and number of years spent in school would lead to increase in the annual income of marketers. The positive coefficient of number of years spent in school also suggests that literate marketers may be more enterprising than their illiterate counterparts probably because of their ability to use market information to an advantage which gave credence to the findings of Adeleke and Afolabi, (2012)

Also, the marketing experience (X_2), cost of transportation (X_5) and membership of association (X_6) positively and significantly influenced the income of fresh fish marketers in the study area, which indicates that an increase in these variables resulted in an increase in income of fish marketers. This implies that marketing experience, cost of transportation and membership of association are significant determinants of the income in the study area.

However, the age of the marketers negatively affected the income marketers with t-value of -1.065, which indicates that increase in age resulted in decrease in income of fish marketers. This might be due to the strength required in the marketing of fish. This is supported by the findings of Bassey, Okon, Ibok and Umoh, (2013) that age negatively but significantly influenced the profit of fish marketers.

Also, quantity of fish sold negatively affected the income of the marketers. This is an indication that an increase in the quantity marketed of fresh fish reduces income. This might be probably because the more the quantity of fresh fish in market, the less the marketing price probably because of the perishable nature of fresh fish.

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Table.3: Computation of multiple regression analysis of the marketers

	Linear	**Semi-Log**	**Double-Log**
Variable	Coefficient (t-value in parenthesis)	Coefficient (t-value in parenthesis)	Coefficient (t-value in parenthesis)
Constant	-351119.373 (-1.450)	10.520 (12.790)	-.404 (-.089)
Age (X1)	-3231.459 (-1.065)	-.014 (-1.359)	-.751 (-1.747)
Marketing Experience (X2)	4441.984 (.749)	.004 (.214)	.079 (.653)
No of YearsSpent in School (X3)	13985.915** (2.602)	.025 (1.368)	.062 (.545)
Cost of Purchase (X4)	.633*** (8.381)	1.67E-006 (6.496) ***	.326*** (5.225)
Cost of Transportation (X5)	.158 (.267)	9.64E-007 (.481)	.010 (.188)
Membership of Association (X6)	22244.821 (.454)	.086 (.515)	.003 (.011)
Price of fish (X7)	617.897*** (3.158)	.002*** (3.267)	1.832*** (2.953)
Quantity of fish sold (X8)	-64.057 (-1.642)	-5.78E-005 (-.436)	-.162** (-2.464)
R^2	0.581	0.481	0.386
SE	137891.60	0.46487	0.51134
F	11.981	7.993	5.261

Source: Analysis of Field Survey 2017 ****Significantat1% **significant at 5% and*significant at10%*

Marketing Efficiency of Fresh Fish

Results in Table 4 show that, efficiency figure is far greater than 100% (i.e. 517.0%) whereas an efficiency ratio of 100% (or 1.0) indicative of efficient trading/marketing activities. Thus, fish marketing activities among fresh fish marketers is highly efficient. The result also, indicate that an increase in the cost of performing marketing service by 100 percent will give a more than proportionate increase of 417.0 percent in the level of satisfaction derived from a kilogram of fresh fish sold in the market.

Table.4: Computation of marketing efficiency of fresh fish marketing

Efficiency Variables	**Fresh Fish**
Total Cost of Marketing	
Cost of Produce	14,455,638
Transportation	576,500
Labour	8,800
Bowl	186,000
Bracket	0
Wire gauze	0
Knife	33,000
Salting	0
Association fee	3,150
Storage	154,500
Rent	272,250
Security	16,000
Utility	11,500
Marketing cost by ith trader	1,261,700
Total Cost of Marketing	**15,531,338**
Absolute margin	
Selling Price (Ps)	17,914,000
Total cost of marketing (Mc)	15,531,338
Buying Price (Pb)	14,455,638
	-12,072,976
Producer Share	
Price of buying fish	14,455,638
Price of selling fish	17,914,000
Producer share	0.80
Percentage of Producer share	80%
Marketing Efficiency	
ME	5.17
ME%	517.0%

Source: Analysis of Field Survey 2017

IV. CONCLUSION

The study showed that more female (81.25%) and more (90%) youth within the age bracket of 21-50 years with majority (57.5%) of married are involved in fresh fish marketing. There was an uneven distribution of income in the market with majority (82.5%) of fish marketers having low funding for their marketing activities with very high (0.78) Gini coefficient value. It further revealed that an average gross margin of #27,458.28 implying that fresh fish marketing was profitable. The marketing efficiency of 571.14% was revealed indicating a high efficiency. It is recommended that government should encourage marketers in the business by giving them soft loans.

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Productivity of Maize Varieties intercropped with Cassava in Lafia and Makurdi Locations, Southern Guinea Savanna, Nigeria

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Abstract— *Two experiments were conducted from 2015 to 2016 at the Teaching and Research Farm of the Federal University of Agriculture, Makurdi [Latitude 07º 45' - 07º 50' N, Longtitude 08º 45'- 08º 50' E, elevation 98 m] in Benue State and the Research and Teaching Farm of the College of Agriculture, Lafia (Latitude 08.33N and Longitude 08.32E) in Nasarawa State, all located in Southern Guinea Savannah of Nigeria. The experiments sought to determine the performance of maize varieties when intercropped with cassava.The experiment was laid out as split-plot in randomized complete block design (RCBD) with three replications. The main plot treatment comprised of two cropping systems [sole cropping (maize, cassava) and row intercropping (maize + cassava)] while the sub-plot treatment was 3 maize varieties [Quality Protein Maize (QPM), Suwan 1-1 and the Local]. Each sub plot consisted of 5 ridges spaced 1m apart and 4m long and the net plot was the three middle ridges, 3m long. Intercropping severely depressed plant height at harvest, leaf area index at harvest, cob circumference, cob length, number of rows per cob, number of seeds per row, cob weight, grain yield and 100-ssed weight in Lafi and Makurdi. The highest grain yield of maize was produced when QPM was planted as a sole crop in Lafia (2.95t/ha) and Makurdi (2.99t/ha). However, values obtained from LEC and LER showed intercrop advantage. Similarly, intercropping decreased the growth and yield (plant height at harvest, root circumference, root length, number of marketable roots per plant, number of unmarketable roots per plant and root weight) of cassava in both locations. Intercropping cassava with Local maize produced the highest root weight in Lafia (8.50t/ha) and Makurdi (9.02t/ha) among the treatments intercropped. All LER and LEC values were above 1.0 and 0.25 respectively in both locations. Values obtained for competitive ratio showed that maize was mnore competitive than cassava probably due to its height advantage.*

Keywords— Maize Varieties, Lafia and Makurdi, RCBD.

I. INTRODUCTION

Maize (*Zea mays* L.) is an important annual cereal plant cultivated worldwide and it belongs to the (Hugar and Palled, 2008). It is extensively used in Nigeria. Maize is ranked second to wheat among the world's cereal crops in terms of total production, use and price relative to other cereals. It is used to produce a large variety of food and non-food products (Raemaekers, 2001). The total world production of maize is estimated at about 1,016,736,092 tons, with the United States, China, and Brazil being the highest world producers (FAOSTAT, 2013). In Africa, maize plays a valuable role in human diet, animal ration and as raw material for agro-based industries. Africa is a minor producer of maize accounting for only about 7% of global maize production (FARA, 2009). The largest producer of maize in Africa is Nigeria, accounting for about 14% of Africa's total production and about 1% of the total world production (FAOSTAT, 2013).

Cassava is a perennial woody shrub that generally grows from one to three meters in height (Onwene, 1978; Hershey, 2005). It is grown by poor resource farmers, many of them women, as main source for food security and income generation (FAO, 2002).The total world production of cassava is about 276.7 million tonnes FAOSTSAT (2014). Africa accounts for 58% of the total world production while Nigeria accounts for 34.2% of Africa's total production and 20% of the total world production. Nigeria produces 54 million tonnes of the total world production making it the world's largest producer. Other large scale producers of cassava in the world include Democratic Republic of Congo, Ghana Tanzania and Mozambique (FAOSTAT, 2014).

Intercropping is a very common practice in the Southern Guinea Savannah ecological zone of Nigeria. It is the growing of two or more crop species simultaneously on the

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same field (Andrews and Kassam, 1976).The success of any intercropping system depends mainly on selection of component crops (Vishwanatha *et al.*, 2011). When two or more plants with different rooting systems, a different pattern of water and nutrient demand and a different above ground habit are planted together, water, nutrient and sunlight are used more effectively. One of the most important reasons to grow two or more crops together is the increase in productivity per unit of land (Preston, 2003).Information on the yield advantage and competitive abilities of maize/cassava intercropping systems in Southern Guinea Savanna of Nigeria is lacking. This study reported here sought to bridge this knowledge gap. The objectives of the study were:

i. To evaluate the suitability of three maize varieties for intercropping with cassava in Lafia and Makurdi.
ii. To determine the productivity of the maize/cassava intercropping in Lafia and Makurdi.

II. MATERIAL AND METHODS

Experimental Locations

Two experiments were conducted from 2015 to 2016 at the Teaching and Research Farm of the Federal University of Agriculture, Makurdi [Latitude 07º 45′ - 07º 50′ N, Longtitude 08º 45′- 08º 50′ E, elevation 98 m] in Benue State and the Research and Teaching Farm of the College of Agriculture, Lafia (Latitude 08.33N and Longitude 08.32E) in Nasarawa State, all located in Southern Guinea Savannah of Nigeria. The experiments sought to determine the performance of maize varieties when intercropped with cassava. Thirty core samples of soil were collected from different parts of the field from 0-30cm and bulked into a composite sample and used for the determination of physical and chemical properties of the soil (see Table 1) before planting. Both the physical and chemical analyses were done in the Soil Science Laboratory of the University of Agriculture, Makurdi.

Table.1: Physical and chemical properties of the surface soil (0-15 cm) at the experimental sites in Makurdi and Ibi in 2015

Parameters	**Makurdi**	**Lafia**
Sand (%)	72.20	73.10
Silt (%)	12.20	11.30
Clay (%)	14.40	13.50
Textural class	Sandy loam	Sandy loam
pH (H_2O)	5.93	6.30
Organic Carbon (%)	0.72	0.80
Organic Matter (%)	1.25	1.36
Total Nitrogen (%)	0.70	0.78
Available Phosphorus (ppm)	3.60	2.90
Cal^{2+} Cmol kg^{-1} soil)	3.41	3.57
Mg^{2+} (Cmol kg^{-1} soil)	1.62	1.70
K^{+}Cmol kg^{-1} soil)	0.29	0.30
Na^{+}Cmol kg^{-1} soil)	0.60	0.52
CEC Cmol kg^{-1} soil)	6.25	6.40
Base Saturation (%)	94.40	95.00

Treatment and Experimental Design

The experiment was laid out as split-plot in randomized complete block design (RCBD) with three replications. The main plot treatment comprised of two cropping systems [sole cropping (maize, cassava) and row intercropping (maize + cassava)] while the sub-plot treatment was 3 maize varieties [Quality Protein Maize (QPM), Suwan 1-1 and the Local]. Each sub plot consisted of 5 ridges spaced 1m apart and 4m long and the net plot was the three middle ridges, 3m long.

Crop Husbandry

The experimental site was cleared and ridged using cutlasses and hoes. Maize and cassava were sown either as sole crop or intercrop on ridges on the same day in both experimental locations (18 April, 2016 and 18 June, 2016 in Lafia and Makurdi respectively). Maize seeds were dressed with Apron Plus® 50DS (10% metalaxy, 1.34% furanthiocarb, 61% carboxin) at the rate of one sachet per three kilogrammes of seed. Three maize seeds were planted per hill by the side of the ridge. Cassava cuttings measuring 30cm were planted at an angle of 45° at the top of the ridge a spacing of 100cm within rows. Maize was thinned to 2 seedlings/stand at 10 days after planting (DAP) while supplying was done to cassava at 14 DAP. Intercropping had a 1:1 (maize:cassava) row proportion. Fertilizer was applied to maize at the rate of 30kg N, 30kg P2O5 and 30kg K2O per hectare (BNARDA, 2003) obtained from NPK 15:15:15 in split doses at 3 and 6 WAP by side placement. At 4 W.A.P, cassava plots in both sole and intercropped were top dressed with 200kg of NPK 15:15:15 by side placement (BNARDA, 2003). Two manual weedings were done at 3 and 7 weeks after planting (WAP) respectively. This was followed by remoulding at 12 WAP. All these operations were carried out by hoe. Hand pulling of the weeds in the experimental plots was done when necessary. 'Best'® (Cypermithrin 10% EC) at a dose of 60 ml in 10 litres of water was used for the control of insect pest on

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maize and this was repeated at fortnightly interval.Harvesting was done as each component crop reached physical maturity. In all cases local implements (knives, cutlasses and hoes) were used for harvesting. Maize cobs were cut and sundried before threshing and winnowing.

Data Collection

Parameters measured for maize component included plant height at harvest, cob length, number of rows per cob, number of seeds per row grain yield and hundred seed weight. The characters measured for the cassava component were plant height at harvest, root circumference, root length, number of saleable roots per plant and weight of saleable roots per hectare. Saleable roots were fresh roots ≥ 150g.

Measures of intercrop productivity was determined by using land equivalent ratio (LER) as described by Ofori and Stern (1987) and land equivalent coefficient (LEC) as illustrated by Adetiloye *et al*. (1983). Competitive ratio (CR) which indicates the number of times by which one component crop is more competitive than the other was calculated using the formula proposed by Willey *et al*. (1980).

Standard procedures were followed in collecting all data and analysis was done using GENSTAT statistical software. Whenever differences between treatment means were significant, means were separated by Fishers Least Significant Difference at 5% level of probability.

III. RESULTS

Maize Component

Plant Height at Harvest

The main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety on the plant height of maize at harvest was significant (P≤ 0.05) in Lafia and Makurdi.

Data from Table 3 showed that irrespective of the cropping system, Suwan 1-1 gave the highest plant height of maize at harvest in both locations. The lowest plant height of maize at harvest was produced when Local maize was intercropped (Table 3).

Sole cropping generally gave higher plant height of maize than intercropping in Lafia and Makurdi. Suwan 1-1 produced the highest plant height of maize in both locations among the varieties evaluated (Table 2).

Leaf Area Index at Harvest

The leaf area index of maize at harvest as influenced by the main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety in Lafia and Makurdi was significant (P≤ 0.05).

QPM produced the highest leaf area index of maize at harvest in both locations when it was planted as sole and the difference was significantly higher than that produced by any other treatment. The lowest leaf area index of maize at harvest was produced when Local maize was intercropped with cassava (Table 3).

On a general note, sole cropping produced significantly higher leaf area index at harvest than intercropping in Lafia and Makurdi. QPM gave significantly higher leaf area index of maize than Suwan 1-1 which in turn produced significantly higher leaf area index than Local maize (Table 2).

Cob Circumference

The main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety was significant (P≤ 0.05) on the cob circumference of maize in Lafia and Makurdi.

Values obtained for cob circumference of maize in Makurdi were higher than those of Lafia. In both locations, sole QPM gave the highest cob circumference of maize and this was significantly higher than that produced by any other treatment except when Suwan 1-1 was planted as sole. Local maize gave the lowest cob circumference in Lafia and Makurdi when it was intercropped (Table 3).

Sole cropping generally produced significantly higher cob circumference than intercropping in both location. QPM gave the highest cob circumference of maize in Lafia and Makurdi among the varieties but this was only significantly higher than Local maize (Table 2).

Cob Length

The main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety was significant (P≤ 0.05) on the cob length of maize in Lafia and Makurdi.

Data presented in Table 3 showed that in Lafia, Suwan 1-1 produced the longest cob length when it was planted as sole but this was not so in Makurdi where Suwan 1-1 produced the highest cob length when it was intercropped. In Lafia, intercropped QPM gave the lowest cob weight of maize while in Makurdi, Local maize produced the shortest cob weight of maize (Table 3).

Generally, sole cropping produced significantly higher cob length of maize than intercropping in Lafia and Makurdi. Irrespective of the location, Suwan 1-1 produced significantly higher cob length of maize (Table 2).

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Table.2: Effect of Cropping System and Maize Variety on the Pant Height, Leaf Area Index Cob Circumference and Cob Length of Maize in Lafia and Makurdi.

Treatment	Plant Height at Harvest		Leaf Area Index at Harvest (cm^2)		Cob Circumference (cm)		Cob Length (cm)	
	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi
Cropping System								
Intercropping	162.00	184.82	174.97	194.14	12.98	13.91	24.22	27.83
Sole Cropping	181.27	192.20	191.23	213.40	15.51	15.88	27.46	28.57
F-LSD (0.05)	3.54	4.32	6.75	6.92	1.33	1.37	1.54	1.05
Maize Variety								
QPM	169.72	182.93	195.15	214.39	14.89	15.59	25.19	27.84
Suwan 1-1	174.97	198.99	184.20	204.49	14.30	14.97	27.50	29.70
Local	170.22	183.62	169.95	192.44	13.55	14.13	24.83	27.07
F-LSD (0.05)	3.54	4.95	7.55	7.32	1.19	1.25	1.31	1.44

Table.3: Interaction Effects of Cropping System x Maize Variety on the Pant Height, Leaf Area Index Cob Circumference and Cob Length of Maize in Lafia and Makurdi.

Cropping System	Maize Variety	Plant Height at Harvest (cm)		Leaf Area Index at Harvest (cm^2)		Cob Circumference (cm)		Cob Length (cm)	
		Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi
Intercropping	**QPM**	161.23	180.43	186.50	196.55	13.77	14.67	22.95	27.00
	Suwan 1-1	164.67	193.43	179.90	195.37	12.73	13.93	25.43	30.17
	Local	160.10	180.61	158.50	190.50	12.43	13.13	24.28	26.33
Sole Cropping	**QPM**	178.20	185.43	203.80	232.23	16.00	16.50	27.43	28.67
	Suwan 1-1	185.27	204.54	188.50	213.60	15.87	16.00	29.57	29.23
	Local	180.33	186.63	181.40	194.37	14.67	15.13	25.38	27.80
F-LSD (0.05)		3.54	3.99	7.64	7.74	1.32	1.71	2.11	2.24

Number of Rows per Cob

The main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety was significant (P≤ 0.05) on the number of rows per cob of maize in Lafia and Makurdi.

Data presented in Table 5 showed that in Lafia, QPM produced the same number of rows per cob and this represented the highest number of rows per cob in Lafia. In Makurdi, QPM produced the highest number of rows per cob when it was planted as sole but the difference was not significantly higher than that produced when Suwan 1-1 was also planted as sole crop (Table 5).

Sole cropping gave significantly higher number of rows per cob than intercropping in both locations. QPM gave the highest number of rows per cob among the varieties evaluated but the difference was only significantly higher than Local maize (Table 4).

Number of Seeds per Row

The number of seeds per row as influenced by the main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety in Lafia and Makurdi was significant (P≤ 0.05).

Regardless of the location, the highest number of seeds per row was produced when Suwan 1-1 was planted as a sole crop. In Makurdi, the number of seeds per row produced by sole Suwan 1-1 was not significantly different from that produced by sole QPM and intercropped Suwan 1-1. Intercropped Local maize gave the lowest number of seeds per row in both locations (Table 5).

Sole cropping largely gave higher number of seeds per row than intercropping in all locations and the difference was significant. Suwan 1-1 produced the highest number of seeds per row among the varieties evaluated (Table 4).

Cob Weight

The main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize

variety was significant (P≤ 0.05) on the number of rows per cob of maize in Lafia and Makurdi.

Cob weight values obtained from Makurdi were higher than those of Lafia. QPM produced the highest cob weight when it was planted as a sole crop in both locations but the difference was not significantly higher than that produced when Suwan 1-1 was planted as a sole crop. Local maize gave the lowest cob weight of maize when it was intercropped with cassava in Lafia and Makirdi (Table 5).

Sole cropping produced significantly higher cob weight in both locations than intercropping. QPM produced the highest cob weight among the varieties evaluated but the difference was only significantly higher than that produced by Local maize (Table 4).

Grain Yield

The grain yield of maize at harvest as influenced by the main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety in Lafia and Makurdi was significant (P≤ 0.05).

Data presented in Table 5 revealed that Makurdi location produced higher grain yield values than Lafia location. In both locations, QPM gave the highest grain yield of maize when it was planted as sole but this was not significantly different from that produced when Suwan 1-1 was planted as sown and when QPM was intercropped (Table 5).

Sole cropping produced significantly higher grain yield of maize than intercropping in all locations. Irrespective of the location, QPM gave the highest grain yield of maize but this was only significantly higher than that produced by Local maize (Table 4).

100-Seed Weight

The main effect of cropping system and maize variety as well as the interaction effects of cropping system x maize variety was significant (P≤ 0.05) on 100-seed weight of maize in Lafia and Makurdi.

Data presented in Table 5 showed that in Lafia, Local maize gave the highest 100-seed weight of maize when it was planted as a sole crop but this was not so in Lafia where Suwan 1-1 gave the highest 100-seed weight of maize when it was planted as a sole crop. In Lafia, Local maize gave the lowest 100-seed weight of maize when it was intercropped while intercropped Suwan 1-1 gave the lowest 100-seed weight in Makurdi (Table 5).

Sole cropping generally gave higher 100-seed weight of maize than intercropping in Lafia and Makurdi. Among the maize varieties evaluated, Local maize gave the highest 100-seed weight in Lafia and Makurdi (Table 4).

Table.4: Interaction Effects of Cropping System and Maize Variety on some Yield and Yield Parameters of Maize in Lafia and Makurdi

Treatment	Number of Rows per Cob		Number of Seeds per Row		Cob Weight (t/ha)		Grain Yield (t/ha)		100-Seed Weight (g)	
	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi
Cropping System										
Intercropping	16.01	15.00	23.11	26.46	3.83	4.13	2.06	2.35	30.42	31.20
Sole Cropping	16.71	18.70	25.69	28.02	4.28	4.44	2.35	2.56	34.85	35.36
F-LSD (0.05)	0.42	1.45	1.57	1.93	0.36	0.22	0.24	0.19	1.54	1.83
Maize Variety										
QPM	17.67	18.78	24.12	26.93	4.52	4.86	2.60	2.93	32.08	31.52
Suwan 1-1	17.30	17.50	26.00	28.54	4.32	4.43	2.52	2.56	32.42	34.00
Local	14.12	14.27	23.09	26.25	3.33	3.58	1.50	1.89	33.41	34.33
F-LSD (0.05)	1.54	1.93	1.67	1.88	0.53	0.34	0.23	0.45	1.03	1.13

Table.5: Interaction Effects of Cropping System x Maize Variety on some Yield and Yield Parameters of Maize in Lafia and Makurdi

Cropping System	Maize Variety	Number of Rows per Cob		Number of Seeds per Row		Cob Weight (t/ha)		Grain Yield (t/ha)		100-Seed Weight (g)	
		Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi
Intercropping	**QPM**	17.67	17.33	24.00	25.43	4.14	4.75	2.25	2.87	31.00	29.70
	Suwan 1-1	17.36	15.00	23.33	28.31	4.11	4.21	2.28	2.35	29.32	29.57

	Local	13.00	12.67	22.00	25.63	3.24	3.44	1.65	1.84	30.95	34.33
Sole Cropping	**QPM**	17.67	20.23	24.23	28.42	4.90	4.96	2.95	2.99	33.15	33.33
	Suwan 1-1	17.24	20.00	28.67	28.77	4.52	4.64	2.75	2.76	35.52	38.43
	Local	15.23	15.87	24.17	26.87	3.41	3.71	1.34	1.94	35.87	34.33
F-LSD (0.05)		1.25	1.32	1.22	1.32	0.46	0.34	0.45	0.23	1.34	1.76

Plant Height at Harvest

Cropping system and maize varieties had significant (P≤ 0.05) effect on the plant height of cassava at harvest. In all locations, sole cropping generally gave higher plant height of cassava at harvest than intercropping. Among the cassava treatments intercropped, cassava produced the highest plant height in Makurdi and Lafia when it was intercropped with QPM (Table 6).

Root Circumference

The root circumference of cassava as influenced by the main effect of cropping system and maize variety was significant (P≤ 0.05) in Lafia and Makurdi. Irrespective of the location, sole cassava produced the highest root circumference and this was significantly higher than that produced by any other treatment. Cassava intercropped with Local maize and cassava intercropped with Suwan 1-1 gave the highest and lowest root circumference of cassava in both locations respectively (Table 6).

Root Length

The root length of maize as influenced by the effect of cropping system and maize variety was significant (P≤ 0.05) in Lafia and Makurdi. In all locations, sole cassava produced significantly higher root length than cassava intercropped with Local maize which in turn gave significantly higher root length than cassava intercropped with QPM and Suwan 1-1 respectively (Table 6).

Table.6: Plant Height, Root Circumference and Root Length of Cassava as Influenced by Cropping System and Maize Variety in Lafia and Makurdi

Treatment	**Plant Height Harvest (cm)**		**Root Circumference (cm)**		**Root Length (cm)**	
	Lafia	**Makurdi**	**Lafia**	**Makurdi**	**Lafia**	**Makurdi**
Cassava + QPM	130.73	158.67	16.00	17.93	38.67	42.33
Cassava + Suwan 1-1	120.90	128.33	13.67	14.37	33.00	37.33
Cassava + Local	117.90	140.90	17.00	19.33	43.78	47.00
Intercrop Mean	123.18	142.63	15.56	17.21	38.48	42.22
Sole Cassava	147.18	163.18	24.00	25.80	53.28	55.53
Grand Mean	129.18	147.77	17.67	19.36	42.18	45.55
F-LSD (0.05)	5.54	65.43	2.34	2.65	4.74	4.32

Number of Marketable Roots per Plant

Cropping system and maize varieties had significant (P≤ 0.05) effect on the number of marketable roots per plant in Lafia and Makurdi. Regardless of the location, sole cassava produced the highest number of marketable roots per plant and this was significantly higher than that produced by any other treatment. In all locations, no significant difference was observed among the cassava treatments intercropped (Table 7).

Number of Unmarketable Roots per Plant

The number of unmarketable roots per plant as influenced by the effect of cropping system and maize variety was significant (P≤ 0.05) in Lafia and Makurdi. In both locations, sole cropping had the highest number of unmarketable roots per plant and the difference was significant. No significant difference was observed on the number of marketable per plant among the treatments intercropped (Table 7).

Root Weight

The root weight of maize as influenced by the effect of cropping system and maize variety was significant (P≤ 0.05) in Lafia and Makurdi.

Sole cassava produced significantly higher root weight in both locations and this was significantly higher than that produced by any other treatment. Cassava intercropped with Local maize gave the highest root weight of cassava among the treatments intercropped and the difference was significant (Table 7).

Table.7: Effect of Cropping System and Maize Variety on the Number of Marketable and Unmarketable roots per Plant and Root Weight of Cassava in Lafia and Makurdi

Treatment	Number of Marketable Roots per Plant		Number of Unmarketable Roots per Plant		Root Weight (t/ha)	
	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi
Cassava + QPM	9.00	9.30	2.00	1.50	6.58	7.09
Cassava + Suwan 1-1	9.50	9.67	1.67	1.67	6.73	7.60
Cassava + Local	9.67	10.67	1.17	1.33	8.50	9.02
Intercrop Mean	9.39	9.88	1.61	1.50	7.27	7.90
Sole Cassava	15.43	16.00	3.83	3.50	12.35	12.88
Grand Mean	10.90	11.41	2.17	2.00	8.54	9.15
F-LSD (0.05)	2.43	2.03	1.43	1.55	1.54	1.65

Assessment of Measures of Intercrop Productivity

Table 8 presents the results of measures of intercrop productivity [Land Equivalent Ratio (LER), Land Equivalent Coefficient (LEC)] and measures of competitive interactions [Competitive Ratio (CR)] between the intercrop components of maize and cassava in Lafia and Makurdi.

All intercrop combinations had LER figures above 1.0 and LEC values above 0.25 in both locations. CR values of maize were consistently higher than those of cassava in all intercrop combinations. The combination of cassava and Local maize had higher values of LER and LEC than the other combinations (Table 8).

Table.8: Land Equivalent Ratio (LER), Land Equivalent Coefficient (LEC) and Competitive Ratio (CR) of Intercropped Maize Varieties with Cassava in Lafia and Makurdi

Treatment	LER		LEC		CR Maize		CR Cassava	
	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi	Lafia	Makurdi
Cassava + QPM	1.41	1.65	0.50	0.67	1.17	1.38	0.85	0.72
Cassava + Suwan 1-1	1.44	1.49	0.51	0.54	1.35	1.33	0.74	0.75
Cassava + Local	2.11	1.83	1.08	0.84	1.40	1.07	0.71	0.93
Grand Mean	1.65	1.66	0.70	0.68	1.31	1.26	0.77	0.80
F-LSD (0.05)	0.13	0.24	0.14	0.16	1.07	1.25	0.34	0.23

IV. DISCUSSION

The depression in plant height at harvest, leaf area index at harvest, cob circumference, cob length, number of rows per cob, number of seeds per row, cob weight, grain yield and 100-seed weight of maize as compared to sole crop resulted from inter-specific competition. Egbe and Adeyemo (2006) had also reported reduction in growth and yield of some component crops in mixtures. These authors opined that inter- specific competition for light, nutrients, water, air and other growth resources often resulted in depressed yields of the intercrop components.

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Growth and yield of maize varied with variety. In all locations, Suwan 1-1 generally produced the highest plant height at harvest, cob length and number of seeds per row. QPM gave the highest leaf area index at harvest, cob circumference, number of rows per cob, cob weight and grain yield in Lafia and Makurdi. Suwan 1-1 produced the highest 100-seed weight of maize in Lafia while Local maize gave the highest 100-seed weight of maize in Makurdi. The result obtained from this study suggests that selection for these characters would be effective for further selection and improvement. The superior performance of these varieties with respect to various parameters was due to their genetic makeup. Differences in their anatomical, morphological and physiological structures enabled them to compete effectively with the component crop, absorb nutrients and water, effectively carry out photosynthesis and store photosynthates which other varieties could not. Plants respond differently to environmental factors based on their genetic makeup and their adaptation capability indicating variability among species (Agbogidi and Ofuoku, 2005; Agbogidi and Egho, 2012).

The decrease in growth and yield (plant height at harvest, root circumference, root length, number of marketable roots per plant, number of unmarketable roots per plant and root weight) of intercropped cassava as compared to sole cropping could be credited to interspecies rivalry for both under- and above-ground growth resources (water, nutrients, light, air, etc.). The taller maize component sheltered the low canopy cassava thus decreasing light availability for optimum photosynthetic activity and subsequently culminating in the low yields of cassava. Sharing of growth resources among components crops under intercropping can limit growth and accumulation of dry matter compared to sole cropping where competition exists (Dasbak and Asiegbu, 2009). The better performance of cassava under intercropping with Local maize over other varieties of maize with respect to root circumference, root length, number of unmarketable roots per plant and root weight suggest that this variety was more suitable than the other varieties for cultivation with cassava in Lafia and Makurdi environment.

LER values were greater than unity in all treatments in both locations, indicating the advantage of intercropping over sole stands in regard to use of environmental growth resources. All LEC values were above 0.25 in Lafia and Makurdi. This further indicates that all intercropping combinations were better in resource use efficiency compared to growing the two crops separately. Adetiloye *et al*. (1983) stated that the minimum expected production before a yield advantage is obtained in a two-crop mixture is an LEC greater than 0.25 (Egbe *et al.,* 2010). The highest LER and LEC in both locations was obtained when Local maize was intercropped with cassava. Intercropping thus, can be the most realistic cropping system to increase crop productivity in Lafia and Makurdi environments. Maize was the more dominant component of the maize/cassava intercropping systems, probably because of its height advantage.

Fujita and Ofosu-Budu (1996)stated that the non-legume growth is severely suppressed due to depression of photosynthesis through decreases in irradiance.

V. CONCLUSION

Intercropping severely depressed plant height at harvest, leaf area index at harvest, cob circumference, cob length, number of rows per cob, number of seeds per row, cob weight, grain yield and 100-ssed weight in Lafi and Makurdi. In both locations, Suwan 1-1 generally produced the highest plant height at harvest, cob length and number of seeds per row. QPM gave the highest leaf area index at harvest, cob circumference, number of rows per cob, cob weight and grain yield in Lafia and Makurdi. Suwan 1-1 produced the highest 100-seed weight of maize in Lafia while Local maize gave the highest 100-seed weight of maize in Makurdi. Intercropping also decreased the growth and yield (plant height at harvest, root circumference, root length, number of marketable roots per plant, number of unmarketable roots per plant and root weight) of cassava in both locations. All LER and LEC values were above 1.0 and 0.25 respectively in both locations. Maize had higher competitive ratio values than cassava.

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Effect of Variety and Sowıng Density on Some Mıcroelements Content and Grain Yield of Chickpea (*Cicerarietinum* L.)

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Abstract— *The objective of this study was to determine the effects of cultivars in different sowing densities on microelementsiron (Fe), nicel (Ni),zinc (Zn)and sodium (Na) and grain yield of chickpea (Cicerarietinum L). Field experiment was performedin research farm at the University of Bingol(Turkey) in 2016. A complete blocks design in two varieties i.e. Arda and ILC-482 were in main plots, whereas five chickpea seeding density (20, 30, 40, 50 and 60 seed m^{-2}) were in sub plots.The results indicated that seeding densities significantly affected grain yield and Ni content while Fe, Ni and Zn were not affected significantly.Variety ILC-482 produced the maximum grain yield (86,26 kg/da) by 60 seed/m^{-2} and Arda gave the lowest grain yield (19,80 kg/da) by 30 seed m^{-2}.The highest Ni conten has been obtained from ILC482 variety (6.66 ppm) andthe lowest Ni content has been obtained from Arda variety (6.20 ppm).*

Keywords—Chickpea, microelements, seeding density, variety.

I. INTRODUCTION

Chickpea (*Cicerarietinum L.)* is an annual grain legume or pulse crop sold into human food markets. Chickpea is the third most important food legume crop and India is the largest producer contributing to 65% of world's chickpea production (FAOSTAT, 2012). According to Akhtarand Siddiqui (2009) during last decade the production of chickpea have declined. Its foundation is believed to be in south-eastern Turkey neighboring Syria and Iran (Ladizinsky, 1975).The earliest residue of chickpea seeds date back to around 7000 B.C in Syria and Turkey. In Turkey, it occupies about 388.518 hectare area with production of 450.000 tonnes and an average productivity of 1158.2 kg ha^{-1} (TUIK 2014). Inspite of the importance of this crop in human daily diet and in agricultural production, productivity of thiscrop is low in Turkey.Hulse (1991) reported that legume is one of the oldest groups of agricultural plants and food legumes are the second most important human's food supply after the cereal grains, which their grain contain 38 to 59% carbohydrate, 4.8 to 5.9% oil, 3% ash, 3% fiber, 0.2% calcium, and 0.3% phosphorus. In general, pulsecropscontain a range of nutrients including low digestible carbohydrates, protein, essential amino acids, fattyacids, and a range of micronutrients (Bhatty, 1988). Bueckert et al (2011) reported that chickpea seeds contained from 29 to 52 mg/kg Zn, 77–112 mg/kg Fe, 1,448–2,457 mg/kg Mg, 1,211–2,457 mg/kg Ca, to 3.8–9.0 mg/g phyticasid. Cereals like wheat and rice combined with pulses are major dietary components for billions of people, and the potential for microelemetbiofortifcation of pulses is high. Chickpea is an important source of microelements like Fe, Zn, Mg, and Ca in vegetarian diets (Abbo et al., 2000; Ereifej et al., 2001).Whereas, limitedinformation is available on chickpea (*Cicerarietinum* L.) mineral biofortification.Micronutrient concentrations in the pulse lentil may vary depending on the geographical location, plant genotype, soil factors, temperature, and other growing season conditions (Thavarajah et al., 2010; Bueckert et al., 2011).The use of high plant density in chickpea production decreases soil water evaporation late in the growing season when plant cover closure is low. In difference, low plant density may allow weeds to grow more aggressively and limit crop yield possible. Plants grown at lower plant density are usually shorter and branchy, which increases losses during combine harvest (Turneret al., 2001).In a study in Canada, a plant population density of 55 plants m^{-2} produced a 23% to 49% seed yield above that of the recommended plant population density of 44 plants m^{-2} (Vanderpuye, 2010).Plant population is a type component of the production of chickpea. The yield of chickpea can be improved by planting of best density of chickpea cultivars. The objectives of this study were to determine the effect of plant density and varieties on grain yield and some microelemntscharacteristics of chickpea under agro-climatic conditions of Bingol, Turkey.

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II. MATERIALS AND METHODS

The present study was conducted throughout spring season in 2016 at the research experiment field of the Bingol University (Turkey) located at 38º 48 N latitude and 40º 32 E longitude (10 km South Bingol) and at an altitude of 1090 m. Experimental field location receives annual rainfall of 938 mm. During the study in 2016, the lowest minimum temperature was fell down below to -5.6ºC in January. Total rainfall level of 2016 is lower than the total precipitation level of previous years. But during the first half of 2016, the total of precipitation was higher than the previous years. The amount of rainfall on the chickpea products was 98.4 mm (Figure 1, Figure 2 andFigure 3).The soil of experiment field is loamy, with contents of organic matter of around 1.9% and pH 6.57. Microelement values were taken from Demir (2016). The soil analysis result for physical and chemical characteristic of the study area are given in Table 1.

Table.1: Soil analysis result for physical and chemical characteristic of the study area

Soildepth	Soiltexture	pH	Salt Content	OrganicMatter	P_2O_5	K_2O	Lime	Fe	Zn	Na
Cm			%	%	Kg/ha		%	ppm		
0-30	Loam	6.57	0.0315	1.905	7.91	24.51	0.36	14.15	0.33	0.78

Two registired cultivars kabuli type(Arda and ILC-482) , adapted to South Easten AnatoliaTurkey were chosen with a morphologicaltraits (Table 2). The seeds were drilled 5-8 cm deep in previously opened furrows on 05th April 2016. In this study,thewholedose of P (6 kg P da^{-1}) withhalf of dose of nitrogen (5 kg N da^{-1}) wereapplied at sowing time and there maining nitrogen (5 kg N da^{-1}) was top-dressed as Ammoniumnitrate (%33) with flowering time on 26 July.2016. Rhizobium bacteria nodules were not observed in the roots of chickpea parsels.Weeds were controlled by hand after germination.

Table.2: Name andgeographicalorigins of investigatedcultivars

Cultivar	Plant height cm	Firstpodheight cm	100 kernelwight g.	Plant type	Origin
Arda	64-85	33-37	34-40	Erect	GAPUTAEM
ILC-482	40-45	20-26	28-31	Semi-prostrate	GAPUTAEM

Experimental design and management: Two factorial trial was set up as a split-plot design (RCBD) with two comparing chickpea varieties (Arda and ILC-482) as main plots and fiwe seed densities (20, 30, 40, 50 and 60 seed m^{-2}) as split-plots. The main plots were randomised in a block design with three repilications. The density treatments were randomised in the main plots. Each variety was sown in four-row plots of 5 m length with between- and within-row spacing of 30 cm. Plot size was 1.2 m x 5 m (6 m^2). Spacing of 0.4 m and 1 m were allocated between plots and blocks, respectively

Microelemnts analysis: Fe, Ni, Znand Nacompositions of whole chickpea flour were determined by the method of Hwang et al.(1997) and; Choi et al. (2013) with slight modifications. One gram of chickpea flour was wet-digested in a mixture solution of HNO_3(10 ml) and H_2SO_4(10 ml) with heating on a hot plate. After extraction cooled, in hood opening carefully to pass the gas and put it to another tub that contain nearly 5ml of distilled water slowly and completed to 25ml by distilled water. This solution was ready for using to determine elements. Fe, Ni and Zn minerals determined by atomic absorption spectrophotometry (Perkin Elmer, AAS 800)

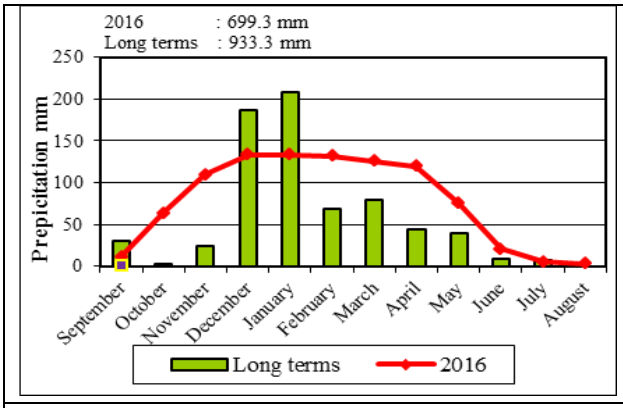

Fig.1: Total prepicitatiom(mm) in the growing

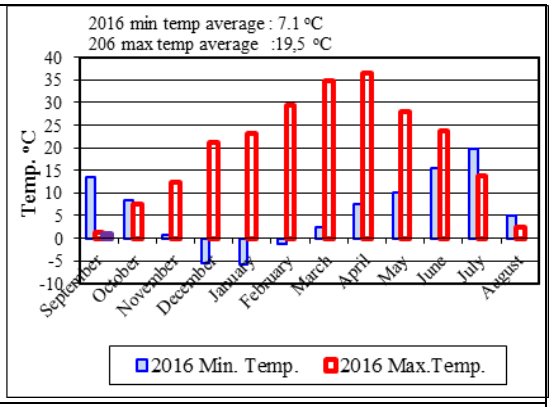

Fig.2: Max and Min temperatures (°C) in the

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environment	*growing environment*

Statistical Data Analysis: Results were evaluated to analysis of variance SAS (Statistical Analysis Systems) program (SAS Institute 1999) and mean separation was performed by Fisher's least significant difference (LSD) test when F test was significant at P<0.05.Regression analysis was conducted to estimate linear and quadratic effects of plant density when results of the analysis of variance indicated these effects were significant at P<0.05.

III. RESULTS AND DISCUSSION

Table.3: Analysis of variance of grain yield (kg/da) of different chickpea varieties and densities.

Sources	DF	Mean Squares				
		Grain yield	**Fe**	**Ni**	**Zn**	**Na**
Replication	2	15.2629	4,6972	0,1256	0,8051	1,6032
Variety	1	8768 *	25,5948 ns	1,6147*	2,0981ns	2,5667 ns
Replication*variety&Random(Error1	2	90.035	6,8840	0,0045	1,3785	4,54870
Density	4	958.483**	2,4586 ns	0,0753 ns	0,7354ns	0,3814 ns
Variety*density	4	407.078**	1,4538 ns	0,0994 ns	1,7764*	0,5810 ns
Error-2	16	61.24	2,5505	0,0896	0,5715	1,8078

*: Significance at 5 % probability, **Significance at 1 % probability,ns = non-significant

Grain yield (kg/da): Table 3 and Table 4 revealed that there were highly significant (P<0.01) differences among the varieties and seed densities. The interaction between the two factors was, however, significant. Variety ILC-482 produced the maximum grain yield (86,26 kg/da) by 60 seed/m^{-2} and Arda gave the lowest grain yield (19,80 kg/da) by 30 see/m^{-2}. Gan*et al*. (2003) concluded that increasing yield of chickpea at high density and they found strong positive relationship between seed yield and plant population densities. Bahr (2007) also noticed that high plant density (50 plants m^{-2}) gave higher seed yield as compare to low plant density (26 plants m^{-2}) in chickpea. Grain yield was increased with increasing in seed density was presented by regression equation in Figure 4 and Figure 5. These results were in line with those of Valimohammadi et al. (2007) reported that plant density has no significant effect on yield. While, Shamsi (2011) and Gana et al. (2007) reported that density does not have a significant effect on yield of chickpea. Regression analysis revealed that the grain yield increased linearly (R^2 = 0.48, 0.82) with seed rate for Arda and ILC-482 (Figure 4 and Figure 5).

Table.4: Effect of plantingdensityandvariety on thegrainyield, Fe, Ni, ZnandNacontents of chickpea

Traits	Cultivars	Densities(Seeds m^{-2})					Means
		20	30	40	50	60	
Grain yield kg/da	Arda	22.48 de	19,80e	24,43de	25,69de	33,41cd	**25,16B**
	ILC-482	32.71cde	46,02c	62,86b	68,93b	86,26 a	**59,36A**
Means		**27.59 C**	**32.91 C**	**43.64 B**	**47.31 B**	**47,31B**	**59,84A**
Fe (ppm)	Arda	3.73	3.78	3.14	5.99	4.31	**4.19**
	ILC-482	5.82	5.62	6.00	6.20	6.53	**6.03**
Means		**4.77**	**4.70**	**4.57**	**6.09**	**5.42**	
Ni (ppm)	Arda	6.30	6.21	5.85	6.1 5	6.48	**6.19 B**
	ILC-482	6.56	6.63	6.69	6.73	6.69	**6.66 A**
Means		**6.43**	**6.42**	**6.27**	**6.44**	**6.59**	
Zn (ppm)	Arda	3,57 ab	3,56 ab	1,91 b	3,32 ab	3,84 ab	**3.24**
	ILC-482	4,30 a	3,17 ab	4,08 ab	3,98 ab	3,30 ab	**3.76**
Means		**3.94**	**3.37**	**2.99**	**3.65**	**3.57**	
Na (ppm)	Arda	3.67	3.7 8	3.85	4.25	4.67	**4.04**
	ILC-482	4.73	5.03	4.10	4.89	4.38	**4.62**
Means		**4.20**	**4.40**	**3.96**	**4.57**	**4.53**	

*:Means within columns or rows with the same letters are not significantly different at 5% level.

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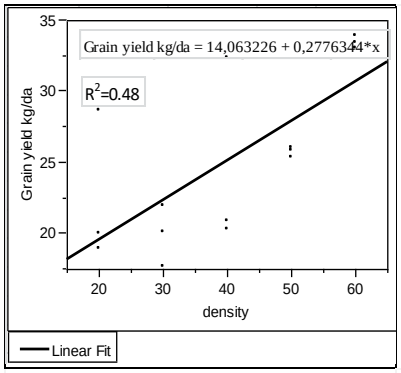

Fig.4: Regression of grain yield of variety Arda with different seed on densities

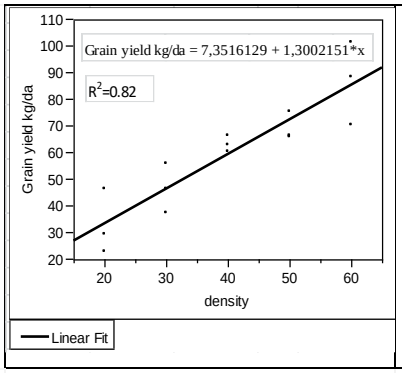

Fig.5: Regression of grain yield of variety ILC-482 with different seed on densities

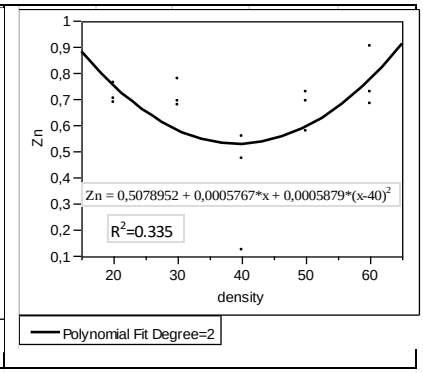

Fig.6: Regression of Zn content of variety Arda with different seed on densities

1

Extraction minerals seeds component: Chickpea (*Cicerarietinum* L.) belonging to the family Leguminosae, is one of the world's most important pulse crops. Chickpea seeds are nutrient-dense foods providing rich content of protein and certain dietary minerals such as iron and phosphorus, thiamin, vitamin B6, magnesium and zinc contents are also present in Khatoon and Prakash (2004).The chickpea is a good source of protein and carbohydrate and its protein quality is better than other legumes such as pigeon pea, black gram and green gram. It also supply some minerals (Ca, Mg, Zn, K, Fe, P) and vitamins like thiamine and niacin (Vilche et al. 2003). In our study researched and foundation percentage of (ppm) some metal such as (Fe, Ni, Na and Zn).

Iron (Fe): In this study, variety, density and interaction ($P<0.05$) had non significant effect on Fe elemen. The summerised Fe values are showed in Table 3 and Table 4.It was observed that the concentration and peak intensity value of iron (Fe) element. The highest value has been obtained from ILC-482 (6.53 ppm) by 60 seed m^{-2}. The lowest value of Fe has been obtained from Arda variety (3.14 ppm) by40 seed m^{-2}. Haytowitz and Matthews (1983) reported that cooking in boiling water caused great lossesof K (24%), Cu (15%) and Fe (8%).According to regression analyses, there was neither linear nor quadratic relationship between fe content and seed rate for both cultivars.

Nicel (Ni): The results of the (Ni) element are presented in Table 3 and Table 4. The main effect of variety was significant ($P<0.05$) but density and interaction had non significant. In our study was working in laboratory center in Bingol university to finding overage of Ni element contain. The highest value has been obtained from ILC-482 (6.73 ppm) by 50 seed $m^{-2.}$ The lowest number has been obtained from Arda variety was (5.85 ppm) by 40 seed m^{-2}.Micronutrient availabilityfor the plant depends, among other factors, texture organic matter and mainly soil(Ali et al., 2002). According to regression analyses, there was neither linear nor quadratic relationship between Fe content and seed rate for both cultivars.

Zinc (Zn): The results of variance analysis of Zn element value of different sample chickpea seed varieties are givenin Table 3 and Table 4. The main effects of variety and the interaction effects of variety x density had non-significant influence on the Zn element. Table 39 and Table 40 suggests that the highest average of Zn has been obtained ILC-482 variety (4.30 ppm) by 20 seed m^{-2}. Wherease, the lowest value was obtained Arda verity (1.91 ppm) by 40 seed m^{-2}. Zn plays an important role in plant reproductive development for initiation of flowering, floral development, male and female gamete genesis, fertilization and seed development (Liu et al., 2005).(Khan, 1998; Ahlawatet al.,2007). A comparisonbetween several crop species has shown that chickpeais more sensitive to Zn deficiency than cereal and oil seeds.Arda showed a quadratic trend ($R^2 = 0.335$) for Zn content for the different seeding rates. while regression equation was not significant in ILC-482 (Figure 6)

Sodium (Na): The results of the Na element are presented in Table 3 and Table 4. The main effect of variety, density and interaction had nonsignificanteffct by ($P<0.05$). The highest Na value of chickpea has been obtained from ILC-482 variety (5.03 ppm) in 30 seed m^{-2}. While, the lowest value of Na has been obtained from Arda variety (3.67 ppm) by 20 seed m^{-2}.(Ali et al., 2002). Micronutrient availabilityfor the plant depends, among other factors, texture,organic matter and, mainly, soil pH. Micronutrient availability for the plant depends, among other factors, texture, organic matter and mainaly soil pH. According to regression analyses, there was neither linear nor quadratic relationship between fe content and seed rate for both cultivars.

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IV. CONCLUSION

Theresults of thisresearchshowedthatmaximumyield of grainwasobservedwithILC-482 relatedto 60 seed/m^{-2} density.The main effects of variety and the density had nonsignificant influence on the Fe, Zn and Na elements, except Ni. However, density x variety interaction was significant only in Zn element.

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Proximate and Mineral Analysis of Coconutrhinoceros Beetle (*Oryctesrhinoceros* Linnaeus 1758) Larva Meal

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Abstract**—** *Insects are an important resource in an ecosystem needed for crop pollination, nutrient recycling, natural selection and nutrition for other animals. Edible insects such as coconut Rhinoceros Beetle can be utilized as a feed resource. Its larva consumes decomposing organic matter and can be converted into meal form for ease of handling, prolonging shelf-life and elimination of harmful microorganisms.*

This study aimed to determine the proximate and mineral analysis of coconut Rhinoceros Beetle larva meal. Results revealed that coconut Rhinoceros Beetle larvae are very efficient in converting low nutrient coconut fiber into a nutrient-dense larva meal. Its rate of recovery from fresh larva to meal form is 50%. Coconut RBLM could be incorporated in swine and poultry rations with developmental stage/s that needed low protein and high calcium and phosphorus mineral requirements.

It is recommended, however, that future studies involving coconut Rhinoceros Beetle larva should include emptying and cleaning of its gut before processing into meal form.

Keywords**— *proximate analysis, Coconut Rhinoceros Beetle Larva Meal.***

I. INTRODUCTION

Insects are an important resource in an ecosystem. Insects are vital in the pollination of most crops, nutrient recycling, natural selection, nutrition for other animals, etc. Insects are a natural source of food of other insects, birds, reptiles and some mammals. The study attempted to explore the nutritional value of an insect species.

Edible insects in their fresh form are a complete food. They contain water, protein, carbohydrates, fats, vitamins, minerals and fiber. Converting them into a meal form results in the absence of water but is still packed with life-sustaining nutrients. However, transforming insects into a meal has some practical and favourable applications such as the elimination of harmful microorganisms that can be vectored by these insects, prolonging its shelf life, ease in handling and packaging, etc.

An insect species that is abundant but neglected in coconut-producing areas is the Coconut Rhinoceros Beetle. This insect species is under the order Coleoptera and family Scarabaeidae. Contrasting portrayalscan be said of the adult and immature stages of coconut Rhinoceros Beetle. Adults have a hand in destroying standing coconut palms by burrowing into the crown to feed on its sap. Resulting burrows will resultto damaged unopened leaves and can attract other beetle species and harmful microorganisms that are destructive to palms (Catley, 1969; Barlow and Chew, 1970; Young, 1975; and Giblin-Davis, 2001). Its larvae, however, feed only on rotting or decomposing organic matter (Bedford, 1980; Giblin-Davis, 2001; Muniappan, 2002) and are beneficial in nutrient recycling. In times of along rainy season, drought or scarcity of food, rodents consume theselarvae for sustenance or nutrient supplementation. This study aimed to determine the proximate and mineral analysis of coconut Rhinoceros Beetle larva meal.

Knowledge of proximate and mineral analysis of coconut Rhinoceros Beetle larva meal (RBLM) will advance its use on food and feed. It could then be used either as a sole or a supplemental nutrient source for certain domestic farm animals.

II. MATERIALS AND METHODS

A. Meal Preparation

Live coconut Rhinoceros Beetle larvae were gathered from decaying/decomposing coconut trunks in coastal communities in Barangay Poblacion, Bislig City, Surigaodel Sur. Gathered larvae were placed in a container having substrates of decaying coconut fiber where larva were found and fattenedfor a week. 3rd instar larvae from the container were collected, washed, rinsed, and weighed. A kilogram of these larvae were 'pan-fried' and cut into pieces.

The rate of recovery regarding percentage (%) was computed using the formula:

$$\text{RR} = \frac{Weight\ of\ Rhino\ Beetle\ Larva\ Meal}{Fresh\ Weight\ of\ Rhino\ Beetle\ Larvae} X100$$

Samples of decaying coconut fiber where larvae were found and coconut Rhinoceros Beetle larva meal were freeze-dried for chemical analysis.

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B. Chemical Analysis

Freeze-dried samples of coconut fiber and coconut Rhinoceros Beetle larva meal were submitted to F.A.S.T. Laboratories in Cagayan de Oro City for chemical analysis.

Proximate analysis was done using Standard AOAC Method (AOAC, 2012). Calcium and Phosphorus were analyzed using Atomic Absorption Spectrophotometer and Colorimetric Method, respectively.

III. RESULTS AND DISCUSSION

Results revealed that rate of recovery of coconut Rhinoceros Beetle larvae was 50%. A kilogram of fresh larvae yielded half a kilogram (0.5 Kg) of larva meal.

Coconut fiber and coconut RBLM samples had high moisture contents of 79.4 and 76.6%, respectively, as shown in Table 1. Water crystals during freeze-drying possibly contributed to its high moisture contents.

Coconut Rhinoceros Beetle larva is very efficient in converting low nutrient coconut fiber into a nutrient-dense meal.

Table.1: Proximate and mineral analysis of coconut fiber and coconut RBLM samples

Parameters	Coconut Fiber	RBLM
1. Moisture, g/100g	79.40	76.60
2. Ash, g/100g	0.94	2.26
3. Crude Fat, g/100g	0.08	2.20
4. Crude Protein, g/100g	2.74	13.70
5. Crude Fiber, g/100g	6.81	4.50
6. Calcium, mg/100 g	42.20	76.00
7. Phosphorus, mg/100 g	17.20	57.30

*F.A.S.T. Laboratories, Cagayan de Oro City

Coconut Rhinoceros Beetle larvae efficiently converted the coconut fibers into body mass such as the ash content by 240% from 0.94 g into 2.26 g, 2,750% or 27.5x increase in fat content, 5x or 500% increase in protein content,180% increase in calcium and 3.33x or 333% increase in phosphorus content. Moreover, crude fiber was decreased by 66% from 6.81% in coconut fiber to 4.5% in coconut RBLM.

The efficiency of the larvae from converting low nutrient substrates into nutrient-rich body mass is by the findings of Taylor (1979). Efficiency in converting fibrous organic matter is due to the larva's presence of cellulytic and hemicellulolytic bacteria in its gut (Sari et al., 2016). These bacteria can digest cellulose and hemicellulose and hydrolyze them into fermentable sugars as an energy source (Shi et al., 2011).

Cellulolytic and hemicellulolytic bacteria can be found in phytophagous or herbivorous insects (Anand et al., 2010; Zhou et al., 2008; Geib et al., 2010; Ferreira et al., 2001; Cazemier et al., 2003). These insects degrade and digest plant biomass with the aid of these microorganisms in their gut.

However, in this study, coconut RBLM had low crude protein (CP) content (13.7%) compared to that of findings of Egba et al. (2014) having 33.97%, Xiaoming et al. (2010) with 23-66%, and Oluwu et al. (2012) having 48%. Low CP content of coconut RBLM was realized possibly due to processing. Preparation and processing methods applied influence nutritional composition of edible insects (FAO, 2013). In this study, processing of Rhinoceros Beetle larvae into meal form involved only in the killing and pan-frying of the larvae without emptying and cleaning its gut before pan-frying. The undigested fiber in its gut possibly contributed to low CP content of RBLM.

With regards to protein, calcium and phosphorus contents, coconut RBLM in this study could be incorporated into poultry and swine rations, as shown in Table 2. It could safely be included in rations of animals having low protein and high Ca and P requirements such as in swine (growers - ≥50Kgs, gestating and lactating sows and breeder boars) and in poultry (grower, breeder and layer turkeys, breeder ducks, grower and layer chickens, meat-type breeder chickens, breeder ducks and grower - ≥6 wk old and layer quails).

Table.2: Nutrient requirements of swine and selected poultry species

Nutrient	Nutrient Requirements											
	Swine				Poultry*							
					Turkey			Duck	Quail**	Chicken		
	Growers (≥50Kgs)	Gestating	Lactating	Breeder Boars	Growers (20-30 wks)	Breeders	Laying Hens	Breeders	≥ 6 wks	6 wks to 1st egg	Laying	Meat-type breeders
Protein	13.20-15.50	12.0-12.9	16.3-19.2	13.0	14.00	12.00	14.00	15.00	19.00	15.0-17.0	12.5-18.8	12.0-15.0
Calcium	12.28-13.84	13.9	39.4	15.0	0.55	0.50	2.25	2.75	3.00	0.8-2.0	2.7-4.06	0.90

Phosphorus	4.89-4.61	11.1	31.5	12.0	0.28	0.25	0.35	-	0.45	0.3-0.35	0.21-0.31	0.45

Source: National Research Council (1998)
*National Research Council (1994)
**FAO (2013)

IV. CONCLUSION AND RECOMMENDATION

In this study, coconut Rhinoceros Beetle larvaeare very efficient in converting low nutrient coconut fiber into a nutrient-dense larva meal. Its rate of recovery from fresh larvae to meal form is 50%. Coconut RBLM having low protein and high calcium and phosphorus contents could be incorporated in swine and poultry rations with developmental stage/s that needed low protein and high mineral requirements such as calcium and phosphorus.

It is recommended, however, that future studies involving coconut Rhinoceros Beetle larva should include emptying and cleaning of its gut before processing into meal form.

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Status of Blue Duiker (*Cephalophus monticola)* and Bushbuck (*Tragelaphus scriptus*) in Kom - Wum Forest Reserve, North West Region, Cameroon

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Abstract— *The study titled "Status of Blue duiker (Cephalophus monticola) and Bushbuck (Tragelaphus scriptus) in Kom-Wum Forest Reserve, North West Region-Cameroon, was realized from the 15th of January to March 31st, 2015. The general objective was to contribute to the conservation of Blue duiker and Bushbuck by establishing a baseline data in Kom-Wum Forest Reserve which will serve as guide for management decisions. The methodology used was "reconnaissance walk, questionnaires, semi structured interviews and focused group discussions. Results obtained indicated that twelve species of mammals were recorded through direct and indirect bio-indicators. The family of Cercopithecidae was the most represented (41.7%) (Putty nosed (Cercopithecus nictitans), Vervet (Cercopithecus aethiops), Patas (Erythrobus patas), Mona (Cercopithecus mona) monkeys and Olive Baboon (Papio Anubis). The results equally revealed that Chimpanzees (Pan troglodytes ellioti), Red duiker (Cephalophus dorsalis), Blue duiker and Bushbuck were the most abundant animals with encounter rates of 3.8, 2.91, 2.41 and 1.93signs per km respectively. The GIS distribution maps showed that Blue duikers and Bushbucks were more in the North East and South West potions of the reserve respectively. The mean encounter rate of anthropogenic activities (hunting, agriculture and logging) stood at 0.94 sign per kilometer. Hunting was most preponderant with an E.R of 1.41 sign per km (50%), followed by agriculture 1.0 sign per km (36%) and lastly logging 0.41 sign per km (14%). Encounter rates of anthropogenic activities plotted against those of mammals gave a coefficient of determination (R^2) =0.058 hence, mammal distribution is only slightly affected by human activities. Up to 65% of the respondents expressed negative attitudes towards conservation of resources in the reserve for the fact that it is their natural heritage and they should not be restrained from exploiting them. A majority (80%) of the respondents however agreed that the reserve is owned and controlled by the government. Though plagued by human interference, the reserve still harbours some Bushbuck and Blue duiker. We therefore recommend that the council, government, NGOs and the local community to step up conservation efforts.*

Keywords— Anthropogenic activities, Bushbuck, Blue duiker, Conservation, Encounter rate.

I. INTRODUCTION

Following the Earth Summit of Rio de Janeiro in Brazil in 1992, and the recommendation to its parties for tracking progress towards the 2010 target of halting biodiversity loss, the number of protected areas in Cameroon increased substantially (Mesmin, 2001). In 2010, 10.6 percent (5 million hectares) of the area of Cameroon were covered by protected areas. Of these, 45 percent (2.2 million hectares) of protected areas coverage were designated after Cameroon signed the CBD. National parks cover 3.1 million hectares corresponding to 61 percent of the area protected with 11 of the 20 parks classified under IUCN category II (IUCN, 2010). Forest and wildlife reserves comprise 940242 and 869428 hectares or 18 percent and 17 percent of land protected respectively. However, insufficient financial support and weak law enforcement have resulted in encroachment of those protected areas by human activities (illegal logging, poaching unsustainable agriculture) and settlements (COMIFAC, 2005). Bush meat trade, wildlife medicine and habitat loss are considered as the biggest threats to wildlife in tropical forests. For example, it is the root cause of the decreasing of African ape populations (Pearce & Ammann, 1995), the commerce of bush meat is particularly critical in Centre Africa. In the Congo Basin, between 1 million and 3.4 million tons of wild meat are

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consumed each year (Wilcox & Nambu, 2007). In West and Central Africa, the amount of antelopes killed for bush meat is widely recognized as unsustainable (Bowen-Jones, 2002). The blue duiker (*Cephalophus monticola*) especially represents a very high percentage of animal species killed for meat across West and Central Africa (Nasi and Vliet, 2011). In his Review of the Commercial Bush meat Trade on Central/West Africa, Bowen-Jones (1998) listed Cameroon as the country with the most references, representing 21% of the literature out of nine countries.

The Bamenda Highlands is the most diversed and important area in Western Cameroon after mount Cameroon and mount Kupe (Sedlacek *et al*., 2007). Within the eco-region of the Cameroonian Highlands, several taxa are endemic to the Bamenda Highlands (Ingram and Nsom, 2007; Ndenecho, 2009) and particularly to its highest peak: Mt Oku. These mountains are well-known for their richness in birds (Ndenecho, 2011) with several endemic species (Ingram and Nsom, 2007), including the banded wattle-eye (*Platysteira laticincta*) and the Bannermans turaco (*Tauraco bannermani*) an emblematic bird for local communities but highly localized and threatened by hunting (Ingram and Nsom, 2007). Several species of primates, including Nigeria-Cameroon chimpanzees and Preuss's guenons (*Cercopithecus preussi*), (both taxa considered as endangered by the International Union for the Conservation of Nature (IUCN), and endemic to the Bight of Biafra: IUCN, 2013), live in the remaining patches of sub-montane forests of the Bamenda Highlands (Ingram and Nsom, 2007).

The Kom-Wum forest reserve which is a biodiversity hot spot recently handed to the councils of Fundong and Wum by MINFOF and being an integral part of the Bamenda high lands will certainly habour some of these important species. The Kom-Wum forest reserve (17000 ha) is one of the largest remaining patches of the Bamenda highland montane forest. It is predicted to have the largest population density of chimpanzees and antelopes in the region (Chuo and Tsi, 2017e). It has been described as an exceptional priority conservation site for Nigeria-Cameroon chimpanzees (Morgan *et al.*, 2011). Despite the importance of this reserve, over hunting and habitat loss are the major causes of fauna loss which are secondarily triggered by the conversion of forest to pasture and agriculture (Chuo and Tsi, 2017e). This conversion has been dramatic and the landscape has changed considerably over the last century, with just a few fragmented forests remaining that hold remnants of flagship species like the Nigerian- Cameroon chimpanzee (*Pan tronglodytes ellioti*) and antelope species (CAEPA, 2014). As such, Reconciling development and biodiversity conservation remains a hard nut to crack. Since the inhabitants in and around the reserve depend on the forest for their livelihoods (Chuo and Tsi, 2017c). This therefore means that, sustainable management of such a reserve must involve the support of the local communities through their active involvement in wildlife management operations and hence decision making (Hulme and Taylor, 2000). Despite the fact that research has not been done on medium size mammals like the blue duiker and bushbuck in this reserve and incomplete knowledge on their abundance and distribution exist. The blue duiker and bushbuck are over hunted for subsistence and commercial purposes by local hunters in and around this reserve does the need to adopt necessary means to conserve the remaining species around the study areas.

II. MATERIALS AND METHOD

2.1. Description of Study Area

The Kom –Wum forest reserve is located between latitude 6° N and 7° N and longitude 9° E and 10°E and is situated in Wum Subdivision in Menchum Division and a reasonable potion extends to Boyo Division of the North West Region of Cameroon. Bounded by Wum to the North West, Bafut to the South West and Fundong to the South East and North East. This reserve was created in 1951, and has a surface area of about 17000 hectares (Morgan *et al.*, 2011). It has an altitude of about 900m to 2140m above sea level in the mountains and about 200m to 600m in the valleys. It is situated towards the western boundary of the region which stretches along the international border between Cameroon and eastern Nigeria. The main rivers that flow through this area are the rivers Ivin, Menchum, Nzele and Kimbi. All of these join the Kasina-la, which flows into Kasina-la State, Nigeria. Figure 5 shows the map of Kom–Wum forest reserve in Cameroon.

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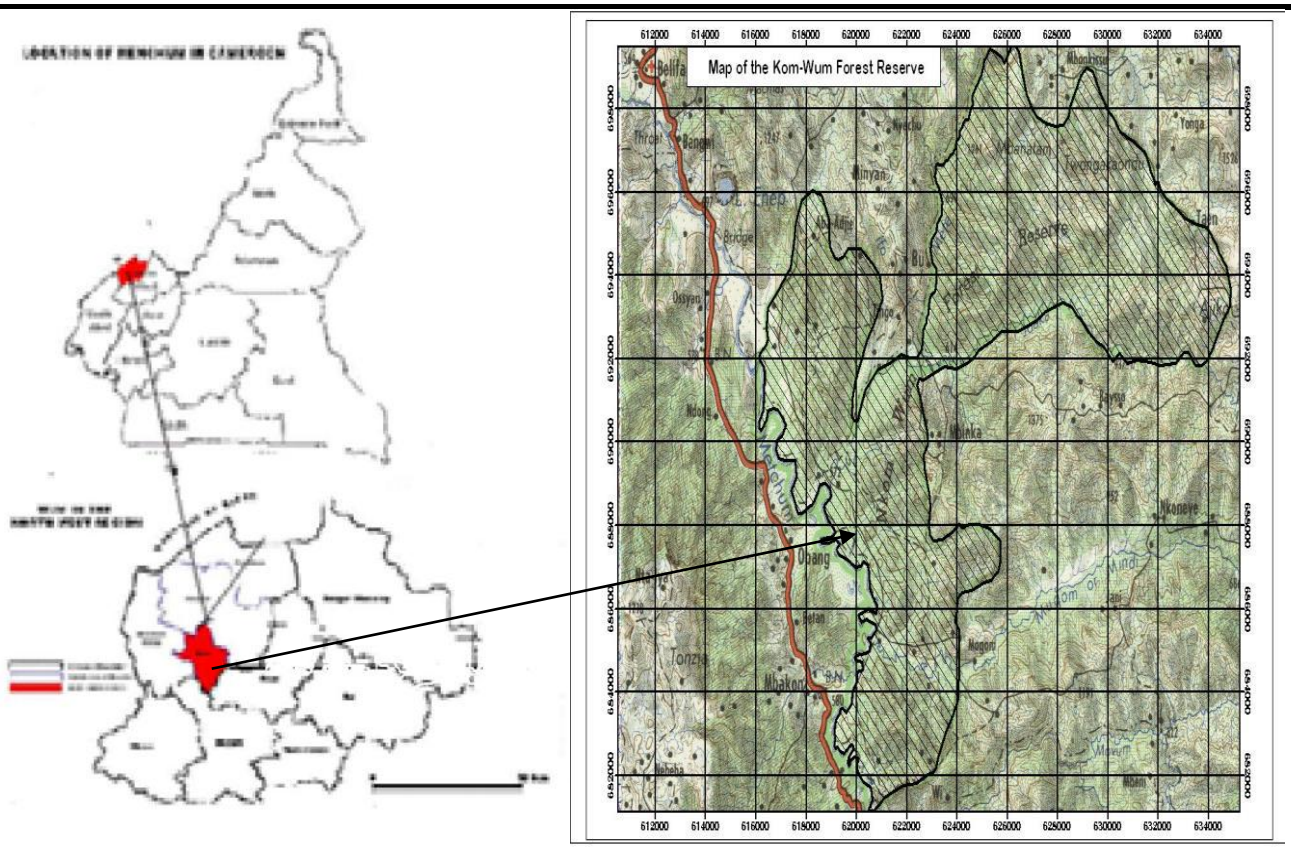

Fig.1: Location of the KWFR in the North West Region of Cameroon (COMINSUD,2011)

2.2. Data collection

Data collection in the Kom-Wum forest reserve was carried out from the 15th of January 2015 to 20th of March 2015. During this period, the "recce walk" was used. A recce is a path of least resistance through an area following a compass bearing (e.g. north-south, southeast-northwest, east-west). The "distance transect method, "despite its wide use (Beck and Chapman, 2008), presents disadvantages which turned to be exacerbated on this study site due to the characteristics of Kom-Wum forest reserve. Firstly, although several factors essential for the transect method (such as the length of line transects, perpendicular distances and their orientation) should be based on data from pilot studies (Buckland *et al*., 1993), no studies on any other mammals have been done in this forest. Moreover, the terrain is mountainous with steep escarpments which made it difficult for transects to be set up. For these reasons, the recce walk method was preferred for this study. The zone was subdivided into quadrates of 2km x 2km giving a total of 23 quadrates. Inside each quadrate, data was collected on recces of 2km long oriented in the East-West direction. A total of 23 recces of 2km each were covered giving a total distance of 46km as shown on the sampling plan on figure 2. Recces were oriented to cut across major vegetation types (, primary forest, secondary forest, gallery forest and Savannah) and drainages feature (rivers and streams) in order to have a representative sample of the reserve. The starting point of each recce was randomly generated using a random number table. A Global position system (GARMIN 62CSx) was used to determine the start and end point of each recce in the field. The "Tracklog" and "Waypoints" of the device were activated. The first element was programmed to record the location every 500m, creating a track which was later transferred to the software Garmin® MapSource®. It also helped the team not to use the same path twice. Unlike the "Tracklog" feature, the "Waypoints" program allowed the researcher to mark independent points. Figure 2 below shows the sampling plan with distribution on Recces in KWFR.

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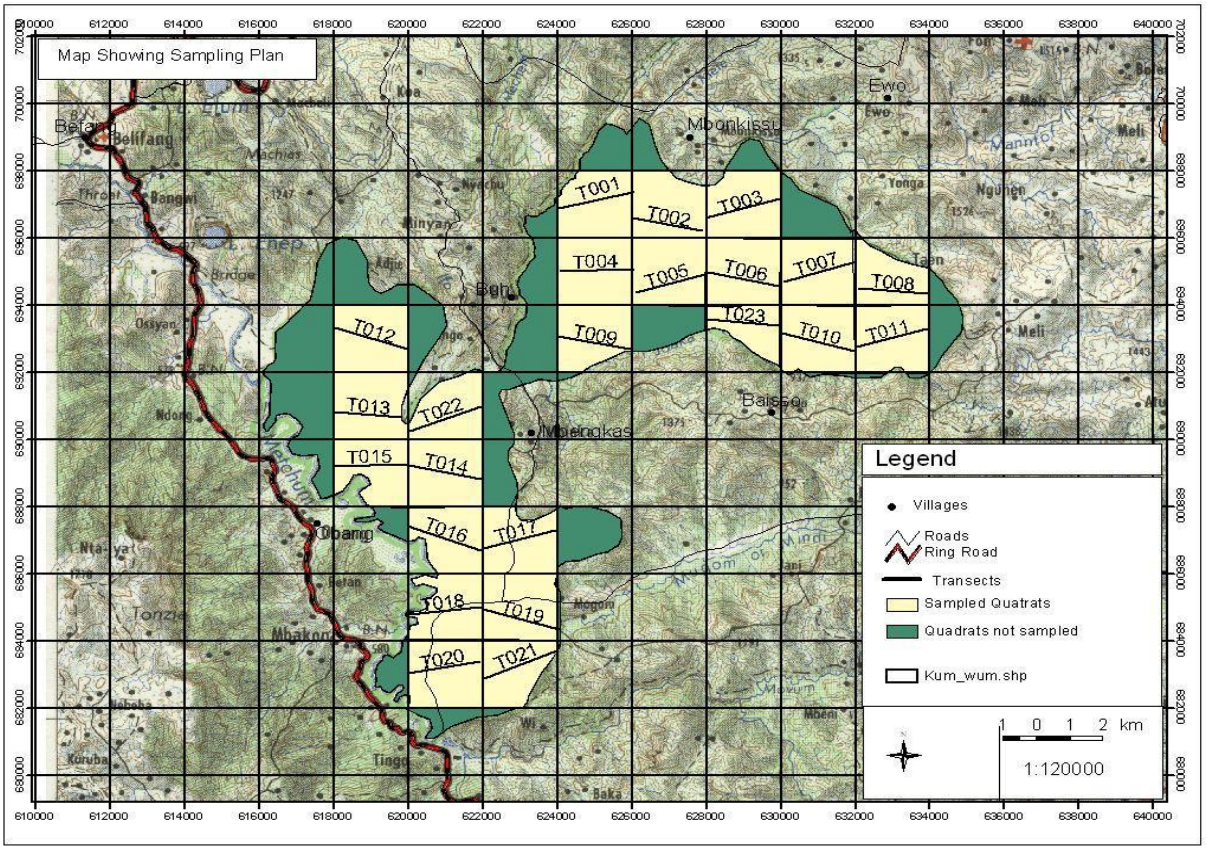

Fig.2: GIS Map showing representation of Recce- transects for animal inventory in KWFR

Data collection was carried out by a team of four individuals: a team leader, two field assistants and one hunter. The team leader carried a compass and GPS to guide the team along recces, the first field assistant carried a pair of binoculars and recorded all observation in a data sheet, the other field assistant helped the team leader in searching for signs while the hunter helped as field guide due to his familiarity with the forest. All mammals sightings, vocalizations, signs (dung, nests, foot prints, carcasses, tracks and food remains) and the signs of anthropogenic activities such as farms (active or abandoned), machete cuts, snares, shot gun shells, honey extraction sites and hunting camps (active or abandoned) along recees were recorded.

Table.1: Shows the stages used for the classification of dung

Index	Observation	Age
Dung:	**Fresh** – boli intact, still warm, strong smell, shiny fatty acid sheen glistering on exterior	1-2days
	Recent – boli intact, odour when boli is break, flies, fatty acid sheen disappear	3-5 days
	Old – no odour, dung form still intact although boli may be partly or completely broken down into anamorphous mass;	6- 14 days
	Very old – dispersed, flattened, tending to disappear	14 days and more

A survey to determine local people perception towards the KWFR was undertaken in five out of the eight villages (Baiso, Mbinkas, Mbonkissu, Bu and Aguilli) with a population of 18.000 inhabitants purposively selected based on their closeness to the reserve. One focus group discussion was conducted per village guided by questions related to animal presence, type of animals hunted, reasons for hunting, animal movement, habitat preference, usefulness of animals, population trend and hunting, traditional role of antelope's meat, awareness of reserve existence as well as the relationships between people and wildlife. Each focus group had at least 6 participants (2 notables, 2 men, a woman and a youth). The turnout of women was very low. The venues for these discussions were at the chief's palace where informants were identified by the chief or quarter head of each village, prior to the administration of questionnaires. Focus group discussions were done on traditional Sundays when most villagers were at home. A wildlife guide for central Africa mammals was used to facilitate the identification of animal species in cases where identification was difficult. Semi-structured interviews were later conducted with every 3 households per village

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depending on the size of each village with the help of an interview guide this was to obtain information on the importance of the reserve, perception about mammal conservation, techniques of hunting and animals hunted by the local population. Two closed ended test questionnaires were designed and administered to two notables in each village after consultation with the chiefs. The aim of this exercise was to identify difficulties and to ensure that the language used was fully understood by respondents before proper administration. A total number of 216 individual out of a population of 18000 were sampled giving a sampling rate of 1.2% (appendix 1).

2.3. Data Analysis

Data collected from the field were summarized and presented using, abundance indices, maps and frequency tables .The Encounter Rate (ER) or Index of Kilometric Abundance (IKA) which represents the total number of observations per kilometer (IKA total = N/L where N is the total number of observations per transect and L is the transect's length in kilometers) was estimated for mammals signs and human activities. The GPS points of Blue duiker and bushbuck indicators and human activities recorded per quadrant were exported to ArcView computer program 3.3 and geo-referenced to produce different spatial distribution maps. The classes of encounter rate were then defined in order to group similar quadrates and represent zones of different concentrations. Different colour bands and corresponding colour intensities were used to represent different encounter rates on the distribution maps. This permitted us to define important zones for mammal species (duikers, bushbuck, chimpanzees etc) in order to determine management strategies for their conservation.

Regression analyses were carried out to test the relationship between the encounter rate of mammals and anthropogenic activities. Encounter rates of these two variables were exported to SPSS (Version17) to produce fitted regression line. The mathematical formula for the coefficient of determination (R^2) and correlation coefficient (r) are given below.

$$R^2 = \frac{\left(\sum XY - \frac{\sum X \sum Y}{N}\right)^2}{\left[\sum X^2 - \frac{(\sum X)^2}{N}\right]\left[\sum Y^2 - \frac{(\sum Y)^2}{N}\right]}$$

$$r = \sqrt{R^2}$$

Where: X: is Anthropogenic activities. , R^2: is the Coefficient of determination

Y: is the Mean encounter rate BD/BB N: is the Number of observation and

r: is the Correlation coefficient.

III. RESULTS AND DISCUSSION

Relative Abundance of Medium to Large Mammals Recorded in KWFR

After the recce walk of 46km, a total of twelve (12) species of medium to large mammals were recorded within the Kom-Wum forest reserve. They belong to five families. The family of Cercopithecidae had the highest number of species represented by 5 species that are the Putty-nosed monkey,(*Cercopithecus nictitans*) (Vervet monkey (*Cercopithecus aethiops*) Patas Monkeys (*Erythrobus patas*) Mona Monkey (*Cercopithecus mona*) and Olive Baboon (*Papio anubis).* These results agree with those reported by (Afuh, 2013) and (Chuo, 2018) who each recorded 14 different species from the Lebialem-Mone-Banyang-Mbo Landscape S.W.R and Black Bush Area of Waindo N.W. R respectively. The Bovidae family followed with four species; the blue duiker (*Cephalophus monticola*), Bushbuck (*Tragelaphus scriptus*) red duiker (*Cephalophus dorsalis*) and Buffalo (*Syncerus caffer*). The families Pongidae, Suidae and Viverredae were each represented by one species; chimpanzee (*Pan troglodytes ellioti),* Red river hog *(Potamochoerus porcus)* and Africa civet (*Viverra civetta*) respectively as seen on table 2 below. The table equally gives the MINFOF current classification of the various animals.

Table.2: Medium to large size mammal species recorded in the KWFR according to family

Family	**Common Name**	**Scientific Name**	**MINFOF Classification**
Bovidae	Blue duiker	*Cephalophus monticola*	**C**
	Bushbuck	*Tragelaphus scriptus*	**B**
	Red duiker	*Cephalophus dorsalis*	**C**
	Buffalo	*Syncerus caffer*	**A**
Pongidae	Chimpanzee	*Pan troglodytes ellioti*	**A**

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Cercopithecidae	Putty-nose monkey	*Cercopithecus nictitans*	**C**
	Vervet monkey	*Cercopithecus aethiops*	**C**
	Patas Monkeys	*Erythrobus patas*	**C**
	Mona Monkey	*Cercopithecus mona*	**C**
	Olive Baboon	*Papio anubis*	**A**
Suidae	Red river hog	*Potamochoerus porcus*	**B**
Viverredae	Africa civet	*Viverra civetta*	**B**

Indices of Mammals Identified in KWFR

The table below summarizes both direct and indirect indices observed in the KWFR.

Table.3: Indices of medium to large mammal species identified in the KWFR

Species	**Indirect observations**							**Direct observations**	**Total**
	D	FP	T	FR	N	C	V		
Blue duiker	111	40	8	_	_	_	_	8	**167**
Bush buck	89	30	6	_	_	_	_	5	**130**
Red duiker	134	47	12	_	_	1	_	11	**205**
Buffalo	11	_	_	_	_	_	_	_	**11**
Chimpanzee	_	_	_	5	174	_	3	_	**182**
Putty-nosed monk	_	_	_	_	_	_	43	80	**123**
Mona Monkey	_	_	_	_	_	_	9	37	**46**
Patas Monkeys	_	_	_	_	_	_	7	1	**8**
Vervet monkey	_	_	_	_	_	_	19	_	**19**
Olive Baboon	_	_	_	_	_	_	32	_	**32**
Red River Hog	_	6	_	_	_	_	_	_	**6**
Africa Civet	11	_	_	_	_	_	_	_	**7**
Total									**942**

Legend: Dung (D), Nest (N), Foot Prints (FP), Tracks (T), Food Remains (FR), Carcass (C), Vocalisation (V)

Both direct and indirect signs were used to identify mammals in the field. Three antelope species were seen directly (blue duiker, bushbuck and red duiker). Monkeys were recorded via direct sightings and vocalizations while Chimpanzees were identified by nests, vocalizations and food remains. Four species of monkeys were seen directly Putty nosed, Mona monkey Patas and Vervet monkeys. Dung was mostly used to identify antelope species (blue duiker, red duikers and bushbuck) because it was very difficult to distinguish them from their foot prints, food remains and tracks. Vocalisations of Chimpanzees (*Pan troglodytes ellioti*) were heard, five groups of Putty-nosed guenon (*Cercopithecus nictitans*), four groups of Mona monkeys (*Cercopithecus mona)*, and 3 groups Olive baboons *(Papio anubis*) were also heard. Dung piles and pellets of blue duiker, Red duiker and bushbuck were recorded within the Kom-Wum forest reserve. Most dung encountered ranged from the ages fresh, recent, with very few old droppings. For the convenience of identification of duiker's presence by signs, closely related duikers such as the black-fronted, Peter's and bay duikers are grouped as red duikers. 174 chimpanzee nest sites were recorded during the survey. Fresh, recent, old and very old arboreal nests were recorded. Most nests were recorded on Recce transects crossing old abandoned roads. Generally, tracks of *Cephalophus monticola* and *Tragelaphus scriptus* were regularly seen close to marshy forest areas.

Relative abundance of medium and large mammals in study area using direct sighting

This refers to animals that were seen directly during inventory. Their encounter rates are calculated on the table below.

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Table.4: Encounter rate of medium to large mammal species sighted in kom-Wum forest reserve

Common Name	Family	Scientific Name	NS	TDC(km)	ER
Blue Duiker	Bovidae	*Cephaluphus monticola*	8	46	0.17
Bush Buck	Bovidae	*Tragelahus scriptus*	5	46	0.11
Red Duiker	Bovidae	*Cephalophus dorsalis*	11	46	0.24
Mona Monkey	Cercopithecidae	*Cercopithecus mona*	50	46	1.10
Putty-nosed monkeys	Cercopithecidae	*Cercopithecus nictitans*	80	46	1.74
Patas monkey	Cercopithecidae	*Erythrobus patas*	1	46	0.02
Mean			**155**	**46**	**0.56**

Legend: Encountered rate (ER), total distance covered (TDC), Number of species (NS**)** 0 = No observation, 0.1- 0.5 = Weak, > 0.5 = High

Up to six species of small to medium size mammals were seen directly in Kom-Wum forest reserve. Eight blue duikers were seen while five bushbucks were seen, 4 groups of Putty-nosed (*Cercopithecus nictitans)* of at least eight individuals, 2 groups of at least 20 individuals and one group of at least 2 individuals were seen during the survey. Other species seen were Patas monkey and three species of duiker**.** In all, the total numbers of duikers seen were higher for red duiker then blue duiker while the total number of individual primates seen per kilometre was highest for Putty-nosed, followed by Mona monkey and then Patas monkeys. Vervet monkey and chimpanzee were not seen, there were only heard. The encounter rate of blue duiker (0.17) and bushbuck (0.11) from direct sighting is therefore weak in KWFR since the ER is Weak between 0.1- 0.5 and high when ER > 0.5 = (Tsi *et al*, 2006). Generally, the mean encounter rate of medium to large size mammals seen directly was high (0.56 Sign/km).

Relative abundance of medium to large mammals using indirect indices

After 46 km survey effort on these recces, a total of 774 indirect signs were recorded. The table below shows the relative abundance of medium to large size mammals in Kom- Wum Forest Reserve.

Table.5: Encountered rate of indirect signs of medium to large mammal species recorded in KWFR

Common Name	Family	Scientific Name	TNI	TDC (km)	ER
Blue Duiker	Bovidae	*Cephalophus monticola*	111	46	2.41
Bushbuck	Bovidae	*Tragelahus scriptus*	89	46	1.93
Red Duikers	Bovidae	*Cephalophus dorsalis*	134	46	2.91
Buffalo	Bovidae	*Syncerus caffer*	11	46	0.24
Chimpanzee	Pongidaea	*Pan troglodytes ellioti*	174	46	3.78
Putty-nosed monkeys	Cercopithecidae	*Cercopithecus nictitans*	43	46	0.93
Mona Monkey	Cercopithecidae	*Cercopithecus mona*	37	46	0.80
Vervet monkey	Cercopithecidae	*Cercopithecus aethiops*	19	46	0.41
Olive baboon	Cercopithecidae	*Papio anubis*	32	46	0.70
Red river hog	Suidae	*Potamochoerus porcus*	6	46	0.13
Africa civet	Viverredae	*Viverra civetta*	11	46	0.24
Patas monkey	Cercopithecidae	*Erythrobus patas*	7	46	0.15
Mean			**774**	46	**1.22**

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Table 5 above shows that chimpanzees (3.78sign/km) were most abundant mammals in KWFR followed by the red duikers (2.91sign/km), blue duiker (2.41sign/km) and then bushbuck (1.93sign/km). The red river hog was the least abundant mammal with an encounter rate of 0.1sign/km. The red duiker was the most abundant species in the family Bovidae. While the chimpanzee was the most abundant primate recorded in the KWFR. The overall Relative Density of large mammals in the Kom-Wum forest reserve was estimated to be 1.22 signs per km (Table 5). In other words, one would identify at least one medium to large mammal sign for every kilometer covered in the study area. Kom-Wum Forest Reserve appears therefore to be poor in mammals.

Geo-Spatial Distribution of Medium to Large Size Mammals in KWFR

Generally, the distribution of medium to large Mammals species in KWFR is highly affected by vegetation type (primary forest, secondary forest, gallery forest and open savannah). Chimpanzees, Mona and Putty-nosed monkeys prefer mature forest; most chimpanzee nests were sighted at high altitudes with very poor topography. Blue duiker, Patas and Vervet monkeys prefer open savanna, forest edges and gallery forest edges while Bushbuck prefer secondary forest with thick under growth gallery forests, they could also be sighted along water sources. According to Tsi *et al.*, (2006) determining animal distribution permits managers and researchers to locate protected and unprotected areas of high biological diversity targeting specific areas for protection or areas to allow improved management.

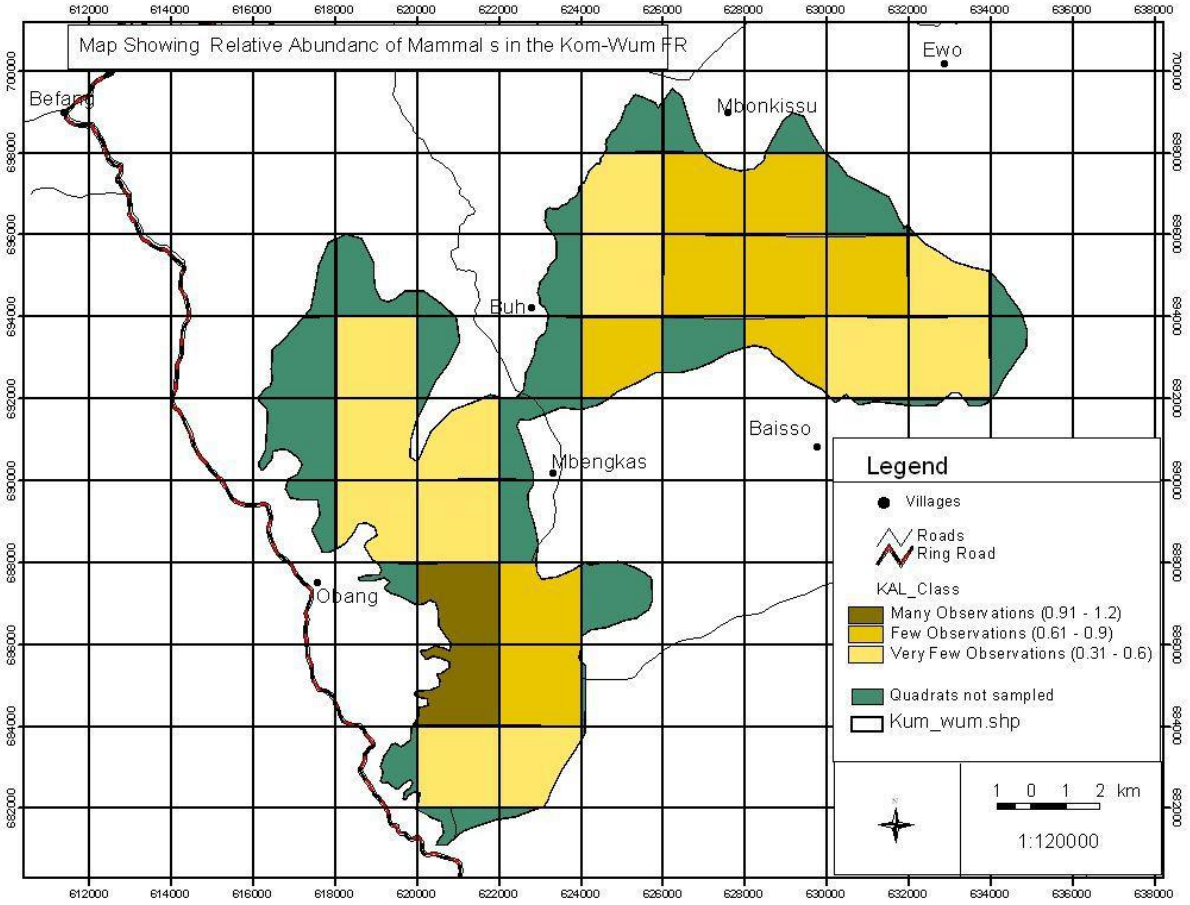

Fig.3: GIS map showing geo-spatial distribution of medium to large mammals in KWFR

Figure 3 shows that most mammals' species are abundant in the North East section of the reserve. The highest population concentration is found in a small portion in the South West section of the KWFR. This is a biodiversity 'hotspot'. High concentrations here could be attributed to the rough nature of the terrain that limits human interference, the presence of River Menchum that acts as a natural barrier confining animals around this area. The North West potion is generally poor in animals probably due to high anthropogenic activities from the high population of Bu village (8000 inhabitants).

Spatial Distribution of Blue Duiker in KWFR

Food availability, habitats and preponderance of predators greatly affected the distribution of blue duiker and bushbuck in KWFR. Figure 4 below the spatial distribution of Blue duiker in KWFR.

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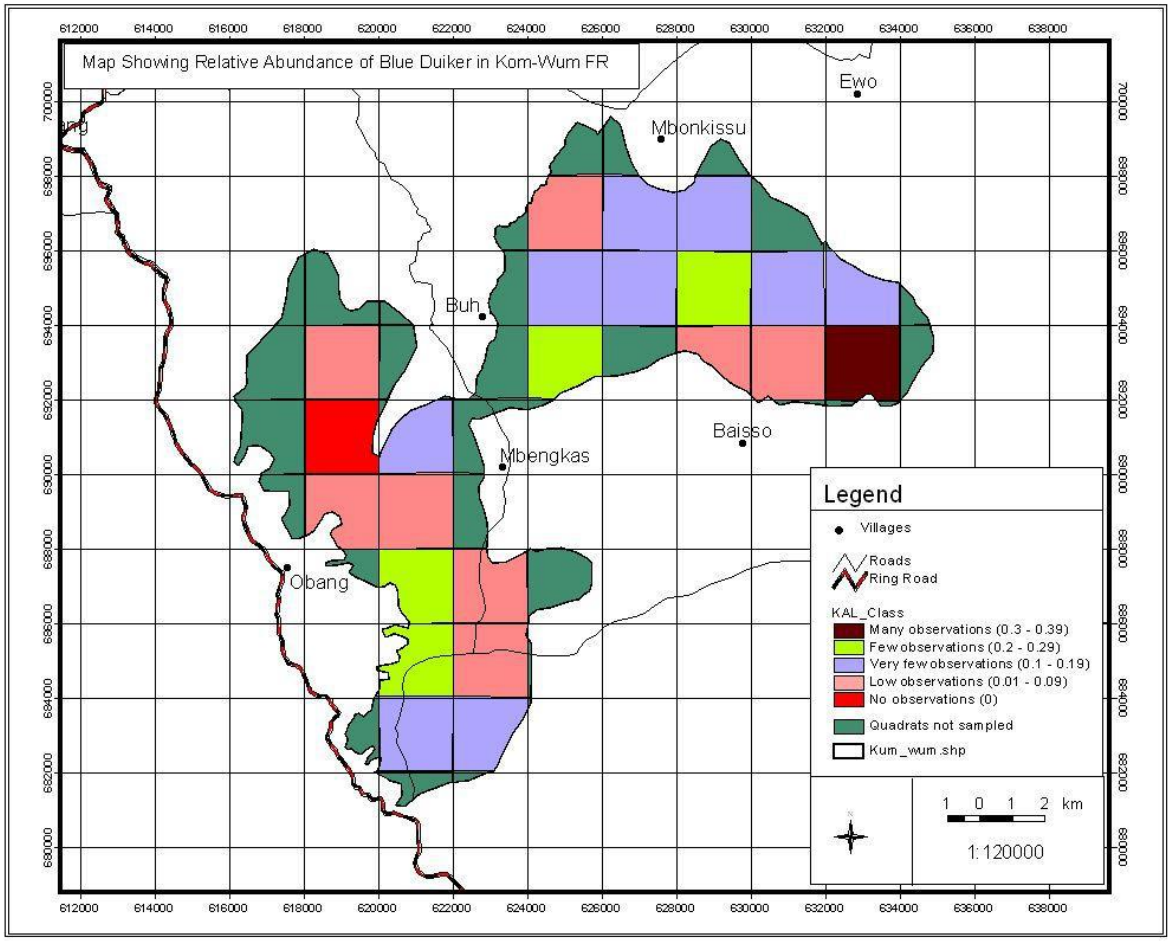

Fig.4: GIS map showing spatial distribution of Blue duikers in the KWFR

Figure 4 above shows that blue duikers have high relative densities in the North east (ER=0.3-0.39). Few observations were recorded in the South West and central potions of the reserve. This could be attributed to the presence of their food, habitat suitability and absence of predators. They have high relative densities in open savanna vegetation bordering gallery forests with fruit trees. Blue duikers showed low densities in primary forest.

Geo-Spatial Distribution of Bushbuck in KWFR

The spatial distribution of bushbuck in Kom-Wum forest reserve was also influenced by availability of food, habitat presence and preponderance of predators (figure 5).

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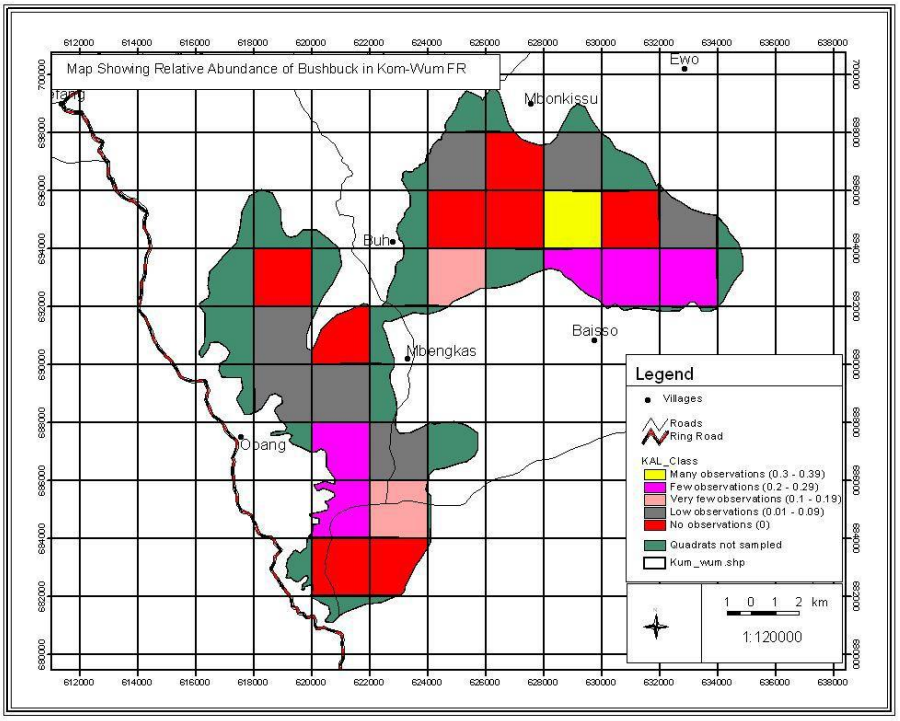

Fig.5: Geo-spatial distribution of Bushbuck in KWFR

Bushbuck had high densities in the North East mostly around swampy areas and in vegetation dominated by young to mature trees, with an under storey more or less dense. Few observations were also noticed around the peripheries of the East and South West. They could also be found in the swampy forest galleries and along water courses.

Anthropogenic Activities in KWFR

Abundance of Anthropogenic

Rapid population increase in and around the reserve over the past two decades has as consequence an increase demand for forest wood, NTFP, animals for food, farming land etc this has tremendously increase the rate of forest degradation. Anthropogenic activities were grouped into three main types, hunting, agriculture and logging. Figure 6 shows the different anthropogenic activities recorded in the KWFR

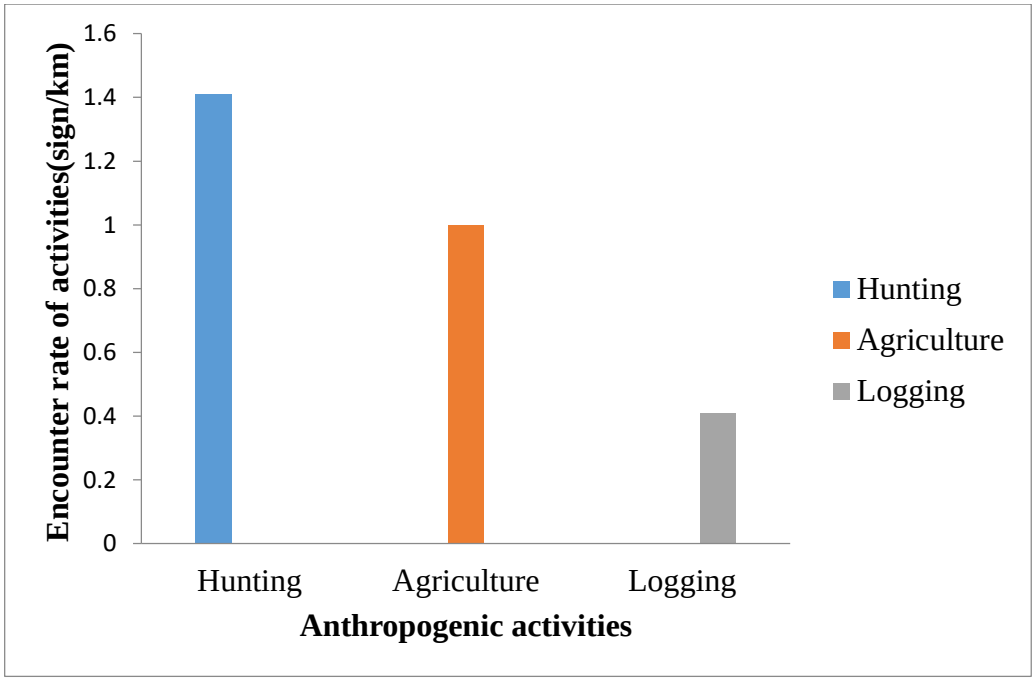

Fig.6: Anthropogenic activities recorded in KWFR

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Figure 6 above shows that hunting (1.41sign/km) (50%) was the most prevalent activity in the KWFR closely followed by agriculture (1.00sign/km) (36%) and then logging (0.41sign/km) (14%). The setting of snare traps was the most common form of hunting in the kom-Wum forest reserve. It is followed by the presence of shotgun shells, and then hunting camps, other signs (abandoned dresses, fireplaces, dishes, and honey extraction sites) and logging activities. Hunting is at the moment the only lucrative means through which the local people derive direct economic benefits from the forest. Wildlife species do not only provide an important source of protein but also a major source of income for the local people surrounding the concession. Wire snare trapping was observed as the most common form of hunting where mostly artiodactyls (duikers, bushbucks and brush-tailed porcupines) are captured and represent the most important species in terms of income. Encounter rates of abandoned cable snares were high especially along hunting tracks. However, the highest percentage of all primate captured in KWFR is made by the use of shotguns. These results agree with those recorded by Fotang, (2014) from Mbi crater and Ekobo (2008) 1.46 sign/km from Nguti Council forest S.W.R of Cameroon.

Geo-spatial distribution of anthropogenic activities

Figure 7 shows the spatial distribution of the different anthropogenic activities identified in the KWFR

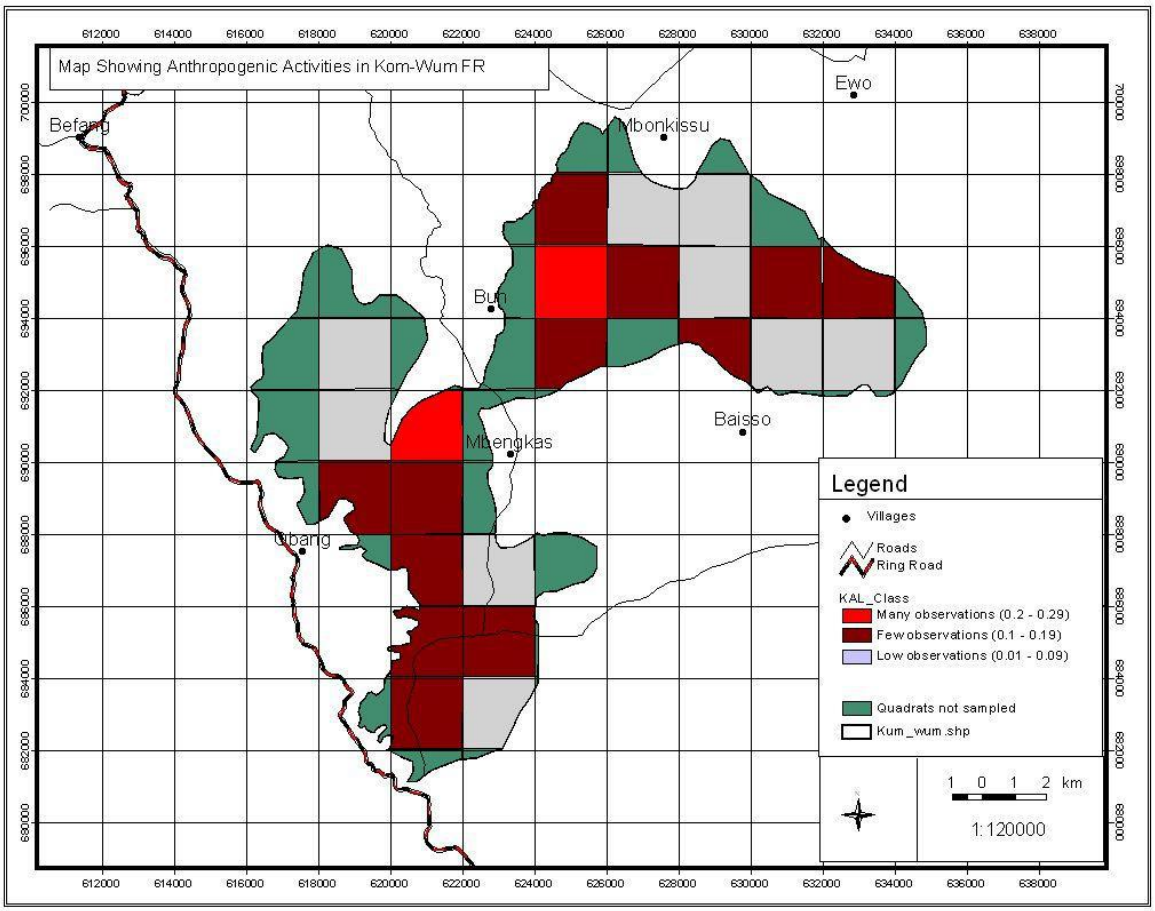

Fig.7: GIS map showing spatial distribution of anthropogenic activities in KWFR

The map above shows that anthropogenic activities are high in the South West and North of the KWFR. Logging had high densities in the Centre Southwest around Moghom while trapping and gun hunting had high relative densities in the North East of the reserve around the villages of Obang and Mbakong (lower Bafut). This could probably be explained by the high population density on the Bamenda-Wum stretch of the ring road. Cocoa, banana, corn and plantation farms were common in the north east of in the reserve.

Effects of anthropogenic activities on the distribution of mammals in the KWFR

Using the encounter rate of mammals and anthropogenic activities, the coefficient of determination R^2 was calculated. The scatter diagram of the fitted regression line for the encounter rates of medium to large size mammals and anthropogenic activities is presented on figure 8.

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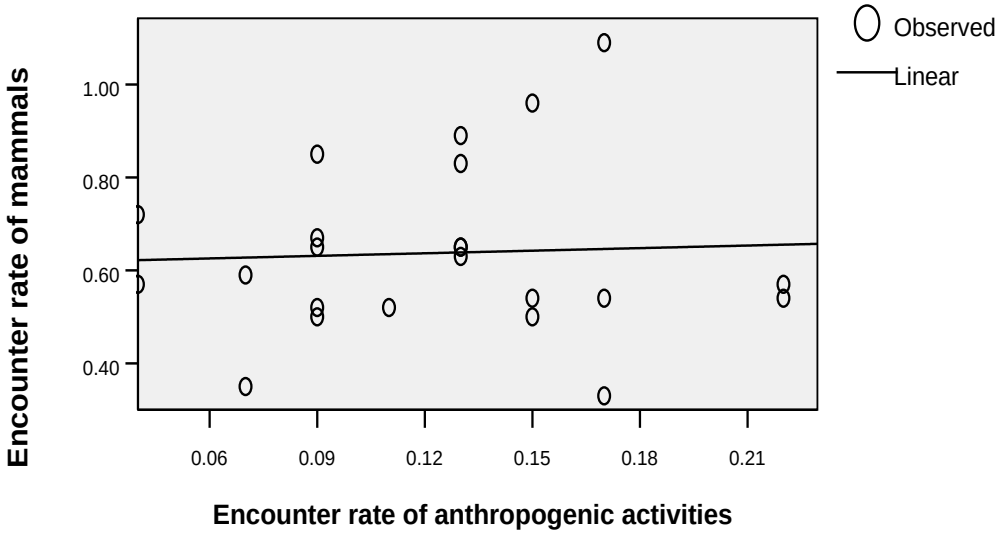

The equation= a+b1X+B2X+B3X + Error

$$Y = 0.587 + 0.885X1 - 0.294X2 + 0.498X3 + 0.107$$

Where Y=Mammal, X1=Hunting, X2=Agriculture, x3=Logging

Fig.12: Fitted regression line of the encounter of mammals and anthropogenic activities in the KWFR

Figure 8: above shows a weak relationship between the medium to large size mammals and human signs in KWFR. This coefficient of determination (R^2 = 0.058) shows that only 5.8% of changes in mammals distribution are provoked by changes in hunting, agriculture and logging. Fonkwo *et al.* (2011) also had slightly similar results in the Bakossi landscape where only 2.33 % of variation in mammal distribution was provoked by a variation in anthropogenic activities. Fotang (2014) reported an R^2 of 0.375 from Mbi crater in the North West region. Among these anthropogenic activities, hunting has the highest effect on the distribution of medium to large size mammals in KWFR followed by logging and then agriculture as shown on the regression equation. Hunting using snares had the highest influence with an ER of 1.4sign/km (50%).This is in line with Ekobo (2008) who reported an. 1.46 sign/km for hunting from the Nguti Council forest S.W.R of Cameroon.

Perception of Local Population towards KWFR

Understanding local peoples' perception is key to improving relationship between people living in protected areas or reserves and management because it can provide a guide for policy decisions (Hill, 1998). The Kom and Wum people have their own picture on KWFR.

Educational Level of respondents

The level of education of a respondent has a remarkable effect on his/her perception of the conservation of biodiversity (Mc Clanahan *et al.*, 2005) Figure 9 below analyses the level of formal education of respondents.

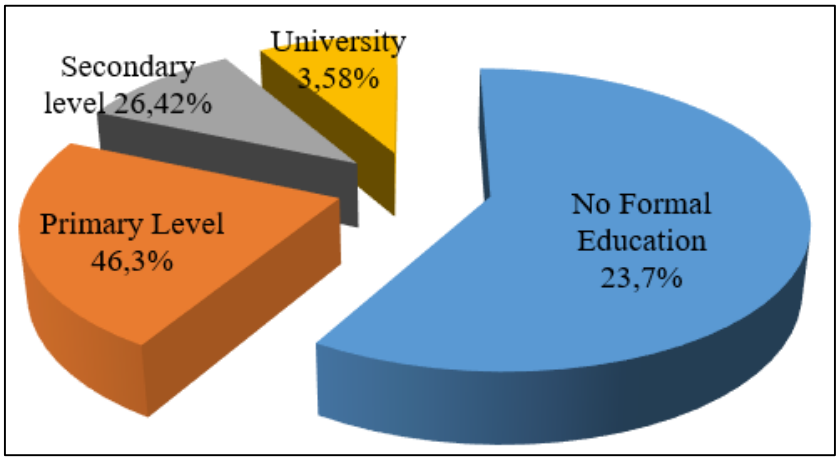

Fig.9: Educational level of the Respondents

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From figure 9, 76.3% of the respondents were literate. The bulk of the literate people ended at the level of primary school. This was noticed in their inability to fill questionnaires. This result agrees with those reported by Fotang (2014) from Mbi Crater who recorded 80.7%.

Occupation of Respondents

The occupational structure of the people living in and around the KWFR has an effect on the people's activities and perception vis-à-vis the forest. Figure 10 below shows the occupation of respondents below.

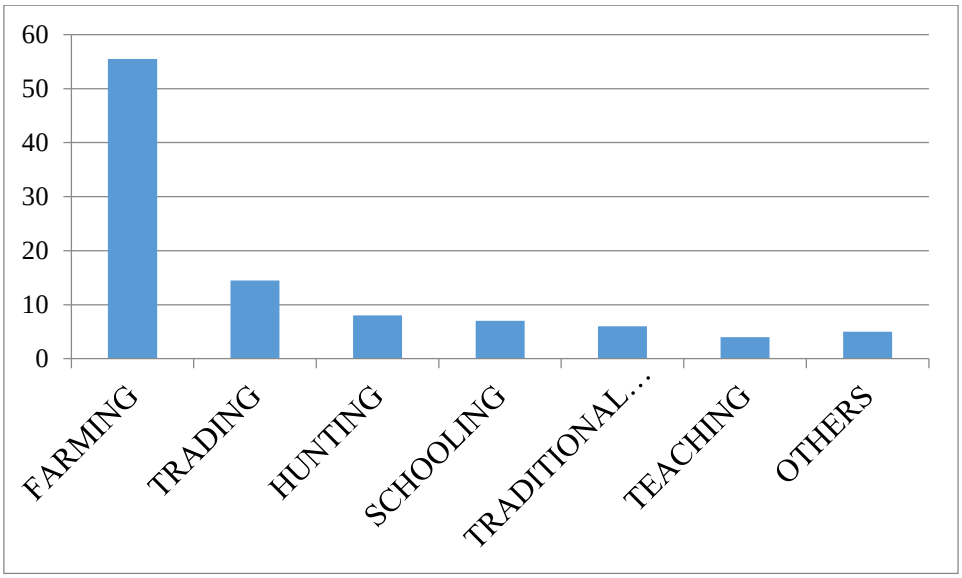

Fig.10: Distribution of respondents by occupation

More than half of the population in and around the reserve is farmers (55.5%). This reveals that farming is an important economic activity in the area. Encroachment into the reserve is therefore eminent if appropriate measures are not taken. After farming, the next economic activity is trading (14.5%). Hunting is equally an important activity although is represented by only 8% of the respondents. This could probably be because some hunters did not want to identify themselves as hunters for fear of the unknown. 7% were students this depicts the high illiteracy levels that were noticed during discussions with the population.

Awareness, ownership and control of KWFR

What the people of Fundong and Wum perceive as to who owns and control the KWFR is very important for its conservation. Figure 11 below shows findings concerning the Kom–Wum indigenes perceptions on awareness, ownership, and control of the reserve.

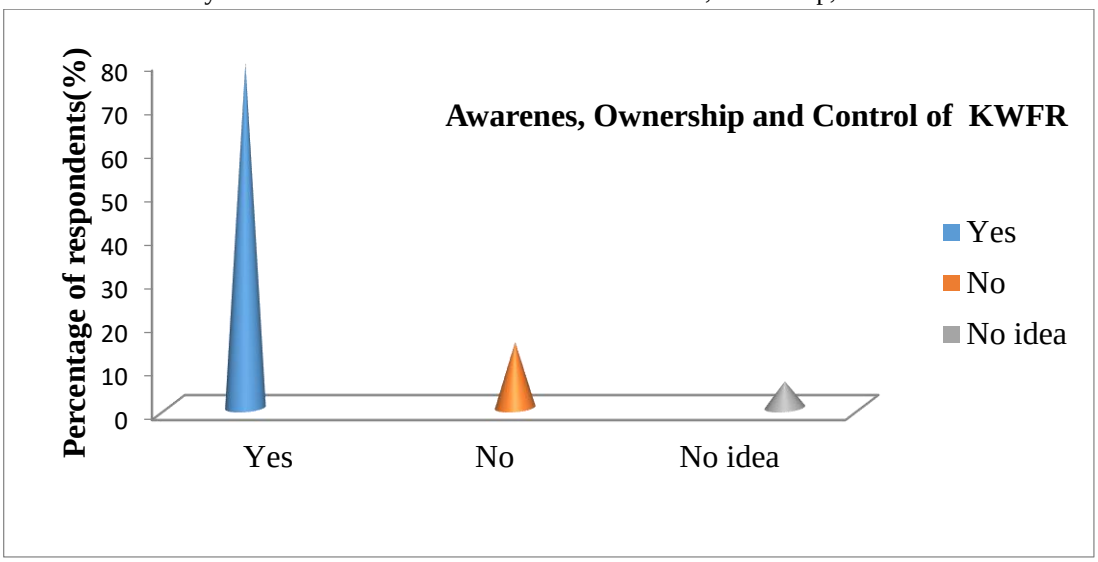

Fig.11: Awareness, ownership and control of KWFR as perceived by local population

Figure 11 reveals that a vast majority of respondents (80%) were aware that the reserve is owned and controlled by the government of Cameroon. They also indicated that they have access but do not respect the

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boundaries which prohibit entering and hunting in the reserve. Access here is due to the absence of law enforcement officers in the reserve (Forest guards). Focus group discussions further revealed that ownership of the reserve was perceived as vested on the government though the presence of government authorities is not felt. The recent handing over of the reserve to the councils of Wum and Foundong has however changed the situation as boundary demarcation has been done and reforestation in the degraded North West potion.

Attitude and willingness to participate in the conservation of mammals in KWFR

Free acceptance of indigenous people in conservation ventures usually facilitates the task of management (Tsi *et al*., 2006) as objectives are easily attained. Unwillingness of some stakeholders like indigenes frustrates conservation efforts. The figure 12 summarizes the altitudes of people in and around the KWFR.

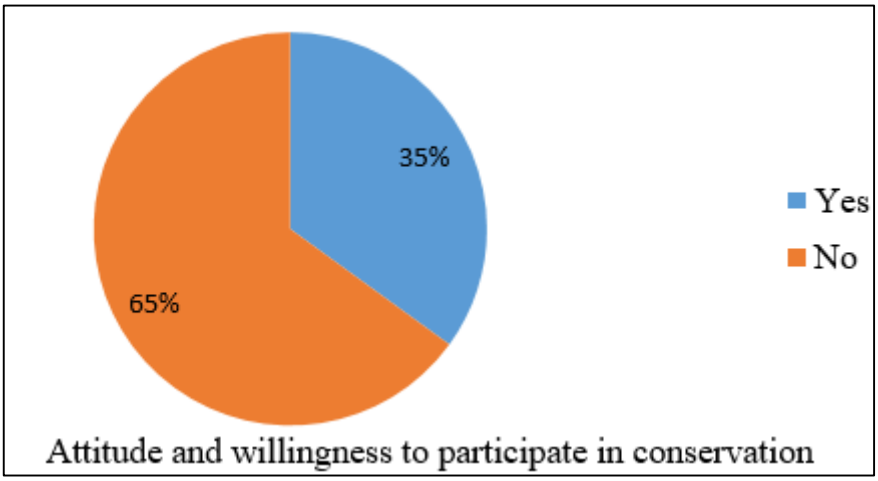

Fig.12: Attitudes of the respondents towards conservation

The results show a most (65%) of the respondents held negative attitudes towards the conservation of resources in KWFR. This could be attributed to high levels of illiteracy, increased number of crop farmers demanding more farmland, low participation in conservation awareness programs and past experience of human wildlife conflicts. Many crop farmers complained that animals especially monkeys (destroy maize) and civet (eat up domestic fowls around the village) are destructive. Almost all hunters interviewed had negative impressions concerning conservation. Conservation of wild life according to them will deprive them of their livelihood as they cannot have access to the fertile soils in the forest.

Animal frequently hunted in KWFR

A question was designed to find out the animals commonly hunted for bush meat. The results are presented on figure13 below.

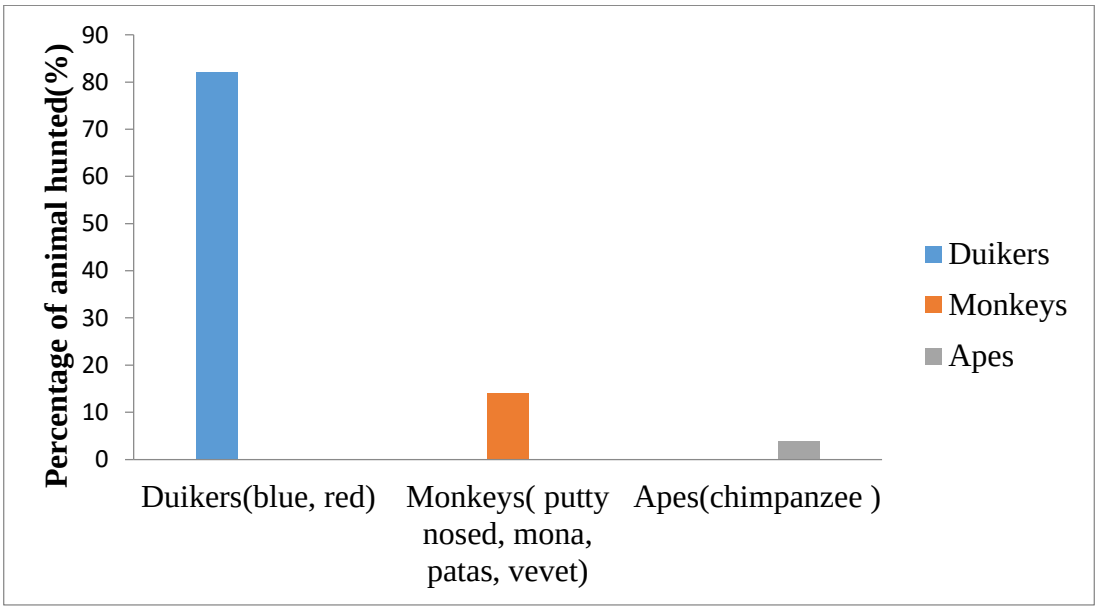

Fig.13: Animals hunted for bush meat in KWFR

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From the figure above, duikers (blue duiker and red duikers) were the most hunted animal species. This was followed by Monkeys (putty nosed, mona, and patas) and then chimpanzee appearing as the least hunted animals species. Results during focus group discussions revealed that the duikers were ceremonial species, highly demanded during the royal hunt festivals where they are used in rituals and in the preparation of special dishes. This high demand was also related to their use in marriage, death and birth celebrations. Similar results were recordents by Lahm, (1993) in three villages of North-eastern Gabon where artiodactyls (Bushbuck and Blue duiker) accounted for 57.5% of animal hunted with the Blue duiker being the most common species hunted by villagers. During focus group discussions, respondents said that Chimpanzees are not hunted because the penalty reserved for culprits is exile in the villages of Mbengcas and Mbakong .A few also mentioned the firm prison sentence some responding said the chimpanzee flesh is very hard, very difficult to cook and has a lot of long bones. This may be the reason for viable populations of chimps in the area. Others regard monkeys as totems and that when killed the person concern will die. Other reasons like chimpanzee and monkeys are difficult to die and have human feelings were also raised. Therefore, taboos, taste and availability are factors that greatly affect bush meat preference and consequently hunting level for wild animals in the study area.

Perceptions on the economic potentials of the KWFR

The people of the KWFR perceive three major economic benefits from the KWFR.

Table 6 below shows the economic benefits perceived by the population living around the KWFR.

Table.6: Economic benefits perceived by respondents

Economic benefits	**Percentage (%)**
Trees for timber	38.8
Non timber forest products	31.2
Touristic potential	18.65
Provision of meat (protein source)	11.35

The table above reveals that most respondents (38.8%) consider timber products as the most important economic benefit they derived from the KWFR. Thirty percent (31.2%) said they harvest bush pepper, rattan cane, Jangsang, bush mango, medicinal plants. They also hunt animals like chimpanzee, monkeys and antelopes in general for their protein source. They build houses with timber from the forest. They don't buy timber from elsewhere. There are numerous touristic attractions that are likely to boost the development of tourism in the area. It has the famous waterfalls and cascades in highlands, a rich, unique and diverse cultural heritage in neighboring villages, attractive landscape and flagship species. It is one of the best reserve still harbouring suitable habitats for chimps with the highest number of chimpanzee (Morgan *et al*., 2011; Chuo and Tsi, 2017e) in the North West region as compared to Fungom, Mbember and Kimbi game reserve. Flagship species are species that can be used as the focus of a broader biodiversity conservation marketing campaign based on its possession of one or more traits that appeal to the target audience. Though ecotourism flagships are frequently charismatic megafauna, which are aimed at attracting tourists such as the giant panda (*Ailuropoda melanoleuca*) and the African elephant (*Loxodonta africana*), Kom and Wum people tend to appreciate species that have strong cultural or local values. These include primates such as the chimpanzee (*Pan troglodytes elliotti*) and birds such as Barnama tauroco (*Tauroco bannermani*) and green tauroco (*Tauroco persa*). Feathers of the Tauraco are widely used in the region for cultural activities such as traditional dances.

IV. CONCLUSION

The results of this study show that 12 species of medium to large mammals were recorded with one flagship species, the Chimpanzee. Chimpanzees, Red duikers and Blue duikers are the most abundant animals in the reserve with encounter rates of 3.78, 2.91 and 2.41 respectively. The mean encounter rates of mammals in the reserve stood at 1.22 sign/km meaning that one will expect to see at least one mammal for every kilometer covered in the reserve. The relative densities of Blue duikers and Bushbucks were high in the North East and South west respectively. Anthropogenic activities were classified under hunting, agriculture and logging and with an encounter rate of 0.94 sign per kilometer. Hunting was most preponderant with an E.R of 1.41 sign per kilometer (50%). Encounter rates of Anthropogenic activities plotted with those of mammals through regression analyses gave a coefficient of determination of R^2=0.058 (5.8%) meaning mammals distribution is only slightly affected by human activities. These results revealed that the local people in and around the KWFR (65%) have negative attitudes towards wildlife conservation. They expressed strong utilitarian attitude with little or no

ecological sentiments towards the reserve. As a result, local people perceive wildlife conservation as a problem rather than an economic and social status advantage, thus making wildlife conservation efforts to be perceived as contradictory to socio-economic welfare of the local communities. In fact, some youths in Baiso and Mbonkessu villages vehemently declared that "conservation is the white man's idea". The species richness of this forest is low thus the status as a community forest is okay for now. More conservation effort has to be mobilised so as to conserve the natural resources in this reserve.

V. RECOMMENDATIONS

To research institutions

- Research on the ecology and distribution of chimpanzee in the Kom- Wum Forest Reserve should be carried out since they are the most abundant mammals.
- Study the status of blue duiker and bush buck in the rainy season to compare the results with those obtained during the dry season.

To the government

- The management status should be reviewed because KWFR has recently been handed over to the councils of Wum and Fundong. The surface area is too big for the council to effectively manage.
- Train and deploy forest guards and related forest management staffs so as to enforce legislation.

To non-governmental organization

- Income generating activities should be sponsored so as to diversify the economy and deter local inhabitants from encroaching into the forest.
- Encourage the rearing of domestic animals like goats, cattle, fowls etc.

CONFLICT OF INTEREST STATEMENT

We declare that there is no conflict of interest regarding the publication of this paper.

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I will like to extend great thanks to all those who contributed in one way or the other to the success of this work. Firstly, to God Almighty for giving me courage and determination will to move on with this work despite all difficulties. Immense thanks and appreciation to the councils of Wum and Fundong and wildlife authorities that harbor the Kom-Wum Forest Reserve and their dynamic staff for their encouragement and provision of secondary data. Sincere gratitude to the chiefs and communities of Baiso, Mbonkesu, Bu, Agulli and Mbinkas for their cooperation and unconditional support during my field work. God will certainly bless the hunters of these villages for guiding me through the thick forest during inventory.

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Appendix: Field Pictures

Old dung bushbuck

Fresh dung of Bush buck

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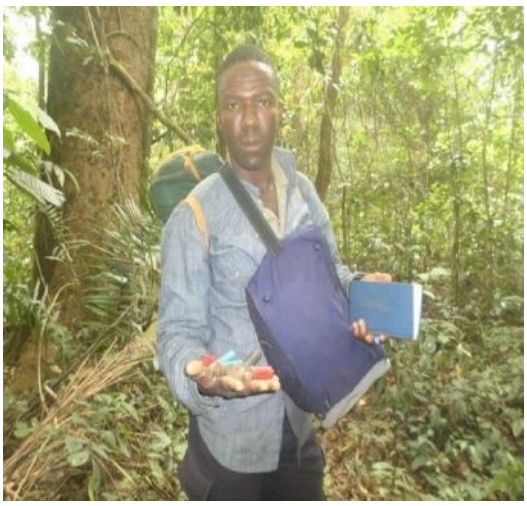
Fresh dung of blue duiker

Chimpanzee nest



Researcher with used cartridges

Discussions at Baisso village

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An Agent-Based Computer Simulation on Banana Bunchy Top Disease

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***Abstract*—** *Banana Bunchy Top Disease* (*BBTD) is an aphid-transmitted virus disease of banana plants. Banana Bunchy Top Virus (BBTV) is the causal microorganism of this disease. Infected plants rarely produce a fruit bunch after infection and do not fruit in subsequent years. This paper studied the factors that defined the rate of BBTD spread in this 2 X 3 factorial simulation experiment. Results indicate that within four months at the onset of infection, the presence of vector aphids with mild (25%) and severe (75%) infectiousness of BBTD can infect slightly more than half and almost all banana plants in a hectare plantation, respectively.*

***Keywords*— banana plants, Banana Bunchy Top Disease (BBTD), Banana Bunchy Top Virus (BBTV), mortality, infectiousness, symptoms.**

I. INTRODUCTION

The word "banana" is a broad term embracing some species or hybrids in the genus Musa of the family Musaceae. The banana plant is a large herb having a pseudostem with a cylinder of leaf-petiole sheaths reaching a height of 20 to 25 feet originating from a fleshy rhizome or corm. They are primarily produced for their fruit (technically a 'berry') (Morton, 1987).

Bananas are among the world's major food crops. It is considered as the poor man's fruit crop in tropical and sub-tropical countries. They are essential plants in the subsistence diet of the poor millions. They are also important export commodities of some developing countries in Africa, Latin America, Asia and the Pacific Regions (FAO, 2004). However, its production is hampered by pests and diseases such as the Banana Bunchy Top Disease (BBTD).

BBTD is an aphid-transmitted virus disease of banana plants. Banana Bunchy Top Virus (BBTV) is the causal microorganism of this disease. Leaves formed after infection are narrow, short with upturned margins and become stiff and brittle. The leafstalks are short and unbending and remain erect, giving a 'rosetted' appearance. The leaves of suckers and three (3) youngest leaves of mother plant show yellowing and waviness or margins. The youngest leaves exhibit very narrowly, dark-green, interrupted ("dot-and-dash") lines on the underside (Morton, 1987).

Diseased plants rarely produce a fruit bunch and do not fruit in succeeding years. Plants infected late in the growing period may fruit once, but the bunch stalk and the fruit will be small and distorted. In plants infected very late, the only symptoms present may be a few dark green streaks on tips of flower bracts (Thomas et al., 1994).

Studies above have shown that the spread of BBTD in a banana plantation can be influenced by several factors. These factors include sanitation, the infectiousness of BBTV and population of aphid vectors.

The model of the study was generated from the software called NetLogo (version 5.2.1) (Wilensky, 1998), an agent-based programming language and integrated modeling environment (Kornhauser et al., 2007). The study aims to define BBTD spread on banana plants in a hectare plantation.

II. MODEL DEFINITION

The BBTD Model relies on the standard planting density of banana plants in a banana plantation, standard labor requirements, management practices, etc. It identifies the potent infecting nature of BBTD where it can infect 100% of banana plants.

The model relies on the following basic assumptions:

- that banana plants are planted in a standard planting density (2,000 hills/hectare)
- that aphids are vectors of the disease and are already present in the plantation
- that aphids can transmit BBTV in 2 hours
- that BBTD can infect 100% of the plants
- that symptoms could appear about a month after infection
- that mortalities could be observed about 4-6 months after onset of symptoms

The model simulates the scenario starting with the aphid vector that gets inside the banana plantation. The aphid with the BBTV infects a healthy banana plant. The banana plant acquires the virus and eventually shows

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the symptoms. Other aphids feed on the diseased plant and acquire the virus. They then infect other healthy plants as they forage for nutrients. This scenario is shown below:

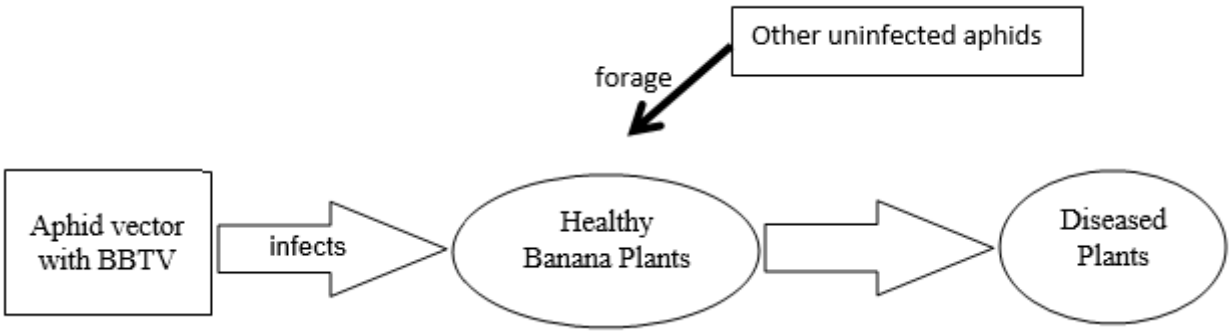

Fig.1: Schematic Diagram of the Scenario

PARAMETERS

This study used existing Virus Model of Uri Wilensky, found in the net logo models library with the following changes in parameter definition, shown in Table 1.

There are four (4) parameters that are included in the Virus Model. These parameters are Number People, Infectiousness, Chance-Recover and Duration.

One (1) parameter was added to the BBTD Model. The parameters in the BBTD Model are Initial Number of Hills, Density of Vector Aphids, Infectiousness, Chance-Recover, and Duration.

The Density of Vector Aphids was included because it is a means wherein BBTD infection could be disseminated in a plantation.

Table.1: Analysis on the Parallelism of Parameters Used in Different Model

Parameters in Virus Model	Parameters in BBTD Model
Number People	Initial Number of Hills
	Density of Vector Aphids
Infectiousness (%)	Infectiousness (%)
Chance-Recover (%)	Chance-Recover (%)
Duration (weeks)	Duration (weeks)

III. RESEARCH DESIGN AND METHODS

In this study, the variable of interest is the percentage of banana plants infected with BBTD virus at any given time. This shows the virulence of BBTD and its infection rate in a hectare plantation.

Since the study suggests that the rate of BBTD spread is influenced by the density of vector aphids and its infectiousness, we set up a computer simulation experiment. In this simulation, we controlled the indicators that are necessary for the movement of BBTD such as (A) density of vector aphids and (B) infectiousness and observed the percentage of BBTD infection under each combination of indicators. Chance-Recover and Duration indicators were fixed at 25% and 52 weeks, respectively.

Indicator (A) density of vector aphids is set at three (3) levels: low (3%), medium (10%) and high (30%). Indicator (B) infectiousness is set at two (2) levels: mild (25%) and severe (75%). An initial number of banana plants was fixed at 2,000 hills.

This experiment is a 2 X 3 factorial. This factorial experimental design yields six (6) treatment combinations. Thirty (30) observations were generated to allow approximation of interaction effects of various levels of indicators. This brings the total sample size to 180 random observations.

For each combination of the two factors, the simulation generates an initial number of infected banana plants. This number is obtained from a uniform probability distribution on the interval [3, 10 and 30 for (A) and 25 and 75 for (B)].

The data obtained were subjected to a two-way analysis of variance (ANOVA) with interaction effects. Two (2) main effects were measured. A and B interaction effects were observed which was A X B. Each of these effects specified the combination of indicators that led to faster BBTD infection rates.

IV. RESULTS AND DISCUSSION

Table 2 shows the data collected from the simulation of BBTD infection rate in banana plants using the two indicators.The Density of Vector Aphids (A) with three (3) levels and Infectiousness (B) with two (2) levels.

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Table.2: Data Collected from the Simulation of Agent-Based Model on BBTD in Bananas

		Density of Vector Aphids (A)														
		Low (3%)					Medium (10%)					High (30%)				
Infectiousness (B)	Mild (25%)	41.9	39.9	56.8	65.4	57	62.4	64.4	60	51.8	57.1	52.8	52.8	55.3	59.6	43.2
		67.9	60.9	48.1	48.9	56.3	46.4	60.8	52.6	49.1	62.1	50.1	65	58.5	59.2	49.3
		54	53.5	66.2	45.1	49.9	65.6	49.8	45.3	66.9	41.1	53.8	53.9	51.4	51.2	57.2
		52.6	53.5	48.2	49.5	51.9	60.9	50.7	49.5	60	49.3	54.2	60.7	65.6	52.8	65.7
		47.9	67.1	71.4	60.5	63.8	41.3	45.9	62	61.9	54.3	61.9	48.3	62.4	47.3	53.7
		57.3	54.4	57.1	49.1	60	58.7	50.2	60.9	55.7	49.9	57.8	58.3	63.9	59.9	74
	Severe (75%)	100	100	100	100	99.9	100	100	99.6	99.8	99.7	99.8	99.9	99.9	100	99.8
		99.9	100	99.8	99.9	99.9	99.9	99.8	100	100	99.9	99.8	99.9	99.9	99.7	100
		99.9	99.9	99.6	99.9	98.7	99.3	99.9	99.9	99.9	99.8	100	100	99.8	99.8	99.9
		100	100	99.9	99.9	98.7	99.8	99.6	99.9	100	99.9	99.7	99.9	100	100	100
		100	99.9	99.8	99.9	99.8	99.6	99.8	100	99.9	99.9	99.9	99.9	100	99.5	100
		99.8	99.8	99.9	99.9	99.9	99.8	100	99.6	99.2	99.3	99.9	100	100	100	99.9

Table 3 shows the two-way table for the effects of Factor A (density of vector aphids) and Factor B (infectiousness).

Table.3: Effect of Density of Vector Aphids and Infectiousness(%) on Rate of BBTD Spread

		Density of Vector Aphids (A)		
		Low (3%)	Medium (10%)	High (30%)
Infectiousness (B)	Mild (25%)	55.7	54.9	56.7
	Severe (75%)	99.9	99.8	99.9

Results show that high density of vector aphids (30%) coupled with mild infectiousness (25%) resulted in a 56.7% spread of BBTD in an hectare of banana plantation after 4 months at the onset of infection followed by low density (3%) of vector aphids with mild infectiousness (25%) having 55.7% and medium (10%) density coupled with mild infectiousness with 54.9% BBTD spread. Moreover, these vector aphids retain the virus for weeks and could cover long distances through wind and rain dispersion (Balcaen, 2016). However, the different levels of density of vector aphids have no significant effect on the rate of infection.

Severe infectiousness (75%) coupled with either low (3%) or high (30%) density of vector aphids resulted in 99.9% BBTD spread. Severe infectiousness coupled with medium density (10%) of vector aphids resulted to 99.8% disease spread and is not significantly lower than having low (3%) and high (30%) density of vector aphids.

Table.4. Two-way ANOVA for Banana Bunchy Top Disease

Sources	*df*	SS	MS	*F*	*p* –value
Main Effects					
Density of Vector Aphids (A)	2	26.4	13.2	0.48	0.621
Infectiousness (B)	1	87516.5	87516.5	3167.56	0.000
Interaction Effects					
Treatment A x B	2	21.0	10.5	0.38	0.685
Error	174	4807.4	27.6		
Total	179	92371.3			

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Table 4 shows the two-way ANOVA for BBTD spread. Different levels of density of vector aphids (A) have no significant effect on the rate of BBTD spread as shown by the P-value (0.621) that is greater than the F value of 0.48.

With the presence of vector aphids even with only mild infectiousness (25%) of BBTD, 55 to 56% infection rate in a banana plantation after four months will have a significant economic impact on its productivity.

The levels of infectiousness have a significant impact on the rate of BBTD spread, i.e. P-value of 0.000 is less than its F value of 3167.56. Severe infectiousness (75%) showed significantly higher BBTD spread (99.9%) compared to mild infectiousness (55.8%). Mild infectiousness is expressed in some banana cultivars and other Musa species characterized by the absence of dark-green leaf and petiole streaks (Magee, 1953).Interaction (A X B) of the density of vector aphids (A) and infectiousness (B) have no significant effect on the rate of BBTD spread in a banana plantation.

V. CONCLUSION AND RECOMMENDATIONS

Within four months at the onset of infection, the presence of vector aphids with mild (25%) and severe (75%) infectiousness of BBTD can infect slightly more than half and almost all banana plants in a hectare plantation, respectively.

The rate of BBTD spread can be averted with quick management response to minimize disease spread. Integrated pest management (IPM) can be used to rid of vector aphids. The combination of eliminating diseased plants (physical) and usage of virus-tested planting materials (cultural method), aphicides (chemical) and use of parasitoids (biological) are some of the methods that can be applied to control aphids that vector BBTD.

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Geomatic Approach and Geophysical Interpretation of the Hydrogeological Basin of the Hassi Naga Region (Algerian Southwest)

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Abstract**—***In this work, we propose a model of Geomatics in the Hassi Naga region, which is located in the Hamada of Tindouf, southwestern Algeria, about 70 km to the northwest of the region. This approach is based on prospecting and thematic analysis of the distribution of Geoelectrical measurements in order to better estimate and manage the Hydrogeology of this region of the Tindouf basin. The results of the geophysical survey allow us to design a complete model that meets the needs of Hydrogeology, whose methodology we have applied consists of decomposing our subject into three classes of entities: Geomatic, geophysical and hydrogeological, discovering the relational links, doing the thematic analysis and ending with results that help to solve the problem of water (Hydrogeology) of the area studied.*

Keywords— Geomatic, Geoelectrical, Geophysics, hydrogeology.

I. INTRODUCTION

The area of Hassi Naga belongs to the northern flank of the Tindouf basin, a vast asymmetric Syncline made up of a set of sedimentary formations of Palaeozoic age unconformably covered by continental deposits of the Neogene [1]. This study shows the interest of Geomatics for geoelectrical exploitation in the Hassi Naga region and facilitates spatial interpretation. The result of this research is the establishment of a Geospatial model allowing the impact of the measurements in Hydrogeology of the Hassi Naga zone [1,2]. The geophysical results make it possible to confirm that there are formations that are likely to be aquifers, and have a hydrogeological interest at the level of the Neogene complex, which brings out three classes:

- A shallow class of thickness does not exceed 140 m.
- A middle class (800 m), corresponding to the silty clays of the Upper Carboniferous.
- A class that concerns a very resistant, dry and deep substratum (≥ 800 m).

Geomatics enabled us to carry out a synthesis mapping, allowing a simulation of the position of the drill holes of the zone of Hassi Naga. The main objective of this study is the analysis of electrical resistivity for the recognition of groundwater resources in the Hassi Naga area, located in the northwest of the Tindouf (SW - Algerian) basin. Taking into account all the existing studies and the confrontation with the results of geophysics by prospection based on the electrical method. By measuring the resistivity of the layers, from which the lithology and the structure of the region are determined to define the aquifer levels, their geometry and their thickness. In the field of mapping and cognitive mapping, the geomatics techniques are important for the study of the surface of the earth [3]. The data are essential to any surveillance, photographic analysis and morphological modeling, whatever the scale. The new solutions are attractive, fast, usable in any type of morphological configuration and provide easily integrable data in geographic information systems, at resolutions ranging from ten meters to centimeters [4,5].

In several fields of interest in cognitive geomatics plays a central and an inescapable role on:

- Spatial uncertainty;
- Implementation of Geomatics within organizations;
- Dissemination;
- Viewing and navigating.

Geomatic parameters for geology and Geotechnics are used in geophysical measurements [6], Hamouda M and al. [7] in order to implement cognition in Hydrogeology. In this work, emphasis is placed on the contribution of topography, Photogrammetry and GIS [8,9,10], to develop thematic maps and synthesis maps. Geographical location of the study area is defined in Figure 1.

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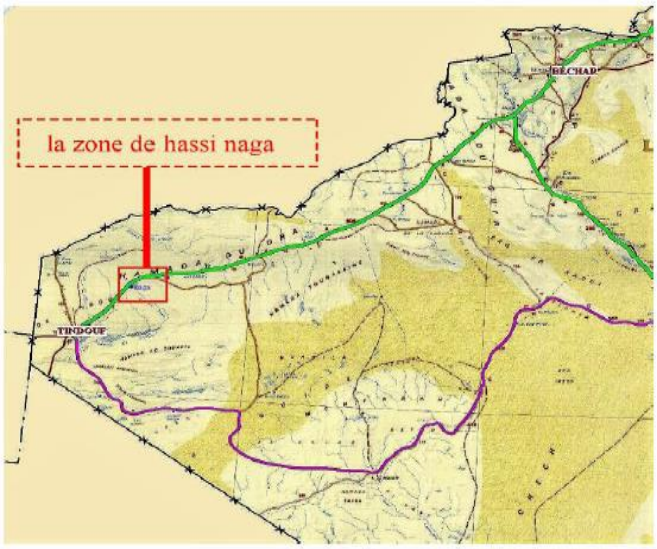

Fig. 1: Geographical location of the study area (ANRH (2015))

II. METHOD

The method of vertical electric soundings involves injecting a continuous electrical current into the ground using two stainless steel electrodes and measuring the potential deference created between the terminals of two other copper electrodes [11,12,13]. The assembly constitutes a quadrupole as it is in the following figure, a resistivity meter is used to measure the electrical current and the potential difference; and allows us to determine the apparent resistivity of the medium according to the scheme and the following formulas [14,15]:

There are several devices of the models of quadruples; the most used are those of Winner and Schlumberger.

• The Wenner: all the electrodes are equidistant, AM = MN = NB = AB / 3

• The Schlumberger: The distance MN is small compared to AB. In general MN <AB / 5

2.1. Mathematical model

The electrical method involves injecting a continuous electrical current into the ground using two stainless steel electrodes and measuring the potential deference created between the terminals of two other copper electrodes. The assembly constitutes a quadrupole as shown in the following figure; an ammeter is used to measure the electrical current and a voltmeter to measure the potential difference (Figure.2):

The idea is to move the four (AMNB) electrodes together and thus to produce profiles and resistivity maps. The devices are numerous and varied, the quadripole remains the most widespread. The source of current is typically 90-volt batteries, more rarely a gasoline generator with a rectifier or a car battery, the new devices walk with ten (10) batteries in series [16, 17].

This method makes it possible to measure the difference of potential ΔU and the electric current injected and recovered in the subsoil I, it remains for us to calculate the resistivity ρ in the one medium considered with two poles A and B, the action conjugate of A and B will give [18]:

- Potential in M:

$$U_M = \frac{\rho I}{2\pi}\left(\frac{1}{AM} - \frac{1}{BM}\right) \quad (1)$$

- Potential in N:

$$U_N = \frac{\rho I}{2\pi}\left(\frac{1}{AN} - \frac{1}{BN}\right) \quad (2)$$

- Difference potential between M and N:

$$\Delta U = U_M - U_N = \frac{\rho I}{2\pi}\left(\frac{1}{AM} - \frac{1}{BM} - \quad \frac{1}{AN} + \frac{1}{BN}\right) \quad (3)$$

From where :

$$\rho = \frac{k \cdot dU}{I} \quad (4)$$

With:

$$k = \pi \cdot \frac{AM \cdot AN}{MN} \quad (5)$$

- The resistivity in the medium in (ohm-meter) is calculated by:

$$\rho_a = \frac{k \cdot \Delta U}{I_{AB}} \quad (6)$$

With:

k: a geometrical factor which depends only on the relation of the electrodes and is expressed by (5);

ΔU: potential difference across the electrodes MN;

I_{AB}: intensity of the electrical current flowing in the circuit AB.

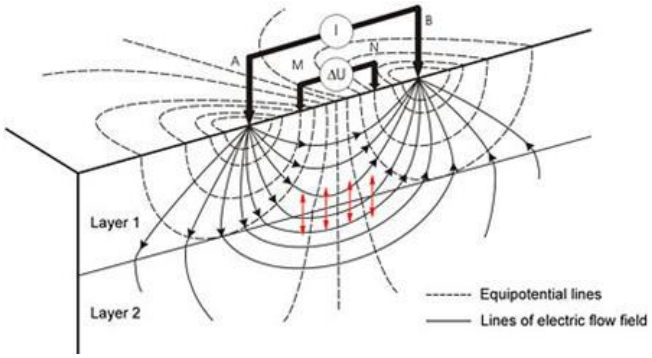

Fig. 2: Schlumberger scheme chosen for the prospecting of the present work

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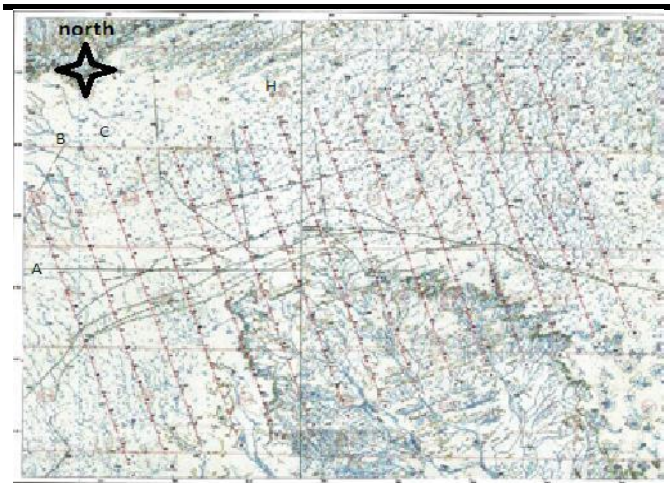


Fig. 3: Profile map (A-P) and points of the field soundings

Cuts and geophysical profiles allowed us to construct two levels of analysis by aggregation of the resistivity's, level 1 (R1, R2, R3 and R4) and level 2 (R5, R6, R7 and R8). These two levels are shown in Figure 4.

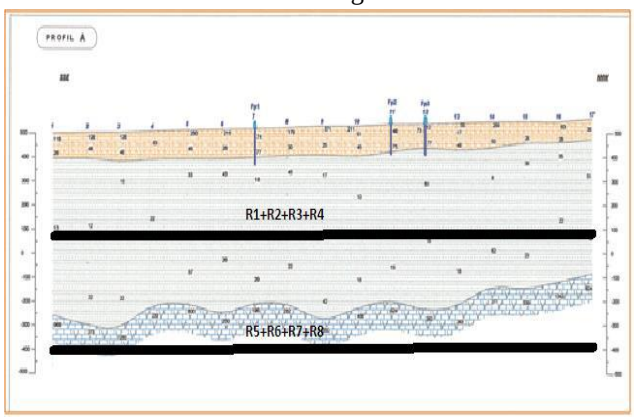


Fig. 4: Profile representing resistivity levels varying between R1 and R8

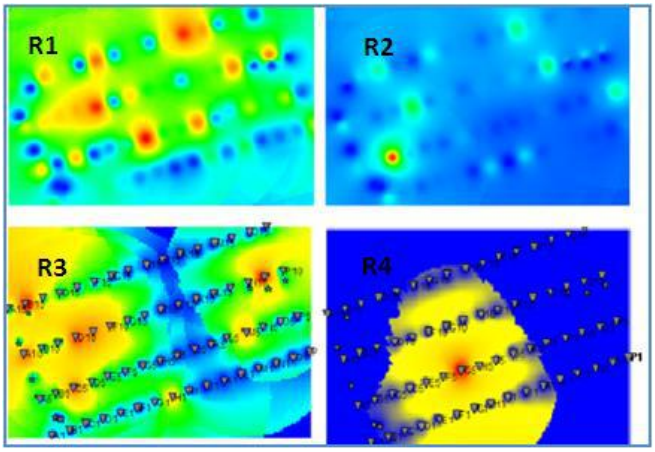


Fig. 5: Spatial distribution of resistivity, level 1 (R1, R2, R3, R4)

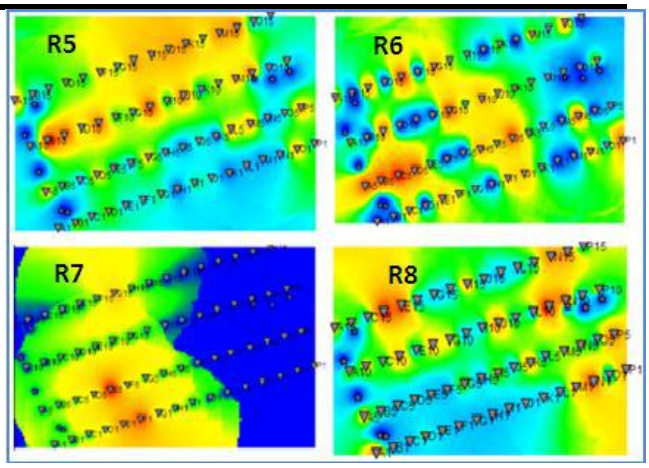


Fig. 6: Spatial distribution of the resistivity, level 2 (R5, R6, R7, R8)

III. RESULTS AND DISCUSSION

The result is based on the interpretation of the maps of apparent resistivity's:

The results of the interpretation are expressed as interpretive geoelectrical cuts and maps of apparent resistivity Figures 5 and 6. All the profiles are oriented South-South-East / North-North-West with similarities in their monotonous appearance, in the thicknesses of the formations Geological features, and by the nearly similar recorded resistivity. On the surface, the resistivity's are high, reflecting, due to the characteristics of the hard and compact limestones covering a large part of the Hamada. Geoelectric Cups of profile A: Depending on the resistivity and the thicknesses, three classes are to be considered:

Classs **#1**: 120 m thick with a first resistivity range of 118 to 571 Ohm-m, relative to the limestone crust of about 1m thickness, and a second range of 25 to 77 Ohm-m corresponds to the formation Neogene of Hamada (clay, sandy clay with limestone intercalation.

Class **#2**: 600 to 700 m thick, with a resistivity of 8 to 62 Ohm-m. Land of the Upper Carboniferous.

Class **#3**: 700 to 800m, with resistivity from 196 to 1342 Ohm-m. Relative to the lower Carboniferous (Upper Viséan), their thickness is 578m.

The summary of class resistivity is defined in Table I.

3.1. Comments and discussions

- Map of apparent resistivity's (AB = 450 m)

The depth of investigation for a line AB equal to 450 m will be located at a depth of 100 to 150m, which characterizes the nature of the Neogene Hamada. The values of the apparent resistivity's are divided into two orders of magnitude:

A relatively conductive range: of which (Ro < 60 Ohm-m) in the majority of the electric soundings would suggest low clay levels.

A relatively high range: at the soundings of which: (Ro > 80 Ohm-m), in the majority of the electric soundings would suggest low clay levels: (Ro > 120 Ohm-m). Would be clayey sandy levels.

- Map of apparent resistivity's (AB = 2000 m)

The depth of investigation for a line AB equal to 2000 m will be located at a depth of 300m to 400m, which characterizes the nature of the Upper Carboniferous represented by silty clays. The values \u200b\u200bof the apparent resistivity's are divided into two orders of magnitude:

Relatively conductive range: of which (20 < Ro < 60 Ohm-m) throughout the map.

Range of which: (Ro > 80 Ohm-m) indicates the existence of sandy levels within this clay-silty complex.

- Map of apparent resistivity's (AB = 6000 m)

The depth of investigation for a line 6000 m AB corresponding to the lower Carboniferous resistant substratum (Viseen higher 1200 m to 1500 m) of dolomitic limestone type with anhydrite intercalation. The values \u200b\u200bof the apparent resistivity's are divided into two orders of magnitude:

Conductive plate of which: (Ro <60 Ohm-m) which corresponds to dolomitic limestones with a high proportion of anhydrite.

A relatively high plateau of which: (Ro> 80 Ohm-m) corresponding to dolomitic limestone with very low intercalations of anhydrite.

- Top Carboniferous Roof Map

The Upper Carboniferous roof forms the Hercynian discordance which constitutes the very thick impermeable substratum of the Neogene's Hamada. In the area of Hassi Naga is escaped from all violent tectonics in general and their roof is at shallow depths (320m and 480m). The ascent of the roof gradually proceeds from South to North.

- Lower Carboniferous roof map (Upper Viséan)

We note the existence of numerous undulations due to the high resistivity's of the depths of the Upper Viséan; the values range from 450m in the south towards 100m in the north, indicating the elevation of the substratum in the northern part of the study area.

3.2. Confirmation and verification of tests

The structure and nature of the subsoil were developed and considered according to the calibration of the tests. The latter is done in accordance with the oil drilling data NG1 [6], located in the study area and the available hydrogeological information on the region. The comparison of the results with existing boreholes offers a good check and justify the continuity of the geological formations and lithological conformity with the classes envisaged by the geophysics. The geoelectric sections of **A** to **D** show a calcareous substratum with intercalation of anhydrite resistant to very strong dry belonging to the upper Viséan, a very thick clay-silty formation, the Heterogeneous Neogene more heterogeneous changes of lateral facies important. The sections from **E** to **H** show the structure that **A** and **B**, with the following features: resistant substratum, the Upper Carboniferous is very clayey, a remarkable dip of the structures towards the South and a slight thickening of the Hamada from the Neogene to the South. The same geological pattern is observed on sections **I** and **L**, with the following two elements: a relative thinning of the upper Carboniferous clay-silty complex in the northern part of the study area. A remount of the resistant substrate towards the north. The geological sections from **M** to **P** in the eastern part of the study area show a continuity of the same geological structure observed in the previous sections with a deep resistant substratum.

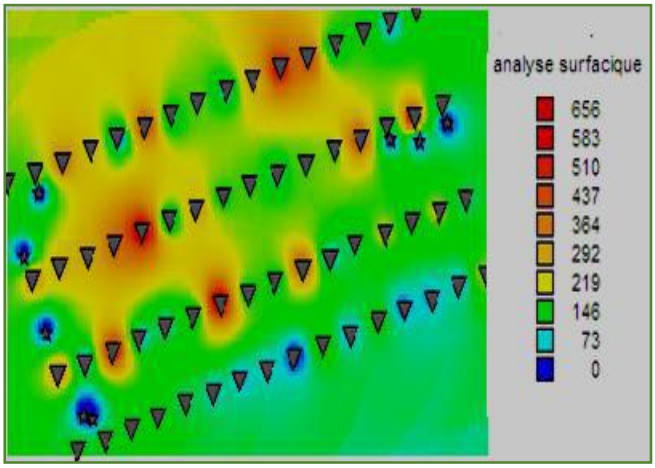
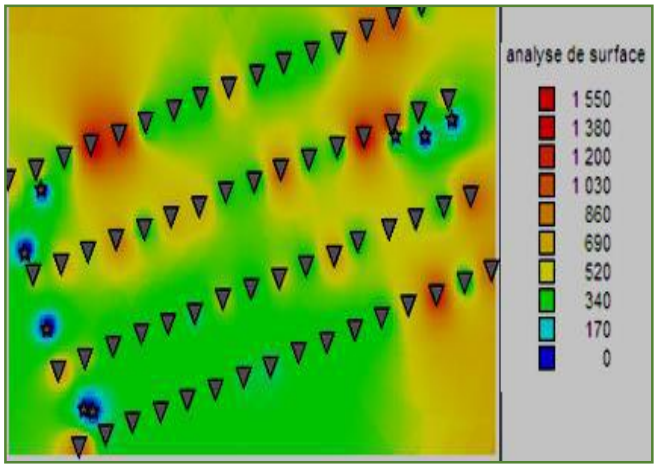


Fig. 7: Aggregation of the resistivity for R1-R4



layers (0-656)

Fig. 8: Resistivity aggregations for the R5-R8 layers (0-1550)

Figures 7 and 8 show maps using the aggregation function (sum) for two levels (N1 and N2). The zones in blue show the possibility of a positive drilling and the zones in red express a hard and deep base.

IV. CONCLUSION

This work is a contribution of geoelectrical prospecting for the needs of hydrogeology. The balance sheet is structured around a model that takes into account three classes highlighting three facies. Topography, geophysics and hydrogeology have allowed us to structure geological information. The spatial relations between these classes gave us thematic maps to locate the presence of water in the aquifer belonging to the Neogene formations and other horizons, especially in the Upper Carboniferous. The resistivity's of the latter are of the order of 90 Ohm-m and more, is likely to indicate aquifers. The spatial mapping allowed us to delimit the zones in relation to the topographic surface and to visualize the shape of the level surface as a function of the geoelectrics data. The integration of other geophysical methods, taking into account all the geological parameters, and the geomatics data available in the region in hydrogeological matters, can help us to develop and modernize the proposed analysis model towards a good exploitation and integrated water resources management.

ACKNOWLEDGEMENTS

Our acknowledgement also goes to the managers of the Algerian water laboratory and the water resources services of the Tindouf region for the consultation of the data's.

Table.1: Summary of distribution of resistivity.

Profiles	**Class 1 (C1)** (Ohm-m)	**Class 2 (C2)** (Ohm-m)	**Class 3 (C3)** (Ohm-m)	**Observations**
B	31-116	18-52 / 70-102	190 -1342	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous
C-D	≥ 200	10 – 57	234 - 1453	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous
E-F	20 - 200	15-50 / 65-73	205 - 1216	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous..
G-H	21 - 90	11 – 41 / 52 - 57	≥ 229	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous..
I-J	≥ 129	< 100	< 50	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous
K-L	77-115	< 50	≥ 350	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous
M-N	< 43 /58 - 265	< 50 / ≥ 400	≥ 350	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous
O-P	< 30 / ≥ 45	10 - 46	≥ 200	**C1:** Neogene of the Hamada **C2:** Carboniferous Superior **C3 :** Lower Carboniferous

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Nitrogen Removal in Mangroves Constructed Wetland

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***Abstract*—** *The potential use of Mangroves Constructed Wetland (MCW) as a low cost, efficient and suitable method for nitrogen removal from sewage in coastal zone of urban cities was examined in Dar es Salaam, Tanzania. In-situ examinations were done in horizontal surface flow Mangrove Constructed Wetland situated at Kunduchi beach area in Dar es Salaam. A wetland of 40 meters by 7 meters was constructed to receive domestic sewage from septic tank of Belinda Beach Hotel and was operated in an intermittent continuous flow mode. The wetland employed the already existing mangrove plants known as Avicennia Marina. The plants had an average breast height of 4 meters during commencement of experiments. The wetland collected the mixture of sewage and seawater at strength of 60% to 40%, respectively. The treatment efficiency of the wetland in nitrogen removal from sewage was determined. The observed removal rates of nitrogen inform of ammonia nitrogen (NH_3-N) and nitrate nitrogen (NO_3^--N) were 85% and 76%, respectively. Mangrove Constructed Wetland has a potential in nitrogen removal from sewages and it is suggested to be used for sewage treatment in coastal areas.*

Keywords*— *Constructed Wetlands, Coastal Area, Mangroves, Sewage, Treatment Performance.

I. INTRODUCTION

Mangroves are woody trees, palm or shrubs that occupy shallow water and grow at the interface between land and sea in tropical and subtropical coastal regions. They are characterized by muddy or fine sediment substrata. These plants, and the associated microbes, animals, and abiotic factors (like nutrients, minerals, water, oxygen, carbon dioxide, and organic substances) constitute the mangrove ecosystem [1]. Naturally, mangrove ecosystems play important role in preventing pollutants from entering the water body by up-taking of pollutants and creating conducive environments for growth of decomposing microorganisms [2, 3]. Many tropical cities that are built around natural harbors or waterways are lined by mangrove swamps. Examples from Africa are: Mombasa, Dar es Salaam and Maputo [4]. However peri-urban mangroves (*A. Marina*, *R. Mucronata* and *S. Alba*) of most coast cities examples are Tudor and Mtwapa creeks in Mombasa, and the Msimbazi River and Kunduchi beach area in Dar es Salaam [5], are recipients of sewage-polluted rivers and are extensively used for sewage dumping. The consequence is a potential risk to human health and ecosystems of estuaries and oceans [1]. This could be attributed to lack of adequate sewage treatment facilities in these cities. For example in Dar es Salaam, the coverage of sewerage system is 7% [6] of service area. Upgrading of the sewage infrastructure therefore, is urgently required in developing countries, where majority of these cannot afford conventional wastewater treatment systems in order to protect receiving environment. The Waste Stabilization Pond and Constructed Wetland (WSP and CW) Research Group at the University of Dar es Salaam in Tanzania has been developing low-cost technologies such as waste stabilization ponds and constructed wetlands [7]. These systems use nature to treat waste, are easy to maintain, are simple to construct and they are very effective technologies in treatment of wastewater [7]. Mangroves constructed wetlands are therefore considered ideal to protect peri-urban mangrove ecosystem. The mechanisms of natural mangrove wetlands to remove nitrogen and other pollutants from sewage are similar to wetland treatment systems using other types of vegetation. Therefore, it is expected that by applying appropriate engineering design and construction, the natural mangrove wetland can be used as an efficient sewage treatment system of the wastes generated from urban and peri-urban areas located along the coast as example in Thailand and China [8]. It is known that mangroves wetlands elsewhere intercept land-derived nitrogen and limit their spreading offshore and hence preventing risk to estuaries' and oceans' ecosystems [9], however their treatment performance on the removal processes of nitrogen, varies widely due to influence of various forcing functions like pH, temperature, Dissolved Oxygen Furthermore. In Tanzania, no efforts have been made to examine the performance of Mangroves Constructed Wetland in the treatment of sewage; as a result no information is available on removal of nitrogen in this kind of treatment system. However, similar studies of nitrogen removal have been conducted in Tanzania the

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differences are; one study was conducted on small scale constructed mangroves cells (microcosms) operated in batch [10] while others conducted the study by using subsurface constructed wetlands planted with terrestrial plants [11].

II. MATERIALS AND METHODS

Mangrove wetland treatment system was constructed at Kunduchi beach area in Dar es Salaam to perform secondary treatment of domestic sewage discharged from septic tank. The climate of the area is typically tropical. The site area inhabits a changeable environment with tides (at low tides the area is just wet and flooded during high tide). The area receives maximum tide range at new and full moon (spring tides) and minimum tide (neap tides) in between full moon and new moon. Also, the site area is dominated by mangrove type - *Avicennia marina* which had an average height of 4 meters. The sewage from septic tank of Belinda Resort Hotel was collected in sewage pond and the seawater from nearby ocean was collected in 10,000 litres tank.

2.1 Experimental Set-up and Operation of Surface Flow Mangrove Constructed Wetland

A wetland cell (unit 01) of *40m x 7m* was designed and constructed (Fig. 1) and its design criteria and features are shown in Table 1.

As presented in Fig. 2, the wetland cell received a mixture sewage and seawater from sewage pond and seawater tank, respectively. The liquid mixture was flowing by gravity at a rate of 5 m^3/day through a 100 mm diameter pipe. To make a mixing ratio of 60% sewage and 40% seawater, the sewage was flowing at a rate of 3 m^3/day while seawater at a rate of 2 m^3/day. The employed mixing ratio was established from pilot experiments that were carried by Pamba [10]. Since the study area is dominated by mangrove of type *Avicennia marina;* this mangrove specie was used as macrophytes for the wetland cell.

In order to imitate the natural phenomenon of alternating flooding with seawater and drying up of mangroves, the wetland cell was operated in an intermitted continuous flow mode of 3 days (inundation time) flooding with sewage and 3 days drying up cycles. The depth of sewage flow was kept 4 centimeters to enable mangrove roots to respire.

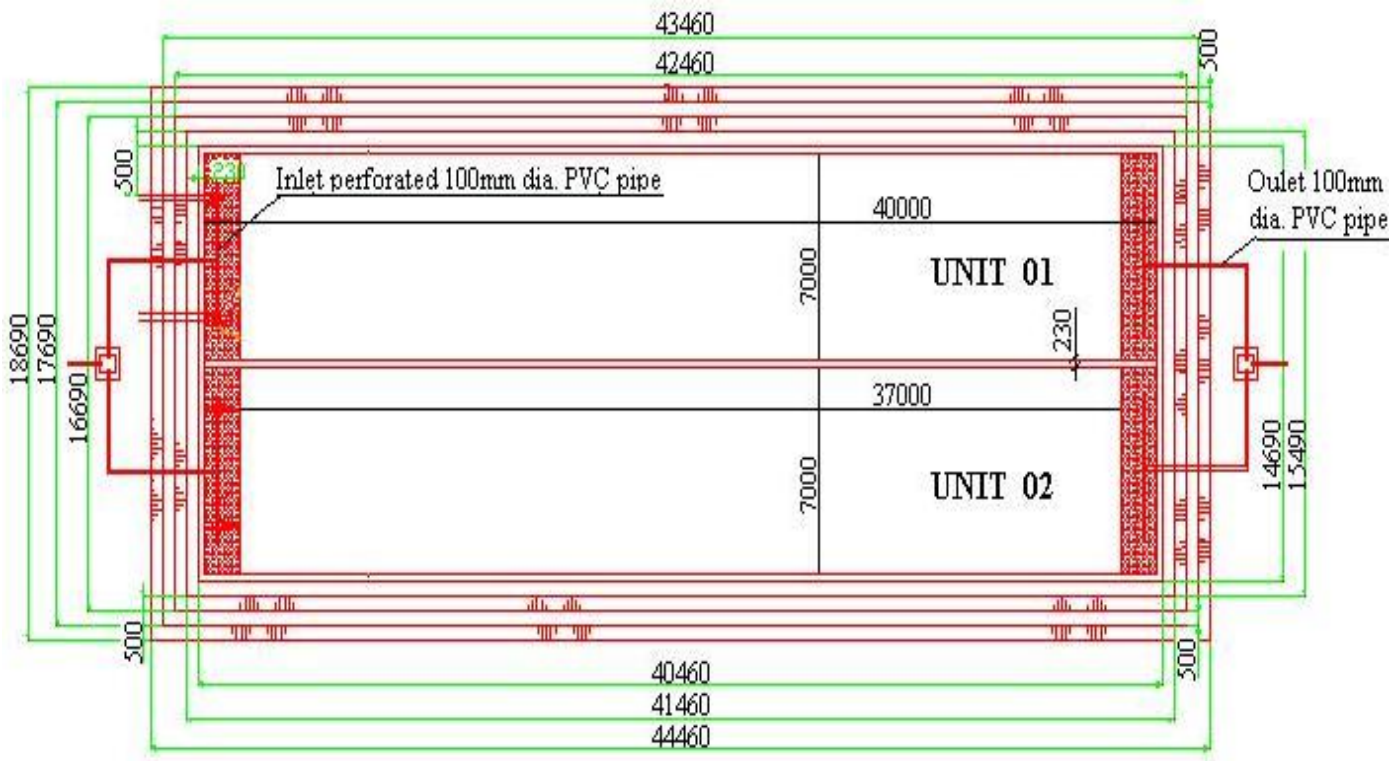

Fig.1: Floor plan for horizontal surface flow Mangroves Constructed Wetland (units are in mm)

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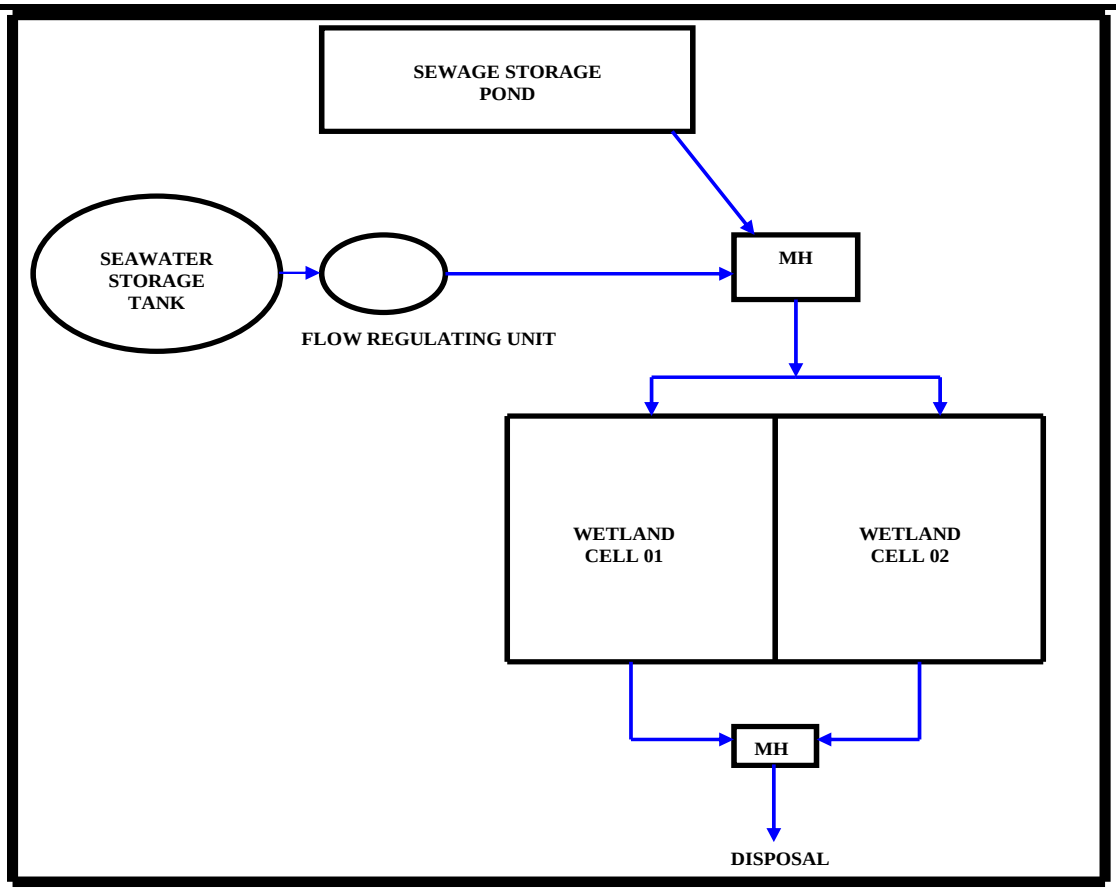

Fig.2: Experimental Set-Up

Table.1: Design Criteria, Dimensions and Characteristics of MCW system

FACTOR	DESIGN CRITERIA FOR SURFACE FLOW WETLANDS	WETLAND CELL DESIGN DIMENSIONS AND CHARACTERISTICS
Length (m)	-	40
Width (m)	-	7
Substrate (soil) depth (m)	Maximum, 1	0.6
Water depth (cm)	Maximum, 10	4
Organic Loading Rate, OLR (kg/ha.d)	Maximum, 80	44.64
Hydraulic Loading Rate, HLR (cm/d)	7-60	4.5
Aspect ratio (L/W)	2-10	
Retention time (d)	5 – 14	7
Slope (%)	Maximum 1	1

2.2 Sampling procedure for physical and chemical parameters

The samples were collected twice per week on the first day when wastewater enters into the wetland cell and on the last day (3rd day) when the wastewater gets out of the wetland cell. The samples were collected at 6:00 am in the morning, 12:00 noon, 6:00 pm in the evening and 11:30 pm in the night. Five (5) sampling locations were established inside the wetland cell. In this manner the cells were divided into four sections and the sampling locations were designated "Inlet, A1, A2, A3 and Outlet". The distances from each sampling location was 10 meters and at each sampling point a composite sample was taken crosswise the cell.

2.3 Analysis of physicochemical parameters

Analysis of physicochemical parameters was according to standard procedures for analysis of water and wastewater [12]. The physical parameters such as Dissolved Oxygen (DO), salinity, water temperature,

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pH and depth of water flow, were measured in situ, then the samples were covered, stored in a box and transported from the site to the laboratory for analysis of chemical parameters: Ammonia Nitrogen (NH_3-N) and Nitrate Nitrogen (NO_3-N). In order to remove probable particulate matters that might interfere with analysis of nitrogen, samples were filtered before analysis by using a filter paper (Whatmen No. 42). The majority of samples upon reaching the laboratory were immediately analyzed. Samples which were not able to be analyzed on the same day of sampling were preserved by being acidified and stored in the refrigerator.

2.3.1 Analysis of physical parameters

Temperature and pH were measured by the pH probe meter (WTW ino-Lab, pH Level 1 type, German, Accuracy is ± 0.01). DO was measured by DO probe meter (WTW inoLab type, German, Accuracy is ± 0.5% of the value). Salinity measured by WTW Cond probe meter (inoLab, Cond Level 1 type, German, Accuracy is ± 0.5% of the value).

2.3.2 Analysis of chemical parameters

Ammonia Nitrogen was determined according to American Standard Test Method [12] by a method known as Phenate method (the accuracy was ± 0.01 mg/L. Nitrate Nitrogen was determined according to American Standard Test Method [12] by a method known cadmium reduction method (Accuracy is ± 1.1% of the value).

2.4 Determination of the treatment efficiency of Mangrove Constructed Wetland in removal of NH_3-N and NO_3-N

For determination of the efficiency of Mangrove Constructed Wetland in wastewater treatment, the influent and effluent wastewater samples on the first and last day of the specified inundation (retention) time were analyzed for ammonia and nitrate and the removal percentages were determined according to equation (1).

.Removal efficiency $= \left(\frac{C1-C2}{C1}\right) X\ 100\%$ ... ... (1)

Where; C1 is concentration of ammonia or nitrate in the influent and C2 is concentration of ammonia or nitrate in the effluent.

III. RESULTS AND DISCUSSION

3.1 Sewage characteristics

The sewage characteristics during loading to the system are presented in *Table 2.*

Table 2: Sewage Characteristics at the Inlet of a Wetland Cell

Parameters	**Mean ± SD**
pH	7.36±0.31
Salinity (ppt)	11.46±10.03
DO (mg/L)	0.54±0.35
Temperature (^{o} C)	29.3 ±0.87
NH_3-N (mg/L)	4.70±1.87
NO_3-N (mg/L)	0.0126±0.0102

3.2 Dissolved Oxygen, DO

The average DO concentrations as a function of location along the wetland are presented in Fig. 3. The average inlet DO was 0.54±0.35 mg/L and the average outlet DO was 1.22±0.99 mg/L. The average DO within a wetland cell (i.e. along the location A1 – A3), was 1.55 mg/L. The levels of DO within the wetland cell were significantly lower than the ones reported in the newly planted mangrove wetlands (experimental microcosms, DO was 18.75±2.82 mg/l) [10]. This may attributed to the lack of sunlight penetration into the water column and wind effect due to plant cover over the trial wetland which was not the case with microcosms. Oxygen is introduced into water column during photosynthesis process of algae and mangroves. Since average DO in wetland system was 1.55 mg/L during the day, this DO creates aerobic conditions that favor growth of aerobic bacteria. Aerobic bacteria are responsible for nitrification processes.

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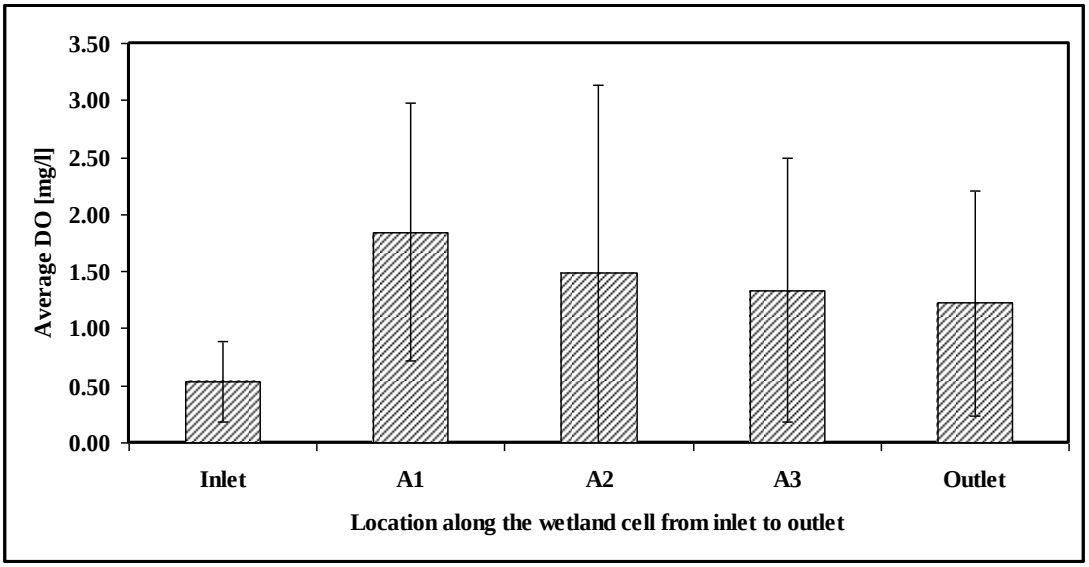

Fig.3: Variation of Average DO Concentration along the Wetland Cell from Inlet to Outlet

3.3 pH

The variation of pH with location along the wetland is shown in Fig. 4. The average pH value in the inlet was 7.36±0.32 and it improved slightly through the wetland to a pH of 7.90±0.23 in the outlet. The average pH within the wetland cell (i.e. along the location A1 – A3), was 7.75. Comparing the pH values obtained in this wetland cell to the values obtained in the experimental microcosms with new-planted mangroves *Avicennia marina* [10], it is noticed that the later was slightly higher (pH was 8.26 ±0.37). Difference of pH between these two systems could be due to decomposition of detritus plant tissues on forest floor and the penetration of light through plant canopy. In new-planted system where plants were small and not fully covered the area, the light could penetrate to the bottom and make the water treatment to be more photosynthetic driven system which is accompanied with pH rise [8, 13]. Usually, the microorganisms work better at certain ranges of pH values. Most bacteria operate well at the pH range of 7.0 to 9.5 [14]. Since average pH in the wetland system was 7.75 during the day, this pH favors most of the decomposing bacteria to decompose nitrogen.

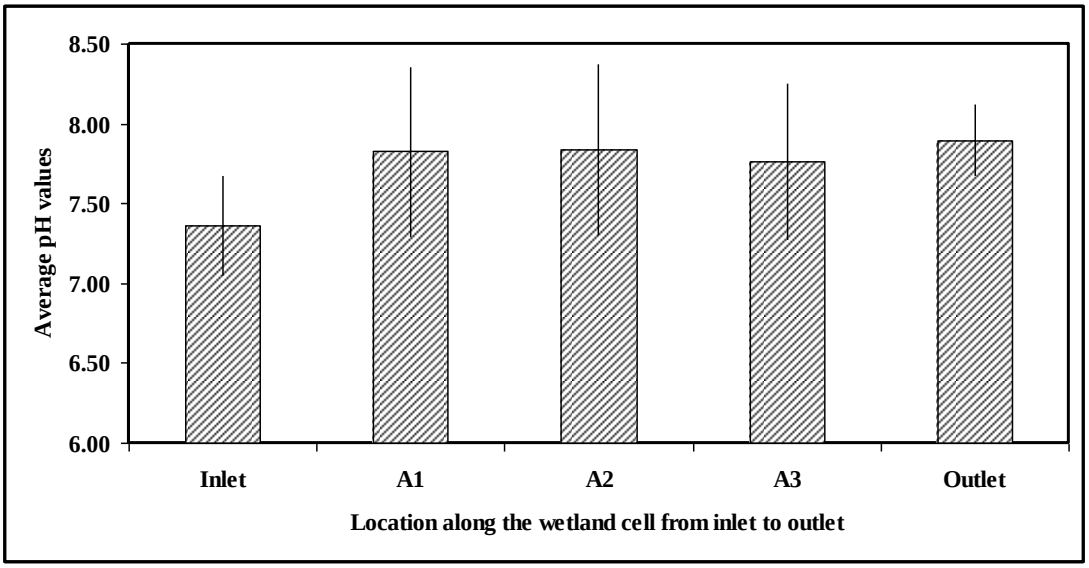

Fig.4: Variation of pH Values along the Wetland Cell from Inlet to Outlet

3.4 Salinity

The average salinity concentrations as a function of location along the wetland are shown in Fig. 5. There was a gradual increase of salinity from 7.21 ± 5.24 ppt in the inlet to 13.75±3.10 ppt in the outlet. The average salinity within the wetland cell (i.e. along the location A1 – A3), was 10 ppt. The low salinity in the inlet is explained by dilution of the sewage by seawater. As the sewage was

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travelling through the wetland salinity increased most likely due to evapo-transpiration from the fully grown *Avicennia* mangrove plants.

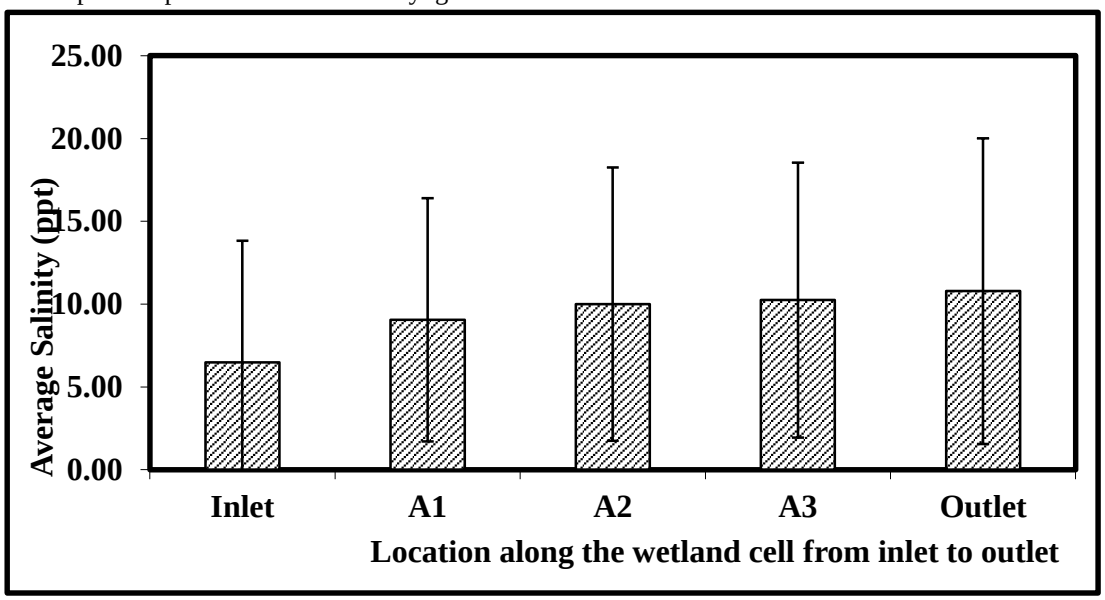

Fig.5: Variation of Average Salinity along the Wetland Cell from Inlet to Outlet

3.5 Temperature

Variation of temperature is as presented in Fig. 6. During the day, average inflow temperature was 30.1 ± 0.22 and average temperature in the system was 29.3 ± 0.27. During the night, average inflow temperature was 29 ± 0.45 and average temperature in the system was 28.9 ± 0.46. Generally temperature inside the wetland cell was slightly lower during the day and night compared to influent water temperature because of the shading effect of plants. The growth rate constants of decomposing bacteria are influenced by temperature changes within the wetland system. The optimum temperature for the growth of nitrifying bacteria ranges from 28 to 36° C [11]. Since average temperature in wetland system was 29.3 ° C during the day, this temperature favors the decomposing bacteria to decompose nitrogen.

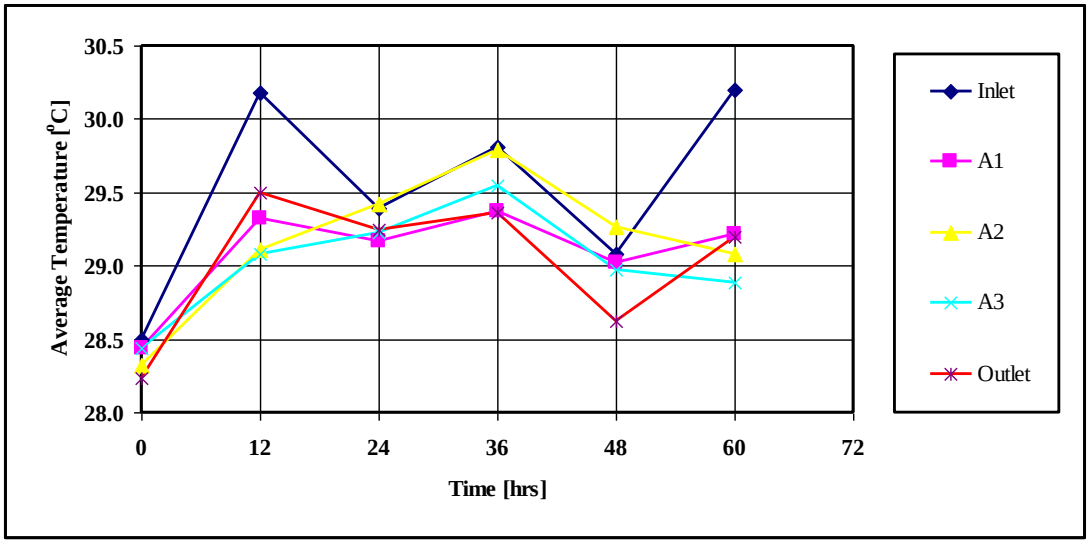

Fig.6: Variation of Average Temperature with Inundation Time

3.6 Ammonia Nitrogen (NH_3-N)

Fig. 7 shows the variation of average NH_3-N concentration with time at different sections in the wetland and Fig. 8 shows the variation of NH_3-N along the wetland cell. It is clear from these figures that the concentration of NH_3-N in the water column typically varied with time and distance from the inlet to the outlet. This observation was made from the five sampling points namely Inlet, A1, A2, A3 and Outlet. The distribution of NH_3-N along the mangrove wetland was less uniform

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within the wetland region indicating that there was an inefficient surface circulation pattern.

High NH_3-N concentration were determined at the inlet and tended to be varying with time due to non-uniform quality of the feed. At the inlet the average concentration of NH_3-N was 4.7 ± 1.87mg/l. The average outlet concentration of NH_3-N after 60 hours was 0.7 ± 0.9 mg/l and the average percentage removal was 85.11.

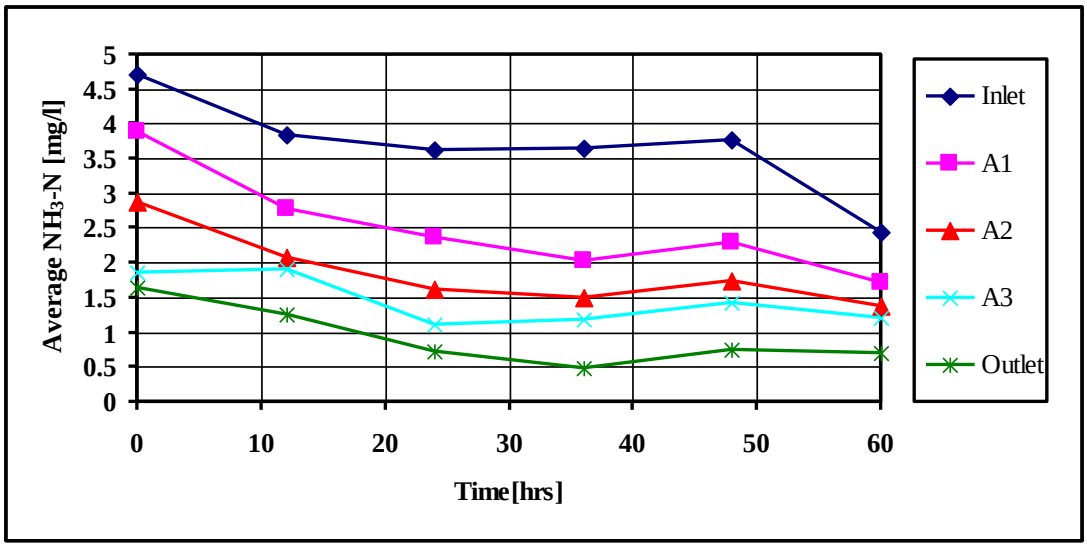

Fig.7: Variation of Average NH_3-N Concentrations with Inundation Times

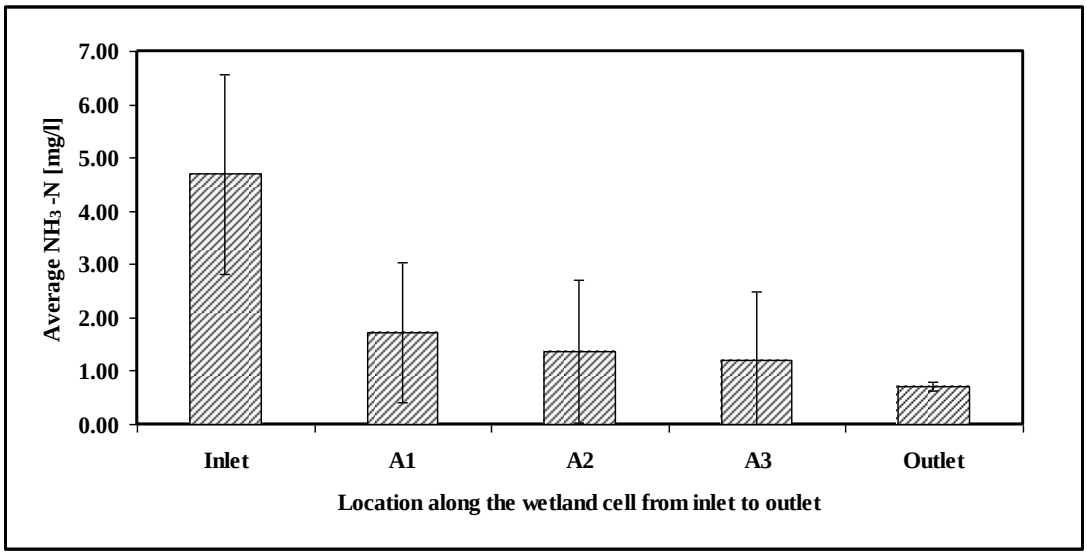

Fig.8: Variation of Average NH_3-N Concentrations along the Wetland Cell from Inlet to Outlet

Ammonia showed very good distribution from inlet to outlet (Fig. 8). The average effluent NH_3-N concentration from the system was 0.7 mg/l which is within the allowable discharge limit used for design (1.5mg/l). The calculated NH_3-N removal rate of the system ranged from 0.31 to 0.57 kg/ha.d with an average removal rate of 0.49 kg/ha.d. The removal of NH_3-N might be achieved by mangroves and algal uptake, volatilization, nitrification and sedimentation [11]. Based on the results of NH_3-N distribution it is evident that, despite the non-steady state operation of the wetland, the continuous removal of NH_3-N over the cell within three days indicated satisfactory performance of the system.

By increasing the water retention time up to about 7-14 days, it is expected that the system will reach equilibrium and the ammonia removal rates would be even better [9].

3.7 Nitrate Nitrogen (NO_3-N)

The average concentration of NO_3-N at the inlet was 0.126 ± 0.1 mg/l while at the outlet after 60 hours was 0.055 ± 0.007 mg/l (Fig. 9). The average percentage removal for the NO_3-N was 76.2 and the removal rate was 0.26 kg/ha.d. By observing variations of nitrate with time

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(Fig. 9), NO_3-N showed non-uniform behavior within the wetland, since there was a net production of NO_3-N in the system (Fig. 9). However, good removals were observed along the wetland cell (Fig. 10). Non-uniform behavior of NO_3-N in the wetland cell was also observed by Mayo and Bigambo [11]. This could also be caused by co-existence of aerobic and anaerobic areas in the bed that encourages losses of nitrogen through nitrification and denitrification process [15].

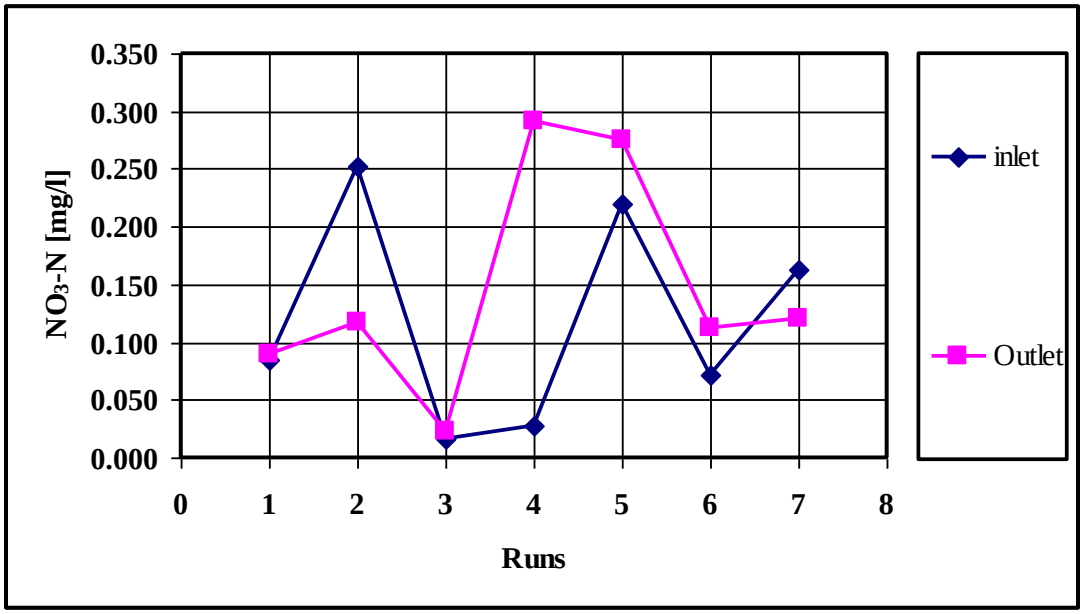

Fig.9: Variation of NO_3-N concentration with inundation time

The removal of NO_3-N in system is mainly through denitrification, plant and algal uptake. According to Vymazal *et al.*, [16], the optimum pH for *Nitrosomonas* (i.e. bacteria that oxidize NH_3-N to NO_2-N) and *Nitrobacter* (i.e. bacteria that oxidize NO_2-N to NO_3-N) is 8.3, and that the nitrification rate falls almost to zero at a pH of 9.6. Experiments by Mayo and Bigambo [11] showed that the optimum pH range for *Nitrosomonas* was from 7.5 to 8.5 and that for *Nitrobacter* was from 8.3 to 9.3. Vymazal *et al.*, [16] quote the optimum pH for *Nitrosomonas* as 8.3 and affirm that the nitrification rate falls to zero at a pH of 9.6. About 90% of the maximum nitrification occurs between pH 7.8 and 8.9. The maximum average pH obtained in this research was 7.9, therefore according to others researcher's results indicate that the pH of 7.9 favored more *Nitrosomonas* oxidation than *Nitrobacter* oxidation and hence it means there was net productivity of NO_2-N in the system by which it shortly oxidize to NO_3-N [17].

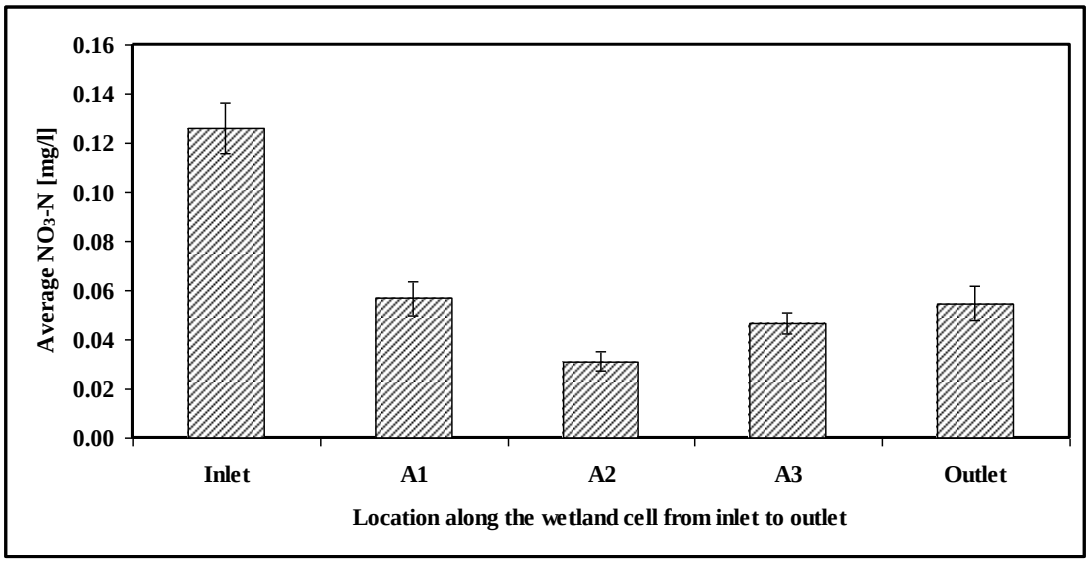

Fig.10: Variation of Average NO_3-N Concentration along the Wetland Cell from Inlet to Outlet

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IV. CONCLUSION AND RECOMMENDATION

Based on the results presented, reduction in concentration of nitrogen was observed. The removal rates of NH_3-N, and NO_3-N were 85%, 76%, respectively. The removal processes were attributed by the forcing functions pH, temperature and DO with averages of 7.75, 29^{o}C and 1.55 mg/L, respectively.

For optimization of system treatment performance with respect to nitrogen and other pollutants removal, it is recommended that, inundation time (retention time) should be long enough (> 5 – 15 days) to allow the system to operate more in a steady state conditions for treatment of sewage to acceptable levels for safe use or discharge. For mangrove root-nodes to respire, water depth must be as low as possible (< 10 cm). Since raw sewage may cause anaerobic conditions in which may produce Hydrogen Sulphide gas (H_2S) which is harmful to mangroves, only primary treated sewage should be used. The primary treatment can be achieved in oxidation ponds, septic tanks, UASB reactors e.t.c. For maintaining of saline conditions, the wetland system should be located in a point where it can receive tidal seawater both neap and spring tide. It is recommended that further research on nitrogen removal in Horizontal Surface Flow Mangrove Constructed Wetland should be conducted on long term basis in order to establish a best database, which will be useful for design of Mangroves Constructed Wetland in coastal zones in tropical and subtropical countries.

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Influence of soil texture on nature of mangrove vegetation in Sundarbans Tiger Reserve forest of India

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***Abstract*—** *Quality of bottom soil forms an important component for any kind of mangrove reforestation as well as coastal rehabilitation program. Since textural composition plays an important role in determining the soil quality, a study was undertaken to assess the influence of variations in soil texture on distribution and abundance of different mangrove species across five zones of Sundarbans Tiger Reserve, India.This study showed these mangrove soils to exhibit good occurrences of sand particles. However,the effects of sands on textural quality were frequently masked by moderate occurrence of finer soil particles which rendered the soils to be largely loamy in texture.Such soil conditions favored establishment of mangrove vegetation. Species wise also, mangrove vegetation was found to be more diverse in loam, clay loam, sandy loam and sandy clay loam soil textures respectively while a very few species were observed in clay, sandy clay and silty clay soils.*

Keywords*— *Sundarbans Tiger Reserve forest; soil texture; mangrove vegetation; distribution; abundance.

I. INTRODUCTION

Mangrove forests cover an area of approximately 160,000 km^2 all over the world, in which the largest forest areas are found in Malaysia, India, Bangladesh, Brazil, Venezuela, Nigeria and Senegal (Giri&Muhlhausen, 2008; Alongi, 2009). Indian Sundarbans falls under the jurisdiction of North 24-Parganas and South 24-Parganas districts in West Bengal comprising of 19 rural blocks covering a total area of 9630 sq. km. Since 1973, about 2585.10 sq. km area of south-eastern part of Sundarbans mangals were declared as Sundarbans Tiger Reserve. This area of Sundarbans Tiger Reserve includes the land area of 1680 sq. km. while the estuarine rivers, creeks and canals cover about 905 sq. km. This entire area under the Sundarbans Tiger Reserve has been further demarcated as "Core Area" (1330.12 sq. km.) and "Buffer Area" (1255 sq. km.). The Indian Sundarbans is bestowed with the highest floral diversity in the form of mangroves, coastal wetland flora, beach flora, marsh and swamp flora (Chanda, 1977; Naskar & Guhabakshi, 1987; Naskar, 1993; Naskar &Mondal, 1999). Ghosh *et al*, 2003 have reported about 110 plant species to occur in this forest of which about 25 species to be true mangroves. Ghosh, *et al* (2003) also studied on floral diversity of mangroves and mangrove associated species in the Indian Sundarbans.Physico-chemical properties of different mangrove soils in Sundarbans Tiger Reserve have been studied with relation to species diversity(Dasgupta *et al* 2003).Further studies have shown that mangrove soils vary considerably as compared to the non-mangrove soils even in same locations and also with other mangrove soils under different locations with regard to some properties (Dasgupta *et al*, 2018).Patterns of tidal inundation also influence soil characteristics that control species zonation of mangrove forest (Banerjee, 1987; Naidoo, 1980; Saha &Choudhury, 1995). While studying mangrove zonation pattern, several workers have shown that physiological adaptations to such variations may appear to be useful for explaining the observed zonations of mangroves (Smith, 1992, Satyanarayana *et al*, 2002).

II. METHOD

The objective of determining the textural composition of soils is to know the percentage of soil particles contained in different grain size fractions viz. sand, silt and clay and to classify the soils under different textural groups so that the dominant grain sizes present in the soil can be identified easily. The collected soil samples were air dried at a temperature of about25^0-30^{0}C and relative humidity of about 20%-60% in the laboratory. This was ground with the

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help of wooden mortar and soil texture was determined by International pipette method (USDA,1966).

During the first phase of the study, regular collections of soil samples were done from the Indian part of Sundarbans covering 15 blocks of Sundarbans Tiger Reserve and adjoining areas. These blocks were divided into five major zones viz. (i) Eastern Zone (ii) Western Zone (iii) Central Zone (iv) Northern Zone and (v) Southern Zone according to their occurrence (Table -1). Soil samples, collected extensively from different zones, were processed and analyzed for textural composition by following the procedures mentioned above.

During the second phase of the study, the textural composition of rhizosphere soils under different mangrove vegetations were assessed. Twenty three mangrove species commonly occurring in these soil zones were identified and soil samples were collected from their root zones to assess the preferredtextural nature of soil required for the specific mangroves for their growth and survival.

III. RESULT AND DISCUSSION

The textural compositions of the mangrove soils in the Indian Sundarbans Tiger Reserve have been presented in Table-1. As observed from the table, the sand particles occurred in high concentrations in most of these soils. However, the direct effects of sands were frequently countered by moderately good occurrence of comparatively finer particles like clay which rendered these soils to be largely loamy in texture. Since mangrove soils occur in intertidal zones and finer soil particles are generally transported to these zones by tidal water, such accumulation of finer particles in such alluviated soils may be expected. Occurrence of these kinds loamy texture may be helpful to maintain a loose soil condition in mangrove soils encouraging good rooting of mangrove vegetation in such soils. In addition, these soils are likely to provide better plant nutrition to the mangroves than the soils which are coarser in texture. In the present study, only two soil zones *viz.* Pirkhali in northern zone and Mayadwipin southern zone showed clayey soil texture. Although these two zones are situated in almost opposite situations but they have a similarity that both of them are characterized by dense mangrove vegetation. This might have trapped larger amount of finer soil particles from the tidal water which increased the clay content of the soils. Since variations in textural compositions are associated with differences in nutrient status, chemical and physical properties and also the biological properties of soils (Brady, 1980), such changes in textural compositions are likely to influence the nature of mangrove vegetations under different textural zones.

Textural compositions of rhizosphere soils for different mangrove species commonly found in Sundarbans Tiger Reserve have been presented in Tables 2 to 6. In the present study, species wise occurrence of different mangrove vegetation appeared to be influenced by the soil textural groups. In Table-7, an effort has been made to identify the mangrove species which were found to be associated with different textural sub-groups of the soils. As observed from the table, loamy textures under different subclasses *viz.* loam, clay loam, sandy loam and sandy clay loam harbored more diverse occurrence of mangrove vegetation while in clay, sandy clay and silty clay textural groups, only a few species of mangroves survived. Since loamy soils constitute a large share of the variations in textural compositions of mangrove soils of Sundarbans, these soils may be, in general, considered to be conducive for good occurrence of diverse mangrove species.

IV. CONCLUSIONS

The study showed thatloam and sandy loam soils showed most diverse mangrove species in Indian Sundarbans Tiger Reserve whereas clay, sandy clay and silty clay showed least diversity.Although sand particles often occurred in appreciable concentrations yet the textural composition of these soils were observed to be mostly under different loamy subgroups. This may be due to transportation of finer particles to these estuarine soils through intertidal water.

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Table.1: Textural compositions of the mangrove soils of Sundarbans Tiger Reserve Forest.

Zone	Block	Textural composition (%)		
		Sand	Silt	Clay
Eastern Zone	Arbesi	**49.1** 41.7-58.5	**28.1** 25.8-33.5	**22.6** 7.9-31.5
	Khatuajhuri	**41.6** 35.1-48.1	**27.1** 21.1-33.1	**31.2** 18.7-43.7
	Harinbhanga	**44.3** 40.6-48.1	**26.8** 23.6-30.1	**28.7** 21.7-35.8
Western Zone	Matla	**40.7** 36.1-46.1	**28.1** 26.1-32.1	**31.0** 21.7-37.8
	Netidhopani	**41.4** 34.8-48.1	**26.5** 22.9-30.1	**31.9** 21.7-42.2
	Chottohardi	**42.6** 36.1-55.7	**29.3** 26.1-31.0	**22.7** 14.5-37.7
Northern Zone	Jhilla	**49.6** 15.0-80.6	**23.9** 8.2-34.5	**32.3** 12.5-75.7
	Pirkhali	**36.8** 25.8-47.8	**10.9** 0.96-20.9	**52.2** 51.2-53.2
	Panchmukhani	**51.8** 29.5-60.5	**35.3** 30.2-49.2	**16.1** 1.58-22.0
Central Zone	Chamta	**50.2** 41.6-59.5	**30.8** 25.6-36.5	**18.8** 3.9-32.7

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	Chandkhali	**50.5** 38.1-61.0	**29.7** 24.5-33.0	**19.6** 6.0-34.9
	Goasaba	**42.9** 34.7-55.7	**26.2** 21.7-36.6	**31.2** 4.72-42.5
Southern Zone	Bagmara	**39.9** 26.2-48.2	**28.1** 23.8-40.2	**31.8** 14.9-40.3
	Gona	**48.2** 42.1-55.8	**28.3** 26.0-29.8	**24.0** 14.3-29.0
	Mayadwip	**35.0** 28.1-43.5	**25.0** 21.4-30.5	**39.9** 26.0-48.8

Table.2: Textural composition (%) of mangrove rhizosphere soils in eastern zone of Sundarbans Tiger Reserve.

Sp. Name	***Eastern Zone***								
	Arbesi			*Khatuajhuri*			***Harinbhanga***		
	Sand	*Silt*	*Clay*	*Sand*	*Silt*	*Clay*	*Sand*	*Silt*	***Clay***
Rhizophora mucronata Lamk	*35.7*	*27.3*	*29.3*	*60.7*	*24.4*	*19.7*	*46.0*	*25.0*	*29.0*
Rhizophora. apiculata Blume	*36.8*	*29.1*	*32.2*	*58.7*	*17.3*	*23.1*	*46.0*	*25.0*	*29.0*
Bruguiera gynmorhiza (L.)Lamk.	*56.0*	*8.0*	*36.0*	*47.7*	*21.5*	*30.7*	*45.6*	*23.4*	*31.5*
Bruguiera cylindrica (L.)Blume	-	-	-	-	-	-	-	-	-
Bruguiera sexangula (L.)Poir	*57.0*	*32.0*	*13.3*	*61.5*	*37.5*	*0.98*	*59.6*	*34.2*	*15.7*
Bruguiera parviflora W. & A.	-	-	-	-	-	-	*55.1*	*35.3*	*26.7*
Kandelia candel (L.) Druce	*49.5*	*3.98*	*17.0*	*57.5*	*8.0*	*34.5*	*46.1*	*16.1*	*37.7*
Ceriops decandra (Griff.)Ding. Hou.	*54.7*	*27.7*	*17.5*	*43.3*	*34.8*	*19.8*	*48.5*	*32.5*	*18.9*
Ceriops tagal (Perr.)Robin	*52.3*	*25.6*	*18.6*	*63.5*	*30.5*	*10.9*	*46.8*	*30.2*	*16.7*
Avicennia alba Blume	*54.7*	*27.7*	*22.9*	*52.6*	*28.7*	*12.4*	*44.8*	*27.9*	*14.7*
Avicennia officinalis L.	*41.7*	*31.5*	*26.7*	*38.5*	*25.6*	*14.6*	*58.5*	*34.5*	*6.9*
Avicennia marina (Forsk.)Vierh.	*51.4*	*32.4*	*17.8*	*50.1*	*33.1*	*16.7*	*54.0*	*31.0*	*15.0*
Sonneratia apetala Buch. Ham.	*49.7*	*27.7*	*0.96*	*56.7*	*30.7*	*12.5*	*41.8*	*29.3*	*18.8*
Sonneratia caseolaris (L.) Engler	-	-	-	-	-	-	-	-	-
Sonneratia griffithii Kurz.	*35.*	*12.1*	*643.1*	*37.3*	*18.9*	*44.7*	*26.3*	*19.1*	*47.8*
Xylocarpus granatum Koen.	*43.1*	*21.4*	*31.2*	*40.1*	*27.1*	*32.7*	*45.9*	*30.9*	*23.0*
Xylocarpus mekongensis Pierre	*54.7*	*27.7*	*17.5*	*56.0*	*11.0*	*9.9*	*55.5*	*34.5*	*33.0*
Aegiceros corniculatum (L.) Blanco.	*41.1*	*24.1*	*34.7*	*54.0*	*8.25*	*37.7*	*45.8*	*31.3*	*22.8*
Aegialitis rotundifolia Roxb.	*49.5*	*33.5*	*17.0*	*58.7*	*36.7*	*12.1*	*51.3*	*34.5*	*17.3*
Heritiera fomes Buch. Ham.	*48.0*	*23.1*	*28.7*	*48.9*	*21.0*	*29.9*	*56.2*	*12.9*	*30.7*
Nypa fruticans (Thunb.) Wurmb.	*46.8*	*27.3*	*25.8*	*59.9*	*30.8*	*14.2*	*58.7*	*35.6*	*23.4*
Phoenix paludosa Roxb.	*51.2*	*37.8*	*10.1*	*49.2*	*29.4*	*8.6*	*60.5*	*34.5*	*5.0*
Exoecaria. agallocha L.	*49.0*	*29.0*	*21.9*	*47.1*	*29.1*	*23.7*	*52.2*	*31.7*	*16.0*

Table.3: Textural composition (%) of mangrove rhizosphere soils in western zone of Sundarbans Tiger Reserve.

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Sp. Name	***Western Zone***								
	Matla			*Netidhopani*			***Chottohardi***		
	Sand	*Silt*	*Clay*	*Sand*	*Silt*	*Clay*	*Sand*	*Silt*	***Clay***
Rhizophora mucronata Lamk	*46.1*	*21. 7*	*32.1*	*43.2*	*19. 3*	*29.7*	*48.9*	*23.4*	*38.7*
Rhizophora. apiculata Blume	*42.4*	*19.7*	*28.0*	*39.7*	*18.1*	*26.4*	*45.6*	*19.5*	*35.7*
Bruguiera gynmorhiza (L.)Lamk.	*45.6*	*25.6*	*31.4*	*41.1*	*22.1*	*26.7*	*47.7*	*24.1*	*28.1*
Bruguiera cylindrica (L.)Blume	*52.3*	*34.5*	*11.6*	-	-	-	*54.5*	*35.4*	*10.8*
Bruguiera sexangula (L.)Poir	-	-	-	-	-	-	*61.2*	*39.8*	*30.2*
Bruguiera parviflora W. & A.	*58.7*	*37.8*	*21.2*	*60.6*	*23.1*	*16.1*	*48.2*	*35.2*	*23.5*
Kandelia candel (L.) Druce	*47.8*	*24.5*	*31.6*	-	-	-	*51.2*	*26.8*	*37.4*
Ceriops decandra (Griff.)Ding. Hou.	*49.3*	*35.7*	*25.4*	*51.2*	*47.8*	*22.5*	*40.4*	*33.4*	*26.1*
Ceriops tagal (Perr.)Robin	*42.1*	*31.6*	*26.1*	*44.3*	*29.7*	*23.1*	*49.5*	*29.2*	*21.1*
Avicennia alba Blume	*55.1*	*28.0*	*13.4*	*50.2*	*29.0*	*14.1*	*40.3*	*35.8*	*23.8*
Avicennia officinalis L.	*43.0*	*25.2*	*30.0*	*35.5*	*25.1*	*24.3*	*48.5*	*34.0*	*26.9*
Avicennia marina (Forsk.)Vierh.	*46.5*	*30.2*	*23.2*	*51.2*	*47.8*	*22.5*	*48.2*	*28.2*	*23.5*
Sonneratia apetala Buch. Ham.	*53.0*	*42.9*	*24.3*	*51.2*	*47.8*	*22.5*	*42.6*	*33.2*	*24.2*
Sonneratia caseolaris (L.) Engler	-	-	-	-	-	-	-	-	-
Sonneratia griffithii Kurz.	*35.6*	*29.1*	*39.1*	*31.2*	*26.1*	*38.2*	*37.2*	*23.2*	*39.6*
Xylocarpus granatum Koen.	*41.3*	*35.6*	*25.2*	*45.9*	*31.5*	*22.9*	*42.3*	*31.1*	*26.0*
Xylocarpus mekongensis Pierre	*41.3*	*35.6*	*25.2*	*46.5*	*31.2*	*27.1*	*42.6*	*33.2*	*24.2*
Aegiceros corniculatum (L.) Blanco.	*40.2*	*23.1*	*33.7*	*51.2*	*47.8*	*22.5*	*39.2*	*25.2*	*35.6*
Aegialitis rotundifolia Roxb.	*55.0*	*31.0*	*14.0*	*51.1*	*30.0*	*13.7*	*56.2*	*29.8*	*15.3*
Heritiera fomes Buch. Ham.	*44.8*	*19.8*	*35.3*	*43.5*	*16.0*	*31.6*	*45.0*	*18.3*	*34.0*
Nypa fruticans (Thunb.) Wurmb.	*57.8*	*22.3*	*20.1*	*59.1*	*21.9*	*18.9*	*56.2*	*21.0*	*17.8*
Phoenix paludosa Roxb.	*49.5*	*32.9*	*17.3*	*48.1*	*32.1*	*18.1*	*39.0*	*25.0*	*13.3*
Exoecaria. agallocha L.	*45.0*	*28.0*	*24.3*	*45.5*	*28.0*	*26.3*	*40.2*	*29.2*	*29.5*

Table.4: Textural composition (%) of mangrove rhizosphere soils in central zone of Sundarbans Tiger Reserve.

Sp. Name	***Central Zone***								
	Chamta			*Chandkhali*			***Goasaba***		
	Sand	*Silt*	*Clay*	*Sand*	*Silt*	*Clay*	*Sand*	*Silt*	***Clay***
Rhizophora mucronata Lamk	*49.6*	*22.2*	*30.6*	*42.1*	*20.1*	*26.3*	*47.1*	*23.7*	*29.1*
Rhizophora. apiculata Blume	*29.3*	*23.5*	*27.3*	*31.1*	*25.4*	*29.7*	*33.8*	*27.8*	*31.4*
Bruguiera gynmorhiza (L.)Lamk.	*48.5*	*21.5*	*29.8*	*59.1*	*5.7*	*35.1*	*61.2*	*15.4*	*39.8*
Bruguiera cylindrica (L.)Blume	*56.7*	*35.7*	*9.8*	*63.4*	*39.7*	*11.8*	*61.0*	*33.0*	*6.0*
Bruguiera sexangula (L.)Poir	*46.5*	*27.5*	*25.9*	-	-	-	*59.7*	*34.5*	*25.6*
Bruguiera parviflora W. & A.	*53.2*	*30.2*	*16.5*	*58.7*	*33.7*	*7.5*	*62.1*	*36.9*	*23.4*
Kandelia candel (L.) Druce	*54.1*	*28.7*	*34.1*	*59.5*	*33.5*	*36.5*	*56.3*	*28.3*	*37.6*
Ceriops decandra (Griff.)Ding. Hou.	*49.1*	*33.1*	*17.9*	*54.1*	*32.1*	*15.9*	*50.2*	*36.7*	*29.8*
Ceriops tagal (Perr.)Robin	*60.5*	*34.5*	*4.9*	*49.5*	*30.5*	*7.5*	*58.0*	*30.0*	*4.0*
Avicennia alba Blume	*56.5*	*29.5*	*13.9*	*59.0*	*36.7*	*15.7*	*61.0*	*33.0*	*6.0*
Avicennia officinalis L.	*53.2*	*30.2*	*16.5*	*55.8*	*32.1*	*17.8*	*61.0*	*33.0*	*6.0*
Avicennia marina (Forsk.)Vierh.	*30.2*	*23.2*	*46.5*	*43.8*	*35.2*	*24.1*	*45.0*	*31.0*	*24.0*
Sonneratia apetala Buch. Ham.	*53.0*	*42.9*	*24.3*	*50.7*	*46.9*	*23.6*	*42.0*	*33.7*	*26.1*

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Sonneratia caseolaris (L.) Engler	-	-	-	-	-	-	-	-	-
Sonneratia griffithii Kurz.	*32.2*	*13.0*	*55.0*	*33.1*	*16.0*	*58.7*	*61.0*	*33.0*	*6.0*
Xylocarpus granatum Koen.	*55.9*	*31.2*	*12.9*	*52.3*	*31.5*	*16.0*	*58.9*	*34.5*	*12.0*
Xylocarpus mekongensis Pierre	*47.5*	*31.2*	*17.4*	*58.7*	*33.7*	*7.5*	*62.3*	*19.6*	*14.8*
Aegiceros corniculatum (L.) Blanco.	*47.5*	*31.2*	*17.4*	*41.6*	*25.6*	*32.7*	*44.5*	*21.1*	*35.9*
Aegialitis rotundifolia Roxb.	*55.0*	*31.0*	*14.0*	*57.6*	*32.0*	*15.1*	*59.1*	*34.2*	*15.6*
Heritiera fomes Buch. Ham.	*44.8*	*19.8*	*35.3*	*44.0*	*20.1*	*27.8*	*43.5*	*26.0*	*31.6*
Nypa fruticans (Thunb.) Wurmb.	*53.1*	*39.7*	*5.6*	*59.5*	*36.5*	*3.98*	*61.1*	*40.2*	*10.5*
Phoenix paludosa Roxb.	*39.5*	*32.9*	*17.5*	*40.1*	*28.8*	*13.4*	*44.5*	*28.9*	*13.0*
Exoecaria. agallocha L.	*46.2*	*25.0*	*24.6*	*45.5*	*28.0*	*26.3*	*48.0*	*26.4*	*22.1*

Table.5: Textural composition (%)of mangrove rhizosphere soils in northern zone of Sundarbans Tiger Reserve.

Sp. Name	***Northern Zone***								
	Jhilla			*Pirkhali*			***Panchmukhani***		
	Sand	*Silt*	*Clay*	*Sand*	*Silt*	*Clay*	*Sand*	*Silt*	***Clay***
Rhizophora mucronata Lamk	*42.2*	*22.9*	*34.8*	*47.8*	*28.3*	*36.7*	*49.8*	*31.1*	*39.5*
Rhizophora. apiculata Blume	*46.0*	*20.7*	*36.7*	*42.2*	*22.9*	*34.8*	*46.2*	*29.1*	*37.7*
Bruguiera gynmorhiza (L.)Lamk.	*44.3*	*16.3*	*28.7*	*42.1*	*10.1*	*25.4*	*47.6*	*13.4*	*29.8*
Bruguiera cylindrica (L.)Blume	-	-	-	*47.8*	*28.3*	*36.7*	*58.6*	*36.6*	*14.7*
Bruguiera sexangula (L.)Poir	*40.3*	*29.7*	*21.8*	-	-	-	*47.8*	*37.8*	*27.6*
Bruguiera parviflora W. & A.	-	-	-	*43.2*	*32.1*	*26.7*	*47.1*	*36.6*	*27.9*
Kandelia candel (L.) Druce	-	-	-	*45.2*	*21.3*	*32.1*	*49.4*	*23.6*	*33.4*
Ceriops decandra (Griff.)Ding. Hou.	*43.7*	*35.1*	*21.1*	*49.8*	*28.8*	*21.3*	*50.4*	*34.5*	*26.8*
Ceriops tagal (Perr.)Robin	*42.3*	*33.4*	*18.7*	*46.7*	*26.7*	*19.8*	*55.0*	*34.4*	*6.8*
Avicennia alba Blume	*44.5*	*33.2*	*14.2*	*47.8*	*28.3*	*36.7*	*48.5*	*35.1*	*15.1*
Avicennia officinalis L.	-	-	-	-	-	-	-	-	-
Avicennia marina (Forsk.)Vierh.	*47.4*	*35.6*	*13.1*	*48.5*	*35.2*	*15.2*	*48.0*	*35.0*	*17.0*
Sonneratia apetala Buch. Ham.	*45.2*	*25.7*	*12.1*	*46.7*	*30.2*	*13.6*	*41.2*	*28.3*	*18.8*
Sonneratia caseolaris (L.) Engler	-	-	-	-	-	-	-	-	-
Sonneratia griffithii Kurz.	*32.5*	*19.4*	*44.1*	*34.8*	*22.9*	*42.2*	*33.5*	*20.3*	*46.7*
Xylocarpus granatum Koen.	*42.5*	*19.7*	*31.6*	*41.1*	*26.5*	*31.9*	*48.1*	*30.1*	*21.7*
Xylocarpus mekongensis Pierre	*44.5*	*25.7*	*17.1*	*46.0*	*20.3*	*13.0*	*45.7*	*24.5*	*13.0*
Aegiceros corniculatum (L.) Blanco.	*44.5*	*21.8*	*37.5*	*47.8*	*28.3*	*36.7*	*45.0*	*25.6*	*36.8*
Aegialitis rotundifolia Roxb.	*39.8*	*34.1*	*23.4*	*47.8*	*28.3*	*36.7*	*40.5*	*35.6*	*24.7*
Heritiera fomes Buch. Ham.	*45.0*	*21.5*	*33.6*	*43.2*	*22.4*	*35.0*	*46.5*	*25.7*	*31.1*
Nypa fruticans (Thunb.) Wurmb.	*51.1*	*36.5*	*13.5*	*50.5*	*33.2*	*14.7*	*49.4*	*32.1*	*14.0*
Phoenix paludosa Roxb.	*48.1*	*33.1*	*18.7*	*39.5*	*27.5*	*13.3*	*45.6*	*31.1*	*18.5*
Exoecaria. agallocha L.	*45.1*	*29.1*	*25.7*	*41.2*	*29.4*	*26.7*	*46.1*	*27.8*	*25.8*

Table.6: Textural composition (%) of mangrove rhizosphere soils in southern zone of Sundarbans Tiger Reserve.

Sp. Name	**Southern Zone**		
	Bagmara	**Gona**	**Mayadwip**

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	Sand	Silt	Clay	Sand	Silt	Clay	Sand	Silt	Clay
Rhizophora mucronata Lamk	45.6	25.5	32.1	47.8	26.1	30.3	48.8	23.1	28.1
Rhizophora. apiculata Blume	46.7	26.6	34.5	51.1	28.9	37.1	49.7	26.9	36.2
Bruguiera gynmorhiza (L.)Lamk.	48.6	29.1	36.7	53.4	32.1	41.2	50.8	31.2	38.5
Bruguiera cylindrica (L.)Blume	-	-	-	-	-	-	49.1	32.1	10.3
Bruguiera sexangula (L.)Poir	-	-	-	-	-	-	-	-	-
Bruguiera parviflora W. & A.	44.3	36.7	25.4	46.7	37.9	29.4	38.2	31.7	17.3
Kandelia candel (L.) Druce	32.7	10.5	56.7	49.1	24.7	35.6	44.4	27.4	18.2
Ceriops decandra (Griff.)Ding. Hou.	45.6	34.5	27.6	49.7	35.6	28.1	43.3	36.2	25.5
Ceriops tagal (Perr.)Robin	44.1	31.2	20.6	46.7	34.5	23.5	45.1	33.4	21.4
Avicennia alba Blume	45.7	28.1	26.1	45.7	29.1	25.7	44.4	27.4	18.2
Avicennia officinalis L.	43.2	24.2	16.5	42.1	25.0	17.0	41.0	23.0	16.0
Avicennia marina (Forsk.)Vierh.	52.3	29.1	17.2	53.8	30.8	14.1	44.4	27.4	18.2
Sonneratia apetala Buch. Ham.	40.1	25.0	16.2	42.3	26.2	17.1	44.4	27.4	18.2
Sonneratia caseolaris (L.) Engler	23.7	36.5	39.7	-	-	-	-	-	-
Sonneratia griffithii Kurz.	30.7	13.6	55.7	31.1	12.0	56.1	34.5	20.0	55.3
Xylocarpus granatum Koen.	43.1	25.7	35.5	44.1	26.2	37.1	45.0	26.2	35.2
Xylocarpus mekongensis Pierre	41.5	33.0	25.1	40.4	38.7	24.3	42.6	33.2	24.2
Aegiceros corniculatum (L.) Blanco.	53.2	23.9	48.2	39.0	24.1	35.2	41.1	24.0	38.7
Aegialitis rotundifolia Roxb.	42.2	34.8	22.9	42.1	34.1	23.4	39.2	36.1	26.1
Heritiera fomes Buch. Ham.	44.0	22.6	35.7	44.2	22.3	35.0	53.6	24.5	30.5
Nypa fruticans (Thunb.) Wurmb.	50.0	35.0	14.7	50.6	36.3	14.6	49.4	32.7	16.0
Phoenix paludosa Roxb.	49.6	35.3	17.4	42.3	37.1	19.7	45.6	33.1	18.1
Exoecaria. agallocha L.	44.6	29.0	26.5	43.6	27.7	26.1	45.0	27.1	25.4

Table.7: Relationship between the soil texture and the different species of mangroves in different zones of Sundarbans Tiger Reserve

Clay	Sandy Clay	Silty Clay	Clay Loam	Sandy Clay Loam	Loam	Sandy Loam
S. griffithii	*H. fomes**	*S. caseolaris*	*R. mucronata* *R. apiculata* *X. granatum* *P. paludosa* *E. agallocha*	*B. gymnorrhiza* *K. candel* *A. officinalis* *S. apetala* *X. mekongensis* *A. corniculatum* *H .fomes*	*B. cylindrica* *C. decandra* *C. tagal* *A. alba* *A. marina* *X. mekongensis*	*B. sexangula* *B. parviflora* *A. rotundifolia* *N. fruticans*
			*B. gymnorrhiza** *C. decandra** *A. alba**		*B. sexangula** *B. parviflora** *S. apetala** *A. corniculatum** *A. rotundifolia** *N. fruticans**	*B. cylindrica** *C. tagal** *A. alba** *A. marina** *X. granatum** *X. mekongensis** *P. paludosa** *E. agallocha**

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Performance of *In Vitro* Cassava (*Manihot esculenta* Crantz) Plantlets Weaned with Locally Sourced Substrates

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Abstract— *The performance of in vitro cassava plantlets weaned on different locally sourced substrates was evaluated. Nodal cuttings were excised from healthy six weeks old OG 001cassava variety in the culture room of tissue culture laboratory of National Root Crops Research Institute, Umudike, Abia State, Nigeria. The explants were washed, sterilized and cultured in vitro. The resulting plantlets were weaned on the following substrates - top soil (TS), river sand (RS), saw dust (SD), rice hull waste (RH), 2:1 top soil plus river sand (TS +RS), 2:1 river sand plus saw dust (RS + SD), 2:1 river sand plus rice husk (RS + RH), 2:1 top soil plus saw dust (TP + SD), 2:1 top soil plus rice husk (TP + RH), 2:1 saw dust plus rice husk (SD + RH) and 2:1 peat pellet plus vermiculite (PP + VE), which served as the control. Completely randomized design was used with ten replications. Results showed that plantlets weaned on PP + VE performed better than the other treatments at the end of the weaning period with significantly ($P < 0.05$) highest survival rate (98%), plant vigour (2.6), number of leaves (5) and number of nodes (8). This was closely followed by RS with survival rate, plant vigour, number of leaves and nodes of 63%, 1.4, 1.7 and 3.5, respectively. Plantlets weaned on the other substrates performed poorly. Although plantlets weaned on peat pellet + vermiculite mixture out-performed the other substrates, river sand if properly handled could be a potential substitute for the conventional substrate in weaning cassava plantlets.*

Keywords— Cassava, In vitro, local substrates, peat pellet, vermiculite, weaning.

I. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is among major food and income security crops in sub-Saharan Africa [1]. It is a major food security crop providing about 500 calories per day for 800 million people in sub-Saharan Africa and other regions of the world [2]. Cassava is the third most important source of calories, after rice and maize for many populations in the humid tropics [3]. According to FAO [4], millions of small-scale farmers in more than 100 countries in the world now cultivate cassava with the major global production area being in Africa.

It is widely grown by farmers because of its remarkable characteristics such as reliability and cheap source of available year-round food, reasonable yields on marginal soils, tolerant to major pests, diseases and drought [5]. As a major carbohydrate crop, it is a versatile resource with potential for creating diverse products such as chips, broken dried roots meal, starch, flour and ethanol [6; 7]. The role of cassava as a traditional food for human consumption is rapidly changing to that of an efficient industrial crop in some parts of Africa. Dried cassava roots and meal are used as raw material for animal feed, while cassava starch is used for industrial purposes [8].

Cassava is traditionally propagated vegetatively using stem cuttings. High seed dormancy characteristic of cassava seeds and delayed germination limit seed propagation. Using traditional stem cutting causes loss of superior genotypes and decreases productivity as a result of low multiplication ratio (1:10) and viral, fungal and bacterial diseases [9; 1]. Yield losses of over 20% have been reported due to diseases [10; 11], hence the development and use of efficient micro-propagation techniques to produce healthy planting materials [11].

Micro-propagation techniques are used for rapid clonal multiplication of selected genotypes of diverse plant species [12]. In vitro culture has contributed significantly to crop improvement by overcoming certain limitations associated with conventional techniques [13]. Culturing of an organized tissue in the form of very small shoots or meristem has allowed the most valuable application of plant tissue culture in order to eliminate virus from infected mother plant [5]. Sesay [1] stated that until production constraints are reduced in high-yielding cassava varieties

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and cassava producers have access to disease-free planting materials the full potential of cassava will not be realized.

Tissue-cultured propagules are extremely vulnerable to environmental stress. This is due to the fact that they are produced under controlled environment, therefore the plantlets produced have small juvenile leaves with reduced photosynthetic capacity and malfunctioning stomata [14], poor vascular connection between roots and shoots and thus reduced water conduction and poorly developed cuticle or waxy layer [15]. These problems have been overcomed by weaning/ hardening of the plantlets [16].

Bonilla Morales [17] reported up to 90% loss of cassava plants from transplanting vitroplants to ex vitro environment. Nowak and Pruski ([14] reported that gradual adaptation of plants to the *ex-vitro* environment (which occurs during weaning) improved plants survival upon transfer to soil. The ultimate success of in vitro propagation lies in the successful establishment of plants in the soil [18].

However, the high cost of using peat pellet and vermiculite for weaning of plantlets has opened research on the use of alternative substrates that will be efficient and cost effective. Bonilla Morales [17] evaluated different organic substrates for acclimatization and hardening of vitroplants of cassava and reported that the substrate solid humus + husk dry rice in the ratio of 1:1 allowed the survival and adaptation of 80% of the vitroplants in comparison with solid humus + shaving (32.5%) and Bocashi (0%). Ubalua and Okoroafor [19] investigated the use of locally available substrate (river sand, saw dust and rice mill waste) as alternative to conventional substrate (jiffy peat) for hardening of sweet potato and reported 100% survival of the in vitro plants regardless of the substrate or substrate combination used. They also reported non-significant difference in plant height, number of leaves and number of nodes produced by the potato plants weaned with combination of 2:1 river sand/saw dust and jiffy peat.

Little work has been done on the use of local substrates for weaning cassava plantlets. Hence, this work was aimed at evaluating the performance of in vitro cassava plantlets weaned with locally available substrates with a view to providing alternative means of weaning cassava plantlets.

II. MATERIALS AND METHODS

2.1 Study location

This work was carried out at the Plant Tissue Culture Laboratory, Biotechnology Research and Development Center, National Root Crops Research Institute (NRCRI), Umudike, Umuahia, Abia State, Nigeria.

2.2 Plant material

Nodal cuttings measuring 1 cm long were excised from healthy six weeks old cassava variety (OG 001) in the culture room of the tissue culture laboratory of NRCRI. The explants were washed, sterilized and cultured in vitro following standard procedures in tissue culture exercise. Plantlets resulting from the culturing process were weaned using different substrates.

2.3 Preparation of substrate bags

The substrate bags were prepared using clear transparent polythene sheets of 0.28mm thickness. The sheet was cut out to a size of 20cm by 7.5 cm with a pair of scissors. Its two open horizontal ends were sealed using the impulse heat sealer. The two sealed ends were infolded and held with pins and then one of the vertical ends was sealed up using the impulse heat sealer, leaving only one vertical end open. The pins were detached and the polythene substrate bags were punctured to allow drainage of water and air passage.

2.4 Construction of humidity chamber

A clear transparent polythene sheet of width 186cm and length 125cm was cut using a scissors and rolled to a white glossy plywood board measuring 50cm in diameter. The transparent sheet was held to the edge of the board using thumb pins. The small overlapping polyethylene sheet at the base of the board was glued to the board so as to close the gaps and to avoid air passage into the humidity chamber.

2.5 Treatments and experimental design

The substrates used for the weaning process included top soil, river sand, saw dusk, rice husk, peat pellet and vermiculite. These substrates were used singly and in combination as follows: top soil (TS), river sand (RS), saw dust (SD), rice hull waste (RH), 2:1 top soil plus river sand (TS +RS), 2:1 river sand plus saw dust (RS + SD), 2:1 river sand plus rice husk (RS + RH), 2:1 top soil plus saw dust (TP + SD), 2:1 top soil plus rice husk (TP + RH), 2:1 saw dust plus rice husk (SD + RH) and 2:1 peat pellet plus vermiculite (PP + VE), which served as the control. Thus a total of 11 treatments were used. These treatments were arranged in a completely randomized design and replicated ten times.

2.6 Weaning of the cassava plantlets

The substrates were properly mixed with water and sterilized by autoclaving at 121^0C for 20 min. The *in vitro*-raised cassava plantlets were carefully removed from the culture vessel and then gently washed with distilled water to remove adhering medium on the roots. Thereafter, the cassava roots were transplanted with care onto the different sterile substrates contained in transparent polybags. One plantlet was transplanted per substrate bag. Immediately

after transplanting, the polybags containing the substrates plus cassava plants were placed in the humidity chamber and sprayed with distilled water. The humidity chamber was made airtight by closing all the open ends. The airtight clear transparent polythene humidity tent was kept under a shade to reduce water loss and irradiance during the acclimatization period.

In the morning of the fourth day, three holes about 1 cm in diameter were made on the sides of the humidity chamber to reduce the relative humidity of the chamber. In the morning of the fifth day, the relative humidity was further lowered by making an opening (window) on the lower side of the tent using a scissors. The plantlets and the humidity tent were then sprayed with water in the morning and evening of the same day. A second window was opened on the opposite side of the tent in the morning of the sixth day. A wash bottle was filled with water and six grains of NPK 20:15:15 fertilizer was added. This was sprayed on the plantlets in the morning and evening of the sixth day. On subsequent days, the plants were sprayed with water morning and evening for the period of the study.

2.7 Assessment parameters

The following parameters were determined at weekly interval for a period of six weeks: percentage survival rate, plant vigour, number of leaves and number of nodes.

A scale of 1 – 3 rating was used to determine the vigour of the plants, where:

1 represents not robust

2 represent fairly robust

3 represent robust

2.8 Statistical Analysis

Data generated were analyzed statistically using SPSS version 16.0 at 5% level of significance and significant means were partitioned using Duncan Multiple Range Test.

III. RESULTS

Plantlets survival rate was significantly ($P < 0.05$) affected by the substrates used in all the weeks sampled. Plantlets weaned with locally sourced substrates significantly had lower survival rates in relation to the conventional substrate (2:1 peat pellet plus vermiculite) with the exception of plantlets weaned on river sand only, which had comparable values with the control from weeks 1 – 4 (TABLE 1). From week 5 into the weaning period, all the plantlets had died except those weaned on peat pellet + vermiculite and river sand only. However, by the end of the weaning period, plants weaned on peat pellet + vermiculite had percentage survival rate of 98%, river sand alone had 63% while the least percentage survival of 2% was recorded in 2:1 top soil plus rice hull waste, 2:1 saw dust plus rice hull waste, top soil only and rice hull waste only.

In the case of vigour of plantlets, plantlets weaned on 2:1 peat pellet plus vermiculite consistently had significantly higher vigour in comparison with those weaned on the other substrates with the exception of plantlets weaned on river sand only at weeks 1 and 2 (TABLE 2). In the same vein, plantlets weaned on river sand alone consistently had significantly higher vigour than those on the other locally sourced substrates, which had comparable vigours.

Number of leaves produced by the cassava plantlets also differed significantly ($P < 0.05$) among the different substrates used from week 2 into the weaning period (TABLE 3). Significantly, the highest number of leaves was produced by plantlets weaned on 2:1 peat pellet plus vermiculite in all these weeks. However, plantlets weaned on river sand alone produced number of leaves that was significantly ($P < 0.05$) higher than those weaned on the other locally sourced substrates, which had statistically similar values. Aside the plantlets weaned with 2:1 peat pellet plus vermiculite, 2:1 top soil plus river sand, 2:1 river sand plus rice hull waste and river sand only, plantlets from the other substrates lost their leaves and died by week 3 into the weaning period. By week 5, the plantlets have lost their leaves and died except those weaned on 2:1 peat pellet plus vermiculite and river sand only.

Significant effect of substrates on number of nodes produced by the cassava plantlets was found in all the weeks sampled (TABLE 4). In weeks 1 and 2, plantlets weaned on peat pellet + vermiculite and river sand alone produced comparable number of nodes that was significantly higher than those on the other substrates. By week 6, plantlets weaned on 2:1 peat pellet produced significantly the highest number of nodes, followed by plantlets weaned on river sand only while the number of nodes from plantlets weaned on the other substrates did not differ significantly.

IV. DISCUSSION

The weaning of in vitro raised plants is essential for better survival and successful establishment. Direct transfer of tissue-cultured plants to sunlight (field condition) causes leaf charring and wilting of the plants [20; 16]. Weaning therefore determines the survival percentage of the plantlets in the field.

In the present study, results revealed that plantlets weaned on 2:1 peat pellet plus vermiculite showed 98% survival rate. This was significantly higher than the percentage survival recorded in the locally sourced

substrates. Following this was river sand alone, which gave survival rate of 63 % that was significantly higher than the other locally sourced substrates that recorded survival rates between 2 and 20 %. Afreen-Zobayed [21] stated that the percentage survival of in vitro plantlets during acclimatization is controlled by the nature of the supporting substrates and the intrinsic quality of the micro propagated plantlets among other factors. Studies have shown that a variety of materials including saw dust, top soil and rice mill waste are detrimental to or have no benefit for plant growth [22].

Ubalua and Okoroafor [19] reported 100% survival rate of sweet potato plantlets grown on sterile substrates (river sand, saw dust, rice mill waste, river sand/rice mill waste, river sand/saw dust, saw dust/rice mill waste and jiffy peat) and 58% survival rate on unsterilized substrates. Similarly, Bonilla Morales et al. [17] reported survival rates of 80%, 32.5% and 0% of in vitro cassava plantlets (Brazilian variety) hardened with 1:1 solid humus + husk dry rice, 1:1 solid humus + shaving 1: 1 and Bocashi and concluded that the 1:1 solid humus + husk dry rice substrate is considered suitable both nutritional level and structural component of the soil for having an adequate porosity for rooting of plant and an ideal adaptation field phase.

Plantlets on 2:1 peat pellet and vermiculite were more vigourous compared to the plantlets on the other supporting substrates where as plantlets on river sand alone had more vigour than the other locally sourced substrates. This may be attributed to the porosity of these materials. Ubalua and Nsofor [23] reported that acclimatization and growth of in vitro raised plantlets is highly correlated with substrate porosity. Savangikar [24] stated that generated plants must be vigorous and capable of being successfully transplanted in the field, and must have high field survival. It has been found that the production of high quality and vigorous plants through *in vitro* culture requires the enhancement of post-transplanting ability for water management, efficiency of photosynthesis and resistance to diseases. This is achieved by using suitable substrate for weaning process.

Overall, the plant growth parameters differed significantly ($P < 0.05$) among the various substrates used. Highest number of leaves and nodes were recorded in plantlets weaned with the conventional substrate (peat pellet/vermiculite). River sand alone weaned plantlets produced the second highest number of leaves and nodes at the end of the weaning process. Plantlets on saw dust alone lost their leaves from week 1into the weaning period. Apart from the plantlets weaned with 2:1 peat pellet plus vermiculite, 2:1 top soil plus river sand, 2:1 river sand plus rice hull waste and river sand only, plantlets on the other substrates lost their leaves and died by week 3 into the weaning period. By week 5, the plantlets have lost their leaves and died except those weaned on 2:1 peat pellet plus vermiculite and river sand alone. This is in support of the earlier report made by [22] that a variety of materials including saw dust, top soil and rice mill waste are detrimental to or have no benefit for plant growth.

Plantlets on peat pellet/vermiculite and river sand alone had competitive results on number of nodes produced at weeks 1 and 2 after which significant differences were recorded between them. Ubalua and Nsofor [23] reported enhanced rooting and growth of cassava plantlets variety TMS 98/0505 on river sand/sawdust and peat pellet/vermicultite and attributed it to the porosity of these substrates. Nodal increase of plants is associated with increases in growth. The retardation in growth as evidenced in the number of nodes recorded in the locally sourced substrates may be attributed to the low drainage property of these substrates leading to accumulation of water and consequently depletion of oxygen. The exhaustion of oxygen increases microbial activity and interferes with plant-soil-water relationship [25]. This has toxic effect on the morphological and anatomical aberration in the number of leaves. This could be the reason for the abortion of leaves, fungal attack, wilting and death of plantlets observed in the locally sourced substrates. On the contrary, peat pellet + vermiculite mixture provides good drainage and aeration to plant roots, which aided better performance of the cassava plantlets. Our findings is in agreement with the report of [19] who reported better performance of plantlets on peat pellet but disagrees with the report of [26] and [27] who reported low effectiveness of vermiculite as a substrate for acclimatization. It has been reported that good quality propagules with well developed roots and leaves are easy to acclimatize to the external environment and that any successful acclimatization protocol must ensure that the plants maintain active growth during the entire weaning period [28].

V. CONCLUSION

Survival, vigour and growth of the cassava plantlets were significantly influenced by the type/quality of the substrates used, their physical nature and possibly their nutrient composition. The leaf abortion, fungal attack, wilting and subsequent death of plantlets observed in the locally sourced substrates (excepting river sand alone) showed the inefficiency of these substrates in weaning cassava plantlets. However, plantlets grown on peat pellet + vermiculite mixture performed better than plantlets grown

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on the locally sourced substrates. Amongst the locally sourced substrates, river sand alone weaned plantlets performed better and could be a potential substitute for the conventional substrate if properly handled.

VI. TABLES

Table.1: Survival of in vitro cassava plantlets weaned on different substrates at different weeks

	Weeks						
Treatment	1	2	3	4	5	6	% Survival
PP + VE	1	1	1	1	1	1	98
TP	0.1	0	0	0	0	0	2
RS	0.8	0.8	0.6	0.6	0.5	0.5	63
SD	0.2	0.1	0	0	0	0	5
RH	0.1	0	0	0	0	0	2
TS +RS	0.6	0.4	0.1	0	0	0	18
RS + SD	0.7	0.3	0	0	0	0	16
RS + RH	0.7	0.5	0.4	0.1	0	0	28
TP + SD	0.4	0.1	0	0	0	0	8
TP + RH	0.1	0	0	0	0	0	2
SD + RH	0.1	0	0	0	0	0	2
LSD (0.05)	0.3	0.6	0.5	0.4	0.01	0.00	25

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk

Table.2: Vigour of cassava plantlets weaned on different substrates at different weeks

	Weeks					
Treatment	1	2	3	4	5	6
PP + VE	2.4	2.4	2.4	2.6	2.5	2.6
TP	0.1	0	0	0	0	0
RS	2.0	1.9	1.8	1.9	1.9	1.4
SD	0.3	0.1	0	0	0	0
RH	0.1	0	0	0	0	0
TS +RS	0.8	0.3	0.1	0	0	0
RS + SD	0.7	0.1	0	0	0	0
RS + RH	1.0	0.6	0.2	0.1	0	0
TP + SD	0.5	0.1	0	0	0	0
TP + RH	0.1	0	0	0	0	0
SD + RH	0.1	0	0	0	0	0
LSD (0.05)	0.8	0.5	0.1	0.0	0.0	0.0

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk

Table.3: Number of leaves produced by cassava plantlets weaned on different substrates at different weeks

	Weeks					
Treatment	1	2	3	4	5	6
PP + VE	2.7	2.7	3.5	3.6	4.7	4.6
TP	0.1	0	0	0	0	0
RS	1.7	2.0	2.3	1.9	1.7	1.7
SD	0.3	0.1	0	0	0	0
RH	0	0	0	0	0	0
TS +RS	0.8	0.4	0.1	0	0	0
RS + SD	1.0	0.3	0	0	0	0
RS + RH	0.8	0.9	0.3	0.1	0	0
TP + SD	0.4	0.1	0	0	0	0
TP + RH	0.3	0	0	0	0	0
SD + RH	0.1	0	0	0	0	0
LSD (0.05)	NS	0.5	0.1	0.1	0.1	0.0

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk

Table.4: Number of nodes of cassava plantlets weaned on different substrates at different weeks

	Weeks					
Treatment	1	2	3	4	5	6
PP + VE	3.8	4.9	5.7	7.1	7.9	8.0
TP	0.5	0	0	0	0	0
RS	4.0	4.2	4.5	3.8	3.5	3.5
SD	0.6	0.6	0	0	0	0
RH	0.6	0	0	0	0	0
TS +RS	1.7	1.3	0.6	0	0	0
RS + SD	2.1	0.5	0	0	0	0
RS + RH	2.5	1.6	0.6	0.6	0	0
TP + SD	1.2	0.5	0	0	0	0
TP + RH	0.4	0	0	0	0	0
SD + RH	0.5	0	0	0	0	0
LSD (0.05)	0.5	0.8	0.2	0.1	0.0	0.0

PP + PV = 2:1 peat pellet plus vermiculite; TS = top soil; RS = river sand; SD = saw dust; RH = rice hull waste; TS + RS = 2:1 top soil plus river sand; RS + SD = 2:1 river sand plus saw dust; RS + RH = 2:1 river sand plus rice husk; TP + SD = 2:1 top soil plus saw dust; TP + RH = 2:1 top soil plus rice husk; SD + RH = 2:1 saw dust plus rice husk .

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Utilization of Guinea corn (*Sughurm vulgare*) Husk for Preparation of Bio-based Silica and its Characterization Studies

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Abstract*—Bio-based silica was prepared from Guinea corn Husk within the context of sustainable chemistry. The Guinea corn Husk was leached in dilute HCl, washed, dried and calcined at 650 ^oC for 4hr. The resultant ash was digested in 2M NaOH solution, precipitated by adding H_2SO_4 and then washed to remove sulphate by-product. The siliceous materials were characterized using SEM-EDX, FTIR and XRD to investigate its composition and morphology, functional groups and the phase respectively. The elemental investigation revealed Si as major element in the ash. The presence of Si-O-Si (siloxane) and Si-OH (silanol) were confirmed by FTIR of the silica. The SEM micrograph of the silica also showed agglomeration of regular spherical particles in the morphology. The XRD pattern of the siliceous material indicated an amorphous form of the product. Guinea corn Husk as an agricultural waste can be a sustainable means for silica and other siliceous materials.*

***Keywords*— Agricultural waste, Bio-based, Guinea corn Husk, Silica, Sustainable chemistry.**

I. INTRODUCTION

The concerns of sustainable chemistry have lead to the production of bio-based materials. Bio-based materials refer to products that mainly consist of a substance (or substances) derived from biomass and either occur naturally or are synthesized, or it may refer to products made by processes that use biomass (Curran, 2010). Sustainable chemistry has one of its objectives to be the use of renewable feed stocks and byproduct in the production processes (Karagolge and Gur, 2016). Sustainable means are used to find alternatives to conventional chemical syntheses and transformations (Varma, 2014). Scalet *et al.*, (2015) posited that this sustainable means play a significant role in effectively addressing the global challenges, such as economy, climate change, limited natural resources, dependency on decreasing fossil resources.

Agriculture practice is a means of sustainable development as it contributes majorly to the economy of some countries in the world (Chongela, 2015). Recently more attention is given to agricultural production in most developing countries. In Nigeria, part of the efforts of the government to diversify economy of the nation from non-renewable oil is by boosting agricultural production (Anyawu *et al.*, 2013). Waste from agricultural practices have been investigated and reported to have a greater potential for bioresource like bioenergy (Simonyan and Fasina, 2013) and biogas (Ngumah *et al.*, 2013).

Guinea corn Husk (GcH) is an agricultural waste from milling of Guinea corn (*Sughurm vulgare*). Guinea corn is an important food crop grown abundantly in northern part of Niger and Benue Rivers in Nigeria (Ndububa and Nurudeen, 2015). Akinloye *et al.* (2014) reported that about 1.5 million tones of guinea corn husk is generated annually which has potential to increase as the economy is diversified to agriculture. Guinea corn Husk has been utilized in the production of bioethanol (Oyeleke and Jibrin, 2009) and its ash as partial replacement in Ordinary Portland Cement in concrete (Ndububa and Nurudeen, 2015) with major constituent of SiO_2.

Silica, SiO_2 is present in abundance on earth crust and plant root tissues (Ghorbani *et al.*, 2013). It exists in amorphous and crystalline structures consisting of inter-linked SiO_4 unit in a tetrahedral arrangement (Nandanwar, 2013; Le *et al.*, 2013). It is an important basic raw material in many industrial finished products such as electronics, ceramic, pharmaceutics, detergents, adhesives and polymer materials. It also has many technological applications as thixotropic agents, thermal insulators, composite fillers, e.t.c. (Ghorbani *et al.*, 2013; Sun and Gong, 2001). Some biomass materials have been reportedly used for synthesis

of silica among which are rice husks (Wang, 2012; Le *et al.*, 2014), wheat husks (Shaik and Shaik 2013), coconut shells (Sivasubramanian and Sravanthi, 2015), sedge (Ghorbani *et al.*, 2013), cow dungs (Rani, *et al*., 2014).

Consequence of the expansion in agricultural production is an increase in the level of agricultural waste. These agricultural wastes encompass waste from unutilized (excess) and biomass residue from processing of agricultural products (Edewor-Ikuponiyi and Amuda, 2013). Often, these wastes pose threat to the environment, resulting in bad odour and also affecting the quality of groundwater. However, agricultural wastes embedded with various useful constituent which can be harnessed as raw materials for further production of materials.

Attention of researchers is now shifted to the utilization of biomass material towards a sustainable development of chemicals. Therefore, the focus of this research is on the possibility of preparing siliceous materials from Guinea corn Hush.

II. MATERIALS AND METHODS

2.1 Materials

Guinea corn Husk (GcH) was sourced locally from farmer as an agricultural waste and was used as a feedstock for the preparation of silica. Chemical reagents used including sodium hydroxide, sulphuric acid, hydrochloric acid and barium chloride are of analytical grade and were used as purchased.

2.2 Methods

2.2.1 Preparation of Silica from Guinea corn Husk

The preparation was done using slightly modified method reported by Sivasubramanian and Sravanthi, (2015). The guinea corn Husk (GcH) was washed thrice with de-ionized water to remove some foreign particles from it after which it was air dried. The air dried GcH was pre-treated by leaching in 0.1 M HCl for 2 hr and washed with de-ionized water to remove some other metallic oxides. The pre-treated GcH was calcined in muffle furnace at 650 ^{o}C for 4 hr to produce Guinea corn Husk Ash (GcHA). Silica extraction was done by adding 2 M NaOH solution to the ash and stirred on magnetic stirrer plate at a constant temperature of 100 ^{o}C for 8 hr. Afterwards, the mixture was filtered and the sodium hydroxide extract in form of sodium silicate (eqn. 1) was further used for the preparation of silica. The sodium silicate was reacted with 2.5 N H_2SO_4 to precipitate pure silica (SiO_2) (eqn. 2). The white siliceous precipitate formed was aged in an autoclave for 5 hr, filtered and washed severally with de-ionized water to remove sulphate impurities.

$$SiO_2 + 2NaOH \rightarrow Na_2SiO_3 + H_2O \quad (1)$$

$$Na_2SiO_3 + H_2SO_4 \rightarrow SiO_2 + Na_2SO_4 + H_2O \quad (2)$$

2.2.3 Characterization Studies

The elemental compositions of the guinea corn husk ash (GcHA) were determined by X-Ray Fluorescence (XRF) and Energy Dispersive X-ray Spectroscopy (EDX). The bio-based silica prepared from the GcHA was characterized for its surface functional groups, morphological properties with elemental compositions and to assess the phase, the degree of crystallinity using FTIR and SEM-EDX and XRD respectively.

III. RESULTS AND DISCUSSIONS

Guinea corn Husk Ash and Silica

The composition of the acid-leached Guinea corn Husk Ash (GcHA) determined by XRF is presented in Table 1. The results showed that the ash contains 93.83% of SiO_2 as the major component; Al_2O_3 and CaO were present as minor components while K_2O, Fe_2O_3 and P_2O_5 as trace components. This indicates that guinea corn husk (GcH) is a good precursor for silica comparing with value reported for rice husk ash (99.08%) by Le *et al.*, (2013). In a related investigation on the same guinea corn husk by Nurudeen and Ndububa, (2015), 78.17% of SiO_2 was reported. However, 93.83% obtained from this study is likely due to the pre-treatment in HCl which reduced the content of other metallic oxides.

Table.1: Chemical composition of the GcHA

Element	**Al_2O_3**	**SiO_2**	**K_2O**	**CaO**	**Fe_2O_3**	**P_2O_5**
% Weight	1.182	93.83	0.069	1.897	0.146	0.247

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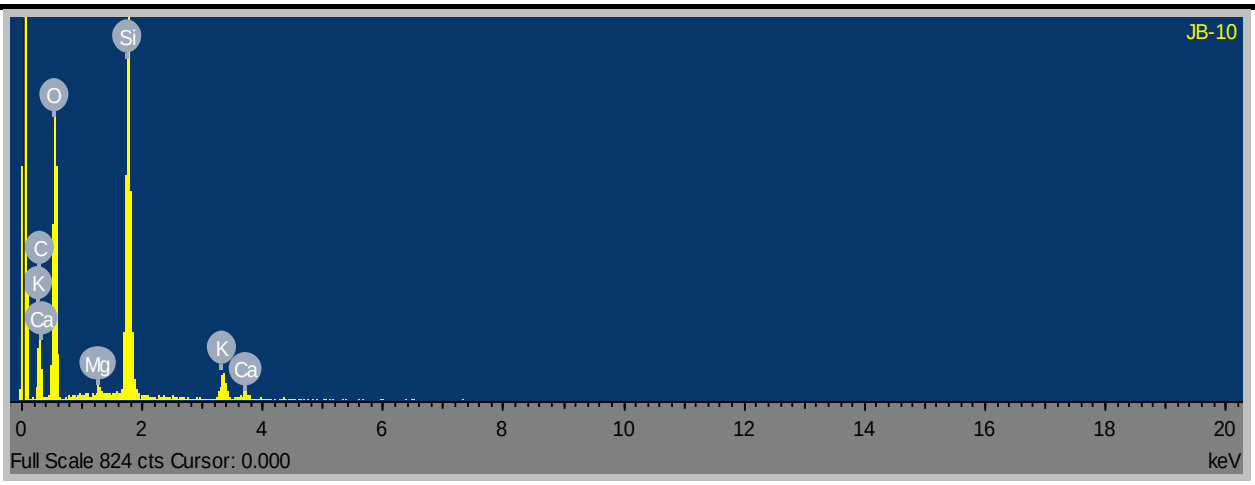

Fig.1: EDX spectrum of Guinea corn Husk Ash (GcHA)

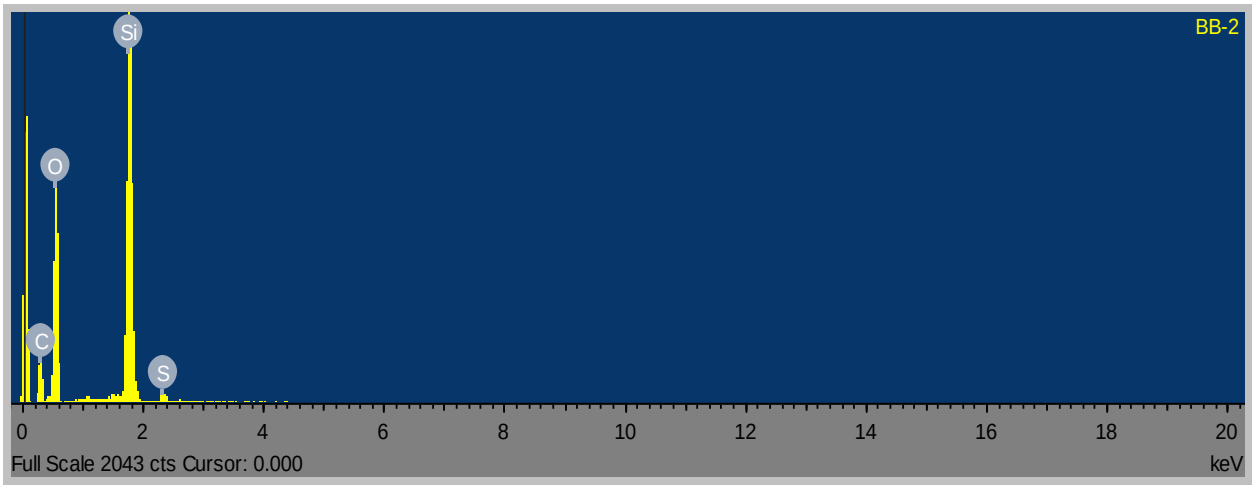

Fig.2: EDX spectrum of the prepared silica from GcHA

The comparison of elemental compositions of the guinea corn husk ash (GcHA) and the prepared silica as revealed by Energy Dispersive X-ray spectroscopy (EDX), (Figs. 1 and 2) with XRF results showed that the results were in close agreement. It is observed that silicon with the highest intensity is the major element present in the ash**.** This agrees with the XRF analysis result reported in Table 1. Consequence to this result, it is evident that silica can be prepared from GcHA. The guinea corn husk ash (GcHA) contains majorly silicon with little impurities of other elements. The intensity of the silicon (Si) in the prepared silica appears to be stronger (Fig. 2) than that of GcHA (Fig. 1). The disappearance of some minor elements like magnesium, potassium and calcium in the silica (Fig. 2) which were earlier present in GcHA (Fig. 1) suggested more purity of the silica. Therefore, purer silica has been extracted from the ash using sodium hydroxide (Shaik and Shaik, 2013). However, the presence of carbon and sulphur are likely to be contaminant during the analysis.

The FTIR spectroscopic study revealed the two major functional groups of silica, silanol (Si-OH) and siloxane (Si-O-Si) (Fig. 3). The summary of the observed functional groups is presented in Table 2. The broad absorption band at between 3433 cm^{-1} is assigned to –OH of silanol and absorbed water (Rani *et al*., 2014). The peaks at 954, 795 and 463 cm^{-1} correspond to asymmetry, symmetry and bending mode of SiO_2 respectively (Le *et al.*, 2013). The siloxane group of the silica is attributed to 1083 cm^{-1} (Chee and Yaacob, 2010). The surface functional group from FTIR analysis confirmed the material to be silica.

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Table.2: FTIR peaks of silica and the assigned bond

Assigned bond	Wave number (cm^{-1})
O-H	3433
Si-O-Si	1083
SiO_2 asymmetry mode	954
SiO_2 asymmetry mode	795
SiO_2 bending mode	463

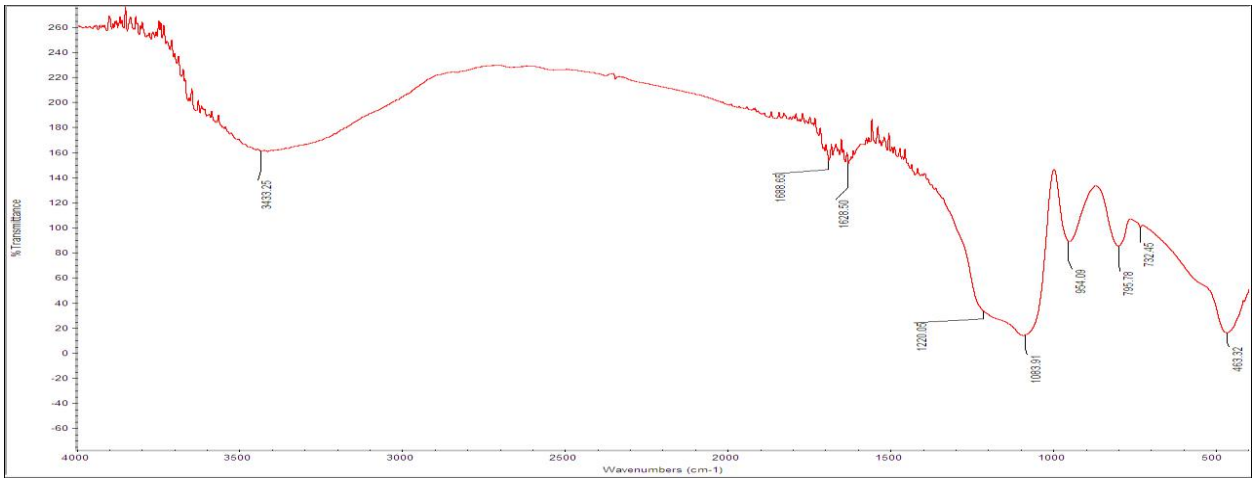

Fig. 3: FTIR spectrum of the prepared silica from GcHA

The morphology studied by Scanning Electron Microscopy (SEM) is shown in Figs. 4 and 5 for the GcHA and extracted silica respectively. The micrograph (Fig. 4) revealed flake like and irregular particles. These irregular particles of the ash morphology suggested different components of the ash. The Fig. 5 however shows agglomeration of regular and spherical shape particles but rough surface. This indicates assembly of similar particles of silicate ions as compare with the morphology of the ash which was irregular. Ndubuda and Nurudeen, (2015) reported the direct use of such ash in partial replacement of cement in concrete. It evident that higher quality and purity of silica can be extracted from ash contained silica by treatment with aqueous sodium hydroxide followed by precipitation using acid (Shaik and Shaik, 2013).

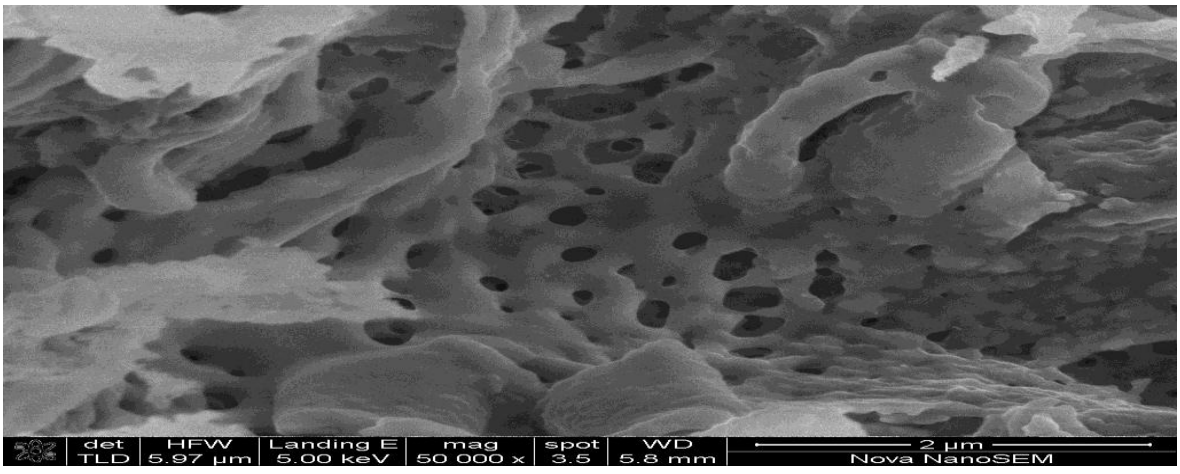

Fig.4: SEM micrograph of the GcHA

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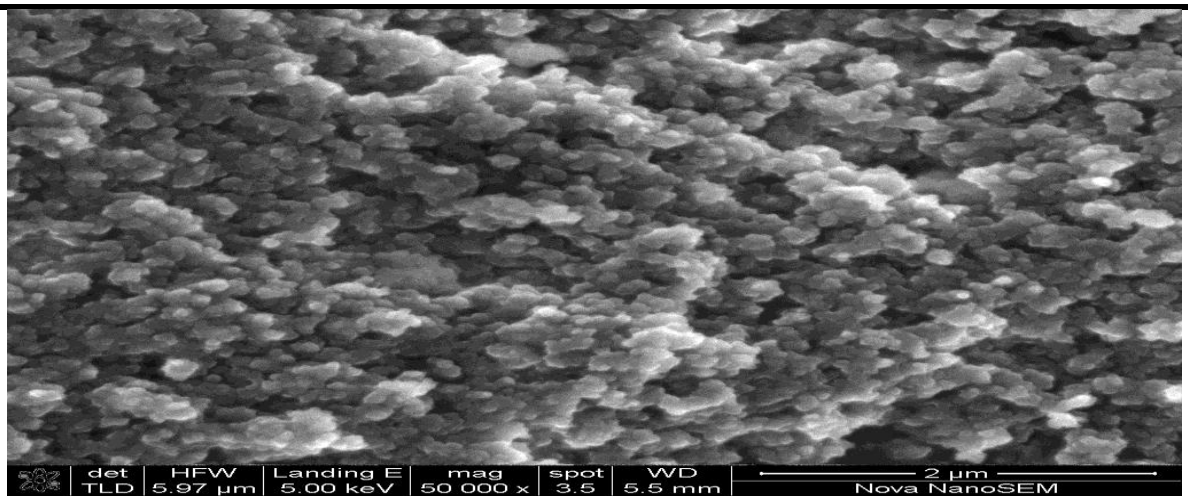

Fig.5: SEM micrograph of the prepared silica

The XRD pattern of the silica is presented in Fig. 6. From the figure, it can be said that the silica is an amorphous since the characteristic peak at 2θ cannot be seen (Le *et al*., 2013; Geetha *et al*., 2016).

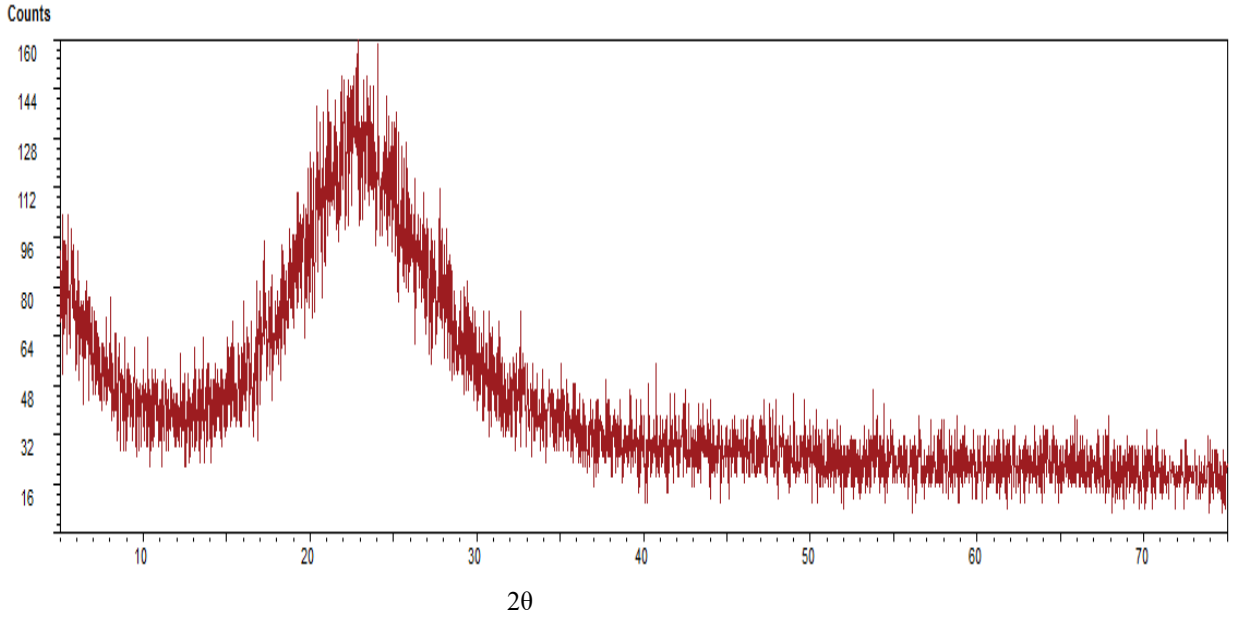

Fig. 6: XRD pattern of the prepared silica

IV. CONCLUSION

This study has successfully demonstrated the use of guinea corn husks, an agricultural waste for the preparation of silica. The characterization studies using FTIR and SEM-EDX confirmed the prepared material to be silica. Also XRD pattern indicated the amorphous form of silica. Therefore, it is found that amorphous silica, an important material which has numerous applications can be prepared in accordance with sustainable chemistry.

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Comparative evaluation of microbiological and nutritional qualities of various cereal-based paps (*Ogi*) in Ondo State, Nigeria

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Abstract*—This study was carried out to determine the microbiological, proximate and elemental analyses of maize-, millet- and sorghum-based Ogi in Ondo State, Nigeria. Samples were monitored at points of preparation from 0 to 96 hours of fermentation. Selected dilutions were inoculated by spread-plate method on appropriate medium for isolation of aerobic bacteria, staphylococci, enterobacteria, lactic acid bacteria (LAB) and fungi. Further identification was done by API 50 CHL, API 50 CHB and API 32 ID kits for LAB, aerobic bacteria and fungi, respectively. Proximate and mineral compositions were in accordance to standard procedures. One-sample t-test, paired-wise sample t-test and Analysis of Variance were used to analyze data. The microbial load gradually increased from 0 hour and attained optimum at 24 – 48 hours of fermentation, before declining at 72 to 96 hours. LAB were persistent and most predominant. Twenty-four bacterial species were isolated. Occurrence of Lactobacillus plantarum (10.3%) was highest while Mucor mucedo (0.86%) was lowest. There were no significant differences in the microbial loads, proximate and elemental compositions of products.Thisstudy revealed the distribution of fermentative microorganisms and few contaminants which were not directly associated with fermentation process. The study also showed significantly acceptable proximate and elemental compositions of the products.*

Keywords*— *Cereals, fermented food, microorganisms, nutritional composition.

I. INTRODUCTION

Fermentation technology has lived as long as mankind [1,2]. It is, thus, an integral traditional practice in many communities in Africa and other continents of the world. Fermentation of food has been described as age-long culture which has been under-documented particularly in West Africa, where absence of writing culture made its origin difficult to trace [3]. Fermentation of food typically involves the application of microorganisms (either from the environment i.e. spontaneous process or inoculated in a controlled environment) that produces certain enzymes which changes the chemical attributes of the food from its original form/state. Fermentation is a desirable biochemical modification process of main food matrix brought about by microorganisms and their associated enzymes [4]. The changes that occur during fermentation could either be deleterious (producing toxins) or beneficial (producing food products with superior or distinct attributes).

The Nigerian indigenous fermented foods constitute a group of foods that are produced in homes, villages and small-scale cottage industries. They are sold to the rural populace who buy them for food and social ceremonies. Roots, legumes, cereals, fruits, oil seeds, nuts, meat, fish, milk and palm tree sap are some of the substrates from which fermented foods are derived. One of the popular indigenous cereal-based fermented foods in Nigeria is *Ogi*, a kind of pap, which is a fermented cereal porridge made from maize (*Zea mays*), sorghum (*Sorghum vulgare*) or millet (*Pennisetum typoideum*). Pap can be simply described as a kind of diet that does not require chewing. The cereal-based pap (*Ogi*) is very smooth in texture and has a sour taste reminiscent of that of yoghurt. Typically, *Ogi* has a distinct aroma and fine texture. The colour of the *Ogi* is mainly depending on the type of feedstock used for the processing. It could either be consumed as porridge (pap) or as a gel-like product (*agidi*) in some West African countries [5,6].

Sorghum, maize and millet beverages in Africa possess similar features in which the lactic acid bacteria

fermentation plays a key role in safety and acceptability of these products in tropical climate. Cereal beverages are popular in Africa because of the social, religious and therapeutic values associated with them. The consistency of the pap varies from thick to watery depending on choice. The pap can be sweetened with sugar and milk; it is then eaten with bean cake. The pap is used as the first native food for weaning babies [7,8].

It also serves as breakfast meal for pre-school, school children and adults. In a more concentrated form it is boiled into a thick gel and then allowed to set stiff in leaf moulds as "*eko*" or "*agidi*". In either form, it is usually preferred to many other indigenous foods by the aged and the convalescence. The stages of traditional *Ogi* production include: washing of grains, steeping for 3 days at ambient temperature (28 ± 2°C), wet-milling, wet-sieving with a hand sieve or muslin cloth with about 300 µm pore size and sedimentation/souring of the filtrate for 1–3days. Thereafter, the water is decanted and the wet, clean sediment (*Ogi*) is collected and stored for personal use or sold to consumers in its wet form in small units packaged in leaves or polypropylene bags [9,10].

The traditional method of *Ogi* processing is accompanied by severe microbial contamination and nutrient losses, the magnitude of which depends on the hygienic practices, quality of water, type of cereal grains and the fermentation or souring periods and the milling method used. This study was, therefore, carried out to determine the microbial quality of fermented maize-, millet- and sorghum-based Ogi in Ondo State, Nigeria.

II. MATERIALS AND METHODS

2.1Sample Collection

Three samples each of sorghum-, millet- and maize-based *Ogi* were monitored at the points of preparation, from different locations over a period of four days, from zero (0) to 96 hours of fermentation of the cereals within Ondo West Local Government Area. The samples were collected in sterile polythene bags and transported to the laboratory for analysis.

2.2Sample Preparation

Ten grams each of the paste-like samples was weighed and introduced in 90 ml 0.85% (w/v) sterile physiological saline and homogenized in a stomacher lab-blender (Panasonic, Model MX-GX1021, China) for 1 min. These were serially diluted to obtain dilution factors of up to 10^9.

2.3Microbiological analysis

One mil each of randomly selected dilutionswas prepared on appropriate agar media by spread-plate method for isolation and enumeration of microorganisms. Aerobic bacteria, staphylococci and enterobacteriaceae were cultivated and enumerated on Plate Count Agar (PCA) (Oxoid England), mannitol salt agar (MSA, Oxoid) and MacConkey agar (Oxoid), respectively. Plates were incubated at 30 °C for 48 hrs, morphological characteristics on plates examined and the number of colony forming units (CFU) for each morphotype recorded separately. Potato dextrose agar (SDA, Oxoid) containing 50 mg/L chloramphenicol and 50mg/L chlortetracycline, to inhibit bacterial growth, was employed for the cultivation of fungi. Incubation was at 25 ^oC for 3 to 5 days. Lactic acid bacteria (LAB) were grown on de Man Rogosa and Sharpe (MRS) agar (Oxoid) incubated under anaerobic conditions in an Anaerobic Gas-Pack system at 30°C for 48–72 h. Colonies were counted and recorded as logarithms of the numbers of colony forming unit per gram (cfu/g). Pure isolates were stocked for further characterization.

2.4Identification of isolates

Bacterial isolates were examined for Gram's reaction, catalase production and sporulation (incubation in nutrient broth plus 50 mg/l MnCl2 for 7 days). Presumptive LAB isolates on MRS agar were examined for Gram's reaction, catalase production, gas production from MRS-broth containing inverted Durham tubes [11] and growth at 15°C and 45°C in MRS broth. Cell morphology and motility were examined by microscopic observation of cells grown in broth for 24 h. Identification of filamentous fungi was carried out following the taxonomical keys of Schipper [12] and Hesseltine [13]. Fermentation and assimilation of carbon compounds were determined using API 50 CHL kits for LAB, API 50CHB kits for aerobic bacteria and API 32 ID kits for fungi according to the manufacturer's instructions (BioMerieux, Marcyl'Etoile, France). The results were recorded visually and analysed by APILAB Plus V3.2.2 software (BioMerieux).

2.5Analysis of Proximate composition

Moisture content was determined by weight loss of 2 g of sample after heating in an oven (MAXI, Model No. PSC31G2-GI, Turkey) at 105 ^oC for 3hrs. The ash content was measured by heating the sample at 550 ^oC until the difference between two successive weights was less than 1 mg. Protein content was determined by multiplying total nitrogen, estimated by standard Kjeldahl method by 6.26. Fat content was determined by ether extraction

method using a glass soxhlet. The crude fibre content was determined using fibretec extraction. The carbohydrate content was determined by differences:

% Carbohydrate=100- (%Moisture+%Fat+%Ash+% Crude fibre+%Crude protein).

2.6 Mineral Composition

A fraction of 0.3 g of each of the paste-like sample was wet digested in a 50-ml beaker using 30 ml of HNO3-HClO4 acid solution (2:1 volume) on a hot digestion system to obtain a colourless solution after heating. At the completion of digestion, the solution of each sample was transferred into a 50-ml calibrated sample bottle and the solution was diluted to the mark with distilled water. Calcium (Ca), Magnesium (Mg), Iron (Fe) and Zinc (Zn) in the samples were determined by flame atomic absorption spectrophotometer. Sodium (Na) and Potassium (K) in the samples were determined by flame photometer using a working standard of 10 ppm for each of the species [14].

2.7 Statistical Analysis

The data obtained were analyzed using statistical one-sample t-test, paired-wise sample t-test and Analysis of Variance (ANOVA) at 95% level of confidence ($P \leq 0.05$) employing the statistical package for social sciences (SPSS) version17.

III. RESULTS

Table 1 showed microbial load during and after fermentation of maize, millet and sorghum for *Ogi* production in Ondo State, Nigeria. Sorghum-based *Ogi* had the highest aerobic bacteria count of 4.3 x 10^5 CFU/g at zero (0) h of fermentation which increased to 1.71 x 10^6 CFU/g at 24th h of fermentation. However, the aerobic bacteria count started decreasing at the 48th h and at the 96th h, no aerobic bacteria was detected on the plate count agar medium. This was also the case with maize-based *Ogi* which had aerobic bacteria count of 3.8 x 10^5 CFU/g at the zero (0) h of fermentation, increased at the 24th h, reduced thereafter and at the 96th h of fermentation, no aerobic bacterium was detected. The millet-based pap had 3.7 x 10^5 CFU/ml at zero (0) h with no bacterium detected at the 96th h of fermentation on PCA medium after following same pattern of growth at 24th, 48th and 72nd h. Staphylococci counts for the maize-based *Ogi* were 2.5 x 10^2, 2.7 x 10^2 and 1.8 x 10^2 CFU/g at the 0, 24th and 48th h of fermentation, respectively. For the sorghum-based *Ogi*, staphylococci counts at the 0, 24th and 48th h of fermentation were 2.3 x 10^2, 3.2 x 10^2 and 1.1 x 10^2 CFU/g, respectively; and 1.8 x 10^2, 2.1 x 10^2 and 1.3 x 10^2 CFU/g respectively for the millet-based *Ogi*. All staphylococci had been eliminated in the sample at the 72nd and 96th h of fermentation of the three cereal-based *Ogi*. The predominant set of microorganisms were the lactic bacteria which kept increasing from the 0 to 96th h of fermentation. LAB counts ranged from 2.9 x 10^4 to 2.93 x 10^8 CFU/g; 2.1 x 10^4 to 1.67 x 10^8 CFU/g and 2.9 x 10^4 to 2.01 x 10^8 CFU/g for the maize-, sorghum- and millet-based *Ogi*, respectively. Counts of members of family Enterobacteriaceae from maize-, sorghum- and millet based *Ogi* at 0, 24th and 48th h of fermentation were 5.2 x 10^2,3.7 x 10^3 and 2.6 x 10^2 CFU/g; 4.1 x 10^2, 4.9 x 10^3 and 1.7 x 10^2 CFU/g, and 4.8 x 10^2, 4.1 x 10^3 and 2.6 x 10^2 CFU/g, respectively; and fungal counts were 2.7 x 10^2, 3.2 x 10^3 and 2.1 x 10^2 CFU/g; 3.5 x 10^2, 2.8 x 10^3 and 1.9 x 10^2 CFU/g; and 2.2 x 10^2, 2.1 x 10^3 and 1.5 x 10^2, respectively.

Table.1: Microbial load during and after fermentation of maize, millet and sorghum for Ogi production in Ondo State, Nigeria

Medium		Fermentation period (hours)				
	Cereals	0	24	48	72	96
Aerobic bacteria count (CFU/g)	Maize	3.8 x 10^5	1.71 x 10^6	2.62 x 10^4	5.4 x 10^2	-
	Sorghum	4.3 x 10^5	1.08 x 10^6	1.23 x 10^4	3.7 x 10^2	-
	Millet	3.7 x 105	1.12 x 10^6	1.02 x 10^4	4.1 x 10^2	-
Staphylococci count (CFU/g)	Maize	2.5 x 10^2	2.7 x 10^2	1.8 x 10^2	-	-
	Sorghum	2.3 x 10^2	3.2 x 10^2	1.1 x 10^2	-	-
	Millet	1.8 x 10^2	2.1 x 10^2	1.3 x 10^2	-	-
LAB count (CFU/g)	Maize	2.9 x 10^4	1.51 x 10^6	2.62 x 10^7	2.71 x 10^8	2.93 x 10^8
	Sorghum	2.1 x 10^4	1.43 x 10^6	2.11 x 10^7	1.22 x 10^8	1.67 x 10^8
	Millet	2.9 x 10^4	1.31 x 10^6	2.43 x 10^7	1.85 x 10^8	2.01 x 10^8
Enterobacteriaceae count (CFU/ml)	Maize	5.2 x 10^2	3.7 x 10^3	2.6 x 10^2	-	-
	Sorghum	4.1 x 10^2	4.9 x 10^3	1.7 x 10^2	-	-

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	Millet	4.8×10^2	4.1×10^3	2.6×10^2	-	-
Fungal count (CFU/ml)	Maize	2.7×10^2	3.2×10^3	2.1×10^2	-	-
	Sorghum	3.5×10^2	2.8×10^3	1.9×10^2	-	-
	Millet	2.2×10^2	2.1×10^3	1.5×10^2	-	-

Table 2 showed the distribution of microorganisms in fermented maize-, millet- and sorghum-based *Ogi* in Ondo State, Nigeria. *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus amylovorus*, *Corynebacterium* spp, *Staphylococcus aureus*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Candida tropicalis* and *Aspergillus niger* were found in the three cereal-based *Ogi*. *Streptococcus lactis* and *Bacillus licheniformis* were present in millet- and sorghum-based *Ogi*. *Micrococcus luteus*, *Escherichia coli*, *Penicillium* sp and *Fusarium oxysporium* were isolated from maize- and sorghum-based *Ogi*. *Aspergillus flavus* was encountered in maize- and millet-based *Ogi*. *Lactococcus lactis*, *Enterococcus faecalis*, *Pseudomonas alkaligenes* and *Bacillus cereus* were isolated only in maize-based *Ogi*. *Pseudomonas aeruginosa* and *Mucor mucedo* were present in millet-based *Ogi* only. *Rhizopus stolonifer* was encountered in only sorghum-based *Ogi*.

Table.2: Distribution of microorganisms in fermented maize-, millet- and sorghum-based Ogi in Ondo State, Nigeria

Microorganisms	Maize-based *Ogi*	Millet-based *Ogi*	Sorghum-based *Ogi*
Lactobacillus delbrueckii	+	+	+
L. plantarum	+	+	+
L. fermentum	+	+	+
L. amylovorus	+	+	+
Lactococcus lactis	+	-	-
Streptococcus lactis	-	+	+
Enterococcus feacalis	+	-	-
Pseudomonas aeruginosa	-	+	-
Pseudomonas alkaligenes	+	-	-
Corynebacterium spp	+	+	+
Escherichia coli	+	-	+
Micrococcus luteus	+	-	+
Staphylococcus aureus	+	+	+
Bacillus subtilis	+	+	+
B. cereus	+	-	-
B. licheniformis	-	+	+
Saccharomyces cerevisiae	+	+	+
Candida tropicalis	+	+	+
Rhizopus stolonifer	-	-	+
Aspergillus niger	+	+	+
Aspergillus flavus	+	+	-
Penicillium sp	+	-	+
Mucor mucedo	-	+	-
Fusarium oxysporium	+	-	+

Figure 1 showed percentage occurrence of microorganisms associated with fermented maize-, millet- and sorghum-based *Ogi* in Ondo State, Nigeria. Twenty-four (24) bacterial species were isolated from the cereal-based food. *Lactobacillus plantarum* had the highest percentage frequency of 10.3 %, followed by *Lactobacillus fermentum* (7.73%), *Corynebacterium* spp (7.3 %), *L. amylovorus* (6.87 %), *Lactococcus lactis* (6.87 %), *Streptococcus lactis* (6.01%), *Saccharomyces cerevisiae* (6.01%), *Lactobacillus delbrueckii* (5.15 %), *Candida tropicalis* (5.15%), *S. aureus* (4.72 %), *Rhizopus stolonifer* (4.29 %), *Micrococcus luteus* (3.43%), *B. licheniformis* (3.43%), *Enterococcus faecalis* (2.58%), *B. subtilis* (2.58 %), *Penicillium* sp (2.58 %), *Fusarium oxysporium* (2.58 %), *P. aeruginosa* (1.72 %), *E. coli* (1.72 %), *A. flavus* (1.72 %), *B. cereus* (1.29 %) while *Mucor mucedo* (0.86 %) had the lowest percentage occurrence.

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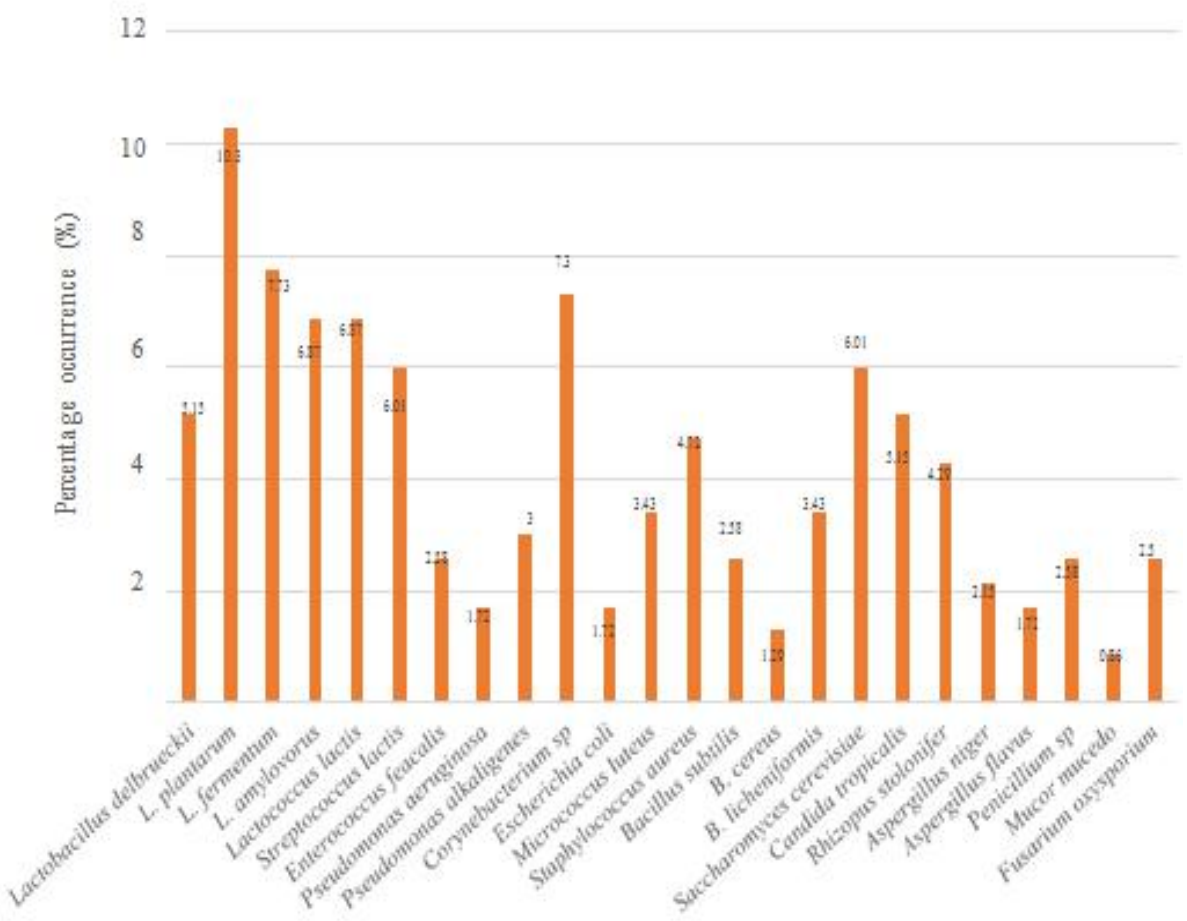

Fig.1: Percentage occurrence of microorganisms associated with fermented maize-, millet and sorghum-based pap (Ogi) in Ondo State, Nigeria

The proximate composition (%) of fermented maize-, millet- and sorghum-based *Ogi* in Ondo State, Nigeria was shown in Figure 2. Carbohydrate (starch) was present in the three cereal-based *Ogi* in quantities higher than any other. Sorghum-based *Ogi* had the highest % of carbohydrate (74.89 ± 0.671) %, followed by maize-based (74.43 ± 0.050) % and millet-based *Ogi* (71.30 ± 0.326) %. The % moisture contents in maize-, millet- and sorghum-based pap were (9.22 ± 0.140) %, (7.98 ± 0.005) % and (7.11 ± 0.004) % respectively; % protein contents were (9.01 ± 0.002) %, (12.11 ± 0.002) % and (11.45 ± 0.040) % respectively while the % fat compositions were (2.54 ± 0.040) %, (2.32 ±0.040) % and (2.42±0.035) % respectively. The % fibre compositions of fermented maize-, millet- and sorghum-based *Ogi* were (3.03 ± 0.040) %, (3.76 ± 0.030) % and (2.15 ± 0.050) % respectively while the % ash contents were (1.77± 0.020) %, (2.53 ± 0.006) % and (1.98 ± 0.005) % respectively. There were no statistical differences among the three cereal-based *Ogi* in relation to the percentage compositions of moisture, protein, fat, fibre, ash and carbohydrate ($P < 0.005$).

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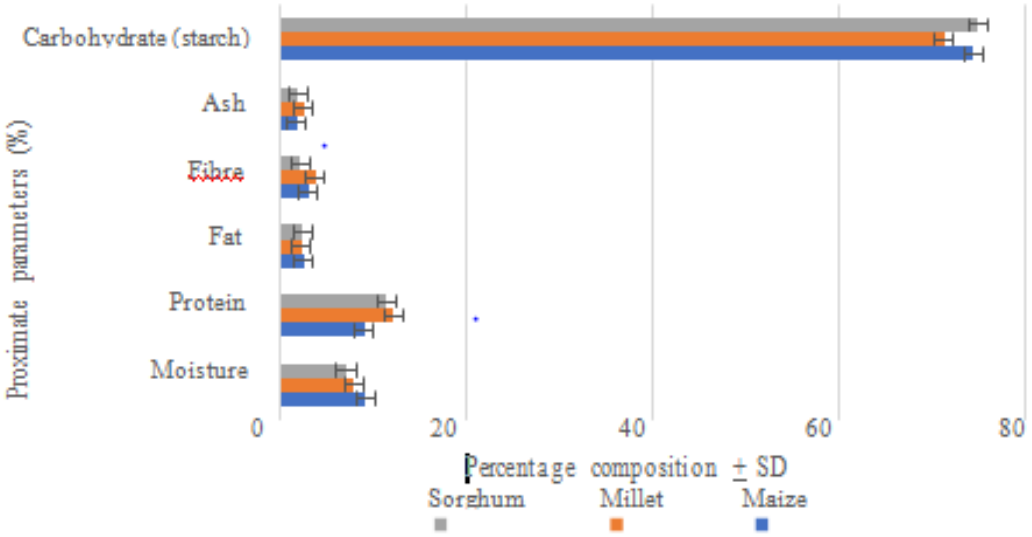

Fig.2: Proximate composition (%) of fermented maize-, millet- and sorghum-based pap (Ogi) in Ondo State, Nigeria. Values represent means of data + SD. Data was statistically analyzed at 95% level of confidence (P < 0.05). SD = Standard deviation

Table 3 showed mineral compositions (mg/100 g) of fermented maize-, millet- and sorghum-based pap (*Ogi*) in Ondo State, Nigeria. The calcium (Ca) compositions of fermented maize-, millet- and sorghum-based pap were 14.01, 10.11 and 28.92 mg/100 g respectively. The Zinc (Zn), Sodium (Na), Iron (Fe), Magnesium (Mg) and Potassium (K) compositions in the three cereal-based *Ogi* ranged from 7.87 to 9.72 mg/100 g, 302.37 to 352.33 mg/100 g, 45.77 to 52.63 mg/100 g, 80.01 to 99.33 mg/100 g and 310.20 to 426.08 mg/100 g, respectively. There was no statistical difference in Ca composition between maize- and millet-based Ogi (P > 0.05) but the compositions of the former two were significantly different from sorghum-based Ogi (P < 0.05). Composition of Zn in maize-based *Ogi* was statistically different from millet- and sorghum-based *Ogi* while the latter two showed no statistical difference. There were also significant differences in compositions of Na, Mg and K among the three cereal-based *Ogi*. There were no statistical differences between Na and Mg compositions in the three cereal-based *Ogi* and their recommended values (t = 2.007, P = 0.183 and t = 2.646, P = 0.118, respectively).

Table.3: Mineral composition (mg/100 g) of fermented maize-, millet- and sorghum-based pap (Ogi) in Ondo State, Nigeria

Cereal	Ca	Zn	Na	Fe	Mg	K
	Mean of data + Standard deviation					
Maize-based	14.01^{a}±0.090	7.87^{a}±0.002	302.37^{a}±0.001	52.63^{a}±0.001	80.01^{a}±0.005	310.20^{a}±0.001
Millet-based	10.11^{a}±0.003	9.72^{b}±0.002	352.33_{b}±0.002	49.22^{a}±0.001	99.33^{c}±0.003	350.66^{b}±0.001
Sorghum-based	28.92^{b}±0.003	9.28^{b}±0.002	321.04^{c}±0.002	45.77_{a}±0.001	95.97^{b}±0.004	426.08^{c}±0.001
Recommended value	60.00	> 3.20	296.00	> 16.00	76.00	516.00

Values represent means of data. Mean values with the same superscript along same column had no statistical difference. Level of confidence = 95% (P < 0.05)

IV. DISCUSSION

Microorganisms play both essential and deleterious roles in food products. In the fermentation industry, the attributes of the food products produced is largely due to the type, age, composition of the microorganisms employed. To a large extent, both population and diversity play a role in the fermentation of products. Table 1 showed microbial load during and after fermentation of maize, millet and sorghum

for *Ogi* production in Ondo State, Nigeria. The microbial load gradually increased from the first day (0 hour) and attained optimum at 24 – 48 hours of fermentation, before beginning to decline from 72 to 96 hours. The density of the microbes for lactic acid bacteria culture using MRS agar is second to aerobic culture [15]. This suggests that lactic acid bacteria play a significant role in the fermentation of grains in *Ogi* production.

The population of microbes of the Enterobacteriaceae family was low during fermentation of grains used for the preparation of *Ogi*. These groups of microorganisms that grow on MacConkey agar medium including *E. coli* and *Enterococcus faecalis*, isolated in this study, do not normally participate in fermentation process. A significant reduction in the growth of *E. coli* and *Klebsiella aerogenes* towards the end of fermentation has been reported by Oyelana and Coker [16]. Hence, their occurrence in fermentation medium of the grains, under study for *Ogi* production, could result from the water used for fermentation or as normal flora of the grains prior to fermentation. This also explains the presence of *S. aureus* in the medium at the beginning of fermentation. *S. aureus* is ubiquitous, and as a normal flora of the skin and nasal cavity of man, it might have been unhygienically introduced during washing of grains and other activities which led to its introduction as contaminant.

The fungal load ranged from 1.5×10^2 to 3.2×10^3 CFU/g, being far lesser than the population of lactic acid bacteria and general aerobic viable counts. This suggests that most of the microbes that participate in the fermentation of grains for *Ogi* production are mainly bacteria, despite the fact that some yeast also participate actively in the fermentation process [15]. The differences in population of the various classes of microbes (i.e. lactic acid bacteria, aerobic bacteria, family of Enterobacteriaceae, and fungi) could be connected to the acidic nature of the medium. It has been previously reported by various authors that as fermentation proceeds the acidity of the medium increases (pH tending towards 0) and the titratable acidity is enhanced [17].

This is, however, as a result of continual increase in population of lactic acid bacteria throughout the fermentation process. LAB usually turn medium acidic and, thus, antagonizes the occurrence or proliferation of other groups of microorganisms. This explains the gradual decrease in microbial load and elimination of the aerobic bacteria, staphylococci, enterobacteria and fungi during fermentation process in this study. This is supported by the study of Adesokan *et al*. [18] who reported that this trend could lead to production of lactic acid bacteria that are responsible for fermentation of *Ogi.*

The distribution of microorganisms associated with fermentation of different grains for *Ogi* production was shown in Table 2 while Figure 1 showed percentage occurrence of the microorganisms. Basically, different microbes tolerate acid medium differently, to some it encourages their growth while in others it antagonizes and leads to their death. Microbes found in food products occur through several means including exposure, handling, use of contaminated utensils for preparation. Several groups of bacteria (coliforms, lactic acid bacteria, aerobic bacteria etc) and fungi participate in the fermentation of steeped grains for *Ogi* production.

Maize had the highest % moisture content (9.22 %) and lowest in sorghum (7.11 %). The lower moisture content value of the sorghum indicates its higher keeping quality than the other cereals under consideration. This is because moisture is important for the proliferation of food-spoiling microorganisms. Scientific investigation has reported that low moisture content in food samples increased the storage periods of the food products [19]; while high moisture content in foods encourage microbial growth; hence, food spoilage [20]. Protein was highest in millet (12.11%) followed by sorghum (11.03%) and lowest in maize (9.01%) implying that the cereals are not devoid of protein as many people presume. This implies that the cereal-based *Ogi* also contain reasonable amounts of body building nutrient. This is similar to the percentage protein content in the range of 8.58-12.39 % as reported by Izah *et al*. [15].

A study also found the percentage protein content of three maize varieties grown in Nigeria in the range of 10.67-11.27 % for the maize grains [21] while another reported mean percentage protein content of 10.8 %, 11.1 % and 10.5 % for the maize samples analyzed [22]. Oko *et al*. [23] reported protein content ranging from 1.17- 7.94% among 20 varieties of rice, with a mean value of 4.99 ± 1.37 %. The protein composition of whole wheat flour ranged from 10.13 to 14.74 % among different Pakistani wheat varieties as reported by Khan and Zeb [24]. Three sorghum varieties analyzed by Mustapha *et al*. [25] revealed that the protein ranged from 14.51 to 14.80 %. According to Pearson [26], plant foods that provide more than 12 % of its calorific value from protein are considered good source of protein.

Highest crude fat (oil) content was exhibited by maize (2.54 %) and lowest in millet (2.32 %). This low percentage of crude fat indicates that prolonged storage of the grains may not affect the quality as poor storage causes rancidity

(peroxidation of polyunsaturated fatty acid) that would impact unpleasant odour and reduced intake of food and nutrient. In a study conducted by Ikram *et al*. [27] to determine fat content of maize, values ranged between 3.21% and 7.71%. Similarly, the results on sorghum by Mustafa *et al*. [25] revealed a range of 3.58 to 4.47%.

Fat contributes to the energy value of these grains, thereby providing essential fatty acids for optimum neurological, immunological and functional developments in children [15]. In the case of crude fibre in this study, millet was highest (3.76 %) followed by maize (3.03 %) and sorghum (2.15 %). The high fibre content of these samples can have some biological beneficial effects such as laxative effect on the gastrointestinal tract (GIT), increased faecal bulk and reduction in plasma cholesterol level [28]. Studies have shown that percent crude fibre ranged from 0.80-2.32% [27].

Ijabadeniyi and Adebolu [21] reported slightly higher values (2.07-2.77%) of the fibre content for the maize varieties grown in Nigeria. Iken *et al*. (2002) observed that the average crude fibre value for the Improved White Dent (IWD), Improved Yellow Flint Dent (IYFID) and Local Floury (LF) varieties was lower than the average value of 9.5% as reported by Watson [29]. The Proteins Advisory Group [30] of the United Nations suggested an upper limit of 5.0% crude fibre in supplementary foods. Thus, the values obtained in this study (2.15-3.76 %) fell within the recommended ranges for infants.

The ash content, which is an index of mineral contents, was found in the range of 1.77 % to 2.53 %. Millet, having the highest value contained a greater proportion of non-endosperm material because ash values indicate the level to which non-endosperm components are present [31]. Carbohydrates are the major food component of the grains. It was found in the range of 71.30 % for sorghum to 74.89 % for maize. Ikram *et al*. [27] observed that carbohydrates are the major chemical components of the maize grains as they reported a range of 69.659-74.549 %. Ijabadeniyi and Adebolu [21] reported slightly lower values (65.63-70.23 %) of the carbohydrate content for the maize varieties grown in Nigeria. Carbohydrate in sorghum was reported by Mustafa *et al*. (2003) to be between 68.34 to 69.65 %. The principal carbohydrate of all cereals is starch, representing 56 % (oats) to 80 % (maize) of the grain dry matter [32].

FAO reported that staple foods such as millet, maize and sorghum are high in starch which makes them absorbed a lot of water during cooking. This makes them bulky and, hence, infants need to consume large quantities to get enough energy and nutrients but it is difficult because they have small stomach. The problem is, however, solved if families feed children with weaning foods prepared from germinated cereal flour and enrich bulky foods. Malting reduces viscosity of the foods and hence a child can eat more at a time [33,34].

Mineral compositions of the samples were shown in Table 3. The Ca composition ranged from 10.11 to 28.92 mg/100 g. The Zinc (Zn), Sodium (Na), Iron (Fe), Magnesium (Mg) and Potassium (K) compositions in the three cereal-based Ogi ranged from 7.87 to 9.72 mg/100 g, 302.37 to 352.33 mg/100 g, 45.77 to 52.63 mg/100 g, 80.01 to 99.33 mg/100 g and 310.20 to 426.08 mg/100 g, respectively. According to FAO/WHO [35], minerals such as iron and zinc are low in cereals but the addition of legumes can improve the iron content. Cereals that are particularly rich in iron and calcium will be useful in reducing prevalence of iron deficiency and assist in bone development in children respectively. Potassium helps maintain fluid balance, and high intake improves blood pressure, according to the American Heart Association [36].

V. CONCLUSION

The results will clear the air as regards the preferences of consumers as to which of the products possesses best nutritional benefits based on the type of cereal grain used for the preparation of the product. This study revealed the distribution of fermentative microorganisms and some contaminants which were not directly associated with fermentation of the cereal grains for production of *Ogi*. There were no significant differences in the proximate and elemental compositions of the maize-, millet- and sorghum-based pap (*Ogi*). The study showed significantly acceptable percentage compositions of crude protein, fibre, ash, fat and carbohydrate. Low moisture content and persistence of lactic acid bacteria in the products are considered responsible for the prolonged shelf-life the products are known for. The variations in elemental compositions of the three cereal grains were not also significant. However, maize-, millet- and sorghum-based *Ogi* could be fortified with products of higher nutrient composition to increase the acceptability of the diet among people of all ages and classes.

AUTHOR CONTRIBUTIONS

OOB and TKB conceived and designed the experiments; OOB and OTA performed the experiments; OOB analyzed the data; YOA contributed reagents/materials/analysis tools;

OOB wrote the paper. All authors read and approved the final manuscript.

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Effect of climate change and some agrotechnical factors on the yield and nitrogen- and water-use efficiency in winter wheat (*Triticumaestivum L.*) production

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Abstract—*Winter wheat is a very important cereal crop in Hungary (~25% of Hungarian arable land). In the last decades in conventional wheat production used huge industrial, external inputs to increase the yields which caused a lot of harmful environmental effects. In long-term experiments different ecological (crop year), genetical (variety) and agrotechnical (fertilization, crop rotation) factors were studied on chernozem soil in Eastern Hungary. The fertilizer responses of wheat varieties depended on crop year (6.5-8.9 t ha^{-1} maximum yields in 2011-2015 years) and the genotypes (in 2012 the difference was ~3 t ha^{-1} among varieties). The optimum N(+PK) doses varied between 30-150 kg ha^{-1} in different crop years. In wheat production the fertilization resulted the highest yield surpluses in average crop years (2.8-5.5 t ha^{-1}) comparing with dry ones (2.9-3.7 t ha^{-1}), respectively. The optimum fertilization could improve WUE in wheat production.*

Keywords—crop rotation, crop year, efficiency, fertilization, wheat.

I. INTRODUCTION

Significant yield increases of small grain cereals (including wheat) have been achived from 1970's years in the developed and developing countries (called "green revolution"). These yield incensements were based on the huge industrial, chemical inputs (fertilizers, pesticides, gasoline etc.). This "industry-like" crop production resulted high yields and enormous harmful environmental effects and less agronomy and energy efficiency [1-3]. Traditional cereal production uses a lot of external inputs to achieve high yields [4]. Winter wheat has a determinative role in Hungarian crop production. The sowing area of wheat is about 1.0 million hectars (~25% of Hungarian arable land) and the country-average yield varies from 3.5-5.5 t ha^{-1} depending on crop years. Many foreign and Hungarian experimental results proved that climatic conditions of crop years strongly modified the yield of wheat [5-6]. The yield-losses and yield fluctuation of wheat caused by crop year (climate change) depended on soil conditions, the stress-tolerance of genotypes [7] and the agrotechniques. According to literature [8-9] the yield decreases of cereals varied between 2-55%. In sustainable wheat production nutrient supply, fertilization is a key agrotechnical element [10-13]. It is possible to reduce the unfavourable, negative agrotechnical, weather effects by using optimum nutrient supply, fertilization and appropriate variety-selection [9]. Because of climate change the water saving crop management and water use efficiency are especially important in cereal production. [14] built up a conceptual model of the factors impacting on water use of different users, including drivers and barriers to water saving.

The aim of this study was to evaluate the long-term experimental date on chernozem soil in Eastern-Hungary and to show the effect of climatic conditions (crop year) and nutrient-supply (fertilization) and genotype (variety-selection) on the yield of wheat. We wanted to study the nitrogen- and water-use efficiencies in wheat production.

II. MATERIAL AND METHODS

Our long-term experiment was set up in 1983 on a chernozem soil in Hajdúság (East-Hungary). The experimental location is found on Látókép Research Farm, 15 km of Debrecen (latitude: 47°30', longitude: 21°30', elevation above the Adriatic see: 118 m).

Analytical data for initial soil conditions showed that as regards its soil physics the area can be classified as having loam soil with nearly neutral pH value (pH_{KCl} 6.46). It has medium humus content (2.76 % in the 0-0.2 m upper soil layer) and a humus layer of about 0,8 m. Its phosphorous and potassium supplies can be regarded medium (AL-P_2O_5 133 mg kg^{-1}) and good (AL K_2O 240 mg kg^{-1}), respectively. The long-term experiment had a split-plot arrangement with four repetitions. In the experiment control treatment and equidistantly increasing

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NPK doses were applied (the basic dose was N=30 kg ha^{-1}, P_2O_5=22.5 kg ha^{-1}, K_2O=26.5 kg ha^{-1} and its two-, three-, four- and five hold).

The other long-term experiment was set up in 1983 on chernozem soil on the Látókép Research Station of the University of Debrecen in the Hajdúság region (Eastern Hungary). The following factors were examined in the long-term experiment:

- crop rotation: biculture (maize, wheat), triculture (pea-wheat-maize)
- fertilization: control, N = 50 kg ha^{-1} P_2O_5 = 35 kg ha^{-1}, K_2O = 40 kg ha^{-1}, and 2-3-4 folds of this dose
- irrigation: irrigated and non irrigated.

III. RESULTS AND DISCUSSIONS

The basic element of sustainable wheat production is to select the suitable, adaptable genotypes into agroecological and agrotechnical conditions. The nutrient supply and fertilization have the key-role in the sustainable wheat production because on the one hand fertilization directly and indirectly modifies all other agrotechnical factors (crop protection etc.) and the other hand the over-optimum fertilization causes different harmful effects (NO_3-N accumulation in different soil layers etc.). Our long-term experimental results proved that weather conditions (mainly the rainfall quantity and its distribution) strongly modified the yields of winter wheat genotypes even on chernozem soil characterized by excellent water- and nutrient husbandry. In the average of wheat varieties and crop years the yield was 7631 kg ha^{-1} but the yields varied depending on the crop years (Table 1). The minimum yield was in 2013 (6514 kg ha^{-1}) and we got the maximum yield in 2015 (8921 kg ha^{-1}). The winter wheat genotypes could differently adapt to the crop year. According to our long-term experimental data we could state that the differences among the varieties were about 3 t ha^{-1} in the same agrotechnical conditions (in 2012 the yields varied between 6075-8919 kg ha^{-1}). The crop year (mainly the water supply during the vegetation period) can modify the optimum N+PK doses, too. In crop year characterized by average water supply the optimum N+PK doses varied between N=90-150 kg ha^{-1} +PK and in crop year after very mild winter the N_{opt} +PK dropped down to N=30-60 kg ha^{-1} +PK (because of very high mineralization of organic matter in the chernozem soil).

Table.1: Fertilizer response of winter wheat genotypes in different crop years
(Debrecen, chernozem soil, 2011-2015)

Variety	2011(N_{opt})	2012(N_{opt})	2013(N_{opt})	2014(N_{opt})	2015(N_{opt})	Average
GK Öthalom	6819 (150)	6175 (150)	5983 (150)	8713 (30)	8862 (150)	7310
Pannonikus	8123 (90)	8139 (150)	6576 (150)	7996 (30)	8864 (90)	7940
Euclide	9586 (150)	8919 (150)	7590 (150)	-	-	8698
GK Csillag	-	7263 (150)	6562 (150)	8350 (60)	9150 (150)	7831
Bitop	-	6075 (150)	6089 (120)	6663 (30)	-	6276
GK Békés	-	7917 (150)	6281 (120)	7915 (30)	8809 (90)	7731
Average	*8176*	*7415*	*6514*	*7927*	*8921*	*7631*
Yield interval, t/ha	6.8-9.6	6.1-8.9	6.0-7.6	6.7-8.4	8.8-9.2	6.3-8.7
Min-Max, %	83-117	82-120	92-117	84-105	99-103	82-114
Interval of yield fluctuation, %	34	38	25	21	4	32
Interval of N_{opt} kg ha^{-1}	90-150	120-150	120-150	30-60	90-150	90-128
$LSD_{5\%}$	457	355	600	674	614	-

The winter wheat is one of the best fertilizer-responding field crops. Our long-term experimental data proved that the fertilization of wheat resulted good yield surpluses on chernozem soil characterized by excellent natural nutrient stock (Table 2). The yield surpluses of wheat varied between 2659 kg ha^{-1} (2013/2014 crop year) and 6020 kg ha^{-1} (2015/2016 crop year). The yields of control treatment proved the excellent natural nutrient avaibility of chernozem soil (1816 kg ha^{-1} and 5897 kg ha^{-1}). The other meteorological parameters could modify the yield surplus of wheat genotypes (in 2013 the strong and long frosting period in March decreased the yields, in 2014 the very mild winter period accelerated the N-mineralization in chernozem soil).

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Table.2: Effect of crop year on the control and maximum yield of winter wheat
(Debrecen, 1999-2017) (average of varieties)

Crop year	Control yield kg ha^{-1}	Maximum yield kg ha^{-1}	Yield-surplus kg ha^{-1}	Rainfall in veg. period (mm)	Rainfall deviation from 30 year average (mm)	N_{opt} (+PK) kg ha^{-1}
2010/2011	4023	8043	4020	340.9	-60.0	133
2011/2012	3906	7303	3397	320.7	-80.2	144
2012/2013	1816	6674	4858	480.2	+79.3	145
2013/2014	5897	8556	2659	284.0	-116.9	49
2014/2015	4662	9024	4362	350.9	-50.0	110
2015/2016	3927	9947	6020	561.7	+160.81	115
2016/2017	5226	8028	2802	379.6	-21.3	133

Wheat is a sensitive arable crop to agroecological and agrotechnical factors. Our multifactorial long-term experimental data (between 1986-2017) proved that the effects of fertilization were different depending on the crop rotation and the weather of crop year. In Eastern Hungary characterized by continental climate the precipitation quantity and its distribution are the decisiveagroecological factor on chernozem soil. The effects of crop year were significant on the yields of wheat in different (bi- and triculture) crop rotation (Table 3). We obtained the strongest effect of crop year in biculture (the yields of wheat varied between 1892-3162 kg ha^{-1} in control and 5419-8029 kg ha^{-1} in N_{opt} +PK, respectively). In diversed crop rotation (triculture) the yield-fluctuations of wheat were less (in control 4426-5763 kg ha^{-1}, in N_{opt} +PK 6190-8600 kg ha^{-1}, respectively). The efficiency of fertilization was modified by crop year and crop rotation. The highest yield surpluses of wheat were obtained in average crop year in different crop rotation, but the efficiency of nutrient supply was much higher in biculture (5513 kg ha^{-1}) comparing with triculture (2837 kg ha^{-1}). The optimum N (+PK) doses were much lower (N_{opt} = 50-100 kg ha^{-1} +PK) in triculture than in biculture (N_{opt} = 150-200 kg ha^{-1} +PK) because of peas forecrop.

Table.3: Effect of crop year, crop rotation and fertilization on the yield of wheat in long-term experiment (Debrecen, chernozem soil, 1986-2017)

Crop rotation	Yield kg ha^{-1} Dry crop year 9 years (28%)		Average crop year 18 years (56%)		Rainy crop year 5 years (16%)	
Biculture (after maize)						
Control	1892 f	3698*	2516 ef	5513*	3162 e	2257*
N_{opt} +PK^{xx}	5590 cd		8029 ab		5419 cd	
Triculture (after peas)						
Control	4426 de	2853*	5763 cd	2837*	5763 cd	1305*
N_{opt} +PK^{xxx}	7279 b		8600 a		8600 a	

* yield surplus of fertilization (kg ha^{-1})
a, b, c, d, e, f Letters are significantly different at P ≤ 0,05 level
^{xx} Nopt +PK = 150-200 kg ha^{-1} +PK in biculture
^{xxx} Nopt +PK = 50-100 kg ha^{-1} +PK in triculture

Our long-term experimental data proved that the using optimum fertilizer doses (N+PK) can increase the water use efficiency (WUE = kg yield/1 mm rainfall in vegetation period) of wheat both in dry and average crop years (Table 4). In different crop rotation the WUE of control varied between 6.00-16.57 kg mm^{-1} in dry and 6.00-09.27 kg mm^{-1} in average crop years, respectively. In optimum N+PK treatment the WUE values were much higher (21.31-24.04 kg mm^{-1} and 24.71-29.48 kg mm^{-1}, respectively).

Table.4: Water use efficiency (WUE) of wheat in different crop years
(Debrecen, chernozem soil, non irrigated)

Crop rotation	Fertilizer treatment	Dry crop year	Average crop year
		yield kg/1 mm rainfall in vegetation period	
Biculture	Control	6.03 d	6.00 d
	N_{opt} +PK	21.31 bc	24.71 b
Triculture	Control	16.57 cd	19.27 c
	N_{opt} +PK	24.04 bc	29.48 a

a, b, c, d Letters are significantly different at P ≤ 0,05 level

IV. CONCLUSIONS

Our long-term experiments proved that we have to harmonize the ecological, biological and agrotechnical factors to increase the nutrient- and water-use efficiency and decrease the harmful environmental effects in wheat production. According to our fidings there were huge differences among the maximum yields and the optimum N+PK doses of winter wheat genotypes. The wheat varieties differently responded to the N+PK fertilizer doses and they differently utilized the natural nutrient sources of chernozem soil. The yields of wheat varieties varied between 6075-9586 kg ha^{-1} and the Nopt +PK doses fluctuated between N = 30-150 kg ha^{-1} +PK depending the crop year (mainly water supply) and genotypes. So under climatic change the optimum fertilization is a key-element to change the conventional wheat production into a sustainable one [1-2, 5, 15]. Monitoring the sustainability of wheat production needs different indicators [16]. The nutrient- and water-use efficiency were modified by crop year, crop rotation and fertilization. We obtained the highest yield surpluses of wheat in average crop year, in diversified crop rotation with using less N_{opt} +PK doses (N = 50-100 kg ha^{-1} +PK) comparing with the dry and rainy crop years, simplified crop rotation (biculture) simiraly to [17], [18] and [13]. A nutrient (mainly nitrogen) efficiency was modified by climatic factors, genotypes and agrotechnical elements [19-22]. The water use efficiency of wheat (WUE) was better in triculture and Nopt +PK treatment (in control 16.57-19.27 kg mm^{-1}, in Nopt +PK 24.04-29.48 kg mm^{-1}) than in biculture (6.00-6.03 kg mm^{-1} and 21.31-24.71 kg mm^{-1}, respectively) crop rotation. The optimum N+PK fertilization could increase the WUE of wheat.

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Biochemical and transcriptomic evaluation of the toxic effects of aloin contaminated agricultural soils on the earth worm *Eisenia andrei*.

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***Abstract*—** *In the present study, we investigated the response of oxidative stress markers and related gene expression in worms exposed to Aloe vera crude exudates (aloin) contaminated agricultural soils for 7 and 14 days. Four sublethal concentrations ranging from 10, 50, 100 and 200 g/kg soils (corresponding to 0.125, 0.625, 1.25 and 2.5 g aloins.Kg-1 soils) were tested. Acetyl cholinesterase activity was evaluated to assess the potential neurotoxic effect of aloin. Lysosomal membrane stability (LMS) was evaluated after the exposure periods in worm's coelomocytes as indicator of cytotoxicity. Our data indicated a significant increase in the antioxidant enzyme activities termed as catalase (CAT), and glutathione-S-Transferase (GST) and caused a pronounced increase of malonedialdehyde accumulation (MDA). Similarly, LMS was highly affected by aloin particularly at higher concentrations and after 14 days of exposure. Cat and gst, gene expression levels showed a significant increased pattern in animals exposed for 7 and 14 days to the aloin concentrations compared to the control condition. ACHE activity was inhibited in animals exposed to C2, C3 and C4 particularly after 14 days of exposure. Our data provide new insights into the cytotixcity effect of aloe vera crude exudates on the earthworm Eisenia Andrei; one of the principal components of agricultural soil biofertility and sustainability.*

***Keywords*— Aloin crude exudates, Toxicity, Eisenia Andrei, Agricultural soils, sustainability.**

I. INTRODUCTION

Aloe vera is a plant that has been used as a constituent of several prescriptions in the conventional medicine worldwide [1,2,3]. Many biological activities like, immunostimulative activity, antiviral and antibacterial as well as antioxidant activity [4,5] have been reported in this plant extracts. Moreover, Aloe vera extracts, rich in polyphenols has increased its use as food supply [1,5] and therefore related agricultural surfaces dedicated to its cultivation increased substantially over the past decade.

Aloin is the main anthraquinone (10-glucopyranosyl-1,8-dihydroxy-3- Hydroxylmethyl-9(10H)-anthracenone) in Aloe vera shoots, which occurs naturally as a mixture of two isomers aloin B and aloinA. In addition to these compounds, other compounds including isoaloesin, aloenin B and aloenin have been described to be involved in the biological properties of Aloe vera extracts [6]. Aloin is related to aloe emodin, which lacks a sugar group but maintain aloin's biological properties [7].

Usually and for human consumption purposes, aloin content in food based products such as juices must be very low, thus during the preparation process aloin is separated due to its noxious properties. Indeed, aloin was reported to provoke pathological modifications and changes the composition of microbiota in the large intestine of rats [8]. Moreover, [] demonstrated that aqueous extract of Aloe vera was able to affect DNA alteration and to generate reactive oxygen species in a dose-dependent manner.

Earthworms are usually used as test animals in terrestrial ecotoxicology due of their burrowing habits. Earthworms highly influence and control soil processes, thus maintaining the soil structure and maintain the dynamics of organic matter. Moreover, these organisms have been used so far as indicators of environmental changes [10, 11]. Among the earthworms, the Eisenia species has been wildly employed for biomonitoring soil's quality [12,13]. A panel of toxicity tests have been performed using these species, generating a great variety of data covering behavioral [14], cellular [15] and molecular [16] aspects.

This investigation aims first, to evaluate the cytotoxicity effects of contaminated agricultural soils with four sublethal concentrations of aloin throw the evaluation of the anti-oxidant stress enzymes activities CAT and GST, the malonedialdehyde (MDA) accumulation and the lysosomal membrane stability (LMS) on the earthworm

Eisenia andrei. Neurotoxicity of aloin was tested using acetylcholinesterase enzyme activity (ACHE). Second, we investigated the gene expression pattern of the glutathione S-transferase (gst) and catalase (cat) genes using quantitative reverse-transcription technique.

II. MATERIAL AND METHODS

2.1. Soil test and Aloe vera leaf latex.

The agricultural soil test was obtained from an agricultural local farming [10]. Leaves from Aloe vera (var. barbadensis) were obtained from 5 years aged plants supplied by a local production activity. Briefly, Aloe vera leaf latex was obtained by stripping away the outerleaf rind, and collecting it [17]. The obtained latex was further added to soils to achieve final concentrations of 10, 50, 100 and 200 g/kg soils corresponding to 0.125, 0.625, 1.25 and 2.5 g aloins.Kg-1 soils. Chemical analysis of Aloe vera latex revealed the presence of aloenin, aloenin B, isoaloesin, aloin A andaloin B at a total amount of 12.5 g.Kg-1latex.

2.2. Animals

E. andrei specimens [18] were cultured as according to OECD guidelines [19,20]. Worms were chosen from a synchronized culture with a homogeneous age structure. Adult animals with clitellum of similar size and weight (400 to 500 mg) were employed in the experiments.

2.3. Worm's Exposure

Worms were removed from the culture medium to the experimental soils. Earthworms were kept during the exposure period (7 and 14 d) in 3-L containers with constant light and 35% humidity at 20±1°C (20). Each condition was conducted in triplicate and a total of 10 worms per condition were randomly utilized for the biomarkers analysis. After the 7-or 14-day exposure period, the animals were placed on moist filter paper for 24 h to allow gut clearance before molecular and biochemical analyses.

2.4. LMS determination

The LMS was assessed in coelomocytes as described by Sforzini et al [21,22,23]. Slides were scored at 630× magnification using an inverted photo-microscope (Zeiss Axiovert 100M) equipped for fluorescence microscopy with a rhodamine emission filter.

2.5. Biochemical analysis

2.5.1. Antioxidant enzyme activity

Worms (0.5–1 g; n=10) were homogenized in ice-cold extraction buffer (pH 7.5) containing 250 mmol.L^{-1}sucrose,50 mmol L^{-1}Tris-HCl, 1 mmol L^{-1} EDTA, and 1 mmol L^{-1} DTT at a1/4 w/v ratio. Proteins in the S9 fraction were quantified according to the Bradford method [24].

The GST activity was measured in DG cytosol by the method of Habig et al. [25]. The CAT activity was determined according to Clairbone's method [26].

2.5.2. MDA determination

Worms (0.5–1 g; n=10) were homogenized in two volumes of 20mM Tris-HCl (pH 7.4) and 0.1% mercaptoethanol buffer. The homogenate was centrifuged at 18,000× g at 4°C for 20 min. MDA level was evaluated as described by Gérard-Monnier et al.[27].

2.6. Gene expression

Total RNA was extracted from the worms using acid phenol-chloroform precipitation according to Chomczynski and Sacchi [28] using TRI-Reagent (Sigma-Aldrich). The abundance of the mRNA of the genes encoding gluthathione-S-transferase and catalase were evaluated in multiplex Taqman assays according to Negri et al. [29] and Banni et al., [30]. cDNA (25 ng RNA reverse-transcribed to cDNA) was amplified in a CFX384 Real-Time PCR detection system (Bio-Rad Laboratories) using iQTM Multiplex Power mix (Bio-Rad Laboratories).

cDNA was amplified in the presence of 1X iQTM Multiplex Power mix using 0.3 µM of each primer and 0.1 µM of each probe in a final volume of 10 µL. Gene expression data were geometrically normalized against ribosomal protein riboS13 (BB998368.1) an invariant actin isotype (DQ286722.1) and 18S rRNA (AB558505.1) (29). Probes, sense primers and antisense primers are reported in table1.

Statistical analyses were performed on the group mean values using a random reallocation test [31]. The LMS, enzymatic activity, and MDA content data are presented as the mean ± SD of 10 samples. Statistica Software, version 6.0 (Statsoft. Inc. 2002) was used for statistical analysis. For multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data along with Tukey's test.

III. RESULTS

Figure 1 shows the effects of exposure to the increasing concentrations of aloin crude extracts for 7 and 14 days on coelomocyte LMS. The LMS was altered in all of the conditions compared to the control group being time and concentration dependent. The highest LMS destabilization was observed in animals exposed for 14 days to highest aloin concentration.

Lipid oxidative alteration was investigated by assessing MDA accumulation evaluated as thiobarbituric acid reactive species (TBARS) (Fig. 2).This biomarker increased significantly in all animals exposed to aloin for

7 and 14 days. Exposure to C2, C3 and C4 rendered a significant increase in MDA accumulation being significantly different over the two exposure periods. The highest MDA level was observed in animals exposed to C4 for 14 days.

The antioxidant activity of CAT and GST significantly increased in worms exposed to aloin after 7 days compared to control animals (Fig.3). This enhancement was maintained for CAT after 14 days being significantly different from the response depicted after 7 days. For GST activity, the significant increase was maintained after 14 days of exposure for anialms exposed to C1, C2 and C3. However we observed a strong reduction in GST activity in worms exposed to C4 after 14 days exposure. The later reduction was even lower than the control value (71.74±9.54 nmole/mn/mg proteins) with 36.55±8.21nmole/mn/mg proteins.

Transcriptomic data highlighted significant up-regulation in the mRNA levels of the gst and cat genes in worms exposed to aloin crude extracts compared to control animals (Fig. 4). For cat the maximum expression levels was observed after 14 days in animals exposed to C3 (4.89-fold increase). Regarding gst, the maximum was depicted after 7 days in worms exposed to C4 (6.17-fold increase). It is important to note that gst expression levels decreased after 14 days exposure compared to 7 days even if it remains significantly higher than control.

The response of AChE activity in earth worms exposed to increasing aloin concentrations is reported in figure 5. AChE activity was unchanged in animals exposed to C1 for the two exposure periods. However, ACHE was significantly inhibited after 7 days and 14 days in worms exposed to C2, C3 and C4when compared with control animals. Interestingly for C2 we noticed a recover in ACHEactivity after 14 days when compared to those after 7 days even if they remain inhibited respect to control. However, in animals exposed to C3 and C4 ACHE activity significantly decreased after 14 days when compared to the values obtained in the same condition after 7 days.

IV. DISCUSSION

The utilization of different types of agricultural and agro-food industries' wastes through composting are important for environmental sustainability and restoring soil quality. However, in some cases a toxicity effects can be observed if persistent toxic molecules are introduced in the soils. The important increase of land surfaces dedicated to Aloe vera cultivation due to its important use in food, cosmetic and pharmacological applications rendered a consequent production of aloin, the principal anthraquinone present in this plan leaves. The later compound is usually re-introduced in agricultural soils.

Earthworms are increasingly considered as a good choice for ecotoxicological testing in terrestrial habitats due to their cosmopolitan characteristics, widespread in many soils, and are excellent indicators of land use and soil fertility. Due to their life-style, earthworms are the first to be exposed to soils contaminants and, therefore, environmental xenobiotics occurs earlier and its effects are more pronounced than in other organisms [32,16,10,11,15].To our knowledge, this is the first report that aims to investigate the effects of aloin exposure on oxidative stress parameters in a earth earthworms.

In this study we report the sub-chronic and acute effects of aloin concentrations on the cellular responses of a non-target organism, the earthworm E. andrei. We first evaluated the LMS in worm coelomocytes and found that aloin negatively affected LMS in a dose dependent manner. LMS is known to be a sensitive cellular marker for the assessment of environmental contaminants effects on living organisms [11, 21].

Interestingly, the observed effect was maximal at the highest aloin concentrations. Thus, the anthraquinone seems to increase catabolic activity in worm's cells. Previous studies reported positive correlations between high-level ecotoxicological endpoints, such as and cell death and immune response and LMS [33]. From this point of view, it is of interest to point out that in marine mussels, a direct relationship between the scope for growth and LMS is well established. This may link LMS to population alterations [34]. The same effect was observed in earth worms exposed to increasing concentration of the herbicide 2-4-D where LMS decrease was correlated with a loss of body weight after 14 days of exposure [11]. Indeed, in the catabolic organisms, dietary energy is used mostly to rapidly turnover the cellular components damage by the toxic compounds and can be used only in a reduced manner for growth and reproduction [34].

In this study the time course response of the LMS showed a more pronounced toxic effect after 14 days of exposure when compared to the same experimental condition after 7 days of exposure. A completely opposite trend was observed in recent works after the exposure of E Andrei from the same period of times to the herbicide 2-4D and to a polymetallic soils gradient [10,11]. This may support the highly toxic effect of aloin at the tested concentrations that empower over time probably due to its metabolites production.

Our data suggested also that exposure to aloin crude extract increased the level of oxidative alterations in the earthworms cells. This was assessed by evaluating the MDA content in animal's tissues after 7 and 14 days of exposure. 7 and 14 days treatment induced higheer MDA accumulation being concentration dependent. In parallel,

exposure to aloin significantly increased the activities of CAT and GST in all the tested concentrations except for GST in animals exposed to the highest aloin concentration. Interestingly, gene expression analysis of the anti-oxidative stress genes cat and phase II biotransformation related gene gst in worms exposed to aloin showed significant up-regulation of these targets after 7 days. However, expression pattern of gst markedly decreased after 14 days when compared to 7 days even if it remains up-regulated when compared to control.

Oxidative stress can be induced by numerous stressors either by alterations in antioxidant defense mechanisms, or by over production of free radicals [35]. Our data provided evidences of physiological alterations in worm's cells that resulted in the accumulation of ROS. In addition to the direct production of ROS, other factors involved in aloin toxicity can be evoked. Indeed as a PAH, aloin may be bio transformed by organisms, and it or its metabolites may affect normal biotransformation process as GST activity was down regulated at higher aloin concentrations. Indeed, it has been suggested that cleavage of the glycosyl bonds by the intestinal microflora of humans and animals may results in the production of aloe-emodin[1,8-dihydroxy-3-(hydroxymethyl)anthraquinone] and other free anthraquinones and anthrones [36]. Moreover, the International Agency for Research on Cancer classified the whole leaf extract of aloe vera as possibly carcinogenic to humans (IARC Monographs, 2014). More recently, [37] reported the activation of aloe-emodin from aloe vera whole leaf extracts to biologically electrophiles compound sand their involvement in the carcinogenicity of exposed organisms.

AChE activity of different worm species ranging from soil to marine ones has been found to be modulated by environmental contaminants other than organophosphate and carbamate insecticides, such as metals and hydrocarbon compounds [38,39,40,41]. Data reported in the present work showed a significant inhibition of AChE activity in worms exposed to C2, C3and C4.This negative effect was significantly pronounced after 14 days of exposure when compared to 7 days. Although anthraquinones have not been so fare involved in the neurotoxicity events of exposed organisms our data may suggest that the observed effect is rather due to the general toxic effect at the body level of exposed worms to aloe vera leaves exudates. Similar effect was observed in marine mussels exposed to the polyaromatic hydrocarbon benzo[a]pyrene at relatively higher levels [42].

V. CONCLUSION

The results of this research stress for the first time the risk due to the introduction of aloe vera exudates in agricultural soils and their harmful effect on the earth worm Eisenia Andrei. Our data provided clues about the occurrence of alterations at cellular level of worms exposed to increasing aloin concentrations, thus highlighting a possible mechanism of action of the toxic compound through a relevant alteration of the activity of the lysosomal vacuolar system and a disorder in the antioxidant balance at protein and gene expression level. Finally a neurotoxic effect of the plant exudates was demonstrated. More in deep investigation of the single component of the tested exudates have to be performed to better understand the observed toxic effect.

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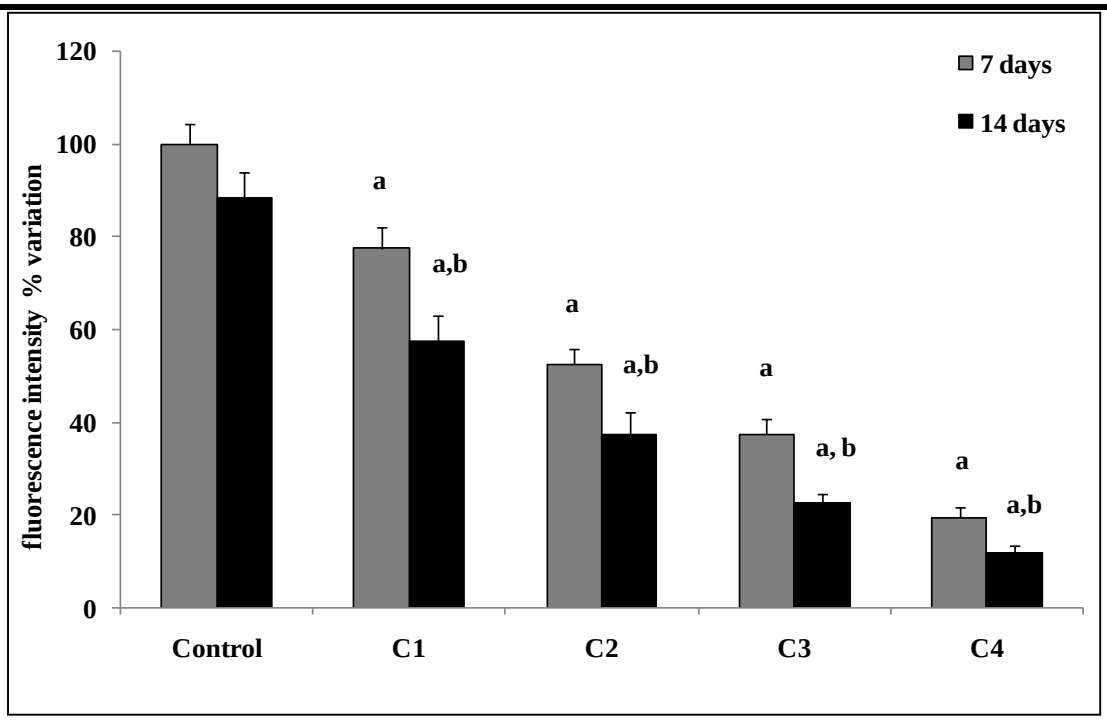

Fig1. Lysosomal membrane stability in E. andrei colemocytes after exposure for 7 and 14 days to aloin crude extract (10, 50, 100 and 200 g/kg soils). Data expressed as % variation in fluorescence intensity (n=10), were analyzed by ANOVA + Tukey'spost test. a: Statistically significant differences (P<0.01) in comparison with control condition. b: Statistically significant differences (P<0.01) in comparison with animals exposed to the same condition for 7 d.

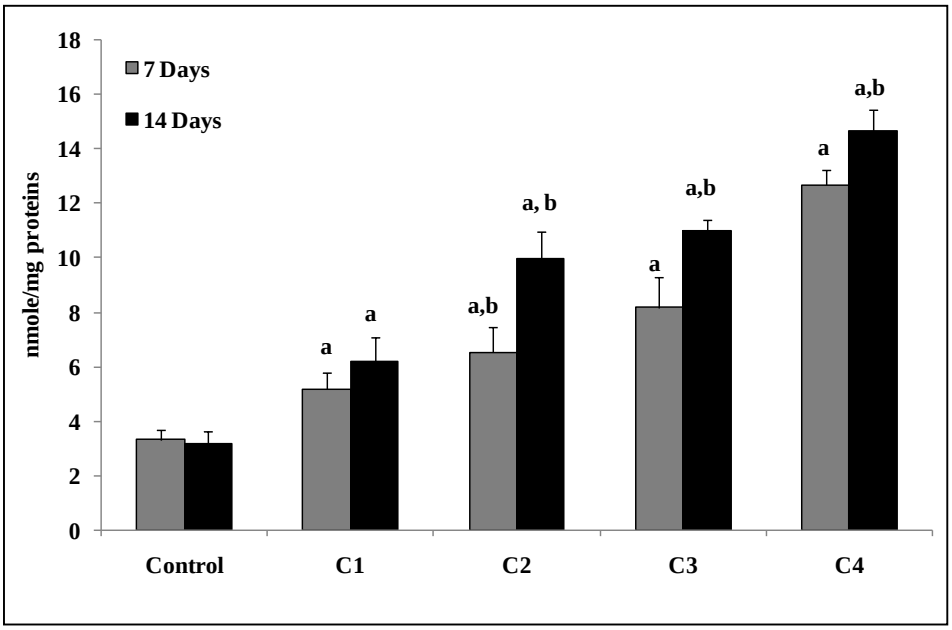

Fig 2. MDA accumulation in E. andrei exposed for 7 and 14 d to aloin crude extract (10, 50, 100 and 200 g/kg soils). Data, expressed as nmole/mg proteins (n = 10), were analyzed by ANOVA + Tukey's post test. a: Statistically significant differences (P<0.01) in comparison with control condition. b: Statistically significant differences (P<0.01) in comparison with animals exposed to the same condition for 7 d.

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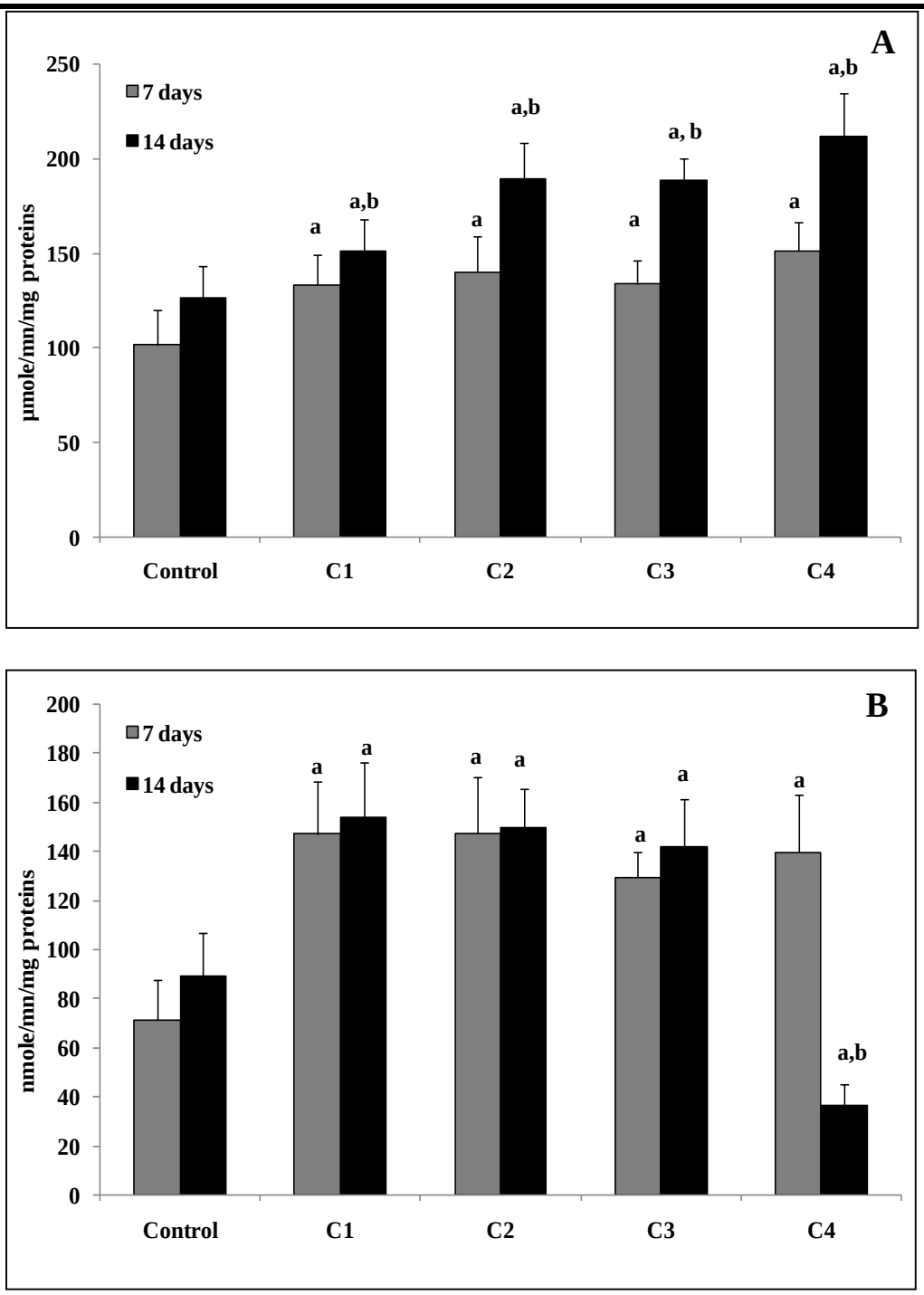

Fig 3: CAT (A) and GST(B) activities in E. Andrei exposed for7 and 14d to aloin crude extract (10, 50, 100 and 200 g/kg soils). Data, µmole/mn/mg proteins for CAT and as nmole/mn/mg proteins GST (n=10), were analyzed by ANOVA + Tukey'spost test. a: Statistically significant differences (P<0.01) in comparison with control condition. b: Statistically significant differences (P<0.01) in comparison with animals exposed to the same condition for 7 d.

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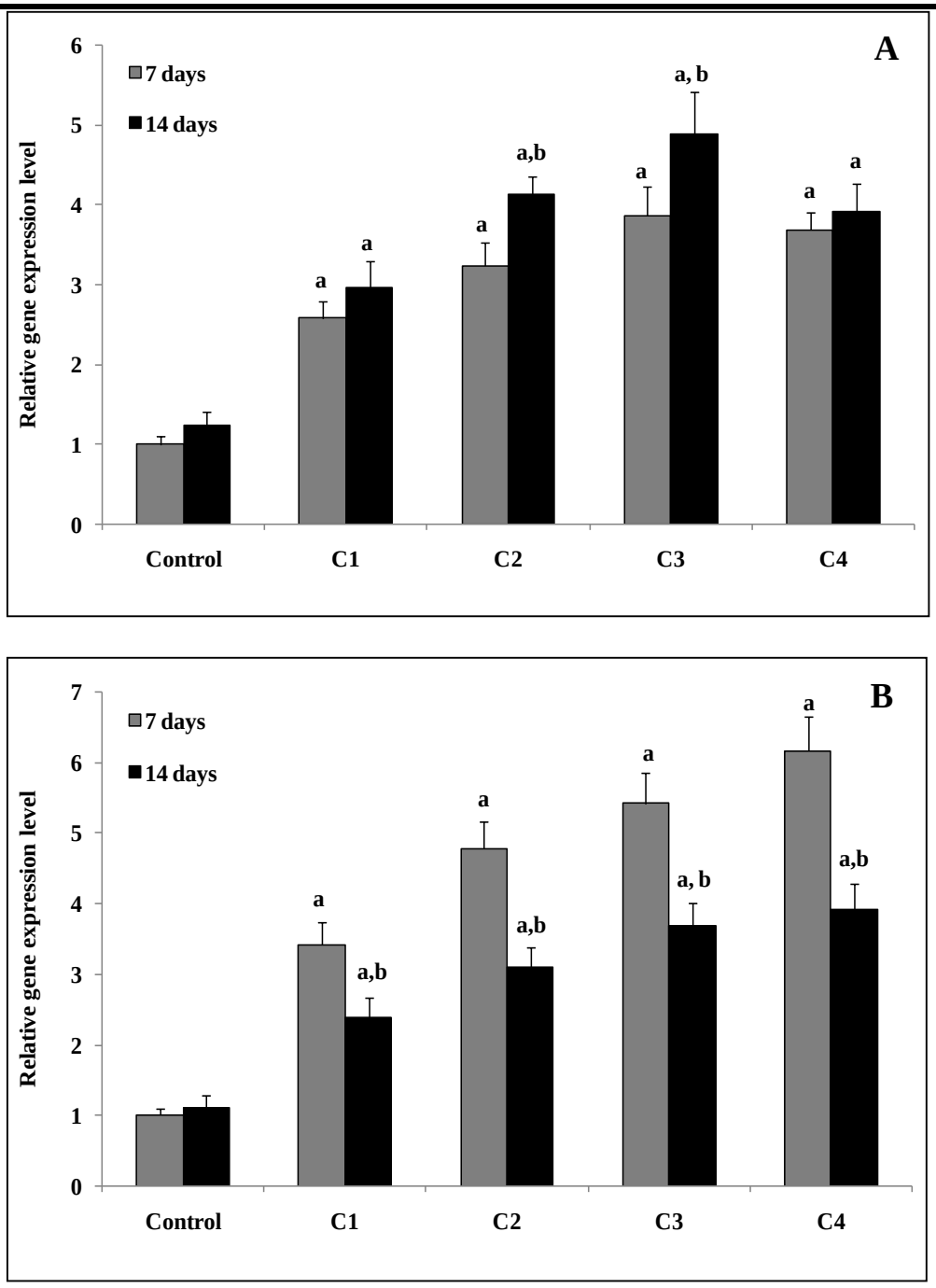

Fig 4. QPCR data of cat (A) and gst (B) targets in worms exposed for 7 and 14 d to aloin crude extract (10, 50, 100 and 200 g/kg soils). Gene expression was performed respect to the control condition (Soil without aloin supply); and was normalized against Actin, 18S and Ribo-S13. A: Significantly different from reference condition, b: Statistically significant differences in comparison with animals exposed to the same condition for 7 d. ($p < 0.05$) threshold cycle random reallocation test according to Pfaffl et al. (2002), n=4.

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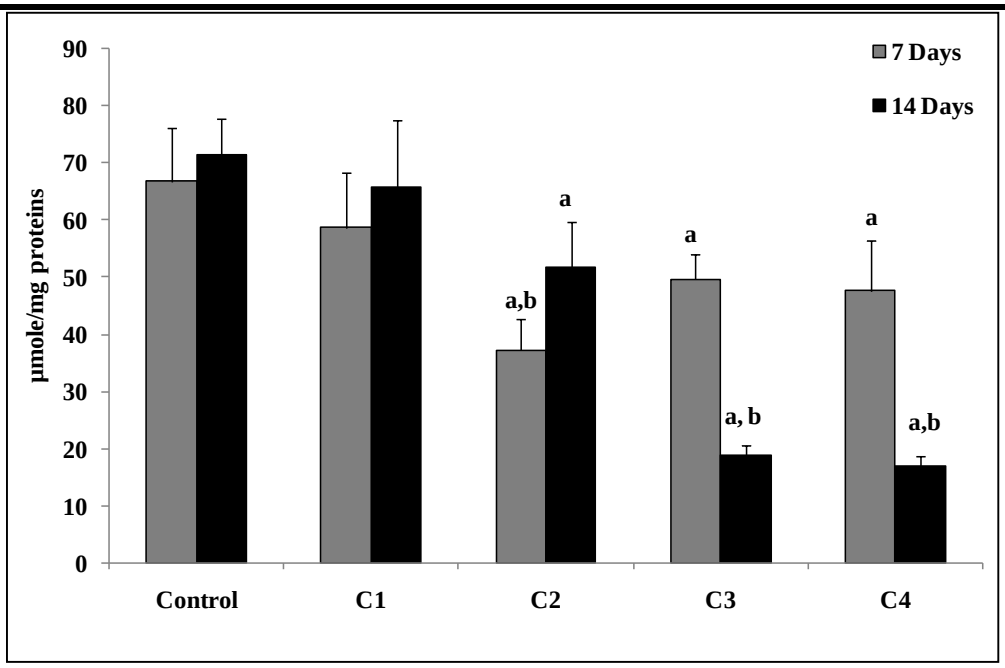

Fig5: ACHE activity in E. Andrei exposed for7 and 14d to aloin crude extract (10, 50, 100 and 200 g/kg soils). Data, µmole/mn/mg proteins (n=10), were analyzed by ANOVA + Tukey'spost test. a: Statistically significant differences (P<0.01) in comparison with control condition. b: Statistically significant differences (P<0.01) in comparison with animals exposed to the same condition for 7 d.

Table.1: Q-PCR primers and Taqman probes from Hattab et al. (2015)

Gene name	Probe	Sense Primer	Antisense Primer
18S	CGCCGACAGAGTGCCATCGACGAA	AATTCCGATAACGAACGAGACTCT	GCCACTTGTCCCTCTAAGAAGTTA
β - Actin	AGTCCGGGCCATCCATCGTCCACA	GGATCAGCAAGCAGGAGTACG	TGGTCATTGATAATGGAGGCACTT
RiboS13	TCGCATGGTGTCGCTCAGACCCGT	TCACAGATTGGTGTTATCCTTCGA	GCAAGACCCTTAGCCTTCAGG
gst	AGCGGAGTGCCTGACCACGACCTC	GGTGTCCGATAGAATTCCTGCTAT	CTCCAGACCATTGTCTACAGCTAA
cat	TGCCTTGTCTCTTGCCGCCATCGT	CTCGATTTCGTCTTATTCTTCGCC	CTTGTATTCGTTGAGTTGCTCGG

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Living Fences, a Widespread Agroforestry Practice in Sri Lanka: Two Cases from Dry and Intermediate Zones

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Abstract*—The study was conducted to examine the structure and composition of live fence agroforestry practices in two regions of Sri Lanka and to identify key ecosystem system services provided by them. The studies were conducted in the Katupotha in Kurunegala district and Hingurakgoda in Polonnaruwa district. Species composition including dominance, diversity and sinusial formation were evaluated.

*Highest average relative importance, relative frequency and dominance values were obtained by Wetahira (Gliricidia sepium), Wetaendaru (Jataropha curcus) and Sudu araliya (Plumeria obtusa) at Katupotha and Teak (Tectona grandis), Wetahira (Gliricidia sepium), Ipil-ipil (Leucaena leucocephala), Neem (Azadiracta indica) and Yakadamaran (Syzygium zeylanicum) at Hingurakgoda. The RIV value shows that live fences of Katupotha was dominated by typical (structural) live fence trees (Over 90% dominance) whereas live fences at Hingurakgoda was dominated by high value timber trees (Over 60% dominance). The results indicate that living fences have high species diversity. A total of 72 species were recorded from the living fences in two sites. Live fences at Hingurakgoda were often more diverse than Katupotha although the total number of species recorded at Katupotha site (68) was more than that of Hingurakgoda (25). 21 out of 25 (84%) species recorded at Hingurakgoda were also recorded from Katupotha. Hence species reported at Hingurakgoda is almost a subset of species identified from living fences at Katupotha. The Index of Similarity for two sites (plant communities) was 0.58 as 21 out of 72 (29%) species were found common to both sites.

The study clearly shows that live fences in addition to acting as protective structures against theft of homegarden produce, entry of stray animals and encroachments also could make further contributions to the environment and mankind due to high biodiversity. They include provisioning of timber, food, medicine, fruits, vegetables and fodder for livestock regulatory functions such as shade, windbreak and enrichment of soil fertility and cultural services such as visual amenity due to having ornamental plants. Further this study indicates that there is lot of potential for further enriching these live fences to better perform ecosystem services. Since live fences are a common farming practice spanning all agro-ecological regions of Sri Lanka, they could serve as a place for conservation of species and tool for identification and evaluation of species for different regions and purposes.

Keywords*—*Agrobiodiversity, agroforestry, Gliricidia, homegardens, live fences.

I. INTRODUCTION

Live fencing is a widespread agroforestry practice in Sri Lanka where trees or shrubs are established to demarcate boundaries of plots of land such as homegardens and farmlands. In addition they perform some vital ecosystem functions such as, protecting from animals, trespassing and encroachments. Their ramifying roots underground will check soil erosion. Living fences can serve as habitats, corridors, or stepping stones for plant and animal species, adding structural and floristic complexity to the agricultural landscape and enhancing landscape connectivity (Forman & Baudry, 1984; Multipurpose Trees Species Research Network [MPTSRN], 1996; Harvey, Tucker & Estrada, 2004).

Although live fences are deliberately established now, it is believed that live fencing have originated out of different type of forest remnants found in the traditional villages of Sri Lanka. With the reduction of natural forests, incorporation of resources of forest origin in land use practices have become all the more important to meet man's demand for plant products and services.

The boundary fences in general are made out of barbed wire with live wooden, dead wooden or cement posts. They are mainly planted with species that can be propagated using stumps or live sticks. These sticks are planted close to each other to form the live fences. The growth of these fencing plants is kept under control by regular pruning and replanting to fill gaps. In areas where

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land holdings are small, utility plants for timber, fodder, green manure, medicinal and food too are established on the boundary fences.

Studies on live fences are available from many parts of the world including Costa Rica (Sauer, 1979; Budowski, 1987), Cuba (Crane, 1945), Kenya (Oteng, Stigter, Ng Ang, & Mungai, 2000), Mexico (Nabhan & Sheridan, 1977), Honduras, (Zahawi, 2005) and many states in India including Kerala (Chandrashekara, Sanker, Shajahan, Biowfield & Boa, 2000) and Eastern Ghats (Choudhury, Rai, Patnaik & Sitaram, 2005). Mishra, Vasudevan and Prasad, (2011) classified the biofences based on the type of area protected. Except for few recent studies (Jayavanan, Pushpakumara & Sivachandran, 2014), live fence practices in Sri Lanka remains relatively less studied and documented.

The objectives of this study was to examine the structure and composition of live fence agroforestry practices found in the low country intermediate and dry zones of Sri Lanka and to identify the key ecosystem services performed by them.

II. MATERIALS AND METHODS

Sites for studying live fences were selected randomly from well-established homegardens in the Katupotha and Hingurakgoda Divisional Secretory Divisions in the Kurunegala and Polonnaruwa districts, respectively. Summary of the agro-ecological setting and geographical information of the two sites are outlined in the Table 1.

Table.1: Summary of agro-ecological and geographical setting of Katupotha and Hingurakgoda sites.

Characteristic	**Katupotha**	**Hingurakgoda**
Agro-ecological region (AER)	IL1 (Low country intermediate zone)	DL1c (Low country dry zone)
Rainfall	Pattern is bimodal (Peaks in October-November and April-May)	
Annual Rainfall (mm)	1682 mm.	1554mm
Elevation (m)	152m	74m
Soil type	Red Yellow Podzolic	Reddish Brown Earth
Average Annual Temperature	27 ^{0}C	27 ^{0}C
Number of homegardens selected for the study	31	25
Geographical area	Kurakkanhenegedara, Dalupothagama, Nelumkanuwa, Pallewela and Thorapitiya	Kimbulwala Grama Niladari division

Source: Punyawardena, (2008)

A vegetation survey was conducted to identify the structure and composition of the live fences. Tree individuals recorded in the live fence were identified and their diameters at the breast height (DBH), total height, crown diameter and length of fences were measured. Clinometer was used to measure the tree height. DBH was measured using diameter tape and crown diameter by using the shadow of the tree during the mid-day.

Because of the presence of large number of individuals from same species of similar dimensions (for basal diameter, total height and crown diameter), size classes were defined and species were classified based on the physiognomic classes during the vegetation survey. Samples of each class were used to measure various dimensions of trees.

Collected data were used to evaluate various aspects of composition and structure of live fences. Composition, dominance and diversity of species were estimated through calculation of following indices:

Relative frequency (RF) is expressed as the percentage of plots in which a species is represented at least once.

$$\text{Relative frequency} = \frac{\text{Number of plots in which species was recorded} \times 100}{\text{Total Number of plots}}$$

Relative importance value (Myers & Shelton, 1980; Mueller-Dombois & Ellenberg, 2003) is the expression of domination of a species in different forest line formations and incorporates four measures:

Relative Importance Value (RIV) = ¼ x (Relative density + Relative basal area + Relative tree height + Relative crown diameter)

$$\text{Relative density} = \frac{\text{No. individuals of species A} \times 100}{\text{Total number of all individuals of all species}}$$

$$\text{Relative Basal Area} = \frac{\text{Sum of DBH of individuals of species A} \times 100}{\text{Sum of DBH of all individuals}}$$

$$\text{Relative Crown Diameter} = \frac{\text{Sum of crown diameters of individuals of species A} \times 100}{\text{Crown Diameter of all individuals}}$$

$$\text{Relative Height} = \frac{\text{Sum heights of individuals of species A} \times 100}{\text{Sum of heights of all individuals}}$$

Similarity or association of species between two sites were estimated using similarity index:

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$$\text{Index of similarity} = 2 \times \frac{\text{Number of common species for both communities}}{\text{Total number of species in both communities}}$$

Menhinick's Diversity Index was used to measure the species diversity of the live fences evaluated during the study. It is based on the ratio of number of species (S) and the square root of the total number of individuals (N).

$$\text{Diversity Index} = \frac{\text{Total number of species recorded}}{\text{Log value of individuals counted}}$$

Trees in the live fence were categorized into four vertical strata (*sinusia*) using a scheme developed after careful evaluation of the vertical structures of live fences (MPTSRN, 1996) as shown below:

- Herbaceous horizon (under cover): Up to 1.83 m in height providing ground level protection with small shrubs, under shrubs and other herbaceous perennials.
- Shrub horizon (sub canopy): multi-branched woody perennials, low growing trees and shrubs providing mid-level cover up to 7.62 m
- Tree horizon (canopy): Up to 7.62 - 15.25 m in height with selected trees based on their uses as well as canopy characteristics
- Emergent horizon (above canopy): tree species taller than 15.25 m

Further socio-economic characteristics of farmers practicing live fences at Katupotha were studied using questionnaire based survey. The information collected from the included occupations of land holders, the extent of homegardens and the length of live fences.

III. RESULTS AND DISCUSSION

3.1 Composition and Dominance

Table 1 shows the frequencies of the twelve most common tree species recorded from live fences in the Katupotha area. Wetahira and Wetaendaru were recorded in all plots giving 100% relative frequency value. Relative frequency of Sudu araliya was 96%. The relative importance (dominance) of the species in live fences in the Katupotha area also shows the similar trend as the relative frequency. Wetahira shows the highest importance (29.72%) followed by Wetaendaru (29.55%) and Sudu Araliya (22.69%).

Table.1: Predominant species recorded from the live fences in the Katupotha area.

Botanical name	**Common name**	**No. of individuals**	**Relative frequency**	**RIV (%)**	**Species rank**
Adathoda vasica	Pavatta	21	16.67	1.35	8
Anacardium occidentale	Cadju	24	37.50	1.00	11
Azadiracta indica	Kohomba	27	20.83	1.82	7
Berrya cordifolia	Halmilla	35	16.67	0.59	12
Ceiba pentandra	Kotta Pulun	60	58.33	2.52	5
Chukrasia tabularis	Hik	27	50.00	1.06	10
Erythrina indica	Katu erabadu	185	41.67	2.13	6
Gliricidia sepium	Wetahira	2272	100	29.72	1
Jatropha curcus	Weta endaru	4109	100	29.55	2
Nerium oleander	Kaneru	513	66.67	6.66	4
Plumeria obtuse	Sudu araliya	2138	95.83	22.69	3
Streblus aspera	Gas nithul	50	45.83	1.10	9

Key: RIV-Relative Importance Value.

Table 2 shows the frequencies of the ten most common tree species of the live fences in the Hingurakgoda area. According to these results Wetahira was recorded in all plots recording 100% relative frequency value as in the case of Katupotha. Relative frequency of Teak and Neem were 96% and 92%, respectively. The RIV values shows that teak (17.09%) was the most dominant species and it is followed by Wetahira (15.16%), Ipil-Ipil (12.30%), Neem (11.81%) and Yakadamaran (10.75%). This shows that most live fences in the Hingurakgoda are planted with high value timber species including Thekka (Teak) and Kohomba (Neem). They are also among the most dominant species ranking first and fourth, respectively based on the Relative Importance Value. Also it is significant to note that almost one half (59.6%) of the live fences have been taken up by the high value timber species.

Table.2: Predominant species recorded from the live fences in the Hingurakgoda area.

Botanical name	Common name	No. of individuals	Relative frequency	RIV (%)	Species rank
Artocarpus heterophyllus	Kos	105	64	6.07	9
Azadirachta indica	Kohomba	302	92	11.81	4
Gliricidia sepium	Wetahira	772	100	15.16	2
Leucaena leucocephala	Ipil ipil	390	68	12.30	3
Mangifera indica	Amba	252	72	7.76	6
Tectona grandis	Thekka	350	96	17.09	1
Syzygium zeylanicum	Yakada maran	325	76	10.75	5
Berrya cordifolia	Halmilla	212	64	7.06	7
Pterospermum suberifolium	Welan	173	48	6.82	8
Ficus racemosa	Attikka	122	56	5.18	10

Key: RIV-Relative Importance Value

3.2 Floristic Richness in the Live Fences

The live fences at Katupotha and Hingurakgoda recorded 68 and 25 species, respectively (Annexure 1). A total of 72 species were recorded from the living fences in two sites. 21 out of 25 (84%) species recorded at Hingurakgoda were also recorded from Katupotha. Hence species reported at Hingurakgoda is almost a subset of species identified from living fences at Katupotha. The Index of similarity was estimated to compare the two plant communities. It was 0.58 as 21 out of 72 (29%) species were found common to both sites. The index of similarity ranges from 0-2 and it also an indicator of the degree of species association with the site.

68 plant species recorded from Katupotha was belonging to 29 families and 63 genera whereas 25 species recorded from Hingurakgoda were belonging to 16 families and 24 genera (Table 3). The Floristic Richness Index (FRI) was calculated for the live fences in the two sites and the values were 160 and 65 for Katupotha and Hingurakgoda, respectively. This shows that floristic richness was much higher at Katupotha when compared to Hingurakkgoda.

Of the families recorded, Fabaceae was represented by most number of species at both sites that is by 9 and 4 species, respectively at Katupotha and Hingurakgoda. The other families represented by high number of species were Apocynaceae, Euphorbiaceae, Meliaceae, Rutaceae and Moraceae.

Table.3: Floristic richness of live fences at Katupotha and Hingurakgoda.

Site	Species	Genera	Families	FRI
Katupotha	68	63	29	160
Hingurakgoda	25	24	16	65

Key: FRI-Floristic Richness Index

3.3 Species Diversity of Live Fences

Species diversity of live fences were measured through recording occurrence of different species in live fences (Table 4) and by calculating diversity index (Table 5). The occurrence of different species in live fences shows that 35% and 60% of live fence plots at Katupotha and Hingurakgoda, respectively have recorded more than 10 species per live fence plot (Table 4).

Table.4: Tree diversity in live fences (Occurrence of species).

Number of species per plot	Number of plots	
	Katupotha	Hingurakgoda
1-5	3 (9.5)	-
6-10	17 (55)	10 (40)
11-15	7 (22.5)	12 (48)
15-20	4 (13)	03 (12)
Total	31 (100)	25 (100)

Key: Number given in the parenthesis is the percentage.

The diversity index (DI) values estimated for live fences are given in the Table 5. This shows that only 13% of live fences recorded DI more than 5 at Katupotha whereas it was 56% at Hingurakgoda. Hence results indicates that live fences at Hingurakgoda were often more diverse than Katupotha although the total number of species recorded at Katupotha site was more than that is recorded from Hingurakgoda.

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Table.5: Tree diversity in live fences (Diversity index).

Diversity Index Range	Number of plots	
	Katupotha	Hingurakgoda
0 – 3	7 (22.5)	3 (12)
3 –5	20 (64.5)	8 (32)
5 – 7	02 (6.5)	13 (52)
7 – 9	0	1 (04)
More than 12	02 (6.5)	0
Total	31 (100)	25 (100)

Key: Number given in the parenthesis is the percentage.

3.4 Uses of Live Fence Trees

Tree species recorded from live fences were categorized based on their main uses (Table 6). The common uses of live fence tree species include firewood, food, handicraft, fence post, medicinal, ornamental, timber and multi-purpose trees. Of the species recorded in live fences highest number (about 32%) fell under the category of timber at both sites.

Table.6: Categorizing tree species occurring in live fences at Katupotha and Hingurakgoda, based on main uses.

Main use	Number of species occurring in live fences	
	Katupotha	Hingurakgoda
Firewood	03 (4.5)	-
Food	07 (10.25)	04 (16.0)
Handicraft	03 (4.5)	01 (4.0)
Live Fence Structural	07 (10.25)	03 (12.0)
Medicinal	15 (22.0)	03 (12.0)
Ornamental	08 (11.5)	03 (12.0)
Multipurpose	03 (4.5)	03 (12.0)
Timber	22 (32.5)	08 (32.0)
Total	**68 (100)**	**25 (100)**

Key: Number given in the parenthesis is the percentage.

3.5 Tree Arrangement (Physiognomy)

Number of species recorded from different vertical layers in the live fences is shown in Table 7. According to the results, the tree horizon (Canopy: 7.62-15.25 m) recorded the highest number of species when compared to the other three sinusium identified in the live fences.

Table.7: Number of species at different layers.

Class	Horizon	Katupotha	Hingurakgoda
1	Herbaceous Horizon (understory) up to 1.83m	15 (22)	06 (24)
2	Shrub Horizon (sub canopy) up to 7.62m	16 (24)	07 (28)
3	Tree Horizon (Canopy) up to 15.25m	23 (34)	10 (40)
4	Emergent Horizon more than 15.25m	14 (20)	02 (08)
Total number of species		68 (100)	25 (100)

Key: Number given in the parenthesis is the percentage.

3.6 Socio-economic Characteristics

Following facts were unveiled from the questionnaire based survey conducted with farmers who were selected for the live fence study from the Katupotha area:

Land use:

The length of live fences and the extent of homegarden protected by them are shown in the Table 8. This shows that 84% of the homegardens were below 1.5 acres in extent and they cover about 62% of the total extent of the homegardens selected for the study. Further it is observed that all these smaller homegradens had intercrops in addition to the coconut which is the main crop of the area. Further it is found that most of these small homegardens are well managed also their live fences. The larger homegardens were found planted with monocultural coconut plantations and most of them were poorly maintained. About three quarter of the live fences in the study sample were found fortified with barbed wire.

Table.8: The extent of homegardens and the length of live fence established to protect them.

Land extent (Ac)	Number of plots	Total extent (Ac)	Total length of the fence (m)
0.5-1	14	10.75	1597.69
1-1.5	12	16.88	2762.69
1.5-2	-	-	-
>2	5	17.25	2067.29
Total	31	44.88	

Employment:

The main employment of the land holders are shown in the Table 9. This shows that about 30% of land holders were full-time farmers while others were involved in some form of off-farm employment.

Table.9: Employment of land holders.

Employment	No. of households	Percentage (%)
Farmers	10	32.3

Businessmen	6	19.4
Mason / carpenter	3	9.6
Teachers	3	9.6
Grama niladhari (Village Secretary)	2	6.5
Other	7	22.6
Total	31	100

IV. CONCLUSIONS

The results shows that Wetahira (*Gliricidia sepium*), Wetaendaru (*Jataropha curcus*) and Sudu araliya (*Plumeria obtusa*) were the most common and dominant species at Katupotha whereas Teak (*Tectona grandis*), Wetahira (*Gliricidia sepium*), Ipil-ipil (*Leucaena leucocephala*), Neem (*Azadirachta indica*) and Yakadamaran (*Syzygium zeylanicum*) at Hingurakgoda. Live fences of Katupotha was dominated by typical (structural) live fence trees such as Wetahira (*Gliricidia sepium*) however live fences at Hingurakgoda was dominated by high value timber trees. Live fences at Hingurakgoda were often more diverse than Katupotha although the total number of species recorded at Katupotha (68) was more than Hingurakgoda (25).

Growing and use of Wetahira (Gliricidia) is widely promoted by many Governmental, Non-governmental and private companies for green manure, vine support for pepper and fuelwood (including for dendro thermal power generation). Kaneru (*Nerium oleander*) plants should be discouraged as the seeds are a readily available poison.

It appears that selection of plant types for live fences depended on the properties including easy propagation, free availability of propagules, not being subjected to be eaten by stray cattle (except Wetahira), fast growth, low spread and aesthetics (e.g. *Nerium oleander*). Some of the tree species would have been avoided due to the wide spread crowns. But such trees with proper silvicultural practices could serve as sources of biomass energy and timber.

The study also shows that live fences in addition to acting as protective structures against theft of homegarden produce, entry of stray animals and encroachments also could make further contributions to the environment and mankind due to high biodiversity. They include provisioning of timber, food, medicine, fruits, vegetables and fodder for livestock regulatory functions such as shade, windbreak and enrichment of soil fertility and cultural services such as visual amenity due to having ornamental plants.

This study also shows that there is lot of potential for further enriching these live fences to better perform the ecosystem services. Since live fences are a common farming practice spanning all agro-ecological regions of Sri Lanka, they could serve as a place for species conservation and tool for identification and evaluation of species for different regions and purposes.

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Annexure 1. Tree species recorded in the live fences at Katupotha and Hingurakgoda and their uses.

No	**Botanical name**	**Family**	**Com. Sinhala name**	**Main use[1]**	**Katupotha**	**Hingurakgoda**
1	*Albizia lebbeck*	*Fabaceae*	*Mara*	T	X	X
2	*Albizia odoratissima*	*Fabaceae*	*Sooriya mara Huree mara*	T	X	-
3	*Alstonia macrophylla*	*Apocynaceae*	*Havarinuga*	T	X	-
4	*Alstonia scholaris*	*Apocynaceae*	*Rukattana*	H (T, M)	X	-
5	*Anacardium occidentale*	*Anacardiaceae*	*Kadju*	F	X	X
6	*Annanas comosus*	*Bromeliaceae*	*Wal annasi*	LFS	X	-
7	*Annona muricata*	*Annonaceae*	*Katu anoda*	F	X	X
8	*Artocarpus heterophyllus*	*Moraceae*	*Kos (Jak)*	MPT	X	X
9	*Atalantia ceylanica*	*Rutaceae*	*Yakinaran*	M	X	-
10	*Atalantia ceylanica*	Rutaceae	Yakinaran	M (FW)	-	X
11	*Azadirachta indica*	*Meliaceae*	*Kohomba*	M (T)	X	X
12	*Berrya cordifolia*	*Tiliaceae*	*Halmilla*	T	X	X
13	*Borassus flabellifer*	*Arecaceae*	*Thal (Palmyrah palm)*	H (O)	X	X
14	*Bridelia retusa*	*Euphorbiaceae*	*Ketakela*	T (M)	X	X
15	*Calohpyllum inophyllum*	*Clusiaceae*	*Domba*	T (M)	X	-
16	*Caryota urens*	*Arecaceae*	*Kithul*	MPT	X	X
17	*Cassia fistula*	*Fabaceae*	*Ehela*	O (T, M)	X	X
18	*Ceiba pentandra*	*Bombacaceae*	*Kotta (Pulun imbul)*	LFS (T)	X	X
19	*Cerbera manghas*	*Apocynaceae*	*Kaduru*	M	X	-
20	*Chukrasia tabularis*	*Meliaceae*	*Hulanhik*	T	X	-
21	*Croton laccifer*	*Euphorbiaceae*	*Keppetia*	M (FW)	X	-
22	*Diospyros ferrea*	*Ebenaceae*	*Kalumediria (Habaraliya)*	T	X	-
23	*Diospyros malabarica*	*Ebenaceae*	*Thimbiri*	T (M)	X	-
24	*Erythrina indica*	*Fabaceae*	*Katuerabadu*	LFS (M)	X	-
25	*Ficus benghalensis*	*Moraceae*	*Mahanuga*	O (M)	X	X
26	*Ficus racemosa*	Moraceae	Attikka	M (FW)	-	X
27	*Garcinia quaesita*	*Clusiaceae*	*Goraka*	F (M)	X	-
28	*Gliricidia sepium*	*Fabaceae*	*Wetahira*	LFS	X	X
29	*Glycosmis pentaphylla*	*Rutaceae*	*Dodampana*	M (FW)	X	-
30	*Grewia damine*	*Tiliaceae*	*Damunu*	T	X	-

	(G. tilifolia)					
31	*Jatropha curcas*	*Euphorbiaceae*	*Wetaendaru*	LFS	X	-
32	*Justicia adhathoda (Adathoda vasica)*	*Acanthaceae*	*Pavatta*	M	X	-
33	*Leucaena leucocephala*	*Fabaceae*	*Ipil-ipil*	MPT (FW)	X	X
34	*Limonia acidissima (Feronia limonia)*	*Rutaceae*	*Divul*	F	X	-
35	*Litsea glutinosa*	*Lauraceae*	*Bomi*	M	X	-
36	*Macaranga peltata*	*Euphorbiaceae*	*Kenda*	T (FW)	X	X
37	*Madhuca longifolia*	*Sapotaceae*	*Mi*	M (T)	X	-
38	*Mangifera indica*	*Anacardiaceae*	*Amba*	F	X	X
39	*Manihot glaziovii*	*Euphorbiaceae*	*Gasmanyokka*	LFS	X	X
40	*Margaritaria indicus (Phyllanthus indicus)*	*Phyllanthaceae*	*Keraw*	T (FW)	X	-
41	*Melia dubia*	*Meliaceae*	*Lunumidella*	T	X	-
42	*Microcos paniculata (Grewia microcos)*	*Tiliaceae*	*Kohukirilla*	FW (M)	X	-
43	*Mitragyna parvifolia*	*Rubiaceae*	*Helamba*	T	X	X
44	*Moringa oleifera*	*Moringaceae*	*Murunga*	F (LFS, M)	X	X
45	*Nauclea orientalis*	*Rubiaceae*	*Bakmee*	T (M)	X	-
46	*Nerium oleander*	*Apocynaceae*	*Kaneru*	O (LFS)	X	X
47	*Opuntia dillenii*	*Cactaceae*	*Katupathok*	LFS (O)	X	-
48	*Pagiantha dichotoma*	*Apocynaceae*	*Divikaduru*	M (H)	X	-
49	*Pamburus missionis*	*Rutaceae*	*Pamburu*	M	X	-
50	*Pandanus tectorius*	*Pandanaceae*	*Watekeya*	H (M)	X	-
51	*Pedilanthus tithymeloides variegatus*	*Euphorbiaceae*	*Kepumkeeriya*	O	X	-
52	*Phyllanthus myrtifolius*	*Phyllanthaceae*	*Gangawerella*	O (LFS)	X	-
53	*Phyllanthus polyphyllus*	*Phyllanthaceae*	*Kuratia*	FW	X	-
54	*Plumeria obstusa*	*Apocynaceae*	*Suduaraliya*	O	X	-
55	*Pongamia pinnata*	*Fabaceae*	*Magulkaranda*	M (T)	X	-
56	*Premna tomentosa*	*Verbenaceae*	*Bu-seru*	M (FW)	X	-
57	*Pterocarpus marsupium*	*Fabaceae*	*Gammalu*	M (T)	X	-
58	*Pterospermum suberifolium*	Sterculiaceae	Welan	T	-	X
59	*Sansivieria trifasciata*	*Agavaceae*	*Sensivieria (Snake plant)*	O	X	-
60	*Schleichera oleosa*	*Sapindaceae*	*Kon*	T (F)	X	-
61	*Sterculia foetida*	*Sterculiaceae*	*Thelambu*	T (M)	X	-
62	*Streblus aspera*	*Moraceae*	*Gasnithul*	FW	X	-
63	*Swietenia macrophylla*	*Meliaceae*	*Mahogani*	T	X	-
64	*Syzygium*	*Myrta*	*Damba*	T	X	-

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	gardneri	*ceae*		(M)		
65	*Syzygium zeylanicum*	Myrtaceae	Yakadamaran	T (FW)	-	X
66	*Tamarindus indica*	*Fabaceae*	*Siyambala*	F (T)	X	-
67	*Tectona grandis*	*Verbenaceae*	*Thekka*	T	X	X
68	*Terminalia bellirica*	*Combretaceae*	*Bulu*	M (T)	X	-
69	*Terminalia catappa*	*Combretaceae*	*Kottamba*	O (T)	X	-
70	*Thespesia populnea*	*Malvaceae*	*Gansooriya*	T (LFS, M)	X	-
71	*Vitex negundo*	*Verbenaceae*	*Nika*	M	X	-
72	*Walsura pisciadia (W.trifoliolata)*	*Meliaceae*	*Kirikon*	T (M)	X	-
	No. of species				68	25

Key: Firewood (FW), Food (F), Handicraft (H), Live Fence Structural (LFS), Medicinal (M), Ornamental (O), Multipurpose (MPT), Timber (T)

[1]**Other uses are given in the parenthesis**

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Effects of Housing Modifications on the Management of Pigs and Growth Performance

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Abstract— *Pig industry in Nigeria is an important arm of the livestock sub-sector in the overall agricultural sector. The comfort of Pigs is determined by effective environmental temperature. It combines the effect of air temperature, flooring, and bedding. The aim of this study is to investigate the impact of different intensive housing systems on the feed consumption level, weight gain and welfare of pigs fed with the same feed (diet) under different housing systems for 8 weeks. Nine Pigs were purchased from a reputable commercial farm and were divided into 3 treatment groups, T_1, T_2 and T_3. T_1 is a well built pen with cemented wall and floor. T_2 is a pen built with bamboo wall, and cemented floor. T_3 is built with bamboo wall without cemented floor – bare loamy soil. The Pigs were raised for eight weeks. The feed given and weight gained for eight weeks were recorded and analysed using ANOVA. Considering the consumption levels of all treatments, T_2 had the lowest feed intake compared to other treatments. T_2 had the highest weight gain while T_3had the lowest. It is hereby concluded that T_3 presented the best result as regards feed consumption, cost of construction and ease of management. Although it presented the lowest weight gain which is attributable to the initial weight and tipping of the feed trough (feed wastage). However, feed wastage can be minimized by using firmer feed trough.*

Keywords— Pigs, ANOVA, Nigeria.

I. BACKGROUND

With ever increasing human population in Nigeria and virtually static agricultural productivity, animal protein consumption among Nigerians has worsened in the past few years (Okpor, 2009). Many Nigerians feed on carbohydrate, this is because the average man cannot afford the cost of animal protein which is richer in amino acid. The deficiency of animal protein in the diet of so many people is often attributed to the low number of livestock (Cattle, Pigs, Poultry, Goats, Sheep and their products), and the activities connected with their production which are not efficient (Morrison, 2001). Ugwu (2006) observed that animal protein apart from its palatability is essential for normal physical and mental development of man. He stated that its deficiency in the diet exerts adverse effect in terms of reduced human productivity due to abnormal development. Equally, he noted that animal protein and energy deficiency causes high incidence of infant mortality, pronounced malnutrition and diseases.

Pig production has been ticked as a panacea to protein inadequacy due to certain attributes which Pigs possess that are absent in other domestic livestock.

According to FAO (2001), pork is believed to be the most popular meat consumed in the world today. Forty four percent of world meat consumption is derived from pork and pork products (FAO, 2001).

Livestock production in Nigeria is carried out under different systems broadly classified as extensive, semi intensive and intensive. The extensive system can also be called the free range system, the animal roam and look for food. It is unspecialized and traditional system which is predominant among small scale farmers. While this may be the cheapest system of production, it also has the highest disadvantages ranging from theft to parasitic infections which render pork gotten from this housing system unwholesome for human consumption when subjected to veterinary inspection.

The semi intensive system gives room for good control of feeding, proper management and animals are more protected under this system than the free range.

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Under the intensive production system, animals are raised in total confinement and this system enables them to fully express their genetic potentials. Adequate nutrients are provided; this helpsin satisfying dietary requirement which culminate in efficient feed conversion and growth (Devandra and Fuller 1989). This system has a lot of advantages over the extensive and semi intensive system in terms of disease and breeding control as well as adoption of improved technology in animal production. This system prevents reckless grazing, destruction of farm crops and curbs animals from becoming nuisance on the street.

Pig industry in Nigeria is an important arm of the livestock sub-sector in the overall agricultural sector. This assertion is derived from the fact that Pig production, among other species has a high potential to contribute to high economic gain in three ways.

First, Pigs have high fecundity, high feed conversion efficiency, early maturity, short generation interval and relatively small space requirement.

Second, they are multipurpose animals providing about 40% of cooking fat, bristles and meat in the world market. Pig is equally important for agro-based industries like feed mills. They provide bone and blood which are used for production of bone meal and blood meal. This isa good source of calcium in animal nutrition. In addition, pig manure is an excellent fertilizer for enriching, replenishing poor soils and provision of biogas. Pig skin is also useful for light leather production (Babatunde&Fetuga, 1990).

Third, it is produced under varieties of production systems ranging from simple backyard piggery to large scale integrated Pig industries with sophisticated bio-safety measures.

The comfort of Pigs is determined by effective environmental temperature whichcombines the effect of air temperature, flooring and bedding.

The aim of this study is to investigate the impact of different intensive housing systems on feed consumption level, weight gain and welfare of Pigs fed with the same feed (diet) under different housing systems for 8 weeks.

II. METHODOLOGY

Location of the experimental site

The experiment commenced on 10th of May, 2016. Nine (9) Pigs (crosses of large white and land race) were allotted into 3 housing systems. This project work lasted for 8 weeks and was terminated on 5th of July, 2016. This research was conducted at the piggery unit of Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.

Experimental design

Three housing systems were studied, which are; T_1, T_2, T_3.Nine (9) Pigs (crosses of large white and land race) were purchased from a reputable commercial farm and were raised for eight weeks. The three treatments have different housing systems:

T_1: This is a well built pen with cemented walls and floor.

T_2: This is a well built pen with bamboo walls, and cemented floor.

T_3: This is a well built pen with bamboo walls without cemented floor – bare loamy soil. Each treatment has three replicates, and each replicate contained one animal.

Experimental procedure

Daily Routine

The daily routine practice in the farm includes; cleaning of pen and its surrounding, washing the drinking trough and cleaning the feeders. Feed and water were given to them throughout the experiment. The weight of the feed leftover as well as feed intake was recorded.

Occasional Routine

All through the period of this research, Pigs were weighed on weekly basis.

III. RESULTS AND DISCUSSION

Table.4.1:Feed Consumption Level

Parameter	Treatment 1	Treatment 2	Treatment 3
Feed intake	569.86±*225*.36[a]	562.98±209.08[a]	602.41±222.45[a]
Leftovers	68.51±73.11[b]	102.38±91.91[a]	57.23±64.15[b]
Feed given	740.00±111.24[a]	740.00±111.24[a]	736.10[a](109.70)

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Table.4.2: Weight Gain

Treatment	Weight gain (Mean±SD)
Treatment 1	9.92±2.35[a]
Treatment 2	9.97±1.58[a]
Treatment 3	9.69±1.90[a]

Table.4.3: Classification of housing parameters, general pig management and health parameters

Parameter	Treatment 1	Treatment 2	Treatment 3
Construction type	Asbestos roof, well cemented wallsand floor	Asbestos roof, bamboo walls and cemented floor	Asbestos roof, bamboo walls and non-cemented floor
Ease of management	Difficult to clean because it is tedious to wash the floor and wall stained with faeces.	Less difficult because it is needless to wash the wall.	It is the easiest to clean. The floor can easily be swept without been washed.
Ease of effluent disposal	Easy	Difficult	More difficult
Pig general outward appearance	Often clean	Partially clean	Often dirty
Skin lesion	Abundance of mange on the back	Few mange on the back	Absence of mange on the animal
Floor condition	Often dry	Occasionally dry	Mostly wet
Labour	Highly Intensive	Moderately Intensive	Least Intensive

The result shown above reveals housing parameters, general pig management and health parameters.

Considering the construction type, Treatment 1 has Asbestos roof, well cemented wall and floor. Treatment 2 has an Asbestos roof, bamboo fence, and cemented floor, while Treatment 3 has an Asbestos roof, bamboo fence and non-cemented floor. This shows that Treatment 3 is the cheapest housing system.

Consideringease of management, Treatment 1 is difficult to clean and takes time, because it involves sweeping and washing of the floor as well as cleaning of the walls. Treatment 2 is less difficult because it only involves sweeping and washing of the floor, without cleaning of the walls. Treatment 3 is the easiest to clean because it only involves sweeping of the floor.

Considering skin lesion, Pigs in treatment 1 were affected by mange (at the back), and Pigs in treatment 2 were mildly affected by mange, while Pigs in treatment 3 were not affected at all.

Considering ease of effluent disposal, effluent in Treatment 1 is the easiest to dispose because of the construction style which enhances proper disposal. It is mildly difficult to pack and dispose effluent in Treatment 2, while effluent in Treatment 3 is extremely difficult to dispose.

Considering general outward appearance, Pigs in Treatment 1 were the cleanest because they were placed on cemented floor. Pigs in Treatment 2 were partially clean, while Pigs in Treatment 3 were extremely dirty because they were placed on non cemented floor.

Considering floor condition, it was observed that the floor condition was often dry due to clean floor in Treatment 1. In Treatment 2, the floor condition is occasionally dry, while Treatment 3 is often wet because the animals often tip the watering trough; therefore, the floor is often wet.

Considering labour intensity, Treatment 1 was the highest because it is a well built pen. Therefore, it requires thorough cleaning and hygiene. Treatment 2 was moderately intensive because it was a partially built pen with bamboo

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walls which doesn't require cleaning. Treatment 3 has the lowest labour intensity because both walls and floor were not cemented.

Table 4.1 reveals the consumption levels of the treatments. Treatment 2 had the lowest feed intake compared to other treatments. Although the differences between treatments were statistically insignificant, this may be as a result of housing modification which was not conducive for pigs in Treatment 2, thereby reducing feed intake (Ugwu, 2006). Treatment 3 has the highest feed intake and this may be as a result of access to available nutrients in the soil which could have enhanced their appetite.

Table 4.2 reveals the weight gain for each treatment. Treatment 2 had the highest weight gain while treatment 3 had the lowest. The observation above maybe due to the difference in the initial weight of the experimental animals.

IV. CONCLUSION

It is hereby concluded that treatment 3 presented the best result as regards feed consumption, cost of construction and ease of management. Although, Treatment 3 presented the lowest weight gain which could be as a result of the initial weight, tipping of the feed trough (feed wastage). However, feed wastage can be minimized by using firmer feed trough.

RECOMMENDATION

It is therefore recommended that farmers can incorporate this experimented low cost housing system. Sanitation, hygiene and good general management practice must be efficiently implemented in order to make it a productive housing system.

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