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Editorial Board

FOREWORD

I am pleased to put into the hands of readers Volume-3; Issue-1:Jan-Feb 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
Date: March, 2018

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Isolation and identification of microbial and fungal flora from female hair samples in Riyadh Saudi Arabia

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***Abstract*—** *The human hair harbors several species of fungi and also bacteria. The present study was performed to determine the prevalence of keratinophilic fungi and bacteria from hair samples of femalesfrom November 2016 to April 2017. A total of 50 human hair samples were examinedusing hair-baiting techniques for isolation. After the incubation period, the number of colony forming unit was counted. The microorganisms were identified based on the colony morphology from culture and microscopic features. After purification, each representative colony was gram-stained and examined for cell morphology and gram reaction under a light microscope. Fungal isolates included were Aspergillus niger, Aspergillus flavus, Penicilliumspp, Alternaria alternata, Chrysosporium keratinophilum. Cladosporium cladosporioides and Trichosporon mucoides. Isolated bacterial species included gram positive bacteria such as Leuconostoc mesenteroidess spcremoris, Kocuriarosea, Staphylococcus haemolyticus, and the gram negative bacteria Kocurikristinae, Stenotrophomonas maltophilia, and Micrococcus luteu/ lylae. Human hair samples from females studied were found have several fungal and bacterial isolates, some of which can cause some serious disease in humans. Health authorities need to heighten up their health information campaigns that will include not only prevention and treatment of serious illnesses but also body hygiene.*

Key words*— *keratinophilic fungi, microbial and fungal flora,female hair.

I. INTRODUCTION

The human hair is one part of our body that is always exposed to environmental pollutants, and also to fungal and bacterial contamination. In Saudi Arabia, women wear the "hijab" to cover their hair. Fungal disorders are emerging significant infections in the world (WHO, 2005). In recent years, they have become an important clinical condition that deserves public health attention because of the fact that some of them are potentially harmful to human health (Anbu, 2004; Ganaie 2010; Deshmukh, 2010; Lee *et al*., 2011). Keratinophilic fungi are usually isolated from the soil and from keratinous tissues such as the skin, hair and nails. This includes the dermatophyte*Microsporumgypseum* (Shukia*et al*, 2003), and some species of *Aspergillus*, *Fusariumsolani*, and *Bipolarisspicifera*. (Shadzi, 2002; Gherbawy, 2006; Anbu, 2004;Ganaie, 2010, Ali, 2008; Zarrin, 2011; Chepchirchir, 2009; Kannan, 2006; Ali-Shtayeh, 2001)

Bacteria, on the other hand were known to reside in the hair follicles, in which 85% of the bacterial population if found in the superficial layers of the skin and hair follicles (Lange-Asschenfeldt et al., 2011) Bacteria such as *Micrococcaceae* represents the most common isolated specie. (Lange-Asschenfeldt et al., 2011) The human hair is also a reservoir of bacterial including *Staphycoccusintermedius* and coagulase-negative *Staphylococci* (Mase et al, 2000), and *Staphylococcus aureus* (Jappe, 2003).

There were very limited reports on keratinophilic fungi and bacterial colonization on the hair. This study aimed to determine the prevalence of keratinophilic fungi and bacteria in the hair of females in Riyadh, Saudi Arabia.

II. METHODS

Collection of human hair samples

Participants were recruited from various areas in Riyadh, Saudi Arabia fromNovember 2016 until April 2017. Participants were informed about the aim and objectives of the study and consent forms were obtained. The study protocol was reviewed and approved by the Princess NourahbintAbdulrahman University Research Ethics Committee. Hair samples were collected from consenting participants aged 14 to 50 years old.

Isolation of fungi from hair samples

Hair samples were placed separately in clean plastic bags and then transferred directly to the laboratory, and kept in a cool place (3-5°C) until fungal assay was performed. Two different techniques were used: hair baiting as recommended by Vanbreuseghem and described by Sharma in 2003. (Sharma, 2003) Fragments of hair samples (10 cm in length) were sprinkled on the surface of double sterilized soil. The soil was moistened with sterilized distilled water and remoistened whenever necessary and incubated at 28 ° C

for three months.The plates were examined every week. The moulds that appeared on the hair were transferred onto a Sabouraud`s Dextrose Agar which contained (g/l): glucose, 20; peptone, 10; agar, 20 and chloramphenicol 40. (Ellis et al., 2007)The other technique used was the direct plating of the hair onto Sabouraud`s Dextrose agar which contained chloramphenicol. (Gherbawy et al., 2006) Blood agar plate for bacteria Plates were incubated at 28°C for 2-10 days and the cultures were examined periodically for fungal and bacteria growth.

Bacterial isolation and identification

After the incubation period, the number of colony forming units was counted using the CFU/mL. The microorganisms were identified based on the different types of colonies.Colony morphologies were recorded and purified to obtain pure colonies for the identification purposes. Each representative colony was gram-stained and examined for cell morphology and gram reaction under a light microscope. Fungi samples were all identified on the basis of their morphological characteristics, whereas the bacterial isolates were identified by the use of Vitekanalyzer (bioMerieux, UK).

Preparation of plant extract

One gram of henna powder, Ziziphusspina-christipowder, roselle powder (*Hibiscus sabdariffa*) and Trigonellafoenum-graecum) were mixed in 10 ml. of distilled water. The content of the flask was then filtered through antibacterial filter to obtain clear infusion of 1 ml. fresh Garlic, Daber oil were used directly. The fungal inoculum was prepared by incubating samples in old culture grown on Potato dextrose agar medium for 5 to 10 days. The petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. A final concentration of approximately 1 ml of each fungus was then spread onto the surface of SDA plate.

Plant extracts which suppressed the fungal growth were tested for their efficiency against the fungi isolated from hair by tested the disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in millimeters.

III. RESULTS

Fifty females participated in the study. The mean age was 27.5 years old. A total of 27colonies of different keratinophilic fungi were isolated from 50 hair samples. The isolated keratinophilic fungi included *Aspergillus niger, Aspergillus flavus, Penicilliumspp, Alternaria alternate, Chrysosporiumkeratinophilum. Cladosporiumcladosporioides, Trichosporon-mucoides.*(Tables 1 and 2)

The isolated bacterial species included gram positive bacteria such as *Leuconostocmesenteroidessspcremoris, Kocuriarosea, Staphylococcus haemolyticus*, and gram negative bacteria including *Kocurikristinae* , *Micrococcus luteu/ lylae,*and *Stenotrophomonas maltophilia*. Dual infection with both gram positive and gram-negative bacteria was also seen. (Table 3)

Table 4 shows the bacterial count in different clinical subsets of females. It was observed that high bacterial count, was found in females who were having dandruff, who were (and were not) using antibiotics and those who were using corticosteroids. Henna users and those using antibiotics had lower bacterial counts. Table 5 represents the antifungal activity of plant extracts by disc diffusion. Henna extract and Dabur oil gave most promising results and were protective against fungal infection.

IV. DISCUSSION

The presence of keratinophilic fungi in different soil has been reported from all over the world. (Anbu, 2004; Ganaie, 2010; Deshmukh, 2010, Lee, 2011, Mahmoudabadi, 2008) Keratinophilic fungi are small, well defined and important group of fungi that colonize various keratinous substrate and degrade them to components of low molecular weight. These fungi are present in the environment with variable distribution patterns.Keratinolytic fungi are associated with human and animal mycoses 26-30 (FilipelloMarchisio, 1996; Shadzi, 2002; Zarrin, 2011; Chepchirchir, 2009; Nakagawa, 1999) Very few studies are reported regarding isolation of *keratinophilic* fungi from human hair samples. (Kannan, 2006; Ali-Shtayeh, 2001)

This study shows the most prevalent isolate both in terms of its percent occurrence and frequency of occurrence *Aspergillus niger*, which some of the isolates are found to be pathogenic to humans. It can cause fatal invasive aspergillosis and pulmonary disease in immunocompromised patients and they are associated with the production of oxalate crystals in clinical specimens. (Atchade et al., 2017; Oda et al., 2013) *Aspergillus flavus* was also isolated in this study. *A. flavus* was reported to have keratinase activity and a strong producer of extracellular keratinase. (Kim, 2007)

On the other hand, bacterial isolates that included *Leuconostoc mesenteroides ssp cremoris, Kocuriarosea, Staphylococcus haemolyticus, Kocurikristinae, Micrococcus luteu/ lylae,* and *Stenotrophomonas maltophilia*. *Leuconostoc mesenteroides* were known to cause nosocomial outbreaks and brain abscess. (De Boni*et.al.*,2011 ,Albanese et al., 2006)

*Kocuriarosea*has been found to cause a significantly wide spectrum of human infections including peritonitis. (Purty et al., 2013) *Staphylococcus haemolyticus* is an opportunistic bacteria that is highly resistant to antibiotics and can cause meningitis, skin and soft tissue infections, endocarditis and bacteremia. (Falcone et al., 2007)*Kocuriakristinae* on the other hand are found to cause urinary tract infection among catheterized children (Chen et al., 2015) *Stenotrophomonas maltophilia* cause respiratory infections (Dignani et al., 2003)

The present research gave us a recent insight about the existence of keratinophilic fungi in the hairs. In many clinical and epidemiological studies, fungal infections of the skin and scalp represent a relatively common problem especially in the tropical and subtropical regions of the world where warm and humid climate provides a favorable environment for fungi. They have become a significant health problem affecting children, adolescents and adults They (these diseases) are transmitted from person to person directly infected (infecting) skin scales or hairs(hair follicles). They can also be acquired by humans from infected animals and by direct exposure to infected soils.

The fungal and bacterial contaminations in the surrounding atmosphere affects the health of human beings and needs knowledge, awareness and maintenance of hygiene to avoid the development of disease. Keratinolytic activity of fungi is important ecologically. The impact of keratinophilic fungi on human health seems unexplored. Knowledge of the frequency and extension of etiological agents of humans and animal mycosis and other potentially pathogenic fungi on the healthy hairs is of prime importance for understanding of epidemiological cycle of these fungi, apart from ecology point of view. Therefore hygiene protocol should be taken to prevent the spread of pathogenic fungi in these environments as there is a risk of fungal infections of human.

V. CONCLUSION

A variety of keratinolytic fungi and pathogenic bacteria exists in the hair. The hair could serve as a vector for disease transmission of pathogenic microorganisms and fungal elements.There is a need for a hygiene protocol to prevent the spread of pathogenic fungi, and also invasion of the deeper structures of the head including the meninges and the brain parenchyma. These findings should be taken into consideration and necessary treatment methods should be taken up periodically.

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Table.1: Frequency of fungal isolates from 50 human hair samples on Sabouraud`s Dextrose Agar:

Sample no.	AGE	Fungal species (SDA)	Number	Percentage
9	20	*Aspergillus niger*	1	3.7
19, 28	29	*Aspergillus niger*	2	7.4
33	31	*Aspergillus flavus*	1	3.7
16	23	*Penicillium spp.*	1	3.7
20	30	*Cladosporium cladosporioides*	1	3.7
35	26	*Trichosporon mucoides*	1	3.7
39	19	*Alternaria alternata*	1	3.7

Table.2: Frequency of fungal isolates from human hair samples of 50 Females grown on sterile soil

Age	Fungal species	n	incubation period	%
29	*Penicilliumspp*	1	50	3.7
26	*Chrysosporium keratinophilum*	1	60	3.7
31	*Chrysosporium keratinophilum*	1	81	3.7
38	*Chrysosporium keratinophilum*	1	50	3.7

Table.3: Bacterial isolates from hair samples

Age	Bacterial Type	Gram stain
29	*Leuconostoc mesenteroides ssp cremoris*	(+ve)
28	*Kocuri kristinae*	(-ve)
16	*Stenotrophomonas maltophilia*	(+ve)
22	*Kocuriarosea*	(+ve)
38	*Micrococcus luteu/ lylae*	(-ve)
23	*Staphylococcus haemolyticus*	(+ve)

Table 4. Frequency of different baseline characteristics within the sample and corresponding mean microbial counts:

Variable	Henna Users	Non-henna Users	with dandruff	No dandruff	receiving antibiotic	Not receiving antibiotic	Using corticosteroids	Not using cortico steroids	Suffering from asthma	No asthma
Number subjects within sample (%)	26%	74%	42%	58%	24%	76%	6%	94%	6%	94%
Mean of total microbial count (units)	11.9	21.5	21.1	17.4	15	20.2	36	17.9	13.3	19.3

Table.5: Antifungal activity of plant extracts (1/10 ml) , and plantpowder by disc diffusion

Zone of inhibition (mm)	Henna powder	water extracts of henna	Cidir Ziziphus spina-christi powder	water extracts of Cidir (Ziziphus spina-christi)	Roselle (Hibiscus sabdariffa) powder	water extracts of Roselle (Hibiscus sabdariffa)	Garlic fresh	Fenugreek Seeds Powder	Daber oil
Aspergillus niger	(-)		(-)		(-)		(-)	(-)	(-)
Aspergillus flavus	2.5mm	(-)	(-)		(-)		(-)	(-)	(-)
Penicillium spp.	2mm	(-)	(-)		(-)		(-)	(-)	2.2mm
Alternaria alternata	(-)		(-)		(-)		(-)	(-)	(-)

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Texture evaluation of whey protein concentrate incorporated ice cream by Back Extrusion technique

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Abstract*—-Back extrusion technique was employed to evaluated texture properties of partial substituted whey protein concentrate (WPC) with milk solids not fat (1, 2, 3 and 4%) in ice cream formula.*

There was no remarkable effect of adding WPC on total solids or fat %. Total protein increased, while ash, and lactose content were significantly decreased.

Back-extrusion results represented a decrease in hardness values of resultant ice cream, while, during storage, there was a slight increase. Energy input values decreased by increasing substitution levels of WP. Although, energy output inversely correlated with substitution levels of WP it correlated with storage period indicating a strong structure for stored ice cream. Load at target deformation (50%) applied to the samples when fresh and after 14 days storage showing decreased values proportional to increasing substitution levels of WP. The resilience showed decreased ratio indicating more visco-elastic properties in fresh ice cream. The recovered height and deformation increased with increasing substitution levels of WP and storage period. Therefore, more sticking properties were obtained in resultant ice cream. Adhesive force decreased significantly with increasing substitution levels of WP and storage period. Adhesiveness values were significantly higher in all treatments than control.

The texture of the ice cream became smoother by replacing milk solid not fat with WPC up to 3%. From the data obtained, it could be recommended that ice cream can be produced with high quality by substituting milk solid not fat with WPC up to 3%.

***Keywords*— Ice cream, Texture properties, Texture profile analysis, Whey protein concentrate.**

I. INTRODUCTION

Ice cream is one of the most consumed dairy products in the world [1]. It is an example of complex materials consisting of ice crystals, air bubbles and fat globules contained within a viscous liquid matrix [2].

Nowadays, separation technologies provide the basis for adding value to milk to meet specific needs such as improvement in texture [3]. The high nutritional value, the excellent functional properties and the source of biologically active peptides [4] that WPC contains have increased its use in food industry [5,6,7].

It is challenging to perform rheological measurements due to the complex structure of ice cream. The application of rheology is important when characterizing the behaviour of such complex soft solids [8].

Back extrusion test (sometimes called *annular pumping*) [9], has great potential in food industry, because it is a simple, rapid and low cost method. It's usually used for soft foods such as pastes and liquids, which can be tested in their own packaging to displace viscous liquids or semi-sold products not suitable for traditional viscometers in a controlled manner in order to assess characteristics such as flow, thinning and thickening, consistency, viscosity, adhesiveness and spreadability. Within dairy products such as yogurt and cream, this test will identify spoon-ability and flow properties of finished products. Back extrusion techniques are most commonly applied as they permit measurement of the sample in the container into which it was deposited or formed in. This removes any risk of disruption when decanting a sample and allows a more objective measure of semi-solids consistency.

However, several authors were used back extrusion test to evaluate the mechanical characterization for many products (i.e., Yoghurt, [10;11;12;13 and 14], Stirred yoghurt; [15;16;17;18;19 and 20], *Misti Dahi*, [21], Whipped creams; [22], Extruded whey protein concentrate (WPC) and extruded whey protein isolate (WPI); [23], Ricotta cheese; [24] Cottage Cheese; [25], Cooked and canned legumes; [26], Sauces; [27], Custard; [28], Mashed potatoes; [29], Molten chocolate; [30], Food hydrocolloids; [31], Starches such as corn, wheat, and cassava; [32].

This study focuses on providing novel bioactive ingredients as WPC, with the objective of replacing the conventional ingredients, to ice cream. Moreover, setting the best ratio of WPC incorporated in ice cream formulas depending on chemical and textural properties measured by back extrusion technique**.**

II. MATERIALS AND METHODS

2.1 Materials

Fresh buffalo's skim (90.9 % moisture, 0.1% fat, 3.4% protein, 4.9% lactose and 0.7% ash) and fresh concentrated cream (29.4% moisture, 67% fat, 1.3% protein, 1.7% lactose and 0.6% ash) were obtained from the herd of Faculty of Agriculture, Cairo University and used for preparing the ice cream mixes. Low heat skim milk powder (SMP) (3.8% moisture, 0.8% fat, 33.4% protein, 54.1% lactose and 7.9% ash) was obtained from Abou El-Hool-Import/Export Co., Cairo, Egypt. Whey protein concentrate powder (4.7% moisture, 5.9% fat, 77.7% protein, 9.1% lactose and 2.6% ash) was supplied by Davisco Foods International, Inc, USA. Commercial grade sugar cane was obtained from the local market, Sodium carboxymethyl cellulose (CMC) as a stabilizer was obtained from Mifad Company, Giza, Egypt. Vanilla was obtained from the local market and used to flavour final ice cream.

2.1.1 Manufacture of Ice cream:

Ice cream mix contained 8% fat, 12% milk solid not fat, 15% sucrose, 0.25% stabilizer. Skimmed milk powder was substituted with WPC at 1.0, 2.0, 3.0 and 4.0% of dried milk solids not fat in the base mix (Table 1).

Table.1: Formulation of different ice cream mixes with WPC as a substitute of MSNF (g/ kg mix).

Ingredients	Control	Level of substitution (g/kg mix)			
		T1	T2	T3	T4
Sugar	150	150	150	150	150
Stabilizer	2.5	2.5	2.5	2.5	2.5
Fresh skim milk	670.38	670.38	670.38	670.38	670.38
Cream	117.69	117.69	117.69	117.69	117.69
Dried skim milk	59.43	48.94	38.16	27.08	17.10
WPC 80	0.00	11.18	22.17	33.05	43.04
Total	1000	1000.69	1000. 9	1000.7	1000.71

T1, T2, T3, T4: Corresponding to 1, 2, 3 and 4% WPC substitution of MSNF.

2.2 Methods

Total solids, total protein content and ash were determined according to [33]. Lactose content was determined according to [34]. Fat content was determined according to [35].

2.2.1 Back Extrusion

The back-extrusion test was carried out using the (TMS-Pro testing machine) equipped with (250 lbf) load cell and connected to a computer programmed with Pro^{TM} texture analysis software. Forty grams of ice cream sample were scooped in a cylindrical glass cup (height 70.1 mm; diameter 43.6 mm) and solidified for 24 hrs. at -18ºC.

Sample surface was flattened to avoid early triggering of the test. A flat probe rod (37.75 mm diameter) was programmed to descend into the sample at a speed of 20 mm/sec to a depth of 50% of original sample high (40 mm) and then ascend back to its original position with a back-off distance of 20 mm. Distance of extrusion was set at 20 mm with a trigger force of 1N. The force encountered by the probe to break contact with ice cream at the start of the ascending (point of maximum force) was measured.

III. STATISTICAL ANAKYSIS

Data were analysed statistically using the MSTAT-C (ver 2.10, MSU, USA.) package on a personal computer. All experiments were carried out in triplicates. Differences were considered significant at $P< 0.05$**.**

IV. RESULTS AND DISCUSSION

4.1. Chemical properties of ice cream mixes:

Fat was adjusted in all mixes to almost 8% for recipe formula during the procedures. A proportional replacement of MSNF with WPC resulted in a significant increase ($P<0.001$) in protein contents of ice cream mixes (Table 2). The increase mainly due to the higher protein content of WPC (77.7%) compared to SMP (33.4%). The total protein content increased in an ascending order with increasing the ratio of WPC substitution being the highest at 4% WPC with a high correlation (0.999). These data agreed with the findings of [36] and [37]. The usage of WPC as a MSNF replacer leads to a significant reduction ($P<0.001$) in ash content (Table 2). This decrease could be due to the differences in ash contents of WPC (2.6%) and SMP (7.9%). Coefficient correlation (-0.985) showed a tight inverse relationship between WPC and ash%. The

obtained results are in a harmony with the findings of [36] and [6].

Table.2: Chemical composition (%) of ice cream mixes with different ratios of WPC.

Treatments	Total solids	Fat	Total protein	Ash	Lactose content
Control	36.96	8.23	4.37^{e}	1.073^{a}	7.98^{a}
T_1	36.37	8.23	4.94^{d}	0.943^{b}	7.24^{b}
T_2	36.24	8.20	5.65^{c}	0.883^{c}	6.51^{c}
T_3	36.16	8.23	6.29^{b}	0.821^{d}	5.81^{d}
T_4	36.29	8.20	6.92^{a}	0.750^{e}	5.42^{e}

(a,b,…) means with unlike superscript letters were significantly different (α=0.05)

Lactose values decreased with increasing the substitution level of WPC (P<0.001) in the mixes. The decrease is due to a lower content of lactose in WPC (9.1%) than in SMP (54%). However, WPC with low lactose content can be safely used at higher levels without concerning of sandiness development defect in ice cream [38].

4.2. Texture properties of ice cream mixes

4.2.1. Hardness

Hardness, defined as the peak force obtained during penetration of the probe through samples during back extrusion test. It gives an indication of the energy required to masticate the ice cream to a state ready for swallowing. However, replacing MSNF by WPC at different ratios decreased (P<0.001) the hardness values of resultant ice cream Table (3) as a negative correlation (-0.891) was obtained. During storage at -18±1˚C for 14 days, there was a significant (P<0.001) increase of ice cream hardness values for control and all treatments (Table 3).

4.2.2. Energy input and output

Energy input is defined as the total amount of energy recorded during down cycle of texture analyzer as an area (the area under the penetration cycle down stroke), or energy input to deform sample during compression. Energy input is an indicative of sample consistency and for example how structure resists mechanical condition imposed. Obtained results revealed that the energy input values decreased (P<0.001) by increasing substitution levels with WPC (-0.739). However data showed that the energy input values were significantly (P<0.001) lower in all treatments with WPC "Fig.1 A" than control (LSD=300.2 at 0.05 α level).

Table 3. Back extrusion measured parameters for the resultant ice cream when fresh and after 14 days of storage at -18±1ºC.

Parameters	Storage	Control	T1	T2	T3	T4
Hardness (N)	Fresh	67.00^{b}	64.5^{d}	54.00^{e}	38.10^{h}	38.00^{i}
	2 weeks	67.13^{a}	67.1^{c}	65.3^{d}	51.3^{f}	44.6^{g}
Position of hardness (mm)	Fresh	16.47^{c}	18.58^{b}	19.79^{a}	19.86^{a}	19.91^{a}
	2 weeks	16.64^{c}	18.93^{c}	19.80^{c}	19.91^{ab}	20.15^{b}
Recovered height (mm)	Fresh	1.56^{g}	1.707^{fg}	1.843^{fg}	3.673^{d}	5.793^{c}
	2 weeks	2.20^{ef}	2.547^{e}	4.173^{d}	7.757^{b}	8.52^{a}
Modulus slop (N/mm)	Fresh	4.26^{a}	3.79^{b}	2.69^{c}	1.93^{d}	1.89^{d}
	2 weeks	4.40^{a}	4.38^{a}	4.06^{ab}	2.64^{c}	2.32^{cd}
Adhesiveness force (N)	Fresh	-14.63^{a}	-14.23^{a}	-11.56^{b}	-11.00^{bc}	-10.16^{bc}
	2 weeks	-13.90^{a}	-13.66^{a}	-11.13^{bc}	-10.11^{bc}	-9.53^{c}
Adhesiveness	Fresh	479.60^{h}	520.36^{g}	737.22^{c}	767.89^{b}	856.33^{a}
	2 weeks	445.27^{i}	483.17^{h}	565.13^{f}	625.99^{e}	668.9^{d}
Mm to break adhesiveness contact	Fresh	13.78^{ef}	17.51^{b}	18.34^{b}	20.89^{a}	21.22^{a}
	2 weeks	11.56^{g}	13.26^{f}	14.62^{de}	15.05^{cd}	16.06^{c}

During storage of ice cream samples, there was a significant increase of energy input values over those of fresh ice cream (P<0.001). Moreover, the interaction of WPC% and storage period affected the input energy (P<0.001) significantly.

Energy output is defined as the total amount of energy recorded during up cycle of texture analyzer as an area, energy returned from sample during decompression. It expresses the strength of the internal bonds of the sample. The more the energy output, the stronger the internal structure of the material. The energy output negatively correlated with WPC% (-0.838) and positively with storage period which indicated a stronger structure of stored than fresh ice cream. While, WPC% significantly

affected energy output (P<0.001), storage period or the two factors interaction didn't affect energy output "Fig.1 B" .

4.2.3. The load at target

The load at target deformation (50%) applied to the samples showed decreased values (P<0.001) proportional to increasing WPC% in ice cream samples for fresh and 14 days storage "Fig. 1 C" that indicated a slight harder ice cream texture. Neither WPC% nor storage period affected time at target deformation (P>0.05).

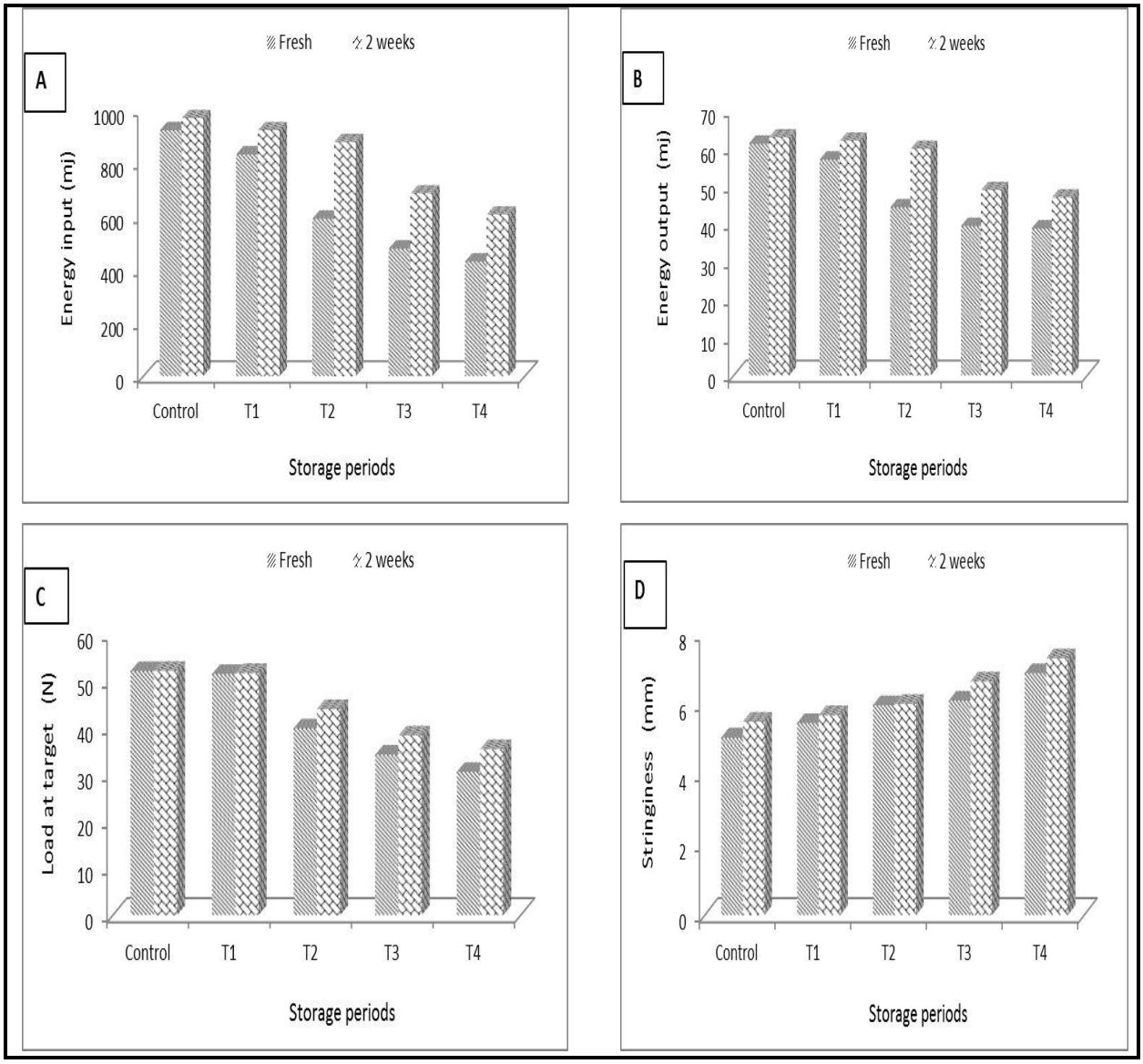

Fig.1: Back extrusion measured parameters; Energy input (A), Energy output (B), Load at target (C) and stringiness (D) values of fresh and stored ice cream with different replacing ratio of WPC

4.2.4. Resilience

Resilience is a subjective measure of how the sample recovers. Ratio of energy input to energy returned. It provides an indication of unrecoverable work from the total deformation. The resilience showed decreased ratio (P<0.001) with a high R^2 (0.941) indicating more visco-elastic properties in fresh ice cream. Stored ice cream showed high data variations with T2 which was more elastic than the rest of the treatments.

4.2.5. Recovered height (mm)

Distance sample remained in contact with test probe during upward cycle before recording negative values of adhesion. Highly viscous sample exhibits no recovery and adhesive properties. Highly elastic sample shows almost instant recovery. Therefore, ice cream samples exhibited visco-elastic property as a recovered height is obtained plus an adhesiveness value. As shown in Table (3) the elasticity of the samples decreased at the expense of viscosity as the recovered height increased (P<0.001) with increasing WPC% and storage period. The

displacement of 4% WPC increased almost 3.7 times that of the control for fresh and 3.8 times for 14 days stored ice cream.

4.2.6. Stringiness

Stringiness length is defined as deformation at given negative force. Stringiness is used to determine maximum 'Stickiness' of sample. The distance a sample is extended during decompression before probe-sample interface is broken. The deformation increased with increasing WPC% and storage period as indicated in (Fig. 1 D). Therefore, more sticking properties were obtained as a result of incorporating WPC or extending the storage period.

4.2.7. Best Fit (mm.N)

Rate at which distance increases per force during test *e.g.* higher distance values indicating softer samples. The best fitting line is drawn between loads at Mod-Point 1 and Mod-Point 2. Mod Point 1 is 5% of target distance and Mod Point 2 is 10% of Target distance. Increasing the distance the plunger travels between Mod 1 and Mod 2 indicated weaker structures of the fresh ice cream treated with WPC or significant lower hardness ($P<0.001$). Number of mm traveled by the plunger in the ice cream fresh and stored samples per Newton increased which indicated softer texture.

4.2.9. Modulus slop (N/mm)

It is the rate at which force increases with deformation or sample resistance increases with increasing stress. Sensorial: rigidity or stiffness of sample in relation to imposed deformation or stress ($M\ L^{-1}\ T^{-2}$ = Mass/Displacement x Time). The data indicated a decrease in rigidity of ice cream samples as a result of increasing WPC% when fresh ($P<0.001$) or after 14 days of storage (Table 3). Additionally, the interaction of the two factors affected the modulus slop ($P<0.05$) significantly.

4.2.8. Adhesive force

Force required for breaking the contact with test probe. Sensorial, it is the stickiness of the sample when compressed between teeth or held in hand. Adhesive force decreased significantly (Tables 3) with increasing WPC% and storage period ($P<0.001$). A decrease of the adhesiveness force with the increase of the WPC% was obtained (-0.875).

4.2.11. Adhesiveness

Adhesiveness is recognized as Work (J) required to break contact between the sample and probe. Total amount of energy input to break attractive forces between the sample and surface onto which it makes contact *e.g.* tongue, teeth, palette, fingers. Adhesiveness of resultant ice cream as affected by replacing MSNF by WPC at different ratios, fresh and after 14 days of storage at -18±1ºC was shown in Table (3). The adhesiveness values were significantly ($P<0.001$) higher in all treatments with WPC than control. During storage of ice cream, the adhesiveness values increased ($P<0.001$) gradually which indicated more stickiness of the ice cream with WPC and that stored for 14 days ($R^2=0.951$).

4.2.12. Start of adhesiveness (mm)

A shifting in the adhesiveness start position for T4 was 7.67 cm compared to the control for WPC fresh ice cream. WPC% caused that shifting in the start of adhesiveness position ($P<0.001$). Additionally, storage period and the interaction between the two factors increased the shifting ($P<0.001$) significantly. The shift for the stored ice cream was 9.82cm. The start of the adhesiveness (mm) increased with increasing WPC% as confirmed with the positive correlation (0.971).

Table 3. Back extrusion measured parameters for the resultant ice cream when fresh and after 14 days of storage at -18±1ºC.

Parameters	Storage	Control	T1	T2	T3	T4
Hardness (N)	Fresh	67.00^{b}	64.5^{d}	54.00^{e}	38.10^{h}	38.00^{i}
	2 weeks	67.13^{a}	67.1^{c}	65.3^{d}	51.3^{f}	44.6^{g}
Position of hardness (mm)	Fresh	16.47^{c}	18.58^{b}	19.79^{a}	19.86^{a}	19.91^{a}
	2 weeks	16.64^{c}	18.93^{c}	19.80^{c}	19.91^{ab}	20.15^{b}
Recovered height (mm)	Fresh	1.56^{g}	1.707^{fg}	1.843^{fg}	3.673^{d}	5.793^{c}
	2 weeks	2.20^{ef}	2.547^{e}	4.173^{d}	7.757^{b}	8.52^{a}
Modulus slop (N/mm)	Fresh	4.26^{a}	3.79^{b}	2.69^{c}	1.93^{d}	1.89^{d}
	2 weeks	4.40^{a}	4.38^{a}	4.06^{ab}	2.64^{c}	2.32^{cd}
Adhesiveness force (N)	Fresh	-14.63^{a}	-14.23^{a}	-11.56^{b}	-11.00^{bc}	-10.16^{bc}
	2 weeks	-13.90^{a}	-13.66^{a}	-11.13^{bc}	-10.11^{bc}	-9.53^{c}
Adhesiveness	Fresh	479.60^{h}	520.36^{g}	737.22^{c}	767.89^{b}	856.33^{a}
	2 weeks	445.27^{i}	483.17^{h}	565.13^{f}	625.99^{e}	668.9^{d}
Mm to break adhesiveness contact	Fresh	13.78^{ef}	17.51^{b}	18.34^{b}	20.89^{a}	21.22^{a}
	2 weeks	11.56^{g}	13.26^{f}	14.62^{de}	15.05^{cd}	16.06^{c}

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4.2.13. Displacement at adhesive force (mm)

Position of adhesiveness force is the biggest negative value from start position of adhesiveness until end of the test. Adhesive force is used to detect the end adhesive contact point prevents confusion in detecting end contact. If we do not set our reference points after the start adhesiveness contact points we will run the risk of detecting very early zero N points due to high data collection rates and changes in sample as probe retracts. In other words position of adhesiveness force is the maximum resistance to break contact with the sample *e.g.* stickiness to pull jaws apart or break contact with a surface. More stickiness of the ice cream samples was expressed by the displacement or the position of the adhesiveness force significantly (P<0.001) brought about by increasing proportionally the WPC% (correlation=0.983), storage period and the interaction between the two factors (Table 5).

4.2.14. Mm to break Adhesive Contact

It is the distance sample remained in adhesive (negative value) contact with test probe during upward cycle before total contact is broken. Also, the distance sample stretches before breaking contact with test probe or test bed. The stretching of the ice cream sample increased with increasing WPC% (P<0.001) and decreased comparing to fresh ice cream with increasing the storage period (Table 3), that might be caused by increasing the stickiness of the ice cream brought about by WPC incorporation in the ice cream mix.

4.2.15. End of adhesiveness (mm)

The end of adhesiveness displacement reaches when the force is equal zero N. This may not be 0.00N if the sample sticks to the probe. In this situation the negative value should be increased to accommodate the weight of sample on the probe. The displacement shifted to the right on the back extrusion chart with increasing values significantly with increasing WPC%, the storage period (P<0.001) and insignificantly (P>0.05) with the interaction of the two factors. WPC% highly correlated with zero N (0.984).

Time at adhesive (sec.) helped in locating the end of adhesive contact *e.g.* break point between probe and sample. Time at adhesive decreased with increasing WPC% and storage period. The stretching of the ice cream sample increased with increasing WPC% (P<0.001) and decreased, comparing to fresh ice cream, with increasing the storage period, that might be caused by increasing the stickiness of the ice cream brought about by WPC incorporation in the ice cream mix.

4.3. Sensory evaluation

Panel evaluation is an important indicator of potential consumer preferences. Panelists scored the T4, the least flavor. T3 was the most acceptable flavor among the ice cream (LSD= 0.4859 at 0.05 α level). Totally adding WPC enhanced the flavor significantly (p<0.001).

V. CONCLUSION

In this work an application of basic back extrusion approaches to an instrumental texture data set was described in the case of ice creams mixtures. As it can be argued from the results, the whole mechanical profiles proved to be able to describe the texture changes of the product and appeared as a helpful screening method to assess the texture of WPC ice creams. The result interpretation and analysis appeared as an operative tool for quantifying the textural attributes. The treatment of the whole mechanical profiles by means of basic back extrusion tools appeared as a promising approach. Obviously, the relevance of fundamental rheological approach could not be substituted: superior analytical performance in routine instrumental texture evaluation is possible through a combination of expertise in current food mechanical properties evaluation and consolidated data. The combination of rheological and sensory techniques can assure the sake of rapidity and simplicity required for the routine quality control operation in the food industry. The present work depicts a study on ice cream fortified with WPC and underlines a possible future direction for the strengthening of routine food objective texture analysis.

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The Strategy to improve the Quality of Cashew Commodities

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Abstract**—** *One of the production centers of cashew nut is East Nusa Tenggara (NTT) province, during the period of 2011-2015 cashew nut production in this area continues to increase every year. Alor District is one of the cashew-producing regions in the province with total production reaching 2,000 tons. However, the resulting quality of production is considered poor. This research aims to formulate strategies to improve the quality of cashew nuts in alor district. This research was conducted in Pantar Tengah subdistric and West Pantar Subdistrict which can represent and describe the distribution of cashew nut production in Alor District in May to March 2017. Data analysis method used is SWOT to know the internal and external conditions which will then be used as a basis for designing work strategies and programs. The results showed that the main priority strategy for the development of cashew nut quality in Alor district is by forming a group of joint enterprises in the form of cooperatives owned by farmers.*

Keywords**—** ***Strategy, Quality, Cashew.***

I. INTRODUCTION

Cashew Commodities is one of the important plantation commodities to be developed because it is one of the country's foreign exchange earners. The value of cashew cashew nuts reached 78,825,562 $ US in 2011 and then increased 17.34% in the following year to reach 95,362,347 $ US. The decline occurred 2 years in a row in 2013 and 2014 by 5.03% and 72.29% but again increased 71, 42% to 184,395,079 $ US. This increase will continue to grow in line with the policy issued by the government that encourages the export of processed products. Industrial developments should be supported by an increase in the amount of production from both smallholders, state plantations and private plantations (Plantation Statistics of Indonesia, 2016).

The area of cashew plantation in Indonesia continues to show decline year by year. data on the total area in 2011 covering 575.841ha and in 2015 to 522,863 ha. this is due to the number of people who convert the land to non-agricultural land. While the production from year to year is always increasing in 2011 as many as 114,789 tons increased in 2015 as much as 137,580 tons. increased production is influenced by the way of cultivation, maintenance and post-harvest cashew cashew in Indonesia which is good enough.

One of the central areas of cashew producers in Indonesia is the province of East Nusa Tenggara (NTT), during the period of 2011-2015 cashew nut production in this area continues to increase every year. The highest increase in production occurred in 2014, which increased by 10.69% from 39,360 to 44,072 Ton. Almost all districts and cities in this province have cashew nut production. in 2015 the highest production of cashew nut in East Flores district with total production reached 10,737 Ton, in the next sequence is Sikka district with total production 8,696 tons, next is Southwest Sumba District, east sumba and ende with total production respectively 5,035, 3,397 and 3,245 tons. In the sixth place is Alor district with total production reach 2000 tons (East Nusa Tenggara in Numbers, 2016).

Cashew production in Alor district fluctuates, in 2011 the total production reached 1,626 tons, then increased 20.37% in 2012 to 2,042 tons which was recorded as the highest increase during the period 2011-2015. In 2013 it decreased by 5.99% and then again increased in 2014 and 2015 by 0.51 and 2.98%. Pantar Tengah Districts is the largest production area since 2011 until 2015 cashew nut production in this area has reached 400 tons more, the highest production occurred in 2012 which is 486 tons. Almost all sub-districts in Alor district produce cashew nuts, except Pura Island sub-district, this is due to geographical conditions that are not suitable for cashew cultivation. In cashew agribusiness, post-harvest stage plays a very important role in producing quality products (Alor in Numbers, 2016).

The development of cultivation and management of agricultural products for the welfare of the community is very necessary given that most of Alor's community depends on agriculture. In terms of the welfare of farmers need the intervention of local government in managing agricultural products and good supervision for the welfare of farmers can be felt. The sale of cashew plantation commodities has not guaranteed the welfare of farmers because the quality of cashew nuts produced is not so good to be marketed and the price is very low at the level

of farmers. therefore it is necessary attention of local government and other related parties through the implementation of development programs, especially in the case of the development of cultivation and quality improvement of cashew komuditi products so as to improve the welfare of farmers and value-added cashew nuts products (Juran, 1995).

II. METHOD

1. Location and Research Design

This research was conducted in Pantar Tengah subdistric and West Pantar Subdistrict which can represent and describe the distribution of cashew nut production in Alor District in May to March 2017. To know the quality of cashew nuts in Alor District, the respondents consist of 10 respondents of collecting merchants, 5 respondents from large traders/inter island traders. Selection of respondents is done by considering that the respondent knows the condition and development and sales of cashew nuts in Alor District. Thus the total number of respondents is 105 respondents. This research aims to formulate strategies to improve the quality of cashew nuts in alor district.

The research method used is quantitative qualitative. while the type of data used is Primary data and secondary data. primary data consists of data obtained directly from the respondents ie farmers and traders who became the object in this study. Secondary data is data obtained from literature study and other data sources related to the research material

III. METHOD OF COLLECTING DATA

Data collection methods were conducted in this study include (1) Interview, that is by asking directly to the respondent. respondents in question are the farmers, traders and other parties associated with the development of cashew cashew in Alor District, (2) Recording, that is study by recording the necessary data both from respondents and related institutions that have supporting data in this research, (3) Observation, that is by doing direct observation to see the state of object in field and factors influencing performance of development and trade of cashew nuts in Alor District.

IV. DATA ANALYSIS

Data analysis was done by using SWOT analysis and Analytical Hierarki Process (AHP). This analysis used to know is an internal and external conditions of an organization which will then be used as a basis for designing work strategies and programs. Internal analysis includes assesment of strength and weakness. Meanwhile, external analysis includes opportunity and challenge. There are two approaches in SWOT analysis that is qualitative and quantitative approach. Then the results of strategy analysis by using SWOT analysis is continued by using AHP method to know the main priority of the resulting strategy.

Data type in this research is primary and secondary data. Primary data is data obtained directly from respondents ie farmers and traders while secondary data is data obtained from literature studies and other data sources related to the research material.

V. RESULT

1. Characteristics of Farmers Respondents

Characteristics of respondents in this study include age, number of labor, education level, land area, production and income of cashew farmers. The average of respondents is 40-50 years or 47.78% with variation ranging of 20-60 years old. the average amount of labor used by respondents is 1-3 people with 72.22% presentation, with range between 1-5 people. The education level of cashew farmers in Alor Regency is still low. The result showed that the average level of education of farmers is 54% of elementary school. this is due to lack of education infrastructure in the area. The area of cashew farmers in Alor District is 48.89% with an area of 1-2 Ha.Tthe average number of cashew nuts production of respondents in alor district is 50% of them get the production of 500 kg or 5 tons per responden. The selling price of cashew nuts ranged from Rp 11.000 - Rp 15.000 per kilogram. 92.22% of respondents sell their production at a price of 14.000-15.000 per kilogram. The result of cashew nut sales per responden reach five to ten million rupiah with percentage 47.78%.

2. Quality Standards of Cashew Commodities in Alor District

In Indonesia, the quality standard of cashew nut is arranged in SNI 01-4463-1998 which divides cashew nut quality into 3 grade. whereas In Alor District cashew nut quality is only divided into 2 grade that is good and less good that can be interpreted by researcher into quality 2 and quality 3. Good cashew nut is not wrinkle, shiny skin and feels heavy. While the characteristics of cashew nutrient quality is less good is wrinkled skin, not shiny and deflated when pressed and feels lightly held. Cashew farmers in Alor District see cashew nut quality in a simple way called "lelesan". This method is considered as the best method because it can provide the best quality seeds.

3. The Strategy to Improve The Quality Of Cashew Commodities

a. Comparative Advantage Strategy

The strategy of comparative advantage is a meeting of two positive elements of strength and opportunity, in this condition the organization or company, or in this case the Alor district cashew agro industry can use the power to make the most of the opportunities available. One

strategy that can be done is to form a group of joint ventures. By establishing a joint venture group, the strengths they have can be used to allow opportunities to get help from various stakeholders such as government support and technological developments can be better absorbed.

b. Mobilization Strategy

The essence of this strategy is how to direct the strengths (resources) that the organization has to mitigate the threats that can cause losses. One strategy that can be done is to carry out entrepreneurship training. The strategy is allegedly able to optimize resources owned by the organization to further improve efficiency, creativity and innovation.

c. Strategy Divestment / Investment

This strategy is a meeting between the negative internal elements of weakness with the external positive element of opportunity. This strategy focuses on choosing between releasing good opportunities because the organization can not make good use (Divestment) or take advantage of the opportunities available to fix the weaknesses owned (Investment). One form of strategy is to form a Partnership business.

d. Damage Control Strategy

Meetings between threat elements and elements The weaknesses of an organization can be of great disadvantage if not well controlled. Organizational weaknesses such as the quality of unskilled human resources should be improved immediately. The ability to cultivate and post-harvest activities of good cashew nuts can protect the organization from any threat. Determination of strategy done qualitatively by the researcher by carefully analyzing the results of interviews, observations, references of previous research and direction of supervisor. Each Strategy is considered to be so important that it is necessary to determine which strategy will be a priority (Saragih, 2003).

The priority strategy is the earliest strategy to be implemented, the results showed that the strategy of ranks first is to form a Joint Business Group (0,545), then establish a partnership business (0.228), carry out entrepreneurship training (0.12) and improve the Quality of Resources Man (0.107). Experts consider that the most appropriate strategy is the comparative advantage strategy of forming a group of joint ventures.

VI. DISCUSSION

Most respondent farmers are of productive age where the average age of respondents is the ideal age for work and has the ability to increase work productivity, and has a great ability to absorb information but not innovative technology in agriculture. The level of education of farmers is still low which is just completing their education at the primary school. Education is one of the factors that determine the productivity of labor, in this case farmers. Farmers who have higher levels of education have a better ability to understand and apply Integrated Crop Management Technology of Cashew Commodities so that productivity becomes higher. (Luluk et al., 2008).

The quality of cashew nuts based on the Indonesian National Standard needs to be considered in trading the results of plantation commodities, especially the farmers of Alor District because it greatly affects the price of cashew nuts. the number of farmers who do not know the good quality at harvest make lower price on the farmer (Assauri, 1999) level. Several factors affect the level of knowledge are the level of education, the necessities of life, and the difficulty of accessing information. To get a high price at the farm level requires a new strategy to improve the quality of cashew nuts because the quality of cashew nuts can affect the price. by studying the National Standards of cashew nuts and applying them, then the resulting production will be of quality and the farmers will get a high price (Susanto, 2014).value chain is important to get more value than cashew seeds. the thing to do is to conduct postharvest management training that can produce processed products. the cashew nut business opportunity is still wide open due to the abundance of raw materials available in almost all parts of Indonesia (Permadi, 2006).

One of the things that can encourage the development of cashew business is to create a small cashew business group as a group forum to share knowledge and others so that problems encountered in the field can be completed together. Some of the advantages gained in the group, among them is the ease in finding capital and marketing, get coaching and agricultural extension. Through the group is expected to facilitate access to marketing and obtain credit from banks. An enterprise will be more optimal if run jointly, not only can minimize the capital used, the results of joint ventures are usually more profitable than the results of individual efforts. In addition, the knowledge of the work done will be more organized. Joint Business Group (KUB) is a container that collects and manages business together. the business groups get help from the government in the form of capital with the aim to improve the welfare of farmers (Haming, 2007).

VII. CONCLUSION AND RECOMMENDATION

The target of cashew nut development in Alor District is to produce a quality product. The main priority strategy generated in this research is to form a joint business group in the form of koorporasi. This strategy is the best way for farmers to develop the quality of cashew nuts because it is a place to share experiences and get direct counseling training by the local government. There is a need for a

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feasibility study on the establishment of a Joint Business Group and a study on the value chain of cashew nut industry in Alor District to advance the cashew nut industry in the area.

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Molecular Characterization and Study of Genetic Relationships among local Cultivars of the Moroccan fig (Ficus carica L.) using Microsatellite and ISSR Markers

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Abstract*— *Molecular characterization of Moroccan local fig (Ficus carica L.) germplasm was performed on the cultivars present in a collection of the National School of Agriculture of Meknes. A total of 22 fig samples were analysed using 7 ISSR primers and 9 loci S.S.R. A total of 54 I.S.S.R. polymorphic bands with an average of 8 per primers and 42 S.S.R. alleles with means 5 alleles per locus were revealed by these analyses. The ISSR markers allowed distinguishing 22 molecular profiles and S.S.R. loci differentiated between 21 different profiles. Pairwise Comparing, 87% of cultivars pairs were differentiated by 7 to 24 alleles and 89% by 9 to 29 ISSR bands. The statistical analysis and genetic distances have shown a wide molecular diversity in the collection, where the average observed heterozygosity was 0.42. The average similarity between cultivars is 70% using SSR markers and 71.6 for ISSR markers.

The same SSR profile was obtained for Nabout1 and Nabout2 with 0 allele difference. Small differences of 1 to 6 alleles were obtained among cultivars which have the same names, which presumably corresponds to somaclonal variations obtained through intense vegetative propagation over long periods, while the differences over 7 alleles suggests the problems of homonyms.

Keywords*— *Ficus carica L., genetic diversity, ISSR markers, molecular characterization, SSR markers, varietal identification.

I. INTRODUCTION

The common fig (*Ficus carica* L. Moraceae) is one of the oldest fruits grown in the Mediterranean [1]. In Morocco, fig cultivars are very diverse and offer a wide range of cultivars particularly in the north [2]. However, because of numerous cases of synonymy (several names for the same cultivar) and homonymy (several cultivars under the same name), pomological characterization of the fig is insufficient to select authentic cultivars needed for genetic improvement programs. To avoid confusion varietal problems, the use of molecular markers is essential to correctly identify the fig cultivars and establish genetic identity for each cultivar.

Currently there are several molecular marker techniques, isozymes were the first genetic markers used in genetic characterizations and have been applied in several fruits species including figs [3, 4, 5, 6]. However, their utility was limited because small number of isozyme systems available, the low polymorphism level obtained and the influence of environmental factors.

The emergence of several techniques based on the Polymerase Chain Reaction (PCR) has developed several types of molecular markers. Random amplified polymorphic DNA (RAPD), the amplified fragment-length polymorphism (AFLP) and simple sequence repeat (SSR), has given the opportunity for genetic resources characterization. Their advantages are made they are highly polymorphic and are not easily influenced by environmental factors [7, 8, 9, 10]. RAPD. markers have previously been used for the characterization of fig cultivars [11, 4, 12], but because of the use of short arbitrary sequences and hybridization relatively low temperatures, these markers cannot be exchanged between laboratories according to standard protocols [13]. To avoid this limitation, a comparison of previous data obtained by RAPD, ISSR and SSR markers on the analysis of 30 fig cultivars showed that SSR and ISSR

markers are complementary tools for reliable characterization of this species [14].

In this study, ISSR and SSR markers were used to characterize the local fig accessions, preserved in the collection of Meknes National School of Agriculture in Morocco, and furnish a molecular database for the breeding fig projects.

II. MATERIALS AND METHODS

2.1. Plant Material

The fig collection located at the experimental station of the National School of Agriculture (ENA, Meknes, Morocco) was the subject of the study. The collection encloses 22 local cultivars collected in northern and central Morocco (Table 1). In some cases, several cultivars were classified under the same name (see, for example, "Ghoudane"). Molecular characterization was performed on a tree by cultivar.

Table.1: Name, code, use and origin of the studied accessions

Cultivars	Code	use	Origin	Cultivars	Code	Use	Origin
Ounq Hmam	OQH	Fresh	My Driss Zerhoun	Ghoudane2	GHD2	Fresh	Taounate
Hzzat	HZZ	Fresh	My Driss Zerhoun	Hamriya	HAM	Fresh	Taounate
Chaâri	CHA	Fresh	My Driss Zerhoun	Sebti	SEB	Fresh	Taounate
Ournakssi	OUR	Fresh and Drying	My Driss Zerhoun	Nabout 2	NAB2	Drying	Taounate
El Fassi	FAS	Fresh	Taounate	Nabout 1	NAB1	Drying	Taounate
Lemtel2	LEM2	Fresh	Taounate	Ghoudane4	GHD4	Fresh	Taounate
Lemtel1	LEM1	Fresh	Taounate	Ghoudane1	GHD1	Fresh	Taounate
Arguel	ARG	Drying	Taounate	Génotype19	GEN19	Fresh	Taounate
El Beida	BEI	Fresh and Drying	Taounate	Géenotype20	GEN20	Drying	Taounate
El Khoubzi	KHO	Fresh and Drying	Taounate	Oulmessia Hamra	OLMH	Fresh	Oulmes
Ghoudane3	GHD3	Fresh	Taounate	Oulmessia Beida	OLMB	Fresh	Oulmes

2.2. Molecular analysis

a. DNA extraction

The DNA was extracted from 30 mg of freeze-dried leaves using the technique CTAB of Saghai Maroof et *al.* [15] with modifications. Molecular characterization was made in the laboratory of the Research Unit "Plant Breeding and Phyto-Genetic Resources Conservation" of the Regional Centre for Agricultural Research in Meknes based on recent studies of Achtak et *al.* [16].

b. Molecular analysis using ISSR markers

Analysis the ISSR markers was performed using primers selected from seven thirty available at said laboratory: F1, F8, F11, IMA834-Z, IMA 5-3 IMA5-Z, UBC-841 (Table 2). The choice was made, after testing polymorphism, amplification of DNA and the reproducibility of the results on five fig cultivars randomly chosen. PCR Amplification was performed in a final volume of 25 µl containing: 2.5 mM $MgCl_2$, 1X PCR buffer, 0.2 mM of each dNTP, 0.5 mM of each primer, 1 unit Taq DNA polymerase and 20 ng of template DNA. Polymerase chain reaction (PCR) was carried out using a Mastercycler Eppendorf using the following conditions: an initial denaturation at 94 °C for 4 min, followed by 35 successive cycles. Each cycle comprises a succession of three steps: denaturation at 94 ° C for 1 min, a hybridization phase at the optimum temperature according to the primer for 1 min (Table 2) and an elongation at 72 ° C for 1 min. After the end of the cycles, the program is completed by a final elongation step at 72 ° C for 10 min. Amplified products were electrophoresed on 2% of agarose followed by staining with ethidium bromide and visualized using an UV trans-illuminator related to an imaging system.

Table 2 : Sequences and specific hybridization temperature of ISSR primers

Locus	Primer sequences (5'----------3')	Hybridization temperature
F1	AGAGAGAGAGAGAGAGTA	41 °C
F8	AGAGAGAGAGAGAGAGCC	46 °C
F11	CACACACACACACACAAC	41 °C
IMA-5-3	CACACACACACACACATG	45 °C
IMA-5-Z	CACACACACACACACAGT	45 °C
IMA 834-Z	AGAGAGAGAGAGAGAGYT	50 °C
UBC-841	GAGAGAGAGAGAGAGYC R3'	46 °C

c. Molecular analysis using specific SSR loci

We selected 9 SSR locus of 17 primers which have been used by Achtak et *al.* [16]. These loci belonging to the three sets of primers: LMFC [17], MFC [18, 19] ; Ahmed et *al.* 2007), FSYC [19]. Analyses were performed according to the PCR conditions developed by Achtak et *al.* [19]. Amplification reactions were performed in a final volume of 25 µl in the presence of 20 ng template DNA, 4 pmol reverse primer and 0.5 mM of each primer, 0.2 mM of each deoxynucleotide, 2.5 mM MgCl2, 1 U Taq polymerase (Qiagene) and 1x of taq buffer. Polymerase chain reaction (PCR) was carried out using a Mastercycler Eppendorf. After 5 min at 94°, 35 cycles were performed with 45s at 94°C, 45 s at either 55 or 60°C (Table 3) and 1 min at 72°C, followed by a final extension step of 10 min at 72°C.

Electrophoresis was performed on 6% polyacrylamide gel which was prepared from a 40% solution of acrylamide, 7.5 M urea and 1X TBE buffer. The revelation was made according to the steps indicated by Benbouza et *al.* [19].

Table 3: Sequences and specific hybridization temperatures of SSR loci

Locus	Primer Sequences (5'----------3')	Hybridization temperature
LMFC30 -F	TCTTTTTAGGCAGATGTTAG	55 °C
LMFC30 -R	TTGTCCGTTTCTTATACAAT	
MFC2 –F	GCTTCCGATGCTGCTCTTA	55 °C
MFC2 -R	TCGGAGACTTTTGTTCAAT	
MFC3 -F	GATATTTTCATGTTTAGTTTG	55 °C
MFC3 -R	GAGGATAGACCAACAACAAC	
FSYC01 -F	CAAATGAAAAACACAAATTTGCCAAC	55 °C
FSYC01 -R	TGCAAGTACTAATTCCTCTGCCGTG	
MFC9 -F	GGAGGCAAAACGACAAACGACAT	60 °C
MFC9 -R	CAAGGAACCAAGCGGGAGGG	
MFC11 -F	CAAAAGAGAAGACCAGCATC	60°C
MFC11 -R	GACGAGGGAAGGAGAGACAC	
LMFC19 -F	AATGAATGGAAATGATCTTG	55°C
LMFC19 -R	CTTATGAAAACTCGGTAGAAG	
LMFC34 -F	GTATTGGATCTTGATTATGTTT	55 °C
LMFC34 -R	GTTACAAAGTACAGGTAAGCA	
MFC4 -F	CCAAACTTTTAGACAACTT	55 °C
MFC4 -R	TTTCTCAACATATTAACAGG	

2.3. Data analysis

The sizes of the bands produced by ISSR were calculated using the Mesurim pro software and that generated by the SSR loci were manually measured with respect to the marker's size.

Genetic relationships among fig cultivars were studied Genetic relationships between olive genotypes were studied on the basis of a similarity matrix [21]. Genetic distances were calculated using Clustering Calculator program developed by Brzustowski [22] through Simple Matching Coefficient. Thus, based on the comparison two by two of genotypes, a histogram of according to the number of alleles which distinguish them has been established.

Two phenograms were drawn based on the unweighted pair group method with arithmetic mean algorithm using the NTSYS-pc program ver. 2.11g [23].

The index PIC (Polymorphism information content) related to the genetic diversity of each primer used was calculated using the formula of Botsein et *al.* [23]

PIC j = n $(1- \sum P^2ij) / (n -1)$, with j: primer concerned, n: The size of the i band and Pij: frequency of marker i revealed by the primer j through the band sum.

For each SSR locus, observed heterozygosity's values were calculated using the GENETIX 4.0 software [25]. The importance of heterozygosity deficiency was assessed using exact tests of Genepop3 software. Furthermore, a factorial correspondence analysis was performed using the Genetics software 4.0 [26] on the SSR markers matrix.

III. RESULTS AND DISCUSSION

3.1. Molecular characterization of cultivars by ISSR markers

Seven I.S.S.R. primers selected, following their polymorphism and the bands clarity, revealed a total of 54 markers varying between 4 to the primer IMA-834-Z and 11 for the primer IMA-5-Z, with an average value of 8 markers primer (Table 4). This number reflects the high level of polymorphism among cultivars revealed by the selected primers. The result obtained is equal to twice the

mean of markers observed by [27], using four ISSR markers in a comparison study of the efficiency of RAPD, SSR and ISSR techniques on the Mediterranean fig cultivars. This difference could be explained by the number and type of ISSR primers used and the plant material studied. In addition, a study conducted on Asian and European cultivars by Ikegami et *al.* [28] showed that the primer UBC-812, the most commonly used to evaluate the genetic diversity was generated nine band. The highest percentage of primers polymorphism the was obtained for the primer IMA-5-Z (92%) and the lowest percentage was observed for F1 primer (63%) (Table 4).

Tableau 4: List of seven I.S.S.R. primers used, their sequence, repeat type (R.T.), hybridization temperature (HT), bands sizes, number of polymorphic markers (P), monomorphic markers (M) and index of polymorphism information content (PIC)

Name	sequence (5'-3')	H.T. (°C)	bands sizes (pb)	Polymorphism %	Number of bandes		
					P	M	PIC
IMA-5-3	CACACACACACACACATG	45	233 - 1235	88	7	1	0,98
F1	AGAGAGAGAGAGAGAGTA	41	910 - 2566	63	5	3	0,96
F11	CACACACACACACACAAC	41	338 - 2561	90	9	1	0,99
IMA-834-Z	AGAGAGAGAGAGAGAGYT	50	834 - 819	80	4	1	0,91
IMA-5-Z	CACACACACACACACAGT	45	488 - 2824	92	11	1	0,99
UBC-841	GAGAGAGAGAGAGAGYC R3'	46	166 - 2692	82	9	2	0.98
F8	AGAGAGAGAGAGAGAGCC	46	288 - 3764	64	9	5	0,98
				80	54	14	0,97

di : dinucleotide

Generally, the number of I.S.S.R. markers generated is correlated positively with the number of primers used. However, this number can be greatly influenced by the analysed plant species and the nature of the migration gel used [29, 30].

Indeed, in comparison with other species, the percentage of polymorphic bands revealed by ISSR primers was very high in *Asparagus acutifolius* L. (100%) [31], *Lupinus spp.* (99%) [32] and in *Oryza sativa* (80.9%) [29], but lower in *Bombyx mori* (64%) [33].

All primers used generated polymorphic profiles with varying and significant index of genetic diversity (Table 4). Indeed, the index analysed according to the procedure of Botsein et *al.* [24] confirmed the genetic variability of seven primers used. The diversity index, which tends towards one, reveals a significant degree of polymorphism. The more its value tends towards one, the primer is polymorphic and vice versa. Thus, the diversity index is 0.91 for the primer IMA-834-Z which has one monomorphic marker on a total of 4 and maximum of 0.99 in the F11 and IMA-5-Z primers which have given almost 100% of polymorphic bands. However, the average value of the diversity index (0.97 ± 0.205) obtained for all the primers consolidates and justifies the choice of seven ISSR primers to analyse our twenty-two fig accessions. In order to confirm the genetic diversity of the collection, pairwise comparison shows that 89% of cultivars pairs are differentiated by 9 to 29 ISSR bands. Thus, the average similarity obtained by the ISSR is 71.6%.

Getting a high number of ISSR polymorphic primers is an important utility. It increases the reliability of the interpretations of the results; especially in our case where profiles generated are reproducible. The number of the polymorphic primers used to analyze various plant species differs according to the authors. Pradeep et *al.* [33] used seven primers, twelve by Wiesner et *al.* [30], 23 by Sica et *al.* [31], a number of 30 per Talhinhas et *al.* [33] and 41 Young et *al.* [34].

Furthermore, the primers that we used in this study are type di-nucleotide repeat. A similar study by Konate [35] in carob showed that they are more efficient than the primers tri-nucleotide repeat.

3.2. Molecular characterization of cultivars by SSR markers

Forty-two alleles were obtained using nine microsatellite loci. The number of alleles per locus varies from three alleles (FSYC01, LMFC19, LMFC34 and MFC4) to 12 alleles (MFC2) per locus with an average of five alleles. Allele size ranges from 100 bps at the FSYC01 loci to 305 bp in the LMFC19 locus (Table 5).

The observed heterozygosity ranged from 0.045 to 0.772 for FSYC01 and MFC9 loci, and 0.42 as the average for all loci. Compared to the value of expected heterozygosity, a significant excess of heterozygosity was observed for the locus MFC9, probably because a selection effect that would take place within a limited gene pool (Table 5). For the fixation index (Fis), it ranges from 0.078(MFC11) to 0.906 (FSYC01) locus, indicating that the genotypes studied have a heterozygosity

deficiency for the loci, except for MFC9 locus, the Fis is negative (-0.406) with an excess significant heterozygote probability. This situation may be explained by the fact that this collection comes from a prospection directed to cultivars with agronomic performance characters in a limited gene pool [27] have discovered a heterozygosity deficiency for four SSR loci (MFC1, MFC2, MFC3, MFC7) in the molecular characterization study (SSR and ISSR) of Moroccan fig germplasm. However, for a larger study of 277 cultivars prospected in Morocco, 15 of 17 loci showed excess heterozygosity [16]. Allelic frequencies ranged from 0.019 for seven alleles belonging to the locus MFC2, MFC9 and FSYC01, to 0.87 for LMFC19-302 allele, with an average of 0.18 and 0.22 as estimated standard deviation.

Table 5: Number and size of alleles and diversity parameters for each of the nine SSR loci

Locus	Range of size (pb)	diversity parameters					
		N	H_0	H_e	P	F_{is}	PI
LMFC30	250-261	4	0,2273	0,7035	0,0000	0,689	0,143
MFC 2	158-190	12	0,5909	0,7955	0,0000	0,279	0,061
MFC 3	108-114	4	0,5455	0,6746	0,0000	0,214	0,163
MFC 9	190-220	3	0,7727	0,5425*	0,0000	-0,406	0,297
MFC 11	182-206	7	0,7273	0,7696	0,0002	0,078	0,077
FSYC01	100-106	3	0,0455	0,4638	0,0000	0,906	0,376
LMFC19	300-305	3	0,1818	0,2800	0,0000	0,371	0,537
LMFC34	214-219	3	0,3636	0,5010	0,0091	0,296	0,306
MFC 4	240-244	3	0,3636	0,4298	0,0035	0,176	0,369
9 locus	100-305	5	0,42	0,58	0,0000	0,29	0,26

N: number of alleles; **Ho:** observed heterozygosity; **He:** expected heterozygosity; **P:** Hardy-Weinberg Test probability; **Fis:** Fixation Index; PI: Probability of identity; * significant excess heterozygosity.

Compared with other collections, it appears that the ENAM collection has diversity parameters such as 5 alleles per locus, and observed heterozygosity of 0.42 are similar to European and Asian cultivars studied by SSR markers where the number of alleles per locus was 5.2 and an observed heterozygosity 0.44 respectively [28]. Diversity levels close to our results have been seen in other collections in Morocco and elsewhere but with heterozygosity higher values. Indeed, the fig collections No. 1 and No. 2 of the Ain Taoujdate Experimental Field of CRRA-Meknes, have seven and six respectively as the average number of alleles per locus and 0.79 and 0.63 as the average observed heterozygosity. A similar level of diversity was shown in the Porquerolles Mediterranean collection (France), with an average of six alleles per locus and 0.54 observed heterozygosity [16]. In contrast, a low polymorphism (3.9 alleles per locus, and 0.38 observed heterozygosity) was detected in Extremadura Spanish collections [17].

The total number of 42 alleles allowed to distinguish 21 SSR profiles whose two Nabout genotypes the same profile. Among the two-hundred-thirty-two cultivars pairwise comparison, only 19 SSR profile pairs were differentiated by less than six alleles. Other pairs were distinguished by 7 to 24 alleles (Fig. 1).

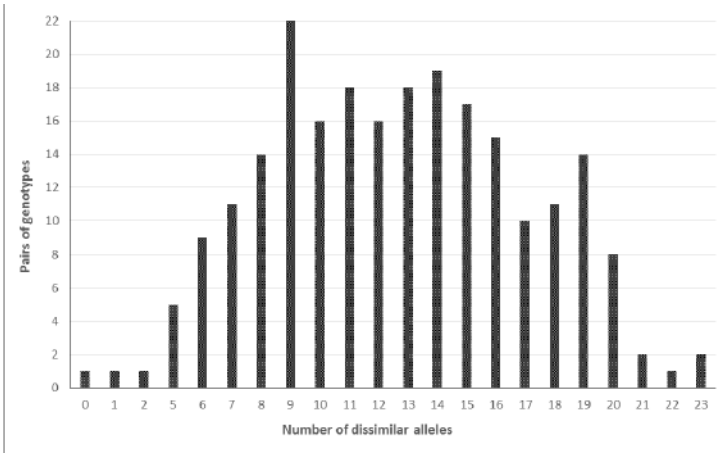

Fig. 1. Frequency distribution of genetic dissimilarity for all pairwise combinations among 22 fig genotypes of ENA Meknes collection

Similar results were reported in a large study of the Moroccan fig [16]. Relatives cultivars (low allelic distinction) presumably correspond to soma-clonal variations via intense vegetative propagation over long periods, while distinct genotypes (over 6 alleles) are issued from seed (sexual reproduction). Getting close cultivars probability, which differ only by 1 to 3 SSR alleles via sexual reproduction, is very low [36]. Thus, descendants analysis of two olive crossbreeding, "Picholine marocaine (Parent female)" x Picholine de Languedoc (Male Parent) " [37; 38] and "Olivière"

(female parent) x "Arbequina" (Parent male) [39] using 36 and 47 SSR loci shows that the descendants are different by at least 16 and 18 alleles respectively. These results indicate that cultivars from the ENAM collection really correspond to prospecting trees belonging diverse populations.

3.3. Cultivars hierarchical classification and factorial correspondence analysis

a. SSR Markers

The SSR data exploitation allowed the all cultivars genetic characterization. Indeed, the dendrogram generated by statistical analysis based on the UPGMA method and similarity distances calculation shows a wide intra-collection molecular diversity (Fig. 2).

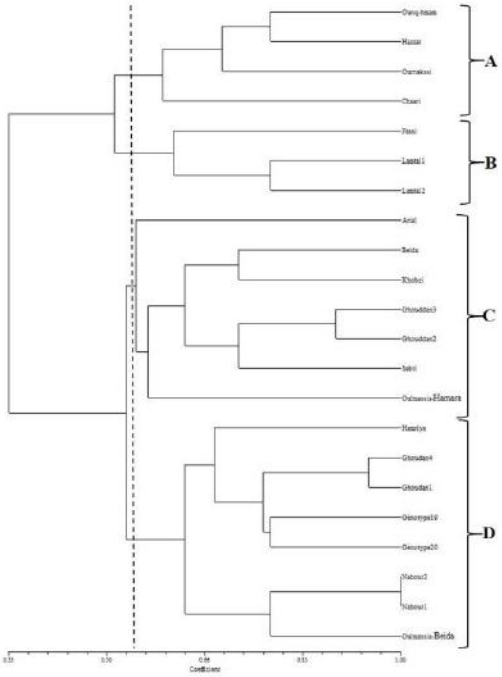

Fig. 2. Genetic relationships among fig cultivars. The dendrogram was based on a similarity matrix UPGMA algorithm of SSR markers.

Approximately 56% of similarity, the resulting dendrogram allowed distinguishing four clusters (A, B, C and D) which each includes 4, 3, 7 and 8 cultivars respectively. Cluster A contains Chaari, Ournaksi, Ounq Hammam and Hzzat cultivars, latter two cultivars belong the same sub-cluster. Similarly, Lemtel1 and Lemtel2 constitute a sub-cluster and belong to the cluster B with the Fassi cultivar. However, Beida and Oulmessia Hamra, Khoubzi and Sebti, Ghoudane2 and Ghoudane3 constitute three sub-clusters and create cluster C with Arial and Hamriya cultivars. Finally, the cluster D contains the rest of cultivars with Oulmessia Beida as single and three sub-clusters constituted by Genotypes19 and Gentypes20 cultivars, Ghoudane1 and Ghoudane4, Nabout1 and Nabout2. The peculiarity of pairs cultivars Lemtel1-Lemtel2, Ghoudane2-Ghoudane3 and Ghoudane1-Ghoudane4 that they are very close and have the same name (Pomological resemblance fruit), suggesting the problems of homonyms and somaclonal variations. The analysis also revealed four cases of homonymy among cultivars (Ghoudan1-Ghoudan, Ghoudan1-Ghoudan3, Ghoudan2-Ghoudan4 and Ghoudan3-Ghoudan4) since they are pairwise genetically and pomology different. Low allelic variations and synonyms and homonyms problems were reported in the fig by Achtak et *al.* [16] and in several other fruit trees [40; 41; 27; 42].

The plot of factorial correspondence for the first, second and third axes, which explained 23.6%, 12.29% and 10.43% of the variance, respectively, giving a cumulative variability of 46.32% (Fig. 3). This plot shows the existence of four similar groups to the four groups revealed by the dendrogram including the same homogeneous cultivars. According to the figure, a clear genetic structure of the studied population (Dendrogram and FCA) is clearly reveals in the analysis. Thus, we find, after this distinction into separate groups, they are from different gene pools and are selected and planted based on their agronomic performance.

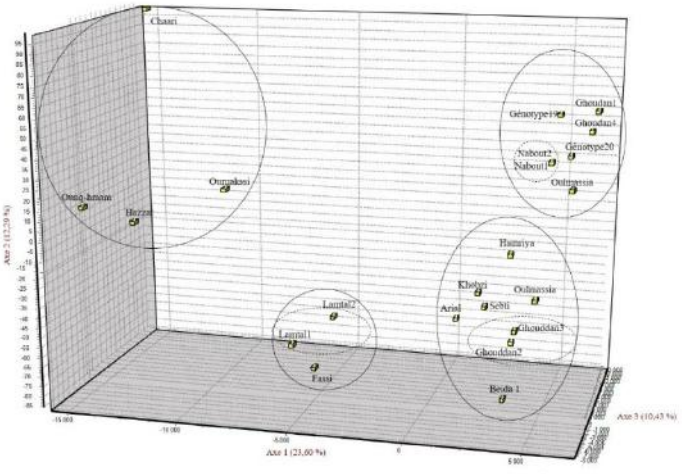

Fig. 3. Factorial correspondence analysis of 22 genotypes as defined in Table 1. the analysis allowed distinguishing for four separate groups.

b. ISSR Markers

The different gels and 54 revealed bands have resulted in the phenogram (Figure 4), which illustrates the genetic relationships existing between 22 fig cultivars. At 77% dissimilarity, the dendrogram below shows four different groups: G1, G2, G3 and G4 respectively containing nine, four, five, and four genotypes (cultivars) that are homogeneous within each group.

Cultivars having the same names lamtal1 and lamtal2, Ghodane2 and Ghoudane3 are very close in pairs, confirming the results obtained using the SSR markers. This confirmation of the molecular similarities shows that the variations between these genotypes are due to the

soma-clonal variations between cultivars with genetic and phenotypic profiles very close.

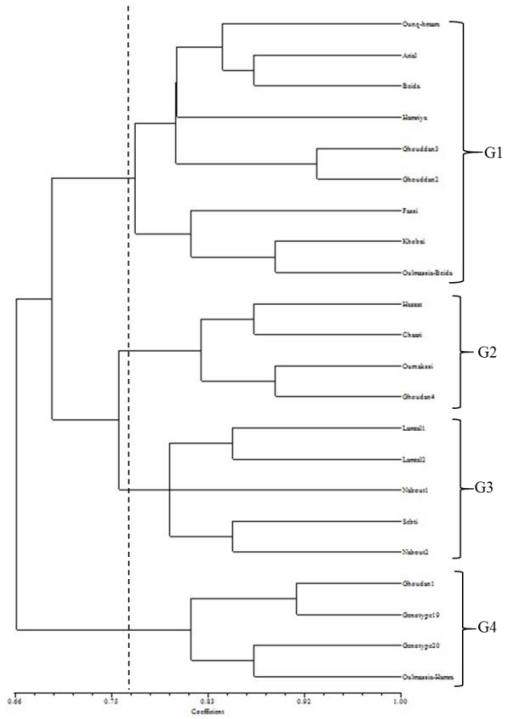

Fig.4. Dendrogram the fig cultivars generated by ISSR based on the UPGMA algorithm and similarity matrix

The dendrogram confirms the high genetic diversity between collection genotypes, however, does not reflect the same homogeneous groups generated by the SSR markers dendrogram (Fig. 4). Our results are in agreement with Ikegami el *al.* [28] studies, on Asian and European cultivars using RAPD, ISSR and SSR markers, which found significant differences in the hierarchical classification generated by the three methods. This can be explained by a low correlation coefficient between the three markers types and different adjustment degrees. Moreover, according to several authors, ISSR give better profiles emanating especially the length of primer sequence, involving a high annealing temperature, compared with the RAPD and RFLP methods, gives thick and reproducible bands [43; 44; 45].

c. Detailed study of cultivars genetic profiles

Pomological characterization, based on 25 qualitative and quantitative traits related to fruit, for the same cultivars realized by Ait Haddou et *al.* [46] has revealed the similarities and differences traits between cultivars having the same denomination (Table 6).

Cultivars are similar for most morphological characters except for Oulmessia Hamra and Oulmessia Beida (Table 6). The molecular marker numbers that does not exceed six alleles per SSR locus for all cultivars except for Oulmessia Hamra and Oulmessia Beida that differ by eight alleles genetically confirms this similarity.

Table 6: Pomological characters most discriminating cultivars bearing the same names in Moroccan collection of ENA Meknes [46].

Accessions	FT	MP	FW (g)	Cal	PL (mm)	FD (mm)	FS1	FS2	SAV	BCE	IPC	PTX	ICF	QS
Lemtel2	B	Midseason	42,37	24	3,97	44,03	Globular	Ovoid	Yes	Light green	Red	Coarse	Small	Medium
Lemtel1	B	Midseason	44,96	23	4,21	44,38	Globular	Ovoid	Yes	Green	Red	Coarse	Without	Medium
Ghoudane3	B	precocious	53,17	19	4,95	47,96	Globular	Ovoid	Yes	Purple	Dark red	Medium	Without	Medium
Ghouane2	B	precocious	55,05	18	4,75	48,20	Globular	Ovoid	Yes	Purple	Dark red	Medium	Medium	Medium
Nabout2	U	Tardive	30,95	34	4,07	39,14	Globular	Ovoid	Yes	Green	Dark red	Coarse	Without	Medium
Nabout1	U	Tardive	31,08	34	3,84	36,28	Globular	Ovoid	Yes	Green	Red	Coarse	Without	Medium
Ghoudane4	B	Midseason	45,73	22	5,03	44,86	Globular	Ovoid	Yes	Black	Dark red	Small	Without	Large
Ghoudane1	B	Midseason	44,56	23	4,92	44,73	Globular	Ovoid	Yes	Black	Dark red	Medium	Without	Large
Genotype19	B	Midseason	42,49	24	5,39	44,30	Globular	Ovoid	Yes	Black	Red	Coarse	Without	Medium
Genotype20	B	precocious	42,39	24	5,53	44,64 44,52	Globular	Ovoid	Yes	Black	Red	Medium	Without	Large
Oulmessia Hamra	U	Midseason	43,52	23	10,9	44,93	Globular	piriformis	No	Violet	Amber	Coarse	Without	Large
Oulmessia Beida	U	Tardive	45,65	22	7,2		Globular	Ovoid	Yes	Yellowish	Dark red	Medium	Small	Medium

FT: fruit type; **PM**: maturity period; **FW**: fruit weight; **Cal**: caliber; **PL**: peduncle length; **FD**: fruit diameter; **FS1**: fruit shape as (width / length); **FS2**: fruit shape depending on where of the maximum width; **SAV**: symmetry along the vertical axis; **BCE**: background color of epidermis; **IPC**: internal pulp color; **PTX**: pulp texture; **ICF**: internal cavity of the fruit; **QS**: quantity of seeds.

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Pairwise molecular profiles comparison of the accessions, having the same denominations, has also identified the differences between cultivars and compared relative to pomological results.

The Lemtel cultivar is represented by two different denominations that have the same characteristics except for pomological fruit weight, the epidermis background color and the fruit internal cavity (Table 6). Indeed, this similarity is confirmed by the molecular profiles that differ only by five SSR markers. These accessions are therefore considered genetically closer. The Nabout cultivar is represented by two similar accessions at all Pomological characters (Table 6). Both accessions are all characterized by globular ovoid figs, a green epidermis and as weight 31g. Furthermore, they have a difference in fruit diameter. This Pomological similarity between the two denominations is validated by the same SSR profile. This result is different from the study of Charafi [47] which shows allelic differences between cultivars of Naboute of Ouazzane Regione cooperative (Janane Rif) and those of the experimental field of Ain Taoujdate (INRA-Meknes).

The cultivar Ghoudane includes four accessories which Ghoudane1 and Ghoudane4 have the same pomological traits and therefore constitute same genotype. However, Ghoudane2 and Ghoudane2 accessions are close together and different than Ghoudane1 and Ghoudane4 by the period of maturity, size, epidermis background color and the seed quantity (Table 6). Given the significant differences between pomological Ghoudane1 and Ghoudane4, firstly, and secondly Ghoudane2 and Ghoudane3, each pair would thus consist of a same cultivar. This distinction is confirmed by molecular results also revealed the similarity among Ghoudane1 and Ghoudane4 with only one SSR allele difference and 2 SSR alleles between Ghoudane2 and Ghoudane3. Ghoudane Polytonality was already demonstrated by Achtak et *al.* [16]. Varietal confusion, synonyms and homonyms problems, low allelic variations related to somaclonal variations, are all very common problems and traits in the fig [16].

Genotype19 and genotype20 are close genetically (5 different alleles), they are also similar for the majority of pomological traits (Table 6), except for the maturity period, texture and the quantity of seeds. Since these characters are not very discriminating, we can consider the genotype19 and genotype20 are derived from the same genetic origin.

Olmessia Hamra and Oulmessia Beida cultivars will differ by the majority pomological characters (Table 6) and 8 SSR markers. Therefore, they can be considered as two separate cultivars and Oulmessia denomination comes from their origin in addition to the genotype color.

IV. CONCLUSION

Molecular characterization allowed characterizing the genetic profiles of 22 fig cultivars from the ENA-Meknes collection. The two cultivars Nabout 1 and 2 have the same SSR profile and pair's accessions Ghoudan1 and Ghoudan4, Ghoudan2 and Ghoudan3, Genotype19 and Genotype20, Lamtel1 and Lamtael2 are genetically very close. This similarity was also confirmed by the pomological characterisation. The remaining accessions are different and therefore can be regarded as separate cultivars. The complementarity of the two approaches is therefore confirmed and the establishment of a genetic identity for each cultivar should consider the polyclonality of some varieties.

The selection of authentic reference cultivars within polyclonal cultivar is not easy when we consider the genetic diversity of plant materi*al.* In addition to the homonymies and synonymies problems and soma-clonal variations which are widespread in this species. For agronomic considerations, we suggest to consider the reference cultivar denomination having the better pomological characteristics. Choosing the most important cultivar is justified by the aboriginal always opted for the best genotypes for this culture. In the same direction, it is essential to make intra-varietal characterizations by combining pomological and molecular characterization using SSR markers to select the best clones of each variety. The collections should contain the selected clones that are references for any breeding program. Thus, follow up these collections will quantify phenotypic variation for this species particularly sensitive to climate variations.

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Production of Ice Cream with Carob Bean Pekmez (Molasses)

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Abstract**—** *Day to day, it is known that people are giving importance to nutrition their diets in terms of health. Whether or not the materials used in the production of food are natural, the amount used in production and effect on human health have become more important. In this study, low-fat ice cream was produced with rich composition carob molasses (pekmez), which has a positive contribution to nutrition, and the effect on ice cream was investigated. Physical and chemical analysis results of ice cream, pH 6,31, dry matter 27,23%, fat 3,50%, protein 3,86%, overrun 18,99% and viscosity (10 rpm-20 rpm-50 rpm) 11.840, 6.560, 3.344 cP, was found. As a result, a new product has been made which can be used in ice cream production of carob molasses.*

Keywords**— Carob, carob molasses, low-fat ice cream.**

I. INTRODUCTION

Ice cream, and all other frozen desserts, usually consist of seven ingredients: Fat, nonfat dry milk, sweeteners, stabilizers, emulsifiers, water and flavoring agents. Although frozen desserts are a very large area, the production of all of them is similar.

Ice cream is a milk product obtained by mixing, pasteurizing, homogenizing, cooling and freezing of pasta ingredients (Goff, 1997: 365, Arbuckle, 1986: 1, Goff ve Hartel, 2013:1). Ice cream is defined as "ice cream, milk and other dairy products, water, sugar and/or additives are mixed at a certain ratio, when desired, by the addition of salep, egg and/or flavoring and which is prepared and packaged according to its technique after being pasteurized and flavoring substances are added in various forms when necessary" at Turkish standards 4265. (TSE, 1992). Marshall and Arbuckle (1996: 315) described the composition of an economical ice cream as 35-37% total dry matter, consisting of 10% milk fat, 10-11% nonfat dry matter, 15% flavor, 0,3% stabilizer and emulsifier.

It is known that instead of synthetically produced products in the world, tendency to natural products increases. Along with consumption of high-energy food, immobile life of mankind triggers obesity. The nutrition diet consists of processed foods, low fiber and high fat saturated fat and sugar content, with low physical activity, poses a serious threat to health and causes important diseases such as obesity, coronary heart disease, diabetes and cancer. (Koeferli vd., 1996:1, Popkin ve Larsen, 2004:2). For this reason, consumers tend to deviate from the consumption of known fatty foods, which have a significant impact on human health.

Carob (Ceratonia siliqua L.) is a plant belonging to the Ceratonia genus of the Leguminosae family and is grown in regions where the Mediterranean climate dominates (Turhan vd., 2007: 417, Tetik vd., 2010: 1, El Batal vd., 2016: 1). Carob has 91-92% dry matter and 62-67% total soluble dry matter when it matures. A significant portion of soluble dry matter forms saccharose (34-42%), fructose (10-12%) and glucose (7-10%) (Karkacıer ve Artık 1995:). Other studies have shown that carob molasses has a total sugar content of 60-70% (Ekşi ve Artık 1986:, Şimşek ve Artık 2002: 465, Turhan vd., 2007: 418, Tetik vd., 2010: 41).

Carob is composed of two parts: fruit pulp (pod) (90%) and seed (10%). From the pulp part of carob, grape molasses, carob flour and animal food products are produced and locust bean gum (LBG), which is used as a stabilizer in food and other areas (which form a very viscous solution even when used in low quantities), is produced from the core part. LBG is synergistic with carrageenan, agar and xanthan gum, creating a very strong and elastic gel (Kumazawa vd., 2002: 373, El Batal vd., 2016: 956). It is estimated that 200,000 hectares per year of the world produce over 300,000 tons of carob fruit (El Batal vd., 2016: 955, Vekiari vd. 2011: 751). It is determined that there are 370.000 carob tree trees in Turkey and that 13,985 thousand tons of carob bean is produced in 2015 (TSI, 2016).

Pekmez (molasses) is produced as a traditional product in Turkey for many years. Pekmez is produced from fruits with high sugar content such as grape, apple, carob, plum, watermelon, apricot, fig, sugar beet, hawthorn, mulberry, raisins, corn, sugar cane (Üstün ve Tosun, 1997:417, Şimşek ve Artık 2002: 460, Özdemir vd., 2004: 33, Karababa ve Işıklı, 2005: 357, Sengül vd., 2007:39).

Grape molasses is defined as "grape pekmez is a thick liquid food which is produced adding honey, cow milk, milk powder, egg, by vacuuming or thickening in accordance with the technique, without reducing the

acidity of the fresh or raisin extract, or by reducing its acidity with calcium carbonate or sodium carbonate, followed by drying with tannin gelatin or suitable enzymes" at TS 3792 (TSE, 1989). Molasses is added to obtain the desired flavor and color in ice cream production, as well as to control the freezing point of ice cream (Temiz ve Yeşilsu, 2010: 539). Although the molasses compositions vary according to the fruit obtained, the basic composition is carbohydrates (Şimşek ve Artık 2002: 460, Karababa ve Işıklı, 2005: 357). The first order of pekmez production in Turkey is grape (Üstün ve Tosun, 1997:417).

Carob molasses is a traditional product produced in Turkey for many years from the fruits of Ceratonia siliqua L. plant. As it is not possible to directly press the fruit which reaches the consumption level, it is extracted with water. For this, the carob is broken down in sizes of 5 and 7 mm, then moistened with water and extruded at 85 ° C for 3 hours. After the extraction, perlite is filtered with molasses soil containing bentonite (containing 50-90% calcium carbonate) to neutralize acidity. After then, the extract is concentrated by evaporation at 85ºC to 65-70º Briks and the extract is pasteurized against microbial growth and deterioration. The final product is filled hermetically in glass jars (Demirözü vd., 2002:330, Turhan vd., 2007: 39,40, Tetik vd., 2010: 418).

As with other molasses, carob pekmez is rich in carbohydrates and mineral substances and is an important food material especially for children in the age of growth, pregnant women, suckling mothers, athletes and workers, who need high energy. It is especially important in terms of mineral substances such as potassium, calcium, phosphorus, magnesium and iron. (Demirözü vd., 2002: 330-333, Şimşek ve Artık 2002:465, 467, Vekiari vd. 2011: 751).

Carob is widely used in the world for industry, afforestation, prevention of erosion, ornamentation, painting, food, animal feed and medical treatment. Carob is widely used medicinally for the treatment of many diseases such as flu, cough, asthma, bronchitis, wound healing, diarrhea, intestinal draining, reflux, nail fractures, anemia, blood disorders, prostate, fatigue, cholesterol, diabetes, urinary infections, liver, kidney, stomach, intestineand lung treatment (Bulut, 2006: 65, Güneş, 2010: 87, Gürdal, 2010: 121,122, Dakia, 2011: 293, Akbulut ve Bayramoglu, 2013: 67, Yıldırım ve Kargıoğlu 2015: 104,106,107).

In this study, a low-fat ice cream was produced using carob pekmez in ice cream production, giving people the opportunity to choose a new variety of products with different flavors and aromas. In the production of ice cream, instead of synthetic materials, xanthan, carrageenan and locust bean gums were used as natural stabilizers and the effect on some properties of ice cream is investigated.

II. MATERIAL AND METHOD

2.1. Material

Used in the production of ice cream, UHT milk, skimmed milk powder, butter, sucrose, locust bean pekmez and LBG, carrageenan and xanthan gum, was given properties Table 1 and Table 2, were supplied from Antalya. Ice cream production was carried out at a milk processing plant operating under the Food Engineering Department of the Faculty of Agriculture, Akdeniz University.

2.2. Method

2.2.1. Production of Ice Cream

Before the production of carob ice cream was carried out for the study, 5 different sugar-molasses mixtures were used in preliminary experiments and molasses and sugar amount to be used by sensory evaluation were determined. Stabilizer usage ratios were determined based on the data obtained from the literature and were determined in the study of Badem (2006: 51). (Marshall ve Arbuckle 1996: 29, 72-75, Vega vd., 2004:). At the end of the study conducted by Badem (2006: 51), the best ice cream from sensory evaluation was selected as carrageenan gum at 0,1%, xanthan gum at 0,1% and locust bean gum at 0,4%. Amounts of the substances involved in ice cream production; For a 2600 gram mix; 2 liters of milk, 300 grams of molasses, 200 grams of sucrose, 70 grams of milk powder, 30 grams of butter. The fat content of the frozen is set at 3,5%.

Table.1: Some properties of ingredients used in ice cream production.

Analyses	UHT Milk	Butter	Milk powder	Sucrose	Carragenan gum	Xsantha n gum	LBG
Dry matter (%)	11,13	-	92,50	98,88	82,00	91,50	90,00
Fat (%)	3,10	82,50	-	-	-	-	-
Protein (%)	3,13	-	-	-	-	-	-
pH	6,38	-	-	-	-	-	-

Ice cream production is started by mixing powdered ingredients. Then, 2 liters of milk heated to 60ºC was added slowly, so that the mixture didn't clump. This mixture was pasteurized at 80ºC for 10 minutes, then rapidly cooled to 30ºC. 300 grams of molasses was added to each mix that was cooled. After matured at 4ºC for 24 hours, produced ice cream mix were frozen at -5ºC with semi-continuous Uğur brand freezing machine. The ice cream processing time was 15 minutes for each mix and was packed. Then, hardening was carried out for 24 hours in the deep freeze at -11ºC. The prepared ice cream was kept at this temperature until analysis was made.

Table.2: Some qualities of molasses used in ice cream production.

Parameters*	(%)
Dry matter	66,91
Carbohydrate	62,50
Lipid	0,41
Protein	4,00
Ash	2,40
Total sugar	62,00
Invert sugar	17,25
Minerals	**(mg/kg)**
Potassium	7040
Calsium	1234
Phosphorus	547
Magnezyum	500
Sodium	203
Zinc	10
Iron	7,6
Manganese	3,0
Copper	0,8

* The information on the table was taken from Kimtek Co. (Antalya).

2.2.2. Analysis in ice cream

pH analysis: After ice cream samples were melted at 20 ° C, they were determined using a Hanna instruments 8519 brand pH meter.

Dry matter analysis: The dry matter content of ice cream samples was determined according to TS 4265 Ice Cream Standard (TSE, 1992).

Protein analysis: Amounts of protein in ice cream samples were determined by the Kjeldahl Method (AOAC, 1999: 13).

Overrun analysis: Overrun in ice cream was determined as Arbuckle (1986: 187) method.

Viscosity analysis: The method given by Chang and Hartel (2002) was used for measuring.Viscosity of mix added pekmez was determined by measuring the Brookfield Viscosimeter (R.V.T.) at 10 rpm, 20 rpm and 50 rpm at 25°C and was measured 30 seconds after the viscosimetric tip was immersed in.

III. RESULT

Physical and chemical analysis results of ice cream produced as described in Material and Method are given in Table 3. The obtained data were given by averaging the replicate analyzes.

Tablo.3: Analysis results of ice cream.

Parameters	Value
pH	6,31
Dry matter (%)	27,23
Fat (%)	3,50
Protein (%)	3,86
Overrun (%)	18,99
Viscosity (10 rpm-cP)	11.840
Viscosity (20 rpm-cP)	6.560
Viscosity (50 rpm-cP)	3.344

IV. CONCLUSION

The effect of pekmez used at 11% and stabilizers (0,1% carrageenan gum, 0,1% xanthan gum and 0,4% LBG) in ice cream production, the values of pH, dry matter, protein and viscosity values obtained in the study, have similarity in comparison with other studies (Koçan ve Koçak, 2002:372, Keçeli ve Konar (2003: 417, Güven vd., 2010: 100, Temiz ve Yeşilsu (2010: 541, 542). Keçeli et al. (1997: 180) investigated the effects of sahlep and some alternative stabilizers on the quality of ice cream produced with goat milk. Accordingly, depending on type of stabilizer used, the properties of ice cream mix have also changed. The highest overrun values were found in ice cream adding LBG and sahlep 36,1% and 35,8%, respectively.

This value was calculated as 27,3% for control ice cream without any stabilizer. Again, Keçeli and Konar (2003: 417) investigated the effect of using LBG, CMC and gelatin in ice cream produced with cow milk. Overrun values were found between 30,65% - 38,17%. The overrun, used LBG 0,5%, ice cream was calculated as 36,93%. Overrun of control ice cream produced (0,25% LBG, 0,02% carrageenan and 0,75% guar gum, 5% fat) by Atsan and Çağlar (2008) was determined as 25,26%. Temiz and Yeşilsu (2010: 542) found that overrun rate decreased as the amount of molasses added to composition increased in ice cream produced using grape and mulberry molasses. In the production of 10% grape molasses and 10% mulberyy molasses were used in ice cream, overrun increase 19%, 21%, respectively. Over-

use of the stabilizers results in harder structured ice cream, so overrun is more limited. Also, LBG, carregen and xanthan gum interaction used limits overrun value (Marshall ve Arbuckle 1996: 34, 73). Overrun in this study, is 18,99%, which is lower than other studies. As a result, it is determined that proportion of molasses used in ice cream production (11%) and amount of stabilizer has effect on ice cream values.

In order to produce a new type of ice cream and to determine physical and chemical properties of ice cream, carob pekmez (molasses) was used as a natural sweetener besides sucrose. It has been determined that carob pekmez can be used for production of a new ice cream because of its high natural, ecological and nutritive value. It is also apparent that the low fat ice cream formulation can be achieved by use of suitable stabilizers.

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Role of *Trichoderma* and *Sinorhizobium* Strains for Improving Growth and Nutritional Status of Alfalfa under Cd Stress

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Abstract— *The plant rhizosphere is a major soil ecological environment for plant- microbe interactions involving colonization of different microorganisms in and around the roots of the growing plant. Plants can be used in the remediation of soils contaminated with heavy metals. The objective of this study was determine the relationship between the effect of Cd on the symbiotic model of Sinorhizobium meliloti – Medicago sativa and the application of Trichoderma sp. on the nutritional status as well as biochemical characterization of the sandy brown forest soil. The effects of biofertilizer Sinorhizobium and coinoculants Trichoderma strains on growth, chlorophyll and N, P and K content of alfalfa growing in soil polluted by cadmium were investigated.*

The results indicate that the presence of the saprobe fungi Trichoderma harzianum further enhanced shoot dry weight, N, P and K content of Sinorhizobium meliloti-alfalfa symbiotic model. The co-inoculation of alfalfa with T. harzianum was more effective for Cd uptake. The effects of the bio-multiple inoculants on the growth of alfalfa were stimulated the colonization of Sinorhizobium strains in the rhizosphere, promoted the nodulation potential and increased the dry organic matter. Sinorhizobium meliloti interacts with alfalfa as a model for rhizobioremediation and Trichoderma strains interact with this model as nodule promotors as well as a partner in the process of cleaning the plant rhizosphere from cadmium metal.

Keywords— Alfalfa (Medicago sativa), heavy metals, Sinorhizobium, Trichoderma fungi.

I. INTRODUCTION

Soil are reservoirs for heavy metals generated by industrial activities e.g. metal finishing, paint pigment and battery manufacturing, leather tanning, mining activities, municipal waste water sludge, urban composts, pesticides, phosphate fertilizer, or from atmospheric depositions (Ariano, 1986, Kabata-Pendias, 1992). Metalliferous or industrial soil, which is heavily enriched with toxic metals, can support the growth of specific plant species called metallophytes, which have long attracted the interest of botanist and are now considered as potential tools for phytoremediation (phytostabilization or phytoextraction). Some of these hypertolerant plants also have the ability to accumulate high concentration of metals in their tissues,(Boularbah *et al*., 2006). Approximately 450 plant species have been classified as hyperaccumulators of heavy metals, (Baker *et al*., 1994; Morel *et al*., 1997).

The health hazards associated with soil contamination with trace elements having toxic effects together with high cost of removal and replacement of polluted soil have prompted to develop alternative and cheaper technologies to recover the degraded land. Current research in this area now includes plants to remediate polluted soils and to facilitate improvement of soil structure, the innovative technique being known as phytoremediation (Brooks, 1998). The possibilities of using such plant species which are easily growing in different climates, and using their biomass in non-food industries, can make them ideal plants for phytoremediation purposes (Linger *et al*., 2002; Khan, 2003). Phytoremediation must be considered as a long-term strategy (Cunningham *et al.,* 1995).

Since 1904, when the term rhizosphere was first coined by Hiltner (1904), rhizosphere processes of plants have been widely investigated, however, little attention has been paid to the microbial community of rhizospheres of plants growing on metal contaminated sites. Soil microorganisms, including plant root associated free-living as well as symbiotic rhizobacteria and mycorrhizal fungi in particular, are integral part of the rhizosphere biota. The overall result of plant-rhizosphere microbe interactions is a higher microbial density and their metabolic activity in the rhizosphere, even in metal contaminated soils (Vander, 1998). Plants also influence the structure of microbial communities through the release of root exudates (Grayston and Campbell, 1996, Grayston *et al*., 1996; Grayston *et al*., 1998; Kozdroj and Van Elsas., 2000) and by providing surfaces for

colonization. However, it is not understood how specific plants increase the remediation of contaminated soil (Kirk *et al*., 2005).

Alfalfa is the most important forage crop in the world. The seed germination and vigor condition of alfalfa seedling are characteristics that determine plant establishment and initial crop development. Many leguminous plants and particularly alfalfa have extensive soil-root system extending as deep as 2m. Rhizosphere or soil-root systems limit the diffusion of solutes in the soil. Hence the plant can serve both as a vehicle for limiting the spread of solutes and by promoting the mineralization of the toxic compound. The general feasibility of rhizosphere approaches to remediation has been previously demonstrated (Anderson and Coat 1994). Frequently, alfalfa development is hampered by environmental conditions, such as metal deficiencies and metal toxicity, as well as by diseases. Previous investigations have shown that common root-colonizing bacteria are plant growth-promoting microorganisms that can improve plant development under a variety of environmental conditions (Reynders and Vlassik 1982, Kapulnik *et al*. 1985, Sarig *et al*. 1988), however, the known mechanisms by which they promote plant growth are quite variable. Some of them promote plant growth mediated by their production of plant regulators (Mordukhava *et al*. 1991). Previous studies demonstrated that alfalfa plants have the ability to geminate and grow in montmorillonite clay individually contaminated with 80 mg/kg of Cd, Cu and Ni and 160 mg/kg of Zn (Peralta-Videa *et al*. 2002). Based on that information, the following research was designed to determine if a correlation exists between the heavy metal tolerance and the growth stage of alfalfa plants. The rhizosphere also affects the availability of heavy metals. Early studies have indicated that there is higher concentration of exchangeable and carbonate bound Cr, Ni, Zn, Cu, Pb and Cd in the rhizosphere than in bulk soil (Wang *et al.*, 2002). Root –induced changes in the rhizosphere are important factors controlling nutrient dynamics in this zone and thus the mineral nutrition of plants. On the other hand, nutrient dynamics also influence the rhizosphere environment (Stratton *et al*., 2001). Rhizobia (e.g. *Azorhizobium*, *Rhizobium* and *Sinorhizobium* species) are soil bacteria able to develop N2-fixing symbiosis with legume plants. *S. meliloti* is a Gram-negative bacterium that can live as a saprophyte in soil or as N-fixing symbiont inside root nodule cells of alfalfa. Fewer studies are the activities of nodulating bacteria in the rhizosphere of the host plants that are important for host infection. These processes include communication between bacteria (Gray *et al*., 1996), competition for access to infection sites on root (Tripplett and Sadowsky, 1992), gene transfer (Sullivan and Ronson, 1998) and the growth in the rhizosphere (Robleto *et al*., 1998).

II. MATERIALS AND METHODS

2.1 Microorganisms: in addition to the new isolates of *Sinorhizobium meliloti* strains (isolated from different root-nodules on root of alfalfa, which grown in 50% sewage sludge amended brown forest soil), two standard strains of *S. meliloti* (heavy metal- tolerant strain GHR94 and salt-tolerant strain GH130) and *Trichoderma* strains (*T. harzianum* (111-2b), *T. viride* (131-2) and *T. koningii* (Cu-3)) isolated from heavy metal contaminated soil Nagyhörcsök, Gyöngyös and Eger, Hungary respectively were obtained from the Gene bank of Culture Collection of the former Research Group of Environmental Microbiology of Hungarian Academy of Sciences.

2.2 Cultural media and test plant: all medial constituents were purchased from Difco Laboratories, Sigma, or Merck Co. Experiments employed the following sterilized media were used for maintained the tested microbial strains as slant cultures as well as carrying out the investigations: Medium for *Sinorhizobium* :(1) Yeast extract mannitol agar: *Sinorhizobium meliloti* isolates were isolated and maintained on yeast extract mannitol agar (YMA) described by Kleczkowska *et al*., (1968). (2) Yeast extracts mannitol broth: YMA medium without agar (YMB).

Medium for *Trichoderma:* (1) *Trichoderma*- selective medium*: Trichoderma* strains were maintained on *Trichoderma*- selective medium described by Askew and Laing ., 1993). (2) Sucrose-yeast extract broth (SYB): for preparation of bioinoculant and to study the sensitivity of the *Trichoderma* strains to Cd salts. Different concentrations of Cd salts were added to SYB medium described by Altomare *et al*., (1999). Alfalfa (*Medicago sativa L.*) seeds of Hungarian origin.

2.3 Soil sample collection and preparation: the experiments were carried out in pots with soil contaminated by cadmium salts (Cd 11.02 mg kg^{-1}). The soil sample used for the present study was collected from a non-cultivated field of the experimental station at Szent István University, Hungary. The soil of sampling area was sandy brown forest of general properties. The soil sample were collected in plastic bags and transferred to the laboratory. The sample was sieved through a 2 mm mesh to remove plant debris and soil fauna and was stored at cool room for plant-microbe interaction.

2.4 Cadmium salt: cadmium chloride ($CdCl_2.6H_2O$) and cadmium sulphate ($3CdSO_4 .8H_2O$) obtained from (Reanal Chemical Company Ltd), with following concentrations used: *In vitro*: 0, 10, 20, 40, 80 and 160µM. *In vivo*: 0, 10, 20, 40, 80 and 160 mg kg^{-1} soil.

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2.5 Isolation, maintenance and growth of *Sinorhizobium meliloti:*

Routine techniques for further studies of *Rhizobium* are described by Vincent, (1970) were used. Comparatively, the isolates were selected according to the intense pink coloration of the colonies. Sub-culturing occurred only once during the experiment and single colonies were selected and each isolate was restreaked to purify (Somesegaran and Hoben., 1994). The bacterial isolates were stored on YMA slant tubes for further investigation. In screw-capped tubes containing YMA with $CaCO_3$ (3g l^{-1}), the strains were streaked and maintained at 4°C.

2.6 Screening isolates for nodulation:

A test of ability of the 55 isolates to form nodules on alfalfa root is the ultimate criterion of authenticity as *Sinorhizobium.* According to Jansen and Strijdom., (1982) that competitive ability as determined in autoclaved soils correlated with the ability to infect plants rapidly. The selection of the strains were depended on the intensity of nodule formation on the root (occupation rate in comparison with control strains), and the presence of leghemoglobin formation inside the root- nodule (the pink color) in which the concentration of leghemoglobin within the nodule bears a direct relationship to the amount of molecular N2-fixed (Jordan and Garrard., 1951).

2.7 Preparation of inoculate: under complete aseptically conditions, the inoculate of tested *Sinorhizobium meliloti* strains were prepared by growing each microorganism of tested bacterial cultures in 250ml Erlenmeyer flask containing 50 ml of YMB medium. The flasks were incubated at 28°C for 2days on a rotary shaker operating at 150 rpm. The bacterial cells from 10 ml cultures were centrifuged (12000 x for 4 min) and washed twice in phosphate buffered saline and re-suspended in 10ml of phosphate buffered. This is the final bacterial suspension which was used as inoculum throughout the following investigations.

Preparation of sinorhizobial inocula for *in vitro*: All *Sinorhizobium* inocula used in in vitro experiments had a cell capacity of $1x10^6$ cell ml^{-1} of saline solution (0.85g NaCl salt dissolved in sterile distilled water and completed to 100ml), this was calibrated by haemocytometer. It was prepared by washing three slant cultures of a 24h old culture, each with 5 ml of sterile 0.85% NaCl solution. The uniform distribution of the cells in the solution was obtained by shaking machine (Bayoumi, 1987). For *in vitro* experiments, *Trichoderma* strains a colony disc of active mycelium of fresh growth colony with 5 mm in diameter was placed carefully in the 250ml Erlenmeyer flask containing 50ml of SYB medium. The flasks were incubated at 28°C for 5 days on a rotary shaker operating at 50 rpm. But, ***in vivo*** for soil-plant inoculation, conidial suspension was prepared by washing the growth colony grown on agar surface with saline solution and the conidial number per ml ($1x10^4$) was calibrated by haemocytometer.

2.8 Ecophysiological selection of S. *meliloti* strains: originally, the total number of isolates was 55, and by the help of nodulation test, the number of root nodule forming strains was reduced to 18 during one month.

2.9 Determination the growth rate of microbial strain under Cd stress

For *Sinorhizobium* strains: two cadmium salts were subjected to establish their effects on the growth rate of 8 *S. meliloti* strains in YMB medium using microfermentor technique (Bayoumi *et al*., 1995a and 1995b) at six concentration (0, 10, 20, 40, 80 and 160mgl^{-1}) of each salt. Using spectrophotometer (DR-2000 model) under different conditions at wavelength 550nm and compared with the rate of growth in control culture.

For *Trichoderma* strains, a colony disc of active mycelium of fresh growth colony with 5mm in diameter was placed carefully in the center of in 250 ml Erlenmeyer flask containing 50 ml of SYB medium contaminated with two cadmium salts at different concentrations (0, 10, 20, 40, 80 and 160mgl^{-1}). The flasks were incubated at 28°C for 5 days on a rotary shaker operating at 50 rpm.

2.10 Phytoremediation of Cd polluted soil:

The soil remediation ability of the plant-bacteria symbiosis was tested using a sterilized soil with or without Cd contamination, which was supplied as $CdCl_2$ or $CdSO_4$ solution to obtain a Cd concentration of 0, 60, and 120$mgkg^{-1}$ dry weight soil. The pots contained a mixture of each 4 kg of soil and approximately $1x10^6$ cells of sinorhizobial strain per seedling. Six seedlings of alfalfa, which were three days old after germination (*M.sativa* seeds were surface-sterilized, sown 0.7% agar plate and incubated for three days in the dark at 25°C), were transferred to one pot containing one of Cd concentration mentioned above in addition to one of *Trichoderma* strains and grown under light (16h photoperiod per a day) at 25-28°C for 8 weeks. Shoots, roots and nodules were harvested from 8 weeks-old plants. Cd concentration was measured directly from the soluble fraction using AAS.

2.11 Monitoring the biological N_2-fixation under Cd stress:

The experiment was designed to detect the interactions between the two cadmium salts amendment and the biological N_2 –fixation in brown forest soil. The study was conducted in pot experiment predicated to different concentrations of cadmium doses. The cadmium salts were applied to the soil before the plantation and plant inoculation. Generally, the following experiments of predicating the impacts of cadmium on the plant-microbe

interactions in addition to coinoculate the system with *Trichoderma* strains (H1-2b *T. harzianum*, 131-2 *T.viride*) were carried out using one Hungarian cultivar of alfalfa, three new isolated strains of *S. meliloti* (GHF-162, GHF-281 and GHF-3153), one standard strain (GHR-94) and two cadmium salts at three concentrations (0, 60 and 120 mg kg^{-1} soil) and performed in the greenhouse.

Seeds were selected for healthy and uniformity without any injury, and surface sterilized with 70 % ethanol followed by acidified 0.2 % $HgCl_2$ for 5 minutes, and thoroughly washed in several changes of sterile distilled water. Seeds were then soaked for 8h at room temperature in sterile distilled water (soaking water was changed every 2 h) and then seeds were germinated on sterile moisted filter paper in large petri dish for 72h in the dark at 28℃ according to Franco and Vincent, (1976). Sterile water was added to the germinated seeds when required. Under a sceptic conditions, eight seedlings were transplanted into each pot and covered with a layer of approximately 2cm of sterile soil. Pots were cellophane covered. Seven days after transplanting, pots were thinned to four seedlings pot^{-1}. Seedling rhizosphere was inoculated with 10 ml of a suspension of biofertilizer *Sinorhizobium* inoculum (GHR-94, GHF-162, GHF-281, or GHF- 3153) prepared as follows: each one of the five biofertilizer *S. meliloti* strains was grown in YMB for 48h at 28℃, to give a final cell concentration $1x10^6$ cell capacity ml^{-1}, using hemocytometer for calibration. Seedlings were watered with sterile tap water when required, and the plants grown under natural illumination 14h photoperiod at around 28±2℃ according to Ta and Faris, (1988). Consequently, the inoculated plant seedlings were coinoculated with one of the following *Trichoderma harzianum* (H1-2b) or *Trichoderma viride* (131-2).

The experiment was conducted in three replicate for each of treatment. Seedlings were watered with sterile distilled water when required. The plants were grown under natural illumination (16h photoperiod) at around 26-18±2℃. The experiment was conducted for 8 weeks, and then plants were carefully uprooted, and washed several times in tap water for farther investigations.

2.12 Plant growth parameters:

Data recorded/ plant as follows: Height of plant shoot in cm, number of root nodules. Dry weight of plants biomass and nodules were determined after oven dried at 75℃ to a constant weight and the values were expressed as g $plant^{-1}$ and mg root nodules $plant^{-1}$. Total N-content (mg $plant^{-1}$) was measured using micro-Kjeldahl method as a criterion of N_2–fixation (Burris, 1974). Cd concentrations were measured after digestion of the air-dried plant samples with HNO_3 + H_2O_2, followed by inductively coupled plasma atomic emission spectrometry (ICP- AES), as described by Mikanova *et al*., (2001).

2.13 Measurement of chlorophyll and total N, P and K contents:

The chlorophyll a and b of alfalfa leaves was extracted with 80% (v:v) acetone at 8 weeks after transplanting and estimated by a procedure as described by Lichtenthaler, (1987). The sample size for this measurement was a 5 mm diameter leaf. The total N, P and K contents in shoots of alfalfa plants were analyzed as described by Mingorance, (2002) using a microwave system after digestion of samples with H_2SO_4+ H_2O_2.Total N and P were determined by colorimetry using automatic air-segment continuous flow analysis and K was analyzed by flame photometry.

2.14 Statistical analyses

Values are mean of three to eight replicates. All data were subjected to one-way analysis of variance. Data were processed by analysis of variance (ANOVA) and Fisher's protected least significant differences (LSD) when appropriate (Sokal and Rohlf 1981).

III. RESULT

3.1 SELECTION OF *S. MELILOTI* STRAINS

Using different microbiological assays and the growth on various growth media and pure cultures, the number of the isolates was reduced. Therefore, a final 18 out of 55 bacterial isolates were collected from different root-nodules of various plant samples and then this number of isolates was reduced by the help of plant inoculation to become 8 according to the faster and early development of root nodules on the roots of alfalfa plants.

3.1.1 Performance of nodulation formation and plant dry weight

Table 1 shows the differences between the strains on the competitive ability in the nodule formation (nodule number per plant). When the strains have the ability to form nodule, it means that they are able to survive and multiply in the environment under normal conditions (without stressed factors). These results gave an account on the mode of the strains to colonize the rhizosphere and the roots of the host plant.

Table 1. Plant dry weight and number of nodules per plant as criteria for the ability to form effective N2-fixing nodules with the host plant (authentication).

Inoculation with isolates	Plant dry matter (mg)	Number of nodules per plant
Uninoculated plant	153	0
Standard: GHR-94	231	10
Standard: GH-130	254	10
GHF-131	241	10
GHF-162	412	14
GHF-1120	225	9
GHF-1141	228	9
GHF-214	244	10
GHF-230	312	13
GHF-243	267	10
GHF-270	281	11
GHF-281	457	14
GHF-290	288	11
GHF-2100	315	13
GHF-2130	309	12
GHF-321	297	11
GHF-353	326	13
GHF-372	317	13
GHF-3111	303	12
GHF-3150	331	13
GHF-3153	461	14

3.2 In vitro tolerance of inoculant strains to Cd salts

3.2.1 For sinorhizobial biofertizer inoculants:

The effect of cadmium in chloride and sulfate forms on the relative growth rates of the selected *Sinorhizobium* strains are presented in Fig 1 & 2. The percentage relative growth of sinorhizobial strains was obtained in comparing with the control. Results clearly demonstrate that the addition of cadmium affected growth conditions differentially with the concentrations of the salt. It was found that by increasing the Cd concentration in the growth medium the growth rate of the strains decreased. But, GHR-94, GHF-162, GHF-281 and GHF-3153 strains of *Sinorhizobium* were the most tolerant against the concentrations of $CdCl_2$ (Fig 1). Similar results were obtained in case of treated the growth medium with $CdSO_4$ (Fig 2). This figure shows that the relative growth rates of the strains were more inhibited by concentrations of the salt investigated. Comparatively, it was found that the effect of sulfate form of the cadmium salt was more toxic to the Sinorhizobium strains.

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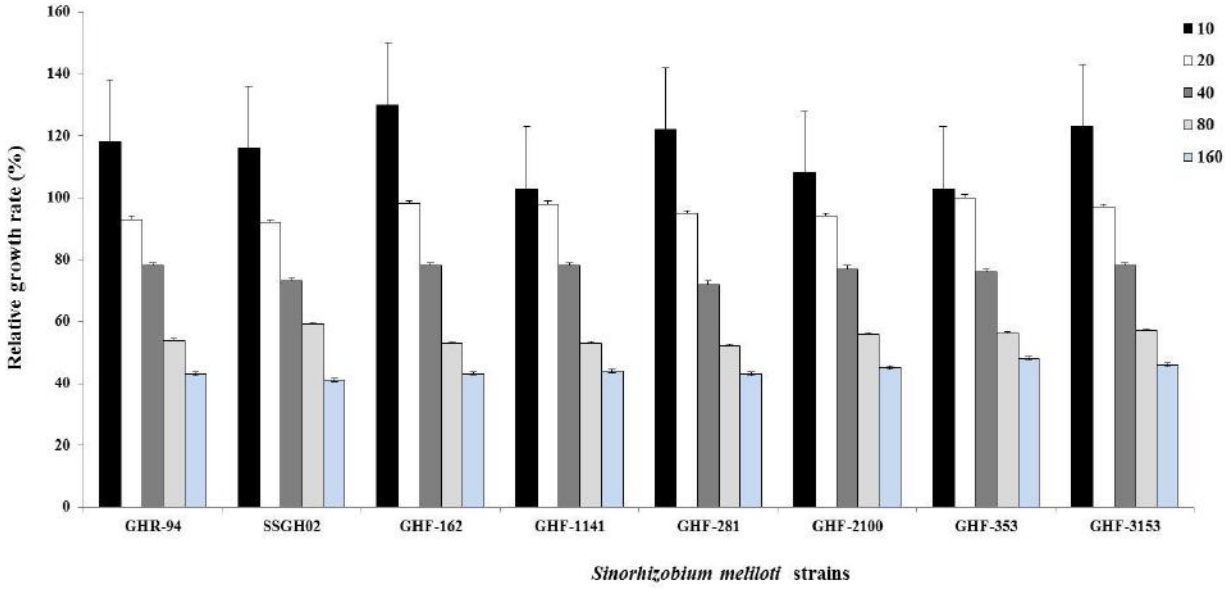

Fig. 1: Effect of cadmium chloride concentrations on the relative growth rate Sinorhizobium meliloti strains.

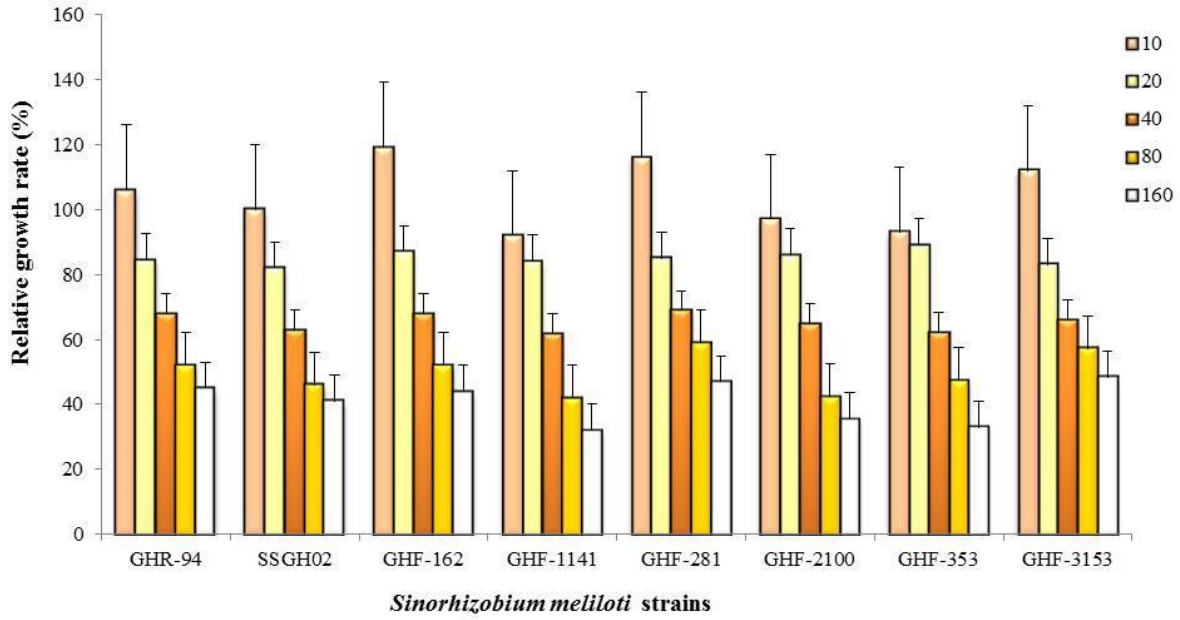

Fig. 2: Effect of cadmium sulfate concentrations on the relative growth rate Sinorhizobium meliloti strains

3.2.2 For Trichodermal coinoculants:

In SY broth medium, effect of different concentrations (0, 10, 20, 40, 80 and 160 mgl^{-1}) of Cd salts were tested and the results showed that the *Trichoderma* strains were tolerated the low concentrations in term of dry weight of fungal colony. But by increasing the concentrations the dry weight of fungal colony was decreased. Fig 3 shows that no significant differences among the strains toward $CdCl_2$ concentrations, and the *T. harizanum* (H1-2b) was more tolerant strain andCu-3 of *T. koningii* was more sensitive to the $CdCl_2$. Fig 4 demonstrates that *T. harizanum* was the most tolerant strain to $CdSO_4$ than *T. viride* and *T. koningii*. Comparatively, the results of this investigation showed that $CdSO_4$ had more toxic effects on the colony dry weight of the three Trichoderma strains than $CdCl_2$.

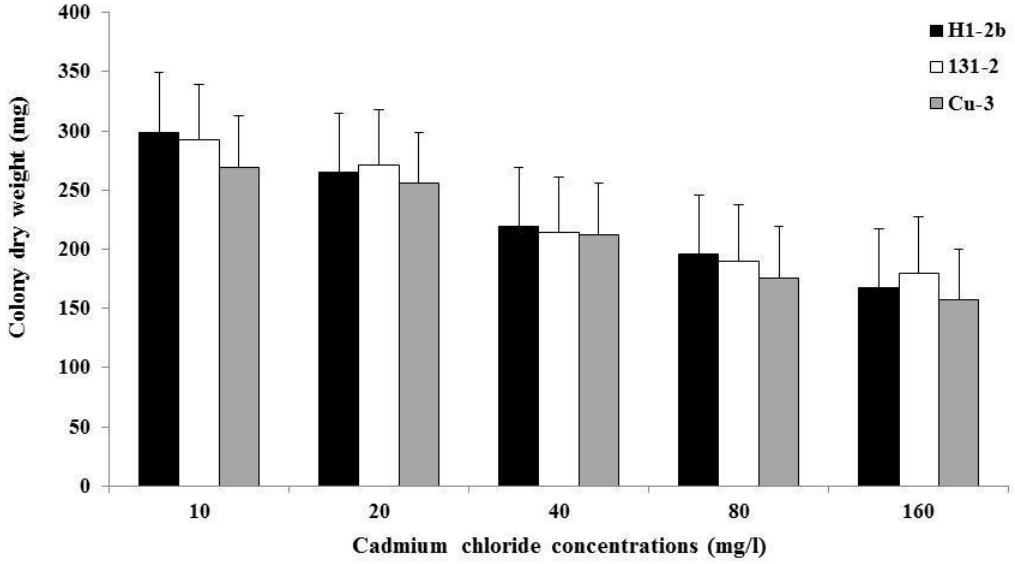

Fig. 3: Effect of cadmium chloride on the colonial dry weight of Trichoderma strains

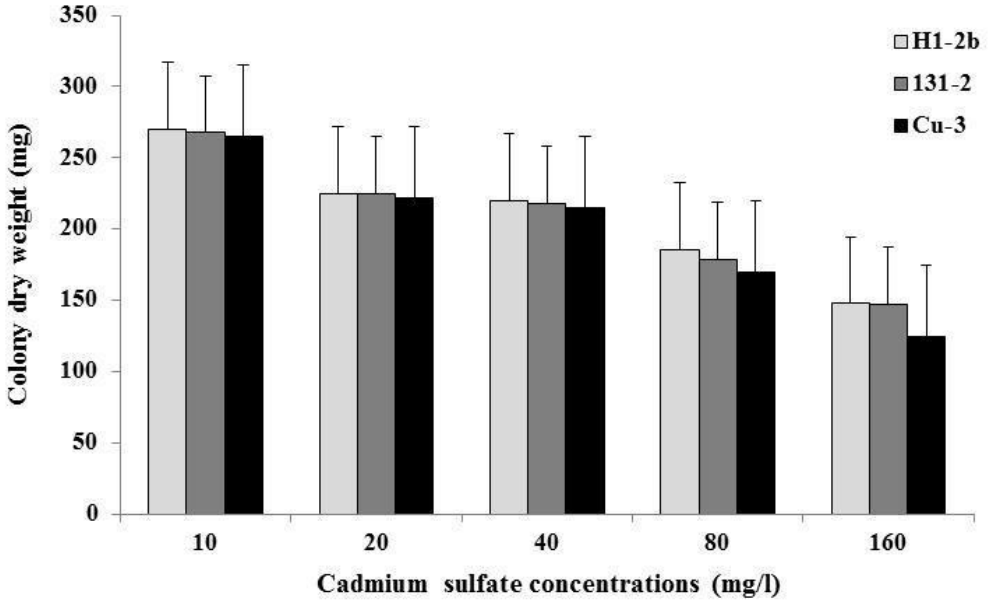

Fig. 4: Effect of cadmium sulfate on the colonial dry weight of Trichoderma strains

3.3 In vivo experiments

3.3.1 Effect of Cd on plant biomass

Generally, the addition of *Trichoderma* strains to the Cd-treated soil as co-inoculating agent improved the plant biomass (shoot and root) of the plant inoculated by four *S. meliloti* strains. The results for the effect of cadmium on the biomass of the alfalfa plants with the inoculants and coinoculants are present in Table 2. All plants were grown even in the presence of the highest concentration (120mg Cd/kg soil). In all cases of the Cd, the biomass per plant varied directly with the metal concentrations applied to the soil and depended on the biofertilizer and coinoculants strain. There were significant differences between the biomass throughout the all plants and the highest concentration of Cd applied to the soil. Moreover, that biomass which was measured at 60mg/kg was significantly increased compared with those at 120mg/kg. The results of this study showed that the coinoculation with *T.harzianum* gave more nodule numbers compared with those coinoculated with *T. viride* strains especially when the plant inoculated by GHF-3153 followed by GHF-281, GHF-162 and GHR-94. It was noted that the R/S ratios were high in all cases which were coinoculated with *Trichoderma* strains and this gave evidence that the *Trichoderma* strains are proved to be plant growth promoting agents. Previously, in the present work, it was

found that the two strains of *Trichoderma* are proved also to be nodule promoting agents due to the increasing the nodule numbers compared with those grown on the roots of plants only inoculated with the biofertilizers. The results indicated that the soil amended with $CdSO_4$ had more toxic effect on the investigated parameters of shoot and root dry weight and R/S ratio than $CdCl_2$.

Table.2: Shoot and root dry weight of Medicago sativa grown in Cd-contaminated soil at concentrations 60 and 120 mg/kg soil and inoculated with Sinorhizobium meliloti in presence or in absence of Trichoderma harzianum and Trichoderma viride strains

Treatments	Shoot dry weight (g)		Root dry weight (g)		R/S ratio	
	T.harzianum	***T. viride***	***T.harzianum***	***T. viride***	***T.harzianum***	***T. viride***
Control	1.76	1.72	0.42	0.41	0.21	0.21
Control+60mg $CdCl_2$	2.02	1.95	0.55a	0.54a	0.3a	0.28
Control+120mg $CdCl_2$	1.96	1.94	0.59	0.57a	0.32a	03a
GHR-94+60mg $CdCl_2$	2.24a	2.16a	0.67a	0.61a	0.4a	0.38a
GHR-94+120mg $CdCl_2$	2.11a	2.03a	0.61a	0.62a	0.35a	0.29a
GHF-162+60mg $CdCl_2$	2.25a	2.34a	0.66a	0.64a	0.41a	0.33a
GHF-162+120mg $CdCl_2$	2.08a	2.02	0.69a	0.63aa	0.4a	0.38a
GHF-281+60mg $CdCl_2$	2.68a	2.61a	0.76a	0.72a	0.45a	0.41a
GHF-281+120mg $CdCl_2$	2.58a	2.49a	0.74a	0.64a	0.48a	0.45a
GHF-3153+60mg $CdCl_2$	2.77a	2.68a	0.81a	0.70a	0.49a	0.48a
GHF-3153+120mg $CdCl_2$	2.72a	2.64a	0.77a	0.69a	0.49a	0.48a
Control+60mg $CdSO_4$	1.85	1.81	0.52	0.5	0.26	0.26
Control+120mg $CdSO_4$	1.76	1.74	0.49	0.47	0.27	0.26
GHR-94+60mg $CdSO_4$	1.95	1.86	0.57a	0.51a	0.3a	0.28
GHR-94+120mg $CdSO_4$	1.91	1.83	0.53a	0.52a	0.27	0.26
GHF-162+60mg $CdSO_4$	1.95	1.94	0.56a	0.54a	0.31a	0.30a
GHF-162+120mg $CdSO_4$	1.98	1.92	0.59a	0.53a	0.34a	0.32a
GHF-281+60mg $CdSO_4$	2.08a	2.01	0.66a	0.65a	0.35a	0.33a
GHF-281+120mg $CdSO_4$	2.25a	2.22a	0.71a	0.65a	0.38a	0.35a
GHF-3153+60mg $CdSO_4$	2.47a	2.38a	0.82a	0.77a	0.39a	0.38a
GHF-3153+120mg $CdSO_4$	2.62a	2.54a	0.67a	0.61a	0.42a	0.42a
LSD (*P*=0.05)	0.32	0.31	0.11	0.09	0.08	0.08

Column values followed by the (a) are significantly different with control according to Fisher's LSD test (*P*=0.05).

3.3.2 Effect of Cd on nodulation

Generally, the addition of *Trichoderma* strains to the Cd-treated soil as co-inoculating agent improved the nodulation potent of the four *S. meliloti* strains. The data for the effect of cadmium on the nodulation potential of alfalfa plants with the inoculants are present in Table 3. All plants were nodulated even in the presence of the highest concentration (120mg Cd/kg soil), but with a few number of nodules. In all cases of the Cd, the number of nodules per plant varied directly with the metal concentrations applied to the soil. There were significant differences between the number of nodules throughout the all plants and the highest concentration of Cd applied to the soil. Moreover, that number of root nodules which was counted at 60 mg/kg was significantly increased compared with those at 120 mg/kg.

The results of this study showed that the coinoculation with *T.harzianum* gave more nodule numbers compared with those coinoculated with *T. viride* strain especially when the plant inoculated by GHF-3153 followed by GHF-281, GHF-162 and GHR-94. Similarly, it was found that the nodule dry weight was decreased in plants inoculated with the biofertilizers compared with those of inoculated with biofertilizers and coinoculated with *Trichoderma* strains. The root nodule biomass on the roots of plants coinoculated with *T.harzianum* was higher than those coinoculated with *T. viride* strain in presence of any of biofertilizers.

The data in Table 3 demonstrates that the best nodule number and nodule dry weight was found at the combination of plant grown in soil amended with 60 mg Cd ($CdCl_2$)/kg inoculated with *Sinorhizobium meliloti* strain GHF-3135 and coinoculated with *T.harzianum*

strain. While the lowest nodule number and nodule dry weight was found at the combination of plant grown in soil amended with 120 mg Cd ($CdSO_4$)/kg inoculated with *Sinorhizobium meliloti* strain GHR-94 and coinoculated with *Trichoderma viride* strains.

Table. 3: Number of nodules and nodules dry weight of Medicago sativa grown in Cd-contaminated soil inoculated with Sinorhizobium meliloti in presence or in absence of Trichoderma strains.

Treatments	Number of nodules/plant		Nodules dry weight (mg)	
	T.harzianum	***T. viride***	***T.harzianum***	***T. viride***
Control	0	0	0	0
Control+60mg $CdCl_2$	0	0	0	0
Control+120mg $CdCl_2$	0	0	0	0
GHR-94+60mg $CdCl_2$	32	29	121	118
GHR-94+120mg $CdCl_2$	26	21	99	78
GHF-162+60mg $CdCl_2$	41a	34	143	174a
GHF-162+120mg $CdCl_2$	34	29	175a	122
GHF-281+60mg $CdCl_2$	54a	42a	211a	171a
GHF-281+120mg $CdCl_2$	43a	33	176a	134
GHF-3153+60mg $CdCl_2$	65a	51a	224a	199a
GHF-3153+120mg $CdCl_2$	51a	39a	196a	162a
GHR-94+60mg $CdSO_4$	28	25	116	98
GHR-94+120mg $CdSO_4$	23	19	92	81
GHF-162+60mg $CdSO_4$	37a	31	129	122
GHF-162+120mg $CdSO_4$	31	26	123	102
GHF-281+60mg $CdSO_4$	49a	38a	196a	155a
GHF-281+120mg $CdSO_4$	39	30	156	121
GHF-3153+60mg $CdSO_4$	58a	46a	248a	201a
GHF-3153+120mg $CdSO_4$	51a	39a	202a	162a
LSD (*P*=0.05)	15.5	14.8	74.8	62.5

Column values followed by the (a) are significantly different with control according to Fisher's LSD test (*P*=0.05).

Comparatively, the results showed high significant differences (at *P*=0.05) in the plant inoculated by either GHF-3153 or GHF-281 in the presence of *Trichoderma harzianum* strains and grew in soil polluted with 60 mg Cd/kg**.**

3.3.3 Effect of Cd on total chlorophylls content

The results of the present study showed that the addition of *Trichoderma* strains to the Cd amended soil as co-inoculating agent improved the chlorophyll content in the plant leaves especially those incoculated with the four *S. meliloti* strains compared with the control plants. The data for the effect of cadmium on the chlorophyll content of the alfalfa plants with the inoculants are present in Fig 5. All plants were grown even in the presence of the highest concentration (120 mg Cd/kg soil), and the leaves were more healthy than those of control pots or even better than those of only inoculated by biofertilizers or coinoculated with *Trichoderma* strains only. In all cases of the Cd, the chlorophyll content per plant varied directly with the metal concentrations applied to the soil as well as with the microbial inoculation. There were significant differences between the chlorophyll content throughout the all plants and the highest concentration of Cd applied to the soil. Moreover, that chlorophyll content at 60 mg/kg was significantly increased compared with those at 120mg/kg.

The results of this study showed that the coinoculation with *T. harzianum* increased the chlorophyll content compared with those coinoculated with *T. viride* strain especially when the plant inoculated by GHR-94 followed by GHF-3153, GHF-281 and GHF-162. The lowest chlorophyll content was at control plant grown in untreated soil and control plant grown in soil amended with 120mg Cd/kg.

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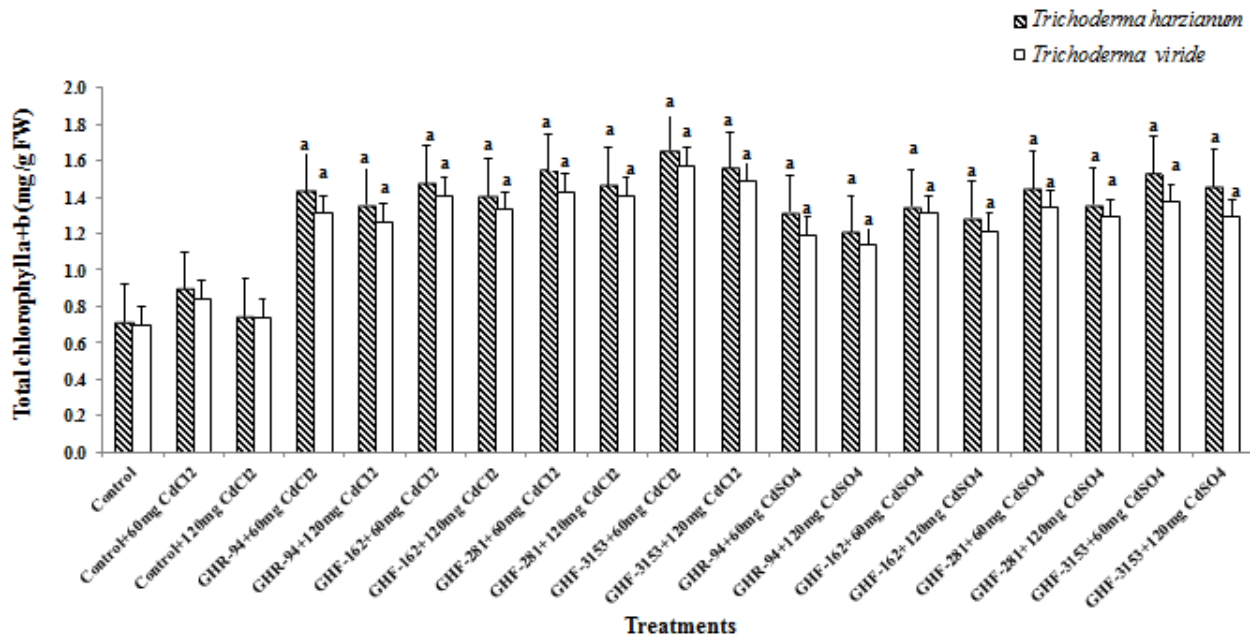

Fig. 5: Total chlorophyll of Medicago sativa grown in Cd-contaminated soil inoculated with Sinorhizobium meliloti in presence or in absence of Trichoderma strains.

3.3.4 Effect of Cd on shoot N, P and K content

Generally, it was found that $CdCl_2$ more stimulated the bioaccumulation of N, P and K in plant shoot more than those plants grew in soil polluted with $CdSO_4$. Table 4 shows that the all parameters of the investigations recorded in $CdCl_2$ soil pollution were higher in N, P and K content in plant shoot than those measured in soil polluted with $CdSO_4$. Also, the higher investigated parameters were registered in plants inoculated with GHF-3153 or GHF-281 and coinoculated with *T.harzianum* strain and grew in 60mg Cd/ kg polluted soil. The results of the present study showed that even in $CdSO_4$ or in CdCl2 polluted soils, plant nutritional status give significant differences under most of the treatments at P=0.05 compared with the control.

Table. 4: Shoot N, P and K content of Medicago sativa grown in Cd contaminated soil and inoculated with sinorhizobium meliloti in presence or in absence of Trichoderma strains.

Treatments	**N content (mg/plant)**		**P (mg)**		**K (mg)**	
	T.harzianum	***T. viride***	***T.harzianum***	***T. viride***	***T.harzianum***	***T. viride***
Control	114	97	31	29	42	38
Control+60mg $CdCl_2$	125	121	37	33	46	43
Control+120mg $CdCl_2$	146	137	45a	43a	55a	52a
GHR-94+60mg $CdCl_2$	187a	174a	52a	46a	61a	58a
GHR-94+120mg $CdCl_2$	177a	165a	45a	44a	56a	54a
GHF-162+60mg $CdCl_2$	201a	189a	66a	61a	67a	62a
GHF-162+120mg $CdCl_2$	187a	179a	52a	49a	62a	59a
GHF-281+60mg $CdCl_2$	235a	224a	74a	69a	76a	71a
GHF-281+120mg $CdCl_2$	211a	208a	54a	51a	59	55a
GHF-3153+60mg $CdCl_2$	254a	247a	82a	76a	82a	74a
GHF-3153+120mg $CdCl_2$	239a	232a	56a	52a	66a	63a
Control+60mg $CdSO_4$	115a	111	32	30	41	39
Control+120mg $CdSO_4$	137	131	41	39	35	31
GHR-94+60mg $CdSO_4$	167a	154a	52a	42	51a	48a
GHR-94+120mg $CdSO_4$	155	147a	44	41	50	47a
GHF-162+60mg $CdSO_4$	181a	179a	56a	51a	57a	52a
GHF-162+120mg $CdSO_4$	176a	165a	53a	45a	52a	49a

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Treatments	N content (mg/plant)		P (mg)		K (mg)	
	T.harzianum	*T. viride*	*T.harzianum*	*T. viride*	*T.harzianum*	*T. viride*
GHF-281+60mg $CdSO_4$	224a	218a	61a	56a	72a	70a
GHF-281+120mg $CdSO_4$	219a	206a	56a	53a	69a	65a
GHF-3153+60mg $CdSO_4$	244a	232a	72a	66a	72a	64a
GHF-3153+120mg $CdSO_4$	229a	221	65a	64a	68a	61a
LSD (*P*=0.05)	43.5	43.6	13.4	12.6	12.3	11.5

Column values followed by the (a) are significantly different with control according to Fisher's LSD test (P = 0.05).

3.3.5 Rhizobioremediation and Cd accumulation in shoot:

Fig 6 demonstrates the Cd uptake by plant shoot throughout the interactions between the rhizomicroorganisms and the absorption surfaces of the plant root. The present study showed that the Cd uptake by plant roots with the association with the biofertilizer Sinorhizobium and Trichoderma as coinoculating agents was higher in soil polluted with $CdSO_4$ than in soil polluted with $CdCl_2$. The more Cd accumulation in the shoot of the alfalfa plant was more in plant roots coinoculate with *Trichoderma harzianum* than those coinoculated with *Trichoderma viride*. Also, the amount of Cd detected in the shoot of the plant was more in those plants grown in the soil polluted with 120 mg Cd/kg than in soil polluted with 60 mg Cd/kg. The roots inoculated with GHF-3153 and GHF-281 has the ability to absorb Cd in high polluted soil better than the soil polluted with low dose of Cd. From the Fig 6 below, the results can summarized as following: Highest Cd accumulation in alfalfa shoot is found the case of: (a) Inoculationg the plant roots with GHF-3153 or by GHF-281 and coinoculating with *Trichoderma harzianum* and grow in soil polluted with 120 mg Cd ($CdSO_4$)/kg. (b) Inoculationg the plant roots with GHF-3153 or by GHF-281 and coinoculating with *Trichoderma harzianum* and grows in soil polluted with 120 mg Cd ($CdCl_2$)/kg.

Lowest Cd accumulation in alfalfa shoot is found the case of: (a) Control and (b) Plant grown in soil of low Cd concentration. The use of term rhizobiremediation her can be replace the term phytoremediation because that the complete system of cooperation is interacted together to tolerate the system against the free Cd ions and allow some of them to be bioabsorbed and bioaccumulated in the plant parts as well as accumulated in the cells of *Sinorhizobium* or *Trichoderma*.

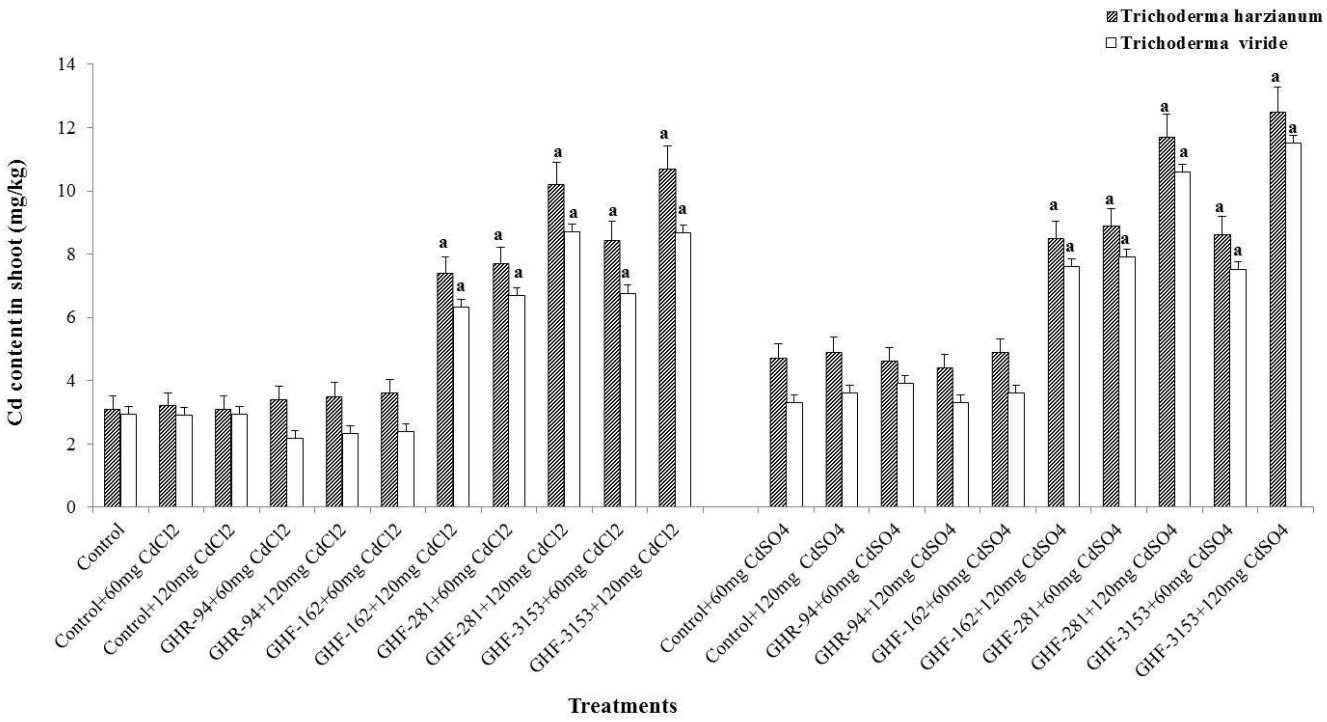

Fig. 6: Shoot Cd content of Medicago sativa grown in Cd amended soil and inoculated with Sinorhizobium meliloti in presence or in absence of Trichoderma strains. Bar values indicate by (a) are significantly different with control according to Fisher's LSD test (P= 0.05).

IV. DISCUSSION

Morrissey *et al*., (2004) reported that one major challenge for the twenty-first century will be the production of sufficient food-the United Nations Population Fund estimates that the global human population may well reach 10 billion by 2050. This means increasing agricultural productivity of food crops, as plants form the basis of every food chain. The introduction of metal pollutants in various forms in the environment can pose a severe threat to the ecological system due to their negative impact on most life forms (Jaiswal and Malik 2000, Gavrilesea 2004). Although, some amounts of heavy metals are required by all life forms, however, there is a threshold limit to this requirement (Cervantes and Corona 1994). At high concentrations, heavy metal ions react to form toxic compounds in cells (Nies 1999, Choudhury and Spain 2003). Another major problem with metals is their persistence as they tend to persist indefinitely in the food chain (Gupta *et al*. 2000, Aleem *et al*., 2003). The conventional treatment procedures used for removal of metals are uneconomical (Say *et al.*, 2001). Therefore, there is a need to develop rapid, economical and environmentally benign technology for the removal of metals from industrial effluents. There are certain microorganisms, which can survive in high concentrations of metals and have the potential to accumulate different metals. This is achieved by the virtue of covalent interaction of metal at cell surface or within the cell by different processes (Gadd and White 1993, Bhanoori and Venkateswerlu, 2000). These microbes can be of immense significance in the clean-up of heavy metals from the environment.

Naár and Biró (2006) established that the rate of Cd pollution significantly correlated with the frequency of four of six *Trichoderma* spp. presumably, the Cd tolerance of the isolates from differently contaminated plots is similar. This fact was supported by an earlier study, in which Cd tolerance of *T. harzianum, T. virens, T. viride* isolates, originating from soils with different Cd pollution levels were studied. The authors said that no considerable difference was found in the minimum inhibitory concentration for *in vitro* growth rate on Cd containing medium. Our *in vitro* results showed that the Trichoderma strains can tolerate the different concentration of Cd in broth medium. Harman *et al*. (2004) stated that *Trichoderma* spp. Are free –living fungi that are common in soil and root ecosystems. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients. Kleifeld and Chet (1992) mentioned that the fungus *T. harzianum* which was applied to pathogen-free soil induced an increase in emergence of seedlings, plant height, leaf area and dry weight. The results of the present study confirm the remarks and we are in agreement with the authors regarding to the increase of the plant dry weight. Bayoumi *et al*., (1995b) studied the effects of different abiotic factors (acidity, salinity, nitrate and temperature) on growth rate of root-nodule bacteria (*Rhizobium* and *Bradyrhizobium*) strains were investigated *in vitro*. Strains isolated from *Vicia faba L*., *Coronilla varia* L. and *Lupinus albus* L. exhibited a large variation in tolerance of the above mentioned factors. These bacteria should be screened under stimulated conditions for enhanced survival before selection to be used for commercial inoculant production. Linear correlation matrix data were useful to find the appropriate concentrations for the selection of the tolerant strains. This conclusion can be applied to the data obtained during the experimental records. The statistical examinations carried out on the collected results confirm also that conclusion of Bayoumi *et al*., (1995b). Alfredo *et al*., (2006) hypothesized that the remediation measures would reduce heavy metal solubility, increase soil fertility and enhance soil microbial functionality. Also they hypothesized that the addition of different amendments and the development of a root system might induce shifts in the microbial community structure among the different treatments. We are in agreement with these concepts with the regard to the increases of nutritional status in the alfalfa biofertilized and coinoculated by functional microbial groups. Naár *et al*., (2002) mentioned that the rate and direction of correlations, however, varied with the type of heavy metals when pot experiment was carried out to test the relation between two beneficial microorganisms in Cd, Zn and Ni polluted soil. The recorded results are in agreement with these conclusions respecting the mode of action of the microbial functions on the plant production and the nutrient content in the plants. The most remarkable result of this study is the survival of *Sinorhizobium* strains in the heavy metal contaminated soil which appear to be sensitive to high concentrations of applied cadmium salts.

These results were in agreement with the conclusion of Pálagyi *et al*., (2004) mentioned that the success of a sinorhizobial inoculant in the soil depends to a large extent on its capacity to compete against indigenous strains. GSM03, a *S. meliloti* strain with enhanced competitiveness for nodule occupancy, was recently established in soil amended with heavy metals containing high doses of Cd, Cu and Pb ion concentrations. The results allowed us to differentiate between alterations in the microbial community apparently caused by inoculation and by the rhizosphere effect induced by the alfalfa plants and by the environment. Only moderate inoculation-dependent effects could be detected, while the

alfalfa plants appeared to have a much stronger influence on the microbial community. There are increasing evidence of adverse effects on microbial processes related to nutrient cycling in these types of soils. Applications of organic fertilizers with low concentrations of heavy metals with improve soil fertility in reclaimed soils. Furthermore, the legume-root nodule symbiosis can be used as an effective parameter for ecotoxicological evaluation of contaminated soils, Smith, (1997). However, in our experiment these factors were similar for all treatments.

Our results clearly demonstrate that small concentrations of Cd in soil cannot cause reductions in the number of rhizobia and indirectly in the nodule number too. These results are in agreement with Giller *et al*., (1993). Al-Kahal *et al*., (2001) mentioned that metal uptake by grains seemed to be directly related to the concentration of heavy metals and was greater in the case of an individual metal added separately than in combination. The results of the present study support this conclusion, but our results showed that the accumulation of Cd in the plant shoot was depended on the microbial inoculation and the amount of Cd present in the contaminated soil.

V. CONCLUSION

The results indicate that *Medicago sativa* is suitable to grow and rehabilitate Cd-polluted soils when inoculated specifically with *Sinorhizobium meliloti* tolerant to cadmium salts at various concentrations. Some other soil microorganisms like and *Trichoderma* fungi can further improve the chances to recover these contaminated sites and bring them back into cultivation. The association of *Medicago sativa* with Cd-resistant sinorhizobial strains can further help to improve the resistance of alfalfa to Cd. It can be assumed that such legumes will also support the N nutrition of the *Medicago sativa* provided that an effective *Sinorhizobim-Medicago* association can be established under Cd-polluted conditions.

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Immuno-Modulatory Activity of Aqueous Leaf Extract of *Moringa Oleifera* in Brioler Chickens

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***Abstract*—** *This experiment was conducted to investigate the immuno-modulatory activity of aqueous leaf extract of Moringa oleifera on immune response of broiler chickens to Newcastle disease (ND) vaccinations. The performance, blood parameters and serum biochemistry of the birds were also determined. A total of one hundred and twenty (120) day-old commercial broiler chicks were randomly allotted to 3 dietary treatments of 4 replicates each. Each replicate had 10 birds. The treatments: T1 – Control group in which the birds were not given any extract; T2 and T3 – birds in these groups were given the prepared stock solution of Moringa oleifera leaf extract at dose rate of 2500mg/kg and 5000mg/kg of body weight in drinking water. The experimental birds were vaccinated with ND vaccines using a stipulated vaccination regime. The Moringa oleifera leaf extract exhibited significant ($p \leq 0.05$) influence on final body weight of the experimental broiler chickens with birds in T3 having an average weight of 1947.43g and birds in T1had 1733.33g. The immune modulating effect of the leaf extract was insignificant ($p \geq 0.05$) though it elicited higher antibody titre of $Log_2 7$ and $Log_2 9$ in birds in T3 compared to $Log_2 6$ and $Log_2 8$ of birds in control group after the first and second ND vaccinations respectively. The leaf extract caused significant ($p \leq 0.05$) increase in white blood cells and leucocytes count. The study concluded that the plant extract had slight immune stimulatory effects on response to ND vaccinations and improved the growth performance of broiler chickens.*

Keywords*— *Antibody, aqueous, growth, immune response, Moringa oleifera.

I. INTRODUCTION

Poultry production provides base for the socioeconomic advancement in the majority of developing countries and this has led to increased demand for poultry products especially broiler meat. This is because consumers perceive that it is a healthy product that contains less fat, predominantly unsaturated fatty acids, and particularly polyunsaturated fatty acids, compared to beef or pork products.

The continuing survival and growth of the broiler chicken industry in developing countries of the world depends on its ability to compete globally, which is largely dependent on the efficiency of its production system. It is a common practice in the management of poultry to administer antibiotics in drinking water as growth promoters and to prevent or control infectious bacterial diseases. The benefit of such practice is to maintain good health, suppress mortality of birds, and to support maximal growth via improved utilization of nutrients and ultimately improve profit (Zeweil *et al.*, 2006). However, the use of synthetically-produced substances especially antibiotic growth promoters was soon found to have objectionable side-effects (Makanjuola *et al.*, 2014). This has led to antibiotics growth promoter being banned mainly due not only to cross-resistance but also to multiple resistances.

Therefore due to the desire for improved economic status in poultry production, researchers revolutionized the application of feed and water additives by focusing on organic or natural supplements instead of using synthetic medicament (Zeweil *et al.*, 2006). The use of medicinal plant either alone or in group (combination) as possible therapeutic measures has become a subject of active scientific investigation (Oyewole, 2012). Some medicinal plant products are known to enhance natural resistance of host to infection due to the presence of bioactive phytochemicals or phyto-nutrients (Soetan and Oyewole, 2009).

Moringa (*Moringa oleifera* Lam.) is a multipurpose tropical tree and it has been dubbed the "miracle tree" or "tree of life" in popular media (Bosch, 2004; Orwa *et al.*, 2009; Radovich, 2013; FAO, 2014) mainly because it is used for food and has numerous industrial, medicinal and agricultural uses, including animal feeding. Moringa leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidant compounds such as flavonoids, phenolics and carotenoids (Anwar and Bhanger, 2003). It is one of the herbs containing bioceutical agents that could substitute synthetic growth enhancers and supplements in broiler and other livestock production since it possesses important medicinal properties which include antibacterial and antifungal activities (Nickon *et al.*, 2008).). In Nigeria, leaf preparations of *Moringa oleifera* is widely used in

folklore for the treatment of immune system related disorders (Oyewo *et al*., 2013).

This study was done to investigate the effect of aqueous leaf extraction of *Moringa oleifera* in drinking water of broiler chickens on their growth performance and immune response to Newcastle disease vaccinations.

II. MATERIALS AND METHODS

2.1 Study site

This study was approved by the Research Committee of the Department of Animal Production and Health, The Federal University of Technology, Akure (FUTA) Nigeria. The field trial was conducted at the Poultry unit of the Teaching and Research Farm of FUTA, Nigeria. The laboratory analyses were done at the Microbiology Laboratory of the Department of Animal Production and Health and Central Research Laboratory, FUTA.

2.2 Aqueous Leaf Extraction of *Moringa oleifera*

Fresh leaves of the plant were harvested and air-dryed under normal environmental conditions. The air-dried leaves were ground before extraction and soaked in distilled water for 24 hours using ratio 1:2 (weight/volume). The preparation was then filtered to separate the debris and filtrate using Whatman's filter paper. The filtrate was collected, the solvent was removed using rotary evaporator and the residue obtained after evaporation was weighed. The concentrated stock solution of *Moringa* leaf extract was prepared by dissolving 500g of the residue in 1 litre of sterile distilled water and stored at 4^{o}C. The concentrated extract at calculated doses was administered in fresh drinking water which was served to the birds on a daily basis during the period of study.

2.3 Experimental design and Animal Management

A total of one hundred and twenty (120) day-old broiler chicks of the Abor acre breed purchased from a reputable hatchery in Akure, Ondo State, Nigeria were used for the study which lasted for a period of 6 weeks. The birds were reared on deep litter using routine management procedures as outlined by the Teaching and Research Farm of FUTA and feed and water provided *ad libitum.* The chicks were divided into three treatment groups (T1, T2 and T3) with four replicates of 10 birds each using a completely randomized design. Birds in control group (T1) were not given any extract while birds in T2 and T3 received the prepared stock solution of *Moringa oleifera* leaf extract at dose rate of 2500mg/kg and 5000mg/kg of body weight respectively in their drinking water. The experimental chickens were vaccinated with Newcastle disease vaccines (NDV) - NDV intra-ocular (Hithner B1 strain) at 3 days old and NDV LaSota via the oral route at 28 days old. The study was conducted for a period of 6 weeks.

2.4 Performance criteria measurement:

Initial weight of birds was measured at day old, then on a weekly basis for the final weight. Thereafter weight gain for each week over the trial period was measured as the difference between the initial weight and the final weight. The feed consumption was recorded per replicate and the feed conversion ratio calculated as a ratio of feed consumed to weight gain of birds per replicate.

2.5 Blood and Sera collection:

Samples of blood for the purpose of serum analysis were collected from 3 birds per replicate in each treatment group before the trial commenced via the heart to determine baseline maternal antibody titre levels against Newcastle disease. The birds were sedated using chloroform before the bleeding exercise. Thereafter, in each treatment 12 birds (3 per replicate) were randomly selected and blood was collected 14 days after administering each of the ND vaccines through the jugular vein for serological analysis to determine the antibody titre values. At the end of the 6 weeks experimental period blood was also collected for haematological and serum protein biochemistry analysis from 12 birds in each treatment.

2.6 Laboratory Analysis

2.6.1 Haematological Analysis

Erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (HB) and white blood cell differentials were analysed as described by (Lamb, 1981). The Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were also calculated accordingly.

2.6.2 Haemagglutination and Haemagglutination Inhibition Test (HA/HI Test)

Serum samples taken from the experimental broiler chickens were analysed using beta (β) micro haemagglutination inhibition technique as described by Thayer and Beard, (1998) to determine the antibody titre levels. The antibody titre is a measure of the humoral immune response elicited in the experimental birds to Newcastle disease vaccinations.

2.6.3 Serum Biochemical Analysis

Diagnostic kits (Randox Laboratories, UK Test Kits) was used to analyse serum biochemical parameters (Total protein, Globulin, Albumin, Alanine transferase, Alaninie phosphatise, Aspartate transaminase) of the experimental broiler chickens.

2.7 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA). Where significant differences were observed, the mean were separated using the SAS statistical package (2012).

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III. RESULTS

3.1 Performance Characteristics

Table 1 presents the performance characteristics of the experimental broiler chickens. The administration of the *Moringa oleifera* leaf extract resulted in significant ($p \leq 0.05$) increase in the FBW and WG of experimental birds. The FBW for birds in treatment 3 (1947.43g) was the highest and significantly ($p \leq 0.05$) different from that of birds in treatment 1 (1733.33g). The WG result followed a similar trend with birds in T3 gaining an average weight of 1911.43g and birds in T1 having 1733.33g. The table also shows that the FBW and WG of birds given the leaf extract (birds in T1 and T2) were not significantly ($p \geq 0.05$) different regardless of the dose. The FI and FCR though not significantly ($p \geq 0.05$) influenced by administration of leaf extract were seen to be highest as the dose increased.

3.2 Immunological Responses

In Table 2 the antibody titre values of the experimental birds in response to ND vaccinations are shown. The table revealed that the birds had a uniform maternal antibody titre value of $Log_2 4$ across the treatments. However after the ND vaccinations, administration of the leaf extracts elicited higher titre values with no significant ($p \geq 0.05$) effect with increasing dose. The birds in T2 and T3 had similar antibody titre values of $Log_2 7$ and $Log_2 9$ while those in T1 had $Log_2 6$ and $Log_2 8$ after the first and second ND vaccinations respectively.

3.3 Haematological Parameters

The blood parameters of the experimental broiler chickens are shown in Table 3. In all the indices measured only the WBC and lymphocyte counts were significantly ($p \leq 0.05$) influenced by administration of the leaf extracts. The WBC values of birds in T2 (2.61×10^6 mm^3) and T3 (2.66×10^6 mm^3) were similar but significantly ($p \leq 0.05$) different from that of birds in T1 (2.51×10^6 mm^3). The lymphocyte counts also followed similar trend. The PCV and MCV values though not significantly ($p \geq 0.05$) different amongst treatments decreased with higher doses of the leaf extracts. It was observed that the ESR, RBC, Hb, MCH, MCHC values increased in experimental birds with no significant ($p \geq 0.05$) differences among the doses.

3.4 Serum Biochemistry

The trend of the serum biochemical parameters of the broiler chickens measured following administration of the leaf extract are presented in Table 4. The leaf extracts did not have any significant ($p \geq 0.05$) influence on serum indices of the experimental birds. An increase was observed in protein indices (Total protein, albumin and globulin) of the birds administered the leaf extract which was not dose dependent.

IV. DISCUSSION

The immuno-modulatory effects of aqueous leaf extracts of *Moringa oleifera* in broiler chickens in response to Newcastle disease vaccinations as investigated in this present study is negligible. However, the birds given the *Moringa oleifera* leaf extract reportedly had the highest immune response when compared with the birds in the control group ascribing from the antibody titre values. This result is in line with the work of Olugbemi *et al* (2010) who reported that giving *Moringa oleifera* aqueous extract had been shown to have beneficial effect on immune response, thus improving the health status of broiler chickens. It is also in line with the work of Madubuike *et al* (2006) that reported that supplementation of *Moringa oleifera* resulted in marked improvement of humoral immunity of broiler chickens. This study is in accordance with the work of Oyewo *et al.* (2013) which suggested that the aqueous leaf extract of *Moringa oleifera* has immune modulation activities. This immune enhancing attribute of *Moringa oleifera* may be due to the phytochemical constituents such as alkaloids and saponins present in aqueous extracts of the plant as reported by Oyewo *et al*. (2013). Also the mineral content of the aqueous leaf extracts like selenium, zinc, iron, manganese and magnesium have been known to contribute to its immuno-modulatory effect (Prasad, 2000; Ravalglia *et al*. 2000; Oyewo *et al.* 2013).

In this study, inclusion of *Moringa oleifera* leaf extract in water of broiler chickens influenced the performance characteristics. The leaf extracts caused improved productive performance of the experimental broiler chickens judging from the trend of the growth performance traits. This was made obvious, in that birds given the highest dose of *Moringa oleifera* extract exhibited the best performance characteristics which reflected in their body weights. This could be attributed to complete amino acids, considerable amount of vitamins, and mineral content; antioxidant, immuno-stimulant and antibacterial properties of *Moringa oleifera* leaf (Makkar and Becker, 1997; Fahey, 2005; Anwar *et al.*, 2007). It could also be related to the effect of the available nutritional components and some growth stimulating constituents of *Moringa oleifera* which probably resulted in the improvement of live body weight of the chickens (Kakengi *et al.*, 2007). In addition, the minute quantity of anti-nutritional factors that affect palatability of feeds were not implicated to compromise the bioavailability of nutrients and growth stimulating compounds present in *Moringa oleifera* leaves (Foidl *et al.*, 2001). It has also being reported that the crude extract of *Moringa oleifera* like other herbal drug may contain digestion enhancing properties which stimulates favorable growth of good bacteria while decreasing harmful microorganisms (Hernandez *et al.*, 2004). However, the

mechanism by which this herbal product influences the growth performance and gut micro flora of poultry are poorly understood (Hernandez *et al.*, 2004).

The aqueous leaf extract of *Moringa oleifera* led to a boost in the total white blood cell and lymphocytes counts in experimental broiler chickens. This further reiterates it's immunomodulation capability, since white blood cells are involved in fighting infection and clearing off injured or dead cells and tissues in body (Jeremy *et al*., 2001; Oyewo *et al*., 2013). The trend in antibodies produced in response to ND vaccinations in experimental birds also supports the result of the total white blood cell and lymphocyte counts. The other haematological parameters were not influenced by the leaf extract and values obtained fell within the normal range for healthy chickens as described by Animashahun *et al* (2006). This indicates that aqueous extract of *Moringa oleifera* leaves did not have any detrimental effect on physiological indices measured. This is however different from the study of Oyewo et al., (2013) where it was reported that the administration of aqueous leaf extract of Moringa oleifera induced anaemia in wistar rats which might have been caused by haemolysis of erythrocytes, due to the levels of saponins and heavy metals (lead) in the aqueous leaf extract. A note of caution was therefore suggested in the use of the crude extract of *Moringa oleifera* in high doses because of the levels of heavy metals in the aqueous leaf extract.

Evaluation of serum biochemical parameters may provide useful information in the assessment of health status of birds and serves to reflect many metabolic alterations of organs and tissues when feeding unconventional feed sources (Kudair and Al-Hussary 2010). In this study, the serum biochemical parameters of the experimental chickens was not influenced by leaf extracts of *Moringa oliefera*. This result of the serum biochemical profile is not in line with the previous work of Adedapo *et al* (2009) who reported significant effect of *Moringa oleifera* extracts on serum ALT, AST and ALP which led him to conclude that high doses of the aqueous extract could lead to liver damage. It is however supported by the work of (Kudair and Al-Hussary 2010) that reported that *Moringa oleifera* leaf has no significant effect on the serum biochemical indices of broiler chickens.

V. CONCLUSION

It can be concluded in this study that *Moringa oleifera* leaf extracts served to moderately boost immunological responses to ND vaccinations, though increasing the dose did not enhance the suggested immunomodulatory activity. It also achieved improved productive performance in the broiler chickens. However, there is need for further investigation to reiterate the capability of *Moringa oleifera* aqueous leaf extract as a substitute to synthetic supplements such as antibiotics in improving broiler production.

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Table.1: Growth Performance of Broiler Chickens birds given varying doses of Moringa oleifera leaf aqueous extract

Parameters	Treatment 1	Treatment 2	Treatment 3	±SEM
Initial weight(g)	35	35	36	0.17
FBW(g)	1768.33[b]	1863.57[a]	1947.43[a]	0.05
WG(g)	1733.33[b]	1828.57[a]	1911.43[a]	0.04
FI(g)	2140.48	2192.86	2259.52	27.15
FCR	1.23	1.20	1.18	0.12

FBW- Final Body Weight; WG- Weight Gain; FI- Feed Intake; FCR- Feed Conversion Ratio
Treatment 1- Birds not given leaf extract of *Moringa oleifera*
Treatment 2- Birds given 2500mg/kg dose of *Moringa oleifera* leaf extract
Treatment 3- Birds given 5000mg/kg dose of *Moringa oleifera* leaf extract

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Table.2: Antibody titre values of the broiler chickens given varying doses of Moringa oleifera aqueous extract following Newcastle disease vaccinations

Treatments	Baseline antibody titre values	Antibody titre Values after 1st NDV	Antibody titre values after 2nd NDV
Treatment 1	$Log_2 4$	$Log_2 6$	$Log_2 8$
Treatment 2	$Log_2 4$	$Log_2 7$	$Log_2 9$
Treatment 3	$Log_2 4$	$Log_2 7$	$Log_2 9$

NDV- Newcastle disease vaccinations

Treatment 1- Treatment 1- Birds not given leaf extract of *Moringa oleifera*

Treatment 2- Birds given 2500mg/kg dose of *Moringa oleifera* leaf extract

Treatment 3- Birds given 5000mg/kg dose of *Moringa oleifera* leaf extract

Table.3: Haematological variables of broiler chickens given Moringa oleifera leaf extracts

PARAMETERS	Treatment 1	Treatment 2	Treatment 3	+SEM
ESR(mm/hr)	3.66	3.80	4.16	0.65
PCV (%)	26.83	26.66	26.16	1.04
RBC($\times 10^6 mm^3$)	256.33	258.13	259.41	10.53
HB(g/100 ml)	8.71	8.89	8.95	0.34
WBC($\times 10^6 mm^3$)	2.51 [b]	2.61[a]	2.66[a]	0.05
MCV(μ^3)	104.66	103.28	100.84	0.33
MCHC (%)	32.46	33.34	34.21	0.40
MCH (Pg)	33.97	34.44	34.50	0.13
LYM (%)	62.26[b]	64.00[a]	64.26[a]	0.04
HETE (%)	19.30	19.33	19.83	1.19
MONO (%)	12.43	12.66	12.50	1.05
BASO (%)	3.33	3.33	3.50	0.22
EOS (%)	1.50	1.66	1.66	0.02

ESR –Erythrocyte Sedimentation rate, PCV – Packed Cell Volume, RBC – Red Blood Cell, HB - Haemoglobin, MCV - Mean Cell Volume, MCHC - Mean Cell Haemoglobin concentration, MCH - Mean Cell Haemoglobin, LYM – lymphochytes, HETE – M0NO – Monocyte, BASO – Basopils, EOS – Eosinophil

Treatment 1- Treatment 1- Birds not given leaf extract of *Moringa oleifera*

Treatment 2- Birds given 2500mg/kg dose of *Moringa oleifera* leaf extract

Treatment 3- Birds given 5000mg/kg dose of *Moringa oleifera* leaf extract

Table.4: Serum metabolites of broiler chickens administered varying doses of Moringa oleifera leaf aqueous extract

TREATMENT	TP(g/dl)	ALB(g/dl)	GLO(g/dl)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
T1	30.79	14.50	16.29	79.31	129.50	109.37
T2	36.76	14.12	22.64	78.03	123.00	114.75
T3	31.27	14.61	16.66	71.67	112.83	118.24
+SEM	4.54	0.83	14.59	0.98	21.75	14.59

TP-Total protein; ALB- Albumin; GLO- Globulin; ALT- Alanine transferase;
AST- Aspartate Transaminase; ALP: Alanine Phosphatase

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Rehabilitation and Reintroduction of wild born orphan chimpanzee (*Pan troglodytes)* within the pongo and okokong islands of the douala-edea wildlife reserve, Littoral Region Cameroon

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Abstract— *This study had as main objective to document on the reintroduction of chimpanzees in the Douala-Edea Wildlife Reserve which appears to be the first case of chimpanzee reintroduction in Cameroon. The study was carried out in the South East zone of the Douala-Edea Wildlife Reserve which holds a small chimpanzee sanctuary under the auspices of "Papaye France" association. Data was collected on the field using semi structured questionnaires, interviews and direct observations alongside a participatory action approach at the sanctuary. Data from discussion guide and questionnaires were descriptively analysed and discussed with respect to our objectives. There were 24 orphan chimpanzees all together present in the zone and being cared for by the association PAPAYE France. This association has released 16 chimpanzees on two Islands of the reserve, the first group made of 9 chimpanzees (6 males and 3 females) were released in 2008 on the Pongo Island and a second group made of 6 chimpanzees (4 males and 2 females) were released on the Okokong Island in 2010; one female was later introduced to this group early 2015. These chimpanzees were released after a rehabilitation process not in line with IUCN guidelines for reintroduction of great apes and not following any developed scientific approach or methodology. Despite this, the released chimps are faring well as new births have been recorded on either Islands, chimps feed, nest, movement and vocalize indicating there have gotten adapted to live on the Islands. It was also noted that the sizes of these islands may not maintain a viable, nutritionally self-sustaining population in the long run hence could better serve as a semi naturalistic sanctuary. It is necessary that the carrying capacities of the islands be determined while larger potential release sites be assessed and prepared for an eventual transfer/reintroduction of these apes in the future.*

Keywords— Rehabilitation, reintroduction, orphan chimpanzee, Pongo and Okokong Islands, Douala-Edea Wildlife Reserve.

I. INTRODUCTION

The common chimpanzee (*Pan troglodytes),* listed as Endangered on the IUCN Red List since 1996 andin CITES Appendix I prohibiting any form of international trade (UNEP-WCMC 2011), is threatened in Cameroon by habitat loss as a result of resource extraction and land conversion, as well as illegal hunting, pet trade, and disease (Oates *et al*. 2008). The cumulative world population of chimpanzees has declined by more than 66 % over the past 40 years passing from 600,000 to less than 200,000 individuals (Butynski, 2001; Kormos *et al*., 2003) and on the other hand, some researchers have estimated that an 80% reduction is likely to occur over the next thirty-three years and this will leave the chimpanzee as "critically endangered" (Walsh *et al.* 2003). To protect chimpanzees from extinction we must address the root causes of numerous threats, including habitat loss, the illegal bushmeat exploitation and the exotic pet trade, armed conflict, and infectious disease; and also provide long lasting solutions of surveillance protection by Park Rangers or Ecological guards in our protected areas.

There is a continual growth in the number of "chimpanzee orphans" especially from the bushmeat trade as commercial bushmeat hunters (poachers) kill many chimpanzees every year. Infants, too small to be killed for meat, are often put on the "black market" for sale as pets or entertainers. The illegal trade of baby chimpanzees has become a source of

significant mortality in wild population not only for the collection of babies but also because the capture of a baby is usually done after the mother and other group members have been killed. For each sale of a young chimpanzee, it is estimated that between 10 and 29 others have perished during the process of capture or transportation (Carter 2003). It can therefore be estimated that the pet trade has potentially affected as much as 7–20% of the wild population in recent years, assuming a total wild population size of 150,000, which is the lower limit reported by Butynski(2001).

Currently, chimpanzees face local extinctions in areas previously considered to be their last strongholds (Walsh *et al*., 2003; Campbell *et al*., 2008; Greengrass, 2009). Given the severity of threats to wild populations, a diverse range of conservation approaches should be considered. Chimpanzee sanctuaries and Reintroduction of chimpanzees from captivity is one strategy to help restore dwindling wild populations (Goossens *et al*., 2005; Beck *et al*., 2007).

The IUCN African Primate Action Plan does not currently recommend reintroduction as a conservation action plan for any primate species (Oates, 1996). But in recognition of the potentially important role of release efforts from captivity for the conservation of chimpanzees and other great apes, the International Union for the Conservation of Nature (IUCN) reintroduction specialist group of the species survival commission (IUCN-SSC/RSG) has elaborated and published specific guidelines for reintroductions of great apes (Soorae and Baker, 2002; Beck *et al*., 2007). Reintroductions typically refer to attempts to re-establish a species within its historic range, in an area where it is locally extinct (Soorae and Baker, 2002; Beck *et al*., 2007).Reintroduction addresses conservation on two different levels. First, animals that are kept illegally as pets are rescued, rehabilitated, and then returned to the wild; and secondly, by reintroducing animals into areas where they are locally extinct, the wild populations are supplemented and potentially more forest can be protected (Cheyne, 2006).

The government of Cameroon seems to opt for these two levels of conservation as in the Douala-Edea wildlife reserve, is an association named Papaye France that runs a small sanctuary caring for orphan chimpanzees. The main goal of this association is to contribute to the conservation of chimpanzees by collecting, rehabilitating young chimps and releasing them into natural habitats where they strive to survive with greater independence though still under strict control and care. This association has attempted to rehabilitate and release some chimpanzees on two Islands in the reserve. This study therefore aims at characterizing the population of released chimpanzees; describing their rehabilitation and reintroduction process; and other management activities of the chimps while pointing out the major problems and opportunities presented in the management of these chimps.

II. METHODOLOGY

Study area

This study was carried out in the Douala-Edea wildlife reserve which is located in the Coastal area of the Littoral Region; in the Sanaga Maritime Division where it covers part of the Edea 1 subdivision, Mouanko subdivision and the Wouri Division where it covers part of Manoka subdivision. Its geographic coordinates lie between latitude 3° 14' and 3°53' N and longitude 9°34' and 10°03' E of the Greenwich meridian. It has an area of about 1,600 km^2 (160,000 ha). Its limits extend from the Atlantic coast for a distance of 35 km inland, with its Eastern boundary along river Dipombé, bounded in the West by the Atlantic Ocean, in the North by river Wouri and in the South by the Nyong River (Nzooh *et al*., 2005).

The reserve is characterized by a typical equatorial climate with average annual rainfall ranging from 3,000 to 4,000 mm.The months of December and January are relatively dry (50mm of rain). Starting in February, rains become more abundant with a peak in June followed by a slight decline variable; a new peak in rainfall occurs from August to October. The monthly average temperature varies throughout the year from 24° C to 29 ° C.

The reserve is located entirely within a sedimentary low plain, from 0 to 60 m (rarely up to 80 m). This plain is crossed by rivers and swamps that provide the only relief to this very flat topography with major rivers being: River Sanaga, Kwakwa, Wouri, Lofe, Mvia and the largest surface water is Lake Tissongo. Also present are other smaller rivers, streams and creeks as well as part of the downward basin of River Dibamba falling into the Wouri estuary (ELF Serepca, 1987).

Like many sites in Cameroon, no systematic inventory of vertebrates has been done (WTG, 2008). However, the presence of a great variety of vegetation among which also extends marine waters provokes a great diversity of land and aquatic fauna. Primates/monkeys typical of the African forest are present in the area. These include: Putty-nosed Mangabey (*Cercopitecus nictitans)*, Colobus monkey (*Colobus satanas*), *Cercopitecus pogoniasgray*i. Chimpanzees (*Pan troglodytes*) and Forest Elephants (*Loxodonta Africana cyclotis*)are also present. Other species

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include Bush pig (*Potamochoeruslarvatus*), Forest antelope (*Tragelaphus euryceros)*, Sitatunga (*Tragelaphus spekii*)and the highly threatened West African Manatee (*Trichechus senegalensis*)(Ajonina *et al.*, 2005).

The vast extended waters of the estuaries, Sanaga River and Lake Tissongo provide habitats favorable to many bird species. This ecosystem has diversified population of birds estimated at more than 35 species divided into 22 families with protected species such as the winged duck (*Pteronetta hartlaubii*) and the African Gray Parrot (*Psittacus erithacus*) (Van der Waarde *et al.*, 2007).

Over 135 fish species in 21 families with 21 endemics have been known from river Sanaga (Ticheler, 2000). Some of the fish species found in the Douala-Edea marine habitats include the Machoirons (*Arius spp*), Carp (*Lutjanus endecanthus*), Tilapia (*Tilapia zilli*) and Mackerel (*Scomberromorus spp*). Some of the reptile species found locally include: Crocodiles (*Tylosurus crocodiles)*, Serpents (*Alligator spp)*, and the fresh water turtles and land tortoises (*Testudinidae* family) (CWCS, 1998; WTG, 2008).

Choice of site

The study was carried out in the South East zone of the Douala-Edea Wildlife Reserve (DEWR) which holds a small chimpanzee sanctuary under the auspices of "Papaye France" association (Figure 1). The association occupies two islands in which chimpanzees have been released and is based in a camp holding younger chimpanzees being prepared for release. The association uses Islands for this process because the chimpanzee movements are limited and access can easily be controlled for security purposes. This area was chosen so as to document on this chimpanzee release carried out by Papaye France which might be the first reintroduction to be realized in Cameroon. Therefore documenting this release process and evaluating its success may contribute to scientific knowledge on chimpanzee rehabilitation and reintroductions.

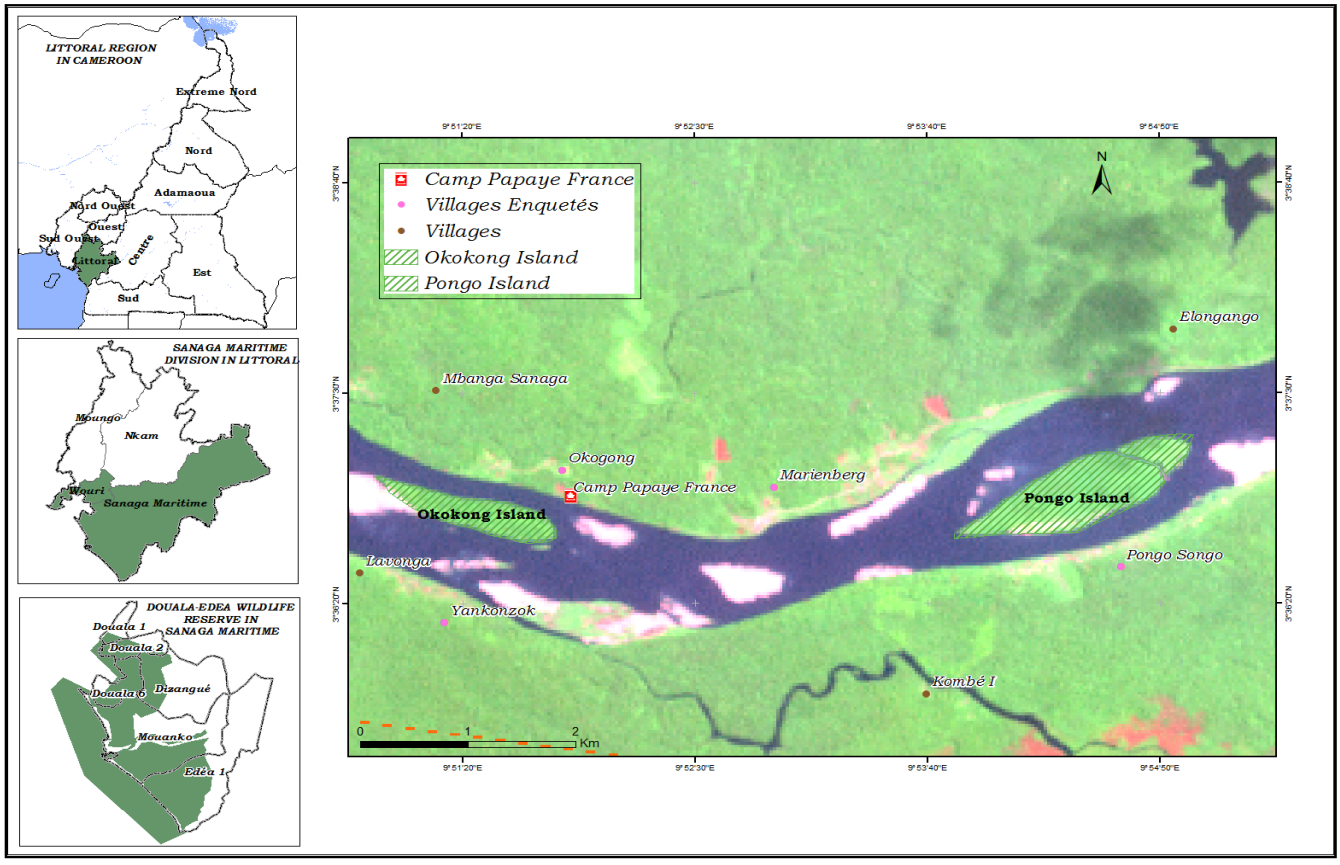

Fig.1: Map of Cameroon showing the location of Littoral Region and that of DEWR showing the two release sites (Pongo and Okokong islands)

Data collection

Primary and secondary data were collected. Secondary data was gotten from past reports, scientific journals and other documents related to the study. Primary data were collected in the field using three main research instruments (interviews, direct observations and participatory action).

Interviews

Interviews were conducted with thepersonnel (manager and caregivers) of Papaye France working at the sanctuary and Forestry agents (Ministry of Forestry and Wildlife) at the conservation service of the reserve. Seven persons all together were interviewed; 4 personnel of Papaye France (manager and caregivers) and 3 forestry agents at the conservation service. These interviews were done with the help of a discussion guide drafted following the guidelines for chimpanzee and sanctuary management (Cox *et al.* 2000). These were conducted in order to get information on the management of the chimps and the sanctuary and also to characterize the population of orphan chimps being cared for.

Direct observations and participatory action

At the level of the rescue center of the association, information was obtained using a participatory approach (that is actively taking part in the daily activities which had to do with the care and follow up of the chimpanzees). This approach gave a better understanding of the management of the orphan chimpanzees, and the functioning of the association. Observations were done at the camp and on the islands. Following the recommendations of Cox *et al* 2000, observations were focused particularly on the important considerations for chimpanzee welfare (feeding regime of the chimps, the rehabilitation process undergone by the young chimps, the health and veterinary protocols and general management of the sanctuary). Also attention was drawn to the relationship between the sanctuary and the surrounding populations as well as human-chimpanzee interaction in the zone. A period of 3-4 days a week for a month was spent at the sanctuary observing and recording their daily activity budgets.

During these field observations, a GPS of mark GARMINE S60 was used to take geographic coordinates in the zone which permitted the mapping of the study area; and also the surface area of the Islands estimated. In order to estimate the area of the islands, a start point was chosen on one of the sides of the island and the coordinate noted. The tracklog option of the GPS was activated while going round the Island on an engine boat (staying as close as possible to the island) and coming back to the starting point. This exercise permitted a closed track whose area was automatically generated by the GPS.

Data analysis

Data from discussion guide were descriptively analysed using the statistical package for social science (SPSS) and discussed with respect to our objectives. Discussions were mainly comparisons and critics based on consulted literature.

Data gotten from the GPS was extracted using MAPSOURCE and transferred into ArcGIS10.0 software which was used to produce the map of the study area showing the Islands and also estimating their surface area.

III. RESULTS

Characterizing the orphan chimpanzee populations

There are presently twenty four orphan chimpanzees being cared for and followed up by the association PAPAYE France. These chimpanzees are divided into three groups (Adult group on the Pongo Island, the adolescent group on the Okokong Island, and the babies or young chimps in the camp). The characteristics of these chimpanzees can be seen on table 1, 2 and 3 below.

Table.1: Characteristics of chimpanzees on the Pongo Island

Identification of chimpanzee	Sex	Age/ Years	Date of arrival in camp	Date of release on Island	Origin of chimpanzee	Observation
Citron	M	23	2003	2008	South	Dominant male
Bambou	M	21	2003	2008	south	Died in February 2015
Tony	M	18	2003	2008	/	
Café	M	19	2003	2008		
Charly	M	23	2003	2008	Kribi and its surroundings	Largest chimp

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Identification of chimpanzee	Sex	Age/ Years	Date of arrival in camp	Date of release on Island	Origin of chimpanzee	Observation
Bobby	M	22	2003	2008	/	
Samba	F	23	2003	2008	/	Nursing mother
Wengue	F	22	2003	2008	/	Nursing mother
Mangue	F	23	2003	2008		Nursing mother
Victoire	M	6	/	/	Born on the Island (2009)	Young of Mangue
Pistache	M	3	/	/	Born on the Island (2012)	Young of Samba
Passion	M	2	/	/	Born on the Island (2013)	Young of Wengue
Cacaouette	M	1.5	/	/	Born on the Island (2014)	Young kid of Mangue

Table 1 shows that there were nine adult chimpanzees (6 males and 3 females) initially released on the Island in 2008. The chimps all came from the South Region of Cameroon (around Kribi, Djoum, Sangmelima and its surrounding localities where the association Papaye France started) indicating they belong to central chimpanzee subspecies (*P. t. troglodytes*). The chimpanzees arrived Pongo from Kribi in 2003 at an average age of 9.5years.These chimps were held in a camp and taken care of till 2008 when they were released on the Pongo Island at an average age of 14.5 years. All the three females released have put to birth with Mangue, one of the females having put to birth twice making 4 kids thus giving a total population of 13 chimps on the Pongo Island.

Unfortunately, one death has been registered on the Island. Bambou, one of the eldest males was lost in March 2015 due to an unpleasant incident of conflict with some villagers who severely injured the chimp with a machete.

Table.2: Characteristics of chimpanzees on the Okokong Island

Identification of chimpanzee (pseudo)	Sex	Age/ years	Date of arrival	Date of release on Island	Origin of chimpanzee	observations
Artimis	F	14	2005	2010	/	Breastfeeding
Nénufar	M	13	2004	2010	/	Dominant male
Etoile	F	13	2004	2010	/	
Patchouli	M	12	2005	2010	/	
Kiwi	M	11	2006	2010	/	
Che Guevara	M	11	2006	2010	/	
Kanel	F	9	2007	2015	/	
Pomme	-	4months	/	/	Born on the island (April 2015)	Newly born to Artimis

There are seven chimps (4 males and 3 females) on this Island of ages varying between nine and fourteen years plus a baby chimpanzee recently born to Artimis (the eldest female on the island). No records could be found on the acquisition of the chimps and their zones of origin which could be an indicator of the subspecies, thus it is possible to have two different subspecies (*Pan troglodytestroglodytes* and *Pan troglodytes ellioti*) cohabitating the island which could lead to the birth of a new hybrid. These chimps were released on the Island in 2010 after a rehabilitation period of 4-5years for most of the chimps.

Theyoungest chimps are found at the center where all new chimps are received and cared for before any subsequent release is envisaged. Table 3 below presents characteristics of the chimps present at the camp.

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Table.3: Characteristics of chimpanzees in the camp

Identification of chimpanzee (pseudo)	Sex	Age	Origin of chimp	Date of arrival
Miel	F	8	/	2011
Banane	M	3	Douala	2013
Guayave	F	5	/	2012
Mandarine	F	4	Douala	2014

There are presently 4 young chimps at the camp, 1 male and 3 female. These chimps receive intense care and follow up by the caregivers preparing them for a subsequent release on one of the Islands.

IV. DISCUSSIONS

Description of the chimpanzee management

The orphan chimps were either obtained from confiscations (law enforcement by MINFOF) or from donors. They are held in the camp where they receive care and undergo a rehabilitation process (without using the IUCN guidelines of 2010) helping them to socialize with other chimps and regain some natural aptitudes vital for their survival and wellbeing after which they are transferred to the Islands. The management of these chimps can be regrouped under three major aspects;

- Diets and feeding regime of the chimps
- The rehabilitation process proper and
- Their health care and hygiene

Diet/Feeding regime

The majority of the diet of the chimpanzees consists of fruits such as banana, pawpaw, pineapple, water melon and mango depending on their availability. Other food items also given to the chimps include sugar cane, coconuts, and occasionally fritters.

The young chimps at the camp are fed at least three times a day with varying diets. They are given food in the morning (6-9 am) usually bananas and other fruits butin case of fruit shortage, they are given cooked food (pap made of corn flour as shown on figure 2a and b below). In the afternoons around 1pm, and evenings between 5 and 6pm. There is no strictly respected feeding protocol for these chimps, as most of the time, they always have something chewing.

Fig.2a and b: Young Chimps (in camp) feeding in a bowl of corn pap (Atanga Roland)

The adolescent chimpanzees on the Okokong Island are given food 2-3 times a day to supplement their diets. Their diet is made up essentially of fruits. They are given at least 4 baskets (equivalent to a 15litre bucket) of a mixture of cut fruits each day. Their food is brought and dropped on the bank of the river or on the sandy shore (Figure 3) when water levels are low. Besides these fruits given to them, they get a good part of their diet on wild fruits (bush mango, figs and cherry) and other foods on the Island based on the plant phenology during the fruiting season (July to September). During this period, they are given less quantities of food as they complement their diet with wild fruits.

On the other side (the Pongo Island), the adult chimpanzees have achieved a greater level of independence as they are able to feed themselves and their young ones particularly

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during the fruiting season. As was noted by one of the caregivers, they also happen to hunt and kill monkeys that are found on the island to supplement their diet, a characteristic known to chimpanzees (Boesch *et al*. 2002). Although these chimps can get their own food, they are brought 2 baskets of fruits every two days and occasionally more when there are tourist visits. This exercise is simply a means of keeping a regular eye on the chimps, to make sure they are all faring well. Nevertheless these chimps demand greater attention during the dry season (between November and March) as the Island alone is not capable of meeting all their feeding requirements.

Fig.3: Chimpanzees feeding on the sandy shore or river bank (Atanga Roland)

Rehabilitation and Reintroduction process

The chimpanzees on the Pongo Island was the first group to be rehabilitated and released. They were brought to the Pongo village in 2003 where they were kept in a camp simulating their natural habitat and being taken care of. These chimpanzee were taken out on regular basis to the nearby forest as a daily exercise for them so they could play, brachiate, feed on wild foods and learn how to nest. This was the first stage in the rehabilitation of the chimps being done without any respected protocols. The Pongo Island was then noticed offering a possibility for these chimps to be free, secured and far from human contact. An approval was gotten from the Minister in charge of Forestry and Wildlife to transfer these chimps on the Pongo Island who to an extent had already acquired certain natural aptitudes from their daily exercise in the forest. It was then that 9 of the chimps of ages between 12 and 16years were taken to the Islands in 2008. An enclosure was built for them where they stayed with their caregivers. These chimps strayed on the islands during the day and came back in the

evenings to sleep in or around this enclosure, but within 6 months, none of the chimps came back to sleep in the enclosure as they began nesting and sleeping on trees. The enclosure was removed and the caregivers left the island only coming back during the day to observe the chimps and provision them with food and medicines. Within a year (that is in 2009), one of the females gave birth and presently 4 births have been registered on the island.

It was the same scenario with the chimpanzees on the Okokong Island, released 6 (4 males, 2 females) in 2010. One female was added to the group in 2015 and one birth was also registered within the same year. The rapid adaptation of these chimps to the islands and the level of independence reach is mainly due to the fact that all these were wild born and had passed sometime in the wild before being captured. This is in line with Ebua *et al* (2013) stipulating that captive wild-born primates fare well in rehabilitation and reintroduction programs as they easily acquire skills vital for their survival in the wild, captive-born individuals.

The young chimps at the camp are submitted to a training or rehabilitation exercise on a daily basis. This exercise aims at helping the chimps acquire natural social and ecological skills that will permit them survive independently (or with greater independence) in the wild when weaned from human contact. They are taken out of their enclosures every morning (after feeding) between 7:30 and 8:30pm for a walk and training exercise in the nearby forest (Figure 4). The spend 3 to 4 hours in the forest, time during which the chimps jump from one tree to another, harvest wild fruits and certain leaves on which they feed, and play around with each other helping to strengthen social bonds between the chimps. This exercise takes place under the supervision and observation of the caregiver. Nevertheless the chimps do not face much difficulties getting acquainted with the natural milieu as they are all wild born chimps and had spent a few months or years with their parents before being captured. This confirms findings that nonhuman primates, wild born individuals fare well during rehabilitation than captive born individuals (Soorae *et al.*, 2002; Ebua *et al*., 2013).

Fig.4: Young chimps taken out for rehabilitation in the forest (Atanga Roland)

Health care management

Many sanctuaries are established in relatively isolated areas with the result that wildlife veterinary and/or medical expertise is seldom easily accessible. For new chimps arriving the camp, there are first taken for checkup and examinations by a veterinary service present in the town of Edea before they can be brought to the camp. It is the same process for any chimp that falls sick or requires medical attention as there is no veterinary service or technician at the site. The chimpanzees are often given anti biotic mostly in the form of syrup mixed in their food or in water.

As for hygiene and cleaning of the chimp enclosure/cage, they are cleaned and disinfected twice a day.

Research and tourism

There are no developed research or education programs carried out by the association but researchers and volunteers are usually received for short periods of time usually varying between 2 weeks and 3 months. Research type accepted at the site is mostly observational and non-invasive research which may have little or no effect on the chimpanzee behavior. Tourism at the site is an important activity and a main source of fund raising for the care of the chimps and management of the sanctuary. Tourists are received throughout the year with most of them from European countries. An average of 7-8 tourists visit the area each week, this number about doubles on average during the dry season between the months of November and February.

Management problems

http://dx.doi.org/10.22161/ijeab/3.1.8 *ISSN: 2456-1878*

- The sanctuary has no adequately trained personnel (wildlife specialist, no veterinary service, or someone with an educational background related to animal care) who could understand biological and ecological aspects of chimp behavior, keep records which could serve subsequent research or researchers and take management decisions which will ensure the long term welfare of the chimps.
- Chimpanzees in the wild are known to have a home range between 5 and 50 km^2 in forest and woodland habitats (Nowak, 1999). The islands are of small sizes (92ha for Pongo and 46ha for Okokong) and can only support limited chimpanzee population sizes. No ecological habitat assessment has been carried out on the islands therefore its suitability as chimp habitat and carrying capacity is not known. But with respect to the IUCN guidelines for reintroduction of great apes stipulating that Islands less than 500ha with densities of more than 0.1 individual per ha cannot maintain a self-sustaining population (Becks *et al*., 2007). With this, the reproduction capacity of these chimps should be monitored and controlled less it extrapolates and exceeds carrying capacity of the Island.
- There are no developed management protocols drafted and followed for chimpanzee care, feeding, health care, tourist visits and research are done without any strictly respected guidelines or policies and is risky both to the chimps, the caregivers and others.
- One of the most difficult aspects of forming and running a sanctuary is the issue of funding. A realistic financial plan is a critical part of the sanctuary planning process. The management of the sanctuary and the chimps rests entirely in the hands of the promoter of the association and a few benevolent and volunteers. From verbal interview, the financial requirements of running the sanctuary are barely met indicating the need for developing adequate finance methods

Management opportunities

- The Islands of Okokong and Pongo could be better exploited to serve as a semi naturalistic sanctuary and a potential rehabilitation site rather than being considered as a permanent released site (as considered by Papaye France) because it cannot sustain viable populations of chimps in the long run. The area is well secured as it is found within a protected area which benefits from legal protection from the states hence security of the chimps and those working with is assured. Nevertheless an ecological habitat assessment must be carried out to know the suitability of these habitats and the maximum number of chimps they can sustain.
- The zone presents a good touristic potential which if coupled with the sanctuary will attract more tourists, therefore could be a significant source of income for the sanctuary management and to the local communities. Therefore ecotourism should be promoted as it could also be used as an opportunity to educate and raise awareness on the issues of conservation to a wide audience of people.
- Scientific research and education are opportunities presented by the sanctuary for studies and research programs on chimpanzees especially on behavioral aspects as the sanctuary provides an adequate milieu where these chimps can be observed for long periods of time. This could be exploited to better understand and document chimpanzee behavior, promote awareness, improve standards for chimp welfare and could also develop medical research.

Weaknesses of the Rehabilitation and Reintroduction process by PAPAYE France

- Firstly, it should be noted that, the rehabilitation and reintroduction of these chimpanzees was done with little or no apprehension of the IUCN guidelines for the reintroduction of great apes drafted in 2007 (Becks et al., 2007). It was done without much pre-release evaluation and with little post release monitoring. It is done without any scientific approach/methodology
- The camp and rehabilitation site did not respect the minimum distance of 22km from the nearest human settlements, this so as to avoid cases of conflicts with local populations
- These individuals need to be exposed to a predatory awareness training because in captivity they tend to lose their natural talents of identifying and recognizing predators.
- There is need for a proper health and veterinary checks to avoid the retransmission of zoonotic infections and the risk of reintroducing individuals capable of surviving due to deformation, and improper ability to locomote.

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V. CONCLUSION

The released population of chimpanzees (9 on the Pongo Island and 7 on the Okokong Island) are all faring well as they are physically healthy. Four births have been registered on the Pongo Island from all released females while one birth had been registered on the Okokong Island. This being a sign of the welfare of these chimps on the Islands.

The rehabilitation and reintroduction process, though not carried out strictly according to recommended norms seems to be a success as they chimps are getting adapted on life on the Islands. They feed on wild fruits, easily nest, vocalize correctly and those on Pongo have even been noted hunting and killing monkeys which they eat. This adaptation is in part due to the fact that all the chimps were wild born and could easily develop aptitudes necessary for their survival; also the absence of natural predators on the island has contributed to this level of success. But knowing that the success of any reintroduction process can only be measured by the establishment of a nutritionally self-sufficient population (Beck *et al.,* 2007), this reintroduction cannot yet be termed as successful as the feeding of the chimps is still supplemented. In other words, this release process could be a form of rehabilitation on a semi naturalistic sanctuary meanwhile it is recommended that potential release sites be assessed where these chimps may strive better and with greater independence; and a reintroduction be carried out following a well-developed scientific methodology.

Nevertheless chimpanzee management and care is quite a complex issue to deal with and for this reason sanctuaries are recommended to have written policies on all aspects of ongoing care and management of chimpanzees. Information on the chimpanzee behaviors should be recorded daily and kept as it could for serve subsequent research.

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Performance and Meat Quality of Growing Pigs Fed Composite Leaf Meal Premix as an Alternative to Commercial Premix

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***Abstract*—** *This trial was designed to study the effects of using composite leaf meal produced from five (5) different leaves: Cassava, Moringa, fluted pumpkin, African basil and bitter leaves as a premix in the diets of growing pigs. Twenty four large white weaner-pigs were used for this trial comprising six treatments and four replicates with one pig per replicate. Six diets were formulated in which composite leaf meal was fed at 0 (2.5% premix), 10 (2.0% premix), 20 (1.5% premix), 30 (1.0% premix), 40 (0.5% premix) and 50 (0.0% premix) g/kg at the expense of a commercial premix and designated diets I, II, III, IV, V and VI. The pigs were then assigned to these 6 dietary treatments which were fed to the pigs at 5% of their body weight for 8 weeks experimental period. Water was supplied ad libitum throughout the experimental period. All data were subjected to analysis of variance. Results showed that, there was no significant difference (P>0.05) in the final weights of the pigs. Highest final live weight (41.67 ± 0.84 kg) and highest feed intake (75.92 ± 0.06) were recorded in animals fed diet II, while the lowest final live weight (37.67 ± 0.84kg) and lowest feed intake (75.57 ± 0.06) were recorded in animals fed diets V and I, respectively. The eye muscle width of carcass was significantly higher (P<0.05) in pigs fed composite leaf meal diets than those fed the control. The eviscerated weights (kg), head (kg), carcass length (cm) and the relative organs weight of liver, kidney, heart, lungs and spleen (g/kg body weight) were not significantly (P>0.05) influenced by the dietary treatments. The longest carcass length was recorded in animals fed diet VI (73.00 cm), while the shortest length was recorded in animals fed diet III (69.33 cm). For the pH of the meat samples, the highest value (5.77) was recorded in pigs fed diet VI, while the lowest pH (5.74) was recorded in pigs fed diet I. However, there was no significant (P>0.05) treatment effect in the pH values. Generally, the cost of the experimental diets (₦/kg) was least in 50 g/kg composite leaf meal diet (₦55.44/kg) and highest in 0 g/kg composite leaf meal diet (₦60.43/kg) and the percentage cost reduction increased as the level of the composite leaf meal inclusion increased (1.44 – 8.26 %). It could be concluded within the limit of this study, that composite leaf meal had high nutrient potentials for pigs and could completely replace commercial premix in pig diets without any deleterious effect.*

Keywords*— *Pig production, Composite leaf meal, Premix, Growth, Carcass characteristics, Relative organs weight, Meat pH, Eye muscle.

I. INTRODUCTION

The search for least-cost rations has led to the replacement of expensive feeding-stuff with cheaper alternatives like agricultural by-products and leaf meal in the formulation of pig rations. The increasing competition between man and animals for available grains is one of the factors causing protein intake shortages in developing nations like Nigeria. The resultant sub-optimal consumption of animal protein by a greater percentage of Nigerian population has challenged not only livestock farmers, but also researchers and policy makers (Iheukwumere *et al.*, 2007). The realization that feeding alone currently accounts for over 75 % of intensive non-ruminant (poultry and pig) production in the third world countries, including Nigeria has stimulated research interest aimed at exploiting different locally available alternative feeding resources (Agbede and Aletor, 2003). The need to harness the potentials of the numerous agro-industrial by-products and green vegetable plants as replacements for the more expensive conventional feed ingredients have been variously expressed (Agbede and Aletor 2004; Adegbenro *et al.*, 2012). Leaf meals not only serve as a protein source but also provide some necessary vitamins such as vitamin A and C, minerals and also oxycarotenoids, which causes yellow colour of broiler

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skin, shank and egg yolk (Abu *et al.*, 2015). Considerable attention has been focused on leaf meals from Cajanus cajan (Damaris, 2007). This study is therefore seeks to evaluate the effect of feeding varying levels of composite leaf meal as a replacement for premix on the performance, carcass and organ characteristics of growing pigs.

II. MATERIALS AND METHODS

Composite Leaf Meal Production: Leaves from five (5) selected plants (Cassava, Moringa, Fluted pumpkin, African basil and Bitter leaves) were "harvest" and air-dried to prevent loss of some vital nutrients. The air-dried leaves were milled using hammer mill and stored in plastic containers prior to use. Thereafter, the leaves were mixed together in the same ratio (1:1:1:1:1) to produce the composite leaf meal.

Experimental Diets and Site: A basal diet was formulated to meet the requirement of swine (NRC, 1994). The quantity of the commercial premix in the basal diet (Diet I) was reduced by 0, 20, 40, 60, 80 and 100 % replaced with 0, 10, 20, 30, 40 and 50 g/kg of composite leaf meal designated Diets I - VI, respectively as presented in Tables 1 and 2. The feeding trial was carried out at the Livestock Section (Piggery Unit) of the Teaching and Research Farm of The Federal University of Technology, Akure, Nigeria.

Experimental Animals and Management: The statistical design of the experiment was a completely randomized design with a total number of twenty four (24) weanling pigs (*Suis* large white) assigned to six (6) dietary treatments, replicated four times with one (1) pig per replicate. The weight of each pig was measured and recorded as initial weight for the animal. Each animal's weight was recorded and later balanced in order to get the average weight for all the treatments. Thereafter, their respective weaners' diets were fed at 5 % of their body weight for the period of four weeks during which the weekly feed consumption and weight changes were measured and feed conversion ratio were calculated. Thereafter, the growers diets were fed to their respective group from the fifth week to the eight weeks at 5 % of their body weight and the same parameters at the weaners' phase were measured.

Slaughtering of Animals: At the end of the feeding trials, all the animals were starved over night and weighed. The animals were hanged suspended upside down on their hind limbs so as to allow for proper bleeding. The animals were then sacrificed through mechanical stunning severance of the jugular vein. Each slaughtered animal was de-haired using cold water, soap and blade and dressed. The animal's lengths were measured after which they were eviscerated and dissected into parts. The following weights were taken; live weight (kg), dressed weight (kg), eviscerated weight (kg), head (kg), liver (g/kg), heart (g/kg), kidney (g/kg), lungs (g/kg) and spleen (g/kg). Also, the following measurements were taken; carcass length (cm), eye muscle (cm), subcutaneous shoulder fat (cm), loin (cm), fat over mid back (cm) and loin side (cm). The head was removed and carcass split longitudinally down the mid line. The length was measured from the anterior edge of the symphysis pubis to the recess of the first rib. Subcutaneous fat depths were measured on each side over the shoulder, mid back and loin. Sides from each carcass were cut across at a point level with the head of the last rib. The maximum width (A) and depth (B) of the exposed *M. longissimus* were measured. The thickness of the subcutaneous fat surrounding the *M. longissimus* was measured at sites P_2 (65 mm from the dorsal mid line), C (over B at right angle to the skin) and K (at the dorso-lateral corner of the *M. longissimus* and at a right angle to the skin) (Gill *et al.*, 1995). The pH of the meat muscles samples was determined using a digital pH meter (DpH-2 ATAGO®). A sharp pointed knife was used to pierce the intact muscles to about 3 – 4cm and the digital pH meter was immediately inserted into the sample muscles to read the pH. The cost of producing the experimental diets were estimated based on prevailing market prices for the ingredients as at the time of the experiment and percentage cost reduction was evaluated.

Statistical Analyses: Data collected were subjected to one-way analysis of variance using SPSS version 13 package and where significant differences are found; the means were compared using Duncan Multiple Range Test of the same package.

III. RESULTS

Performance and Cost of Production Estimate: The influence of composite leaf meal on the performance of swine indicated that there were no significant differences ($P>0.05$) in final live weight and total feed intake among the treatments as presented in Table 3. Highest final live mean weight (41.67 ± 0.84 kg) and highest mean feed intake (75.92 ± 0.06) were recorded in animals fed 10g/kg composite leaf meal, while lowest final live mean weight (37.67 ± 0.84 kg) and lowest mean feed intake (75.57 ± 0.06 kg) were observed in animals fed 40g/kg and 0g/kg composite leaf meal, respectively. The weight gain and the feed conversion ratio of the experimental animals were significantly influenced ($P<0.05$) by the dietary treatments. Highest mean weight gain (23.67 ± 0.51 kg) was recorded in animals fed 10g/kg composite leaf meal,

while the lowest mean weight gain (19.33 ± 0.51 kg) was observed in animals fed 40g/kg composite leaf meal. For the feed conversion ratio, the highest mean value (3.95 ± 0.09) was observed in animals fed 40g/kg composite leaf meal and lowest mean value (3.22 ± 0.09) was observed in animals fed 10g/kg composite leaf meal. Generally, the cost of experimental diets (₦/kg) was least in 50g/kg composite leaf meal diet (₦55.44/kg) and highest in 0 g/kg composite leaf meal diet (₦60.43/kg) and the percentage cost reduction increased as the level of the composite leaf meal inclusion increased (1.44 – 8.26%).

Carcass and Organs Characteristics: The results from the carcass and organ evaluation of swine fed composite leaf meal diets indicated that there were no significant differences (P>0.05) in all the parameters measured as shown in Table 4. The eviscerated weights, head weight, carcass length and the relative organs weight of liver, kidney, heart, lungs and spleen were not significantly influenced (P>0.05) by the dietary treatments. Highest eviscerated weight (38.33 kg) was observed in animal fed diet VI (50g/kg composite leaf meal) while the lowest value (36.00 kg) was observed in animal fed diet III (20g/kg composite leaf meal). Animals fed diet VI (50g/kg composite leaf meal) had the highest value for head (3.95kg) while animals fed diet V (40g/kg composite leaf meal) had the lowest value (3.65 kg). The result on eviscerated weight was not significantly different among the treatment mean values but numerically higher in animals fed diet VI (50g/kg composite leaf meal). Animals fed Diet II (10g/kg composite leaf meal) had the highest live weight per animal (41.67 kg).The longest carcass length was recorded in animals fed 50g/kg composite leaf meal (73.00 cm) while the shortest carcass length was recorded in animals fed diet 20g/kg composite leaf meal (69.33cm). Animals fed diet 50g/kg composite leaf meal had the highest liver value (956 g/kg) while animals on diet 0g/kg composite leaf meal had the lowest value (779.33 g/kg). The kidney of animals fed diet II (10g/kg composite leaf meal) had the highest value (133.67 g/kg) while lowest value was recorded in animals fed diet 20g/kg composite leaf meal (114.67 g/kg). Animals on diet I (0g/kg composite leaf meal) had the highest value for heart (153.67 g/kg) while lowest value was observed in diet 30g/kg composite leaf meal (123 g/kg).

Eye Muscle Measurement, Fat Deposition and pH: The influence of composite leaf meal on the eye muscle, fat deposition and pH of the experimental animals indicated that the eye muscle width and midback fat were significantly influenced (P<0.05) by the dietary treatments as presented in Table 5. However, the eye muscle depths, shoulder fat, loin fat and C, K, P were not affected by the dietary treatments. Highest eye muscle width and depth were recorded in animals fed 10g/kg composite leaf meal (7.48 and 3.35 cm, respectively). The lowest eye muscle width and depth were recorded in animals fed 0g/kg composite leaf meal (6.87 and 3.03 cm, respectively). The lowest sub fat loin was recorded in animals fed 20g/kg composite leaf meal (0.72 cm), while highest was recorded in animals fed 10g/kg composite leaf meal (1.25 cm). For C, K, and P; highest values were recorded in 10g/kg composite leaf meal (1.02, 1.28 and 1.08 cm, respectively). Lowest C, K, P values were recorded in animals fed 30g/kg composite leaf meal and 40g/kg composite leaf meal (0.72, 0.63 and 0.60 cm, respectively). For the pH of the meat samples, highest value (5.77 pH) was recorded in animals fed 50g/kg composite leaf meal while lowest value (5.74 pH) was recorded in animals fed 0g/kg composite leaf meal. However, there was no significant (P>0.05) difference in the pH values.

IV. DISCUSSION

All pigs were in apparently good health during this trial. Feed conversion ratio (FCR) was statistically similar in all dietary treatments. The best FCR was recorded in animals fed 10g/kg composite leaf meal diet, which indicated that animals utilized their feed better than those fed other test diets at this stage. Similar final weight, weight gain and feed conversion ratio across treatments suggested that commercial premix could be replaced completely with composite leaf meal without any serious adverse effect on the growth parameters (Adegbenro *et al.*, 2012). In general, the growth parameters measured in animals fed the control diet and those fed the composite leaf meal diets were identical, thus suggesting the nutritional adequacy of the composite leaf meal in replacing the commercial premix in pig diet in sub-tropical Africa where these leaves are abundant. Thus, in the course of world clamour for sustained food security through enhancement of organic farming, the combination of these leaves could be used as a cheaper replacement of commercial premix in swine diets. The economy of production revealed that the cost of feed per kilogram of feed and cost of feed per gain were affected by the dietary treatments. These cost indicators were highest in the control diet and lowest in the 50g/kg composite leaf meal diet, which suggests plausible economic benefit of this inclusion level in pig production. As a result of an increase in percentage cost reduction as the level of the composite leaf meal inclusion increased, it may be safe to completely replace commercial premix with composite leaf meal as this would result in lower the cost of production, increased meat production and affordability

by the resource poor. For instance, the complete replacements of commercial premix with composite leaf meal at 5% inclusion level reduced about ₦8.26 per kilogram of feed. This translates to a colossal savings of ₦82,600 per tonne by farmers (Adegbenro *et al.,* 2012). This observation could encourage pig farmers to produce more and thereby making meat available for the populace. Carcass has been shown to be an instrument that determines the relationship between the "whole sale" or "retail cuts" of animals. Observation on the eviscerated weight therefore implies that th e of animals may not necessarily be directly proportional to the performance traits. The result on carcass length indicates that the diets promoted the development of identical carcass length percent. Organs are body parts, composed of several types of tissues, capable of carrying out specialized function (Sarojini, 2005). Among the organs measured are; liver, kidney, heart, lungs and spleen. The organs weight measured in this study were not influenced by the treatments, thus suggesting that the diets were not detrimental. The eye muscle width and eye muscle depth increased with increased live body weight of pigs which indicate that pigs with lighter body weight will possess smaller eye muscle width and eye muscle depth (Gill *et al.,* 1995). Shoulder fat, midback fat and loin fat reduces as the level of the composite leaf meal increases but does not follow any particular trend. This could be linked to the fact that composite leaf meal contains some elements that helps in reducing lipogenesis in the animals. The low pH obtained makes the meat better in terms of appearance and keeping quality. The pH of meat is an important for determining its quality characteristics. Anaerobic glycolysis generates lactate that accumulates, lowering the intracellular pH of meat, so that by 24 hours post mortem the pH has fallen to an ultimate pH of about 5.4 – 5.7 (Chalotte *et al.,* 2003). The ultimate pH depends on glycogen concentration at the time of slaughter (Przybylski *et al.,* 2006). From the current work, the pH values ranged from 5.74 – 5.77 which is still in range of the report of Chalotte *et al.* (2003) and the values of 5.3 – 6.9 reported for pigs by FDA (2012).

Conclusion: The quality of meat depends on numerous factors. The attention is most often focused on the effect on nutrition on meat quality such as pH value, fat quality etc. This research has shown that composite leaf meal will not cause any deleterious effects on the quality of meat produced from these pigs considering the fat quality and pH level. Also, inclusion of composite leaf meal reduced the cost of experimental diets which could help to stem over dependence of pig farmers on importation of conventional premix in developing countries like Nigeria.

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Table.1: The Gross Composition (g/kg) of the Weaner Diets

INGREDIENTS	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI
Maize	520.00	510.50	501.00	486.50	472.00	462.50
Wheat offal	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	90.00	90.00	90.00	90.00	90.00	90.00
Groundnut cake	100.00	100.00	100.00	100.00	100.00	100.00
Palm kernel cake	90.00	90.00	90.00	90.00	90.00	90.00
Brewer's dried grain	150.00	150.00	150.00	150.00	150.00	150.00
Bone meal	15.00	15.00	15.00	15.00	15.00	15.00
Oyster shell	5.00	5.00	5.00	5.00	5.00	5.00
Premix	2.50	2.00	1.50	1.00	0.50	0.00
Composite leaf meal	0.00	10.00	20.00	30.00	40.00	50.00
Salt	2.50	2.50	2.50	2.50	2.50	2.50
Vegetable oil	5.00	5.00	5.00	10.00	15.00	15.00
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**
Calculated						
Crude Protein (g/kg)	191.30	193.40	195.50	197.10	198.80	200.90
Metabolizable energy (MJ/kg)	11.87	11.74	11.60	11.58	11.56	11.42
Lysine (g/kg)	7.50	7.50	7.50	7.40	7.40	7.40

Table.2: The Gross Composition (g/kg) of the Grower Diets

INGREDIENTS	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI
Maize	560.00	550.50	541.00	526.50	512.00	502.50
Wheat offal	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	65.00	65.00	65.00	65.00	65.00	65.00
Groundnut cake	100.00	100.00	100.00	100.00	100.00	100.00
Palm kernel cake	90.00	90.00	90.00	90.00	90.00	90.00
Brewer's dried grain	120.00	120.00	120.00	120.00	120.00	120.00
Bone meal	15.00	15.00	15.00	15.00	15.00	15.00
Oyster shell	5.00	5.00	5.00	5.00	5.00	5.00
Premix	2.50	2.00	1.50	1.00	0.50	0.00
Composite leaf meal	0.00	10.00	20.00	30.00	40.00	50.00
Salt	2.50	2.50	2.50	2.50	2.50	2.50
Vegetable oil	20.00	20.00	20.00	25.00	30.00	30.00
Total	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**
Calculated						
Crude Protein (g/kg)	176.80	178.80	180.90	182.60	184.20	186.30
Metabolizable energy (MJ/kg)	12.43	12.30	12.16	12.14	12.12	11.98
Lysine (g/kg)	6.70	6.60	6.60	6.60	6.50	6.50

Table.3: Influence of composite leaf meal on the performance and cost of production estimates of swine

Parameters	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI	±SEM
Initial live weight (kg)	17.67	17.67	17.67	17.67	17.67	17.67	0.54
Final live weight (kg)	39.33	41.67	38.67	39.33	37.67	41.33	0.84
Weight gain (kg)	21.67^{ab}	23.67^{a}	21.00^{ab}	21.33^{ab}	19.33^{b}	23.00^{ab}	0.51
Total feed intake (kg)	75.57	75.92	75.83	75.62	75.72	75.85	0.06
Feed conversion ratio	3.52^{ab}	3.22^{a}	3.63^{ab}	3.55^{ab}	3.95^{b}	3.31^{a}	0.09
Cost of experimental diets (₦/kg)	60.43	59.56	58.69	57.50	56.31	55.44	
Cost of feed consumed (₦/kg)	4566.70	4521.80	4450.46	4348.15	4263.79	4205.12	
Cost of feed/kg gain ((₦)	210.74	191.04	211.93	203.85	220.58	182.83	
% Cost reduction in experimental diets	-	1.44	2.88	4.85	6.82	8.26	

a-b: Mean within rows having different superscripts are significantly different (P<0.05)

Table.4: Influence of composite leaf meal on the carcass and organs of swine

Parameters	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI	±SEM
Live weight (kg)	39.33	41.67	38.67	39.33	37.67	41.33	0.84
Eviscerated weight (kg)	36.67	37.67	36.00	37.00	36.33	38.33	0.76
Head (kg)	3.90	3.88	3.93	3.97	3.65	3.93	0.12
Carcass length (cm)	69.67	71.33	69.33	70.67	71.00	73.00	0.58
Liver (g/kg)	779.33	904.00	800.33	881.33	922.00	956.00	28.95
Kidney (g/kg)	132.33	133.67	114.67	118.67	127.33	118.33	3.09
Heart (g//kg)	153.67	129.33	134.67	123.00	143.00	135.00	4.35
Lungs (g/kg)	311.33	310.33	315.67	374.33	315.33	334.67	9.76
Spleen (g/kg)	54.00	55.67	57.67	63.33	58.67	52.33	2.08

Table.5: Influence of composite leaf meal on the eye muscle and fat deposition of swine

Parameters	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI	±SEM
Eye muscle width (cm) (A)	6.87^{a}	7.48^{b}	7.47^{b}	7.46^{b}	7.40^{b}	7.35^{b}	0.007
Eye muscle depth (cm) (B)	3.03	3.35	3.27	3.10	3.32	3.27	0.05
Shoulder fat (cm)	2.47	2.68	2.27	2.15	1.97	2.17	0.10
Midback fat (cm)	1.02^{ab}	1.23^{b}	0.83^{ab}	0.95^{ab}	0.77^{a}	0.85^{ab}	0.06
Loin fat (cm)	0.93	1.25	0.72	0.82	0.80	0.82	0.09
C	0.80	1.02	0.77	0.72	0.75	0.73	0.05
K	0.92	1.28	0.75	0.63	0.63	0.72	0.09
P	0.80	1.08	0.68	0.80	0.60	0.75	0.06
pH	5.74	5.77	5.76	5.77	5.77	5.77	0.05

a-b: Mean within rows having different superscripts are significantly different (P<0.05)

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Evaluation of the effect of locally produced biological pesticide (АқКөbelek ™) on biodiversity and abundance of beneficial insects in four forage crops in the Almaty region of Kazakhstan

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Abstract— *Using a non-replicated plot design, we experimentally assessed the effects of a locally produced biological pesticide on the abundance, species richness and Shannon diversity of beneficial insects in four forage crops (alfalfa, soybeans, corn, and triticale) in southeastern Kazakhstan. 2-way ANOV tests detected no effect of the biological pesticide treatment on the abundance (N) of either predators or pollinators. However, there were significant differences in pollinator and predator abundances among crops. Pairwise t-tests between the experiment and control plots for each crop detected no significant differences in predator or pollinator Shannon diversity index values (H). Paired t-tests revealed significant differences in diversity index values for both predator and pollinator functional groups among crops within each treatment (experiment, control). Corn and triticale plots had notably similar predator abundance (N), species richness (S) and Shannon diversity index (H) values. Corn, alfalfa and soy-triticale differed in pollinator Shannon H, N and S values, suggesting each contained a distinct pollinator assemblage. A trial rapid assessment for differences using a point-based system for indicator species showed only small difference among crops and between treatment and control plots. This method may be more applicable in situations sampling disturbance needs to be minimized and a rapid but less thorough assessment is required.*

Keywords— Bacillus thuringiensis, beneficial insects, pollinators, biodiversity, forage crops.

I. INTRODUCTION

Anthropogenic impact on the environment leads to a sharp disruption of the existing equilibrium in ecosystems of different levels, including in agricultural systems. Broadly speaking, more biologically diverse communities appear to be more stable in the face of perturbations [1]. In undisturbed communities, abiotic and biotic factors control the number and diversity of organisms. Agricultural systems, with extensive monocultures, disrupt the processes of natural regulation of abundance and diversity of species. As a result, crop systems experience periodic outbreaks of one or more crop pests. As these pest populations grow, they create opportunities for additional opportunistic pest species and pathogens to become established and further destabilize the agricultural system. The common response to pest outbreaks in Kazakhstan and neighboring countries has been to use chemical insecticides of various types. Use of chemical pesticides for pest control has many negative consequences, among the more important are including the loss of critically important but non-target beneficial species (pollinators and pest predators), dramatic declines in agricultural biodiversity and the rise of pesticide-resistant pest populations. In addition, the toxic and teratogenic products of the chemical pesticide decomposition accumulate in the soil, vegetation, and eventually in the tissues and organs of other organisms, including humans and domestic animals. One of the alternatives to the chemical method of control is the use of biological preparations based on entomo-pathogenic viruses, bacteria, fungi, protozoa and nematodes. However, since many biological preparations are polytrophic, i.e. they can affect beneficial and non-target species, it is critical to assess such effects prior to broader use of biological preparations in agricultural systems. As an example, the impacts of widely used *Bacillus thuringiensis* (Bt) based biological preparations

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on beneficial insects have been broadly assessed in the work of researchers from around the world [2-21].

The list of pesticides approved for use in the Republic of Kazakhstan includes biological preparations on the bacterium *Bacillus thuringiensis* (Bt) All of these preparations are rated as non-hazardous to bees (known toxicity to honey bees *Apis*) by Kazakh regulatory agencies. Recognizing the potential risk to beneficial insects, the application of these products is closely regulated (similar to Category II restrictions in the University of California IPM Bee precaution pesticide ratings [22]): application only when wind speed <5-6 m/s, a mandatory minimum 1-2 km border-protection zone, and restrictions of 6-12 hour periods on daytime application in the summer months.

With the increasing use of IPM pest control in Kazakhstan, including use of Bt based biological preparations, it is important to better understand their effects on the critically important pollinator and beneficial predator species. This research focused on a preliminary assessment of the effect of the locally produced Bt-based biological preparation АқКөbelek™ on the broad suite of beneficial insect species (predator and pollinator) in four forage crops commonly grown in southeast Kazakhstan.

II. MATERIALS AND METHODS

The study was conducted at the research farm LLP "Bayserke Agro" (Panfilov district, Almaty region of Kazakhstan). An organic farm research facility, the agricultural complexes support a very diverse and well-studied insect and arachnid fauna [23-35], including several insect species listed in the Red Book of the Republic of Kazakhstan and the Red Book of the Almaty region. These are: the dragonfly *Calopteryx virgo*, the mantids *Hierodula tenuidentata*, and *Bolivaria brachyptera*, the heteropterans *Zicrona caerulea* and *Coranus subapterus*, and the lady beetle *Coccinella sedakovi* (Figures 1-6).

This study was part of a larger 2015-2017 program to assess the environmental effects of a number of IPM practices in forage crop production, specifically looking at how the abundance, species composition and diversity of pest species, their predators and pollinators responded to various practices. One of the 2016 objectives of the project was to evaluate the effect of the locally produced Bt-based biological pesticide on four forage crops, soybeans, alfalfa, corn and triticale.

Two 4-hectare plots were selected in each of the four crop fields, one randomly assigned for the experimental treatment and one for the control treatment. The biological pesticide preparation used a culture of *Bacillus thuringiensis* var. *kurstaki* strain 2123-3k produced by the Kazakh Research Institute for Plant Protection and Quarantine named after Z. Zhiembaev. The experimental application used a concentration of 150 billion life-capable Bt spores/g and a flow rate 2.5 L/Ha, as per national regulatory guidelines[1]. The control solution was an equal amount of distilled water. We used SPC-25 knapsack sprayers (Figure 7-8) to apply the experimental and control treatments. We applied the treatment and control sprays every 14 days May-September 2016 for a total of 10 applications.

We collected insects and other arthropods using a methods previously described for work at the research farm LLP "Bayserke Agro" [24-36], methods developed to standardize entomological research in former Soviet states [37-40]. We used regular transect collection methods to sample foliage dwelling arthropods in treatment and control plots, including vegetation sweeps along randomly placed 1 m wide x 10 m long within-plot transects, beating 10 randomly selected 1 row-meter sections of each crop, and netting visible specimens along established 100 m transects. We collected soil-surface and subsurface arthropods manually along vegetation transects, by beating at selected collection points (ten 1 m crop row sections per plot per sampling period), and by trapping with dry Barber pitfall traps (10 traps/plot) baited with moistened dry pet food. We also collected ground nesting Hymenoptera using artificial nesting sites [28]. We used a novel variation of the traditional Barber trap [41], made from .5 L plastic bottles, for the collection of ground fauna.

Indicator species can be useful in defining distinct communities and have been used successfully to assess community change. We used a point system of relative abundances of indicator species [42, 43] as a relative measure of plot biodiversity. Previous research [44] suggested that changes in such a point system could be useful in identifying potential treatment effects. We counted the individuals of each species captured manually and/or visually noted in each 100 m transect walk, scoring these as follows: 1 point - 1-2 individuals, 2 points - up to 5 individuals, 3 points - 5 -10 individuals, 4 points - 11-20 individuals, 5 points - more than 20 individuals. We confirmed the identity of species from experts and standard references and used published life history information to identify predator and pollinator species [45-70].

Methods – Statistical analysis

The experiment was a randomized block design with only one datum for each combination of factors (crop type, treatment). With only a single treatment and control plot within each crop type, we utilized a 2-factor Analysis of Variance (ANOV) without replication [71] to test the null hypothesis that the abundance of predator or pollinator

[1] List of pesticides against Lepidoptera caterpillars from the family of Noctuidae.

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species was the same in all plots. Factor A (rows) were treatments (experiment, control) and Factor B (columns) was crop type (soy, alfalfa, corn, triticale). The results of the ANOV tests allowed us to test hypotheses about each of the two factors, crop type and experimental treatment. We assumed that the effects of the experimental treatment did not vary by crop type and that there was no significant interaction between factors [71].

We used PAST [72] to calculate the Shannon diversity index (entropy, H), which takes into account the number of individuals as well as number of taxa in compared units. While the ANOV tests for differences in total abundance in all blocks, showing overall block and treatment effects, we can also test for differences in the Shannon diversity index among any pair of samples. We used a post hoc Hutchinson t-test in Excel [73] to make pairwise comparisons between plot pairs. To test for a crop effect on H we compared pairs of crop plots within each treatment (experiment, control) to each other. Comparing Shannon diversity index values within treatments across crop types allowed us to detect underlying differences in diversity index values among crops types, unrelated to treatment effects.

III. RESULTS

We sorted all of the collected specimens into predators and pollinators, based on previously cited published life history information. Any taxa not falling into one of these two groups were discarded as not relevant to the study. Specimens are archived at the Kazakh Research Institute of Plant Protection and Quarantine, Almaty, Kazakhstan.

We recorded 4795 individuals in 84 taxa that we classified as predators (Table 1a). The most species rich taxonomic groups of predators were in the Insecta: Coleoptera, with 25 species, and Hymenoptera, with 20 species. The latter included 4 species of Formicidae. The next most species rich taxon were the spiders (Aranei), with 13 species. 7 families of other insect predators, each with between 1 and 5 species, accounted for the remaining individuals.

The number of individuals (N) in predator taxa ranged from low in the soy plots (447-488) to a high of 702 in the triticale control plot (Figure 7a). Predator abundances for alfalfa, corn and triticale plots were broadly similar (range of approx. 600-700 individuals). The number of predator taxa (S) varied fairly widely among crops for both treatments (Figure 7b). The lowest number of predator species occurred in the soy plots (55, 57 taxa), and the highest number in the corn and triticale plots (63-64 taxa). Predator N values for the alfalfa plot fell between the soy and corn-triticale plots.

We also collected 3075 pollinator individuals, in 58 taxa belonging to four orders of Insecta: 15 species of Lepidoptera, 4 Coleoptera, 29 Hymenoptera (including 1 Formicidae) and 10 Diptera. (Table 1b). Species in several families (Hymenoptera, Coleoptera and Diptera) were listed as both predators and pollinators because they exhibited functional characteristics of both groups.

Patterns in the number of pollinator individuals (N) and in pollinator species (S) among crops (Figure 8a and b) were similar. In general, fewest pollinator individuals and species were reported in the corn plots, and highest reported for alfalfa, with N and S values for soy and triticale falling between these values.

Results of 2-way ANOV without replication (1-tailed, α=.05, Figure 9) indicated that the insecticide preparation (rows) had no effect on either predator abundances ($F=1.49$, $F_{critical}=10.13$) or on pollinator abundances ($F=1.26$, $F_{critical}=10.13$) across crop types. Total abundances of either predators or pollinators did not differ significantly between insecticide treatment and controls for any of the 4 crop types tested. However, we did detect significant column (crop) effects for predator abundances ($F=15.54$, $F_{critical}=9.28$) and pollinators abundances ($F=82.59$, $F_{critical}=9.28$), indicating significant differences in among-crop abundances of both predator and pollinator assemblages.

The numerical data (Tables 1 and 2, Figures 1 and 2) and the ANOV results (Figure 9) suggested crop effects (and perhaps some treatment effects as well) on N and S values of both predators and pollinators. In a without replication design, parametric tests for differences in N values were not possible, so we tested for differences in diversity index values between plots. The Shannon diversity index (H) takes account the number of individuals (N) as well as number of taxa (S) in compared units. We calculated Shannon H values for all crop blocks (Table 2). We then made three sets of pairwise comparisons (2-tailed Hutchinson's t-test, $p<.05$). The first, between experiment and control for each crop, tested for significant experiment effects on H diversity index values. The second and third, between all crop pairs within the Bt experiment and within the control, tested for crop effects on predator and pollinator H values.

Results of these tests are in Table 3, and presented visually in Figure 9. For predators: we detected no significant differences in predator H index values (* = significance) in biological pesticide experiment to control comparisons in any of the four crops tested. Three of four comparisons of pollinator H values detected no significant differences. We detect one significant difference in pollinator H index values, in the triticale plots, but not in soy, alfalfa or corn comparisons. While the t-test test resulted in a significant difference in pollinator H index values in the triticale plots, we do not believe this is a significant result. Examination of the triticale pollinator H index values (Figure 10, Table 2) show very similar experiment and control H values. Based on the closeness of the triticale pollinator H index values and on the large estimated variance in H for these

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plots, we concluded that the detected difference was in error, an artifact of the high variance. This suggests predator and pollinator diversity, as estimated by the H index value, was not affected by the biological pesticide treatment in any crop type.

Within-treatment (experiment, control) comparisons between crops indicated predator H values were generally not significantly different. We detected significant differences in experimental plots between soy and the other three crop plots. Comparisons within control plots showed that predator diversity H values differed significantly between soy and both corn and triticale plots. Predator H values were not different for the soy-alfalfa comparison. Triticale predator H values, for both experiment and control, were significantly greater than predator H values in any of the other crop types.

For pollinators: biological pesticide treatment had no effect on pollinator assemblages in any of the tested crops. Overall pollinator N, S and H index values followed similar patterns across the tested crops: low values for corn, high values for alfalfa and lower values for soy and triticale that were very similar. Pollinator diversity index values did not differ significantly in soy, alfalfa or corn plots in experiment to control comparisons (Table 3). Significant differences in pollinator H values were detected between triticale experiment and control plots. While the test results indicate a treatment effect on pollinator diversity, closer inspection of triticale experiment and control results for pollinator N, S (Table 2, Figure 8) and Shannon H index (Table 2, Figure 10) showed little differences in these values, less than other pair-wise comparisons. We concluded the result was a product of high variances, and treated this result as an artifact.

Tests for crop effects (pair-wise comparisons within treatments) on pollinator diversity index values found significant differences in all but one of the between-crop comparisons (Table 3). The absence of significant differences in pollinator H values for soy and triticale, in both experiment and control plots, suggests that these two crops contained pollinator assemblages of similar diversity. All other pair-wise comparisons showed significant differences in pollinator diversity index values, suggesting that corn and alfalfa contain pollinator assemblages of differing diversity, different from each other and from the soy-triticale pollinator assemblage.

Point-based indicator species data are summarized in Table 4. Indicator species point scores differ by crop type, but not by much. Similarly, differences in scores between the experimental Bt treatment and the control were very small (soya - 230 and 232 points, alfalfa - 320 and 318 points, maize - 246 and 252 points, triticale - 282 and 283 points on the test and control areas respectively).

Most Lepidoptera and Diptera scored highest in the legume plots (soybeans and alfalfa) compared to cereals (corn and triticale). Hymenoptera, combining pollinators and predators, scored slightly lower in the cereals, but did not show clear preferences for crop types. This was particularly true for Hymenoptera known to prefer artificial nest sites. Some predatory beetles, especially moisture loving species of ground beetles, and species found primarily on plant stems and leaves, scored highest in corn plots. Stands of corn provide the most favorable moisture and shade conditions for these species among the four crop types. Some spiders, such as *Argiope bruennichi*, by contrast, scored higher in soybean crops, where favorable light conditions, structure for web construction and higher pollinator insect abundances exist. A related species *Argiope lobata*, a xerophile common in dry in steppes and semi-deserts, scored high in the relatively arid triticale plots, where it had more optimal conditions for existence.

IV. DISCUSSION

Several families (Hymenoptera, Coleoptera and Diptera) exhibited functional characteristics of both predators and pollinators and were included in both groups. Larvae of the syrphid flies (Diptera) are predators, but the adults are recognized pollinators [74]. Many adult forms of Hymenoptera are both predators and pollinators. Large hunting wasps (Sphecidae, Vespidae) prey on various arthropods to feed themselves or to provision their nests. Other Hymenoptera are parasitoids (e.g. Ichneumonidae, Braconidae, Scoliidae some Sphecidae), with adults serving as pollen vectors but with predatory or parasitic larvae. Larvae of the soldier beetles (Cantharidae) are predators, but the adults are pollinators that primarily feed on nectar, pollen and honeydew [75]. Of the ant species we collected (Formicidae), four were identified as predators [76, 77]. Only one ant species, *Lasius niger*, classified as a predator, was also listed as a pollen vector [78].

Evidence for biological pesticide treatment effects on predator abundance (N) or S was inconsistent or not evident (no significant ANOV result). While predator abundance (N) in the experiment plots was lower than in the controls for alfalfa, corn and triticale, the opposite occurred in the soy plots. Predator S values were lower in the soy experimental plot than the control, but the opposite in the alfalfa plots, while predator S values in the corn and triticale plots remained nearly identical. There did not appear to a treatment effect on pollinators, with pollinator N and S values nearly identical in experiment and control plots for all four crops, but a potential crop effect was suggested by the wide differences in N and S values among crops, for both treatment and control plots.

Defined by differences in Shannon diversity, N and S, there appear to be three predator assemblages in the test plots (experiment and control): a similarly diverse predator assemblage in the alfalfa and corn crops, with a less diverse predator assemblage in the soy plots and higher diversity

predator assemblage in the triticale plots. Predator N, S and H values in all corn and triticale plots are notably similar. Corn and triticale can have significant pest populations. Research in Eastern Europe showed 24 species of insects from three orders, Hemiptera, Coleoptera and Diptera, were commonly found as pests on triticale [79]. There are 9 principal pets of corn in Eurasia, 4 Lepidoptera, 2 Coleoptera, 2 Heteroptera and 1 Diptera [80] and multiple minor pests. These prey populations may successfully support diverse and numerous predator populations.

Corn, alfalfa and soy-triticale (Figure 16) each contained apparently distinct pollinator assemblages, defined by their differences in Shannon diversity index values (species composition and relative abundance). The corn pollinator assemblage was notably different, with much lower species number (N), abundances (S) and Shannon diversity (H) index values, than any of the other crop types. This may be a reflection of the wind pollinated nature of corn and the lack of flowers that would attract pollinators.

The points based system using indicator species was of limited use in detecting effects on diversity. As a proxy measure of diversity it showed only small differences (a weak trend) in point scores among crops. It also showed small differences in point scores between experiment and control, which indicated that the biological pesticide treatment had no effect on diversity. However, it was very difficult to determine how much of a difference in point scores should be considered a significant change. In general, this approach may be of greater use as a rapid assessment tool than for experimental studies, which demand more detailed numerical information.

In previous research, we used point score system to evaluate other ecosystems, including protected national parks in Kazakhstan, where the biodiversity assessment was constrained by the need for a rapid methodology and for a method that did the least damage to the environment during the survey. The technique has been used in reserves in the Russian Federation [42]. The use of the points based system was a first trial of its usefulness in an agricultural setting. Broader adoption of this approach must consider the trade-off between the benefits of speed of assessment and minimal damage to the biota and environment against cost of lost information, due to the under-sampling of rarer and less numerous species that are important contributors to overall biodiversity.

Biological preparations based on entomo-pathogenic viruses, bacteria, fungi, protozoa and nematodes are an attractive alternative to chemical pesticides for pest control in agriculture. However, because these preparations do have effects on certain species of arthropods, preliminary assessments of their overall effect are needed. For example, preparations based on the bacterium *Bacillus thuringiensis* are widely used worldwide, and their impact on the non-target fauna of agricultural ecosystems have been evaluated [2-21]. The list of pesticides approved for use in the Republic of Kazakhstan [22] includes 7 biological products based on the bacterium *Bacillus thuringiensis* including locally developed AкKөbelek™. As a local preparation, it holds significant potential for widespread adoption in agriculture in Kazakhstan because it will be a widely available at low cost, providing a viable alternative to both imported Bt preparations and to current chemical pesticides. However, it has not been evaluated previously for its effect on pollinators and predators.

Based on this preliminary assessment, we found AкKөbelek™ to not have significant effects on resident pollinator or predator populations in forage crops and can thus can provide a good biological alternative to chemical control of a variety of lepidopteran pests of forage crops. These results support the use of this Bt preparation for use in both agrarian and forestry applications. We believe AкKөbelek™ can be used in combination with artificial nest sites (used to increase populations of a suite of important solitary bee pollinators) to increase crop yields throughout Kazakhstan.

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Table.1a: Predator taxa and number of individuals recorded from each plot.

Taxon	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticlae Control	
Aranei									
Agelena orientalis	2	2	2	2	0	0	0	0	general predator
Araniella cucurbitina	4	5	10	11	10	11	21	23	general predator
Araneus diadematus	0	0	0	0	0	3	6	7	general predator
Aculepeira armida	0	0	4	4	9	12	14	15	general predator
Heliophanus potanini	0	0	0	0	0	11	7	9	general predator
Argiope bruennichi	11	5	1	2	1	0	0	5	general predator
Argiope lobata	1	3	0	0	0	0	0	1	general predator
Pardosa agrestis	8	10	11	10	4	4	15	16	general predator
Pardosa paludicola	2	2	4	2	12	13	8	10	general predator
Pisaura mirabilis	9	10	20	22	4	4	23	25	general predator
Steatoda paykulliana	0	1	0	0	0	0	3	4	general predator
Thomisus albus	10	11	11	14	3	2	4	11	general predator
Thomisus onustus	3	7	7	8	2	3	3	5	general predator
Xysticus striatipes	12	0	20	21	6	7	11	10	general predator
Insecta: Odonata									
Anax parthenope	1	1	2	2	2	2	5	4	
Calopteryx virgo	0	0	0	0	4	2	0	0	aerial predator
Sympetrum vulgatum	14	13	21	23	7	8	14	16	aerial predator
Platycnemis pennipes	9	10	12	10	20	21	22	23	aerial predator
Enallagma cyathigerum	12	12	6	7	15	17	15	17	aerial predator
Insecta: Mantodea									
Hyerodula tenuidentata	0	0	0	0	3	4	0	0	general predator
Iris polystictica	4	5	2	2	2		4	4	general predator
Mantis religiosa	1	2	2	2	1	1	2	2	general predator
Insecta: Orthoptera									
Decticus verrucivorus	0	0	0	0	0	0	9	10	opportunistic predator
Platycleis intermedia	3	5	10	11	4	5	13	12	opportunistic predator
Tettigonia viridissima	14	16	21	20	8	4	18	21	general predator
Tettigonia caudata	4	11	4	5	2	2	9	11	general predator
Insecta: Dermaptera									
Anechura bipunctata	0	0	9	11	21	16	4	17	opportunistic predator
Labidura riparia	7	2	0	0	13	16	4	4	general predator
Insecta: Heteroptera									
Coranus subapterus	4	6	3	4	2	3	3	4	general predator
Nabis ferus	11	5	10	12	10	22	14	15	general predator
Orius minutus	7	5	4	4	22	20	8	10	general predator

Rhynocoris annulatus	1	2	2	2	1	1	2	2		general predator
Insecta: Coleoptera										
Anchomenus dorsalis	10	15	14	15	23	25	17	25		general predator
Brachinus crepitans	12	10	4	5	11	13	4	6		general predator
Calathus halensis	19	21	22	20	24	20	17			general predator
Callistus lunatus	1	2	0	0	0	0	0	0		general predator
Calosoma denticolle	2	2	2	2	0	0	0	0		general predator
Calosoma auropunctatum	0	0	1		1	1	1	0		general predator
Carabus cumanus	0	0	1		0	0	0	0		general predator
Carabus cicatricosus	0	0	0	4	1	1	0	0		general predator
Carabus nemoralis	0	0	1		0	0	0	0		general predator
Chlaenius spoliatus	0	0	0	0	2	2	0	0		general predator
Elaphrus cupreus	0	0	0	0	23	27	0	0		general predator
Lebia cruxminor	2	2	4	2	2	2	2			general predator
Lebia chlorocephala	1	2	6	4	0	0	0	0		general predator
Nebria aenea splendida	25	27	10	14	21	19	10	12		general predator
Scarites terricola	0	0	0	0	2	2	2	2		general predator
Paederus riparius	15	17	2	2	25	28	4	4		general predator
Pachylister inaequalis	0	0	0	0	1	0	0	0	0	general predator
Cantharis fusca	7	2	5	4	2	4	7	9		larva general predator
Adalia bipunctata	17	2	19	22	22	25	24	26		specialized predator
Coccinella sedakovi	2	2	2	2	7	9	3	2		specialized predator
Coccinella septempunctata	24	26	24	25	27	28	25	25		specialized predator
Coccinula quatuordecimpustulata	14		12	5	18	21	27	21		specialized predator
Harmonia axyridis	0	2	0	0	0	0	0	0		specialized predator
Hippodamia variegata	21	20	25	27	23	10	18	22		specialized predator
Hippodamia tredecimpunctata	2	2	2	2	0	0	0	1		specialized predator
Propilaea quatuordecimpunctata	0	0	13	14	17	22	9	11		specialized predator
Insecta: Neuroptera										
Chrysopa carnea Chrysopidae	11	10	12	10	19	20	11	10		larva specialized predator
Insecta: Hymenoptera										
Ophion sp.	0	3		0	3	4	6	0	0	specialized predator on noctuid larva
Netelia sp.	0	0	0	0	0	2	8	7		parasitoid on Lepidoptera
Ammophila heydeni	0	3	0	0	0	0	0	0		general predator
Eremochares dives	0	11	0	0	0	0	0	0		general predator
Apanteles sp.	2	0	0	0	0	0	0	4		parasitoid on Lepidoptera
Leucospis intermedia	0	0	0	0	0	0	0	2		parasitoid on Lepidoptera
Scolia schrencki	0	0	3	9	1	1	4	4		specialized predator on soil grubs
Pemphredon inornata	0	0	9	10	21	19	21	23		larva specialized predator on aphids
Pemphredon lethifer	0	0	12	14	18	21	17	16		larva specialized predator on aphids
Sceliphron destillatorium	22	10	4	6	0	0	0	0		specialized predator on Aranei
Sceliphron deforme	0	0	2	5	0	0	0	0		
Sphex funerarius	0	2	2	2	3	4	5	6		specialized predator on Orthoptera
Paravespula germanica	8	2	3	4	7	8	4	4		specialized predator Lepidoptera larvae

Polistes dominula	5	2	13	14	21	18	21	23		general predator
Polistes gallicus	17	18	20	25	23	25	23	22		general predator
Polistes nimpha	2	0	0	0	0	0	0	0		general predator
Chrysis ignita	0	0	0	0	1	3	3	4		parasitoid on Hymenoptera larvae
Insecta: Hymenoptera: Formicidae										
Cataglyphis aenescens	2	2	23	25	3	4	23	20		general predator/scavanger
Formica pratensis	9	4	25	29	4	2	10	10		general predator/scavanger
Lasius niger	17	19	17	23	14	16	4	6		general predator/scavanger
Tetramorium caespitum	22	10	20	25	13	17	23	19		general predator/scavanger
Diptera										
Dasisyrphus sp.	7	9	22	23	2	4	9	10		larva specialized predator on aphids
Syrphus ribesii	10	12	21	23	4	4	10	9		larva specialized predator on aphids
Sphaerophoria sp.	26	25	25	27	4	6	16	19		larva specialized predator on aphids
Promachus leontochlaenus	0	0	0	0	0	0	2	4		general predator
Selidopogon diadema	0	0	0	0	0	0	1	1		general predator
total number	488	447	600	654	601	651	652	702	4795	

Table 1b. Pollinator taxa and number of individuals recorded from each plot.

Taxon	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticlae Control
Lepidoptera								
Chazara briseis	4	11	11	10	1	1	8	11
Chazara enervata	7	13	14	15	1	3	15	14
Macroglossum stellatarum	4	6	11	11	1	1	4	5
Melanargia russiae	12	14	21	23	5	6	10	12
Papilio machaon	1	1	1	2				
Colias hyale	11		22	24	3	2	9	10
Colias erate	8	10	20	21	1	1	10	14
Pieris brassicae	10	11	21	10				
Pieris rapae	19	21	23	25			14	15
Pontia daplidice	21	23	21	20	4	4	13	14
Polyommatus icarus	23						19	
Thersamonia thersamon	11	10	16	19			4	6
Nymphalis urticae	12			10			4	4
Vanessa cardui	6	7	11	10			8	
Inachis io			2	2				
Argynnis pandora			5	4				
Coleoptera								
Trichodes hauseri Cleridae	4	2	10	11	2	2	6	
Trichodes spectabilis Cleridae	6	7	3	2	0	0	0	0
Malachius aeneus Cleridae	2	5	11	10	4	4	5	4
Cantharis fusca Cantharidae	7	2	5	4	2	4	7	9

http://dx.doi.org/10.22161/ijeab/3.1.10 *ISSN: 2456-1878*

Hymenoptera								
Ophion sp.		3			3	4	6	8
Netelia sp.						2	8	7
Apanteles sp.	2							4
Leucospis intermedia								2
Scolia schrencki			6	9	1	1	4	4
Ammophila heydeni		3						
Eremochares dives		11						
Pemphredon inornata			9	10	21	19	21	23
Pemphredon lethifer			12	14	18	21	17	16
Sceliphron destillatorium	22	10	4	6				
Sceliphron deforme			2	5				
Sphex funerarius		2	2	2	3	4	5	6
Polistes dominula	5	2	13	14	21	18	21	23
Polistes gallicus	17	18	20	25	23	25	22	22
Polistes nimpha	2							
Paravespula germanica	8	2	3	4				
Chrysis ignita					1	3	3	4
Hylaeus arenarius	3	2	10	21	4			
Andrena cineraria	1	2	4	5	5	6	4	5
Halictus quadricinctus	5	7	20	22	2	4	7	7
Anthidium cingulatum	10	11	11	13	2	3	21	23
Megachile rotundata	11	10	22	25	10	11	19	18
Osmia coerulescens	5	4	19	21	4	4	6	7
Anthophora borealis	2	5	14	14	1	1	4	4
Apis mellifera	2	3	8	9				
Bombus lucorum	2	3	11	12	1	1	3	4
Bombus laesus	1	2	7	8				
Xylocopa valga	2	5	5	7	1	1	4	4
Hymenoptera, Formicidae								
Lasius niger	17	19	17	23	14	16	4	6
Diptera								
Eristalis tenax	19	21	14	15	18	21	12	14
Dasisyrphus sp.	7	9	22	23	2	4	9	10
Syrphus ribesii	10	12	21	23	4	4	10	9
Spirophora sp.	26	25	25	27	4	6	16	16
Lucilia caesar	11	10	11	14	2	3	8	9
Calliphora vicina					6	7	4	4
Sarcophaga haemorrhoidalis			6	6	5	4	7	8
Promachus leontochlaenus							2	4
Selidopogon diadema							1	1
Stratyomis sp.							12	10
Total	358	344	546	610	200	221	396	400

Table 2. Shannon Diversity (H) Index values, number of taxa and number of individuals of predator and pollinator groups in all plots.

Predator taxa	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticale Control
Shannon H	3.67	3.67	3.77	3.76	3.74	37.8	3.87	3.9
No. taxa S	54	56	60	58	62	62	62	63
Individuals N	488	447	600	654	601	651	652	702
Pollinator taxa	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt	Triticale Control
Shannon H	3.42	3.41	3.62	3.65	3.56	3.11	3.56	3.44
No. taxa S	41	40	45	46	35	35	43	42
Individuals N	358	344	546	610	200	221	396	400

Table 3. Results of pair-wise t-tests testing for differences in Shannon H diversity index values a) between treatment vs control pairs within crops, b) between Bt experiment plots for all crop pairs, and c) between control plots for all crop pairs. (*) indicates significant differences in Shannon-Weiner Diversity Index values (2-tailed Hutchinson's t-test, α=.05, tcritical =1.96); (–) indicates plot pairs were not tested (results not useful).

	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticale Control
Predator	Values of t							
Soy Expt.	x	0.01	2.20*	-	1.46	-	4.73*	-
Soy Control		x	-	1.81	-	2.25*	-	5.06*
Alfalfa Expt.			x	0.31	0.72	-	2.57*	-
Alfalfa Control				x	-	0.55	-	3.87*
Corn Expt.					x	0.95	4.06*	-
Corn Control						x	-	3.19*
Triticale Expt.							x	0.80
Triticale Control								x
Pollinator	Values of t							
Soy Expt.	x	0.11	4.54*	-	4.76*	-	0.39	-
Soy Control		x	-	5.42*	-	4.19*	-	0.46
Alfalfa Expt.			x	0.92	7.91*	-	3.57*	-
Alfalfa Control				x		8.25*	-	4.48*
Corn Expt.					x	0.68	6.83*	-
Corn Control						x	-	4.42*
Triticale Expt.							x	2.35*

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Table 4 - Number of pollinator and predator species and total population points in experiment and control plots of forage crops.

Plot №	Crop type	The number of indicator species	The population in points
1	Soybean (experiment)	85	230
2	Soybean (control)	86	232
3	Alfalfa (experiment)	97	320
4	Alfalfa (control)	95	318
5	Corn (experiment)	85	246
6	Corn (control)	84	252
7	Triticale (experiment)	91	282
8	Triticale (control)	92	283

Fig.1: Dragonfly Beautiful Demoiselle Calopteryx virgo Linnaeus, 1758, male and female (Photo by I.I. Temreshev).

Fig.2: Larva of wood mantis Hierodula tenuidentata Saussure, 1869, consuming a moth at a light trap (Photo by I.I. Temreshev).

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Fig.3: Short-winged Bolivaria Bolivaria brachyptera (Pallas, 1773). (Photo by I.I. Temreshev).

Fig.4: Blue Zikrona Zicrona caerulea (Linnaeus, 1758) consuming a leaf beetle larva (Photo by P.A. Esenbekova).

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Fig.5: Short-winged Coranus Coranus subapterus (De Geer, 1773). (Photo by P.A. Esenbekova).

Fig.6: Tien Shan ladybird Coccinella sedakovi Mulsant, 1850 (tianschanica Dobrzh, 1927.) (Photo I.I. Temreshev).

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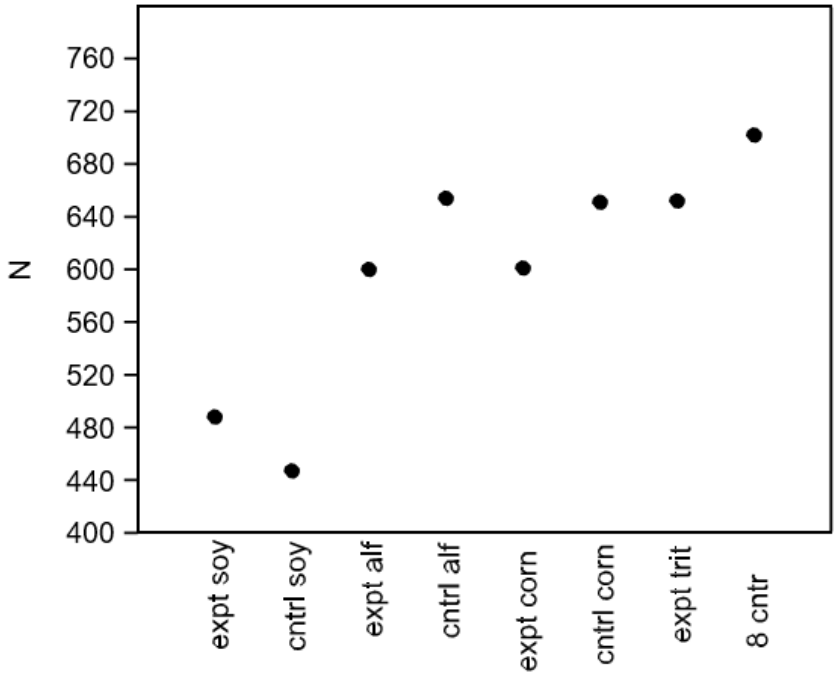

Fig.7a: Number of predator individuals (N) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

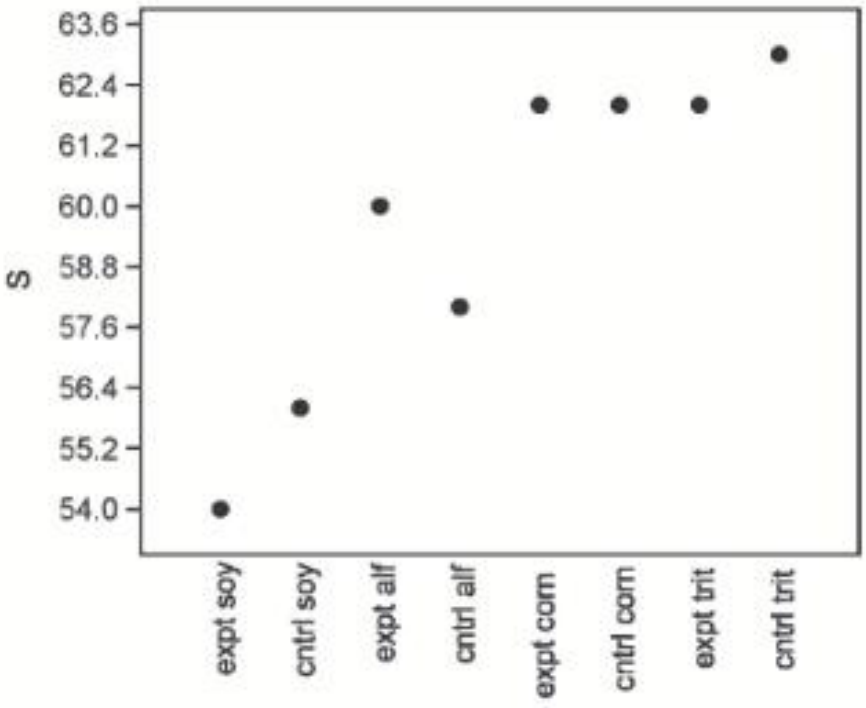

Fig.7b: Number of predator taxa (S) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Com, 7-8 Triticale.

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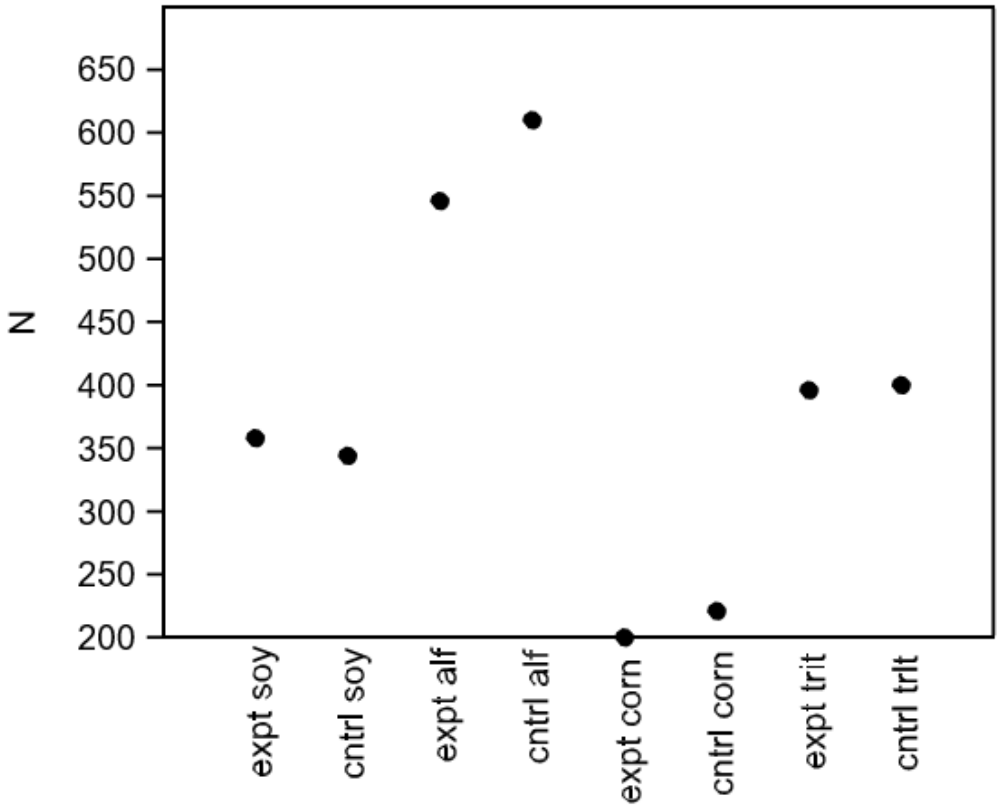

Fig.8a: Number of pollinator individuals (N) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

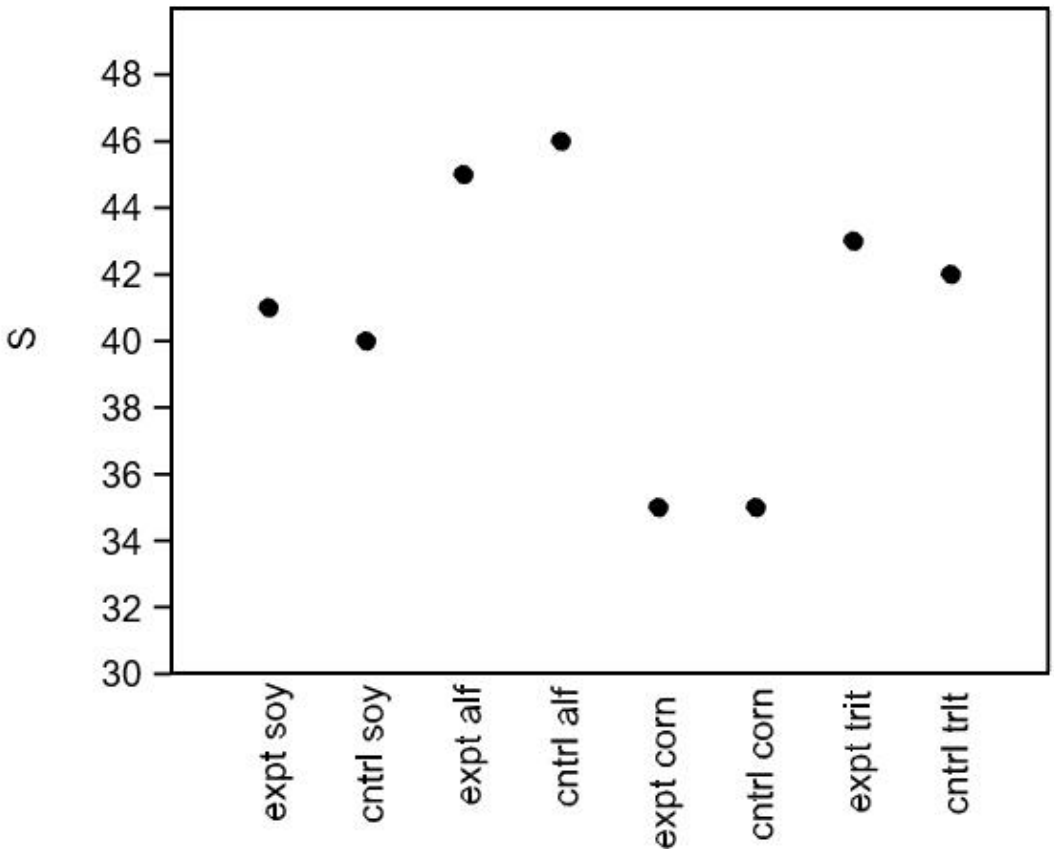

Fig.8b: Number of pollinator species (S) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

A. Result for predator species.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
tmt	4	2341	585.25	4792.917
control	4	2454	613.5	12867
soy	2	935	467.5	840.5
alfalfa	2	1254	627	1458
corn	2	1252	626	1250
triticale	2	1354	677	1250

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	1596.125	1	1596.125	1.495257	0.308702	10.12796
Columns	49777.38	3	16592.46	15.54389	0.024766	9.276628
Error	3202.375	3	1067.458			
Total	54575.88	7				

B. Result for pollinator species.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
tmt	4	1500	375	20198.67
control	4	1575	393.75	26373.58
soy	2	702	351	98
alfalfa	2	1156	578	2048
corn	2	421	210.5	220.5
triticale	2	796	398	8

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	703.125	1	703.125	1.26206	0.34305	10.12796
Columns	138045.4	3	46015.13	82.5939	0.002213	9.276628
Error	1671.375	3	557.125			
Total	140419.9	7				

Fig.9: ANOV without replication. F values with astersiks (*) indicate significant differences in abundances.

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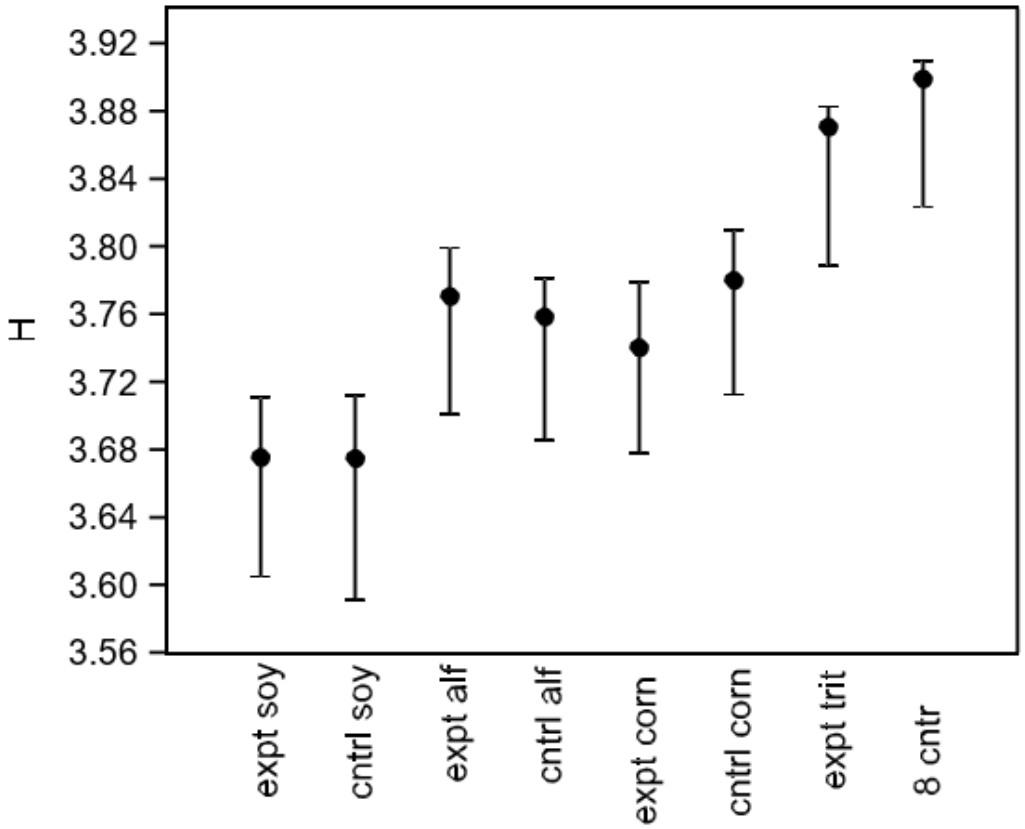

Fig.10a: Predator Shannon diversity index values (H) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

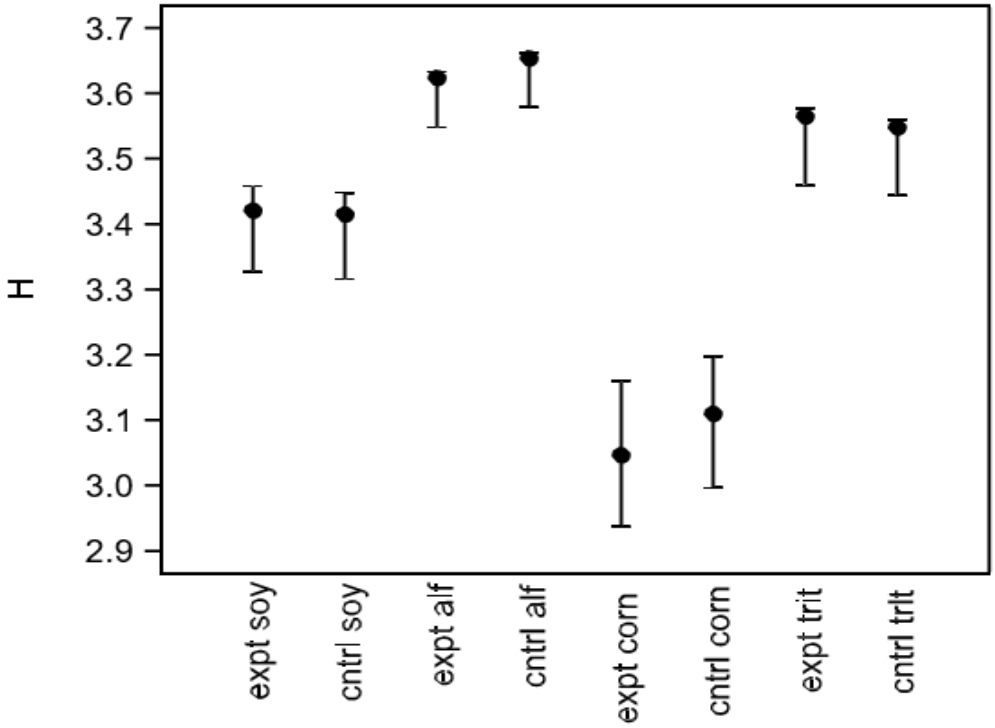

Fig.10b: Pollinator Shannon diversity index values (H) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

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Impact of elevated Carbon Dioxide on two groundnut genotypes (Arachis hypogaea L.) under Open Top Chamber facility

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Abstract— *The impact of enhanced atmospheric CO_2 concentration (550ppm) was assessed in Open Top Chambers (OTCs) facility to identify the growth and yield parameters at different growth stages of two popular groundnut (Arachis hypogaea L.) genotypes- Dharani, K-9. The results showed significant differences between genotypes, CO_2 levels and time intervals for all the characters studied. The experiments revealed that the genotype Dharani recorded higher response for seed weight, harvest index at eCO_2 while K-9 recorded higher response for total biomass.This study is necessary if we are to realize the potential genotype for maximum yield in the future climate change scenario.*

Keywords— *Arachis hypogaea L., Elevated CO_2,Yield, Biomass,Open Top Chamber.*

I. INTRODUCTION

The carbon dioxide (CO_2) concentration in the atmosphere is increasing due to human activities, fossil fuel combustion and energy use scenario [1]. The increased levels of CO_2 have led to an increased threat of global warming and climate change. Changes in climate factors such as increasing CO_2 concentration, increased temperature and changed precipitation pattern may have significant impacts on plant development and metabolism. The concentration of atmospheric CO_2 has been progressively increasing from 280 ppm (pre-industrial era) in the year 1850 to 407 ppm in 2017 [2]. This created an attention towards the crop responses to enhanced CO_2 levels and also to identify the responsive high yielding genotypes in order to enhance the productivity and production.

The agricultural productivity need to be increased to meet the demand for protein-rich diets due to rising global population. The pre-historic records shows legumes are the oldest cultivated crops and at present they are considered as the major nutrient supplying crops for a balanced human diet [3]. Groundnut seed is chiefly used for edible oil and contains nearly half of the essential vitamins and one-third of the essential minerals. So, for the resource poor farmer's groundnut play an important role in nutritional security [4]. Furthermore it is an excellent fodder for livestock. The productivity of groundnut in Andhra Pradesh is very low against Indian productivity (1615 kg ha^{-1}) and world productivity (1676 kg ha^{-1}). The low productivity can be due to erratic rainfall, incidence of pests and diseases in addition to cultivation of low yielding varieties etc. [5].

Keeping in view the climate change scenario and high yielding groundnut crop varieties, an experiment was conducted in Open Top Chamber facility. The present study was conducted:

- To realize the potential genotype of groundnut for maximum yield to elevated/enhanced Carbon Dioxide concentration (550ppm) when compare with ambient CO_2 (400ppm) in Andhra Pradesh.
- To conduct the experimentin Open Top Chambers (OTCs) facility for evaluating the best groundnut genotype.
- To identify the growth and yield parameters at different growth stages of two popular groundnut (*Arachis hypogaea L.*) genotypes- Dharani and K-9.

II. MATERIALS AND METHODS

2.1 **Materials:** The field investigations were carried to study the impact of elevated carbon dioxide on growth, biomass and yield of groundnut crop under the open top chamber (OTC) facility at Central Research Institute for Dryland Agriculture (ICAR-CRIDA), Hyderabad.

The seeds of the groundnut genotypes- Dharani and K-9 were obtained from the Regional Agricultural Research Station, Ananthapur. The seeds were sown and raised in open top chambers (OTCs) at ambient (aCO_2-400ppm) and elevated (eCO_2-550ppm) CO_2 levels during Kharif season 2016. The seeds of two genotypes were sown

directly in the soil and the observations were recorded at regular intervals from sowing to harvest.

2.2 **Open Top Chamber facility:** The OTCs are lined with transparent (90% transmittance of light) PVC (polyvinyl chloride) sheet having 3m x 3m x 3m dimensions. Six OTCs were used for the experimental purpose. Four OTCs were maintained with eCO2 of 550ppm and the other two OTCs served as an ambient control without any additional CO2 supply. Throughout the day within the OTCs, eCO2 concentration was maintained and monitored continuously during the experimental period. Continuous injecting of 100% CO2 from a compressed CO2 cylinder into plenum of OTCs was done to maintain the eCO_2 in OTCs at crop canopy level, where it was mixed with air from air compressor before entering into the chamber. From the centre point of OTCs the air sample from each chamber was drawn at three-minute interval into non-dispersive infrared (NDIR) CO_2 analyzer (California Analytical) and the CO_2 concentration was maintained with an automatic switching solenoid, rotameters, Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA) software [6].But gentle washing was frequently done to maintain the transparency of polythene cover.

Inside the OTCs, the maximum temperatures were 1 to 1.5°C higher than outside except for few days following rainfall while the minimum temperatures remained nearly the same. However, there was no significant difference in their maximum temperatures among the aCO_2 and eCO_2. Likewise, inside the chambers relative humidity (RH) was higher as compared to outside. The light intensity in chambers was 80-95% of outside environment. Continuous growth measurements were made at 30, 60, 75, 90 and 110 days i.e. flowering, pegging, poding and at harvest respectively. The recommended agricultural practices were followed during the crop growth period in the OTCs and also the crop was maintained free from moisture stress, pests and diseases.

The characteristics of the test field includes sandy loam in texture, neutral in pH (6.8), low in available nitrogen (225 kg ha^{-1}), medium to high in available potassium (300 kg K_2O ha^{-1}) and phosphorus (10 kg ha^{-1}). The crop received 662.8 mm rainfall during the crop growth period. Relative humidity and temperature was continuously measured using the sensors fitted inside the chambers throughout the experimental period.

Destructive samples of two groundnut genotypes were drawn from both CO_2 levels at 30, 60, 75, 90, DAS and at harvest (110 DAS) in order to record plant height, leaf area, root length, root volume, pod number and biomass of leaf, stem and root.

The plant height was measured from base of the plant to the tip of main shoot and root length was recorded on main root of plant and expressed in centimetres. The root volume was quantified by water displacement method and expressed as ml. The leaf area at different intervals was measured with photo-electronic leaf area meter (LI-3100, LI-COR) and expressed as cm^2/plant. The dry weights of stem, root and leaf were recorded after thorough drying of plant material in hot air oven at 65°C and expressed as g/plant.

The groundnut crop was harvested at 110 days. Three replications with five plants for each replication in each CO_2 concentration were harvested and used for recording final biomass, fodder yield, seed yield and its components *viz.*, pod number, pod weight, seed number and 100 seed weight. The HI (%) was calculated as (seed yield)/ (total above ground dry mass) * 100.

The data were statistically analyzed using a three way analysis of variance (ANOVA) to test the significance of variability between the genotypes, CO_2 concentrations, time intervals and their interactions using star software.

III. RESULTS & DISCUSSION

The impact of eCO_2 on growth and yield of two groundnut genotypes was found significant. The results of the investigations have revealed that the response of two groundnut genotypes differed at two concentrations of CO_2 namely- aCO_2 and eCO_2 in terms of growth, biomass, yield and harvest index. The mean per se values and analysis of variance of groundnut genotypes (Dharani & K-9) under aCO_2 and eCO_2 conditions at different growth stages were tabulated Table 1, biomass parameters in Table 2 and yield parameters in Table 3. The mean performance of different morphological and biomass parameters was presented in Fig. 1and yield parameters in Fig. 2. The percentage increase of the morphological, biomass and yield parameters due to eCO_2 over aCO_2 was presented in Fig. 3.

Table 1: The mean per se values and analysis of variance of morphological parameters of groundnut genotypes (Dharani & K-9) under aCO_2 and eCO_2 conditions at different growth stages

		Plant height (cm)		**Branches/pl**		**Root length (cm)**		**Root volume (ml/pl)**		**Leaf area (cm^2/pl)**	
		Dharani	K-9	Dharani	K-9	Dharani	K-9	Dharani	K-9	Dharani	K-9
30 DAS	aCO_2	11.8	13.2	3.7	4.0	6.9	6.0	0.5	0.5	180	148
	eCO_2	16.2	16.2	4.3	4.7	11.0	8.7	0.5	0.5	272	159
60 DAS	aCO_2	45.7	44.3	4.3	4.3	11.8	10.7	1.4	0.6	712	265
	eCO_2	61.7	58.9	5.3	5.7	12.7	10.6	1.3	1.5	1120	993
75 DAS	aCO_2	63.0	44.3	5.7	5.7	13.0	12.0	1.4	0.8	1026	772
	eCO_2	64.0	60.0	5.7	6.3	13.7	12.3	1.7	1.5	1682	1286
90 DAS	aCO_2	64.3	46.0	6.3	6.0	13.7	12.0	1.6	0.8	1227	910
	eCO_2	67.3	60.3	5.7	6.3	14.3	12.7	1.7	1.5	2214	1469
110 DAS	aCO_2	71.0	66.3	7.0	6.3	13.8	12.5	1.6	0.9	-	-
	eCO_2	68.8	70.5	7.3	7.0	14.8	14.0	1.9	1.7	-	-
	df										
Time intervals (T)	4	**		n.s.		**		**		**	
CO_2 levels (C)	1	**		n.s.		*		**		**	
Genotypes (G)	1	**		n.s.		*		**		**	
T * C	4	**		n.s.		n.s.		**		**	
T * G	4	**		n.s.		n.s.		*		**	
C * G	1	**		n.s.		n.s.		**		n.s.	
T * C * G	4	n.s.		n.s.		n.s.		n.s.		n.s.	
Error	38	31.5		1.1		5.5		0.0		24392	

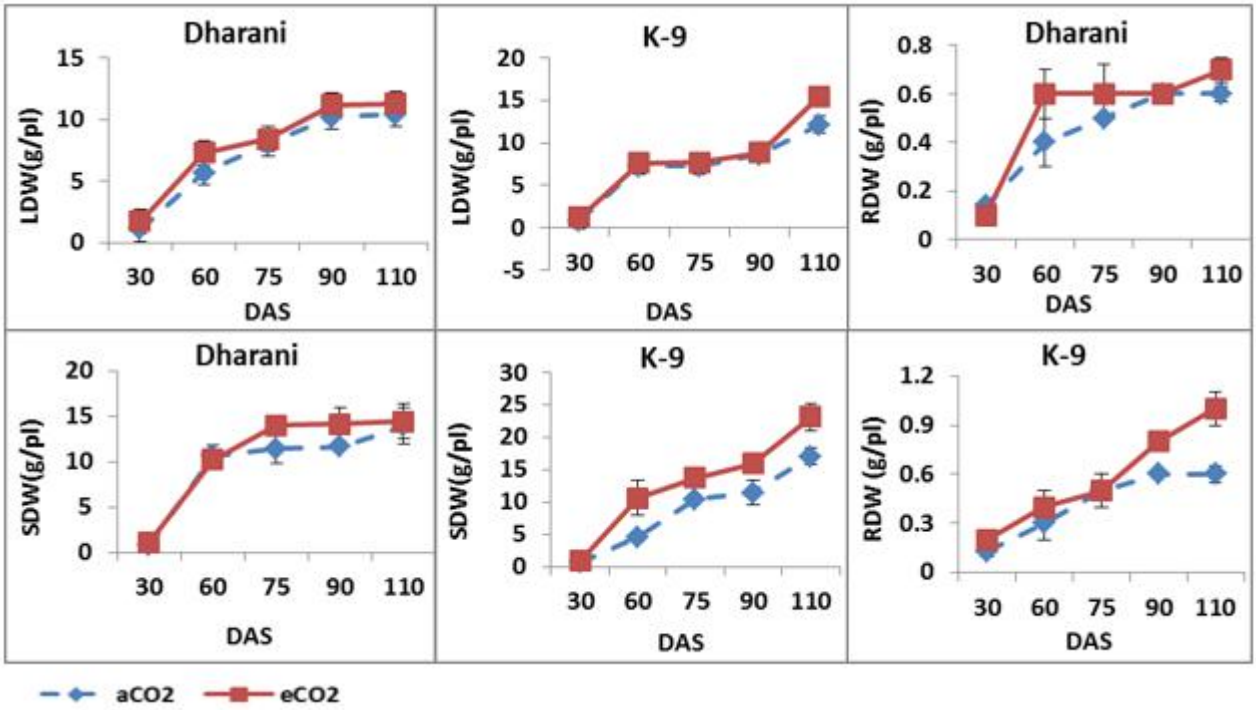

Fig.1: Biomass parameters of two groundnut genotypes at different growth stages under eCO_2& eCO_2 conditions

Table 2: The mean per se values and analysis of variance of biomass parameters of groundnut genotypes (Dharani & K-9)under $aCO_2$and eCO_2conditions atdifferentgrowth stages

		Leaf dry weight (g/pl		Stem dry weight (g/pl)		Root dry weight (g/pl)		Total biomass (g/pl)	
		Dharani	K-9	Dharani	K-9	Dharani	K-9	Dharani	K-9
30 DAS	aCO_2	1.1	0.8	0.8	0.7	0.14	0.13	2.1	1.6
	eCO_2	1.7	1.2	1.1	0.9	0.10	0.20	2.9	2.3
60 DAS	aCO_2	5.7	7.2	10.2	4.6	0.4	0.3	16.7	12.1
	eCO_2	7.3	7.6	10.2	10.7	0.6	0.4	18.1	18.7
75 DAS	aCO_2	8.0	7.2	11.4	10.5	0.5	0.5	28.6	21.9
	eCO_2	8.4	7.7	14.0	13.8	0.63	0.41	33.4	27.7
90 DAS	aCO_2	10.2	8.6	11.6	11.5	0.6	0.6	33.1	21.8
	eCO_2	11.2	8.8	14.1	16.0	0.65	0.81	37.9	32.1
110 DAS	aCO_2	10.4	12.1	13.9	17.1	0.6	0.6	44.2	50.1
	eCO_2	11.3	15.5	14.4	23.2	0.7	1.0	48.4	55.9
	df								
Time intervals (T)	4	**		**		*		**	
CO_2 levels (C)	1	n.s.		n.s.		n.s.		**	
Genotypes (G)	1	**		*		**		**	
T * C	4	**		**		**		**	
T * G	4	n.s.		**		n.s.		**	
C * G	1	**		n.s.		n.s.		**	
T * C * G	4	n.s.		n.s.		n.s.		n.s.	
Error	38	0.5		3.3		0.0		5.5	

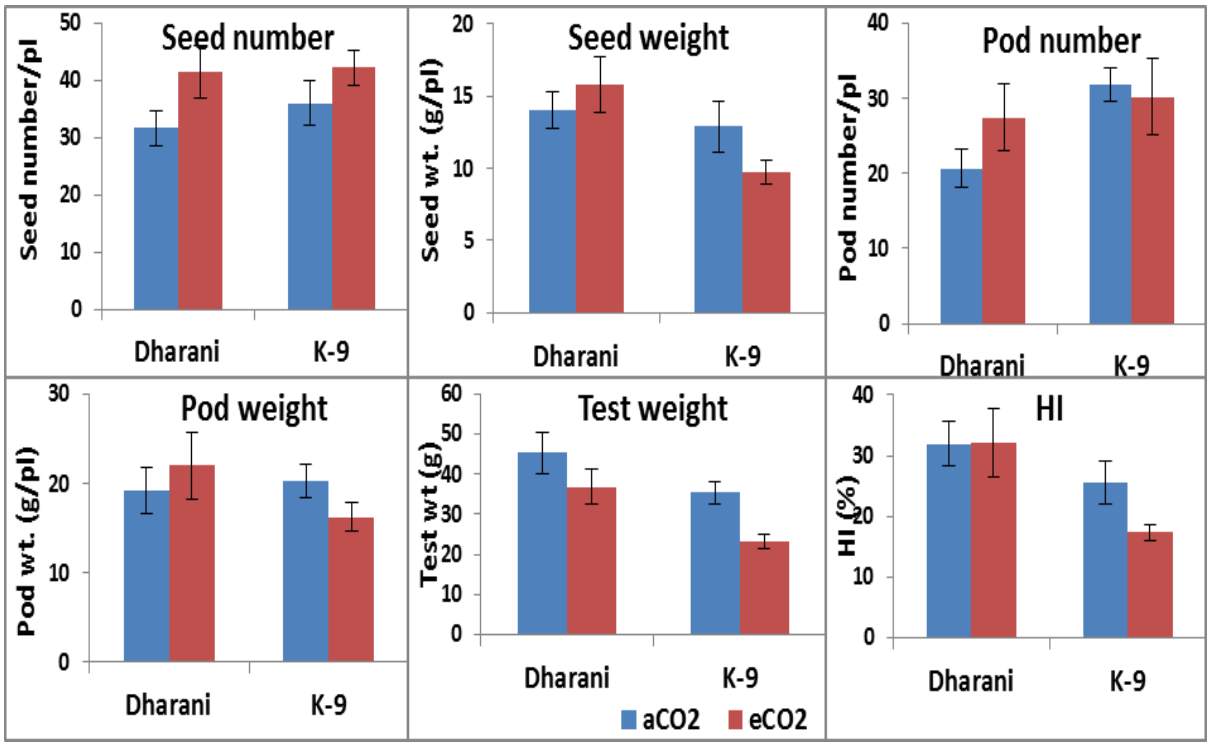

Fig.2: Yield and yield contributing parameters of two groundnut genotypes at eCO_2 and aCO_2 conditions

3.1 Shoot parameters: The maximum plant height of both the genotypes recorded at the harvest stage. The maximum plant height in Dharani was 71cm at aCO_2 and 68.8cm at eCO_2, whereas it was 66.25cm and 70.5cmrespectively with K-9. The maximum increment in plant height with eCO_2 was at 30 DAS in Dharani (37%), at 75 DAS in K-9 (35%) when compared with aCO_2. A significant increase in plant height, leaf expansion in sweet potato and cowpea was reported by Bhattacharya et al. under eCO_2 [7].

At harvest, the mean stem dry weight was 13.9g/plant and 14.4g/plant in Dharani while 17.1g/plant and 23.2g/plant in K-9 at aCO_2 and eCO_2 respectively. The maximum percentage increment in stem dry weight with eCO_2 was observed as 44.6% at 30DAS in Dharani while 131.9% at 60 DAS in K-9. The genotype K-9 showed better response of stem dry weight than Dharani at eCO_2. Similar increase in stem dry weight throughout the crop growth period with eCO_2 in castor was reported by Vanaja et al.[8].

Table 3: The mean per se values and analysis of variance of yield parameters of groundnut genotypes (Dharani & K-9) under aCO_2 and eCO_2 conditions at harvest

Parameters	Dharani		K-9		Mean sum of square			
	aCO_2	eCO_2	aCO_2	eCO_2	CO_2 levels (C) df (1)	Genotypes (G) df (1)	C * G df (1)	Error df (6)
Pod number/pl	20.6	27.4	31.8	30.2	n.s.	n.s.	n.s.	58.1
Pod weight (g/pl)	19.2	22.0	20.3	16.3	n.s.	n.s.	n.s.	8.5
Seed number/pl	31.6	41.4	36.0	42.2	*	n.s.	n.s.	37.9
Seed weight (g/pl)	14.0	15.7	12.9	9.7	n.s.	*	*	5.4
Test weight (g)	45.4	36.7	35.3	23.2	n.s.	n.s.	n.s.	80.5
Harvest Index (%)	32.0	32.2	25.4	17.3	n.s.	**	*	18.6

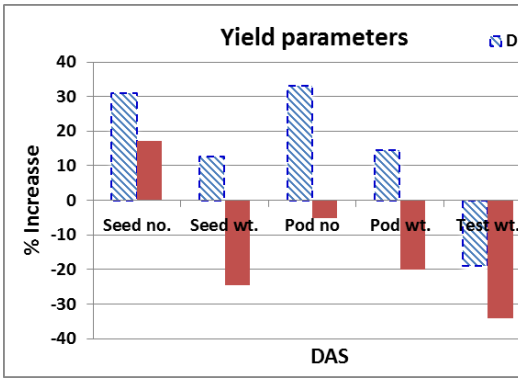

Fig.4: Percentage increase of the yield parameters of two groundnut genotypes due to eCO_2 over aCO_2

3.2 Root parameters: The percentage increment of root length varied from 4 to 59% in Dharani, 3 to 44% in K-9 with eCO_2 and the highest increment in root length was noticed at 30DAS. The root length was 13.8cm and 14.8cm in Dharani whereas 12.5cm and 14cm in K-9 at aCO_2 and eCO_2.The CO_2 enrichment enhances the root growth much more by increasing its length, volume and weight. Increased root length was observed by Madhu et al. with increased CO_2 conditions in groundnut crop [9].

The improved response due to eCO_2 was more significant at initial growth stages of K-9 while a linear increase recorded with Dharani. Higher increment in root volume than root length was observed by Vanaja et al. in rainfed crops [10] under eCO_2. Vanaja et al. observed and reported that eCO_2 significantly increased root volume in sunflower (C3) and maize (C4) crops [11].

The response of root dry weight to eCO_2 was more significant at later growth stages in both the genotypes and the increment was higher in K-9 as compared with Dharani. The maximum increase in root dry weight due to eCO_2 was at 60 DAS in Dharani (50%), whereas it was at harvest in K-9 (66.7%). The increased root volume, root length under eCO_2 infers the possibility of deeper soil penetration and spread to more volume of soil, which would be an advantage in a drier climate. In general enhanced CO_2 strongly enhanced the root growth by increasing its length, volume and weight. Vaidya et al. [12]testified that the biomass of stem, root, leaf and total biomass of groundnut genotypes recorded significant increase at eCO_2.

3.3 Leaf parameters: The maximum incrementin leaf area with $eCO_2$was 80.4% in Dharani while it was 274.5% in K-9. Increased CO_2 tends to accelerate the growth and leaf area per plant, which may increase the total biomass. Leaf area was increased by 46% at eCO_2 compared to ambient grown by Jyothilakshmi et al. in *Vigna mungo* L.[13].

The maximum increase in leaf dry weight was at 30 DAS and it was 47.8% in Dharani, 54.7% in K-9. The leaf biomass increased under eCO_2 was reported in

groundnut. Increased leaf area and leaf biomass are expected to improve total biomass as well as yield of a crop as it can improve the photo assimilation of the plant.

3.4 Total Biomass: Among the two genotypes, Dharani recorded higher total biomass at all growth stages as well as under both aCO_2 and eCO_2 than K-9 while K-9 recorded higher response to eCO_2. It is interesting to observe that the eCO_2 impact on total biomass was more at initial growth stages as total biomass increased by 42.4% in Dharani at 30DAS whereas 53% in K-9 at 60 DAS. The increased total biomass at eCO_2 in both the genotypes was mainly contributed by enhanced stem and leaf biomass at initial growth stages. An increased above ground biomass in pigeon pea under elevated CO_2 conditions wasdetectedby Saha et al. [14].

3.5 Yield parameters: Higher numbers of pods were observed at eCO_2 in Dharani while at aCO_2 in K-9. Similar trend was recorded for pod weight and seed weight. It is excited to perceive that among the yield parameters, eCO_2 improved only seed number with K-9 and this response could not improve seed weight as the seed filling in this genotype was poor which reflected in reduced test weight. It is also noted that in both the genotypes the test weight decreased under eCO_2 and it could be due to the prolonged peg initiation and pod formation with poor seed filling.

Among all the yield parameters, Dharani recorded improved performance for pod number, pod weight, seed number, seed weight with eCO_2 while only seed number improved with K-9. Under aCO_2 more number of pods per plant was recorded with K-9 while higher improvement with eCO_2 was registered with Dharani. The increased pod number in Dharani contributed to increased pod weight and seed weight at eCO_2 while no response was recorded with K-9. The reduced test weight in K-9 indicating poor seed filling at eCO_2 though higher seed number was recorded. It clearly indicating the response of vegetative and reproductive biomass at eCO_2 differed with selected genotypes, as higher improvement in vegetative biomass with K-9 and reproductive biomass with Dharani was observed.

3.6 Harvest index (%) (H): The genotype Dharani maintained similar HI at both aCO_2 and eCO_2 as this genotype was able to proportionate the increased biomass to reproductive components. However the response of K-9 was entirely different and the increased total biomass at eCO_2 was mainly due to improved performance of vegetative components specially leaf and stem biomass and the yield components failed to respond to the eCO_2. This resulted in decreased HI of K-9 at eCO_2 revealing that this genotype was not able to take the advantage of enhanced CO_2 environment in terms of yield. The results which are in tune with previously reported findings revealed a significant increase in the HI in black gram due to their improved partitioning efficiency under eCO_2 conditionby Vanaja et al. [15].

The results obtained from the present study showed an increase in the dry matter production as well as economic yield at eCO_2 level. The seed yield improved by 12.59% in Dharani, whereas the harvest index (HI) increased only by 0.7%. This clearly shows that the eCO_2 improved both biomass and economic yield. Thus it may be concluded that the groundnut genotype Dharani is positively responding to increasing CO_2 not only for biomass but also for seed yield.It was also conveyed by Krishna Reddy et al. that Dharani produced significantly higher pod yield than other genotypes on sandy loam soils of Tirupati, Andhra Pradesh, during early kharif under irrigated conditions [16].

To conclude, the present study reveals that the importance of legume crops which are protein rich could also sustain under climate change to meet the demand. Overall results revealed that the highest response to eCO_2 in terms of seed weight, harvest index was shown by Dharani, biomass in K-9. Hence identification of traits and genotypes to fit in the future predicted climatic conditions is required to sustain and improve the yield.

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Effect of Different Plant Growth Regulators on Callus Induction from Seeds of Chickpea (Cicer arietinum L.)

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Abstract— *The present study was undertaken to develop a reproducible protocol for efficient in vitro callus initiation of chick pea (Cicer arietinum L.). The main objectives of this present study were to develop the optimal concentrations and combination of auxin and cytokinin for optimized callus induction from seeds as explants. Callus induction was initiated from seeds on MS media supplement, which varied according to the plant growth regulators treatment. Among the growth regulator combinations the highest rate of callus induction (85%) was observed in MS medium containing 2 mg L^{-1} of 2,4-Dichlorophenoxyacetic Acid (2,4-D), 2 mg L^{-1} Benzylaminopurine (BAP) showed higher percentage (63%) of callus formation than 1- Naphthaleneacetic acid (NAA), which produced 49% of callus. There were significant differences in percentage of calli fresh/dry weights (g/jar) on the different initiation (seven) medium used were the MS+2,4-D, MS+2,4-D +NAA+ BAP and MS+ BAP had the highest fresh/dry weights (g/jar) in both induction medium.*

Keywords— Chickpea, explants, in vitro, callus, plant growth regulators and sterilization.

Abbreviations— MS = Murashige and Skoog media; 2, 4-D = 2, 4-Dichlorophenoxyacetic Acid and NAA= 1-Naphthaleneacetic Acid BAP = Benzylaminopurine .

I. INTRODUCTION

Chickpea is an important grain legume cultivated worldwide on more than 12 million hectares [1] and representing an important and available protein, phosphorus, iron and soluble vitamins source. It is the third important food legume of the world [2]. The protein content of chickpea is 22 % [3]. It is usually supplemented with cereals to form balanced diet compared to the other sources of protein, pulses are the cheapest source and have been called “Poor man’s meat.” Chickpea proteins are rich in essential amino acid "Lysine" which is generally absent in food grain. It has been cultivated from ancient times in the Mediterranean region, in the Middle East and in Indian subcontinent. However, its production is limited due to many biotic and abiotic stresses. Besides providing protein to the diet, legumes have served the purpose of adding valuable nitrogen and organic matter to the soil and provide rich fodders to the milk and draft animals.

Plant tissue culture plays an important role in the production of agricultural and horticultural plants and in the manipulation of plants for improved agronomic performance. *In vitro* culture of plant cells and tissues has attracted considerable interest in recent years because it provides the means to study the physiological and genetic processes of plants in addition to offering the potential to assist in breeding improved cultivars increasing their genetic variability. Regenerated plants are expected to have the same genotype as the donor plant; however, in some cases, somaclonal variants are found among regenerated plants, e.g. in rice [4, 5, 6, 7]**.** The composition of the medium, mainly the hormonal balance, is another important factor influencing *in vitro* culture initiation and plant regeneration from somatic embryos [**8**]. The auxin 2, 4-dichlorophenoxyacetic acid (2, 4- D), alone or in combination with cytokinins, is widely used to enhance callus induction and maintenance [9]. Genetic factors are considered a major contributor to the *in vitro* response of cultured tissues. Differences in the production of embryogenic calli and regenerated plantlets have been observed, depending on the genotype and explant source [10]. Therefore, plant regeneration from callus culture could provide useful germplasm for plant breeding programmes.

Considering the above facts, the present experiment was undertaken to develop a stable, reproducible, and efficient protocol for callus induction of chickpea *in vitro* conditions and to identify the medium suitable for seeds callus induction.

II. MATERIALS AND METHODS

2.1 Plant Materials

The seeds of chickpea used in this study were purchased from the local market healthy and uniform, Al Bayda – Libya. The definition of the type of seeds through the Herbarium in the Department of Botany

2.2 Seed **sterilization**

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Seeds of chick pea were collected as the source of explants. These collected explants were used for the callus induction. The seeds brought to the laboratory and then they were first cleaned thoroughly under a running tap water for 20 min. The explants were immersed in detergent solution and then rinsed three times with autoclaved water. For further surface sterilization of seeds, first ethanol solution (70%) at 1.5 min and seeds were disinfected with 20 % (v/v) Sodium hypochlorite (5.25% Cl_2) containing two drops of a wetting agent Tween 20 solution at 15 min were applied and then for 3-5 times explants were immersed in double- distilled water. Due to importance of explants inoculation step in tissue culture technique, this procedure was conducted in the laminar airflow. The material preparation was conducted following by the method of Verma [11] with some alteration.

2.3 Media preparation

Different callus induction media consisting of fllu MS basal (Murashige and Skoog) these media supplemented with various concentrations of auxins and cytokines, like), 1- Naphthaleneacetic Acid (NAA), 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Benzylaminopurine (BAP) **Table 1**.

Table.1: Culture media composition (Murashige and Skoog 1962)

MS+2 mg L^{-1} 2,4D	MS+2 mg L^{-1} 2,4D +2 mg L^{-1} NAA
MS+2 mg L^{-1} NAA	MS+2 mg L^{-1} 2,4D + 2 mg L^{-1} BAP
MS+ 2 mg L^{-1} BAP	MS+2 mg L^{1} NAA+ 2 mg L^{-1} BAP
MS+0.5 mg L^{-1} 2,4D +0.5 mg L^{-1} NAA+ 0.5 mg L^{-1} BAP	

Both media contained 30 g L^{-1} sucrose and the pH of the culture medium was adjusted to 5.8 after adding 8 g L^{1} agar , then autoclaved for 20 min., at 121°C and 1.1 kg/cm^2 pressure. In complete aseptic conditions sterilized seeds were cultured on different callus induction media . All cultures were incubated in a controlled growth chamber, and maintained at 25 ±1°C at normal condition . Each treatment was represented by 10 cultures and the experiment was repeated three timesthen all cultures were subcultured using same medium after 3 weeks from incubation. three and five weeks from culture, the frequency of explants producing calli (%), calli fresh/dry weights (g/jar) were recorded respectively.

Calli frequency

Calli frequency was calculated according the following equation:

$$\textbf{Calli}\ (\%) = \frac{\textbf{Number of explants producing calli}}{\textbf{Total number of cultured explants}} X100$$

Calli fresh weight (g/jar)

Weight of callus was taken from each treatment after five weeks were carefully air dried and recorded as fresh weight (g/jar).

Calli dry weight (g/jar)

Weight of fresh derived callus taken from each treatment were dried using oven at 40 °C for 72 h, and recorded as dry weight (g/jar).

Statistical analysis

The test of least significant difference (L.S.D) at the level of 0.05% significance was used to examine differences among treatment means and interactions. Data were statistically analyzed using MSTAT-C software package according to the described method by Freed et al [12].

III. RESULTS AND DISCUSSION

Callus induction and initiation of chickpea seeds were observed after 5 days. All seeds formed callus in both induction medium the data of callus induction frequency were recorded from seed explants on seven different media with different levels of plant growth regulators. Callus was first visible within five and seven days in MS+2, 4-D (2 mg L^{1}) and MS+ BAP (2 mg L^{1}) medium respectively. Callus induction rate, and fresh/dry weights of callus were greatly influenced by both induction medium and seeds explants (Table 2).

Maximum callus induction (85%) was obtained for explants when seeds were cultured on MS medium enriched with MS+2, 4-D (2 mg L^{-1}) medium. Lowest callus of (47%) was observed for seeds, when MS media are supplemented with BAP (2 mg L^{1}). Rao and Chopra [13] have reported that initiation and development of calli were influenced by the medium and chickpea genotypes. These genotypic differences with respect to callus initiation were also observed in many other plants [14, 15, and 16]. Among all the growth regulators used, 2,4-D was found to be the most effective growth regulator for chick pea callus induction either when used alone or in combinations with cytokinins. The highest callus induction from hypocotyl was resulted in [17] expriment on MS medium supplimented by 2 mg L^{1} 2, 4-D + 0.5 mg L^{1} NAA. This result is same as my resultsimilar to me findings.

As well as the obtained results indicated that using 2-4D alone as only growth regulators in the culture medium enhanced of callus . This coincided with other authors **[18]** they affirmed that auxins 2,4-D represent one of the most important classes of signaling molecules involved in the regulation of cell division, cell elongation and cell differentiation in higher plants .

Table.2: Effect of media composition on frequency of callus (%) and calli fresh and dry weights (g) from seeds of chickpea regardless of the culture conditions.

Medium	Callus induction (%)	Weight of callus (g)	
		Fresh	Dry
MS+2 mg L^{-1} 2,4D	85[a]	3.04[a]	0.72[a]
MS+2 mg L^{-1} NAA	49[d]	1.23[c]	0.13[bc]
MS+ 2 mg L^{-1} BAP	63[b]	2.52[b]	0.56[a]
MS+2 mg L^{-1} 2,4D +2 mg L^{-1} NAA	62[b]	1.01[d]	0.20[b]
MS+2 mg L^{-1} 2,4D + 2 mg L^{-1} BAP	62[b]	1.27[c]	0.33[b]
MS+2 mg L^{1} NAA+ 2 mg L^{-1} BAP	55[c]	1.12[c]	0.20[b]
MS+0.5 mg L^{-1} 2,4D +0.5 mg L^{-1} NAA+ 0.5 mg L^{-1} BAP	57[bc]	1.75[c]	0.53[a]

Data scored after 3 weeks (for callus induction) and 5 weeks (for weight of callus) in culture 30 explants per treatment. Means followed by the same letter are not significantly different at the 0.05 probability level.

IV. CONCLUSION

In conclusion, the results of the present study indicated that seeds have good callus induction ability. The *in vitro* protocol reported in this study could be used for clonal propagation of chick pea and to obtain competent target tissue for genetic modification.

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A Review of Landscape Design as a Means of Controlling Gully Erosion

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Abstract**—***Gully erosion is the most visible and devastating form of soil erosion known to be one of the leading causes of land degradation worldwide. Landscape design is one of the techniques used in combating this problem. The objective of this paper is to review the use of landscape design in controlling gully erosion. The method used is a review of academic articles, conference papers, internet materials, textbooks and publicly available materials on landscape design and gully erosion. The results of this study indicated that previous authors whose works were reviewed have a convergent view that the use of vegetative approach, an aspect to landscape design, must be practiced for effective control of gullies. On the other hand, however, previous authors have divergent views on the use of structural approach, another aspect to landscape design, for control of gully erosion. Recommendations of this study include: (1) planting of a combination of woody trees and grasses; (2) use of some locally accessible structures for example, sieve structures; and (3) practise of agroforestry.*

Keywords— Landscape Design, Gully Erosion, Review, Sustainable Development, Structure, Vegetation.

I. INTRODUCTION

Gully erosion has been recognized as the major cause of land degradation worldwide (Musa, Ahmed, Muhammed and Abdul, 2016). According to them, it is fast becoming the most reoccurring disaster in many countries of the world. Gullies are steep sided watercourses, marked by stepped longitudinal profile and commonly an abrupt channel head, subject to intermittent flow of water (Khairulmaini and Fatemeh, 2011). Poesen (2011) observed that gullies are among the morphological indication of long periods of soil erosion revealing the effect of atmospheric adjustment such as heavy rainfall and land use practices in the landscape. Gully erosion has attracted a growing interest as reflected by two recent international conferences: one in Leuven, Belgium (Poesen and Valentin, 2003) and one in Chengdu, China (Valentin, Poesen and Li, 2005).

By removing vegetation cover, the erosion-resisting capacity of the soilbecomes disturbed (Rickson, 2001). He also opined that when the kinetic energy of rainfall splash increases, it results in increases in soilseparation. He further stated that hydraulic surface flow mostly increases with lack of vegetation cover, which inevitablyincreases soil susceptibility to erosion mostly gully, by reducing cohesion and shear strength. Ehiorobo and Audu(2012) reported that gully erosion occurs due to extreme overflow of fluid with a very high speed and energy to remove and transmit soil particles down-hill slope.In most instances, the development of gullies is caused byovergrazing, road construction and urbanization, log haulage, improper farming andirrigation practices (Valentin *et al.*,2005).

The need to understand how to monitor and prevent gulling is particularly acute (Poesen, Nachtergaele Verstraten and Valentin, 2003) and the search forinexpensive, durable, low maintenance techniques to control gully erosion has proven elusive (Norton, Bowannie, Peynetsa, Quandelacy and Siebert, 2002). It is important therefore, that a holistic understanding of gully erosion should involve collaborative inputs from experts in diverse fields like chemists, geologists, biologists and others (Brevik, Cerda, Mataix-Solera, Pereg, Quinton, Six and Van, 2015).

Landscape as an essential part of the environment which includes topography, vegetation and associated plants and soil, water bodies, is one of the most visual needs of people (Zheng, Zhang and Chen, 2011). Williams and Tilt (2006) opined that an effective landscape design can become an integral part of a good community environment. A well defined landscape space can enhance the quality of living areas which meets people`s preferences (VanDerZanden and Rodie, 2008). A multifunctional landscape design solution must embrace the various ecosystem services that have already been bequeathed to a land area. These services include: (1) supporting and biophysical services (e.g., protecting and enhancing biodiversity and water quantity and quality); (2) provisioning services (e.g., production of energy and other utilitarian resources); (3) regulating

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services (e.g., waste reduction and reuse); and (4) cultural and social services (e.g., visual quality, beauty, human health, and recreational opportunity) (Lundy and Wade, 2011). Landscape design is thus defined as the art of modifying an area for aesthetic or practical reasons (David, 2017).

Amangabara(2012) stated that erosion control is an important factor in landscape design and the prevention of erosion must be top priority during the life cycle of planning for soil conservation in any landscape design. The application of landscape design for controlling gullies requires a good knowledge of hydrometeorology and surface hydrology (Professional Landscape Design, 2007).Controlling gully erosion is a multi-approach goal in that there are three main areas to review in any landscape design project involving conservation and they are design consideration, vegetation selection and soil treatments (Blair, 2014).This paper is thus focused on reviewing the use of landscape designs whether vegetation or artificial installations in controlling gully erosion.

1.1 Statement of the Problem

Gully erosion usually has unpredictable impacts that are often serious and flashy(Nyssen, Poesen, Moeyersons, Deckers, Miiku and Lang, 2004).They also asserted that in the Ethiopian highlands, the development ofgullies has led to an enlarged drainage of the inter gully areas, resulting in soil moisturedecrease and a corresponding crop yield reduction on plots located near the gully walls. In tropicalnorth-western Australia, about 80% of the sediment in the Lake Argyle reservoir hascome from gully and channel erosion and less than 10% from the catchment in the area of highly erodible soils formed on sedimentary rocks (Wasson,Caitcheon, Murray, McCullochand Quade, 2002). Gully erosion has also caused loss of farmland leading to drastic decrease in soil productivity, loss of property and threat to life resulting from food shortage and famine (Abdulfatai, Okunlola, Akande, Momohand Ibrahim, 2014).

According to Blair (2014), artificial installations used forerosion control in landscape design are effective to certain extents but may however besubject to decayand become less effective overtime. He was also of the opinion that some of these installations are limited in capacity and that it has been common practice to implant concrete or rock barriers intoerosion gullies in the hope that a solid barrier will counteract erosiveactivity and capture sediment.He further stated that these efforts to control gully erosionwith a solid barrier though helpful at the onset of installation usually fail.An example is the sieve structure which can only slow down gully water flows but not stop them (Layne, BreinDemisachew, Jaldesa, Badasa and Dereje, 2015). Hydrological knowledge needed for effective implementation of landscape designs is most times relegated to the background especially in developing countries,hence, the problem of gully erosion and its negative impacts continue(Amangabara, 2012).

1.2 Objective

The objective of this study is to review the use of landscape design in controlling gully erosion.

II. CONCEPTUAL FRAMEWORK: SUSTAINABLE DEVELOPMENT

This research is based on the concept of sustainable development. Morelli (2010) saw sustainable development as meeting the resources and services needs for current and future generations without compromising the health of the ecosystems that provide and more specifically as a condition of balance, residence and interconnection that allows human society to satisfy its needs while neither exceeding the capacity of its ecosystems to continue to regenerate the services necessary to meet those needs nor by our actions diminishing biological diversity.World Conference on Environment and Development (WCED) (1987) opined that sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs. This research sets to review landscape design as a means for gully erosion control so as to build in sustainability into the management of the phenomenon.

III. METHOD

The researchers gathered a total of 34 materials for this research, but were able to summarize the characteristics of 10 that were deemed more relevant tolandscape design as means of controlling gully erosion and sustainable development. This research made use of academic articles, conference papers, internet materials, textbooks and publicly available materials on landscape design and gully erosion.

IV. LITERATURE REVIEW

Markus, Miloš, Jozef, Štefan and Pavol(2013) stated that erosion as one of the major and most widespread forms of land degradation, poses severe limitations to sustainable agricultural land use, reduces on-farm soil productivity and contributes to water-quality problems from the

accumulation of sediments and agro-chemicals in waterways. They also claimed that gullies are formed mostly after deforestation,the beginnings of agricultural utilization and that they are often controlledby access roads or by other linear artificial landscape elements. They reported further that accelerated water erosion in general includinggulling was most effective when human interference wascombined with colder and wetter climatic fluctuations.

Prolonged erosion causes irreversible soil loss over time, reducing the ecological functions of soil: mainly biomass production, crop yields due to removal of nutrients for plant growth, and reduction in soil-filtering capacity due to disturbance of the hydrological cycle; from precipitation to runoff (Víctor, Dur´an, Carmen and Rodr´iguez ,2008). They further stated that runoff is a fundamental process in land degradation, causing soil erosion and influencing the soil water balance and hydrology of the catchments. They also noted that in a wide range of environments, both runoff and sediment loss will decrease exponentially as the percentage of vegetation cover increases and that soil-erosion resistance increases exponentially with greater root density.

McGarigal (2006) defined landscape as an area that isspatially heterogeneous in at least one factor of interest. He pointed out that the concept of landscape differs from thetraditional ecosystem concept in focusing on groups of ecosystems and the interactions amongthem and that the focus is on spatial heterogeneity and its impact on process.According to Williams and Tilt (2006), landscape design is the art of developing property for its greatest use and enjoyment and canbecome an integral part of a good community environment.They reported that increasing evidence has shown that designcan achieve multifunctional benefits if the rolethat natureplays is taken into consideration. They further opined that landscape design has multiple benefits such as decentralized and naturalized ways of managing stormwater, runoff deduction and water quality enhancement. Musacchio (2009) was of the opinion that there is the need to be an intricate balance between the environmental and socio-economic aspects of a design to achieve sustainability goals. He further claimed that every design has its main focus and project success should be measured based on its main project goal, rather than by a rigid set of metrics.

Katherine, Valerie, Carissa and Eric (2002) stated that understanding gully erosion mechanism is very important to design the gully erosion measurement system and develop its control. They observed that the use of vegetation to control erosion has been practised in many countries for centuries. They enumerated some factors to consider when using vegetation to control erosion which include texture and layering of materials, existing vegetation and surface and groundwater movement from upslope and so on. In the view of Yifan, Yongqiu and Wen (2011), the control of gully erosion can be divided into three approaches. They stated that the firstis to try to stabilize the gully using the vegetation cover method, the second is to control the runoff flow from upstream of the gully and the third is to build some soil conservation works inside the gully to restore the hydraulic balance of the gully. There are two essential components to managing the erosion problem: rehabilitating the landscape to control the source of soil loss, and reducing sediment flow through the gully system (Layne, Brien, Demisachew, Jaldesa, Bedasa, and Dereje, 2015). They argued that gully erosion cannot be stopped completely, especially when gullies receive rushing floods of water from heavy rainfall but that gullies can be treated to achieve long-term suppression of sediment transport, and when combined with better landscape management the erosion can be substantially reduced. In the opinion of Valentin, Poesen and Li (2005), many techniques have proved to be effective for gully prevention and control, including vegetation cover, zero or reduced tillage, stone bunds, exclosures, terracing and check dams. They also opined that aboveground vegetation is known to favour water infiltration and to protect soil from erosion. They further claimed that gully erosion is reduced when soil physical properties such as structural stability and infiltrability is improved by the inherent strength of the tree root mat that binds the surface soils.

Izinyon, Ehiorobo and Adedeji (2013) asserted that stabilization of gullies involves the use of appropriate vegetative measures in the head, floor and sides of the gully. They further stated that once gullies begin to form, they must be treated as soon as possible, to minimize further damage and restore stability and that there are a multitude of physical and biological techniques which can be applied for effective gully treatment. In their opinion, the combination of the two measures (biophysical approach) is the best solution for effective gully control and for productive use of the gully area andthe construction of gully physical structures will be followed by the establishment of biological measures. They also reported that natural regeneration which comes after the gullies are protected and enclosed should also be considered in the overall rehabilitation scheme and attention must always be given to keeping the gully catchment well vegetated. According to

Mwango, Msanya, Mtakwa, Kimaro, Deckers, Poesen, Massawe and Bethuel (2014), roots bind particles in the topsoil, which offer protection to soilthat is under pressure of detachment by sheet flow or concentrated flow. They claimed that the presenceof roots also increases the soil's roughness, thereby providing a greater capacity forinfiltration and for reducing surface runoff velocity. Ken and Wallie (2011) suggested that various artificial installations are useful in controlling gully erosion. They were also of the opinion that plants reduce soil erosion by intercepting raindrops, enhancing infiltration, transpiring soil water and by providing additional surface roughness by adding organic substances to the soil. They further stated that plant roots have a mechanical effect on soil strength and that by penetrating the soil mass, roots reinforce the soil and increase the soil shear strength. They also reported that an important aspect of rehabilitation work, which is most often neglected, is the follow-up maintenance of rehabilitation efforts and after installation, the erosion control structures need constant attention (particularly after rainfall) to ensure that they are still effective and that they will continue to contribute to improvement. Yang, Ming-Han and Shujuan (2013) asserted that increasing tree canopy coverage is not only beneficial in the control of gullies but also mitigates Urban Heat Island (UHI) effect and may reduce the incidence of heat-related diseases**.**

Table.1: Summary of Characteristics of some Studies that describe Landscape Design as a means to Control Gully Erosion

S/N	Author(s)	Topic of Research	Method(s)	Result(s)	Recommendations	Conclusion
1	Katherine, Valerie, Carissa and Eric (2002).	A Property Owners Guide to Controlling Erosion Using Native Vegetation for Arrow Lakes.	Literature review of materials, physical measurement, observation.	The use of live branches is an effective method for ensuring water drains from a slope without removing parts of it.	In order to stabilize gullied slopes that feed into rivers, live branches should be anchored in a trench with a stake and tied up.	Eroded slopes can generally be stabilized using vegetation and bioengineering methods.
2	Ken and Wallie (2011).	Practical Soil Erosion Control and Veld Rehabilitation in the Little Karoo.	Physical measurement, observation.	Hollows facilitate water infiltration on bare capped soil surfaces where very little rain water infiltrates the soil surface.	Existing rooted vegetation should not be disturbed in any landscape as this encourages the occurrence of soil degradation.	Using a combination of methods outlined will be more effective for rehabilitation and also be more cost-effective.
3	Yang, Ming-Han and Shujuan(2013).	Design-with-Nature for Multifunctional Landscapes: Environmental Benefits and Social Barriers in Community Development.	Use of Geographic Information System (GIS), literature review of materials.	Design-With-Nature concept demonstrates benefits in reducing runoff and Urban Heat Island (UHI) effect.	Careful considerations must be paid to human perceptions and cultural values, which shape or reshape the way landscape is valued, appreciated, and managed.	Design-With-Nature approach has environmental benefits on storm water management and Urban Heat Island (UHI) effect.
4.	Victor, Duran, Carmen and Rodriguez (2008).	Soil-Erosion and Runoff Prevention by Plant Covers: A Review	Literature review of materials.	Results showed the impacts of plant cover on eroded Mediterranean soil.	Proven efficiency of the plant covers for the restoration of degraded environment should be	Careful assessment of soil for sustainable management through the use

					considered more widely.	of plant covers will aid the avoidance of catastrophic degradation.
5.	McGarigal(2006).	What is Landscape	Literature review of materials, use of GIS.	Basic approaches for defining a landscape and the importance of landscape definition in resource management planning and analysis.	A formal accuracy assessment should be completed that involves an extensive ground truthing of the maps representing landscapes because this will allow precise estimates of both errors of omission and errors of commission.	The landscape concept differs from the traditional ecosystem concept in focusing on groups of ecosystems and the interactions among them.
6.	Markus, Miloš, Jozef, Štefan and Pavol (2013).	Human Induced Soil Erosion and Gully System Development in the Late Holocene and Future Perspectives on Landscape Evolution.	Literature review of materials.	Demonstration of the interaction between land use, soil erosion, floodplain development, and land use changes of the study area.	Relative significance of slow processes and resultant changes on the complex interconnections between causes and effects of land use change and soil erosion should be observed and understood. .	Today's agricultural potential and possible future land use trajectories are strongly connected with the legacies of past land use changes and soil erosion.
7.	Izinyon, Ehiorobo and Adedeji (2013).	Appraisal of Structural and Non-Structural Approaches to Gully Erosion Control.	Literature review of materials, rainfall reading with rain gauge.	Results showed limitations in the use of structural approaches to curb the advance of gully erosion.	The use of biophysical approach which is a combination of structural and non-structural approaches to controlling gully erosion should be implemented.	The study area is susceptible to gulling due to high rainfall and dispersive nature of soil in the study area hence non-structural methods should be utilized to control and manage the gully.
8.	Valentin, Poesen and Li (2005)	Gully Erosion: Impacts, Factors and Control.	Literature review of materials.	Although many strategies to prevent and combat gully erosion have proved to be effective, they are	Research priorities should include sub-surface flow erosion processes, prediction models,	Gully erosion is not a process limited to badlands, mountainous

				rarely adopted by farmers in the long run and at a large scale in the study area.	and the causes of adoption or not of conservation strategies by farmers.	and hilly regions but a global and serious cause of land degradation affecting a wide variety of soils prone to crusting and/or piping.
9.	Mwango, Msanya, Mtakwa, Kimaro, Deckers, Poesen, Massawe and Bethuel (2014).	Root Properties of Plants Used for Soil Erosion Control in the Usambara Mountains, Tanzania.	Physical measurement, observation, literature review of materials.	Results showed the rooting characteristics of Guatemala grass (*Tripsacumandersonii*), Napier grass (*Pennisetum purpureum*) and Tithonia shrub (*Tithoniadiversifolia*) and their potential for erosion control.	In-depth studies to investigate physical Relative Soil Detachment (RSD) rate for different soil textures are recommended in order to come up with more representative RSD models.	Studies are needed to evaluate more plants growing in various habitats for selection of plant species that can effectively control concentrated flow erosion rates.
10.	Layne, Brien, Norton, Demisachew, Jaldesa , Bedasa , and Dereje (2015)	Sieve Structures to Control Gully Erosion on the Borana Plateau, Ethiopia	Physical measurement, observation, literature review of materials.	Placement of sieve structures and gabions in the gully was successful in controlling gully erosion.	It is important to begin gully remediation at the gully head otherwise erosion will continue to cut back upslope.	It is necessary to adopt a landscape approach that tackles the entire gully network, beginning where the gully erosion starts.

Source: Researchers' design, 2018

V. RESULTS AND DISCUSSION

Landscape designis a highly effective means of controlling gully erosion. Table 1 summarizes the characteristics of some of the studies reviewed in this research. The topics of the studies summarized capture different approaches in landscape designfor controlling erosion and also made use of standard methods for carrying out research such as literature review, physical measurement, observation, Geographic Information System (GIS) and laboratory analysis. All the researchers (e.g., Katherine, Valerie, Carissa and Eric, 2002; Ken and Wallie, 2011; Victor, Duran, Carmen and Rodriguez,2008; Layne, Brien, Norton, Demisachew, Jaldesa ,Bedasa , and Dereje) have a unity of opinion on the use of plants or vegetation as an approach in landscape design for controlling gully erosionalthough some of them object to the use of structures or installationsonly (e.g., Izinyon, Ehiorobo and Adedeji, 2013).

Previous authors made recommendations on different approaches to and concepts for landscape design for controllinggully erosion which include; (1) use of live branches to stabilize gullied slopes (Katherine, Valerie, Carissa and Eric 2002),(2) use of hollows which facilitate water infiltration (Ken and Wallie, 2011), (3) investigation of rooting characteristics of plants within the affected location (Mwango, Msanya, Mtakwa, Kimaro, Deckers, Poesen, Massawe and Bethuel, 2014), (4) design-with-nature concept for landscape design (Yang, Ming-Han and Shujuan,2013); and (5) placement of sieve structures and

other locally constructed structures for the control of gullies (Layne, Brien, Norton, Demisachew, Jaldesa , Bedasa , and Dereje, 2011).

VI. RECOMMENDATIONS

The specific recommendations for the use of landscape design as a means for controlling gully erosion emanating from this paper are:

1. Introduction of vegetation in affected communities should not just be limited to planting of woody trees whose canopies intercept raindrops andform larger ones that have more kinetic energy to detach soil particles, but should include planting of grasses that would absorb the kinetic energy of the larger raindrops formed by the canopies.
2. Use of structures such as terraces which alter existing environmental conditions of the soil in any community should be discouraged. This is because, in the case of terraces, the ground is cemented causing absorptive capacity of the soil to be compromised hence, increasing runoff that detaches and transports soil particles.
3. The use of structures such as sieves which do not degrade the soil and can be easily accessed locally should be introduced to compliment vegetative control of gullies.
4. The practice of agroforestry which is a combination of planting of economictrees and cropping activities should be encouraged. This will encourage control of gullies and generation of income for the affected community.
5. Environmental legislations/regulations which discourage cementing of residences of individuals within gully erosion prone communities should be established and fines levied to offenders.

VII. CONCLUSION

This paper discussed landscape design as a means of controlling gully erosion through a review of works done by previous authors. It reviewed two major aspects of landscape design: use of vegetation and the use of structures or installations in controlling gullies. The authors generally agreed that vegetation helps to curb gully erosion however; some of them were against the use of only structures in controlling gully erosion while others encouraged it. Landscape design is an effective tool in controlling gully erosion even though it may be subject to decay especially where the use of structures is concerned as illustrated by some of the authors' works reviewed.

This study therefore concludes that landscape design should be adopted to prevent and control gully erosion giving special attention to the use of vegetation as this not only helps to control gullies but also has other environmental benefits (e.g. improvement of aesthetic quality of the environment).

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Fungicidal effect of three plants extracts in control of four phytopathogenic fungi of tomato (*Lycopersicum esculentum* L.) fruit rot.

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***Abstract*—** *Fungicidal effect of leaf aqueous extracts of Azadirachta indica, Tithonia diversifolia and Chromolaena odorata were determined on rot causing fungi. In the study, the phytopathogenic fungi isolated from the infected tomato fruit parts and identified based on morphological and cultural characters were: Aspergillus niger Van Tiegh, Fusarium oxysporum Schlecht, Geotrichum candidium Link and Rhizopus stolonifer Ehrenb. ex. Fr. as confirmed by pathogenicity tests. Leaf aqueous extracts of different concentrations (20, 40, 80, 60 and 100 % w/v) of A. indica, T. diversifolia and C. odorata were added to growth media prior to inoculation. All aqueous extracts of the tested plants significantly ($p < 0.05$) reduced mycelial growth of the fungal pathogens and this effect gradually increased with increasing concentration. Fungicidal activity was strongly exhibited by A. indica extract at 100% w/v against all the pathogenic fungi. In the case of T. diversifolia extracts inhibitiory effects at 20, 40, 60, 80 and 100% w/v were greater than those of C. odorata on A. niger , F. oxysporum and G. candidium while for R. stolonifer inhibition, C. odorata produced the highest in the all five concentrations than T. diversifolia extracts. It could be emphatically concluded that the tested plant extracts can effectively control rot causing fungi disease of tomato. This makes them potential biocide in diseases management in that they are cheap and environmentally safe as they showed fungicidal and fungitoxic ability.*

***Key words*— *Aqueous extracts, Fungicidal effect, Inhibitory effects, Phytopathogenic*.**

I. INTRODUCTION

Tomato (*Lycopersicum esculentum* L.) is the second most important vegetable crop in the world because of its special nutritive value. The edible fruit of the tomato plant has a series of usages in different forms. The crop is nutritious and contain high amount of dietary source of vitamins A, B, C, E and nicotinic acid (Kanneh *et al.*, 2015; Godia, 2014). In Sierra Leone and other parts of the world, it is consumed as fresh fruit, salads, soup and stew and often used in other dishes (Osei *et al*., 2014). Its cultivation provides source of employment to many and continue to play a key horticultural role in the sub-region in terms of reducing poverty and food insecurity (Osei *et al*., 2014). Recently, according to Kanneh *et al.* (2015), the Global production of fresh fruit tomato is about 100 million tons cultivated on 3.7 million hectares.

In Sierra Leone as well as other countries in Africa, the average yield on farm is between 7.5-10t/ha (Godia, 2014) which is far below the potential yield of the crop 45-50 M t/ha. Tomato production is seriously affected by over 200 diseases caused by pathogenic fungi, bacteria, viruses and nematodes (Abad Z. G. and Abad J. A., 1997; Koch et al. 1992; Lukyanenko, 1991; Moriones and Navas-Castillo, 2000). Major fungal diseases affecting tomato production are late blight, early blight, septoria leaf spot, fusarium wilt, and verticillium wilt, corky root rot, damping-off, leaf mould and powdery mildew (Panthee and Chen, 2010).

Most farmers control these diseases using fungicides. However, the negative environmental impacts, mammalian toxicity and high costs are making their use unattractive thereby searching for alternatives such as natural plant-based chemicals (Asawalam, 2006). Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan, 1991). These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (Das *et al*., 2010). Many research workers have tried to find out safe and economical control of plant diseases by using extracts of different plant parts (Hasan *et al*., 2005; Bdliya and Alkali, 2008). Hence the objective of the study was to determine to efficacy of aqueous leaf extracts for controlling some important postharvest tomato fruit rot diseases in *in-vitro*.

II. MATERIALS AND METHODS

2.1 Experimental Site and Source of Materials

Experiments were carried in Department of Crop Protection, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta. The tomato fruits with symptoms of rot were randomly collected from two different markets in Ogun State, Nigeria. The markets are Osele main market, Oeda Local government and Kuto main market, Abeokuta South.

2.2 Preparation of culture media

Potato Dextrose Agar (PDA) (BAM Media M127) was prepared by dissolving 39 grams in 1 litre Erlenmeyer flask and then made up to 1 litre using sterile distilled water. The medium was autoclaved at 121^{o}C for 15 minutes at 15 lb. The sterilized medium was allowed to cool to 45°C, before supplemented with streptomycin sulphate (3 grams) and aseptically dispensed into sterilized 9 cm diameter glass Petri dishes.

2.3 Isolation and Identification of Fungal Pathogens

Diseased tomato fruits were randomly collected from the two different Markets. The Chiejina (2008) isolation method was used. Thin sections (2 mm diameter) were cut from the Periphery of diseased tomato fruits and surface sterilized in 0.1% mercuric chloride for 2-3 min, after which they were rinsed in three changes of sterile distilled water. The sections were plated in water agar and mycelium was transferred into clean PDA plates.

The plates were incubated at room temperature (27 ± 2° C) for 6-7 days. Subcultures were made ascetically from the plates into similar clean PDA plates and were incubated under similar conditions until pure cultures were obtained. The identification of the isolated fungi was done macroscopically and microscopically. Macroscopic identification was based on observed culture growth patterns and mycelial colour. Small portions of the fungal cultures were teased and mounted in lactophonel in cotton blue on clean slides, covered with clean cover slips and then viewed microscopically. Fungal identification was confirmed with the aid of books by Barnett and Hunter (1999), Alexopoulos *et al*. (2002), Agrios (2005) and Ellis *et al.* (2007)

2.4 Pathogenicity Test

Each of the fungal isolates obtained from the diseased tomato fruits were tested for their ability to cause the same disease condition previously observed in healthy tomato fruits. Health tomato fruits were washed in sterile distilled water and surface sterilized by dipping into 0.1% mercuric chloride and with the aid of a sterile cork borer, cylindrical cores were removed from each of the tomato fruits. Pure cultures of the isolates of the isolates were introduced into the cores and the cores were replaced and sealed with sterile petroleum jelly. The fruits were kept at room temperature for 7-10 days. On establishment of disease condition, inocula were taken from the infected tomato fruits and cultured. The organisms were re-isolated and identified as previously isolated organisms. This was taken as evidence that they incited the disease.

2.6 Sources of plant materials and Preparation of Extracts.

Leaves of three plant species namely *Azadirachta indica* (A. Juss) (Neem), *Tithonia diversifolia* (Hemsley) A. Gray (Mexican sunflower) and *Chromolaena odorata* (Linn) were used in the experiment. These were obtained within the premises of the Federal University of Agriculture, Abeokuta. Fresh leaves of *A. indica*, *T. diversifolia* and *C. odorata* were washed in tap water then surfaced-sterilized with (1% NaOCl for 5min and rinsed in five changes of sterile distilled water) and air dried at (28 ± 2 °C) for 1h. 20grams, 40grams, 60grams, 80grams and 100 grams of each plant material were grounded using sterilized Brabantia 5-speed blender (Model BBEK 1051) in 100 ml distilled water, and then filtered through a Whatman® No. 9 filter paper separately into a 250 ml Erlenmeyer flask to produce 20 %, 40 %, 80%, 60% and 100% extract concentrations.

2.7 Effect of plant extracts on mycelial growth inhibition of fungal pathogens.

Extract-media mixtures were prepared by mixing 1 ml extract with 9 ml molten PDA prior to solidification for each extract concentration. Media amended with mycelial disc of a 5- day-old cultures of each fungus were placed in the centre of the petri dishes. The control plates consisted of PDA mixed with 1 ml sterile distilled water. Benlate a standard fungicide, at a concentration of 20 mg/ml was used to assess the efficacy of the plant extracts Owolade and Oskkanlu (1999) modified method. All treatments were in three replicates and incubated at 28 ± 2°C. Radial growth in treatments and control were measured at 24 h interval for seven days. This was expressed as the mean growth along two axes on two pre-draw perpendicular lines on the reverse side of each plate. The percentage inhibition of mycelial growth by each extract was computed using formula.

$I = 100\times (C - T)/C$

Where;

I = percentage inhibition of mycelial growth
C = mycelial growth of fungus in control plate
T = mycelial growth of fungus in the treatment

(Sobia *et al*., 2011)

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III. STATISTICAL ANALYSIS

Data were subjected to analysis of variance (ANOVA) and significant means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

IV. RESULTS

4.1 Inhibition of mycelial growth of *Aspergillus niger* by three aqueous plant extracts

The aqueous extracts of the tested plants reduced mycelial growth and inhibition of *A. niger*. However, the inhibitory effect of the plant extracts and benlate solution on the mycelial growth *A. niger* was significantly different (P <0.05) at all the various concentrations. *A. indica* exerted the highest inhibitory effect of (92.0 %) at 100 % (w/v) followed by *T. diversifolia* and *C. odorata* at the same concentration. Fungitoxicity of tested plant extracts against *A. niger* increased as the concentration increased (Table 1).

Table.1: Inhibition of mycelial growth of Aspergillus niger by three aqueous plant extracts

Plant Extracts	*Azadirachta indica*		*Tithonia diversifolia*		*Chromolaena odorata*	
Concentration % (w/v)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)
100	0.90	92.0	1.44	80.5	2.00	75.7
80	2.04	75.5	2.20	70.5	3.34	64.5
60	3.67	60.2	2.52	67.4	3.95	56.5
40	4.70	54.5	3.65	50.9	5.01	45.9
20	6.98	40.7	3.98	44.8	6.83	25.6
Benlate	0.00	100.0	0.00	100.00	1.00	88.9
Contol	9.00	-	8.10	-	9.09	-
LSD (0.05)	0.840	20.25	1.022	8.08	0.840	10.45

4.2 Inhibition mycelial growth of *Fusarium oxysporum* with three aqueous plant extracts

The effect of aqueous tested plant extracts mycelial growth *F. oxysporum* revealed that three extracts produced significant (P<0.05) levels of inhibition of mycelial growth of *F. oxysporum* at various concentrations. The highest inhibitory of mycelial growth was manifested by *A. indica* (87.4%) at 100 % (w/v) concentration while the least (15.5%) inhibition was recorded for *A. indica* at the concentration of 20 % (w/v). However, benlate solution was superior in mycelial growth inhibition (Table 2)

Table.2: Inhibition mycelial growth of Fusarium oxysporum with three aqueous plant extracts.

Plant Extracts	*Azadirachta indica*		*Tithonia diversifolia*		*Chromolaena odorata*	
Concentration % (w/v)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)
100	0.73	87.4	2.00	76.8	3.33	62.7
80	1.11	80.8	2.77	68.5	5.66	40.0
60	3.14	45.0	3.07	64.6	6.34	35.4
40	4.04	30.1	3.40	60.3	6.45	31.0
20	4.87	15.5	3.47	53.8	7.68	25.2
Benlate	0.00	100.0	1.00	89.4	1.02	70.0
Contol	5.70	-	5.01	-	8.85	-
LSD(0.05)	0.440	7.70	0.77	9.13	1.882	24.10

4.3 Inhibition mycelial growth of *Geotrichum candidium* with three aqueous plant extracts.

The three plant extracts showed relatively high fungitoxic effect at 100 % (w/v) concentration on mycelial growth inhibition of *G. candidium*. At 80 % (w/v) concentration, *A. indica* induced the highest (70.6 %) mycelial growth reduction compared to *T. diversifolia* and *C.odorata*. However, benlate solution was high in exerting mycelial growth reduction. (Table 3)

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Table.3: Inhibition mycelial growth of Geotrichum candidium with three aqueous plant extracts.

Plant Extracts	*Azadirachta indica*		*Tithonia diversifolia*		*Chromolaena odorata*	
Concentration % (w/v)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)
100	1.13	79.8	1.50	65.5	1.52	60.9
80	4.52	70.6	2.01	54.0	2.05	50.5
60	2.20	62.5	2.54	45.1	2.50	40.0
40	2.80	50.0	2.82	32.5	2.88	31.9
20	4.40	30.4	3.25	24.7	3.30	19.8
Benlate	1.12	81.6	1.14	60.0	1.00	55.4
Contol	8.01	-	6.01	-	7.09	-
LSD(0.05)	1.73	16.05	0.82	23.82	1.20	21.5

Inhibition mycelial growth of *Rhizopus stolonifer* with three aqueous plant extracts.

Result showed that the tested plant extracts utilized produced significant levels of inhibition of mycelial growth of *R. stolonifer* at various concentrations. However, the highest inhibition was exerted by *A. indica* at 100 % (w/v) while the least inhibition (15.9 %) was exhibited by *T. diversifolia* at 20 % (w/v). As the concentration increased mycelial inhibition growth increased (Table 4)

Table.4: Inhibition mycelial growth of Rhizopus stolonifer with three aqueous plant extracts.

Plant Extracts	*Azadirachta indica*		*Tithonia diversifolia*		*Chromolaena odorata*	
Concentration %(w/v)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)	Mycelial Growth(mm)	Inhibition (%)
100	1.50	64.7	1.42	60.5	1.55	63.6
80	2.07	51.2	2.07	49.2	2.10	51.8
60	2.67	39.7	2.17	35.4	2.18	45.5
40	3.27	30.5	2.67	22.8	2.60	30.4
20	3.87	22.8	2.91	15.9	2.89	22.8
Benlate	1.01	48.2	1.12	44.8	1.20	50.3
Contol	4.27	-	5.89	-	6.98	-
LSD(0.05)	0.74	18.50	0.64	17.01	0.86	18.09

IV. DISCUSSION

The mycelial growth inhibition and of the pathogens by the leaf aqueous extracts of *A. indica T. diversifolia*, and *C. odorata* investigated in this study indicated that, antifungal activity showed by the tested plant extracts had inhibitory effects on the growth of *A. niger, F. oxysporum, G. candidium and R. stolonifer.* These results further revealed that antifungal activities of the extracts were enhanced by increasing the concentration from 20 to 100 % (w/v); hence the inhibition activities of the extracts were concentration dependent. This is in agreement with the report of Ilondu (2012) and Chiejina and Ukeh (2013) who indicated that increase in the antifungal activities had corresponding increase in concentration of plant extracts. *A. indica* exhibited high fungitoxic effect in inhibiting mycelial growth reduction against the four pathogen fungi.

The antifungal activity of *A. indica* conforms to the results of (Ogbebor and Adekunle 2005; Conventry and Allan 2001) that this extract is very effective in inhibiting the growth of *F. moniliforme*, *A. flavus* and *A. niger*. Fungitoxic properties of *A. indica* could be attributed to the presence of sapronin and alkaloid, chemical components which has been identified as antifungal agents in the plant (Kumar *et al*. 2008). The fungicidal effects of plant extracts on different pathogens of crop plants have been widely reported (Amadioha and Obi 1999; Okigbo and Ogbonnaya, 2006; Olufolaji, 1999 and Onifade, 2002).

However, the differences in the efficacy of the extracts could be attributed to the differences their active ingredients (Onifade, 2002; Okigbo *et al*. 2009). Major compounds of plant extracts are phenols, flavonoids, alkaloids, quinones, saponines, tannins and sterols (Halama and Van Haluwin, 2004) and their fungicidal or fungistatic properties against various plant pathogens have been established (Scheuerella and Mahaffee, 2002).

These products might either have direct inhibitory effects on pathogens, exhibiting fungicidal or fungistatic properties. They could help in the establishment of

favorable conditions for antagonistic microbes (Scheuerella and Mahaffee, 2002). Benlate solution at 20mg/ml was found to be more effective than aqueous plant extracts in inhibiting the mycelial growth of the pathogens. This may be as result of the refined nature of benlate and its active ingredients being more concentrated than of the plant extracts.

V. CONCLUSION

This study demonstrated that *A. indica*, *T. diversifolia*, and *C. odorata* should be used as a potential biocide in plant disease management, as they showed fungicidal and fungitoxic ability (at 100% w/v). The utilization of plant extracts to control disease in vegetable filed minimizes or eliminates the risks and hazards of toxic fungicides, especially on freshly consumed vegetables. It is anticipated that further research into these extracts would identify the active compounds responsible for their fungicidal activity.

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Agriculture in Sri Lanka: The Current Snapshot

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Abstract*—Sri Lanka being an island is blessed by nature with ideal environmental factors on the field of agriculture which is being intentionally practiced towards the sustainability. The agriculture sector in Sri Lanka always acts as a major economic strength to the national economy as it ensures the food security, employment and poverty alleviation of rural communities. The sector is mainly driven by variety of distinct sub sectors which include crop cultivations such as paddy, tea, rubber, coconut, vegetables, fruits, export crops and sugar while in addition livestock and fisheries sectors also provide a substantial contribution simultaneously. Negative and positive fluctuations of each above mentioned subsector directly affects on the overall country uplift and the society wellbeing. This paper will be discussing about the present status of the agriculture in Sri Lanka in a concise manner with respect to the recently published official data by country regulatory bodies.*

Keywords*—*Agriculture Production Index, Ceylon, Food Security, Gross Domestic Production, Zoonotic Diseases.

I. INTRODUCTION

The Democratic Socialist Republic of Sri Lanka formerly known as Ceylon is an island located just below the southern tip of India surrounded by the Indian ocean, which is having a population up to date around 21 million. By ethnicity, the majority of the Sri Lankan people are Sinhalese (74.9%) whereas the minorities are represented by Sri Lankan Tamils (11.2%), Sri Lankan Moors (9.3%), Indian Tamil (4.1%) and Others (0.5%). Buddhists are the dominant religious characters (70.1%) while Hindus (12.6%), Muslims (9.7%) and Christians with Roman Catholics (7.6%) are further represented respectively [1]. Being a tropical country, the temperature is usually constant with respect to a certain altitude and there are two monsoons which are locally called as "Maha" Season (November to February) and "Yala" season (May to September) caused by the country rainfall distribution. On this basis, the country has been divided into three climatic zones; Wet Zone, Intermediate Zone and Dry Zone.

Fig.1: Map of Sri Lanka
(Source: www.nationsonline.org)

Wet Zone receives over 2500mm of mean annual rainfall covering the south- west regions including central hill countries and the Dry Zone covers the northern and eastern parts of the country with a mean annual rainfall less than 1750mm while Intermediate Zone lies between these two zones receiving a mean annual rainfall ranging from 1750mm to 2500mm. In addition, the country hasbeen classified into 24 agro-ecological zones based on the rainfall, soil characteristics, forestry and the land use manner and with the advancement of navel technology, these 24 agro-ecological zones have further been divided into 46 sub-regions [2]. Excluding inland waters, the land territory spreads over 62,705 km^2 in which the agricultural lands occupy 20.7% portion contributing around 8% to the National Gross Domestic Production (GDP) and acquiring 28.7% of employment without seconding to the other industries. Main agricultural crops in Sri Lanka are paddy, tea and rubber which consume 1,592,000 hectares [1]. Almost 75% agricultural land is

governed by smallholdings in which around 70% farmers solely practice crop production while the rest is having an integration of crop with livestock and in few cases only the livestock [3].The livelihood of Sri Lankan people has originated with a great agricultural history dating back more than 2500 years. Persisting moderate climate with ideal temperature and rainfall patterns throughout the year, largely extended fresh water network which is consisted with manmade reservoirs, rivers and other freshwater resources along with adjacent fertile soil; the country is renowned as the Pearl of Indian Ocean. As a consequence of having ideal resource base, the agriculture sector in Sri Lanka always acts as a major economic strength to the national economy as it ensures the food security, employment and poverty alleviation of rural communities. But at present, the agriculture activities in Sri Lanka shown a substantial reduction by 4.2% in 2016 in contrast to 2015 which had 4.8% recorded growth rate. This negative growth rate has been resulted due to prevailed adverse weather conditions such as floods due to heavy rain falls and consequent drought conditions throughout the year. Although the livestock production, fishing and other beverage crops made a positive contribution towards the growth, this performance contraction has beenmainly attributed by rice, tea, rubber and fruits during the year. According to the Agriculture Production Index (API) which is the tool for defining the movement of the agriculture and fisheries sector productivity, in 2016 an overall decline of 2.4% has been recorded oppose to 4.4 overall growth rate in 2015 [4].

II. PADDY PRODUCTION

Millions of farm families in world engage with paddy cultivation and majority of them belong to the small scale category [5]. Rice is the most important staple food crop which occupies the majority of total cultivable agricultural lands through which the livelihood activities of people are originated in Sri Lanka [6]. In Sri Lanka, although there are more than 90%of irrigated paddy lands are located in the dry zone, the majority of dry zone paddy farmers have a relatively poor economic status as they are technically inefficient of utilizing the available resources [7]. Various policies have been adapted by the government as to provide incentives to paddy farmers [8] since rice performs as a foundation to uplift the rural economy. Even though the country could achieve 4.4 million metric tons for the year 2016, the total production has declined by 8.3% oppose to the records revealed in 2015 [4].

III. TEA PRODUCTION

Fig.2: Tea Cultivation in Up Country

Tea which is having a history of more than a century; extending up to the British colonial era, plays a bigger role in national economy by obtaining export earnings as to uplift the government revenue and providing employments to people who are struggling to get rid of poverty [9]. In the case of tea, it has been reported that the revenue generated through the tea exportation had been reinvested as to compensate 71% of food import bills indicating the indirect contribution towards the food security performed by the local tea production [10]. Sri Lanka is the third largest tea producer in the world possessing a share of around 23% of the global demand. The annual tea production is around 340 million kilograms per year and the production is possible throughout the year. Ceylon tea is renowned as the best tea in the world due to its intrinsic high quality, aroma and the taste which have been caused to be unique among the other tea producing countries. Sri Lanka mainly exports about 51% of tea as value added products such as green tea, flavored tea, iced tea and so on [11]. In the year 2016, the tea production has made a substantial decline in accordance with the supply and demand. Total tea production in 2016 was reported as 292.6 million kilograms which was contradictive to 328.8 million kilograms recorded in 2015 corresponding to an overall distinct decline by 11%[4].

IV. LIVESTOCK PRODUCTION

Livestock species are the domesticated animals such as cattle, swine, sheep, goat, poultry and horses basically being reared for food purposes. Size of the farm, productive capacity of the land, technology used, financial resource available, knowledge and experiences of the operator, labor availability, location of the farm with respect to the market and the cost of inputs and its availability are the major determinant factors when choosing a livestock enterprise in general. Livestock provides quality food and well-balanced nutrition directly

to human by converting the natural vegetation, crop residues, by-products from food manufacturing, and different organic wastes whereas it also ensures indirectly the food security by increasing crop harvest through supplying organic manure and stabilizes the food supply by acting as a buffer during any fluctuation in crop production [12].

Fig.3: Intensive Cattle Farming

Although the demand for the livestock products is relatively stable in developed countries, the global livestock sector shows a dynamic nature hence there is a continuous increasing demand being performed by developing countries as a consequence of the population growth, urbanization, income growth, increase in animal number and developments of animal health care facilities. At present, many livestock production systems tend to increase their output efficiently in a sustainable manner with the aid of advanced science and technologies [13]. In the point of agriculture, the majority of the land surface has been occupied by the animal husbandry. Most of the people on the earth engage in livestock production even at small scale as livestock integration is a source of income diversification while improving the soil fertility for the crop production and providing draught power and transportation. However, as in all agricultural systems except the benefits there are negative impacts as well. Public concern towards the food safety and healthy diet has been playing a substantial role on the consumption patterns negatively on the livestock products throughout the world whereas inefficient management of farm animals is a source of emitting unacceptable amounts of greenhouse gases hence deteriorating the land and water resources by their effluents [14]. The risks associated with zoonotic diseases which can transmit form humans to animal represent a significant constraint when implementing livestock integration plans. Restrictions are being applied and indispensable health programs are being carried out in order to encourage the international trade and to assure the safety of ultimate consumers [15]. In year 2015, the livestock industry in Sri Lanka contributed a 0.6% to GDP. The cattle and buffalo population has shown a marked increase by 10% and 2% respectively oppose to 2014. Although there was an increase of animal number, the total milk production had decreased by 4% in 2015 as a consequence of FMD (Foot & Mouth Disease) epidemic leading a negative growth of dairy sector. Since there was a decreased international market price for milk derived food commodities, the imports of milk and milk products demonstrated a 22% increase as usual. According to the available data, chicken meat production has increased by 9% and the egg production has increased by 3% in year 2015 compared to 2014. Accordingly, the per-capita availability of eggs has increased by 1.2 kilogram per year and the per-capita availability of chicken meat has increased by 0.63 kilogram per year respectively. The goat population is around 3.5 million and the production still behaves as a traditional practice which is especially centralized in the dry zone. Meat (mutton) is the main output consumedthan the milk. The mutton production was recorded as 135,000 metric tons causing per-capita consumption of 0.09 kilogram per year. Swine production is distinctly practiced along the western coastal belt of Sri Lanka. The total swine population was recorded as 94,612 and this number is corresponding to a 32% of significant increase. The estimated swine meat production was 718,000 metric tons and consequently the per-capita consumption was 0.34 kilogram per year in year 2015 [16]. In the year 2016, the local milk production has been recorded as 384 million liters with a growth of 2.6% respectively. This marked increase has been achieved by importation of productive heifers, affordable prices of raw milk for farmers, increased demand for raw milk and expanding the production capacities of milk factories. Out of total milk production, 82.8% (317.9 million liters) is accounted by cattle milk production while 4.2% (66.1 million liters) is buffalo milk. Although there is an increase in milk production, the milk powder imports were recorded as 94,011 metric tons as an increase of 15% valuing Rs. 33.6 billions. Milk production at National Livestock Development Board (NLDB) contributed 17.9 million liters while MILCO (Pvt) Ltd accounted for 70 million liters of milk collection. Government takes maximum efforts to increase the milk production in the country with an intention of achieving the self-sufficiency in milk. Thechicken production showed a growth of 5.7% marked increase to 173,830 metric tons [4].

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V. COCONUT PRODUCTION

Fig.4: Coconut Cultivation in Dry Zone

Coconut is a perennial crop which is grown especially in tropical regions over 90 countries. In Sri Lanka, Coconut cultivation plays a bigger role for sustaining the livelihoods of large numbers people and it ensures the food security after the paddy cultivation as coconut can withstand adverse climatic fluctuations [17]. The productivity of coconut cultivation is being improved and the emerging most problems are being eliminated by introducing new improved varietieswith the help of latest scientific practices. A total of 394,836hectare of area is under coconut cultivation. Coconut and coconut based products earn substantial amount of foreign exchange from the international market. Products included under edible category of coconut products are desiccated coconut, nuts, coconut oil, coconut water, coconut cream and coconut milk while activated carbon, coir and coir based products and crafts out of shell are classified into industrial category. The distribution of coconut cultivation is highly intensive especially in West and North Western provinces. There are three main districts under these two provinces where the coconut cultivation is obviously dominant known as the "Coconut Triangle" which includes Kurunegala, Puttlam and Gampaha districts [18]. In 2016, the supply of coconut and coconut products appeared as a deceleration. Consequently, there was a decline of 1.5% associated with the estimated value of 3,011 million nuts oppose to the values recorded in 2015. This negative production status has been attributed mainly by prolonged drought period experienced within the growing areas [4].

VI. RUBBER PRODUCTION

The demand for the natural rubber has been increased substantially due to rapid increase in demand for Natural Rubber (NR) in the world thus, investments on rubber sector is said to be highly profitable. Rubber goods have a large market potential in the world. The opportunities should be optimized to encourage the growth of rubber downstream industries to meet the anticipated growth in the industry [19].The history of Sri Lankan Rubber industry originated in 1876 with establishing of the first rubber trees in Henerathgoda Botanical Gardens located at Gampaha. At present, the majority of manufacturing firms are scattered in South West of the country. Since 1876, Sri Lankan natural rubber has a higher demand due to its popular premium quality in the world especially on type called Lanakprene which is having distinguishable intrinsic characteristics such as odorless nature, light colored and clean ideal for producing better rubber derived products. In year 1950, the rubber industry was led by tire re-trading and rapidly expanded due to introduction of free trade policies to the nation in the year of 1970. Since two decades, the country produces ranges of large numbers of value added rubber products such as rubber bands, tires, tubes, industrial components, auto parts, carpets, footwear, bottles, gloves etc. Major markets for manufactured rubber products are USA, Germany, Italy, Belgium, & UK [20].

Fig.5: Rubber Plantation

In 2015, the total rubber production and total exports recorded as 885700 metric tons and 103700 metric tons respectively in which the export value was 3548 million rupee. The local consumption of rubber showed with a value of 1,274,200 metric tons whereas the cost of production per kilogram was Rs. 170.00. Government has already taken up the steps to be achieved in order to

ensure the growth, competitiveness and the sustainability of the rubber industry in Sri Lanka as to capture the global market against its competitors by formulating and introducing a comprehensive strategic plan known as Sri Lanka Rubber Industry Master Plan (RMP) validated for stakeholders which was supported by Asian Development Bank (ADB) and variety of multidisciplinary field experts. This plan is consisted a detail of value chain analysis encouraged with prospective strategies, goals and action procedures and will be worthy up to 2026 with the commencement of the master plan in the year 2017 [21]. It has been recorded that in year 2016, the rubber production showed a significant declined growth rate by 10.7% (79.1 million kilograms) reported in the past 50 years performed especially by the smallholder sector due to reduction of the tapping days as a response to the lower prices [4].

VII. MINOR EXPOT CROPS & OTHER FIELD CROPS

Minor export crops also play a substantial contribution towards the national economy through the agriculture and provide livelihoods for people. Areca nut, betel, cardamom, cinnamon, citronella, clove, cocoa, coffee, ginger, goraka, lemongrass, nutmeg, pepper, turmeric and vanilla are the main minor export crops grown in different locations under varying climates in Sri Lanka. Majority of these are freely available and can be grown easily in villages thus performing a vital role even in Sri Lankan village economy [22]. The production of minor export crops in 2016 declined by 9.5% compared to12.9% growth which was reported in 2015 and the production status of Other Field Crops (OFcs) also showed a simultaneous contraction by 6.4% corresponding to 339,253 metric tons in 2016 from 362,452 metric tons in 2015 due to adverse weather conditions and prevailed fluctuations of seasonal patterns [4].

VIII. VEGETABLE & FRUIT PRODUCTION

As a mean, Sri Lanka produces around 1,250,000 metric tons of vegetables and fruits annually (around 710,000 metric tons of vegetables and 540,000 metric tons of fruits) through already identified 80 different vegetable and fruit varieties grown under varied agro-climatic conditions throughout the island. Temperate vegetables and fruit crops such as leeks, beet, carrot, bean, salad cucumber, tomatoes, bell pepper, tomatoes, Chinese-cabbage, cabbage, strawberries, sukini, salad leaves, cauliflower and cherry are extensively cultivated especially in the hill country as its climate is obviously ideal whereas tropical fruits and vegetables such as green chili, gherkins, lemon, pumpkin, papaya, mango, melon, red onion, bitter gourd, banana types and queen pineapple are broadly cultivated in low country and dry wet areas of the country. Indigenous yam types such as kiriala (*Xanthasomasagittifolium*) and innala (*Lecranthus*), Underwater stems of *Lasiaspinosa* (kohilaala) and *Nymphea lotus* (nelumala), bread fruit, drumsticks (murunga) and young jak fruits are also popular commodities derived from fruits and pods of perennial crops.

Fig.6: Banana Cultivation

Generally, the land extent for cultivations has not exceeded beyond a hectare since most of such vegetables or fruits growers are small level producers or home gardeners. The export market for processed/ value added and fresh fruits & vegetables has been identified as a sector with an extreme potential for further progressive future due to increasing demand coming from international markets. Approximately 65% of fresh vegetable and fruit products are exported to the Middle East and the Maldivian market while 90% of processed vegetable and fruit products are targeted to European market. Saudi Arabia, United Kingdom, Germany, Pakistan, Kuwait, India, Maldives, United Arab Emirates and Qatar are the top fruit & vegetable importing countries from Sri Lanka [23]. In 2016, the total annual vegetable production performed a marginal increase by 1.3% to 1,648,501 metric tons relative to 1,627,592 metric tons reported in 2015. The annual fruit production status demonstrated a marked reduction by 6% in 2016 irrespective to the arising demand posed by exporters, after 15.6% decline observed in 2015 [4].

IX. SUGAR PRODUCTION

Sugar production in 2015 increased by 7% to 55,982 metric tons due to increased extent of cultivation and higher purchase price for sugarcane, blessed by favorable environmental conditions around the country. Out of the total production, about 27,612 metric tons (53.7%) was accounted by Pelwatte sugar factory which is the pioneer of providing domestic sugar requirements to the nations

while SuduOya, Sevenagala and Gal Oya sugar factories were responsible for compensating the rest. Kantale sugar factory has been engaging for cultivation of sugarcane with an intention of recommencing its manufacturing operations in the near future. Although there was an uplift of the production, the sugar recovery rate which indicates the productivity had declined to 7.5% in 2015 from 8% recorded in year 2014. Nevertheless, the overall production in 2015 was quite enough to recover 10% of the total sugar requirement of the country. The sugar imports had been increased by 20.1% to 623,971 metric tons in year 2015 due to low sugar prices showed in international market thus indicating to take sufficient actions for expanding the potentials of the sugar industry as to reduce the amount of foreign exchange incurred in importing sugar [24]. In year 2016, the sugar production in the country showed a marked increase of 10.9% to 62,048 metric tons which was encouraged by cultivation of high yielding sugar cane varieties, irrigation systems, extended planting activities and better field maintenance [4].

X. FISHERIS SECTOR

Food security and nutrition has become a global challenge since hunger, poverty and malnutrition still remain among most of the people in world. The fishing sector worldwide plays a considerable role as a basis of livelihood especially in developing countries for alleviating the poverty, hunger and malnutrition thus ensuring the food security, nutrition of millions of people as well as building up the economic viability among communities. It has been proven that there are about 30,000 fish species living on Earth's hydrosphere and around hundreds of species are caught commercially. Fish contain Omega-3 fatty acids which can reduce the blood pressure and other associated cardiovascular disorders thus avoiding the risk of deaths linked with heart failures. Eating fish may even cause to lower the depression, risk of stroke and mental retardations. Fish intake is very much important especially for mothers who are pregnant or breastfeeding hence it supplies DHA which is a specific type of mega-3 fatty acids beneficial for the brain development of infants [25]. According to the FAO (Food and Agriculture Organization of the United Nations), it has been recorded that more than $50 billion is wasted in each year especially from the marine fishing sector due to numerous malfunctions, around 20-30% of wild fish harvested are used in aquaculture as fishmeal and being consumed by coastal communities. Fish has been classified as the most largely traded food commodity in world of which 50% is represented by developing countries [26].The fisheries sector in Sri Lanka which is comprised with three main subsectors namely coastal; offshore and deep sea; and inland and aquaculture, acts as an essential source of animal protein and plays key economic activities among coastal communities [27].

Fig.7: Marine Fisheries Sector

The sector has been classified as one of main potential areas that can be expanded as to uplift the national economy. Currently, the sector is capable of providing 560,000 employment opportunities directly and indirectly which sustain around 2.6 million people aiding for generating income, foreign exchange and supplying the regular required nutrition in an affordable manner. Being an island, Sri Lanka is rich with aquatic resource bases viz. Exclusive Economic Zone (EEZ) which extends 517,000 km^2 as the marine territory and mainly identified 45 lagoons and estuaries. In addition 489,000 ha is represented by inland water resources such as villus, irrigation reservoirs, seasonal and perennial tanks possessing a greater inland fish mass that is even possible to be expanded. The Sri Lankan fisheries sector contributes around 1.7% for the National GDP. In year 2015, the total fish production was reported as 384,610 metric tons in which 334,390 metric tons were accounted by marine fish production while 50,220 metric tons donated by inland fisheries. The total fish export value was quantified as Rs. 18,458 million and the quantity exported were reported as 12,982 metric tons respectively. Since Sri Lankan people can obtain acceptable protein requirements through intake of fish as a food in an affordable manner, the government of Sri Lanka has already taken steps to increase the national fish production up to 685,700 metric tons as to ensure per capita fish consumption of 22 kilogram per year [28]. In year 2016, the growth of the fisheries sector showed a moderate expansion by 2.1% over 2015 to 530,920 metric tons [4].

XI. ISSUES & CHALLENGES

At present, the country confronts many challenges in the field of agriculture. Many of such challenges and issues are increase of demand on food due to uninterrupted population growth, wasting large amount of money for importing different food commodities which even can be produced locally, Non-adherence of young generation for agricultural practices, economical imbalance of farm households, harmful environmental impacts due to extensive use of agrochemicals, land fragmentations and competition for land with other industries, lack of exposure for applying appropriate latest technologies for food production, export failures caused by malfunctions on complying with international standards, issues resulted on low quality food products, food safety and food security, sudden climatic fluctuations, lack of a consistent and efficient national agricultural policies, No appropriate subsidy schemes for farmers, issues with disseminations of agricultural information, inefficiency of land use patterns and developments, inadequate enforcements of already defined legal frameworks, lack of coordination among other allied organizations and industries, insufficient amount of vital resources such as workable human resources, infrastructures and finance, inadequacy of effective extension services, research and development processes, and proper well-updated databases, High involvement of middlemen, post harvest wastages, lack of good quality raw materials, inadequacy of suitable machineries, poor genetic crop varieties and animal breeds, high costs on labor wages, poor practices on soil conservation, water and land pollution, natural disasters and hazards, poor and inefficient modes of waste management practices, conflicts with wild animals, malfunctions of irrigation systems, poor marketing activities and market access for rural farmers, lack of proper insurance schemes, instability of market demand, supply and prices, No effective coordination among buyers and producers, disease outbreaks and poor early responses and preparations, Inadequacy of awareness program especially on Good Agricultural Practices (GAP) and organic certification, food habits and perceptions on foods of the traditional publics, inadequate linkages between famer based communities, researchers, extension approaches and farmers and so on [29].

XII. CONCLUSION

The overall performance of the agriculture sector in Sri Lanka has shown a negative growth rate in year 2016 with respect to 2015. This decline has been mainly attributed by the changes of anticipated favorable weather patterns prevailed while other existing issues and challenges also affected on the same scenario simultaneously. Although adverse weather effect is not under the human control, appropriate well-established strategic approaches should be applied in order to minimize the negative influences posed by such already identified existing issues and challenges. Policies, regulatory frameworks and guidelines, value chains, farm inputs and logistics, natural resource management, financing, marketing, data availability and its accessibility and reliability, knowledge and awareness on information and services must be well defined and applied in a responsible and applicable manner for the sector efficiently. The authorities which are obviously responsible for uplifting the agriculture sector must attempt to achieve the self-sufficiency of each sub sector as to save the foreign exchange on continuous imports. Food availability should be ensured throughout the year by practicing appropriate management of buffer stocks with an intention to be used whenever necessary. Food commodities should be marketed and promoted within and outside the country through eco-friendly practices and novel innovations. Appropriate latest technologies should be introduced and communicated among all agricultural stakeholders by establishing proper coordinating mechanisms. Thus, focusing on all the potentials and alternatives for establishing a sustainable agriculture system as to uplift the socio-economic stability of the country.

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Evaluation for stable resistance to *Stenocarpella maydis* in tropical maize (*Zea mays* L.)

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***Abstract*—** *Maize ear rots caused by Stenocarpella maydis cause reduction in yield and quality of the maize due to the mycotoxins produced by the pathogen. Breeding for resistance is the most feasible option in managing ear rots. However, to obtain stable resistance to S. maydis has been a challenge partly due to effect of the environment and availability of different isolates. The objective of this research was therefore, to determine the effect of multiple isolate inoculations in breeding for resistance to S. maydis and to identify genotypes with stable resistance. Seven inbred lines were crosses in a 7 x 7 full diallel without reciprocals. The resultant crosses (21) and their parents (7) were planted and evaluated at two sites, Lusaka and Mpongwe, during the 2015/16 cropping season. The experiment was laid out as a randomized complete block design with 3 replications. Treatments were: (1) single inoculation with isolate A, (2) single inoculation with isolate B and (3) a multiple inoculation of two isolates AB and (4) control with no inoculation at all. The mean genotypic scores were found to be 5.52, 4.96, 5.50 and 1 for treatment 1, 2, 3 and 4 respectively. The t-test analysis revealed that treatment 1 had a higher mean disease severity score (5.52) as compared to treatment 2 (4.96) ($P < 0.01$). Equally mean for treatment 2 (4.96) and 3 (5.50) were significantly different ($P < 0.01$). However, there were no significant differences between mean disease severity score for treatment 1 and 3. This indicated that multiple isolate inoculations could give rise to inappropriate genetic information due to the possibility of antagonistic effect between isolates. The genotypes (P2 x P4) and (P3 x P6) crosses were found to have stable resistance to S. maydis. These exhibited consistent significant negative SCA effects ($P< 0.05$) in both locations.*

***Keywords*—Maize, ear rot, Stenocarpella maydis, resistance, mycotoxin, Specific combining ability (SCA).**

I. INTRODUCTION

Maize (*Zea mays* L) is the world's most grown cereal and it is predicted that by 2020 it will surpass both rice and wheat to become the number one cereal in the world (M'mboyi, et al., 2010). The sub-Saharan populace depends on maize (*Zea mays* L) as the main staple carbohydrate source (Fischer et al., 2014). Approximately (15.7 %) 22 million hectares, of the 140 million hectares grown globally, accounts for sub-Saharan Africa (Pingali, 2001). Farmers consider maize, not only to be a major source of energy but also their main source of income.

Maize production is carried out in diverse climates because of its versatility and it is the most productive species of food plants (Dowswell et al., 1996). In terms of soil, maize can be grown in wide range of soils, ranging from deep fertile soils along river bottoms and lake basins to well-drained and easily worked upland soils (M'mboyi et al., 2010).

Maize production is hampered by a number of biotic and abiotic stress factors. The biotic constraints in maize production include insects, weeds and pathogenic infection (M'mboyi et al., 2010). Among the diseases, ear rot caused by an important fungal pathogen, *Stenocarpella maydis* causes yield losses of 10-50 % (Vigier et al., 2001). In pre- and post-harvest maize, the occurrence of mycotoxins is of great concern as they tend to cause health disorders in both livestock and humans who consume contaminated grain (Munkvold and Desjardins, 1997; Miller, 2001).

To control ear rots, a combination of crop sanitation, good agronomic practices and timely harvesting have been used, but with limited success (Munkvold, 2003). To curb this vice, deployment of resistant genotypes through breeding is the most cost effective way especially for the resource poor farmers in Zambia. However, resistance to *S. maydis* is greatly affected by underlying issues of gene interactions and the type of germplasm under study (Mukanga et al., 2011; Tembo et al., 2013). Identification of genotypes with stable resistance across locations can be utilized as the source of resistance in genotypic combinations (Tembo et al., 2013). A higher number of resistant parental genotypes to *S. maydis* in mating combinations are likely to produce a larger proportion of stable resistant off-springs. However, in maize, underlying issues of epistasis and gene interaction may interfere with expected outcome (El-Badawy, 2012) and there is therefore need for individual off-spring evaluation. In addition, it should be realized that effectiveness of breeding for stable resistance may be influenced by the type of isolates and its interaction with the environment (Rossouw et al., 2009). Previous studies have established multiple inoculations of different ear rot pathogens, as not an appropriate breeding strategy due to

antagonistic effects associated with these pathogens (Tembo et al., 2013). Little is known about the effect of multiple isolate inoculations of *S. maydis* in breeding for resistance. Therefore, there is need to investigate that effect. Further breeding for stable resistance will therefore depend on the reaction effect of isolates when multiple inoculated. A previous study indicated that multiple pathogen inoculation should be employed for stable resistance if there are synergetic effects among pathogens (Chilipa et al., 2016) while this cannot clutch for pathogen combination with antagonistic effect (Tembo et al., 2013). The specific objective of this study therefore was i) to determine the appropriateness of multiple isolate inoculation on maize ear in breeding for stable resistance to *Stenocarpella maydis* and ii) to identify genotypes with stable resistance.

II. MATERIALS AND METHODS

Germplasm used in the study

Seven white-kernel parental inbred lines with varying reactions to *S. maydis* (Table 1) were crossed in a 7 x 7 full diallel (without reciprocals) during the 2015/16 off season. A total of 21 progenies (F_1 single cross hybrids) together with their parents (a total of 28 genotypes) were evaluated in this study. The inbred lines were crossed in the 2015/16 off season.

Study sites management and experiment design

The evaluation trials were planted in December 2015/16 cropping season at Lusaka (15^0 24'S; 28^0 04'E, altitude 1216 m) and Mpongwe (13^o 32'S; 28^o 03E, altitude 1206 m). Rainfall received during the 2015/16 cropping season was approximately 811 mm and 897 mm at the trial sites in Lusaka and Mpongwe respectively. Standard agronomical practices such as weeding and fertilizer application were followed. Fertilizer was applied at each site as compound D (N 35 %; P 70 %; K 35 %) 350 kg/ha and 300 kg/ha of top dressing, Urea (46 % N). The trial layout was a randomized complete block design (RCBD), with three replications in each location. The plants were established in two- row plots, 5 m long and 0.75 m apart and 0.25 m between plants. Trials were hand planted with two kernels per hill and later thinned at two weeks to one plant after emergence to a uniform stand of 20 plants per 5 m. The cobs were inoculated with single and multiple isolates of *S. maydis* approximately 3-4 weeks after mid-silking stage (Clements et al., 2003). Details of how the pathogen was cultured and toothpick-inoculated are explained in the following sections.

Pathogen isolation and culture

Isolates used in the study were obtained from Region II, Lusaka (15^o 24'S; 28^o 04'E) and Region III, Mpongwe (13^o 32'S; 28^o 03E) and were confirmed to be distinct in their base morphology colour and spore count per mm^2 as per procedure by Dorrance et al., (1999) and Rossouw et al., (2009). Isolate from region II and III were denoted as Isolate A and B respectively

Potato dextrose agar (PDA) media was prepared by weighing 3.9 % of PDA powder into glass bottles filled with 500 mL of distilled water in order to culture isolate A and B. The mixture was boiled while stirring until the powder dissolved completely. The glass bottles with the solution were then transferred to an autoclave for sterilization. The bottles and contents were autoclaved for 15 minutes at 121^0 C at a pressure of 15 MPa. 50 millimeters of the PDA solution was later poured into each of the 50 jars under the film board and left to cool overnight. 30 (5 cm x 5 cm base and 8cm height) jars were plastic and 20 (9.5 cm diameter, 10 cm height) were glass. 10 petri dishes 8.5 cm in diameter and 1.3 cm height were also filled with PDA solution and left to cool overnight. The petri dishes were used for initial culturing of the pathogen.

Toothpick- inoculum preparation

Toothpick-inoculum preparation was done using the modified procedure by Chambers (1988). A composite sample of *S. maydis* colonized kernels from each region denoted isolate sample A (Region II, Lusaka) and B (Region III, Mpongwe) were each separately sterilized in domestic bleach of the JIK brand that contains 3.5% sodium hypochlorite (NaClO) (Reckitt Benickiser South Africa (Pvty) Limited) solution for three minutes and then rinsed thrice in distilled water. The kernels were blotted on sterilized filter paper to dry and then 3-5 kernels were plated on petri dishes with 3.9 % potato dextrose agar and incubated at 27-30 ^{0}C. After 4-5 days the fungal growths from the separately inoculated isolate (A and B) plates were sub-cultured and ready to be transferred to toothpicks after 5-7 days.

The toothpicks were initially sterilized by boiling in water for 20 minutes and later air dried to room temperature. The toothpicks were then transferred to glass and plastic bottles which were initially autoclaved for 15 minutes and left to cool to room temperature. The bottles were filled with freshly prepared potato dextrose agar (PDA) and left to cool overnight to room temperature. The toothpicks were transferred to the bottles by placing them in an upright position in the bottles under the fume board. The plastic bottles contained approximately 100 toothpicks while the glass jars had between 150-200 toothpicks. Fungal culture plugs from pure cultures of each isolate of *S. maydis* were placed in specific bottles containing sterile toothpicks for ten days to allow the pathogen to fully colonize the toothpicks. Fully colonized toothpicks were then air dried before inoculating the genotypes.

Inoculation of test ears

Inoculation was done by piercing through the base of the test ear at 3-4 weeks after mid silking stage. Four treatments were used. Thus treatment 1, involved single

inoculation using a toothpick colonized separately with isolate A; treatment 2 was done using single inoculation with toothpick colonized with isolate B and treatment 3, multiple inoculation (AB) using two toothpicks colonized by two different isolates A and B as done by Tembo et al, 2013. Multiple inoculation was achieved by inserting, these two toothpicks 5 mm apart into the base of the ear. Treatment 4, control was left without any inoculation at all in the second row. For each treatment five (5) plants were considered for inoculation and these were separated by three un-inoculated plants which acted as borders. Artificial inoculation encourages symptom development and disease progression and thus five plants were considered enough for assessment of the disease.

Single inoculations were performed in the first row with each isolate inoculation separated by three non-inoculated plants. Multiple inoculations were performed in the second row of the plot with the remaining plants treated as control and border plants.

Data collection and analysis

The plants were harvested at maturity and data were collected. Disease severity score was determined visually from all the five (5) inoculated plants per treatment in 5 m long first two row plots. Each inoculated treatment per genotype was harvested separately and the plot number noted. Percentage ear rot (ER) was estimated visually using percentage of ear colonized by the pathogen from the point of infection and the mean severity ratings computed. The rating was done using modified procedure by Tembo et al., 2013 with an *S. maydis* severity rating score as follows: 1= 0-25 %; 2= 26-50 %; 3= 51-74 %; 4= 75-84 %; 5= 85-94 %; 6= 95-99 % and 7= 100 % (completely rotten).

A paired two tailed t-test, was performed to compare the mean differences for *S. maydis* diseases severity scores among the three treatments across locations (Treatments 1 [Inoculation with Isolate A], 2[Inoculation with Isolate B] and 3[Multiple inoculation with Isolate A & B]). This was performed in Microsoft excel 2010.

Diallel analysis was performed using Griffing (1956) method 2, model I, fixed model in GenStat using Restricted Maximum Likelihood (REML) and regression approaches. The relative importance of GCA (general combining ability) and SCA (specific combining ability) effects were estimated.

III. RESULTS

Effect of multiple isolate inoculations

The mean disease severity scores across genotypes for treatment 1 (inoculation with isolate A), treatment 2 (inoculation with isolate B) and treatment 3 (inoculation with isolate A & B) were 5.52, 4.96 and 5.50 respectively. A student's paired t-test (Table 2) performed on comparison of treatment 1 mean disease severity score comparison (MDSC) and treatment 2, indicated highly significant ($P < 0.001$) mean differences. Significant mean disease severity score differences were equally found between treatment 2 MDSC and treatment 3 on disease severity.

Stenocarpella maydis ear rots genotypic disease severity effect

Significant differences were obtained among genotypes with regards to *S. maydis* disease severity scores across inoculation treatments in each location (Table 3) ($P < 0.01$). Similarly, across location data shows interaction between location x genotype and location x isolate were highly significant ($P < 0.01$ and 0.001 respectively). Further analysis per location revealed significant ($P < 0.001$) specific combining ability (SCA) effects across treatments in both locations.

The genotypic mean disease severity effects of each isolate vis-à-vis, treatment 1, treatment 2, treatment 3 and treatment 4 [as the control on the test genotypes] was found to be 5.52, 4.96, 5.50 and 1 respectively (Table 4).

The individual hybrids crosses mean severity scores and there SCA effects are tabulated below (Table 5).

The hybrids (P2 x P4) and (P3 x P6) crosses were found to have stable resistance to *S. maydis* across locations. (P2 x P4) exhibited genotypic means of 3.79 and 2.92 for Lusaka and Mpongwe respectively. (P3 x P6) showed a genotypic mean of 3.65 for Lusaka and 2.88 for Mpongwe. The significant SCA effects for (P2 x P4) were -0.39 and -0.76 for Lusaka and Mpongwe respectively whereas (P3 x P6) exhibited significant SCA effects of -0.54 (Lusaka) and -0.86 (Mpongwe).

IV. DISCUSSION

Breeding for stable resistance against ear rots such as *S. maydis* has been a challenge, primarily due to environmental factors. In addition, breeders have previously bred for resistance to *S. maydis* without particularly taking the aspects of isolates into consideration (Rossouw et al., 2002; Tembo et al., 2013). It remains to be established if isolates have an effect in breeding for stable resistance. It was for this reason that the effect of isolates in breeding for resistance was investigated in this research study. In this study Isolate A (obtained from Lusaka) and Isolate B (obtained from Mpongwe) were used.

A paired t-test revealed that mean disease severity scoring for treatment 1 (inoculation with isolate A) was higher than that for treatment 2 (inoculation with isolate B) (Table 2). The fact that the mean disease severity for treatment 3 was higher ($P < 0.01$) than treatment 2, but not significantly different from treatment 1, indicates that isolate A could have suppressed the virulence effect of isolate B when multiple inoculated. Previous studies on ear rot pathogens discouraged multiple inoculations of ear

rot pathogens as a breeding strategy because of the antagonistic effects (Tembo et al., 2013). This paper, reports the possibility of antagonistic effects of multiple isolate inoculations which have not been fully exploited recognizing that different isolates exists for *S. maydis.* However, contradictory information has been reported in sweet potato and common beans upon multiple infections with sweet potato virus (SPV) and *Collectorichum lindemuthianum* respectively, whereby in this scenario synergistic interactions occurred (Gibson et al., 1998; Gasura and Mukasa, 2010; Chilipa et al., 2016).

It can therefore be deduced that multiple inoculation can either create antagonistic or synergistic effects. Multiple inoculations among different pathogens or isolates of the same pathogen with synergistic effects can be reliable and a beneficial screening approach for breeders. On the other hand, multiple inoculation approach of pathogens with antagonistic effects generates less informative genetic information (Tembo et al., 2013). Across isolate performance to determine stable resistance of genotypes was chosen. This is because isolates occur naturally, hence having genotypes with stable resistance across isolates will be ideal in tackling this challenge and to enhance resistance.

Some genotypes were found to possess significant specific combining ability (SCA) effects in both locations. Specific combining ability (SCA) effects can also assist in ascertaining which parental materials can be utilized in hybridization. In Lusaka six crosses; (P1 x P4), (P2 x P4), (P3 x P7), (P6 x P7), (P3 x P6) and (P4 x P5) had negative significant (Table 5) SCA effects. This implied that these crosses exhibited higher resistance to *S. maydis* in their specific combinations when compared to other crosses with either one of the parents in common. In Mpongwe (P2 x P4) and (P3 x P6) had negative significant ($P < 0.05$) SCA effects. (P2 x P4) and (P3 x P6) crosses were found to have stable resistance to *S. maydis* across locations and as such can be used as parents in three way crosses or marketed as single cross hybrids after further evaluation. These exhibited significant SCA effects in both trial locations (Lusaka and Mpongwe).

V. CONCLUSION

In breeding for resistance to *S. maydis*, multiple isolate inoculation technique was found to be inappropriate due to the possibility of antagonistic effects of the isolates as it could lead to misleading genetic information. The use of individual isolates in breeding for resistance to *S. maydis* will be ideal in this case. (P2 x P4) and (P3 x P6) crosses were found to have stable resistance to *S. maydis* across trial locations (Lusaka and Mpongwe).

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TABLES

Table.1: Germplasm used in the experiment for S. maydis in Lusaka and Mpongwe

Parent	Name	Type	Source	Grain text	Reaction to *S. maydis*
P1	XL 003	Inbred line	Public	F	Resistant
P2	XL 029	Inbred line	Public	SF	Susceptible
P3	XL 057	Inbred line	Public	F	Resistant
P4	XL 071	Inbred line	Public	F	Susceptible
P5	XL 083	Inbred line	Public	SF	Moderate
P6	XL 087	Inbred line	Public	F	Resistant
P7	XL 195	Inbred line	Public	F	Resistant

Where P- parent line, Grain texture, F-flint, SF-semi flint

Table.2: Genotypic mean disease severity score comparisons (MDSC) among treatments

MDSC	Student t-test (P- Value)
Treatment 1 $(5.52)^x$ vs Treatment 2 $(4.96)^y$	< 0.001
Treatment 1 $(5.52)^x$ vs Treatment 3 $(5.50)^z$	0.13
Treatment 2 $(4.96)^y$ vs Treatment 3 $(5.50)^z$	< 0.001

x, y, z mean disease severity score across genotypes for treatments 1, 2 and 3 respectively. Treatment 1, 2 and 3 represents treatments with: single inoculation with isolate A, Single inoculation with isolate B and multiple inoculations of Isolates A & B respectively.

Table.3: Mean squares for Stenocarpella maydis cob rot disease severity scores across two experimental locations and in each individual location evaluated in 2015/16 season.

		Across locations		Individual sites	
Source	df	Across locations	df	Lusaka	Mpongwe
Location	1	241.01**			
Replication/location	4		2	4.01	11.56
Genotype	27	4.69	27	2.26**	5.40**
GCA			6	0.96	3.84
SCA			21	2.64***	5.85**
Isolate	3	770.44***	3	557.54**	247.82**
Location x Genotype	27	2.83**	27		
Location x isolate	3	35.51***	3		
Genotype x isolate	81	1.80	81	0.62	2.52
Gen x Isolate x location	81	1.35	81		
Error	444	1.06	222	0.5	2.02

, * significant at $P \leq 0.01$, and $P \leq 0.001$ respectively, MS, mean square

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Table.4: Effect of treatments on the test genotypes across the locations during 2015/16 cropping season.

Treatment	Mean
Treatment 1	5.52
Treatment 2	4.96
Treatment 3	5.50
Treatment 4	1.00
LSD (α = 0.05)	0.23

LSD, Fishers Protected Least Significant Difference test performed at P ≤ 0.05

Treatment 1, 2, 3 and 4 are: Single inoculation with isolate A, single inoculation with isolate B, multiple inoculations with isolate (A & B) and control without any inoculation respectively.

Table.5: Mean disease severity scores across treatments to Stenocarpella maydis in Lusaka and Mpongwe during 2015/16 cropping season

	Lusaka		Mpongwe	
Cross‡	Mean	SCA Effect	Mean	SCA Effect
P1XP2	3.86	-0.02^{ns}	3.13	0.02^{ns}
P1XP3	4.11	0.04^{ns}	3.55	0.13^{ns}
P1XP4	4.06	-0.40*	3.53	-0.48^{ns}
P1XP5	4.25	0.04^{ns}	3.42	-0.06^{ns}
P1XP6	4.44	0.17^{ns}	4.05	0.28^{ns}
P1XP7	4.17	0.17^{ns}	3.25	0.11^{ns}
P2XP3	3.92	0.12^{ns}	3.51	0.42^{ns}
P2XP4	3.79	-0.39*	2.92	-0.76*
P2XP5	3.81	-0.13^{ns}	2.88	-0.27^{ns}
P2XP6	4.5	0.51**	4.33	0.90**
P2XP7	3.63	-0.09^{ns}	2.5	-0.30^{ns}
P3XP4	5.05	0.67***	4.68	0.70*
P3XP5	4.26	0.13^{ns}	3.34	-0.12^{ns}
P3XP6	3.65	-0.54**	2.88	-0.86*
P3XP7	3.5	-0.42*	2.83	-0.28^{ns}
P4XP5	4.07	-0.45**	3.98	-0.07^{ns}
P4XP6	4.61	0.04^{ns}	4.48	0.15^{ns}
P4XP7	4.83	0.53**	4.17	0.47^{ns}
P5XP6	4.54	0.21^{ns}	3.83	0.03^{ns}
P5XP7	4.26	0.20^{ns}	3.67	0.49^{ns}
P6XP7	3.73	-0.38*	2.96	-0.50^{ns}
	4.14^{x}	0.18^{y}	3.52^{x}	0.37^{y}
	0.57^{z}		1.45^{z}	

‡ Crosses derived from parental inbreds P1 to P7 as described in Table 1. LSD, Fishers Protected Least Significant Difference test performed at P ≤ 0.05. . x - Grand locational mean. y- Standard error of the mean. z- Least Significant difference.

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Potential Impact of Salt Stress on Male Reproductive Development of *Glycine Max* (L.) Merr. (Soybean)

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Abstract *— Product yield and the continuity of the quality of products of plants are in parallel with their ability to tolerate or adapt to environmental factors. For this reason, it is extremely important to determine the changes in the plants under various stress conditions. Male reproductive structures are directly related to product yield and quality, and they're very sensitive to abiotic stress. Stress causes irreversible damage to plants depending on its amount and duration. The aim of this study is to determine the sensitivity of male reproductive structures of soybean seedlings and the critical salt concentration at which fertile pollen grains could be obtained in our soils whose salinity is increasing day by day. The selected soybean seedlings were exposed to increasing salt concentrations (50, 100, 150, 200, 250mM) for 6 months and they were compared with a control group in terms of flowering, pollen morphology (pollen size, exine and intine thickness, aperture structures), pollen viability, pollen germination, and pollen tube length. It was determined that, by affecting the growth process of soybean at varying grades, salt stress causes deformations in the plant's reproductive structures and decreases it's tolerance to salt stress.*

Keywords— crop, flowering, pollen germination, pollen tube growth, pollen viability.

I. INTRODUCTION

Soil salinity inhibits the growth and development of the plants' by reducing their ability to absorb water and micronutrients from the soil and it has been a threat to agriculture for nearly 3000 years [1]. Soil salinity paves the way for physiological drought by causing hyper-ionic and hyper-osmotic stress and irreversibly damages the plants [2]. While the world population is growing faster than ever abiotic stresses such as salinity, drought, heat are shrinking arable areas and significantly reducing product yield and quality [3], therefore mankind might face famine in the future.Therefore, environmental stresses negatively affect plants' vegetative and reproductive stages by hindering their morphological, anatomical and physiological parameters, and threaten the continuity of these cheap and highly cultivable sources [4]. Especially, male reproductive stage is more sensitive to stress factors such as heat, drought, salinity and low light intensity than vegetative stage[5], andthey cause anomalies in reproductive structures of plants and negatively affectsphsyiologicalproccesses of these structures such as flowering, pollination and pollen viability [6]. Reproductive stage is regulated by abiotic factors and it plays an important role in plants' ability to survive. These results indicate that reproductive stage can significantly affect product yield and quality [7].

Legumenssimbiotically balance nitrogen (N) in agricultural ecosystem. They have a wide range of use in industry and medicine, and they are best known as cheap protein source. Among the legumens, soybean (41% protein in dry matter) have the highest protein content [8]. Soybeans sensivity to salinity varies between its developmental stages. Germination rate was more sensitive than other parameters to salinity for soybeans grown in increasing salt concentrations for 45 days [9]. It's product yield decreases under salt stress and this is an indication that reproductive stage of this plant is affected more than other stages [10]. Growth, development and product yield of soybean seedlings grown under salt stress were thoroughly studied [11]. Furthermore, it has been found that salt stress delays anthesis in soybean seedlings with various genotypes and this finding is in parallel with product yield [12]. However, these studies attribute the relationship between product yield and salt stress to growth and development parameters of the seedlings. Whereas, in recent years, it has been found that male development is a lot more sensitive to stress than female development [13], andIt is noteworthy that product losses resulting from the stress effect are directly related to male fertility[14]. Because, the ecological conditions of our world is worsening day by day, and increasing the yield and quality of the foods that have a very important place in the feeding of living beings depend on the successful reproduction and development of the plants which produce them. Therefore, we tried to explain the relationship between salt stress and yield by observing the

changes in male reproductive structures and behaviour. We hypothesize that the negative impact of salt stress on male reproductive structures and behaviour directly affects the product yield. The direct relation between male reproduction structure and product yield [15] has critical importance for managing the future of agricultural production. Therefore, by investigating male reproductive structures of soybean seedlings grown under salt stress, we aim to determine the critical salt concentration at which fertile pollen grains can be obtained, and whether the pollens are suitable for fertilization after pollination.

II. MATERIAL AND METHODS

Experimental design

This study was conducted to investigate the impact of salt stress on the reproductive biology of soybean during flowering stage. *Glycine max* (L.) Merr. cv. Nova (soybean) with simillar sizes were surface sterilized with 0.1% (v/v) sodium hypochlorite and than germinated in moistened vermicutile for a week. 6 different pots, including one for control group, with a 20 l capacity (top and bottom diameters were 20 and 10 cm, respectively) filled with fine sand were prepared. 20 germinated seeds were planted in each pot at 4cm depth. After emergence, seedlings were exposed to salt stress with increasing concentrations (50, 100, 150, 200, 250 mM salt), for one time, with 100 ml. Seedlings were grown in a greenhouse at controlled temperature, humidity, light intensity, and a photoperiod of 16/8h per 24 h, for one year. Plants were irrigated daily with Hoagland's nutrient solution. Experiments were setup in completely randomized design with three replicates.

Measurements:We following exposure to salt stress (50, 100, 150, 200 mM) andcontrol (0) in growthchambers. 5-7 unbloomed flowers were randomly harvested from each pot after treatment with different salt concentrations. Collected flowers were air-dried for 1h in the vacuum furnace (25 ^{0}C) and than fixed in FAA (formaldehyde-acetic acid –alcohol-H_2O, 10-5-50-35, by vol.) fixative (about 5 min) for SEM. Mature pollen grains mounted on aluminium stubs were examined with a LEO Stereoscan 360 SEM to calculate their polar axis (P), equatorial axis (E), aperture diameters, and the P/E ratios. Pollens were prepared according to Wodehouse (1935) [16] method to determine the pollen wall thickness. The slides were observed using Olympus light microscope with X 100 objective using oil immersion.

To evaluate pollen viability, buds were randomly collected from control and treatment groups between 08:00 and 09:00. Anthers of the buds were isolated, placed on a glass slide in petri dishes, and crushed into a fine powder. Pollen grains of each group were stained with potassium iodide (I/KI) solution [17] for 1h. The numbers of fertile and sterile pollen grains were determined using a light microscope (10 X 100). Pollen grains stained in a dark color (brown) were identified as fertile pollens (viable and living pollen) and yellow or light red stained pollen grains were identified as sterile pollens.

In vitro pollen germination and pollen tube growth were determined using a pollen germination medium consisting of 15% sucrose ($C_{12}H_{22}O_{11}$), 60 mgl boric acid (H_3BO_3), and 1% agar dissolved in 100 ml of deionized water [18]. Fresh pollen grains from each pot were randomly collected from anthers of buds in the morning between 08:00 and 09:00 h. Pollen grains were dusted onto 10 ml of the germination medium and the mixtures were poured on microscope slides. Slides were placed on moistened filter paper in petri dishes. Petri dishes were covered with parafilm to maintain high humidity, and were incubated at 22 ±2 ^{0}C for 3 hours. After incubation, pollen grains were examined using a light microscope with a magnification of 10 x 100 to determine the rate of pollen germination. A pollen was considered to be germinated when the pollen tube length was either equal to or greater than the diameter of the pollen [19], and pollen germination percentage was calculated according to Luza et al.,(1987) [20].Pollen tube lengths of 50 randomly selected pollen grains from each group were measured using an ocular micrometer on a light microscope.

Cumulative stress response index (CSRI) [21] was used to reveal the reproductive responses of soybeans grown under increasing salt stress. According to this method, examined parameters of each group were calculated as the sum of the relative individual component.

Statistical analysis

Alldata of pollen parameters were statistically performed to test the significance of examined parameters by Duncan's multiple range test in analysis of variance (ANOVA) using SPSS 14.0. Differences among the mean values of the experimental data were compared with Least Significant Differences (LSD) at $P \leq 0.05$and $P \leq 0.01$. Graphs for all experimental data were constructed to determine whether the differences of the mean values between control and experiments. Data in the figures indicate mean values±standard errors (SD) based on three replicates for each application. Data represent means and the vertical bars represent the standard deviation.

III. RESULTS AND DISCUSSION

Reproductive stage in which plants' male and female structures develop and differentiate begins with the transformation of the vegetative meristem into flower meristem. The fact that the rate of deformation caused by various environmental stresses depend on the developmental stage of the plants can also mean that

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product yield and quality is related to the success of the reproductive stage. We determined the changes in all examined of soybean seedlings caused by salt stress. Salt stress altered the the process of transition between vegetative stage to reproductive stage in soybean depending on the salt concentration. At 50 mM and 100 mM salt concentrations transition was faster than the control (P≤0.05). However, at 150 and 200 salt concentrations transition was significantly delayed (P≤0.05). Even though increasing salt stress shortened the reproductive stage, flower stucturesshrinked. This may be an attempt of the plant to complete its life cycle as soon as possible to produce seeds [22]. These results may seem as positive stress at first, however, it can only be clarified by investigating the characteristics of the reproductive structures of the plants. Salinity negatively affects all metabolic functions during vegetative development stage and this in turn significantly decreases fertility during reproductive stage [23]. The number of flowers decreased by 10%, 30%, 74% and 90% at 50, 100, 150 and 200 mM salt concentrations, respectively, compared to control. 5 different salt concentrations were used in the study. However, soybean could not complete its vegetative development in 250 mM salt concentration. Therefore, there is no data from this treatment concentration (Fig 1). Stress factors such as drought and salinity can negatively affect the development of these structures due to the heterogeneous distribution of toxic ions in flower constructions [24]. While the flower numbers showed a decrease parallel to increasing salt concentration, it was determined that these structures were nearly absent especially at the highest salt concentration (P≤0.05). Excessive Na^+ accumulation in chickpea leaves grown in salt stress significantly delayed flowering and reduced reproductive structures [25].

Salinity causes anomallies during pollen formation and development processes [26]. Pollen size decreased with increasing salt concentrations, compared to control (except for 100 mM salt concentrations). Pollen diameter decreased by 1%, 4%, 4% and pollen length decreased by 2%, 4%, 4% at 50, 150, 200 mM salt concentrations, respectively, compared to control. Mean pollen size of the 100 mM salt concentration treatment group was even higher than the control with a 4% increase in pollen diameter and a 3% percent increase in pollen length (Fig 2a, Fig 5). Increasing salt concentrations caused the pollen shape to change from prolate-spheroidal (P/E=1.14-1) to spheroidal (P/E= 1.14-0.88) at the highest salt concentration (Fig. 2b, Fig. 5).

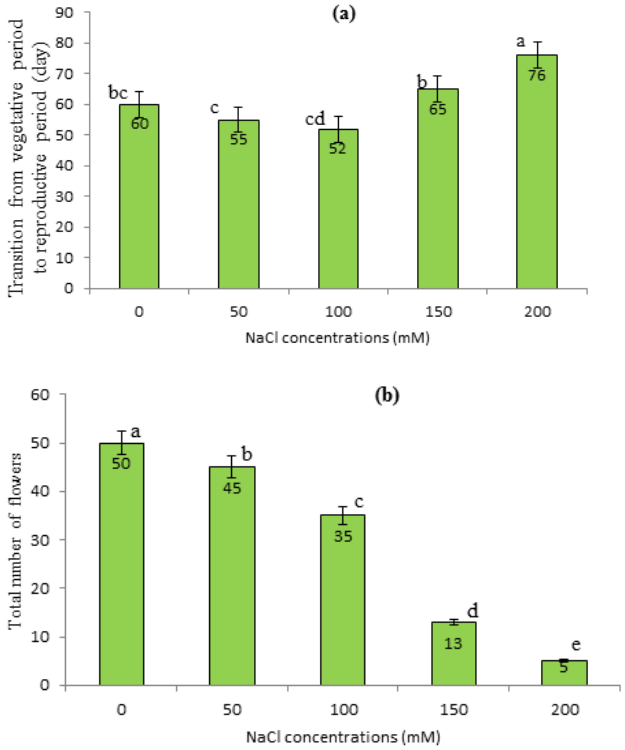

Fig. 1:The effect of increasing salt concentrations on the transition from vegative stage to reproductive stage (a) and the number of flowers of Glycine max (b)

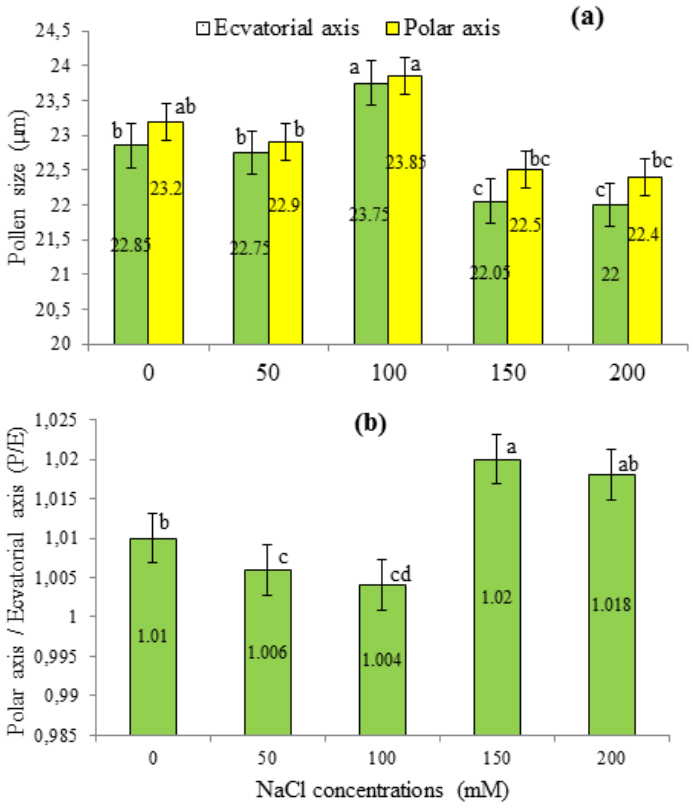

Fig. 2: Pollen size (a) and pollen shape (P/E) (b) of Glycine max exposed to salt stress

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The decrease in pollen size might be caused by the osmotic potential effect of pollen protoplast content as a result of excessive salinity [27]. On the other hand, the decrease in pollen size under the influence of stress factors also negatively affects the germination success of pollen on stigma and pollen tube length[28].

Exine and intine layers, known together as pollen wall, weren't significantly affected by increasing salt concentrations during the reproductive stage which is the most sensitive stage of plants to environmental stresses [29]. However, the fact that the highest applied salt concentration led to an increase in the thickness of both walls, even if too small, reveals the parallelism between the stress factor and the exine thickness. Thickness of the exine and intineslightly increased in parallel with increasing salt stress. Both wall structures of treatment groups were simillar to control group in width ($P \geq 0.05$). However, there was a slight increase at the highest applied salt concentration, compared to control ($P \leq 0.01$) (Fig 3). One of the conditions for becoming a fertile pollen is the presence of a well-developed pollen wall. Because any deformation in the pollen wall causes sterile pollen formation. The increase in exine width under environmental stress reveals the effort of the plant to form fertile pollen [30]. This can be a sign that pollen is protecting itself in the face of increased salt stress. The increase in exine thickness can be considered as an adaptation to the stress environment of the plant.

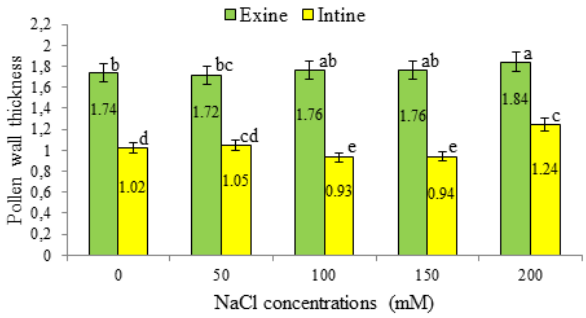

Fig. 3: Pollen wall thickness (exine and intine) of Glycine max exposed to increasing salt concentrations

Pollen germination begins with the emergence of vegetative pollen cell from the aperture. These areas are vulnerable to attack from the outside because they are openings where the pollen wall is the thinnest or non-existent. Salt stress decreased the aperture size of the pollens depending on the concentration, compared to control ($P \leq 0.05$). Aperture size decreased by 11% at 50 mM, 19% at 100 mM, 23% at 150 mM, and 29% at 200 mM salt concentrations (Fig 4 and 5). The aperture sizes in soybean seedlings exposed to salt stress decreased, and as a result pollen germination rate and pollen tube length also decreased. Any adverse effect on pollen aperture structure of plants exposed to various stress conditions also adversely affect pollen viability [31]. At the same time, these gates which provide communication between the pollen and the environment may undergo deformation due to the abnormal development of the pollen wall. This negative effect reduces the size of the apertures and significantly reduces pollen germination [32].

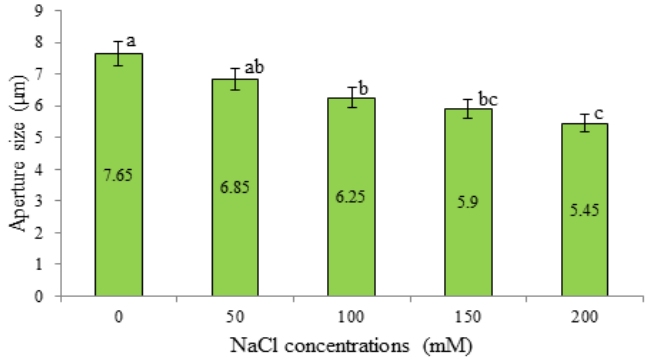

Fig. 4: The effect of salt stress on the pollen aperture size of Glycine max

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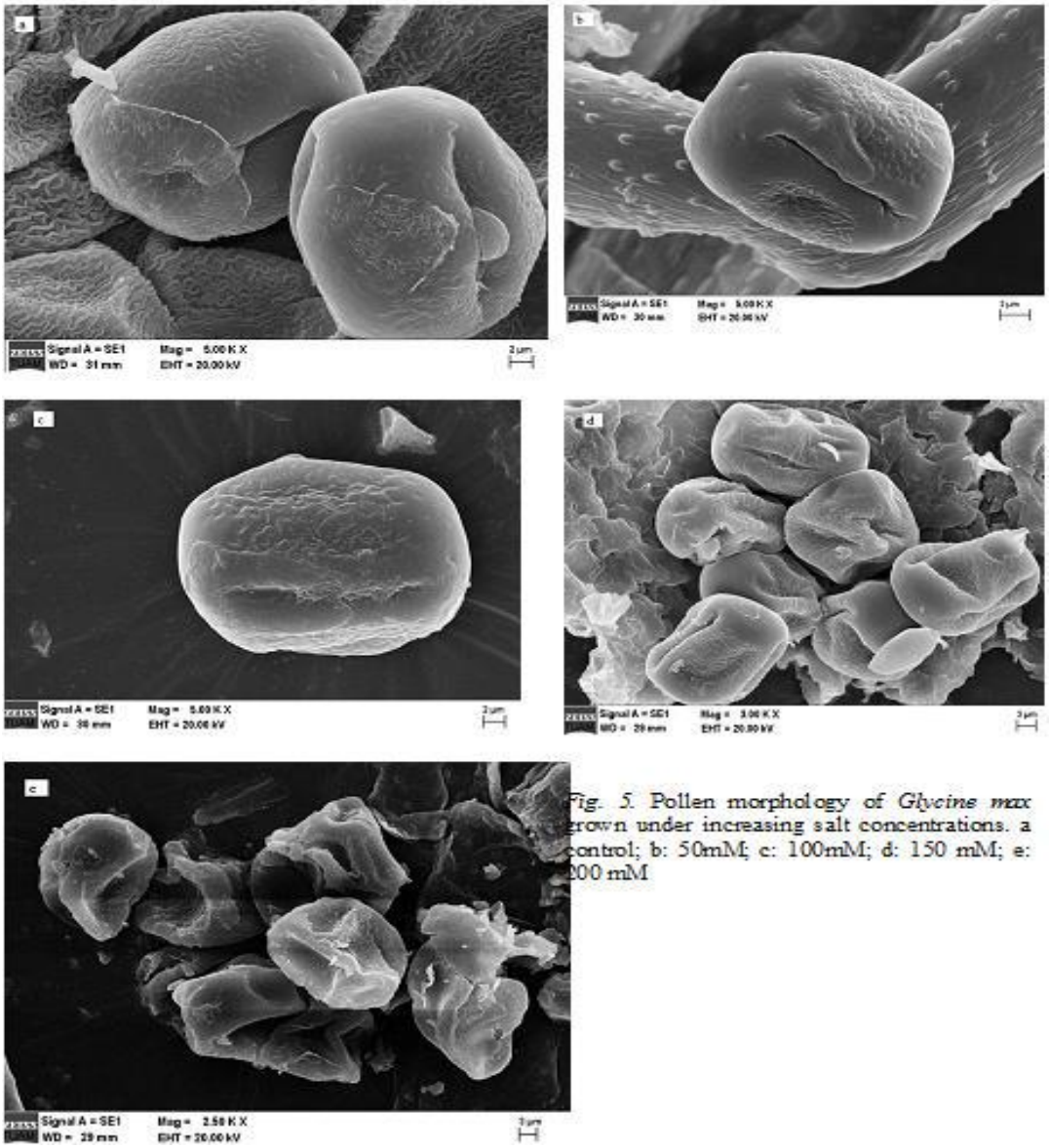

Fig. 5. Pollen morphology of *Glycine max* grown under increasing salt concentrations. a: control; b: 50mM; c: 100mM; d: 150 mM; e: 200 mM

Male reproductive stage in which microsopore main cells undergo meiosis (microsporongenesis) and microspores produce gametes (microgametogenesis) is the most sensitive stagein internal and external stresses [33] and abitoic stress during this stage causes the formation of infertile pollens [34]. Environmental stress significantly increases the recombination rate and this leads to formation of infertile pollens [35]. Pollen viability decreased with increasing salt concentrations. It decreased by 23%, 48%, 62% and 82% at increasing salt concentration, respectively, compared to control ($P \leq 0.05$). Simillar results were observed for pollen germination. However, salt stress had more negative impact on pollen germination rate. At the highest applied salt concentration, germination rate decreased by 96% ($P \leq 0.05$). The decrease in pollen viability in our study parallel to the increase in salt stress is an indication that this stress negatively affects male development in the reproductive stage. On the other hand, various stress factors impedes the passage of nutrients from leaves to anthers and this causes the formation of fragmented or infertile pollens [36]. Decrease in pollen viability causes pollination, fertilization, and product yield to decline significantly [37]. For this reason, the existence of a positive relationship between pollen viability and pollen germination can be mentioned.Because pollens can undergo germination only if they are healthy and well-developed. This is the reason why pollen germination rate (96%) decreased more than pollen viability (82%) at the highest applied salt concentration, compared to control ($P \leq 0.05$). Negative effects of salt stress on pollen viability of soybean seedlings were reflected on pollen germination at the same rate as on pollen tube length. It was observed that pollen tube lengths of successfully germinated pollens decreased as the salt concentration increased. However this negative effect was not as sever as seen on pollen vialibity and germination rate ($P \leq 0.05$). Pollen tube lengths decreased by 12%, 40%, 65%, 80% at

increasing salt concentrations, compared to control (Fig 6). A recent study on grapevines showed a positive relationship between the decrease in pollen tube growth and pollen viability [38]. When pistil structures of Arabidopsis plants exposed to salt stress is examined, no pollen germination is observed at some pistils, and in others pollen tube grows, however, it grows only a tiny amount or it is obstructed from reaching the stylus [39].

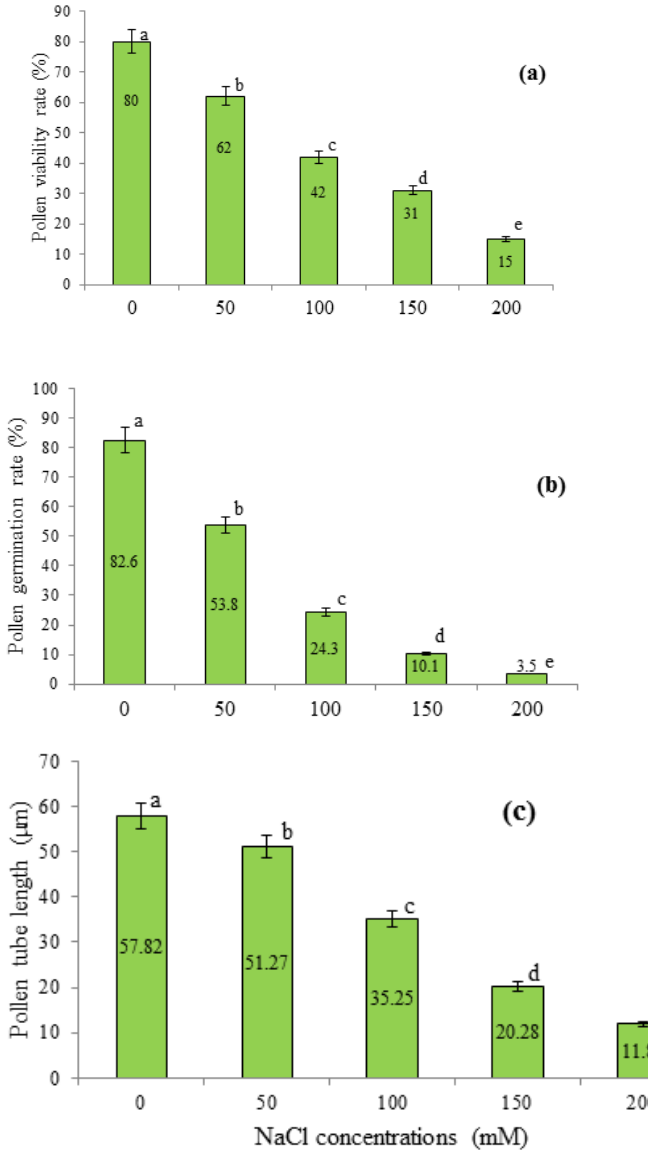

Fig. 6: Examined parameters of the reproductive structures of Glycine max grown under increasing salt concentrations. a: pollen viability; b: pollen germination rate; c: pollen tube length

IV. CONCLUSIONS

The observed decrease in all investigated parameters with increasing salt concentrations in our study was also explained by CRSI. Cumulative stress responses increased by 59%, 68%, 70% at 100, 150, 200 mMNaCI concentrations, compared to 50 mMNaCI. This increase is in inverse relationship with the tolerance level of the plant against the applied stress (Fig7). The fact that the tolerance level of the reproductive structures of *Glysine max* at the highest applied salt concentration was the lowest (-257.01) showed that the tolerance level of the plant was gradually decreasing. Our results show once again that the reproductive period is the most sensitive period to stress and as the stress level rises pollination will decrease. Therefore, it has become a necessity to speed up the work to adapt these nutrients, which have a very place in human nutrition, to our soil which becomes arid with each passing day due to excessive salinity.

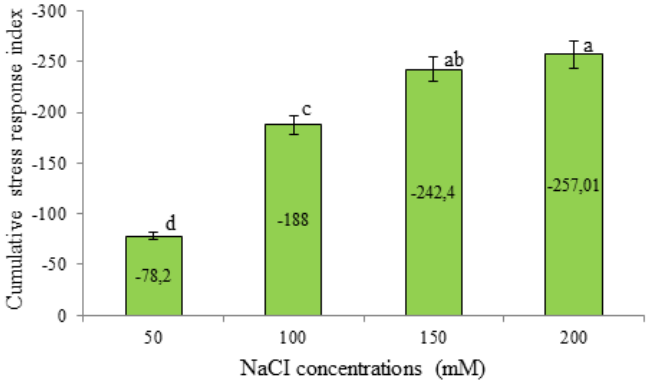

Fig. 7: Cumulative response index of all investigated parameters of Glycine max grown under increasing salt concentrations

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Effect of Different Media Combination on Growth and Biomass Production of Oil Palm (*Elaeis. guineensis*) Seedlings

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Abstract**—** *The study evaluates the effect of different media on growth and vegetative traits of oil palm seedlings. The treatments were T1 Control, T2 100% coco peat, T3 20% soil + 80% coco peat, T4 40% soil + 60% coco peat, T5 60% soil + 40% coco peat. Oxisol soil used for plantation crops was designated as a control evaluation. The new media were filled in polybag size 38cm x 57cm before transplanting the seedlings. The seedlings used were from Calix 600 series (D x P oil palm seeds). The newly produced growth media combination had the equal potential as standard media for oil palm nursery. The treatment (T4) which contained 60% coco peat and soil noticeably, enhanced growth of the seedling like plant height. Root dry weight (g) of seedlings grown in this planting medium greatly impacted plant root. This could have been due to the presence of silica content in the coco-peat which provided good aeration in the medium and indirectly stimulated root expansion. Increased in shoot dry weight of the seedlings grown in T4 was recorded compared to the plants grown in other media. The results obtained generally indicated that compost- based planting medium has the potential to influence seedling as an alternative growth medium.*

Keywords— coco peat, oil palm seedling, media, biomass, Elaeis. Guineensis

I. INTRODUCTION

Agriculture in tropics faces some challenges relating to plantation crops such as cocoa, oil palm and rubber which are grown in a marginal area predominantly covered by Ultisols and Oxisols which are basically low in key cations Noordiana et al., (2007). This has resulted in poor growth of oil palm especially at nursery stage which sometimes leads to plant death or long-term irreparable root and shoots damage. Due to the problems posed by the soils, integrated or alternative growing media are being widely considered in many parts of the world as it may positively influence plant total yield (Paranjpe et al., 2003). Many soils used in the tropics like Malaysia in the plantations including oil palm require a lot of fertilizer for adequate support of plant growth. In view of this, a soilless growing system like coco peat, vermiculite and perlite, especially for young plants, may be considered as an alternative growing medium to the soil (Van and Postma, 2000).

Soilless medium helps to prevent root-infecting pathogen related problems. This is due to its superior physicochemical characteristics coupled with lower infestation rate of pathogenic pests at the initial stage. Strongly weathered and sedimentary soils which are classified as Ferralsols, Nitosols, and Acrisols, (Oxisols and Ultisols) are mostly used for planting perennial crops including oil palm in Malaysia (Sabri, 2009). Thus, better performance of many plantation crops is achieved in Malaysia when it is planted in Ultisols and Oxisols soils which possess some of these qualities and could be found in most oil palm planting areas in the country (Salisu et al., 2013). However, these soils still heavily depend on fertilization and planting of cover crops for a better plant yield (Rantala, 2006). This could be attributed to its low cation exchange capacity (C.E.C) and high aluminium content Yaacob et al., (1992). Apart from crusting and low nutrients, the soils are susceptible to erosion (Eswaran et al., 1992). Despite the obvious weaknesses of the soils, many farmers still largely depend on it for future expansion of agriculture (Joint F.A.O, 2000). The soils have been continuously used for plantation crops like oil palm and rubber with continuous improvement (Shamshudin and Fauziah, 2010). Consequently, Adekunle, (2014) suggested a continuous evaluation of the soils for growth and nutritional need of the plantation crops. Establishment of high-quality seedlings of plantation crops involves different input such as suitable fertilizer rate. The type of growing medium plays a significant role Baiyeri and Mbah, (2006). Traditionally

plants are grown in soils, but its use had caused a significant setback. Objective of this experiment was to evaluate the effect of different media on growth and vegetative traits of oil palm seedlings.

II. MATERIALS AND METHODS

The experiment was carried out at Field 10 of the Universiti Putra Malaysia, Serdang Malaysia for a period of four months. The study consists of five treatments which are different rates of coco peat combinations and soil as a planting media on the oil palm seedlings. The treatments combinations were T1 Control, T2 100% coco peat, T3 20% soil + 80% coco peat, T4 40% soil + 60% coco peat, T5 60% soil + 40% coco peat. Oxisol soil commonly used in plantation in Malaysia was used as a control treatment. The growth media was prepared early before transplanting of seedlings into the large polybag. Large polybag size 38cm x 57cm, according to the standard in oil palm nursery was used. The seedlings used were from Calix 600 series (D x P oil palm seeds) and collected from Sime Darby Seeds and Agricultural Services Sdn. Bhd. Seedling selection was based on average height and fronds sizes. The seedlings were watered before the transplanting in order to reduce the transplanting shock. The seedlings were kept under a shelter house for about 7 days before they were placed in the field plot. Experimental design was a Randomized Complete Block Design (RCBD) with 4 replications. Fertilization was carried out once a month. N.P.K Blue special with composition 12: 12: 17: 6 + TE were used at a rate of 20g/plant. Irrigation was carried out twice a day, which is in the morning and in the evening especially during early plant establishment. Weeding was manually done. In order to control pests, Malathion (insecticides) was applied on the seedlings. Data collection began at second month after planting and was taken once a month until fourth months. Growth parameters such as height were taken monthly using standard measuring tape while girth size was taken using digital Vernier calliper. Leaf chlorophyll content was also measured with SPAD meter-502. For the biomass production, shoot dry weights were collected and determined. Fresh biomass for leaves, stem and roots were oven-dried at 50°C for 48 - 72 weighed (g) to a constant weight 0.01 g. Root: shoot ratio was also determined using an equation proposed by Hunt (1978).

$$RSR = \left(\frac{Total\ root\ dry\ weight\ (g)}{Total\ shoot\ dry\ weight\ (g)}\right)$$

Chlorophyll content was determined by first taken leaf samples with a leaf punch. Portions of the leaf samples (1.0 cm leaf disks) were collected in scintillation bottles containing 15 ml aqueous 80% acetone and kept in dark for two weeks after the extraction, the absorbance was determined at 664 and 647 nm using a light spectrophotometer (UV-2550). Actual total chlorophyll content was determined using described and published equation by Coombs et al., (1987).

III. FOLIAR NUTRIENT ANALYSIS

Nutrient analysis was carried out to determine N, P and K using Kjeldahl method. The plant leaves were dried and grounded with machine. Thereafter, weighed 0.25g and put it into a digestion tube. Then, 5 ml concentrated Sulphuric acid (H_2SO_4) was added. This process was conducted in a fume chamber. Thereafter, the mixture digestion tubes were placed in the digestion block at the temperature 450°C in the Fume Chamber for approximately 45 minutes. The digestion tubes were removed and allowed to cool, after which, 2 ml of 50% hydrogen peroxide (H_2O_2) was added and the heating process was repeated in the Fume Chamber. After the stipulated heating period, the sample in the tube became colourless. The solution was left to cool and later diluted with distilled water to make up 100 ml. The samples were analyzed for N, P, and K using Auto Analyzer, while Mg was analyzed with Atomic Absorption Spectrophotometer (Perkin-Elmer, Model AAS 3110). All data from this experiment were analysed by using Statistical analysis System (SAS) and Analysis of variance (ANOVA). The mean comparisons between the treatments were determined by using Least Significant Difference (LSD) at $p < 0.05$.

IV. RESULTS

There were significant differences between the treatments on the height increment at $p < 0.05$. Figure 1 showed the response of the plants towards different combination of soil and coco peat at different percentage for the second month of planting, whereby the treatment T4 showed the highest height increment with 3.01 cm followed by T5 and significantly different from T1, T2, and T3 1.89 cm, 1.77 cm and 1.74 cm respectively. Treatments T1, T2, and T3 and T5 were not significantly different. Meanwhile, at the third month of planting, different treatments showed growth improvement of the oil palm seedlings. Treatment T4 had the highest mean value with 3.27 cm and significantly different from T2 which gave the lowest mean value of 2.28 cm.

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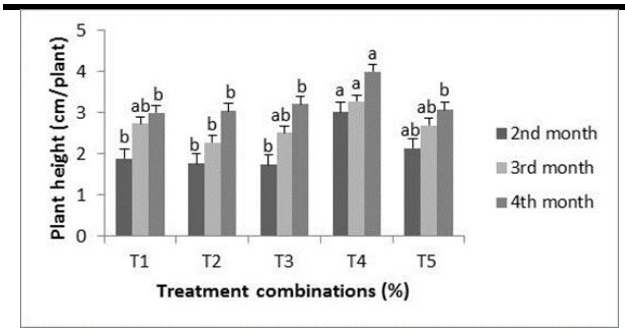

Fig.1: Effects of different treatment combinations of cocopeat on height of oil palm seedlings in each month. Means sharing the same letter in the figure for treatment combinations in each month are not significantly different at p<0.05.

However, the T4 was not significantly different T1, T3, and T5. Different coco peat combinations on the growth of oil palm seedlings at the fourth month after planting indicated that there were significant differences between the treatments on the plant height increment at p<0.05 of the oil palm seedlings. However, the results showed that treatment T4 had significantly higher increment of the oil palm seedling than all other treatments. This indicated suitable suitability of the composition of the treatment T4. There were significant differences as the seedlings demonstrated plant girth increment whereby T4 recorded the highest mean value (4.78cm) followed by the T3 in the third month as shown in Figure 2. The results from the two treatments were significantly different from T1 (3.19cm), T2 (3.35cm) and T5 (2.83cm). Similarly, the results show that treatment T4 had significantly higher girth increment than all other treatments in the fourth month. The remaining treatments were not significantly different from each other.

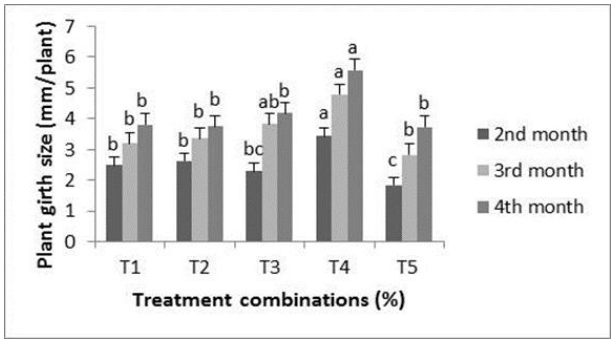

Fig. 2: Effects of different treatment combinations of coco peat on girth size of oil palm seedlings in each month. Means sharing the same letter in the figure for treatment combinations in each month are not significantly different at p<0.05.

There was a significant difference among the treatments in the fourth month of planting in terms of chlorophyll content. Figure 3 show that the treatment T3 and T4 had significantly higher chlorophyll content and significantly different from T1, T2 and T5.

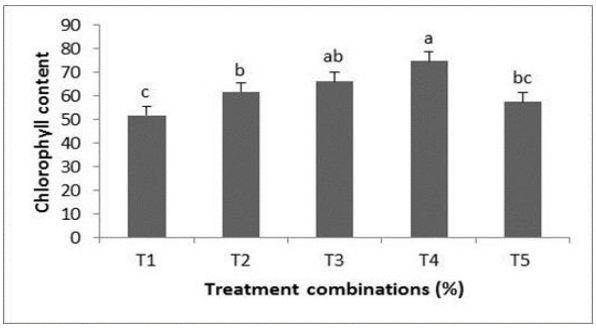

Fig. 3: Effects of different treatment combinations of coco peat on chlorophyll content of oil palm seedlings. Means sharing the same letter in the figure for treatment combinations are not significantly different at p<0.05.

V. PLANT BIOMASS YIELD

At the end of the experiment, the biomass yield of the oil palm seedlings grown on the coco peat combinations at the fourth month indicated that there were significant differences between of the treatments on the shoot dry weight at P < 0.05. The results showed that the treatment T4 had significantly higher shoot dry weight than the seedlings grown on all other treatments as shown in Figure 4.

VI. ROOT-SHOOT RATIO (RSR)

Result of the root: shoot ratio indicated that the effects of the treatments varied significantly at p < 0.05. However, seedlings grown with the treatment T4 (0.37) significantly higher than T1 (0.33), T2 (0.30), T3 (0.29) and T5 (0.33) as shown in Figure 5.

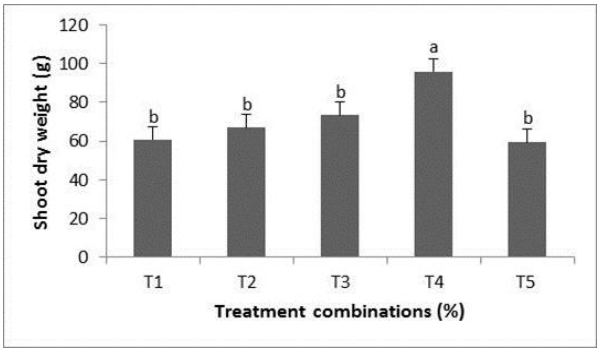

Fig. 4: Effects of different treatment combinations of coco peat on shoot dry weight of oil palm seedlings. Means sharing the same letter in the figure for treatment combinations are not significantly different at p<0.05.

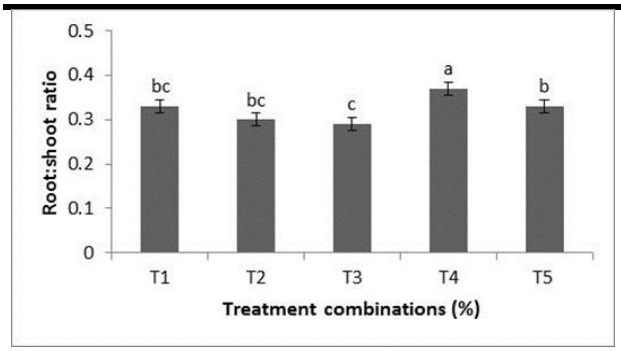

Fig. 5: Effects of different treatment combinations of coco peat on root: shoot ratio of oil palm seedlings. Means sharing the same letter in the figure for treatment combinations are not significantly different at p<0.05.

VII. PLANT NUTRIENT CONCENTRATION

Foliar nutrient analysis showed nitrogen concentration from the various treatments in the oil palm seedlings whereby in plant grown with T1 had significantly higher nitrogen content in the leaves of the oil palm seedlings than plant grown in T2 (Figure 6). Furthermore, T4 had significantly higher phosphorus content in the leaves of the oil palm seedlings than the plants grown with T5 (Figure 7). There was a significantly different among the seedlings to different treatments. Noticeably, potassium concentration in leaves of seedlings grown with T1 was significantly different from seedlings grown on T5.

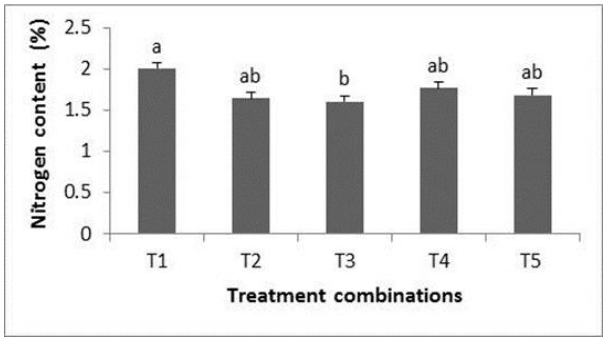

Fig. 6: *Effects of different treatment combinations of coco peat on nitrogen concentration of oil palm seedlings. Means sharing the same letter in the figure for treatment combinations are not significantly different at p<0.05.*

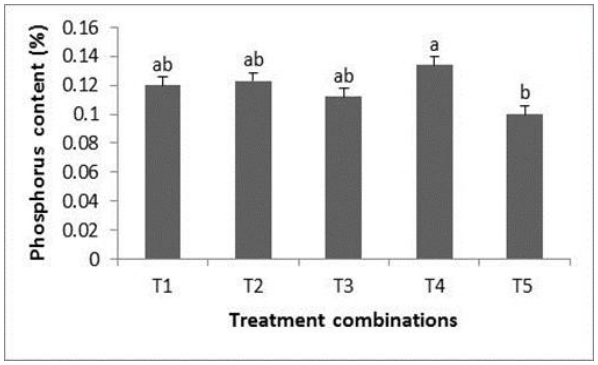

Fig. 7: Effects of different treatment combinations of coco peat on phosphorus concentration of oil palm seedlings. Means sharing the same letter in the figure for treatment combinations are not significantly different at p<0.05.

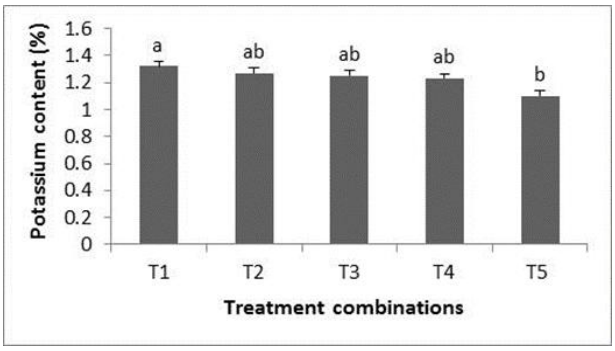

Fig. 8. Effects of different treatment combinations of coco peat on potassium concentration of oil palm seedlings. Means sharing the same letter in the figure for treatment combinations are not significantly different at p<0.05.

VIII. DISCUSSION

The results showed that combination of coco peat with soil, used as a growing medium during planting of the oil palm seedlings, had beneficial effect on plant growth. The combination of coco peat with soil indicated the faster plant growth and development of oil palm seedlings. This may due to good root system and better heat properties of coco peat (Salisu et al, 2017). Hence, it is clearly seems that the combination of coco peat with soil on treatment T4 indicated the best combination growing media for oil palm seedlings growth as shown in height and girth increment. This study was in line with study by Salisu et al. (2016) who found shoot length, growth and development of plant were higher on medium containing coco peat or coconut husk on rubber plant. In term of root dry weight, treatments T4 (40% soil + 60% coco peat) and T5 (60% soil + 40% coco peat) showed the highest mean value which could have been due to coco peat combination with the soil. The positive response was noticed in good root growth and vegetative traits. Noticeably, seedlings grown in treatment T1 had the lowest mean value of root dry weight. This may be due to heavy structure and compaction of the soil which could have caused root restriction which translated into the poor plant vegetative growth. Similar results were also found on shoot-root ratio. This could have been due to good physical and chemical properties of coco peat (Prasad, 1997). The concentration of Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) in finely ground dried sample were determined. Nutrients concentration of the seedlings indicated normal range which could be found in healthy mature tissue of various plant species including oil palm. Noticeably, micronutrient analyzed showed that N.P.K were not negatively affected by the treatments. Consequently,

variation in the growth and vegetative traits of oil palm seedlings observed in this study could have been due to differences in the physical properties of the media. Weight of planting media showed that treatment T4 (40% soil + 60% coco peat) had less 50% of weight (10kg) compared to the treatment T1 (100% soil) had 20kg. This indicated that the materials used for the planting medium composition fulfilled requirements of a suitable planting medium which includes lightweight for easy handling and transportation. This was supported by Asiah et al., (2004) who noted that lightweight of a growing medium is important for commercial or economic purpose. Also, Hashim et al., (1987), observed that use of lightweight potting medium would burden for workers during field planting operation and would certainly reduce the palm distribution time during planting or re-planting exercise.

IX. CONCLUSION

The study showed that the Calix 600 (Sime Darby sources) seedlings have a greater response to utilization of the new growth media combination. For instance, the new growth media combination performed equally as soils. Good planting media management is essential for the production of quality oil palm seedlings. Combination of soil and coco peat significantly enhanced growth of oil palm seedling. The study indicated that various coco peat ratio with soil tends to increase oil palm seedlings growth at nursery stage. The performance is noticeable in plant grown in T4 which contained 60% coco peat and root dry weight. The treatment equally influenced root: shoot ratio of the seedlings. This could have been due to the presence of nutrient concentration in the treatment (T4). The results obtained generally indicated that compost- based planting medium has the potential to influence seedling as an alternative growth medium compared to the soils used in oil palm nursery.

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Effects of wastewater quality on Henna (*Lawsonia inermis* L.) germination and seedling growth: a case study, Tunisia

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***Abstract*—** *Water resources scarcity led countries like Tunisia to adopt a policy of water economy by increasing the use of treated wastewater in agricultural irrigation. In this context, we studied the effects of raw and secondary treated urban and industrial wastewaters and fresh waters (distilled and well waters used as control) on the germination and seedling growth of Lawsonia inermis. The seeds were used untreated or pretreated with 0.5% H_2SO_4, concentrated H_2SO_4 and soaked in distilled water prior to germination. Germination was conducted at 25°C during 7 days under conditions of dark and light. Germination rate, moisture content, root and shoot lengths were measured in different experimental conditions crossing seed pre-treatment, water quality and incubation condition. The R software was used to perform three classification factors ANOVA, Pearson correlation analysis and multiple linear regression analysis. Results showed that the best germination performance was obtained when the seeds were pretreated by 0.5% H_2SO_4, watered by treated urban wastewater and exposed to light. Treated urban wastewater had a best contribution to germination rate considered model than fresh water and the other wastewaters types tested. So at the stage of early growth treated urban wastewater could be considered as potent water for Lawsonia inermis irrigation.*

***Keywords*— Germination, raw and treated wastewaters, R software, seedling growth, urban and industrial wastewaters.**

I. INTRODUCTION

Water resources management in many arid and semi-arid regions had a big challenge for a long time due to water shortage problems [1]. To address this problem, many countries such as Tunisia introduced adaptation policies focused on alleviating pressure on conventional water resources, reuse of unconventional water, transfers between basins, desalination and pollution control [2]. Reuse of treated wastewater began in Tunisia in the early sixties, applied in agriculture and progressively being developed in industrial, municipal and urban applications [3]. Currently a total of 112 wastewater treatment plants (WWTPs) are located throughout Tunisia with a wastewater capacity of 300 million m^3 per year and producing a volume of 243 million m^3 of treated wastewater. Only a volume of 60 million m3 were reused, 33% for agricultural irrigation [3].

Physico-chemical and biological wastewater characteristics reflect its environmental impacts and its effects on crops when it is reused for agriculture irrigation. For this, Tunisia has developed a standard for agricultural reuse, the NT 106.03 [4]. Reuse of treated wastewater for irrigation may have many beneficial effects such as the increase of the organic matter content of the soil and the positive effects on growth and yield of different plant species [5, 6]. Taking into account the benefits and drawbacks of the irrigation with treated wastewater leads to consider that some crops are more prone to contamination than other by Metal Trace Elements (MTE) and pathogens which may still remain in the treated wastewater [7] such as crops with the edible parts exposed to the contaminated soil after wastewater irrigation like leafy and tuberous vegetables [8, 9]. The World Health Organization (WHO) guidelines for the safe use of wastewater in agriculture recommended restrictions for crops, especially those eaten raw [10]. So in several countries around the world (Mexico, Peru, Tunisia, Saudi Arabia, etc.), treated wastewaters are used mainly for irrigation of green areas, forage and industrial crops [11] and also for human food crops knowing that the degree of pre-application treatment is an important factor in the planning, design, and management of wastewater irrigation systems [12]. In Tunisia the 1994 order from the Ministry of Agriculture and Water Resources listed crops that can be irrigated with treated wastewater, including industrial crops such as *Lawsonia*

inermis L., cereals, forages, fruit trees provided that they are not irrigated by spraying, forest trees, flowers and herbs [13]. The effect of treated wastewater irrigation on plants can be evaluated by standardized biotests used as indicators for wastewater reuse [14]. The crop germination inhibition is one of the approaches used to evaluate the toxicity of an effluent used as potential alternative to fresh water especially for water shortage areas [15]. According to [15] and [16], exposure to urban and industrial effluents had an inhibitory effect on germination by delaying it and leading to reduce the fresh weight of seedlings of several plant species namely *Solanum lycopersicum, Trifolium pratense, Triticum aestivum, Secale cereale , Pisum sativum, Trigonella foenum-graecum, Hordeum vulgare, Brassica juncea, Brassica napus, Coriandrum sativum* and *Nigella sativa*.

Henna (*Lawsonia inermis*) is mainly cultivated in India, the Middle East and the Mediteranean [17]. In Tunisia, this plant has been cultivated since 1400 BCE for many traditional and commercial uses. The total area planted with *L. inermis* in this country was estimated to 500 hectares on 5 million hectares of arable land [18]. The present study aimed to evaluate the application effects of raw and treated urban and industrial wastewater on the germination and early growth of Henna *(L. inermis*) seeds pretreated with 0.5% H_2SO_4, concentrated H_2SO_4 or soaked in distilled water. The experiment was conducted under conditions of light and darkness.

II. MATERIALS AND METHODS

2.1 Sampling: plant material, fresh water and wastewater samples

The *L. inermis* seeds used in this study were collected in 2015 year from a local cultivar of the oasis of Chenini in Gabes southeast Tunisia and stored in hermetically sealed containers under ambient laboratory condition with temperature varying from 15 °C to 35 °C respectively in winter and summer seasons. For the germination tests, fresh water types used as control for comparison were distilled water (pH = 7.07; electrical conductivity = 0.38 mS/cm) and well water (WW) set up in the experimental site of Oued Souhil in Nabeul, Tunisia. The wastewater types used in this study were raw (RUW) and secondary treated urban wastewater (TUW) and raw (RIW) and secondary treated industrial wastewater (TIW). Water and wastewaters samples were collected in polyethylene bottles. Urban wastewater samples were effluents collected from a Tunisian WWTP (activated sludge) located in Hammamet region (Fig. 1) with supply water of 90% domestic and 10% touristic origins. Industrial wastewater samples were collected from a WWTP (facultative pond) of Moknine region (Fig. 1) with 70% of water supply from textile industry and 30% of domestic origin. "Fig. 1" presented the inter-annual mean rainfall of the locations of water sampling points in Tunisia and Table 1 summarized the main characteristics of the studied effluents.

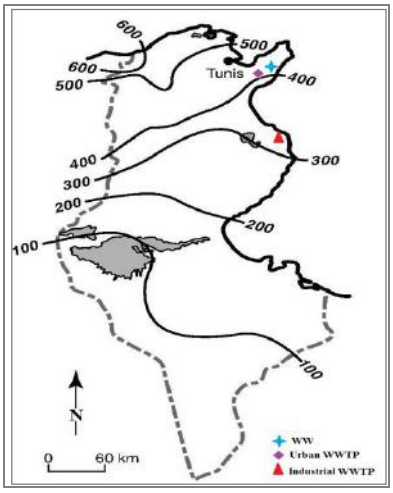

Fig. 1: Map of the inter-annual mean rainfall (mm) of Tunisia with the locations of water sampling points. Notes: WW: well water; WWTP: wastewater treatment plant.

2.2. Physicochemical parameters and methods of analysis

The physicochemical parameters were measured in the Research Laboratory of Valorization of Unconventional Waters in National Research Institute of Rural Engineering, Waters and Forests in Tunis according to Standards Methods. The pH and electrical conductivity (EC) measurements were determined according to the protocols of AFNOR NF T90-008 [19] and NF T90-031 [20] using respectively the pH-meter Adwa 1000 (Romania) and the conductivity-type meter WTW LF330 (Germany). The chemical oxygen demand (COD), biochemical oxygen demand during 5 days (BOD_5) and total suspended solids (TSS) were measured respectively according to the protocols of AFNOR NF T 90-101 [21], NF EN 1899-1 [22] and NF EN 872 [23] in the laboratories of the National Sanitation Utility of Tunisia (ONAS). Chlorides were tittered according to Mohr method [24]. The concentrations of sodium (Na^+) and potassium (K^+) ions were measured by flame spectrophotometry method [25] using the flame photometer Jenway PFP7 (U.K.).

Table.001: Comparison of mean concentrations of physico-chemical parameters of the well water and wastewater samples used for germination tests to agricultural irrigation reuse Tunisian standard NT 106.03 - 1989.

Water samples / Parameters	Well water	Raw urban wastewater	Secondary treated urban wastewater	Raw industrial wastewater	Secondary treated industrial wastewater	Maximum levels for irrigation according to NT 106.03 [4]
pH	7.87	7.01	7.12	7.85	8.01	6.5 - 8.5
EC (mS/cm)	4.35	2.83	2.77	6.21	5.48	7
COD (mg O_2/l)	3.7	425	45	1076	330	90
BOD_5 (mg O_2/l)	5.9	139	7	467	117	30
TSS (mg/l)	0	196	7	440	90	30
Na^+ (mg/l)	547.56	636.18	616.3	1258.58	1178.48	NS
Mg^{2+} (mg/l)	2.82	36.29	30.75	92 .25	49.2	NS
Ca^{2+} (mg/l)	12.252	26.05	25.05	75.15	70.14	NS
SAR	36.66	18.89	19.49	22.97	26.37	NS
K^+ (mg/l)	29.76	26.1	24.5	53.11	50.85	NS
NH_4^+ (mg/l)	3.26	37.03	21.78	40.3	16.33	NS
Cl^- (mg/l)	1050	500	450	1250	1050	2000
HCO_3^- (mg/l)	504.9	1285.2	1101.6	1244	945.72	NS
Cd^{2+} (mg/l)	0.015	0.006	0.006	0.012	0.011	0.01
Co^{2+} (mg/l)	0.033	0.015	0.014	0.031	0.028	0.1
Cr^{2+} (mg/l)	0.104	0.026	0.024	0.052	0.011	0.1
Cu^{2+} (mg/l)	0.016	0.007	0.001	0.132	0.026	0.5
Fe^{3+} (mg/l)	0.048	0.274	0.14	0.201	0.179	5
Mn^{2+} (mg/l)	0.008	0.0084	0.01	0.099	0.062	0.5
Ni^{2+} (mg/l)	0.032	0.024	0.022	0.166	0.058	0.2
Pb^{2+} (mg/l)	0.116	0.038	0.037	0.063	0.054	1
Zn^{2+} (mg/l)	0.042	3.55	3.35	10.7	4.5	5

WWTP: Wastewater treatment plant; EC: Electrical conductivity; COD: Chemical oxygen demand; BOD_5 : Biochemical oxygen demand during 5 days; TSS: Total suspended solids ; NS: not stated ; SAR: Sodium absorption ratio $= \frac{Na^+}{\sqrt{\sqrt{(Ca^{2+}+Mg^{2+})/2}}}$

The concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions were determined by complexometric EDTA (Chem-Lab) titration using basic medium [26]. The bicarbonate ions (HCO_3^-) concentration was performed by volumetric titration using sulfuric acid (H_2SO_4) (Honeywell) and methyl orange (Acros Organics) [26]. The metallic trace elements were performed by atomic absorption spectrophotometry (PerkinElmer A Analyst 400, U.S.A.) [27].

2.3. Germination tests

For germination tests, fresh water and wastewater samples were filtered through Whatman paper No.1 (filter mesh= 11 µm) and kept at 25°C. Healthy and uniform Henna seeds were selected and sterilized with $HgCl_2$ (Prolabo) solution at 0.1% and thoroughly washed with sterilized distilled water to avoid surface contamination. Henna seeds freshly harvested show physiological dormancy and their pre-treatment permit to reduce dormancy [28]. Therefore the seeds were subjected to different pre-treatments: (i) some were treated for few seconds with concentrated H_2SO_4 (Honeywell, 18.1 mol/l) then thoroughly washed with distilled water [29], (ii) others were soaked in 0.5% H_2SO_4 (Honeywell) for 48 h then washed with distilled water [28], and (iii) others were soaked in distilled water for 7 days [28]. Untreated seeds served as control during the germination tests. For each pre-treatment, 40 seeds were placed equidistantly on

soaked filter paper in Petri dishes and were irrigated with 2 ml of fresh water and wastewater samples previously described (Fig. 2a). The seeds, irrigated with distilled water and well water, were taken as control. Six replicate were taken for each pre-treatment. Then Petri dishes were placed in growth chamber (Memmert, Germany) at 25°C for 7 days and maintained in the dark. The same experiment was done under light conditions (Fig. 2b). Germinated seeds were counted and germination rate was calculated and expressed in percentage following the formula: G = [NT x 100]/N, where G is the germination rate, NT the number of seedlings emerging on day and N the day after planting [30]. The root and shoot lengths and fresh weight were recorded after 7 days. Dry weight of seedlings was taken after keeping seeds in hot air oven at 80°C for 24 h. The moisture content was obtained by difference between fresh weight and dry weight.

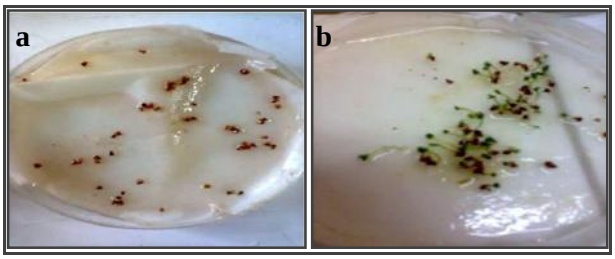

Fig. 2: (a) Lawsonia inermis seeds put in germination; (b) Seeds after 7 days of germination in the light.

2.4. Statistical analysis

The studied statistical model includes three dependant factors (seeds pre-treatment, water quality and incubation condition) explaining the variations of four quantitative variables which are germination rate, moisture content, root and shoot lengths. Data normality of each quantitative variable was assessed through the Shapiro-Wilks test. A three classification factors ANOVA was performed to study the variability of the response variables according to the dependent factors. The relationship between quantitative variables was evaluated by Pearson's correlation analysis. Changes in the germination rate were explained and predicted through a multiple linear regression analysis involving the rest of the studied parameters. The multivariate regression analysis model is developed following the equation:

$y = \beta_0+\beta_1 x1+\ldots+\beta_n x_n+\varepsilon$ [31] where y the dependent variable, xi the independent variable, β_i the parameter and ε the error. Statistical analyses were performed using version 3.3.2 of R software [32].

III. RESULTS

3.1. Characterization of fresh water and wastewater samples used for germination tests

The measured physicochemical parameters and their concentrations obtained in fresh water and wastewater samples used for the germination tests are shown in Table 1. All results were compared with standardized levels for treated wastewater quality found in accordance with the Tunisian regulations NT 106.03 governing the agricultural reuse of TWW [4]. Generally, the wastewater collected at the tested WWTPs is slightly alkaline. The pH varies between 7 and 8 with an average value of 7.57. Electrical conductivity (EC) values varied from 2.7 to 6.2 mS/cm with an average of 4.37 mS/cm. Sodium (Na^+) concentrations varied from 547.56 to 1258.58 mg/l with a mean value of 847.42 mg/l. Chloride (Cl^-) levels were between 450 and 1250 mg/l with an average of 1720 mg/l lower than the critical value of 2000 mg/l for the chloride amount in treated wastewater reused in agriculture according to NT 106.03 [4]. Values of the sodium adsorption ratio (SAR) were varying from 18.89 to 36.66 meq/l with a mean of 24.87 meq/l. Potassium (K^+) concentrations varied from 24.5 to 53.11 mg/l with an average of 36.86 mg/l, bicarbonates (HCO_3^-) levels ranged from 504.9 to 1285.2 mg/l with a mean of 1016.28 mg/l and ammonium (NH_4^+) levels were between 3.26 and 40.3 mg/l with an average of 23.74 mg/l (Table 1). Concentrations of the MTE such as Cd^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} were within the 0.001 – 10.7 mg/l interval. Overall, values were below the thresholds accepted of the Tunisian regulations and the only excess was noted for Zn^{2+} in RIW (10.7 mg/l; Table 1). Results showed also that wastewaters used in the biotests presented chemical oxygen demand (COD) levels which ranged from 45 to 1076 mg O_2/l with a mean value of 469 mg O_2/l, biochemical oxygen demand (BOD_5) levels were between 7 and 467 mg O_2/l with an average of 182.5 mg O_2/l and total suspended solids (TSS) levels varied from 7 to 440 mg/l with a mean value of 183.25 mg/l. Only concentrations of COD, BOD_5 and TSS contained in WW and TUW were with the limit of the Tunisian regulations NT 106.03 [4] (Table 1). The comparison of well water and wastewater types tested throughout this study between them showed that well water presented high pH, SAR and chloride values, low COD, BOD_5 and TSS values and high concentrations of Cd^{2+}, Co^{2+}, Cr^{2+} and Pb^{2+} (Table 1). Analyzed urban wastewaters had low pH, EC and SAR values, high concentrations of HCO_3^- and Fe^{3+}, low COD, BOD_5 and TSS values and low concentrations of Cd^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+} and Pb^{2+} (Table 1). The studied industrial wastewaters presented high concentrations of Na^+, Cl^-, Mg^{2+}, Ca^{2+}, K^+ and NH_4^+, very high COD, BOD_5 and TSS values in addition to high concentrations of Cu^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} (Table 1). Investigation of Table 1 indicates that reuse of TUW is acceptable according to pH, EC, COD, BOD_5, TSS, Cl^- and all analyzed MTE concentrations.

3.2. Water quality effects on germination and seedling growth

The statistical model includes seven variables, three of them are qualitative and four are quantitative. Water quality, incubation condition and seeds pre-treatment explain variations of the independent parameters germination rate, moisture content, root and shoot lengths. "Fig. 3A" showed that the seeds untreated and incubated in darkness did not germinate independently on irrigation water while the maximum germination rate (99.16%) was reached when Henna seeds were pretreated with 0.5% H_2SO_4 under light condition and irrigated with distilled water and treated urban wastewater. The ANOVA test showed that germination rate variation was significant depending on incubation condition ($p < 0.05$) and was not significant on the seeds pre-treatment ($p = 0.07$) and water quality ($p = 0.37$). The interactions between the studied factors water quality, incubation condition and seeds pre-treatment, taken in pairs or thirds, were not significant either ($p > 0.05$).

The moisture content varied from 0 mg when the seeds were untreated and put in germination under darkness condition with all types of irrigation water mentioned above to 0.3 mg when they were pretreated with 0.5% H_2SO_4, put in germination under light condition and irrigated with distilled water (Fig. 3B). Moisture content variations were significant according to the type of seeds pre-treatment (ANOVA, $p < 0.05$) and the incubation condition (at 25°C for 7 days under dark or light) ($p < 0.05$). Whereas there was no significant variations with the irrigation water quality ($p = 0.15$). In addition the interaction of seeds pre-treatment and incubation condition factors had significant effect on moisture content variation of *L. inermis* germinating seeds ($p < 0.05$) but interactions between seeds pre-treatment and water quality; water quality and incubation condition and finally the three studied factors together did not affect significantly moisture content variations ($p > 0.05$).

For root length, the minimum value (0 cm) was obtained when the seeds were untreated and put for germination under darkness condition independently on irrigation water. However pre-treatment of seeds with concentrated H_2SO_4 (18.1 mol/l) for germination under darkness condition and irrigated with distilled water allowed us to reach a maximum length of 0.56 cm (Fig. 3C). The ANOVA test showed that root length did not present significant variations with the three studied factors water quality, incubation condition and seeds pre-treatment taken separately, in pairs or thirds ($p > 0.05$).

Concerning shoot length variations, the minimum value (0 cm) was observed for untreated seeds and put for germination in darkness independently on irrigation water. The maximum value (1.16 cm) was reached for seeds pretreated by distilled water for 7 days, irrigated with distilled water too and germinated under light condition (Fig. 3D). Shoot length varied in a highly significant way with seeds pre-treatment and incubation condition (ANOVA, $p < 0.01$) and significantly depended on water quality ($p < 0.05$). "Fig. 3D" showed that the shoot length of *L. inermis* seeds decreased under all tested wastewater types mentioned above and used for irrigation. Only interaction between seeds pre-treatment and incubation condition factors was significant (ANOVA, $p < 0.05$). Interactions between them and water quality taken in pairs or thirds were found not significant (ANOVA, $p > 0.05$).

Pearson correlation analysis revealed that all quantitative variables (germination rate, moisture content, root and shoot lengths) were significantly correlated to each other. High positive correlations were noted between germination rate and moisture content ($p = 1.2.10^{-12}$, r = 0.81), moisture content and root length ($p = 3.6.10^{-11}$, r = 0.78) and moisture content and shoot length ($p = 2.1.10^{-12}$, r = 0.81). Root and shoot lengths presented very high significant positive correlation between them ($p=2.2.10^{-16}$, r = 0.95).

The examination of the contributions importance of the variables to the model mentioned above revealed that Moisture content showed the best contribution with an absolute value of the coefficient β (beta) of 204.255, followed by the scores received from Root length (β = 49.107), Shoot length (β = 35.198), Incubation condition (β = 27.405), Seeds pre-treatment (β = 2.731) and Water quality (β = 0.302). The regression equation was obtained as follows: Germination rate = –30.63 – 0.302 Water quality + 2.731 Seeds pre-treatment + 27.405 Incubation condition + 204.255 Moisture content – 35.198 Shoot length + 49.107 Root length, where the Germination rate is predicted with certain values of Water quality (DW = 1, WW = 2, RUW = 3, TUW = 4, RIW = 5 and TIW = 6), Seeds pre-treatment (untreated seeds = 1, seeds imbibed in distilled water for 7 days = 2, seeds soaked in 0.5% H_2SO_4 for 48 h = 3 and seeds treated for few seconds with concentrated H_2SO_4 = 4) and Incubation condition (darkness = 1 and light = 2).

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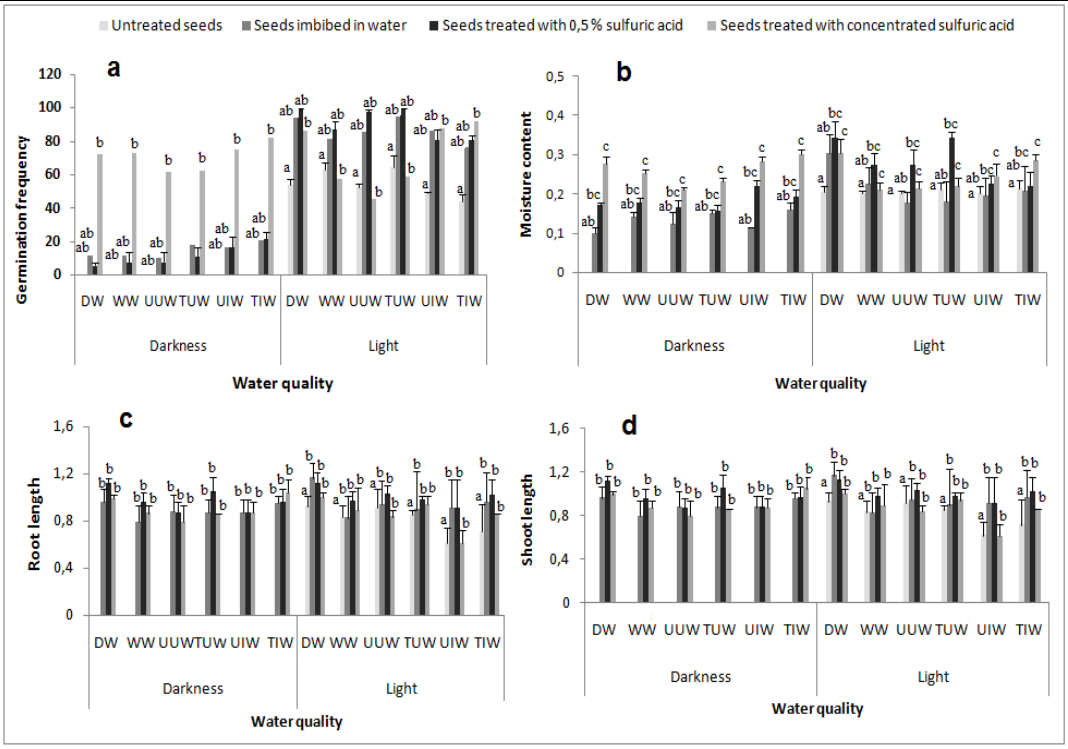

Fig. 3: Effects of water quality, incubation condition (light / darkness) and seeds pretreatment on (a) germination rate, (b) moisture content, (c) root and (d) shoot lengths of Lawsonia inermis seeds.

Notes: DW: seeds irrigated with distilled water, WW: well water, RUW: raw urban wastewater, TUW: secondary treated urban wastewater, RIW: raw industrial wastewater, TIU: secondary treated industrial wastewater. Means (±SD) followed by the same letter are not significantly different at the $p < 0.05$ level, as determined by Tukey's HSD test.

IV. DISCUSSION

Results showed that fresh water and wastewaters used in the germination tests were slightly alkaline with pH values varying from 7 to 8. Park et al. (2014) [33] considered that the shape and availability of nutrients contained in the irrigation water depend on the pH value that it should be between 5.5 and 6.5, values with which the solubility of most micronutrients is optimal. In addition Akinyemi and Souley (2014) [34] reported that irrigation water outside the pH range of 4.5–8.5 can cause nutritional imbalance or contain toxic ions. Electrical conductivity (EC) informs about water total soluble salt concentration (Rhoades, 1996). Based on NT 106.03 electrical conductivity threshold, EC values of the analyzed water and wastewaters allows their use for agricultural irrigation. Sodium (Na^+) is one of the ions contributing directly to total water salinity and can be toxic to sensitive crops such as carrots, beans and strawberries [35, 36]. Na^+ amounts of the studied fresh water and wastewaters samples varied from 547.56 to 1258.58 mg/l. The main problem with a large amount of sodium is its effect on the soil permeability and the water infiltration. Irrigation water with a high concentration of Na^+ hardens the soil and makes it compact and waterproof when dry [37]. Based on the literature, sodium, calcium and magnesium cations can be tolerated with relatively large quantities in the water irrigation but a continuous use of water with a sodium adsorption ratio (SAR) expressing the sodium amount compared to calcium and magnesium of more than 9, such as the fresh water and wastewaters samples used in this study (SAR varied from 18.89 to 36.66 meq/l), entails soil destruction [34] resulting in slower water infiltration and reduced soil aeration [38]. When such soils desiccate, crusting is formed, making plowing difficult and opposes the seed germination and seedling emergence [34]. Tunisian standards NT 106.03 for wastewater reuse did not state the potassium limit values in irrigation water but potassium concentrations of the studied fresh water and wastewaters samples included between 24.5 and 53.11 mg/l exceeded the threshold fixed by Peterson (1999) [39] which is limited to 0.2 mg/l. High concentarations of potassium may introduce a magnesium deficiency and iron chlorosis and an imbalance of magnesium and potassium may be toxic, but the effects of both can be reduced by high calcium levels [40]. The chloride anion (Cl^-) concentration of the tested samples varied from 450 to 1250 mg/l and was in accordance with NT 106.03 Tunisian standards [4]. In contrary Ayers and Westcot (1985) [35] fixed a water chloride content limit for

irrigation equal to 300 mg/l and considered that high chloride concentrations in irrigation water increase the osmotic pressure, reducing crop growth due to low water availability to plant. While Karaivazoglou et al. (2005) [41] reported that using irrigation water with high concentration of chloride, an essential element for plant growth, may be beneficial to plant development. There is no limit bicarbonates (HCO_3^-) values used for agricultural irrigation water [4]. According to Peterson (1999) [39] bicarbonates concentration should not exceed 25 mg/l for annual plants. Consequently, the analyzed water and wastewaters (504.9–1285.2 mg/l) did not respect the recommended Peterson limit. Park et al. (2014) [33] reported that the presence of HCO_3^- ions in excess in irrigation water may harm the plant mineral nutrition through its effects on the uptake and metabolism of nutrients, causing unsightly foliar deposits on leaf tissue and precipitates salts. For trace metallic elements, their concentrations in all types of water mentioned above and used for irrigation, did not exceed the values fixed by the Tunisian standard NT 106.03 [4] governing the treated wastewater reuse in agriculture and Peterson (1999) [39] limits. Well water, used as a control, had higher concentrations in Cd^{2+}, Co^{2+}, Cr^{2+} and Pb^{2+} metallic ions and was more saline than the urban wastewater types tested in the present study. This may be due to the fact that the well from which this water was brought is located in the Oued-Souhil region near Nabeul in northeastern Tunisia, constituting since the eighties a management and control of treated wastewater reuse and sewage sludge amendment pilot site [42]. Hussain and Al-Saati (1999) [43] affirmed that irrigation water is the main source of adding salts to the soil and during irrigation with saline water, a leaching of salts directly related to water movement occurs and crops remove only small amounts of salt [44]. Thus in Oued-Souhil region, the groundwater (water wells), will be increasingly saline and will accumulate the MTE contained in treated wastewater used for irrigation. Scientists estimated that parameters such as BOD_5, COD and TSS can provide useful information depending on the final use of reclaimed water [45]. For this, determination of the organic load in the studied wastewaters was performed through calculation of BOD_5 and COD. Gori et al. (2008) [46] conducted experiments to verify the possibility of reusing industrial wastewater of comparable quality to that used in this study (originated from textile (70%) and domestic (30%) activities) for the irrigation of ornamental shrubs. They found that some of them (*Buxus* and *Pistacia*) showed no signs of toxicity, while others (*Viburnum* and *Photinia*) showed greater sensitivity especially during sprinkle irrigation.

It has been proved in this study that germination rate variation of Henna seeds was not significantly depending on irrigation water quality. Thus, raw and secondary treated urban and industrial wastewaters used for irrigation of *L. inermis* seeds did not affect their germination rate. According to Rodosevich et al. (1997) [47] this will not have influence on their productivity. In contrary, seed germination rate, biomass production and root development of other species such as *Pisum sativum*, *Lens esculentum* and *Cicer arietinum* were reduced under irrigation with 50% (an amount of 50% wastewater dissolved in Tap water) and 100% concentrated urban and industrial wastewaters [48]. It is possible that wastewater organic compounds may alleviate some negative impacts. Panasker and Pawar (2011) [49] considered that 20% wastewater concentration does not inhibit seeds germination and seedling growth but at higher concentrations (60%, 80% and 100% concentrations) they are affected. The parameter moisture content did not show significant variations with the irrigation water quality factor. In the literature, seeds moisture content varied with plants species because the plant species groups have different functional traits related to water use like water content, dry matter content and moisture content due to their phylogenetical differences and their different respective life history characteristics [50]. Huma et al. (2012) [16] revealed that the most decrease of seeds moisture content under raw domestic and industrial wastewaters irrigation was obtained for the species *Coriandrum sativum* and *Nigella sativa*. While, the lowest decrease, was observed for *Brassica juncea* and *Trigonella foenum-graecum*. Root length parameter measured at the end of *L. inermis* germination assays did not show significant variations based on the water quality used for irrigation. In contrary Yasmin et al. (2011) [51] showed that the root length was unaffected when *Lens esculentum* varieties was irrigated with raw industrial effluent at a concentration of 10% and an increase of 6.3–11.64% was detected for 20–60% concentration. Huma et al. (2012) [16] stated that this parameter has decreased for the seeds of the species *Brassica juncea*, *Coriandrum sativum*, *Nigella sativa* and *Trigonella foenum-graecum* under raw domestic and industrial wastewaters irrigation. For shoot length parameter, variations were significant depending on the irrigation water quality. Therefore, *L. inermis* shoot length decreased under urban and industrial wastewaters irrigation in both forms raw and secondary treated. These results are similar to those found by Bazai and Achakazai (2006) [52] who studied the effect of treated urban wastewater on germination and seedling growth of *Lactuca sativa* L. and suggested that shoot length decreased with 75 and 100% concentration.

The multiple linear regression analysis confirmed the ANOVA results indicating that germination rates of

Henna seeds did not differ significantly with the irrigation water quality and seeds pre-treatment factors and had significant variations depending on the incubation condition factor. Thus the regression equation presented the factors of irrigation water quality and seeds pre-treatment with lower contributions to the model considered (having respective β absolute values of 0.302 and 2.731) than the incubation condition factor (β = 27.405). Henna seeds presented higher germination rates under incubation in light condition. This confirmed the results approved by the scientists on the light stimulatory effect on seed germination [53, 54]. Small-seeded species like *L. inermis*. need light to avert germination when they are too deep in soil or under plant shadows that could compete with the seedlings and minimize their chance to survive [55]. Hilhorst and Karssen (1988) [56] explained that during seed germination light induces a chain of events leading to gibberellins biosynthesis and enhances the sensitivity of the seeds to these hormones. Concerning the parameters moisture content, root and shoot lengths, they presented also high contributions to the predictive model of Henna germination rate (with respective β absolute values of 204.255, 49.107 and 35.198) and this can be justified by the significant correlation between all quantitative variables (germination rate, moisture content, root and shoot lengths) as reported by the Pearson test results. For seeds pre-treatment factor, it presented a low contribution to the model considered of Henna germination rate. This can be justified by the fact that *L. inermis* seeds were able to germinate without any pre-treatment when they were exposed to light as it was revealed by the results of this study (mean germination rate varied from 44.16% to 64.58% when the seeds were untreated and incubated in light). Indeed Henna seeds have endogenous non-deep physiological dormancy which requires exposure to light in order to break dormancy [28]. Results showed that pre-treatment of Henna seeds facilitate germination even in absence of light and the maximal germination rate was reached when the seeds were pretreated with 0.5% H_2SO_4 (99.16%). Parihar et al. (2016) [28] had pre-treated differently the seeds of *L. inermis* by heating under different temperature regime (20, 25, 30, 35°C for 6 hours in a day), leaching and soaking seeds in water, allowing a germination rate of up to 90%. For the irrigation water quality factor, the results showed that the highest germination rates (20.83% for seeds soaked in DW/incubated in darkness – 99.16% for seeds pre-treated with 0.5% H_2SO_4/incubated in light) were obtained when Henna seeds were irrigated with treated urban wastewater. This can be explained by the fact that they were less saline and less loaded by MTE than well water and raw wastewaters used in the germination tests. Katembe et al. (1998) [57] and Bojović et al. (2010) [58] showed negative effect of salinity on seed germination and seedling growth of some crop species such as *Atriplex*, *Brassicaceae* and *Solanaceae* and stated that high NaCl concentrations greater than 400 mM inhibit more seed imbibition, germination and seedling root elongation. Panuccio et al. (2014) [59] demonstrated that saline water used for irrigation of quinoa at low concentrations (100–200 mM of NaCl and 13.36–53.46 mM of $MgCl_2$) increased the germination rate like pure water used as control.

V. CONCLUSION

The crossing of different experimental conditions namely seeds pre-treatment, seeds incubation condition and water quality (distilled water and well water used as control for comparison and raw and treated urban and industrial wastewaters) used for irrigation, adopted to study the Henna (*Lawsonia inermis*) germination and seedling growth, showed that well water and secondary treated urban wastewater quality was acceptable for use in agricultural irrigation. Germination rate variation of Henna seeds was not significant based on water quality used for irrigation and seeds pre-treatment and was significant according to incubation condition. In fact, the germination performance of Henna seeds was obtained with seeds pre-treatment by 0.5% sulfuric acid, watering by secondary treated urban wastewater and exposure to the light. While, their moisture content variation was significant according to the type of seeds pre-treatment and the incubation condition and was not significant based on the quality of irrigation water samples tested here. Shoot length of *L. inermis* seeds varied significantly with seeds pre-treatment, incubation condition and irrigation water quality. Whereas, root length parameter did not shows significant variations based on these three studied factors. Statistical study showed that irrigation water quality, seeds pre-treatment, incubation condition, moisture content, shoot and root lengths were predictive of the germination rate score. The low contribution of the irrigation water quality factor to the model considered of the germination rate reflected the tolerance of Henna to different types of irrigation water. So *L. inermis* can be considered as a metal-tolerant plant which can maintain its germination under wastewater irrigation condition. At early growth stage, treated urban wastewater could be considered as potent water for *L. inermis* irrigation. Further studies should be done for monitoring *L. inermis* agronomic behavior and its mineral and MTE composition for the rest of growth stages.

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Effectiveness *Trichoderma* and *Beauveria bassiana* on Larvae of *Oryctes rhinoceros* On Palm Oil Plant (Elaeis Guineensis Jacq.) In Vitro

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Abstract— *O. rhinoceros horn beetle (Coleoptera: Scarabaeidae) is the main pest attacking oil palm crops in Indonesia, especially in palm oil rejuvenation areas. The study was conducted from April to August 2016 in the laboratory of the Faculty of Agriculture, Al-Azhar University, Medan. The materials used in this research are horn beetle pest larvae (O. rhinoceros) originating from PT. Socfin Indonesia, Trichoderma sp fungi originating from the Food Crops and Horticultural Fields of Medan and the B. bassiana fungi are derived from the Plant Seed Plant Protection Center (PBPPTP), aqua pro injection, Tween 80, rice and 96% alcohol. This research uses Completely Randomized Design (RAL) Non Factorial consisting of 13 treatments. The result of mortality of O. rhinoceros larvae on 1-14 DAA observation can be seen in appendix 2-43. Based on fingerprint analysis showed that the application of Trichoderma sp and B. basianna fungi on O. rhinoceros larvae had no significant effect on observation of 1 DAA to 9 DAA, but had a very significant effect on observation of 10 DAA to 14 DAA. The results of germination of Trichoderma sp and B. basianna fungus 4 hours after incubation period can be seen in appendix 61-66. Precentages mortality of the highest larvae of O. rhinoceros to Trichoderma sp fungus with a dose of 20 gr (96.67%) with the application method spread on the larvae. The highest amount of conidial density is found in Trichoderma sp fungi with doses of 60 g x 100-1 ml of aqua pro injection of 7.25 x 106 conidia/ml.Germination level of conidia mushroom highest in Trichoderma sp fungi with dose 60 gr x 100-1 ml aqua pro injection that is as much as 91%.*

Keywords—Effectiveness, Trichoderma, Bauveria, Orycetes, Oil Palm Oil, Vitro.

I. INTRODUCTION

Palm oil is the most productive plant with the highest production of oil per hectare from other vegetable oil producers. Indonesia is the second largest palm oil producer in the world after Malaysia. As many as 85% more world market of palm oil is controlled by Indonesia and Malaysia [39]. One of the obstacles in the cultivation of oil palm crops is the pest attack that can cause damage to the plant to result in decreased levels of palm oil production. Pests can attack palm oil from the pre-nursery stage to the producing stage [23].

O. rhinoceros horn beetle (Coleoptera: Scarabaeidae) is the main pest attacking oil palm crops in Indonesia, especially in palm oil rejuvenation areas. These insects bore oil palm shoots which resulted in stunted growth and damage to the point of growing so deadly plants. In palm oil rejuvenation areas, horn beetle attacks can result in delays in palm oil production up to a year and dead plants may reach 25% [23]. Reducing the use of pesticides in agricultural areas requires the availability of other safe and environmentally friendly methods of control, such as by utilizing natural enemies, such as entomopathogenic fungi, predatory insects, and parasitoids [41].

Groups of fungi that infect insects are called entomopathogenic fungi. The famous entomopathogenic mushrooms are *Namuraea rileyi*, *Metarizium anisopeliae* and *B. bassiana*. *B. bassiana* (Balsamo) Vuillemin (Ascomycota: Hyphocreales) is a facultative entomopathogenic fungus with a wide range of hosts, besides this fungus has the potential to control more than 70 insect pests belonging to different orders, especially lepidoptera pests [18].

In addition, the use of antagonistic fungus is also an alternative control option because this method is considered safe for both users, consumers and the environment. An antagonist fungus that has been widely used as a biological controller is *Trichoderma* sp. *Trichoderma* sp mushroom cultures in applicative media such as bran can be given to the planting area and are bio decomposers as well as bio

fungicides. *Trichoderma* sp also has a very effective biocontrol mechanism in suppressing the development of pathogens such as mycoparasitism, antibiosis, and competition [7].

B. bassiana fungi have been tried to control the *O. rhinoceros* pests in oil palm with concentration of 25g ml-1 [32]. While *Trichoderma* sp fungus has been tried to control P. nicotianae disease on Deli tobacco plants with concentration of 20g / L. Therefore, it is necessary to do research to find out the concentration of *B. bassiana* and *Trichoderma* sp fungi which is most effective to control horn beetle pest larvae on oil palm crop.

This study aims to determine the effectiveness of *B. bassiana* and *Trichoderma* sp fungi with different doses and methods of application to control horn beetle (*O. rhinoceros*) pest larvae in oil palm crops.

II. METHOD

Place and time of research

This research will be conducted at the Laboratory of the Faculty of Agriculture, Al-Azhar University, Medan. The study was conducted from April to August 2016.

Materials and tools

The materials used in this research are horn beetle pest larvae (*O. rhinoceros*) originating from PT. Socfin Indonesia, *Trichoderma* sp fungi originating from the Food Crops and Horticultural Fields of Medan and the *B. bassiana* fungi are derived from the Plant Seed Plant Protection Center (PBPPTP), aqua pro injection, Tween 80, rice and 96% alcohol.

The tool used in this research is haemocytometer, microscope, glass object, glass cover, hand sprayer, test tube, jar, kassa, erlemeyer, name label, beaker glass, scales, tube, aluminum foil and stationery.

Research methods

This research uses Completely Randomized Design (RAL) Non Factorial consisting of 13 treatments.

Where :

T1 = *Trichoderma* sp dose 20 gr B1 = *B. bassiana* dose 20 gr

T2 = *Trichoderma* sp dose 40 gr B2 = *B. bassiana* dosage 40 gr

T3 = *Trichoderma* sp dose 60 gr B3 = *B. bassiana* dose of 60 gr

1 = Application method is dispersed 2 = Application method is sprayed

(Control) = Provision of aqua pro injection

T11 = *Trichoderma* sp dosage 20 gr pertoples of dispersed application method.

T12 = *Trichoderma* sp dosage 20 gr x 100 ml-1 aqua pro injection pertoples application method is sprayed.

T21 = *Trichoderma* sp dosage 40 gr pertoples of dispersed application method.

T22 = *Trichoderma* sp dosage 40 gr x 100 ml-1 aqua pro injection pertoples application method is sprayed.

T31 = *Trichoderma* sp dose 60 gr pertoples of dispersed application method.

T32 = *Trichoderma* sp dose 60 gr x 100 ml-1 aqua pro injection pertoples application method is sprayed.

B11 = *B. bassiana* with a dose of 20 gr peroples of dispersed application method.

B12 = *B. bassiana* with a dose of 20 gr x 100 ml-1 aqua pro injection pertoples application method is sprayed.

B21 = *B. bassiana* with a dose of 40 pertoples of dispersed application method.

B22 = *B. bassiana* with a dose of 40 g x 100 ml-1 aqua pro injection pertoples application method is sprayed.

B31 = *B. bassiana* with a dose of 60 gr peroples of dispersed application method.

B32 = *B. bassiana* with a dose of 60 gr x 100 ml-1 aqua pro injection pertoples application method is sprayed.

Based on the treatment performed, the number of replications can be determined by the following formula (Hanafiah, 2010):

$t (r-1) \geq 15$

$13 (r-1) \geq 15$

$13r - 13 \geq 15$

$13r \geq 15 + 13$

$13r \geq 28$

$r \geq 28/13$

$r \geq 2.15 = 3$ replications [12].

The amount of treatment is 13 treatments, among others:

T11 T12 T21 T22 Controls

T31 T32 B11 B12

B21 B22 B31 B32

But to further strengthen the results of research, then the replication used in the study is as much as 3.

Based on the above description can be arranged the treatment unit as follows:

Treatment amount: 13 treatments

Number of repetition treatment: 3 replications

Number of research units: 39 units

Number of larvae per jar: 10 heads

Number of larvae tested: 390 heads

Distance between jars: 1 cm

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Distance between replicates: 15 cm
Number of jars of *Trichoderma* sp: 18 jars
Number of jars of *B. bassiana*: 18 jars
Number of control jars: 3 jars
Total jars total: 39 jars
The mathematical model used in this research is the Non Factorial Completely Randomized Design (Hanafiah, 2010) which is assumed as follows:

$$Y\_ij = \mu + N\_i + + \varepsilon\_ij$$

Where:
Y_ij: The results of observation of the treatment to the fungus and j-thr
μ: Effect of middle value
N_i: Effect of N treatment on the mushroom stage
Σ_ij: Effect of errors from the treatment of fungi and jth thrust
If the treatment has significant effect, the Duncan test is 5% and 1%.

Implementation of Research

Provision of larvae to be tested

Horned beetle larvae (*O. rhinoceros*) originating from PT. Socfin Indonesia was taken and maintained at Al-Azhar University Laboratory in Medan. The captured larvae are then adjusted in size and size to each other to obtain sufficient quantities to test. The larvae to be tested are instar larvae II.

The supply of entomopathogenic fungi

Trichoderma sp mushroom comes from Balai Proteksi Tanaman Pangan dan Hortikultura (BPTPH) Medan, while the fungus *B. bassiana* is isolated from the pest of *O. rhinoceros* which is derived from Balai Besar Germination Plantation Crop Protection (BBPPTP) Medan. To anticipate the deficiency of the mushrooms that will be used then both mushrooms are propagated in the rice medium each containing 100 grams / pack and incubated at room temperature for 5-7 days. This mushroom removal is done in the incase to avoid contamination. In the event of contamination, re-purification by taking part of the uncontaminated fungus of *B. bassiana* and *Trichoderma* sp. To be grown on rice media until *Trichoderma* sp and *B. bassiana* are completely pure and uncontaminated.

Application of Entomopathogenic Fungi

Application is applied only once in a manner dispersed and sprayed with each type of fungus according to the treatment, on the control only by spraying aqua pro injection alone on instar larvae instar II. Then observed its development.

Observation Parameters

Percentage of Mortality

Observation of larval mortality started one day after application (DAA) weeks 14 days after application. The percentage of larval mortality was calculated by the formula:

$$P = a / b \times 100\%$$

Information:
P = Percentage mortality of *O. rhinoceros* (%)
a = The number of *O. rhinoceros* is dead
b = Total *O. rhinoceros* observed entirely
If on the control found dead larvae then, the percentage of mortality obtained later in the correction using the Abbott's formula is as follows:

$$P = (Po-Pc) / (100-Pc) \times 100\%$$

Where:
P = Percentage *O. rhinoceros* test that died after correction
Po = Percentage of *O. rhinoceros* test that died at treatment
Pc = Percentage of test dead *O. rhinoceros* in control

Number of Conidia

The number of conidia of entomopathogenic fungi is calculated before application is performed. Conidial density calculation was performed by suspension of conidia from isolation treatment of isolate taken as much as 1 ml. Then the suspension is dropped on haemocytometer. Conidial density was calculated under a 400x magnification binoculars microscope using Gabriel & Riyatno [10] formula as follows:

$$C = t / ((n \times 0.25)) \times 106$$

Where:
C: Conidia density per ml of solution
t: Total number of conidia in the sample box observed
n: Number of sample boxes (5 large boxes x 16 small squares)
0.25: Correction factor for use of small-scale sample boxes on haemocytometers

Conidia germination

The conidia granulation from each of the entomopathogenic fungi suspensions used was calculated before application was performed and after 24 hours of inoculation was performed. One drop of suspension of entomopathogenic fungus dripped on the glass object then drops with PDA media and closed with a glass cover, then the amount of

germinated conidia is calculated, so it is not germinated. The calculations are performed on the field of view under a microscope with 400 x magnification, using Gabriel & Riyatno [10] formula as follows:

$$V = g / ((g + u)) \times 100\%$$

Where:
V: Conidia germination (viability)
g: The number of conidia that germinate
u: The number of conidia that does not germinate

III. RESULTS

Percentage of Mortality

The result of mortality of *O. rhinoceros* larvae on 1-14 DAA observation can be seen in appendix 2-43. Based on fingerprint analysis showed that the application of *Trichoderma* sp and *B. basianna* fungi on *O. rhinoceros* larvae had no significant effect on observation of 1 DAA to 9 DAA, but had a very significant effect on observation of 10 DAA to 14 DAA. For more details can be seen in Tables 1, 2 and 3.

Table.1: Average number of mortality Larva O.rhinoceros (%) due to fungus Trichoderma sp and B. bassiana in the 1-5 Days After Application (DAA)

Treatments	Observation of day to-					Mean
	1 DAA	2 DAA	3 DAA	4 DAA	5 DAA	
Control	0,00	0,00	0,00	0,00	10,00	2,00
T_{11}	13,33	23,33	30,00	40,00	40,00	29,33
T_{12}	10,00	13,33	23,33	30,00	33,33	22,00
T_{21}	3,33	20,00	23,33	36,67	50,00	26,67
T_{22}	3,33	6,67	13,33	20,00	23,33	13,33
T_{31}	6,67	16,67	23,33	26,67	26,67	20,00
T_{32}	0,00	0,00	0,00	13,33	23,33	7,33
B_{11}	0,00	6,67	10,00	10,00	20,00	9,33
B_{12}	16,67	23,33	30,00	30,00	36,67	27,33
B_{21}	13,33	16,67	23,33	23,33	26,67	20,67
B_{22}	13,33	13,33	13,33	23,33	26,67	18,00
B_{31}	0,00	0,00	10,00	13,33	16,67	8,00
B_{32}	13,33	13,33	20,00	23,33	26,67	19,33
Mean	7,18	11,79	16,92	22,31	27,69	17,18

The average number of mortality larvae of *O. rhinoceros* (%) due to fungus *Trichoderma* sp and *B. bassiana* 6-9 Days After Application (DAA) in Table 2 . While the characteristics of *O. rhinoceros* larvae that died from *Trichoderma* sp and *B. basianna* fungi can be seen in Figs. 7 and 8.

Table.2: Average number of mortality Larva O. rhinoceros (%) due to fungus Trichoderma sp and B. bassiana in the 6-9 Days After Application (DAA)

Treatmnets	Observation day to -				Mean
	6 DAA	7 DAAA	8 DAA	9 DAA	
Control	10,00	10,00	20,00	20,00	16,67
T_{11}	53,33	6 3,33	76,67	76,67	72,22
T_{12}	43,33	46,67	50,00	56,67	51,11
T_{21}	50,00	53,33	60,00	66,67	60,00
T_{22}	30,00	33,33	36,67	56,67	42,22
T_{31}	36,67	50,00	50,00	53,33	51,11

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Treatmnets	Observation day to -				Mean
	6 DAA	7 DAAA	8 DAA	9 DAA	
T_{32}	23,33	26,67	26,67	33,33	28,89
B_{11}	26,67	36,67	56,67	60,00	51,11
B_{12}	36,67	46,67	53,33	56,67	52,22
B_{21}	26,67	33,33	43,33	60,00	45,56
B_{22}	43,33	50,00	53,33	56,67	53,33
B_{31}	16,67	33,33	46,67	63,33	47,78
B_{32}	26,67	33,33	43,33	63,33	46,67
Mean	32,56	39,74	47,44	55,64	47,61

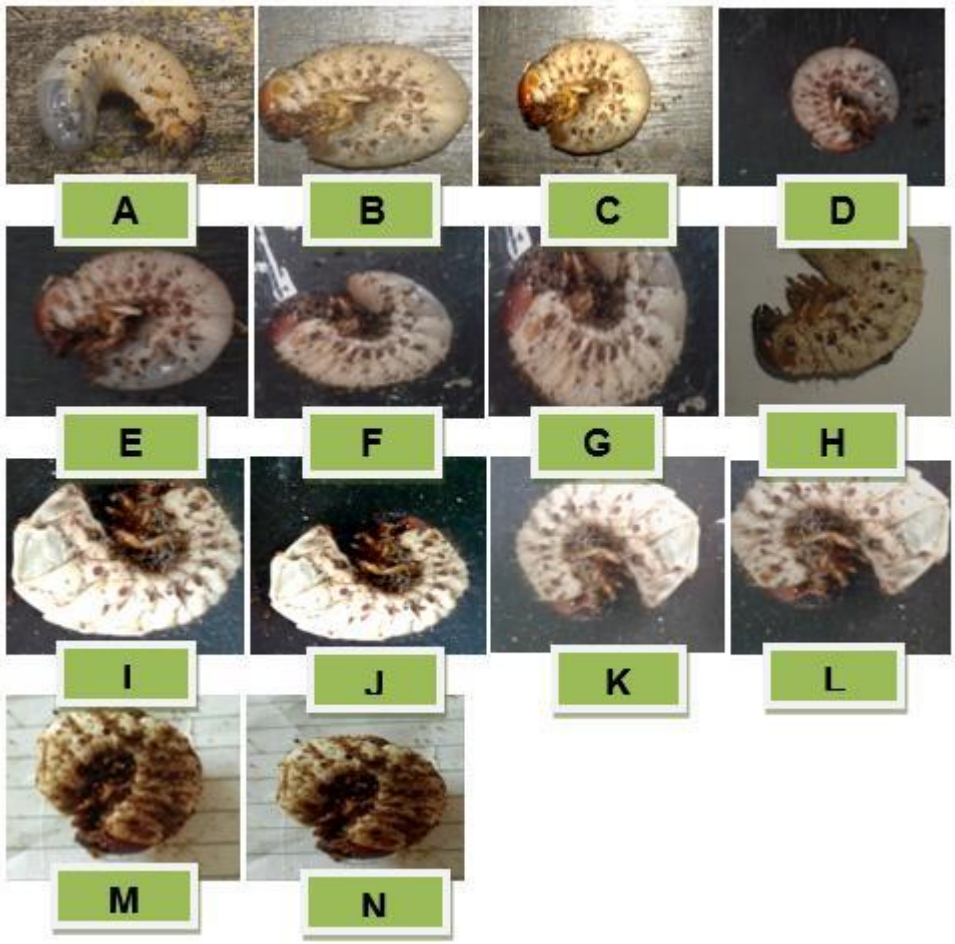

Fig.7: Symptoms of fungal infection of B. bassiana on Larvae of O. rhinocetos

A. Larvae attacked by the fungus *B. bassiana* 1 DAA
B. Larvae attacked by the fungus *B. bassiana* 2 DAA
C. Larvae attacked by the fungus *B. bassiana* 3 DAA
D. Larvae attacked by the fungus *B. bassiana* 4 DAA
E. Larvae attacked by the fungus *B. bassiana* 5 DAA
F. Larvae attacked by the fungus *B. bassiana* 6 DAA
G. Larvae attacked by the fungus *B. bassiana* 7 DAA

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H. Larvae attacked by the fungus *B. bassiana* 8 DAA
I. Larvae attacked by the fungus *B. bassiana* 9 DAA
A. Larvae attacked by the fungus *B. bassiana* 10 DAA
K. Larvae attacked by the fungus *B. bassiana* 11 DAA
L. Larvae attacked by the fungus *B. bassiana* 12 DAA
M. Larva attacked by the fungus *B. bassiana* 13 DAA
N. Larva attacked by the fungus *B. bassiana* 13 DAA

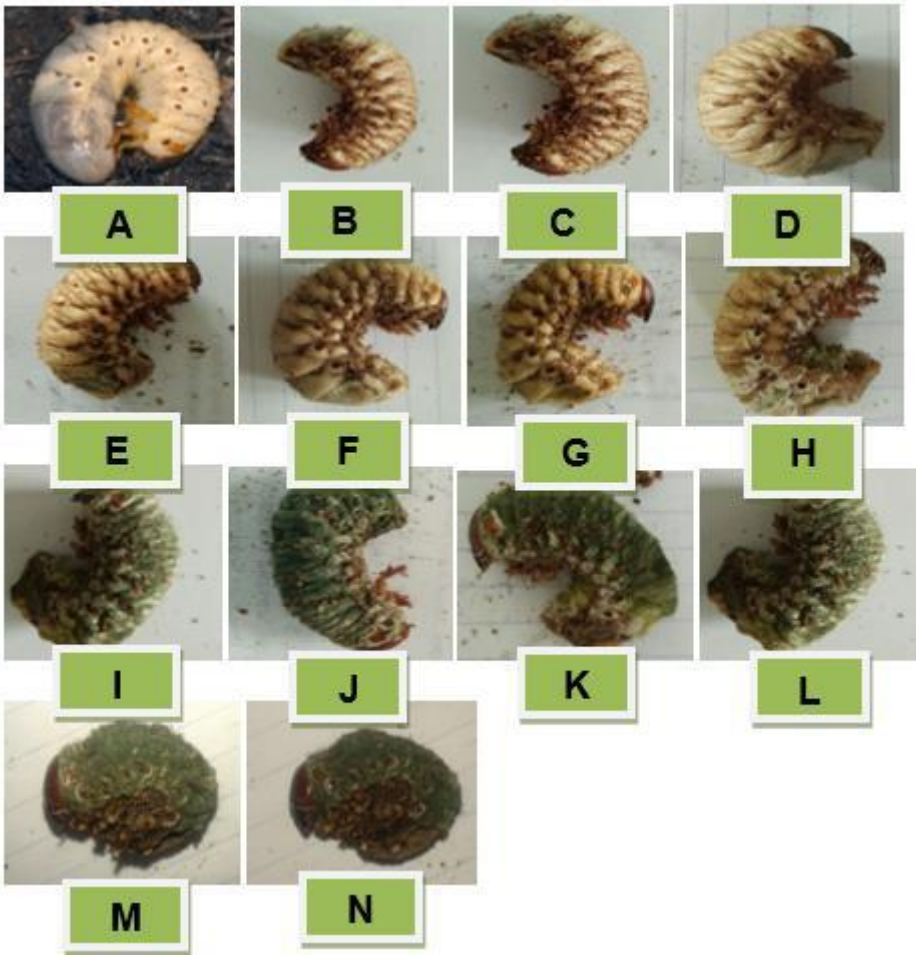

Fig.8: Symptoms of fungal infection of Trichoderma sp on Larvae O. rhinocetos

A. Larvae attacked by the fungus *Trichoderma* sp 1 DAA
B. Larvae attacked by the fungus *Trichoderma* sp 2 DAA
C. Larvae attacked by the fungus *Trichoderma* sp 3 DAA
D. Larvae attacked by the fungus *Trichoderma* sp 4 DAA
E. Larvae attacked by the fungus *Trichoderma* sp 5 DAA
F. Larvae attacked by the fungus *Trichoderma* sp 6 DAA
G. Larvae attacked by the fungus *Trichoderma* sp 7 DAA
H. Larvae attacked by the fungus *Trichoderma* sp 8 DAA
I. Larvae attacked by the fungus *Trichoderma* sp 9 DAA
A. Larvae attacked by the fungus *Trichoderma* sp 10 DAA
K. Larvae attacked by the fungus *Trichoderma* sp 11 DAA

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L. Larvae attacked by the fungus *Trichoderma* sp 12 DAA
M. Larva is attacked by the fungus *Trichoderma* sp 13 DAA
N. Larva is attacked by the fungus *Trichoderma* sp 13 DAA

From Table 3 it was found that in 10 DAA of *Trichoderma* sp and *B. bassiana* in T11 treatment was the highest mortality (86.67%) and the lowest was in control (20%). But the different T11 treatment was not significant with the treatments B21, B22, B31, B32, T12, T21, T22, T31, T32, B21, B22 and T11 differed markedly by control.

Table.3: Average number of mortality Pests Larvae O.rhinoceros (%) due to fungus Trichoderma sp and B. bassiana 10-14 Days After Application (DAA)

Treatments	Obsert=vation day to-					Mean
	10 DAA	11 DAA	12 HAS	13 DAA	14 DAA	
Control	20,00 bC	20,00 cC	20,00 cC	20,00 bB	20,00 bB	20,00
T_{11}	86,67 aA	86,67 aAB	90,00 aA	93,33 aA	96,67 aA	91,67
T_{12}	73,33 abAB	76,67 abAB	83,33 aAB	83,33 aA	96,67 aA	85,00
T_{21}	80,00 abA	90,00 aAB	90,00 aA	90,00 aA	93,33 aA	90,83
T_{22}	70,00 abAB	73,33 abAB	76,67 abAB	80,00 aA	90,00 aA	80,00
T_{31}	60,00 bAB	63,33 bAB	66,67 abB	76,67 aA	83,33 aA	72,50
T_{32}	60,00 abAB	73,33 abAB	76,67 abAB	80,00 aA	83,33 aA	78,33
B_{11}	73,33 abAB	80,00 abAB	83,33 aAB	83,33 aA	93,33 aA	85,00
B_{12}	76,67 abA	80,00 abAB	86,67 aAB	86,67 aA	90,00 aA	85,83
B_{21}	83,33 aA	86,67 aAB	90,00 aA	90,00 aA	93,33 aA	90,00
B_{22}	83,33 aA	90,00 aA	90,00 aA	90,00 aA	93,33 aA	90,83
B_{31}	80,00 abA	86,67 aAB	86,67 aAB	93,33 aA	93,33 aA	90,00
B_{32}	73,33 abAB	86,67 aAB	90,00 aA	93,33 aA	93,33 aA	90,83
Mean	70,77	76,41	79,23	81,54	86,15	80,83

Description: The numbers followed by the same letter in the same column show different results that are not real based on DMRT at f = 5% and very different 1%.

From observation 11 DAA showed that the highest mortality was in T21 and B22 (90%) treatment and the lowest mortality was in control (20%). This can be seen in Figures 9 and Figures 10 where T21 and B22 treatments differed not significantly on all treatments but differed significantly with controls.

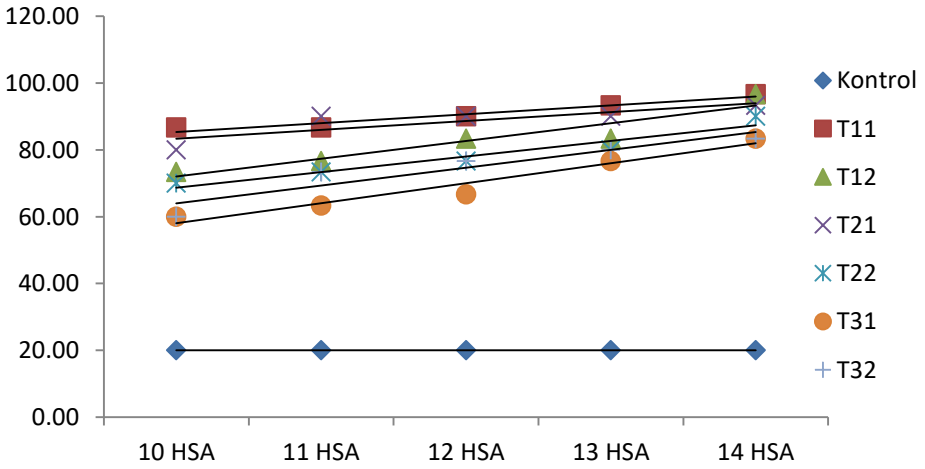

Fig.9: Percentage of Larvae Mortality O. rhinoceros After Application of Trichoderma sp 10-14 DAA.

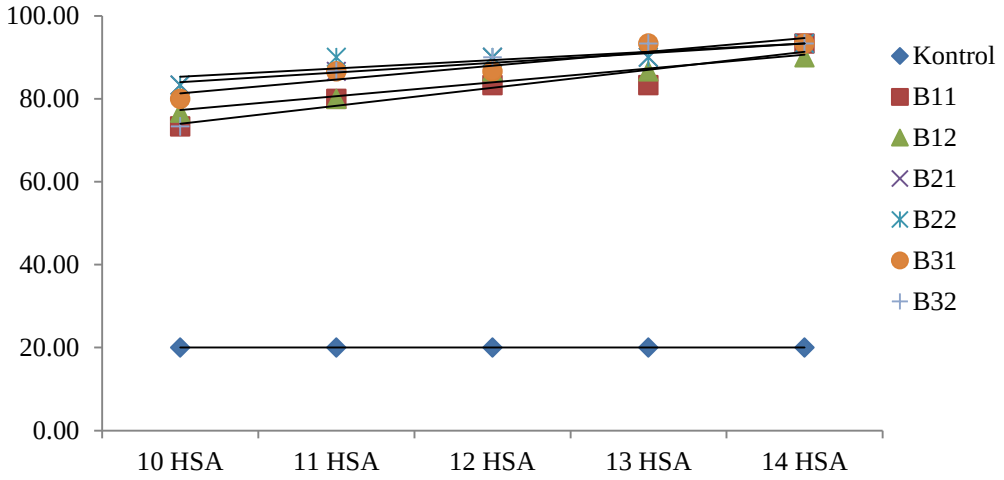

Fig.10: Percentage of Larvae Mortality O. rhinoceros after application of B. bassiana Fungus 10-14 DAA.

Growth of Conidia

Data from conidial density of entomopathogenic fungi Trichoderma sp and *B. basianna* can be seen in appendix 43-60. After the data were analyzed the density was known that the density of conidia from both entomopathogenic fungi showed a difference of Table 4.

Table.4: Conidial density of Trichoderma sp and B. basianna with various doses per ml of aqua pro injection

Types of fungus	Doses	Spore density		
		10^8	10^7	10^6
Trichoderma sp	20 gr x 100-^{1} ml	4,75 x10^6	5,32 x 10^6	5,85 x 10^6
	40 gr x 100 $^{-1}$ ml	5,60 x 10^6	6,22 x 10^6	6,45 x 10^6
	60 gr x 100 $^{-1}$ ml	7,25 x 10^6	6,89 x 10^6	5,25 x 10^6
B. bassiana	20 gr x 100-^{1} ml	5,52 x 10^6	4,38 x 10^6	4,22 x 10^6
	40 gr x 100 $^{-1}$ ml	5,95 x 10^6	5,12 x 10^6	4,68 x 10^6
	60 gr x 100 $^{-1}$ ml	6,33 x 10^6	5,20 x 10^6	4,85 x 10^6

The results of germination of *Trichoderma* sp and *B. basianna* fungus 4 hours after incubation period can be seen in appendix 61-66. After the data were analyzed, it was found that germination of conidia from both entomopathogenic fungi showed differences in Table 5.

Table.5: Conidia germination of Trichoderma sp and B. basianna with various doses per ml of aqua pro injection

Types of fungus	Total spore		
	20 gr x 100-1 ml	40 gr x 100 $^{-1}$ ml	60 gr x 100 $^{-1}$ ml
Trichoderma sp	76%	83%	91%
B. basianna	77%	80%	85%

IV. DISCUSSIONS

Percentage of Mortality

From Table 1 it can be seen that in observation 1 DAA has seen mortality in *O. rhinoceros* larvae, but the mortality is still relativelay low. This low mortality due to fungal conidia that enter and infect is still not maximal, because the fungi entering entomopathogen to infect the insects have a time span, otherwise the fungus of entomopathogen infects and deadly *O. rhinoceros* larvae is highly dependent on the amount of environmental conidia and nutrients that needed. As stated by [25,29] the ability of entomopathogenic fungi to kill larvae is highly dependent on environmental conditions, nutritional fit, pH, from where the fungus grows and develops.

From Table 2 on observations 6 DAA to 9 DAA mortality of *O. rhinoceros* larvae increased even though the different treatments were not real. But the mortality of larvae is still low, both due to the fungus *Trichoderma* sp and *B. bassiana* slow development of the fungus is due to the nutrients that the body is less available. This is in accordance with the stated by [15] states that the development of entomopathogenic fungi influenced the nutritional content of growing media used.

The Table 3 description of the occurrence of differences in mortality rates against *O. rhinoceros* larvae is caused by the number of conidia or concentration of conidia germination as well as different application methods. In this case the more the number of conidia that enter the body of the larvae and the germination of the high, then the death of the larvae will be faster. In addition, the number of conidia that enter the body of the larvae is also influenced by the method of application. Application with the spread method, then conidia that can enter into the body of the larvae will be more and more. Because the conidia that the application does not exist or very few are not on target. Whereas when the application with spray method the possibility of drift is large enough so that the conidia that concerns the body of the larvae is less. This is consistent with that stated by [32,34] also states that, sooner or later mortality in pests depends on the amount of dose used.

This indicates that the application of *Trichoderma* sp and *B. bassiana* fungi with the same dosage applied sprinkled differently is not real by spraying either at the beginning of the observation or until the end of the observation. This is due to conidia applied from both types of fungi the germination and the power of the infection are both high. So the mortality of larvae is also high, as stated by [3, 36, 36] that the higher dose conidia used in the treatment, the larval mortality is also high.

From observation 12 DAA showed that the highest mortality was in T11, T21, B21, B22 and B32 treatment (90%) and the lowest mortality was in control (20%). The treatments T11, T21, B21, B22 and B32 were not significantly different for all treatments but differed significantly with the controls. The high mortality of larvae other than influenced by the amount of conidia is also influenced by temperature/humidity. This is in accordance with the results of [32] study that the optimal temperature for *Trichoderma* sp growth ranges from 22°C-27°C. Meanwhile, according to [1] that germination, growth and optimal sporulation of *B. bassiana* fungi occur at temperatures 25-30°C.

From observation 13 DAA showed that the highest mortality was in T11, B31 and B32 treatment (93.33%) and the lowest mortality was in control (20%). Treatments of T11, B31 and B32 were not significantly different for all treatments but differed significantly with controls. This suggests that the density of conidia effectively controls the mortality of the *O. rhinoceros* larvae. At a higher level of conidia density, more and more mycelium and conidia grow. This is in accordance with [37] assertion that it increases the occurrence of conidial contact with the body of the larvae, thereby providing a better chance for conidia to stick, germinate and penetrate into the body of the larvae.

From the 14 DAA observations it was found that T11 and T12 (96.67%) and B11, B21, B22, B31 and B32 (93.33%) were the highest mortality and the lowest was in control (20%). However, the treatments of T11 and B11 differed significantly from the treatment of T12, T22, T22, T31, T32, B12, B21, B22, B31, B31, T11 and B11. This is because the longer the entomopathogenic fungi are in the body of the larvae, the amount of conidia increases. Damage to body tissue larvae due to fungus attacks will be more severe, so that the mortality rate of larvae will be higher. This is in line with the statement [19,38] the longer the contact time of entomopathogenic fungi and host, the more likely the entomopathogenic fungus to infect the host, resulting in an increase in larval mortality.

From the observation in Fig. 9-10 it is also found that infected entomopathogenic larvae indicate a change in which the larval body becomes hard, mummification and the appearance of fungal conidia around the conidia color tube will appear in accordance with the color of the infecting conidial fungus. This is in line with [37] statement that infected insects of entomopathogenic fungi undergo mummification and after a few days will grow a colony of white-colored fungus around the body. At the beginning of death the larvae have not shown any change. While on the third day the larvae have begun to experience a change in color, where conidia mushrooms have begun to grow on the side of the larvae. On the fifth day until the larval larvae infected the body the larvae are fully satisfied by the entomopathogenic fungal conidia.

Growth of Conidia

Conidia density

Table 4 shows that the highest conidial fungus, *Trichoderma* sp with dose of 60 gr x 100-1 ml of aqua pro injection, is 7.25 x 106 conidia / ml and the lowest conidial density is found in fungus *B. basiaana* at a dose of 20 gr x 100-1 ml aqua pro injection as much as 4.22 106 conidia/

ml. If seen from the number of doses can be concluded that, the higher the dose the number of conidia will also be higher.

Conidia germination

Table 5 shows that the number of conidia of *Trichoderma* sp fungi is higher than that of *B. basianna*. The conidia growth difference of each of these fungi may be caused by unequal nutrients. Each genus or fungal species requires nutrients, pH, water content in medium, optimal temperature, light, aeration, and different incubation periods for growth and development of conidia [24,25,42]. The percentage of conidial germination is also influenced by the amount of conidia and conidial germination. The higher the amount of conidia and higher germination, the faster the mortality of the larvae. As stated by [43] that the higher dose conidia used in the treatment, the more rapid the death.

V. CONCLUSIONS

From result of research can be concluded that:

1. Precentages mortality of the highest larvae of *O. rhinoceros* to *Trichoderma* sp fungus with a dose of 20 gr (96.67%) with the application method spread on the larvae.
2. The highest amount of conidial density is found in *Trichoderma* sp fungi with doses of 60 g x 100-1 ml of aqua pro injection of 7.25 x 106 conidia/ml.
3. Germination level of conidia mushroom highest in *Trichoderma* sp fungi with dose 60 gr x 100-1 ml aqua pro injection that is as much as 91%.

VI. SUGGESTIONS

To know the effectiveness of the fungus *Trichoderma* sp and B. bassianna need dilakuakn further research on application fungus of *Trichoderma* sp and *B. bassianna* directly to the field.

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Development of Biodegradable Board using Water Hyacinth (*Eichornia crassipes*)

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Abstract**—** *The aim of this study was to utilized aquatic weed to become a bio-board with proper mechanical properties. Water hyacinth (Eichornia crassipes) is aquatic weed with rapid growing rate that usually clog the irrigation facility and cover the river surface cause negative impact to environment usually water environment. In this research water hyacinth was used to produced bio-boards through cutting, soaking, refining, molding, and drying process with applied of five different loading pressures (2MPa, 3.5MPa, 5MPa, 6.5MPa, 8MPa) in applied of 110 degree Celsius. In the producing process of bio-board hydrogen bond among the cellulose fiber were used instead of used chemical substance and additional additive. Bio-boards were successfully produced under experimental condition. Mechanical properties of each bio-board were investigated. Result of water hyacinth bio-board density was 1.1691 g/cm· average bending rupture stress 46.21 MPa and tensile rupture stress in average value was 6.64MPa. Bio-board with certain different strength range could be considered to be applied as packaging, seedling pot, mulching or insulating material in advance application*

Keywords**— *agriculture waste, bio-board, utilization, water hyacinth.***

I. INTRODUCTION

About 6 million m^3 wood is used for the timber industry, including the production of pulp each year in Indonesia (Ministry of Environment Republic Indonesia, 2012). Wood has many uses in daily life; it is the main cellulose source for housing, furniture, packaging, paper, and other products. In 2007 about 66.2 percent of industrial forest plantation was planted for pulpwood plantation type. During the period 1993-2006, the pulpwood plantation sharply increased from 29,000 ha in 1989 to 200,000 in 2006 with average of 104,000 ha per year (FAO,2009). Those data indicated that wood demand for pulp industry continues to grow in Indonesia, however local forest production have been unable to meet the increasing demand.

Wood based product demand has been increasing nowadays. Wood based product such as, packaging, wall layer, seedling pots and others product which are daily consume product. The increasing demand for wood based products will impact on increasing the amount of logging, both industrial plantations and forests in general. Intensive use of wood can cause environmental problems such as deforestation, floods, and also global warming.

To overcome these problems, researchers have focused on finding others renewable resources to replace the use of wood for some uses. There is some non-wood which potential resources to be use as pulping material and other wood based product. Intensive use of wood as main cellulose source can be decreased by substituted by other cellulose source material such as biomass.

In this research, water hyacinth (*Eichornia crassipes*) the free-floating aquatic macrophyte growing generally to 0.5 meters that can grow and spread rapidly during rainy season. Water hyacinth is consider as weed (pest) and an unwanted plant. Due to its fast growth and rapid spread, water hyacinth has caused some problem such as the reduction of fish, wide coverage the river surfaces and canals, clogging irrigation facilities and water pollution. Attempts to control or remove the water hyacinth incur high cost and labor and the effect just temporary due to rapid growth.

Based on research conducted by Pasaribu and Swahlita (2007), known that moisture content of fresh water hyacinth is 94.25% with yield of pulp in dry condition of 3.6%. About $1m^2$ of area has 28 kg of fresh water hyacinth, mostly (84%) from those fresh water hyacinth is stem. Water hyacinth is low in lignin content (10%) and contains high amounts of cellulose (60%) and hemicellulose (33%). Water hyacinth currently used as craft and compost (Gunnarson and Petterson ,2007). Recently, bio- board that already developed made from bagasse (sugar cane waste), rice straw, wheat straw and corn straw successfully.

The aims of this research were to utilize water hyacinth to produce bio-board, analyze characteristic of bio-boards from three different biomasses. Physical characteristic such as density, moisture content, bending stress and tensile stress of bio-board were investigated. Producing bio-board with 5 different loading pressures will result in different mechanical properties of bio-boards.

Water hyacinth bio-board produce through a cutting, milling, soaking, refining, soaking pulp and molding

process with applied of five different loading pressures (2 MPa, 3.5MPa, 5MPa, 6.5MPa, and 8MPa) in applied at 110 degree Celsius. Bio-boards were successfully produced under the experimental conditions.

II. MATERIAL AND METHODS

2.1 Bioboard Producing Process

2.1.1 *Raw Material Preparation*

First, the water hyacinth was dried under sunshine for 3 days (8hours a day). Then, materials were cut (1 cm x 1 cm) then milled with a Toshiba miller to obtain smaller pieces before it was immersed under water for 168 hours.

2.1.2 Refining

After soaking in water for 168 hours, materials became soft due to water absorption into the cells. Materials were refined about 10 minutes with refining machine. The key process in this study was the refining. According to Lumaniaen (2010), refining will give several effects to fibers characteristics such as cutting and shortening of fibers, wall structure, delamination, internal fibrillation/ swelling, curling the fiber or straightening the fiber, and redistribution of hemicelluloses from the interior of the fiber to the exterior.

2.1.3 Molding Process

Refined materials were immersed again in water for 7 days to enhance the hydrogen bonding among the cellulose and among the fiber. Furthermore, the materials molded 20 minutes without heat with molding equipment in size 100 mm x 100 mm then molded 60 minutes at 110-degree Celsius. Producing process was showed in Fig 1.

2.1.4 Experimental Condition

In this study water hyacinth was produced with 5 different pressures (2Mpa, 3.5MPa, 5MPa, 6.5MPa, and 8 MPa). The bio-boards were made through 20 minutes without heat molding process, then molded for 60 minutes molding process at 110 ºc. In this study the parameters that investigated are bio-board density, bending stress. To determine bio-board's density, first bio-board thickness, dimension and weight was measured. Then, bio-board thickness, dimension, and weight data were calculated into mass (g) and volume (cm^3) data.

To investigate moisture content, right after test piece was broken by bending test, test piece was inserted into the test container then dried in oven in applied 105 ºc for 24 hours. The test piece was weighed both before and after drying process. Bending test and tensile test were obtained by use of Universal Testing Machine which is connected with sensor and PC to record data automatically during the test. To did bending stress test, 4 specimen tests of each bio-board are prepared at a dimension of specimen test is 50mm x 20mm. 3 specimen tests from each bioboard were prepared to conduct tensile test.

III. RESULT AND DISCUSSION

Five water hyacinth bio-boards were successfully produced under experimental conditions, shown in Fig.2.

3.1. Density

To determine a bio-board's density, first bio-board's thickness, dimension and weight were measured. Then, bio-board thickness, dimension, and weight data were processed into mass (g) and volume (cm3) data. Finally, using equation (1) bio-board density data was obtained and process into Figure 2.

$$\rho = \frac{m}{v} \qquad \text{Equation (1)}$$

Where ρ= density (g/cm^3), m= mass (g), V= volume (cm^3)

Water hyacinth bio-board densities range 1.057 g/cm^3 - 1.279 g/cm^3 which are classified as high-density board based on American National Standard for particle board (1999. It reached highest density at 5MPa pressure while the others achieve the highest density by 8MPa pressure.

Density of 2MPa and 3.5MPa bio-board showed not significant difference similar with density of 6.5MPa and 8MPa which also not significant difference. Uniformly in fiber size of water hyacinth affect the high density of bio-board. In fact, water hyacinth bio-board were the thinnest board among others, which is explain the tight connection among fibers inside water hyacinth bio-board. Water hyacinth bio-board density shown in Fig.3.

According to American National Standard on Particleboard (1999) High density board define with density above 800 kg/m3 (equal with 0.8 g/cm^3). Thus, water hyacinth bio-boards was considered as high-density board.

3.2. Bending stress

Bending strength testing conduct with Universal Testing Machine, (Shimaizu, Japan) connected with load cell sensor device (Kyowa, Japan) and PC to record data during testing. Measurement method set to bending test, calibration between load cell and PC was checked before conduct the test. Specimen was put on the two parts of UTM device and then force applied from upper side until yield point (over the elastic limit of material).

Bending test data was load (N) and the deflection (mm) that was calculated further with i.e (2). However, from Fig .4 it can be seen that the relation between deflections was proportional with the increase of load starting at 0 until approximately 3 mm and then gradually decrease after reach peak load. Since Bio-boards are made from biomass the distribution of the cellulose is not uniformly.

$$\sigma = \frac{3FL}{2bh^2} \qquad \text{Equation (2)}$$

Where *F* is the load (force) at the fracture point (N), *L* is the length of the support span (mm), *b* is width (mm), *h* is thickness (mm).

While the bending test took place, the displacement-load reached its peak at the same time the test piece was broken (permanent damage) and the peak load will represent the maximum load that can be hold by the test piece. Figure below implies at deflection 0 mm to 7 mm the stretching bond happened and when the curve gradually decreases it is implies the material unable to stretch anymore.

Water hyacinth bio-board curve (Fig. 5) show wave pattern when bending strength decrease from 51.75 MPa (2MPa bio-board) to 41.56 MPa (3.5 MPa bio-board) and then increase to 48.12 MPa (5MPa bio-board) slightly decrease to 47.64 MPa (6.5 MPa bio-board) and decrease again until 42 MPa (8MPa). Increasing loading pressure did not have significant effect to bending strength. The best loading pressure to produced high bending strength bio-board with water hyacinth as raw material is 2MPa.

Moisture content analysis for bending and tensile test conducted directly after specimen broken. Water hyacinth bio-board contain of 7 to 9.96 percent moisture content in bending test condition. the moisture content of specimen during the bending test did not give significant impact to the bending strength of material, however the biomass itself that make the different characteristic of material, in this case, bio-board bending strength. This evidence was contrast with Baharoglu *et al* (2012) research result, that state in some level, increasing moisture content was significantly decreased the mechanical strength properties in particleboard case.

3.3. Tensile stress

Tensile strength test was conducted to investigate the maximum force (tensile stress) that bio-board can withstand on before it broke (reach fracture point) and over the elasticity limit..After data recorded data was calculated by follow equation;

$$\tau = \frac{F}{A} \qquad \text{Equation (4)}$$

Where ; τ = tensile strength (MPa) , F = force (N), A = section area of specimen (mm^2)

Water hyacinth bio-board achieves the highest tensile strength 7.61 MPa by 6.5MPa bio-board and the lowest tensile strength is 5.73MPa by 2MPa bio-board (Fig 6) Water hyacinth tensile strength just obtains 10% of bending strength as shown in Fig 5. Water hyacinth specimen for tensile strength crack without fibrous edge, Moisture content analyses also conduct right after specimen broke. The aim of moisture content analyses on tensile condition is to observe the current condition when the specimen broken due to the tensile stress. In generally, moisture content on tensile condition is higher than moisture content on bending condition. The range of moisture content on tensile condition is 10.3 percent to 15.5 percent. The result show that tensile strength was lower than bending strength water hyacinth bio-board .

Based on Suboyejo (2004) the resistant of material to deformation strongly depend on the direction of orientation of the load. Since bio-board are made from biomass, which are the cellulose are bounded by hydrogen bound (Van der Wall's force). This is different from metal material that the atoms are bounded by ionic, covalent or metallic bound, which are stronger than hydrogen bounds.

Natural fibers consist of lignin, hemicelluloses, and cellulose. According to Mishra et al. (2004) the elementary unit of cellulose macromolecule is anhydro-D-glucose, which contains three alcohol hydroxyls (-OH). These hydroxyls from hydrogen bonds inside the macromolecules itself (intramolecular) and also intermolecular as with hydroxyl groups from the air.

IV. CONCLUSION

Water hyacinth bio-board densities range 1.057 g/cm^3 - 1.279 g/cm^3 which are classified as high-density board based on American National Standard for particle board (1999). water hyacinth bio board average bending rupture stress, tensile strength respectively were 46.21 MPa,. 6.64 MPa. For further research the effect of fiber length and distribution could be observed to obtain more data.

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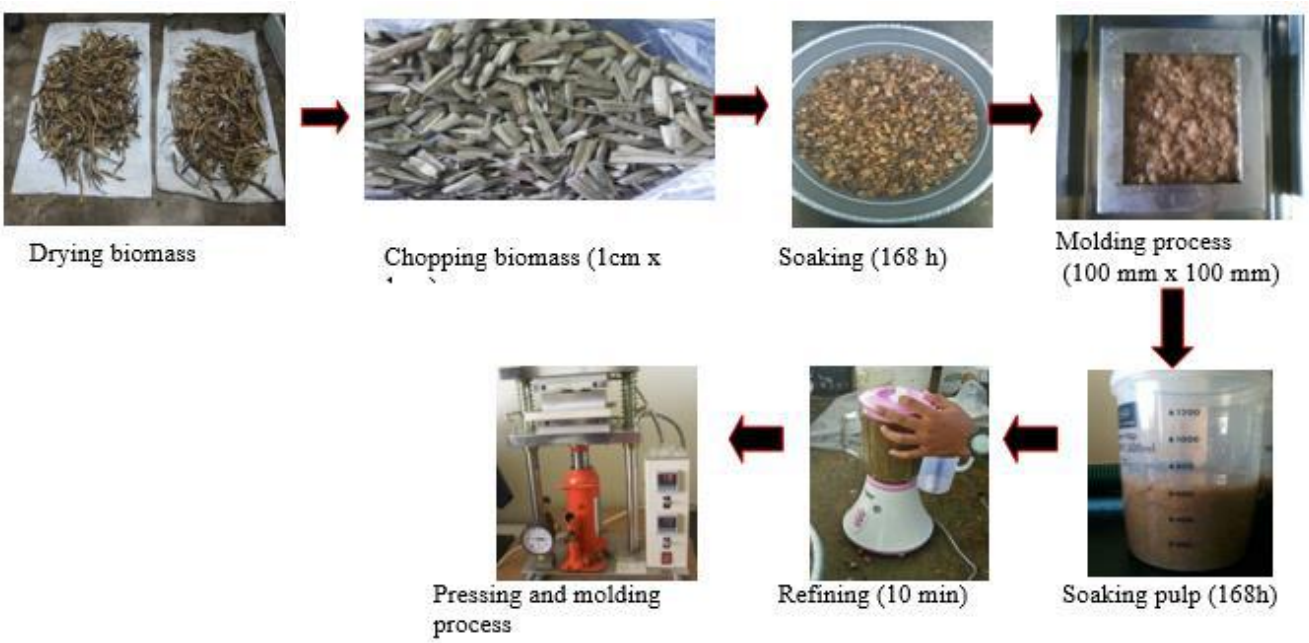

Fig 1: Biodegradabel board producing process

Fig 2. Water hyacinth bio-board successfully produce

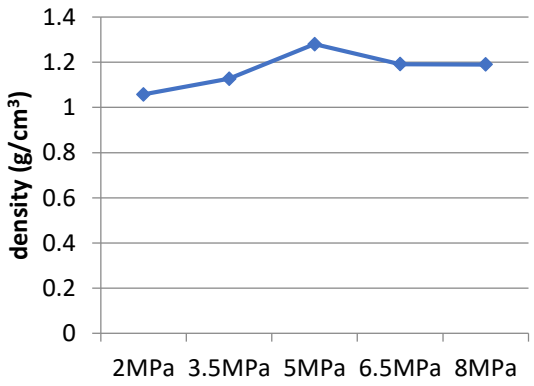

Fig 3. Water hyacinth density

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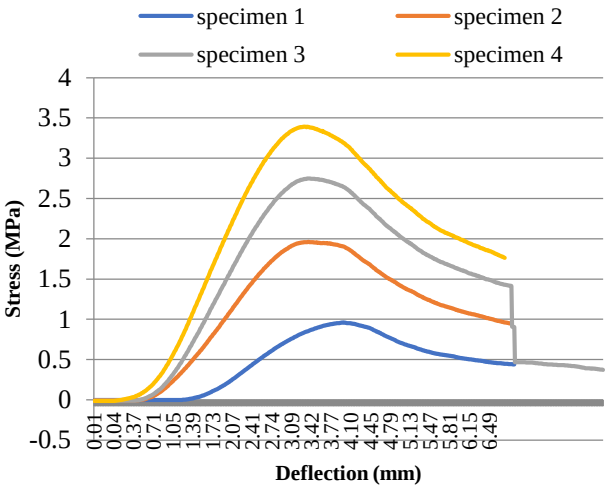

Fig 4. Stress-deflection graph on bending test

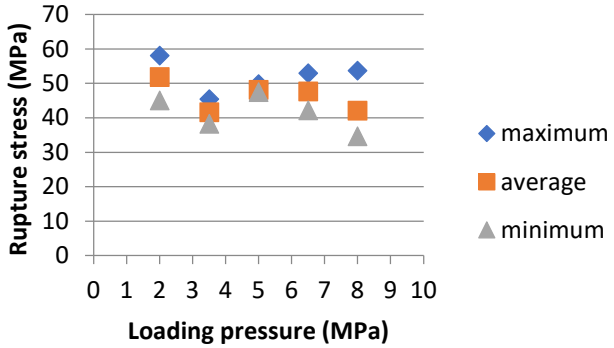

Fig 5. Water hyacinth bio-board rupture bending strength curve

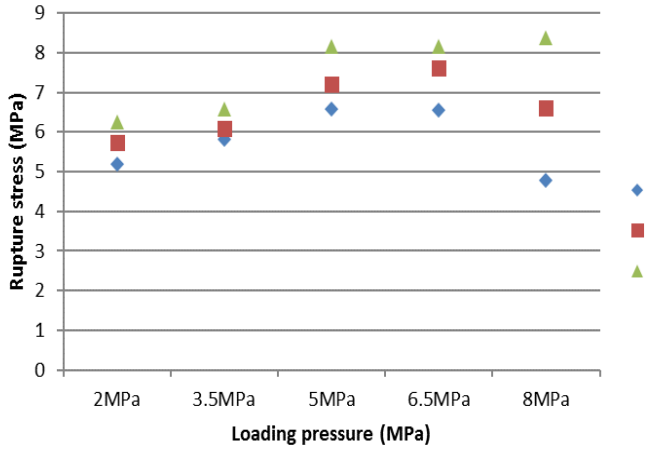

Fig 6. Water hyacinth bio-board tensile strength

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In vitro Propagation of *Adenia hondala* (Gaertn.) de Wilde

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Abstract— *Adenia hondala (Gaertn.) de Wilde belonging to the family Passifloraceae is a perennial climbing herb with potential medicinal value. The possibility of in vitro clonal propagation of Adenia hondala was investigated by the use of nodal explants cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of BAP and KN. Optimum treatment was the combination of 1 mg.L^{-1} BAP and 0.5 mg.L^{-1} KN that enhanced percent response of explants and the number of multiple shoots per explants. An average of 10.23 shoots per explants was resulted after 32 days of culture. In vitro shoots were elongated in MS medium supplemented with 1 mg.L^{-1} KN. Half strength MS medium supplemented with 1 mg.L^{-1} IBA was found to be the best medium for rooting. The rooted plantlets were gradually acclimated ex vitro in mist chamber and successfully established under field conditions with high survival rate.*

Keywords— Adenia hondala, in vitro, multiple shoots, nodal segments.

I. INTRODUCTION

Adenia hondala belonging to the family Passifloraceae is found in the forests of Western Ghats. It has been red listed as vulnerable in South India. *A. hondala* is a perennial climber with tuberous roots, simple tendrils, simple and lobed leaves and circular glands between lobes of leaves. The monoecious flowers have oblong petals, 5 stamens, globular ovary and a trifid stigma. The tuber powder is used to treat cough and it increase lactation in nursing mother. The extract of tuber is used to cure intermittent fever, Anonymous, (1999) and the roots are used for the treatment of skin troubles, Anonymous, (2003).

Ayurveda is a system of Medicine with historical roots in Indian Subcontinent. '*Vidari*', an Ayurvedic drug is an ingredient of more than 50 Ayurvedic formulations like Chyavanaprash and its annual requirement is about 500–1000 Metric Tonnes, Sulaiman *et al*., (2014). Ayurveda correlates '*vidari*' to tubers of *Pueraria tuberosa* (Roxb. Ex Willd.) DC (Fabaceae) and *Kshiravidari* to *Ipomoea mauritiana* Jacq. (Convolvulaceae). However, in Ayurvedic Pharmacopoeia both these species are attributed similar properties and are substituted by each other, Venkatasubramanian *et al*., (2009). Apart from these, tubers of *Adenia hondala* (Passifloraceae) and the pith of *Cycas circinalis* L. (Cycadaceae) are also traded as '*vidari*' ,Ved and Goraya, (2008). '*Vidari*' is used as aphrodisiac, cardiotonic, diuretic and refrigerant, Chopra *et al*., (1992). In traditional and folklore systems, *'vidari'* has been used as a tonic, rejuvenator and galactogogue, Mithila *et al*., (2014). It is reported that use of *'vidari*' is beneficial in persons suffering from or prone to diabetes and coronary disease problems, Sulaiman *et al*., (2014).

Indiscriminate collection, poor seed set and seed germination resulted in the disappearance of this plant from wild habitats. The reason for the diminishing number of *A. hondala* could be the dwindling forests due to excessive urbanization. Having established its potential as a medicinal plant there is a great necessity for large scale multiplication of this plant which is rapid, simple and genetically stable. *In vitro* protocols serve as a viable tool for conservation and propagation of germplasm, especially of endangered and threatened plants. There is a single report on *in vitro* propagation of this plant species (*A.hondala*) which focuses on *in vitro* organogenesis and somatic embryogenesis. The present experiment was conducted to develop a successful protocol for rapid clonal propagation of *A.hondala* through the culture of nodal explants.

II. MATERIALS AND METHODS

2.1 Plant sample and experiment design

Stem cuttings with four to six nodes were collected from three months old *A. hondala* plant. The stem was cut into single node pieces (2 to 3 cm length) and was washed in running tap water for 10 minutes. The nodal explants were then immersed in Bavistin solution (15 g.L^{-1}) for 3 minutes with Cefotaxime (200 mg.L^{-1}) and Tetracycline (200 mg.L^{-1}). After distilled water wash, the explants were sterilized in 1% mercuric chloride solution for three minutes followed by several rinses with double distilled water. The pH of the medium was adjusted to 5.8 before autoclaving at 15 psi

pressure and 121°C temperature for 15 minutes. All these culture vials were incubated in plant growth room at 25±2°C under 16/8 hour photoperiod with 50 µ mol m^{-2} s^{-1} light intensity supplied by cool white fluorescent lamps and 60±65% relative humidity. The nodal explants were then inoculated into MS media (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. The shoot bud initiation and multiple shoot induction were studied by inoculating the explants on MS media supplemented with 6- Benzylaminopurine -BAP (0.25, 0.5, 1.0, 2.0 $mg.L^{-1}$) either alone or in combination with Kinetin-KN (0.25, 0.5, 1.0, 2.0 $mg.L^{-1}$). Subcultures were carried out at an interval of 14 days. The proliferation rate for each of the treatment was observed. Same media compositions were used to study shoot elongation. During elongation, the length of shoot and the number of nodes were observed and recorded. Half strength and full strength MS media with (0.25, 0.5, 1.0 $mg.L^{-1}$) or without Indole Butyric Acid- IBA were experimented for *in vitro* rooting. Percentage of root induction, root length and root number were the parameters recorded for each treatment. The rooted shoots were taken out from the culture bottles and washed thoroughly with running tap water to remove traces of medium. These plants were transferred to plastic pots containing a mixture of soil and sand. Almost all rooted plants were acclimated and transferred to field conditions.

2.2 Statistical analysis

All experiments were performed with three replications, having 30 samples each. The effect of various treatments on selected growth parameters was measured quantitatively and statistically tested using analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) 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

3.1 Effect of BAP and KN on multiple shoot induction and shoot proliferation from nodal explants

Cultures without any growth regulators were taken as control. No shoot induction was observed in control media. The effect of cytokinis on multiple shoot induction was experimented by culturing the nodal explants on MS medium supplemented with BAP and KN either alone or in different combinations. It was observed that, for the same concentration of BAP and KN tested (0.25, 0.5, 1.0, 2.0 $mg.L^{-1}$), percentage of explants showing shoot induction decreased (Table 1) on KN supplemented (75- 78%) media than the media with BAP (81- 83%). Among various concentrations and combinations, best results were recorded on medium containing 1 $mg.L^{-1}$BAP and 0.5 $mg.L^{-1}$ KN noticed 87% of explants proliferated within 7 days (Table 1).This particular combination of growth regulators regenerated 10.23±0.40 shoots, (Fig 1) which was found to be the single optimum treatment that promoted highest number of multiple shoots within 32days. MS medium with different combinations of BAP and KN favoured comparatively high multiple shoot regeneration than the treatments with BAP or KN alone. Combinations of BAP (1.0 $mg.L^{-1}$) with KN (0.25, 0.5 $mg.L^{-1}$) significantly increased the shoot number as compared to other treatments with BAP and KN alone (Table 2). In the present study, increased concentrations of BAP (2 $mg.L^{-1}$) adversely affected the shoot multiplication rate.

3.2 Effect of KN on shoot elongation

Same media composition as that of the shoot induction media were experimented for shoot elongation. Shoots obtained from KN supplemented media were significantly longer among all treatments including their combinations (Table 2). Among all the concentrations and combinations of growth regulators used, MS with 1.0 $mg.L^{-1}$ KN showed better result for shoot elongation after 12 days of sub culturing with an average length of 4.87± 0.15cm and an optimum number of 4.10 ± 0.40 nodes per explants. In the present study, length of the shoots were shorter in BAP as compared to KN either alone or in their combination (Table 3). Shoots of KN supplemented media were significantly longer among all cytokinin treatment. The presence of KN in the medium allowed the *in vitro* shoots to elongate where the morphogenetic response was lower in lower concentrations (0.25, 0. 5$mg.L^{-1}$) as compared to higher concentration (1.0 $mg.L^{-1}$). Moreover higher concentrations of BAP (2 $mg.L^{-1}$) inhibited the shoot length compared to its lower concentrations (0.25, 0.5, 1.0 $mg.L^{-1}$) with or without KN.

3.3 Effect of Media and IBA on rooting

To induce rooting, *in vitro* shoots were transferred to full strength and half strength MS media with (0.25, 0.5, 0.1 $mg.L^{-1}$) or without IBA. The effects of media and IBA treatment on root formation from in vitro shoots were summarized on Table 4. The inclusion of IBA in the rooting media increased the root number. It was observed that half strength MS supplemented with 1.0 $mg.L^{-1}$ IBA showed highest percentage of root induction (83.00±0.50). Half strength MS media supplemented with IBA (1.0 $mg.L^{-1}$) was optimum in inducing an average number of roots (3.83 ± 0.35) reaching up to length 4.43 ± 0.15 cm within two

weeks of culture. As compared to half strength MS, full strength MS supplemented with IBA resulted in a significant decrease in the percentage of root induction, number of roots and length of roots.

Table.1: Effect of BAP and KN on multiple shoot induction using nodal explants

Treatments	MS + Growth regulators		% of explants showing shoot induction	Number of days for shoot induction
	BAP (mg.L^{-1})	KN (mg.L^{-1})		
T_0	0	0	00.000^{i}	00.000^{g}
T_1	0.25	0	83.36±0.41^{c}	8.20±0.26^{d}
T_2	0.5	0	82.33±0.47^{d}	8.43±0.20^{d}
T_3	1	0	81.26±0.20^{e}	8.90±0.10^{c}
T_4	1	0.25	85.73±0.61^{b}	7.67±0.20^{e}
T_5	1	0.5	87.05±0.10^{a}	6.90±0.26^{f}
T_6	2	0	80.57±0.14^{f}	8.86±0.05^{c}
T_7	0	0.25	78.37±0.40^{g}	9.26±0.24^{b}
T_8	0	0.5	77.73±0.25^{g}	9.46±0.05^{ab}
T_9	0	1.0	75.90±0.62^{h}	9.77±0.01^{a}
T_{10}	0	2.0	75.50±0.34^{h}	9.76±0.14^{a}

Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly (P>0.05)

Table.2: Effect of BAP and KN on shoot proliferation after 32 days of culture.

Treatments	MS + Growth regulators		Number of multiple shoots per explant
	BAP (mg.L^{-1})	KN (mg.L^{-1})	
T_0	0	0	00.000^{g}
T_1	0.25	0	3.50±0.30^{e}
T_2	0.5	0	3.97±0.21^{de}
T_3	1	0	4.67±0.25^{c}
T_4	1	0.25	5.93±0.30^{b}
T_5	1	0.5	10.23±0.40^{a}
T_6	2	0	3.40±0.36^{e}
T_7	0	0.25	2.40±0.40^{f}
T_8	0	0.5	3.87±0.11^{de}
T_9	0	1.0	4.23±0.49^{cd}
T_{10}	0	2.0	2.17± 0.50^{f}

Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly (P>0.05)

Table.3: Effect of KN on shoot elongation after 12 days of culture

Treatments	MS + Growth regulators		Shoot length(cm)	Number of nodes
	BAP (mg.L^{-1})	KN(mg.L^{-1})		
T_0	0	0	00.000^{g}	00.000^{d}
T_1	0.25	0	2.50±0.10^{e}	2.20±0.30^{b}
T_2	0.5	0	2.53±0.37^{e}	2.30±0.46^{b}
T_3	1	0	2.80±0.20^{de}	2.23±0.51^{b}
T_4	1	0.25	3.03±0.35^{d}	2.80±0.40^{b}
T_5	1	0.5	3.53±0.40^{bc}	2.50±0.40^{b}
T_6	2	0	2.06±0.15^{f}	1.20±0.10^{c}
T_7	0	0.25	3.23±0.25^{cd}	2.77±0.25^{b}
T_8	0	0.5	3.87±0.06^{b}	3.50±0.36^{a}

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Treatments	MS + Growth regulators		Shoot length(cm)	Number of nodes
	BAP ($mg.L^{-1}$)	KN($mg.L^{-1}$)		
T_9	0	1.0	4.87 ± 0.15^{a}	4.10 ± 0.40^{a}
T_{10}	0	2.0	3.06 ± 0.20^{d}	3.63 ± 0.31^{a}

Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly (P>0.05)

Table.4: Effect of media and IBA on in vitro rooting.

Treatments	Media strength	IBA ($mg.L^{-1}$)	% of root induction	Root number	Root length **(cm)**
T_0	Half strength MS	0	51.03 ± 0.47^{d}	1.27 ± 0.35^{d}	0.90 ± 0.26^{d}
T_1		0.25	66.00 ± 1.00^{c}	1.80 ± 0.36^{c}	1.83 ± 0.42^{c}
T_2		0.5	71.80 ± 0.80^{b}	2.80 ± 0.10^{b}	2.53 ± 0.29^{b}
T_3		1	83.00 ± 0.50^{a}	3.83 ± 0.35^{a}	4.43 ± 0.15^{a}
T_0	Full strength MS	0	40.5 ± 0.50^{d}	0.83 ± 0.35^{c}	0.90 ± 0.40^{b}
T_1		0.25	51.63 ± 0.20^{c}	0.93 ± 0.41^{c}	0.83 ± 0.20^{b}
T_2		0.5	61.00 ± 1.00^{b}	1.63 ± 0.05^{b}	1.53 ± 0.25^{a}
T_3		1	65.9 ± 0.65^{a}	2.40 ± 0.10^{a}	1.93 ± 0.37^{a}

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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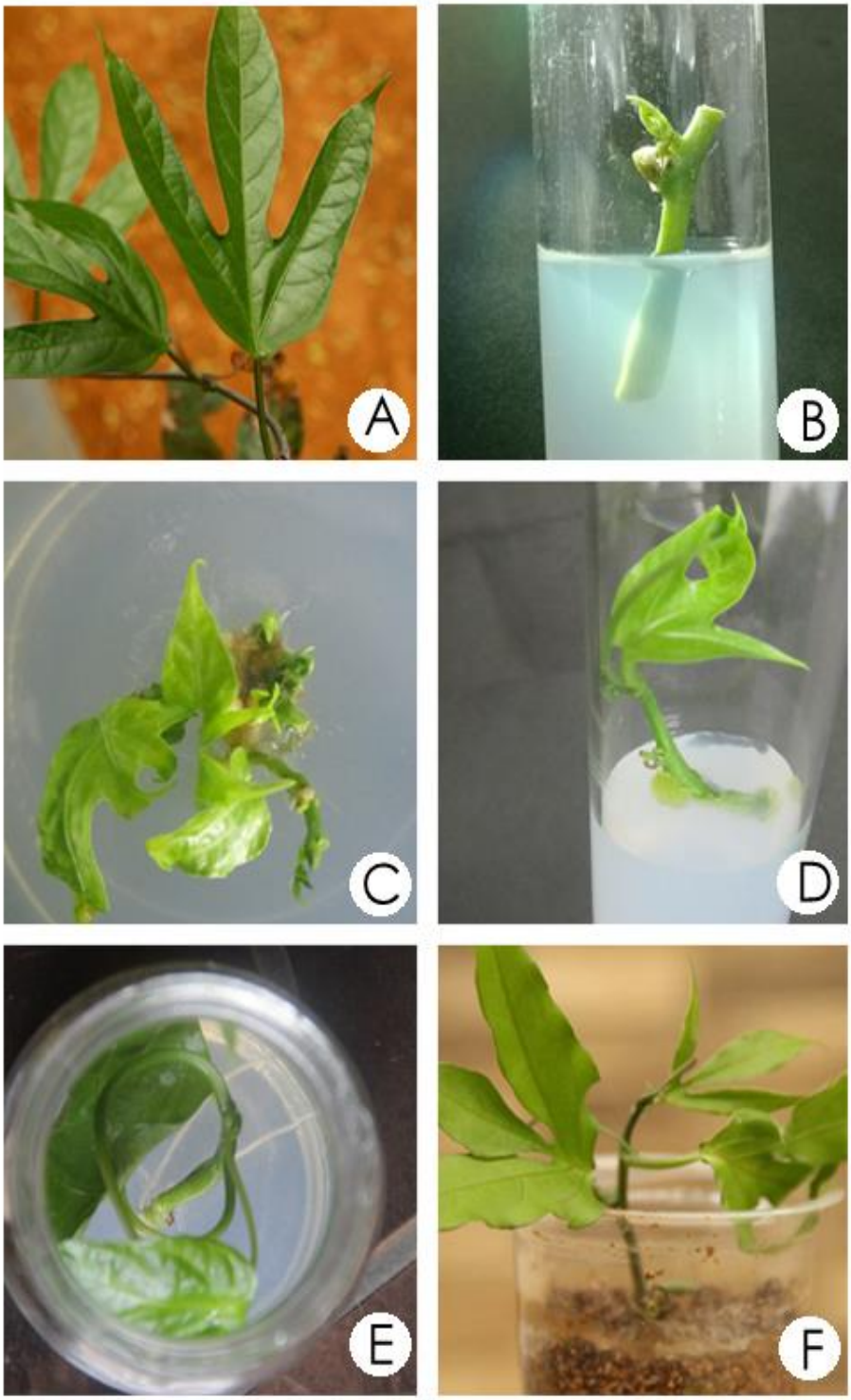

Figure.1.Micropropagation of *Adenia hondala*.A, Habit of *Adenia hondala*.: B, Shoot bud initiation from nodal segments in MS medium supplemented with BAP 1 mg.L-1 + KN 0.5 mg.L-1: C, Mul tiple shoot induction from nodal segments in MS supplemented with BAP 1 mg.L-1 + KN 0.5 mg.L-1: D, Shoot elongation in MS supplemented with KN 1 mg.L-1: E, Rooting in half MS basal media with 1 mg.L-1 IBA: F, Hardened *in vitro* regenerated plant of *Adenia hondala* after acclimatisation

IV. DISCUSSION

Cytokinins have major role on plant development, such as the regulation of shoot induction, shoot multiplication and promotion of cell division, Mok and Mok, (2001). In the present study various concentrations of BAP and KN induced significant differences on shoot induction percentage, number of shoots per explants and shoot length. It was observed that the percentage of shoot emergence per explants increased on media with decreased concentrations of cytokinin. These findings are in line with the findings of Reddy and Saritha, (2013). The synergistic effect of BAP and KN produced high rate of shoot multiplication. The results are in accordance with Mehdi *et al*., (2014); Shirin and Rana, (2007) and Saha *et al*., (2007). BAP enhanced *in vitro* shoot induction and proliferation of many medicinal plant species (Lakshimi and Mythili, 2003) which is in conformity with the results of the present study. BAP is more effective on shoot induction and multiplication and less effective on shoot elongation as compared to KN. These results are in conformity with those of Mehdi *et al*., (2014) and Sharma *et al*., (1993). KN treatment significantly affected on increasing the shoot length and number of nodes as compared to any other concentration or combination of BAP either alone or with KN. The stimulatory effect of KN on shoot elongation was reported by Saha *et al*., (2007). In the present experiment, optimum response for rooting resulted on half strength MS media with 1 $mg.L^{-1}$ IBA than full strength MS media with same hormone concentrations. It has been reported, Zayona *et al.*, (2014) half MS supplemented with auxin enhanced the number of rooted plants in micropropagation of *Paulownia elongata*. The number and length of roots was found to be increased on half strength MS with or without IBA than full strength MS. These results are in accordance with Shekhawat *et al*., (2015) and Lattoo *et al*., (2006).

V. CONCLUSION

In vitro propagation through nodal explants of *A.hondala* is an easy and economic way for obtaining large number of consistently uniform plants. Present protocol holds tremendous potential to select, multiply and conserve this genotypes, which are a potential resource of medicinally important constituents and it reduce the dependence on the natural habitat for the supply of raw drugs.

VI. ACKNOWLEDGEMENTS

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Adaption of Wheat Genotypes to Drought Stress

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***Abstract*—** *Drought can serve to restrict the growth and development of wheat. The current research was conducted to screen for drought-tolerant wheat genotypes through phenotypic markers, including growth indicators and yield. We used a Randomized Complete Block (RCB) design with three replicate sites (about333 m^2 area per replicate). Six wheat genotypes which are frequently grown under rain-fed conditions at the southern highland of West-Bank, Palestine were evaluated for specific phenotypes including stem length, spike with awns length, awns length, number of tillers, total grain, total hay, and mass of seeds (per 100).*

The results showed significant variations among the six wheat genotypes for most of the measured parameters. Yellow-Hetia genotype showed the highest stem length, spike with awns length, awns length, weight of 100 seeds, and yield (grain plus hay). However, the remaining genotypes presented almost similar production ranging from 475-488 kg/dunum. In contrary, Nab-El-Jamal genotype exhibited the lowest grain production and Um-El-Rabee' genotype revealed the minimum hay production. Based on our data, Yellow-Hetia could be a promising cultivar for future breeding programs, especially those involving drought tolerance.

***Keywords*— Wheat genotypes, drought stress, adaptation.**

I. INTRODUCTION

Cereal crops have supplied the means for sustaining human life as direct human food sources as well as indirect sources through livestock and poultry feed. They provide around three-quarters of human caloric intake and are grown on more than two-third of the arable land area (Ahmad *et al*., 1989). FAO's forecast for global cereal production in 2017 is about 2 627 million tonnes comparing with 2 534 million tonnes in last year's production (FAO, 2016).

Wheat (*Triticum aestivum* L., the most important cereal crop)is currently forecast at 754.8 million tonnes for 2017/2018 at 1% lower than the previous year's production (FAO, 2017).This crop is considered as the most widely cultivated plant, as well as the first important and strategic crop for the majority of the world's population.

In West Bank-Palestine, cereals occupy the largest cultivated land after fruit trees with total areas of 33,470 hectares, in which wheat contributes the majority of the total field crops in terms of area covered and total production. More preciously, wheat covers an area of 13,270 hectares, in which 99% of its cultivation is under rain-fed condition with a very low average production of 1,970 Kg per hectare (PCBS, 2013).

In fact, its low productivity might be attributed to a number of reasons including deterioration of the seed cultivars, unsuitable agricultural practices, lack of extension services, and different biotic and abiotic stresses (Basheer-Salimia and Atawne, 2014). Toward this end, drought alone seems to be the most devastating stress since it decreases the overall crop yield more than any other type of plant stress (Ahmad *et al*., 2015), especially during the last two to three decades, mainly as a direct result of global climate change (Basheer-Salimia and Ward, 2014; Basheer-Salimia and Sayara, 2017).Furthermore, water stress tends to impair plant growth and development and reduces optimal productivity (Farooq *et al*., 2011; Ahmadizadeh *et al*., 2013).

Here, detectable climate change has been observed as lower average precipitation rate, more marked changes in the distribution of precipitation from one year to the next, with winter getting shorter and extensive. This situation has produced major fluctuations in wheat yield over the past several years and this variability is expected to get more pronounced in the future. Moreover, wheat is grown in a wide range of geographical and climatical conditions, therefore it is crucial to assess and evaluate the adaptability and suitability of the existing wheat genotypes for each location especially under harsh drought conditions.

II. MATERIALS AND METHODS

Plant Materials, Experimental Sites, Design and Plantation: This experiment was carried out using six genotypes of wheat, namely White-Hetia, Yellow-Hetia, Nab-El-Jamal, Um-Ar-Rabee', Anber, and Sury. Seeds were grown at Al-Beera area (southern part of Al-Dahriya city),

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under rain-fed conditions located at the southern highland of West-Bank, Palestine (Figure 1). The targeted area (elevation: 595 m; latitude: 31° 26' 5" North; longitude: 34° 56' 3" East) is characterized by low average rainfall and humidity at 390 mm and 59%, respectively.

The experiment was laid out in a randomized completely block (RCB) design, with three replicates using the net plot size of 333 area (about 1/3 dunum, 22 m * 15 m) per replicate. To isolate the plots as well as to facilitate the follow-up process (cultural practices, measurements, etc), one meter corridors around all the plots were used. Adoption rate of 5 kg gram of seeds/wheat genotype/plot (equivalent to 15 kg/dunum) were manually sown.

Measured and Evaluated Parameters: Maturation and harvesting date were determined when the moisture content of the seeds reaches 15%. Stem length (from the stem base up-to the stem apex); spike with awns length (from the base of the spike to the apex of the awns); awns length (from the base of the awns to the apex of the awns) and tillering (using randomly one square meter frame-quadrate per plot, and then number of fertile tillers for all plant occurring within each quadrate was recorded); were registered. All data was built on a randomly selecting ten representative plants per replicate.

Concerning yield parameters, total yield (grain plus hay), total grain, and total hay production were also recorded in kilograms/plot/genotype, and accordingly calculated per dunum (1000 m^2). In addition, weight of 100 seeds per genotype was also measured

Data Analysis: The data were statistically analyzed using one-way analysis of variance (ANOVA) and means were separated using the Tukey's pairwise comparisons at a significance level of $P \leq 0.05$ using the MINITAB package system.

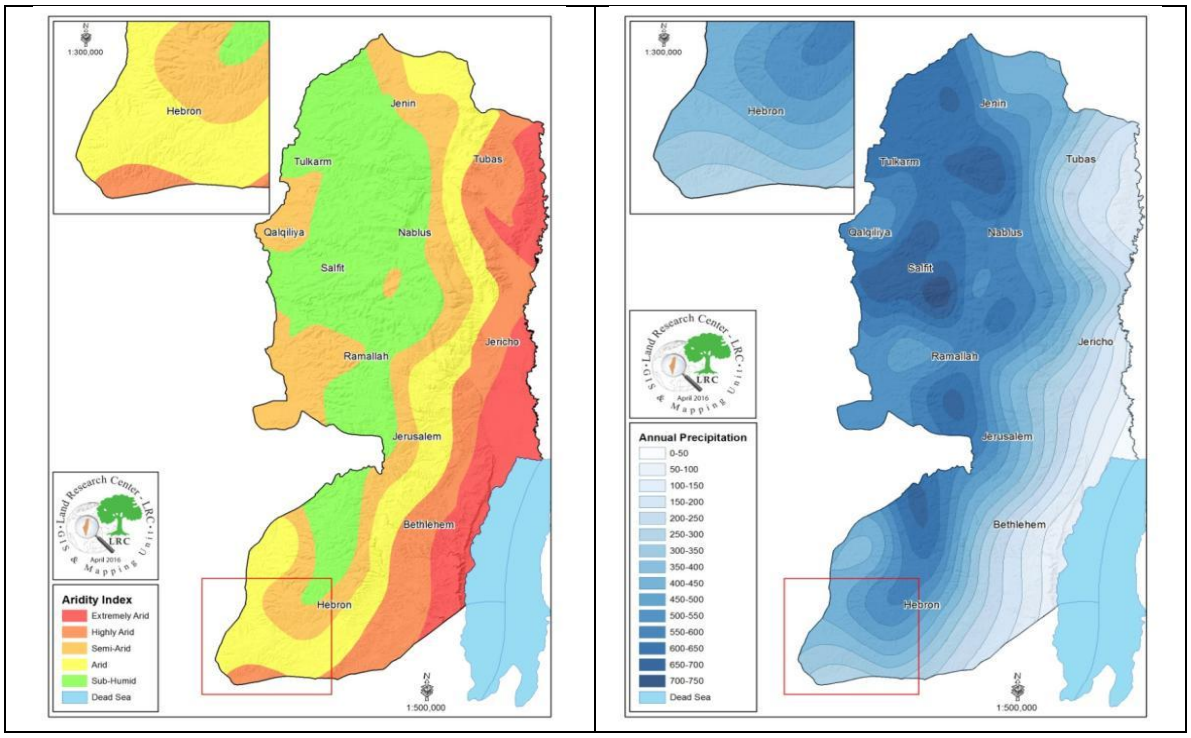

Fig.1: Maps showed the aridity index of targeted study site (the left) and the average annual precipitation (the wright).

III. RESULTS

Morphological parameters: As shown in table (1), significant variation in stem length between the six examined wheat genotypes was observed. Yellow-Hetia genotype presented significant higher stem length, whereas Suri and Anber genotypes exhibited the lowest values, respectively. Meanwhile, the other wheat genotypes revealed almost a moderate stem length.

Concerning the spikes length (spikes and awns) and the awns length, Yellow-Hetia genotype revealed the highest values (20 and 15 cm respectively), whereas White-Hetia presented the lowest ones. The remaining four genotypes were moderate in these two examined variables.

Furthermore, tiller variable was high in Anber genotype and low in White-Hetia.

Table.1: Some morphological parameters of different wheat genotypes grown under drought condition. (Mean± StDev).*

Genotype	Stem Length (cm)	Spike Length with awns (cm)	Awns Length (cm)	Tillers Number
White-Hetia	84.21^{b}±14.03	12.74^{c}±2.08	10.22^{d}±1.73	2.53^{c}±1.35
Yellow-Hetia	102.43^{a}±13.18	19.77^{a}±2.96	15.33^{a}±2.27	4.27^{b}±1.78
Nab-El-Jamal	83.46^{b}±15.75	16.99^{b}±1.49	12.03^{c}±1.15	4.80^{b}±1.63
Um-El-Rabee'	72.94^{c}±10.78	16.90b±0.97	12.29^{bc}±0.96	5.00^{b}±2.10
Anber	64.92^{d}±9.26	18.48^{ab}±2.00	13.43^{b}±2.59	6.33^{a}±2.38
Suri	61.62^{d}±6.72	17.75^{b}±1.34	12.29^{bc}±1.68	4.53^{b}±1.20
P Value	0.000	0.000	0.000	0.000

*: Means within column using different letters are differ significantly at the p≤0.05 level (using one way ANOVA analysis).

Yield parameters: The Yellow-Hetia genotype had the highest significant total yield production (700 kg/dunum), specifically 263 kg/dunum as grain and 436 kg/dunum as hay. However, the remaining genotypes presented almost similar production ranging from 475-488 kg/dunum (Table 2). In contrary, Nab-El-Jamal genotype exhibited the lowest grain production and Um-El-Rabee' genotype revealed the lowest hay production.

Furthermore, significant variations in the weight of 100 seeds of wheat among the six genotypes were observed (Table 2). Indeed, Yellow-Hetia genotype presented the highest average seeds weight, whereas Nab-El-Jamal genotype gave the lowest weight.

Table.2: Different yield parameters of six wheat genotypes grown under drought condition. (Mean± StDev).*

Genotype	Grain/Seeds (Kg/dunum)	Hay (Kg/dunum)	Total Grain+Hay (Kg/dunum)	Weight of 100 Seeds (gram)
White-Hetia	200^{bc}±4.00	275^{c}±2.00	475^{b}±2.00	45^{c}±2.00
Yellow-Hetia	263^{a}±7.55	436^{a}±8.27	699^{a}±11.53	74^{a}±2.08
Nab-El-Jamal	150^{d}±14.42	334^{b}±14.24	484^{b}±27.71	27^{e}±0.58
Um-El-Rabee'	225^{b}±9.17	250^{d}±9.54	475^{b}±7.00	38^{d}±1.53
Anber	200^{bc}±8.19	288^{c}±7.00	488^{b}±8.72	41^{cd}±1.00
Suri	188^{d}±11.24	298^{c}±5.29	486^{b}±12.50	57^{b}±1.53
P Value	0.000	0.000	0.000	0.000

*: Means within column using different letters are differ significantly at the p≤0.05 level (using one way ANOVA analysis).

IV. DISCUSSION

During the last several decades, crops of the Middle-East including Palestine are suffering from low production and productivity in general. In fact, wheat productivity is among the lowest across the region (Basheer-Salimia and Atawnah, 2014). Reason(s) for this catastrophic situation could be either a result of single or multiple factors; however drought seems to be the most important challenge and a major restriction to wheat yield especially in arid and semi-arid regions (Ozturk and Aydin, 2017). Therefore, adaptation of plants to drought stress is a vital and critical issue (Rizhsky *et al*., 2002).

Many researchers reviewed the factors that can affect plant response to drought stress at morphological, histological, physiological, biochemical, and molecular levels (Nezhadahmadi *et al*., 2013). Some of these factors might include but are not limited to the stage of plant development, duration of stress, and the plant genotype (Chaves *et al*., 2003). Toward this end, the observed variation among the six examined wheat genotypes (stem length, spike length, awns length, tillers, and different yield parameters) will serve as useful markers for any future wheat breeding program in the region.

Here, the significant higher lengths of stem, spike with awns, and awns presented at Yellow-Hetia genotype could be attributed either to the genetic variations and/or environmental conditions. Since all of the genotypes were examined at the same environmental conditions, we attribute differences to the genetic make-up of the genotypes. Similar results were reported by Shafi and others (1992) who stated that the genetic make-up of any plant affects its qualitative and quantitative characteristics.

Furthermore, drought was found to reduce the stem length of field crops (Yadavi *et al*., 2000; Sharpe, 2002), however some stem variations existed that appeared as a result of the genetic make-up of the plants.

A similar variation goes also with the tillering (branching) performance for the different examined wheat genotypes. Simane and others (1993), pointed out that the tillering proliferation is one of the first developmental processes, and it occurs during early growth and depends mainly on the availability of water and nitrogen. However, all examined genotypes were under the same environment, therefore the existing variations are likely genetically related rather than environmentally controlled.

Concerning the yield parameters, Yellow-Hetia genotype was significantly dominant and higher than the other genotypes in terms of total grain, total hay and total yield. The superiority of this genotype could be attributed to the adaptability of such a local genotype and its history that may play a significant role in the formation of yield (Austin *et al*., 1980; Agoston, 2009), the compliance of this genotype to an thesis under the harsh conditions which therefore increase the fertile-tillers number and accordingly grain-set and yield (Gooding, 2009; Mollasadeghi *et al*., 2012), and the spikes filling potential and yield.

Furthermore, the highest values of the spike length presented at Yellow-Hetia might explain its higher yield since this feature is considered as the source of assimilates closer to the caryopsis which leads to increases in the accumulated dry matter in the kernels (Thorne, 1974). Furthermore, spikes commonly stay green and functional for a longer time together with the awns (Sharma *et al*., 2003), thereby resulting in higher yield.

Moreover, awns found to have a direct vascular linkage with the spike because head photosynthesis tends to be much higher when awns are well developed (Weyhrich *et al*., 1995). Moreover, awns have been shown to be advantageous during drought stress in the driest of areas (Motzo and Giunta, 2002).

Generally, the grain weight is considered to be influenced by the environmental conditions as well as the agronomic inputs throughout different growth stages of the crop (Edwards, 2010). In fact, drought and limited water availability are the main factors limiting crop production (Seghatoleslami *et al*., 2008). Here, it is obvious that Yellow-Hetia is promising genotypes for future breeding programs since it presents significantly higher yield compared with the other 5 wheat genotypes when examined under the same climatic conditions. This result is in agreement with Jaleel and others (2007), who stated that almost all plants are tolerant to drought stress but the extent of this varies from species to species and even within species.

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Microbial analysis of leafy vegetables in iceless cooling facility

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Abstract*— *Against the background that leafy vegetables stored under ambient conditions are influenced by environmental factors which may cause significant quality loss in terms of freshness, colour, texture and composition, the iceless cooler was used to evaluate microbial load of leafy vegetables. The samples were evaluated for bacterial (coliforms and mesophiles) and fungal (mold and yeast) loads. Results of the microbial test showed that Corchorus olitorius had highest mean microbial load with plate count of 6.7 x 10^4 CFU/g and Hibiscus sabdariffa had the least mean microbial load with plate count of 4.8 x 10^4 CFU/g after five days of storage. Corchorus olitorius show a significant increase of Escherichia coli, Staphylococcus aureus and Streptococci species after five days of storage. The ANOVA results showed that vegetables stored under ambient conditions were significantly different ($p<0.01$) from those stored in the iceless cooler. The results also indicate that Amaranthus dubius recorded the highest mean weight of 1.94kg and maintained its freshness and colour for up to three days compared with Corchorus olitorius with the least weight of 1.84kg.

Keywords*— *Amaranthus dubius, Corchorus olitorius,* Deterioration, *Hibiscus sabdariffa and Storage.

I. INTRODUCTION

Leafy vegetables such as *Amaranthus dubius, Corchorus olitorius* and *Hibiscus sabdariffa* are important traditional vegetables cultivated in the three Northern Regions of Ghana because of their health benefits (Amaglo & Nyarko, 2012). These vegetables contain high sources of proteins, carbohydrates, vitamins and minerals which are essential for a healthy human life (Buyukunal *et al,* 2015). However, leafy vegetables farmers in Ghana do not posses adequate hygienic production information and good agricultural practices (e.g., storage of vegetables) and thus, posing high potential public health risk (Adam *et al.,* 2016). Knowledge and good skills of leafy vegetable storage is very important in order to help maintain the nutrient composition and texture.

According to O'Connor-Shaw *et al.* (1996) microbial spoilage is a limiting factor for shelf life of leafy vegetables and fruit specie stored under controlled atmosphere conditions. Shelf life, including microbial spoilage results in 30–50% shrinkage of fresh-cut fruits and leafy vegetables (Warren, 2005). Contamination sources of fresh-cut fruits and vegetables include raw materials and contact with processing equipment. The microorganisms that exist on the surfaces of raw, whole produce appear to be the major source of microbial contamination and consequent spoilage of fresh-cut fruits and vegetables. Boyette *et al.* (1993) indicate that the microbial decay of fresh-cut lettuce is largely due to the growth of microorganisms originating from pre-harvest environments. Delaquis *et al.* (1999) indicate that the types of microorganisms found on shredded lettuce were highly associated with the microorganisms detected on lettuce before shedding.

Microbial spoilage including off-flavor formation, slimy surface, wetness and soft rot, discoloration, and visual microbial growth/colonies has been used as a main or exclusive objective criterion to determine shelf life of fresh-cut products (O'Connor-Shaw et al., 1994; Sapers *et al*, 2001,). Jacxsens *et al.* (2003) reveal that green, yellow and red bell pepper was unacceptable by day 6 of storage under atmospheric condition of 7°C due to acidic flavor, water loss and texture change. Also, Allende *et al.* (2004) indicate that processed Lollo Rosso lettuce had a shelf life shorter than 7 days at 5°C due to high microbial counts and off-odor formation under MAP. It therefore suggests that leafy vegetables deteriorate quickly due to the active process of senescence and biochemical processes which change the original composition of the crop.

The lack of appropriate vegetable storage techniques often times result in post-harvest loss (Adam *et al.*, 2016). This is because storage of harvested products under controlled conditions is known to retard the growth of postharvest spoilage and pathogenic microorganism (Nguyen & Cardin, 1994). The reasons for the lack of adoption of modern storage technologies by vegetable farmers include huge cost of storage facilities coupled with unstable electricity power (Adam *et al.*, 2016; Gogo *et al.*, 2016).

As an alternative to the modern technologies for storing fruits and leafy vegetables, Nicol *et al.* (1997) suggested the use of iceless coolers by farmers and traders who

cannot afford to acquire improved storage technologies and equipment. The iceless cooler can be made up of a wooden frame with dimensions of 0.7m x 0.7m x 1.2m. The sides of the frame are covered with jute sacks. The top water trough is filled with water regularly to soak the jute sacks at the sides of the coolers. The iceless cooler also has a water trough at the base which collects excess water. The coolers are kept in an open shaded area where there is good flow of air current. Energy from the air current is used in evaporating the water at the sides of the cooler and also to preserve the vegetables.

The arguments for the iceless cooler is that the equipment is less expensive and built on simple home-made technology which can keep fruits and vegetables for longer periods of time. The iceless cooling facility works on simple principles of evaporative cooling to conserve the quality and shelf life of leafy vegetables. However, there is very limited information regarding how changes in atmospheric composition affect spoilage microflora profile during iceless cooling storage of leafy vegetables. The main objective of this study was to assess the effects of iceless cooling facility on microbial development, quality and shelf life of traditional leafy vegetables.

II. LITERATURE REVIEW

The common types of leafy vegetables cultivated in Northern Region of Ghana include *Amaranthus dubius*, *Hibiscus sabdariffa*, *Corchorus olitorius*, bitter leaf, baobab leaves, lettuce, cabbage and asparagus. *Amaranthus dubius (Aleefu)* known as *Amaranth* is a dark green cosmopolitan genus of annual or short-lived perennial leafy vegetable in Africa, India, Bangladesh, Sri Lanka and the Caribbean. The vegetable forms an important source of plant protein (Babalola *et al.*, 2010), which is very useful for treating fever. The vegetable also contains vitamins (A and C) and minerals (potassium, iron, magnesium and calcium) in the diet of people (Smith & Eyzaguirre, 2007; Tweneboah, 2000). *Amaranth* leaves in general are recommended as a good food with medicinal properties for young children, lactating mothers and for patients with fever, haemorrhage, anaemia, stomach ache (Schippers, 2002), constipation or kidney complaints. It is also an excellent nutritional food for AIDs patients especially when *Amaranth* grain porridge (1 cup) is combined with moringa leaf powder (1 table spoon) (Babalola *et al.*, 2010).

Corchorus olitorius (ayoyo) is a native plant of tropical Africa and Asia, and has since spread to Australia, South America and some parts of Europe. It is popularly used in soup preparation and folk medicine for the treatment of fever, chronic cystitis, cold and tumours. Scientists have indicated that drinking vegetable juice freshly made with *Corchorus olitorius* in an empty stomach can prevent and cure Ebola Virus Disease. It is also reported that *Corchorus olitorius* has the power to clear or open the natural ducts of the fluids and secretions of the body, as well as encourages lactation, purgative and tonic solving aches and pains, dysentery, enteritis, fever and tumors. *Corchorus olitorius* leaves are also rich sources of potassium, iron, copper, manganese, zinc and high energy values essential in human and animal nutrition.

Hibiscus sabdariffa (Roselle) is an important annual crop grown successfully in tropical and sub-tropical climates. The plant is cultivated for its stem, fibre, edible calyces, leaves and seeds which are used in making various foods. The commercially important part of the plant is the fleshy calyx (sepals) surrounding the fruit (capsules). It is used for making wine, juice, jam, jelly, syrup, gelatin, pudding, cakes, ice cream and flavours and also dried and brewed into tea, among other things (Solomon, 2013). *Hibiscus* is used for treating loss of appetite, colds, heart and nerve diseases, upper respiratory tract pain and swelling (inflammation), fluid retention, stomach irritation, and disorders of circulation. It is also good for dissolving phlegm. According to Jonadet *et al.* (1990) *Hibiscus* can be used as a laxative and diuretic to increase urine output. Calyces of the red and dark red coloured type are extracted and sweetened to produce a refreshing drink while calyces and leaves of the green type are used for making vegetable stew (Babalola, 2001).

III. MATERIALS AND METHOD

3.1 Research design

The experimental design was a randomized complete block design. Nine samples of *Corchorus olitorius*, *Hibiscus sabdariffa and Amaranthus dubius* were bought from the Tamale central market to serve as control. Samples were also taken from the vegetables cultivated purposely for this research work. Harvested vegetables were weighed and kept inside the iceless cooler facility. Temperature readings and relative humidity inside and surroundings of the coolers were taken thrice a day at 9:00am, 12noon and 3:00pm. Daily records on colour change, freshness and weight loss of the vegetables taken.

3.2 Preparation of smears

A drop of physiological saline was placed on a clean grease free glass slide. A sterile inoculating loop was used to transfer a colony to the slide and emulsified to make a smear. The slides were left to air-dry before heat fixing, using the flame of a Benson burner to properly adhere the smear on the slides as well as preserve microorganisms and prevent smears from being washed from the slides. Smears were allowed to cool before staining.

3.3 Gram staining of cultures

Gram staining was done to help identify the gram status and morphology of organisms. The fixed smear was

covered with crystal violet stain for 60 seconds and rapidly washed off with tap water. The smear was covered again with Lugol's iodine for 60 seconds and washed off with tap water. Acetone- alcohol solution was used to decolorise the stain for only 10 seconds and immediately washed off with tap water. The smear was covered again with neutral red stain (safranin) for 1 minute and washed off with tap water. The slides were placed in a draining rack for the smear to air dry. The smears were examined microscopically with oil immersion objective to report the gram status and morphology of bacteria.

3.4 Microbial load tests

Ten (10) grams of chopped vegetable each was weighed into each sampling bottle containing 90 millilitres (90 ml) of peptone water, making a dilution of 10^{-1}. This was then agitated vigorously for two minutes to obtain homogeneity. One millilitre (1 ml) of the sample inoculum was pipetted from the 10^{-1} dilution into a separate tube containing 9 ml of peptone water to make a dilution of 10^{-2}. The liquids were carefully mixed by aspirating 10 times with a sterile pipette and again 1.0 ml transferred into another dilution tube containing 9 ml of dilution peptone water and mixed to give 10^{-3}. These dilutions were then ready for inoculation.

3.5 Sample analysis

Sterile plates count agars were labeled 10^{-1}, 10^{-2} and 10^{-3} and 0.1ml (100 µl) of the various dilutions inoculated. The inoculum was promptly spread on the surface of the plates using sterile glass spreader (Drigalsky spatulas). The plates were allowed to dry for 15 minutes before incubating in an inverted position at 35ºC – 37ºC for 48 hours. Colonies were then counted and enumerated. All colonies in three plates corresponding to one dilution and showing between 30-300 colonies were counted. Averages of the replicates were calculated and multiplied by their dilution factor. This was then reported as the colony forming unit per gram (cfu/g). Overcrowded colonies were not counted and were reported as "Too Numerous to Count" (TNTC).

3.6 Data analysis

Using the Predictive Analytical Software version 21.0 and Student Edition of Statistix 9.0, the analysis was done to determine treatment effects on the quality parameters of the vegetables. The least significant difference at 5% and 1% level of significance were used to compare means of the vegetables kept under ambient conditions in the same vicinity with the cooling facilities for the same storage period of seven days. Microbial analysis on *E. coli*, *Staphylococcus* and *Streptococcus* was carried out at the Savannah Research Institute's laboratory in Tamale. Each day at 3pm, the weight of the samples were taken using a top pan scale. The weight loss was measured in percentages over the storage period of seven days. Results of microbial population for the different conditions and their interaction effects were subjected to analysis of variance (ANOVA) and coefficient of variation.

IV. RESULTS

4.1 Quality parameters of leafy vegetables

Table 1 presents the results on the quality parameters of leafy vegetables. Before storage of *Amaranthus*, there was 62, 49 and 43 dilution on plate count giving an average colonies count of 51 which gave Cfu/g of 5.1 x 10^4. *Amaranthus* after storage recorded 73, 59, and 34 dilution on plate count, respectively giving an average of 55 and Cfu/g of 5.5 x 10^4. For *Corchorus*, there was 73, 55 and 35 dilution on plate count giving an average colonies count of 54 and Cfu/g of 5.4 x 10^4 before storage. After five days of storage of *Corchorus*, there were 82, 70 and 49 dilution on plate count respectively giving an average of 67 and Cfu/g of 6.7 x 10^4. The results also indicate that *Hibiscus* had 57, 42 and 33 dilution on plate count giving an average colonies count of 44 and Cfu/g of 4.4 x 10^4 before storage and 64, 45 and 34 dilution on plate count, respectively giving an average of 48 and Cfu/g of 4.8 x 10^4 after storage.

Table.1: Dilution on plate count average

	Dilution on plate count					After five days of storage				
Vegetable	10^{-1}	10^{-2}	10^{-3}	M	Cfu/g	10^{-1}	10^{-2}	10^{-3}	M	Cfu/g
Amaranthus dubius	62	49	43	51	5.1 x 10^4	73	59	34	55	5.5 x 10^4
Corchorus olitorius	73	55	35	54	5.4 x 10^4	82	70	49	67	6.7 x 10^4
Hibiscus sabdariffa	57	42	33	44	4.4 x 10^4	64	45	34	48	4.8 x 10^4

Table 2 presents findings on average colonies count of *Escherichia coli, Staphylococcus aureus* and *Streptococci species*. The results of the study show that before storage of *Amaranthus dubius*, there was 143, 53 and 44 average colonies count of *Escherichia coli, Staphylococcus aureus and Streptococci species*, respectively compared with 52, 66 and 153 average colonies count after five days of storage. The findings suggest that there was a significant increase of *Staphylococcus aureus and*

Streptococci species of *Amaranthus dubius* after storage in the iceless cooler.

The results on *Corchorus olitorius* show a significant increase of *Escherichia coli, Staphylococcus aureus and Streptococci species* after five days of storage. The average colonies count before storage was 96 *Escherichia coli*, 52 *Staphylococcus aureus* and 39 *Streptococci species* compared to 142 *Escherichia coli*, 60 *Staphylococcus aureus* and 149 *Streptococci species* after five days storage of *Corchorus olitorius.* The findings on *Hibiscus sabdariffa* showed a decrease of *Escherichia coli* and *Staphylococcus aureus* after five days of storage. The results also show an increase of *Streptococci species* from 142 colonies count before storage to 156 colonies count after storage.

Table.2: Average colonies count of E. coli. Staphylococcus aureus and Streptococci species

Vegetable	Average colonies count before storage			Average colonies count after storage		
	Escherichia coli	*Staphylococcus aureus*	*Streptococci species*	*Escherichia coli*	*Staphylococcus aureus*	*Streptococci species*
Amaranthus dubius	143	53	44	52	66	153
Corchorus olitorius	96	52	39	142	60	149
Hibiscus sabdariffa	139	46	142	138	32	156

Table 3 presents the test of bacteria for the sampled vegetables. The findings show that there was no statistically significant difference between before storage and after storage of the leafy vegetables.

Table.3: Pairwise test of bacteria for treatment varieties

Vegetable	Before storage	After five days of storage	Coefficient of variation
Amaranthus dubius	$5.1x10^4$ (ab)	$5.5x10^4$ (ab)	
Corchorus olitorius	$5.4x10^4$ (ab)	$6.7x10^4$ (a)	14.25
Hibiscus sabdariffa	$4.4x10^4$ (b)	$4.8x10^4$ (ab)	

Table 4 shows the comparison test of *Escherichia coli* for the sampled vegetables. The findings indicate that there is no statistically significant difference between before storage and after storage for *Corchorus olitorius* and *Hibiscus sabdariffa.* However, there was a statistically significant difference between before storage and after storage for *Hibiscus sabdariffa.*

Table.4: Pairwise test of E. coli for treatment varieties

Vegetable	Before storage (cfu/g)	Five days in storage (cfu/g)	Coefficient of variation
Amaranthus dubius	143a	52.0c	
Corchorus olitorius	95.67b	142.00a	6.45
Hibiscus sabdariffa	139.0a	138.00a	

Table 5 presents the test of *staphylococcus* for treatment varieties. The results show that *Amaranthus dubius* and *Corchorus olitorius* tested for *staphylococcus* are not significantly different for before storage and after storage. The findings also indicate a significantly difference for *Hibiscus sabdariffa* before storage and after storage.

Table.5: Pairwise test of Staphylococcus for treatment varieties

Vegetable	Before storage (cfu/g)	After storage (cfu/g)	Coefficient of variation
Amaranthus dubius	53.000ab	66.000a	
Corchorus olitorius	52.000ab	60.000ab	14.43
Hibiscus sabdariffa	46.000bc	32.000c	

Table 6 shows the test of microbes of the leafy vegetables used for the study. The results show that bacteria count had increased from 49667a to 56778a after storage in the iceless cooler, *Staphylococcus aureus* increased from 50.333a to 52.667a and *Streptococci species* increased significantly from 75.00b to 152.44a. The results also show that *Escherichia coli* decreased from 125.89a to 110.67b after five days storage.

Table.6: Microbes test

Iceless cooler storage	Bacteria (Cfu/g)	*E. coli* (Cfu/g)	*Staphylococcus aureus* (Cfu/g)	*Streptococci species* (Cfu/g)
Before storage	49667a	125.89a	50.333a	75.00b
After storage	56778a	110.67b	52.667a	152.44a
Coefficient of variation	14.25	6.45	14.43	4.59

Table 7 shows the mean comparison test of *E. coli*, *Staphalycocus* and *Streptococcus* for the vegetables after days of storage. The results show that there was a statistically significant difference count of *E. coli* for *Amaranthus dubius, Corchorus olitorius and Hibiscus sabdariffa.* The findings did not show a significant difference for *Staphylococcus* and *Streptococcus*.

Table.7: Pairwise test of E. coli, Staphalycocus and Streptococcus for varieties after storage

Vegetable	*E. coli* (cfu/g)	*Staphylococcus* (cfu/g)	*Streptococcus* (cfu/g)
Amaranthus dubius	97.5c	59.500a	98.17a
Corchorus olitorius	118.8b	56.000a	94.00b
Hibiscus sabdariffa	138.5a	39.000b	149.00a

4.2 Trends of deterioration of leafy vegetables

Figures 1-3 show the rate of deterioration of leafy vegetables under ambient condition and in the iceless cooler facility. Figure 1 shows that *Amaranthus dubius* maintained its original weight of 2 kilograms from day zero through to the second day before losing 1.0% of its weight under the iceless cooler. By the fourth day of storage, its accumulated weight lost was 3% and by the fifth and sixth days of storage, the cumulative weight loss was 5.5% and 9.5% respectively. On the 7th day, its cumulative loss was 15%. *Amaranthus dubius* reduced weight by 0.5% on first day of the storage and 2% on the second day under ambient condition. There was a sharp rise in cumulative weight loss of 22% on day four and 97% loss on the seventh day (Figure 1).

Corchorus olitorius maintained its original weight of 2 kilograms from day zero to day 2 before losing1.0% of its weight. By the fourth day of storage, there was a cumulated weight loss of 4% and on the fifth day an increase of weight loss up to 11.5% was recorded. By the sixth day, the cumulative weight loss rose to 20% (Figure 2). However, under ambient conditions, the cumulative weight loss was 4% on the third day and by the fourth day, the cumulative weight loss increased to 29% and on the sixth day it recorded an increase of 57% (Figure 2).

Hibiscus sabdariffa loss a cumulated weight of 2% on the third day under the iceless cooler facility. It also experienced a sharp increase of weight loss of 29% on the fourth day. Under ambient conditions, *Hibiscus sabdariffa* reduced weight by 1% a day after the storage which increased to 4% the second day. By the third and fourth days, the percentage weight loss was 29% and 57%, respectively.

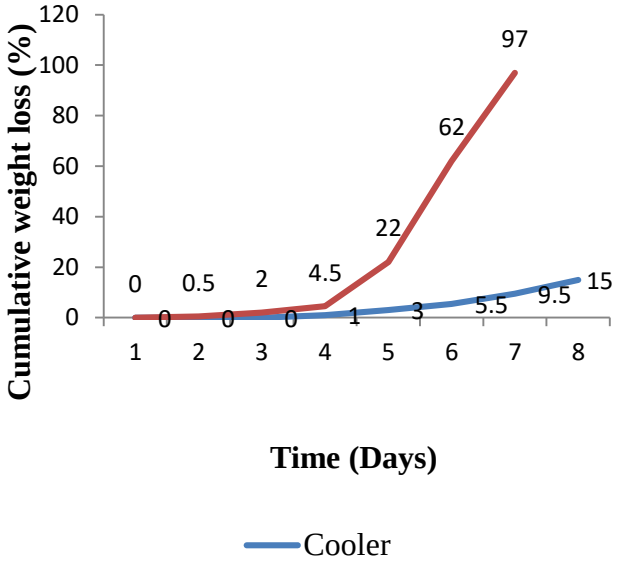

Fig.1: Cumulative weight loss of Amaranthus dubius

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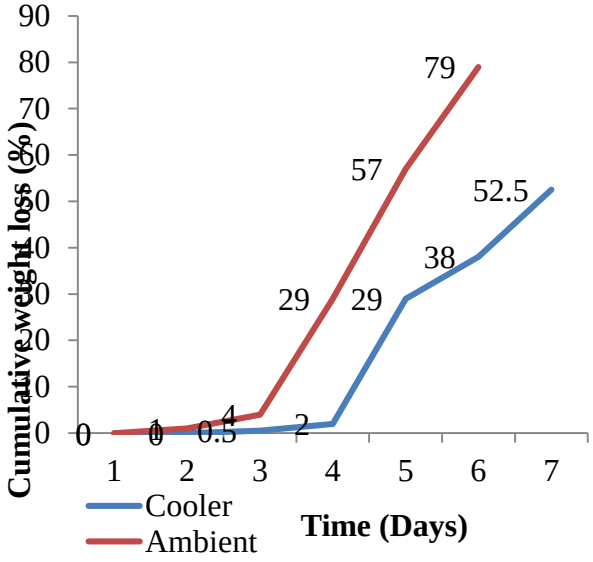

Fig.2: Cumulative weight loss of Corchorus olitorius

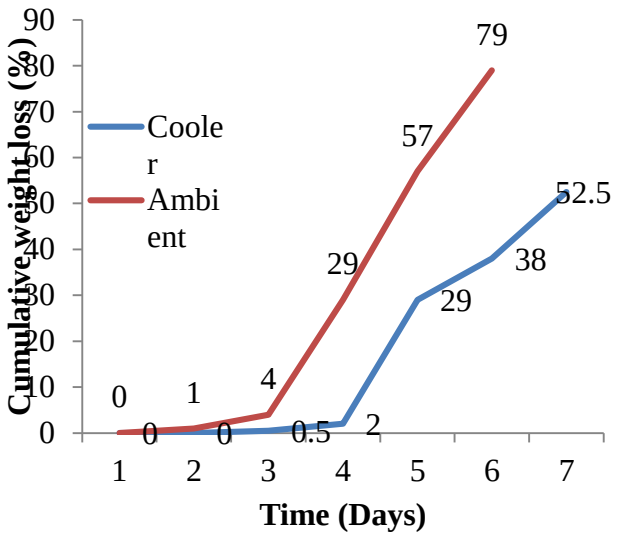

Fig.3: Cumulative weight loss of Hibiscus sabdariffa

Mean weight loss of leafy vegetables

Table 8 presents the results of the mean weight loss of leafy vegetables. The results of the study show that *Amaranthus dubius*, *Corchorus olitorius* and *Hibiscus sabdariffa* were not significantly different ($p<0.01$) between the iceless cooler facility and vegetables stored under ambient conditions. *Amaranthus dubius* recorded the highest weight of 1.94kg at the end of the storage period under iceless cooler and *Corchorus olitorius* had the least weight of 1.84kg.

Table.8: Mean weight (kg) loss of leafy vegetables

Storage facility	*Amaranthus dubius*	*Corchorus olitorius*	*Hibiscus sabdariffa*
Iceless cooler	1.94a	1.84a	1.86a
Ambient conditions	1.42a	1.04b	1.27b
Coefficient of variation	42.05	0.49	4.54

4.3 Freshness rating for leafy vegetables

Table 9 shows the freshness level of the leafy vegetables after five days of storage. The results show that *Amaranthus dubius* and *Corchorus olitorius* stored in the iceless cooler facility showed no significant differences ($p>0.01$) in freshness rating. The results also indicate that there is no significant difference of freshness of the vegetables stored under ambient conditions.

Table.9: Freshness of leafy vegetables

Storage type	*Amaranthus dubius*	*Corchorus olitorius*	*Hibiscus sabdariffa*
Iceless cooler	2.00a	2.83a	2.50b
Ambient condition	3.14a	3.00a	3.14a
Coefficient of variation	27.52	24.38	2.51

4.4. Colour rating

Table 10 shows the colour rating of leafy vegetables under different storage situation. The results show that *Amaranthus dubius* and *Hibiscus sabdariffa* stored under the iceless cooler facility was not different for colour ($p > 0.01$). *Corchorus olitorius* showed a significant difference in colour rating under the iceless cooler ($p < 0.01$).

Table.10: Colour rating for the respective leafy vegetables

Storage type	*Amaranthus dubius*	*Corchorus olitorius*	*Hibiscus sabdariffa*
Iceless cooler	1.86a	2.29b	2.00a
Ambient condition	3.14a	3.29a	3.29a
Coefficient of variation	28.43	2.53	26.74

V. DISCUSSION

The use of iceless cooling facility has a positive impact on the quality of leafy vegetables, even though there were increases of *Escherichia coli* count at all cases. There was a significant difference of total *Escherichia coli* count ($p < 0.01$) between before storage and after storage of the leafy vegetables. This finding is similar to Abdullahi and Abdulkareem (2010) due to field contamination before vegetables are harvested. The study findings also indicate a significant difference ($p < 0.01$) of deterioration of vegetables bought from the market and those cultivated for this study. Even though *Hibiscus sabdariffa* and *Corchorus olitorius* recorded higher counts, the variation in the data set for both market sourced vegetables and vegetables cultivated at home was 4.39.

The findings further indicate that *Amaranthus dubius* deteriorated at a lower rate compared with *Corchorus olitorius and Hibiscus sabdariffa*. While *Amaranthus dubius* stayed in the cooler for more than 6 days and had not deteriorated beyond saleable weight (10% weight loss), *Corchorus olitorius* and *Hibiscus sabdariffa* could not stay such long. Under ambient conditions, it took only 3 days for all the vegetables to stay within saleable weight (5% – 10%). The factors accounting for weight loss of leafy vegetable is water loss. According to Wilson *et al.* (1999), water loss is due to the temperature of the product and air velocity.

The findings on leafy vegetable freshness indicate that *Amaranthus dubius* remained 100% fresh inside the iceless cooler facility for two days, while *Hibiscus sabdariffa* and *Corchorus olitorius* remained 100% fresh for only a day. *Amaranthus dubius* declined in freshness to 75% fresh for the next four days, while *Hibiscus sabdariffa* and *Corchorus olitorius* declined to 75% fresh for the next three days. This predisposed that *Amaranthus dubius* has a high potential of staying longer in the facility compared to the other leafy vegetables. Observation of leafy vegetables stored under ambient conditions show that they loss their freshness within the 1st day and deteriorated to 50% in the 2nd day of storage.

VI. CONCLUSION

Leafy vegetables are vital sources of minerals and vitamins for human health and development, hence, the need to conserve the quality and shelf life of vegetables through the use of iceless cooler facility. The iceless cooler facility has the tendency to retain freshness and colour of vegetables for a short while compared to vegetables stored under ambient temperature. The coefficient of variations confirms the efficiency of the iceless cooler facility which should be adopted by farmers and traders for the storage of leafy vegetables in order to reduce postharvest loss. Given the efficiency of the iceless cooler facility, it is prudent to use it for preserving the quality and shelf life of *Amaranthus dubius*, *Corchorus olitorius* and *Hibiscus sabdariffa*.

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Agriculture urbaine et périurbaine (AUP) et économie des ménages agri-urbains à Dakar (Sénégal)

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Résumé— *Les dynamiques actuelles de l'urbanisation en Afrique sub-saharienne posent d'importants défis en matière d'alimentation et de sécurité alimentaire des populations urbaines. Dans ce contexte, la réflexion sur la fonction alimentaire de l'agriculture urbaine et périurbaine (AUP) trouve toute sa pertinence et de nombreux travaux ont été consacrés à l'analyse de la fonction alimentaire de l'AUP. Par contre, peu de recherches ont porté sur la dimension essentiellement commerciale de l'activité, permettant de générer des revenus susceptibles de répondre aux différents besoins, alimentaires et non-alimentaires, des ménages agri-urbains. La présente contribution s'intéresse à cet aspect particulier de l'activité maraichère pratiquée dans la région de Dakar dans trois zones de production impliquant 214 maraîchers.*

Les résultats obtenus montrent que l'AUP à travers le maraîchage urbain à Dakar constitue une activité commerciale à part entière dont les revenus monétaires sont de loin plus importants que le salaire minimum au Sénégal. Il s'avère que cette orientation presque exclusivement commerciale permet indiscutablement aux ménages agri-urbains de mieux couvrir leurs dépenses alimentaires et non-alimentaires inhérentes à la vie en milieu urbain.

Mots clefs : agriculture urbaine et périurbaine, économie des ménages agri-urbains, Dakar, Sénégal.

Urban and peri-urban agriculture (AUP) and the economy of agri-urban households in Dakar (Senegal)

Abstract— *The current dynamics of urbanization in sub-Saharan Africa pose significant challenges to food and food security for urban populations. In this context, the reflection on the food function of urban and peri-urban agriculture (UPA) is highly relevant and much work has been devoted to analysing the food function of the UAP. On the other hand, little research has been carried out on the essentially commercial dimension of the activity, making it possible to generate income likely to meet the different needs, food and non-food, of agri-urban households. This contribution focuses on this particular aspect of market gardening activity carried out in the Dakar region in three production areas involving 214 market gardeners.*

The results obtained show that the UPA through urban market gardening in Dakar constitutes a fully-fledged commercial activity whose monetary income is far higher than the minimum wage in Senegal. It turns out that this almost exclusively commercial orientation undoubtedly allows agri-urban households to cover their food and non-food expenses inherent in living in urban areas.

Keywords— Urban an peri-urban agriculture, agri-urban households economy, Dakar, Senegal.

I. INTRODUCTION

Avec plus de 7 milliards d'habitants (UNFPA, 2016), la planète est aujourd'hui confrontée à un problème de satisfaction des besoins alimentaires de sa population (Bricas et Seck, 2004). Le problème de la sécurité alimentaire mondiale se pose de plus en plus avec acuité au niveau des villes devenues en l'espace de quelques années le lieu de vie de plus de la moitié de la population (Veron, 2007). La sécurité alimentaire en milieu urbain est encore plus préoccupante dans les villes des pays du Sud, déjà confrontées à un taux de pauvreté important. C'est dans ce contexte que l'agriculture urbaine et périurbaine (AUP) se développe en occupant une place incontournable dans l'approvisionnement alimentaire des villes (Mougeot,

2000, Aubry et al., 2010). L'AUP que nous définirons ici comme l'ensemble des activités agricoles situées à l'intérieur ou en périphérie de la ville (Mbaye et Moustier, 2000) représenterait entre le tiers et le quart de la consommation mondiale de produits agricoles (Padilla, 2005).

La fonction alimentaire de l'AUP a été largement étudiée dans plusieurs recherches le plus souvent consacrées au contexte des villes situées dans les pays du Sud (Moustier et Pages, 1997, Aubry et al., 2010, De Bon et al., 2010, Chagomoka et al., 2015). Dans la plupart des travaux, cette fonction de l'AUP est abordée sous l'angle du rôle qu'elle joue par rapport à la sécurité alimentaire des populations urbaines, en particulier dans les stratégies des ménages urbains pauvres (Casale, 2006, Mfoukou-Ntsakala A. et al., 2006, Olahan, 2010, Chagomoka et al., 2015). Pour autant, dans un contexte urbain marqué par l'omniprésence des échanges commerciaux et monétaires, limiter la fonction alimentaire de l'AUP à son caractère d'activité d'autoconsommation paraît réducteur. Elle se décline également en une activité génératrice de revenus (Maxwell, 2003) permettant aux ménages agricoles urbains de diversifier considérablement leurs régimes alimentaires (Zezza & Tasciotti, 2010). Dans ce cadre, la forme marchande et commerciale de l'AUP est un atout majeur pour les ménages agricoles urbains. Or, bien que beaucoup évoquée (Golhor, 1995, Temple et Moustier, 2004, Smith, 2004, Ba et Aubry 2011), cette question reste peu abordée dans la littérature (Danso et al., 2002, Zezza et Tasciotti, 2010, Dubbeling et al., 2010).

À Dakar, l'agriculture, particulièrement le maraîchage, est une pratique très ancienne et bien ancrée dans l'écosystème urbain et périurbain. D'abord développée du temps de la colonisation (Sposito, 2010), elle a été vite adoptée par les populations locales particulièrement celles issues de l'exode rural et jadis agriculteurs dans leurs villages. Pour autant, le maraichage urbain dans la capitale sénégalaise présente d'importantes particularités si on la compare avec des activités agricoles urbaines menées dans d'autres villes. Que ce soit à Antanarivo, Ouagadougou ou Brazzaville (Madjélia et al., 2016, Aubry et al., 2008, Mfoukou-Ntsakala et al., 2006) l'AUP y est menée et perçue comme une activité d'autoconsommation garantissant aux ménages urbains les plus pauvres une sécurité alimentaire grâce à un accès direct à des aliments frais. Or, l'horticulture maraîchère à Dakar sort de ce stéréotype. Même si elle occupe une place prépondérante en termes d'approvisionnement alimentaire en produits frais et de création d'emplois pour la ville (Temple et Moustier, 2004, Guèye et al, 2009, IAGU, 2011), elle constitue une activité essentiellement commerciale. La faiblesse de la part autoconsommée des productions maraichères s'explique en partie par les habitudes alimentaires observées dans la région de Dakar (Sposito, 2010, GRDR, 2015). A titre de comparaison, si les légumes cultivés dans ce territoire sont consommés par les populations locales, elles ne sont pas par contre des aliments de base dans les repas dakarois contrairement au riz et au cresson cultivé, dans la ville d'Antanarivo (Dabat et al., 2012, Aubry et al., 2008). Le principal aliment de base à Dakar et de manière générale au Sénégal est le riz majoritairement importé des pays asiatiques même si la production locale provenant de la vallée connait de plus en plus de succès. Et cette importance des produits importés ne se limite pas au riz car la part des produits alimentaires dans les importations du Sénégal est d'environ 54% (Wade & Lançon, 2015). Malgré le caractère commercial de l'AUP, l'analyse des revenus issus des activités de maraîchage n'a été abordée que dans deux études relativement récentes (IAGU, 2011 ; Gaye et Niang, 2010). Par contre, la question spécifique de l'impact de ces revenus sur l'économie des ménages et ses implications en termes de sécurité alimentaire n'a pas été traitée. Le présent article ambitionne de mieux documenter la pratique de l'AUP dans la région de Dakar et d'éclairer sa contribution économique à la satisfaction des besoins des ménages agri-urbains. Une première partie détaillera les principales caractéristiques de l'AUP dans la région dakaroise. Une deuxième partie présentera un bilan socio-économique et discutera l'hypothèse d'une vocation essentiellement commerciale de cette activité, dont les recettes permettent aux ménages agri-urbains de mieux répondre à leurs différents besoins non-alimentaires, tout en contribuant au renforcement de la sécurité alimentaire. Une dernière partie traitera finalement des contraintes qui pèsent sur le devenir de l'AU dans la région de Dakar.

II. MATÉRIAUX ET MÉTHODES

Unité de sondage et taille de l'échantillon

L'importance des zones de production maraîchère dans la région de Dakar (Ba, 2008) fait que le choix de l'unité de sondage sur le terrain devient crucial pour la fiabilité des résultats. L'étude s'est fondée sur une discrimination spatiale des sites de production en suivant la subdivision administrative de la région de Dakar. Celle-ci a permis de classer les futurs sites sélectionnés selon un gradient urbain/rural. Vu l'existence de plusieurs sites de production dans chaque niveau, le critère d'importance ou d'envergure du site de production en termes de superficie occupée a été introduit. Le croisement de ces critères a permis de ressortir trois unités de sondage que nous qualifierons indifféremment tout au long de cet article sous le vocable de sites ou zones de production. Ces zones de production sont respectivement la Grande Niayes de Pikine appartenant à la partie intra-urbaine de Dakar, les Niayes de Malika située en zone péri-urbaine et la zone de

Lendeng de Rufisque à une quarantaine de kilomètres dans la partie rurale de la région.

L'échantillon, défini suivant une méthode statistique usuelle[1], se compose de 214 individus, répartis comme suit : 101 maraîchers dans la Grande Niayes de Pikine, 54 à Malika et 59 à Lendeng.

Déroulement des enquêtes

Les données ont été recueillies au moyen de questionnaires et d'entretiens semi-directifs avec 12 focus groupes, entre mars et juin 2016. L'élaboration du questionnaire, la saisie et l'apurement de la base de données a été faite sur Sphinx. L'ensemble des traitements statistiques ont été mené sur Excel et SPSS.

Présentation du cadre de l'étude

La région de Dakar couvre une superficie de 550 km^2, soit 0,3 % du territoire national et sa population est estimée en 2017 par l'ANSD à 3 529 300 habitants soit 49% de la population urbaine du Sénégal. Elle concentre également l'essentiel des services administratifs, du tissu industriel et des établissements commerciaux ainsi que financiers du pays. Cette situation ne fait que renforcer la forte croissance urbaine qu'on enregistre aujourd'hui dans la région de Dakar avec un niveau d'urbanisation de 96%. Malgré ce caractère de région quasiment urbaine, la pratique agricole, notamment la culture maraîchère, constitue une activité majeure dans l'économie de la région, marquée par un secteur informel prédominant et un taux de chômage de la population assez élevé (15,7%) surtout au niveau des personnes de moins de 30 ans (ANSD, 2017). Ainsi, on dénombre à l'échelle de la région de Dakar plus d'une vingtaine de sites ou zones de production agricoles (Ba, 2008) situées pour l'essentiel dans le domaine agroécologique des Niayes.

La Grande Niayes de Pikine

Située dans la partie ouest de Dakar, la Grande Niayes de Pikine est la plus grande zone des Niayes de cette région et couvre une superficie de 750 ha. Elle est limitée au nord par la commune de Golf Nord (département de Guédiawaye), au Sud par la Patte d'Oie (département de Dakar), à l'est par la ville de Pikine et à l'ouest par le village de Cambérène (département de Dakar). À l'image de l'ensemble des Niayes, la Grande Niayes de Pikine alterne un ensemble de systèmes dunaires et de dépressions interdunaires. Si les dunes sont composées de sols à faible capacité de rétention d'eau, celui des cuvettes et marigots interdunaires est essentiellement argileux ce qui explique son fort potentiel agricole. Les Niayes se distinguent également par le caractère affleurant de la nappe phréatique qui permet aux maraichers de s'adonner quasiment toute l'année à leur activité agricole. La Grande Niayes de Pikine est l'une des plus grandes zones de production maraîchère de la région de Dakar et la plus grande située en milieu intra-urbain (Faye, 2010).

La zone de Malika

La zone de production de Malika se trouve dans la commune du même nom située à environ une vingtaine de kilomètres du centre-ville de Dakar dans la périphérie du département de Pikine. Elle fait également partie de l'ensemble des Niayes et connait donc les mêmes problématiques associées à cet ensemble. Malika abrite également la principale décharge d'ordures sauvages de la région de Dakar, Mbeubeuss, sur le lit d'un ancien lac autour duquel se développent les activités agricoles. Par ailleurs, Malika est confronté aux effets du changement climatique avec l'avancée de la mer qui, cumulée au caractère affleurant de la nappe phréatique, font que les ressources hydriques de cette zone sont très vulnérables aux phénomènes de salinisation (Ba et al., 2016), observé sur l'ensemble des Niayes.

La zone de Lendeng

La zone de Lendeng se trouve dans la commune de Rufisque Est dans le département de Rufisque à environ une quarantaine de kilomètres de Dakar. Elle appartient au même écosystème des Niayes, ce qui lui confère des conditions physiques adéquates pour la pratique agricole notamment la production maraîchère. Contrairement aux autres zones des Niayes, la nappe phréatique y est plus profonde car allant de 12 à 36 mètres, ce qui ne facilite pas son accès par les céanes[2] et/ou les puits pour une exploitation agricole. Par ailleurs, la zone agricole de Lendeng s'étend sur une superficie de 56,83 ha dont 40 ha exploitées à des fins de production maraîchères soit 70 % de l'espace disponible (IAGU, 2011). Lendeng est également la plus importante zone de production maraîchère de la région de Dakar en termes de disponibilités foncières et en termes de quantités produites.

[1]Méthode de Bernoulli

[2] Terme désignant un puits peu profond creusé dans le sol avec une forme plus ou moins évasée suivant sa profondeur.

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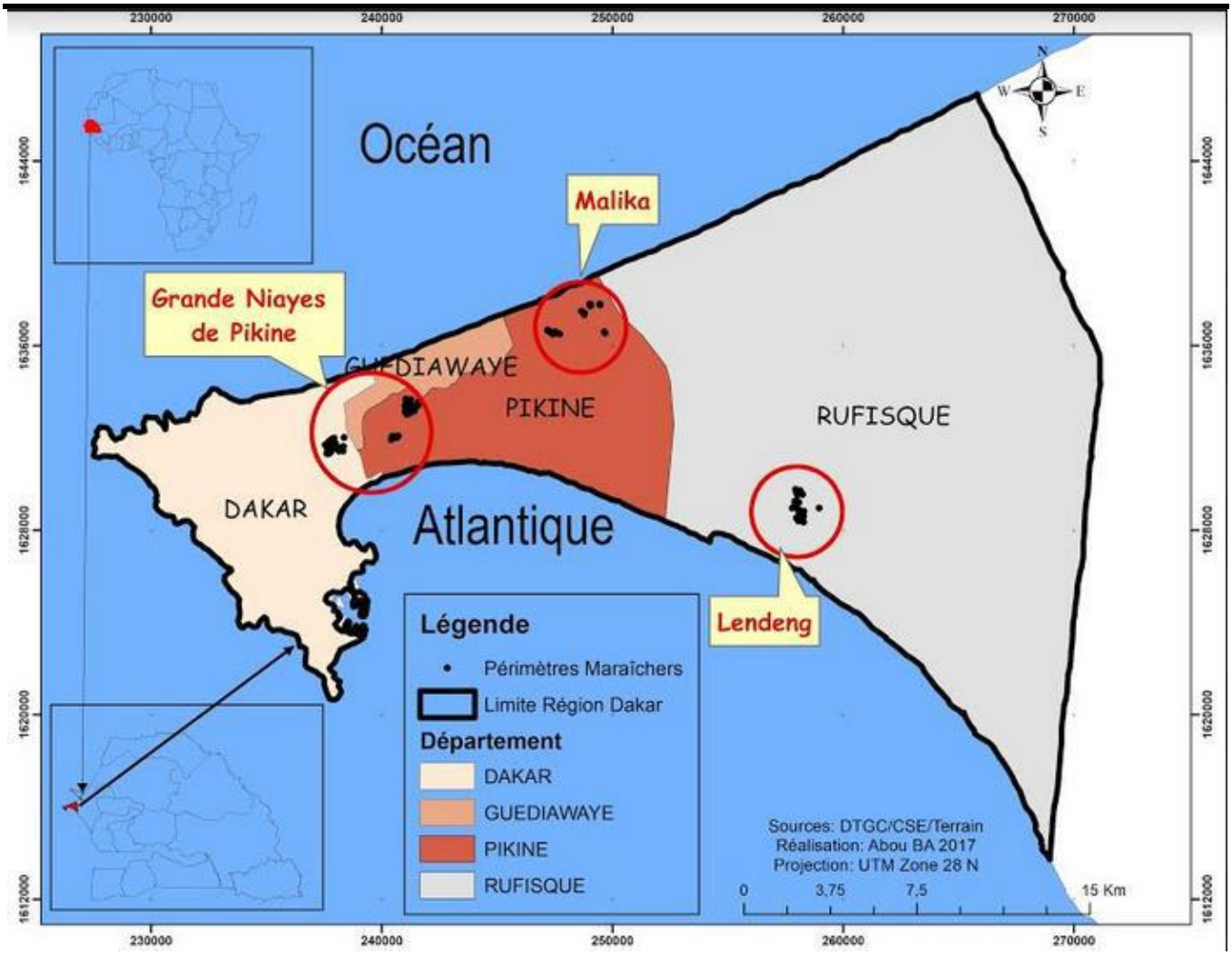

Fig.1: Carte de situation de la région de Dakar et de localisation des zones d'étude

Caractéristiques de l'agriculture urbaine et périurbaine à Dakar

Une activité masculine peu attractive pour les jeunes

L'AUP à Dakar particulièrement l'horticulture maraîchère est une activité presque exclusivement (à 95%) masculine. Cela s'explique par le contexte socioculturel du Sénégal où, d'une part, les femmes ont difficilement accès à la terre agricole et, d'autre part, elles sont généralement spécialisées dans la commercialisation des récoltes (Niang, 2014). Ces agriculteurs sont généralement des adultes avec un âge moyen de 49 ans (âge minimum de 19 ans et maximum de 80 ans). Au-delà de cet âge moyen, on constate que malgré l'importance du chômage chez les jeunes, le maraîchage ne mobilise que très peu cette catégorie de la population (18 % des agriculteurs interrogés). Cette situation résulte d'abord de la précarité de cette activité ensuite de sa marginalisation par les pouvoirs publics (Kedowide, 2010) et enfin de l'omniprésence des séniors, voire des retraités, demandeurs de revenus supplémentaires (Bâ et al, 2016) dans un contexte où le système de retraite est très largement inexistant.

Des acteurs expérimentés et animés par une question de survie

Le maraîchage à Dakar est une activité menée principalement par des agriculteurs très expérimentés. Près de la moitié des maraichers interrogés (46 %) ont une vingtaine d'années révolues dans la pratique agricole en milieu urbain alors que seulement près de 8 % (tableau 1) y sont depuis moins de cinq années. Ceci dénote entre autres du manque de renouvellement des acteurs dans cette filière consécutive probablement à la réduction des espaces agricoles née de l'urbanisation ininterrompue de l'agglomération dakaroise.

Tableau 1 : Nombre d'années passées dans l'activité agricole

Catégorie	[0-5]	[6-10]	[11-15]	[16-20]	[21 et plus]	Total
Effectifs	16	42	26	31	99	214
Proportion (%)	7,5	19,6	12,1	14,5	46,3	100

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Les considérations liées à la survie sont les principales motivations des agriculteurs dakarois à s'adonner à l'horticulture maraîchère. En effet, le besoin de subsistance est le motif le plus cité avec 81 % des réponses enregistrées. Cela est davantage conforté par plusieurs recherches qui voient en l'AUP une activité de subsistance pour les ménages urbains les plus démunis (Olahan, 2010, Chagomoka et al, 2015), même si le contexte dakarois montre plusieurs spécificités.

Une situation foncière précaire et ambiguë

La situation foncière permet d'interroger les modalités d'accès à la terre des agriculteurs urbains de la région de Dakar. Les résultats montrent une typologie du statut foncier très hétérogène entre les agriculteurs. C'est ainsi que la plupart des agriculteurs (51 %) se considèrent comme des propriétaires de leur exploitation contre, 15 % de métayers, 11 % de locataires et 6 % d'agriculteurs se considérant être dans une situation d'occupation anarchique (figure 2). Il convient de noter que sur les 51 % d'agriculteurs se disant propriétaires, moins de 20 % disposent d'actes de propriété. Et parmi ces derniers, une majorité s'avère contestable d'un point de vue juridique, le transfert de propriété reposant généralement sur des actes non reconnus par la législation foncière.

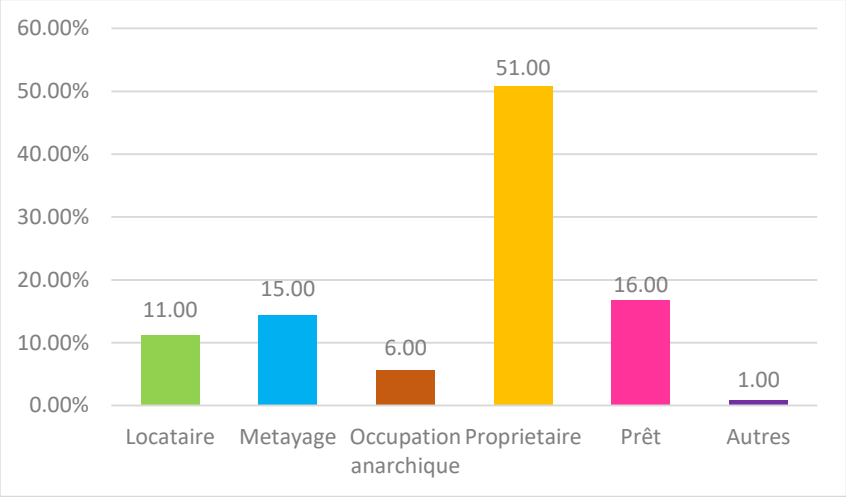

Fig.2 : Répartition des agriculteurs selon leur statut foncier

Par ailleurs, le caractère informel et/ou traditionnel des formes de tenure foncière accentue la précarité de la situation des agriculteurs puisqu'elle ne leur permet pas d'obtenir des crédits au niveau des institutions financières en mettant sous hypothèque ou sous garantie leur exploitation agricole. De plus, l'exiguïté des superficies agricoles exploitées constitue également un frein pour les bailleurs qui préfèrent investir dans les grandes exploitations agricoles. Les activités maraîchères se font en effet sur de petites exploitations avec en moyenne 0,39 ha par agriculteur. La distribution des agriculteurs selon la superficie exploitée est assez hétérogène (écart-type = 0,54 ha) même si près de six (06) agriculteurs sur dix (10) exploitent moins de 0,25 ha (tableau 4).

Tableau 2 : Répartition des agriculteurs selon la superficie cultivée

Superficie cultivée (ha)	Effectifs de producteurs	Proportion (%)
[0-0,25]	124	57,9
]0,25-0,5]	28	13,1
]0,5-0,75]	27	12,6
]0,75-1,0]	5	2,3
]1,0-1,25]	15	7,0
]1,25 et plus]	15	7,0
Total	214	100

Les sources et modes d'irrigation

On recense trois sources d'approvisionnement en eau dans l'AUP à Dakar. La plus répandue est basée sur l'utilisation de la nappe phréatique (Bâ et al., 2016), peu profonde dans la région du fait de son appartenance à la grande zone

agroécologique des Niayes. La deuxième source est le réseau de distribution d'eau potable assurée par la SDE[3]. Celle-ci est beaucoup plus présente dans la partie sud des Niayes à cause de la salinité des sols et des eaux souterraines. Les agriculteurs ayant recours à cette source d'approvisionnement sont soumis à d'énormes contraintes à cause de la concurrence avec les besoins domestiques, même s'ils bénéficient d'un système tarifaire préférentiel contrôlé. La dernière source d'approvisionnement en eau à Dakar est relativement nouvelle et très peu répandue à l'échelle de la région. Il s'agit de l'utilisation des eaux usées traitées (EUT) fournies depuis 2012 par les STEP[4] grâce à un programme initié par la FAO ayant permis l'installation d'un réseau d'adduction allant des STEP aux zones de production concernées (Souré et al., 2012).

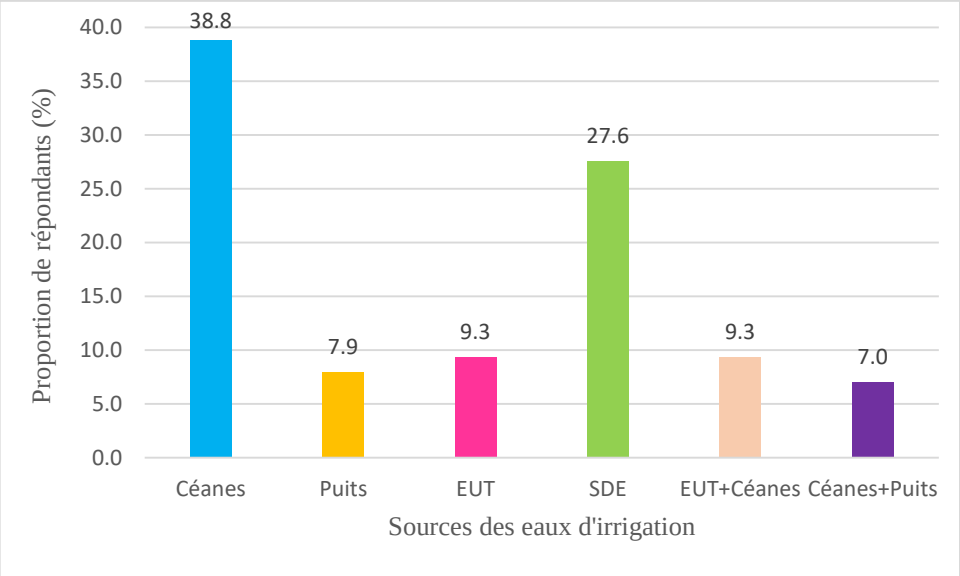

Fig.3: Principales sources d'irrigation

Les déchets organiques urbains comme matières premières pour la fertilisation

L'utilisation des déchets organiques dans le maraichage n'est pas une pratique nouvelle, mais elle occupe de plus en plus de place dans les activités maraîchères et en dehors, car participant à l'amélioration du cadre de vie urbain par le recyclage des milliers de tonnes de déchets produits chaque jour dans la ville (Tounkara, 2015, N´Diénor, 2014). Ces fertilisants sont très divers de par leur origine, mais ce sont les crottins de cheval qui en représentent la plus grande proportion avec 71 %, suivis des fientes de volaille avec 54 % et des bouses de vache utilisées par 22 % des agriculteurs. Cette importance des fertilisants organiques (figure 4) s'explique d'une part par leur disponibilité et d'autre part par la forte intégration de l'horticulture maraîchère au secteur de l'élevage.

Malgré, la pratique répandue de la fertilisation organique, on note toujours la persistance de la fertilisation chimique ou minérale. Cette dernière concerne principalement deux fertilisants : l'urée et le NPK[5], utilisés par l'ensemble des agriculteurs interrogés. Ce constat est similaire à celui fait dans les travaux de Niang (2014) et du IAGU (2011).

[3] Sénégalaise des eaux, société publique de distribution d'eau potable

[4] Station d'épuration

[5] Engrais composé de trois éléments chimiques : azote (N), phosphore (P) et potassium (K)

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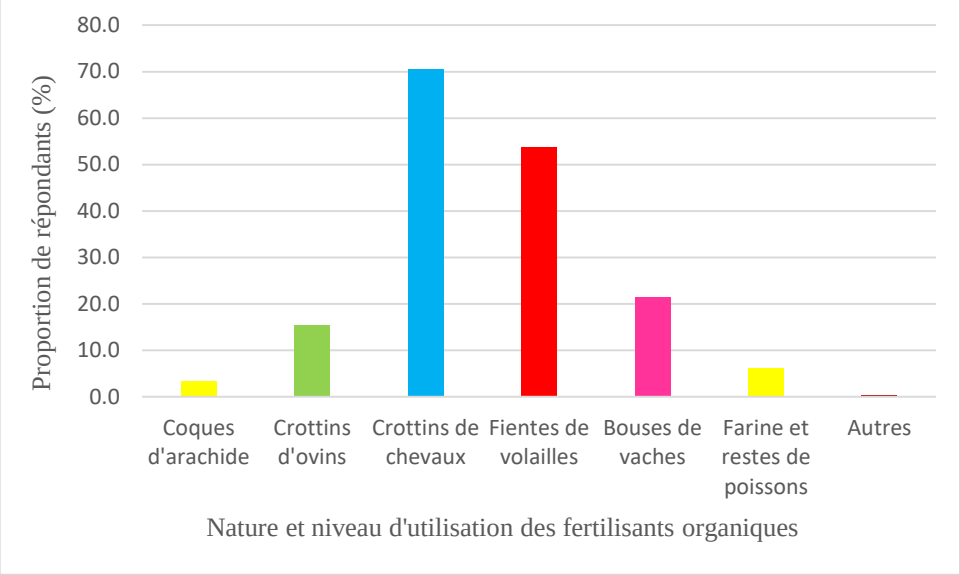

Fig.4: Nature et niveau d'utilisation des fertilisations organiques

Aperçu des principales spéculations cultivées

Du fait de son caractère d'activité urbaine, l'horticulture maraîchère à Dakar est dominée par des spéculations à cycle relativement court. Ces dernières peuvent être regroupées en deux catégories : les légumes à feuilles (les plus cultivées) et les légumes à fruit. Pour les premières, la laitue, le chou et la menthe constituent les principales spéculations avec respectivement 88 %, 34 % et 22 % de part d'activité chez les agriculteurs. Tandis que pour les légumes à fruit, ce sont l'oignon (54 %), le jaxatou[6] (48 %) et le poivron (45 %) qui occupent plus les maraîchers (figure 5).

On constate que la localisation est un élément important dans le choix des spéculations cultivées à cause entre autres de la pression foncière, du besoin de répondre à la forte demande des citadins et de la nécessité d'avoir des revenus en permanence pour financer les activités agricoles. C'est ainsi que dans les zones intra-urbaines (Grande Niayes de Pikine), on a une omniprésence des légumes feuilles à cycle très courts, particulièrement la laitue et la menthe. Par contre, les zones périurbaines présentent une plus grande variété de spéculations avec des cycles de cultures plus longs même si la laitue y occupe à l'instar de toute la région une place de choix dans le calendrier des agriculteurs.

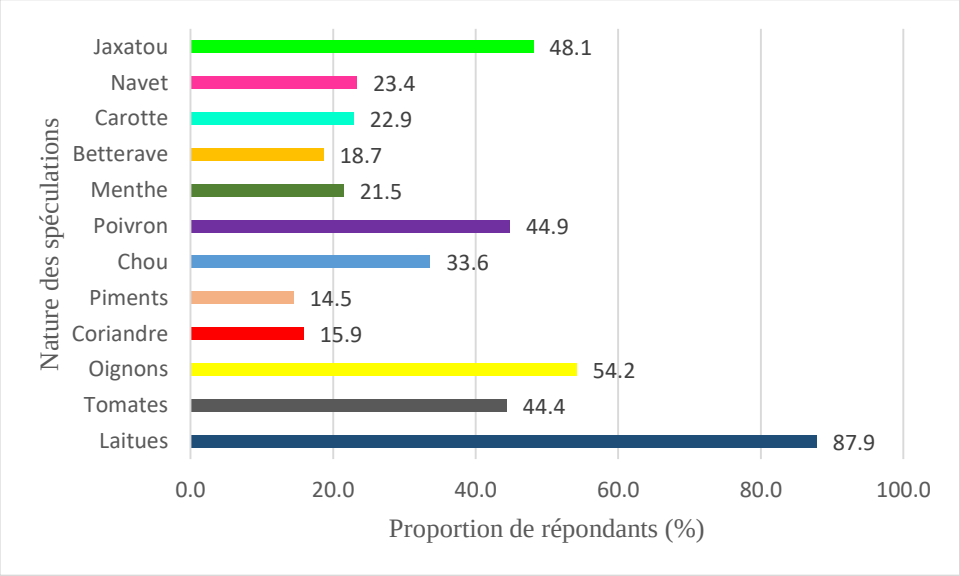

Fig.5: Type de spéculations cultivées

Principales contraintes rencontrées par l'AUP

[6] Aubergine amère

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L'horticulture maraichère, à l'image de toutes les activités agricoles pratiquées dans la région de Dakar, doit faire face à d'importantes contraintes dont la plupart sont inhérentes à son caractère d'activité urbaine. Les trois principales contraintes identifiées (figure 6) par les agriculteurs sont l'insécurité foncière (74 %), le manque d'eau pour l'irrigation (62 %) et la salinisation des eaux et des sols (27 %). Ces résultats corroborent les travaux antérieurs de Gaye et Niang (2010), IAGU (2011) et Niang (2014). D'autres contraintes moins préoccupantes que les trois principales entravent également le bon fonctionnement des activités maraichères. Il s'agit entre autres de l'accès aux systèmes de crédits (19 %), des contraintes climatiques principalement les inondations (18 %) et de la limitation des quotas pour les exploitations faisant l'irrigation avec l'eau de la SDE (15 %). À cela, on peut ajouter d'autres contraintes propres à chaque zone de production comme la cherté de l'eau pour les agriculteurs utilisant l'eau de la SDE et la mauvaise qualité des eaux usées traitées pour les maraîchers de la grande Niayes de Pikine.

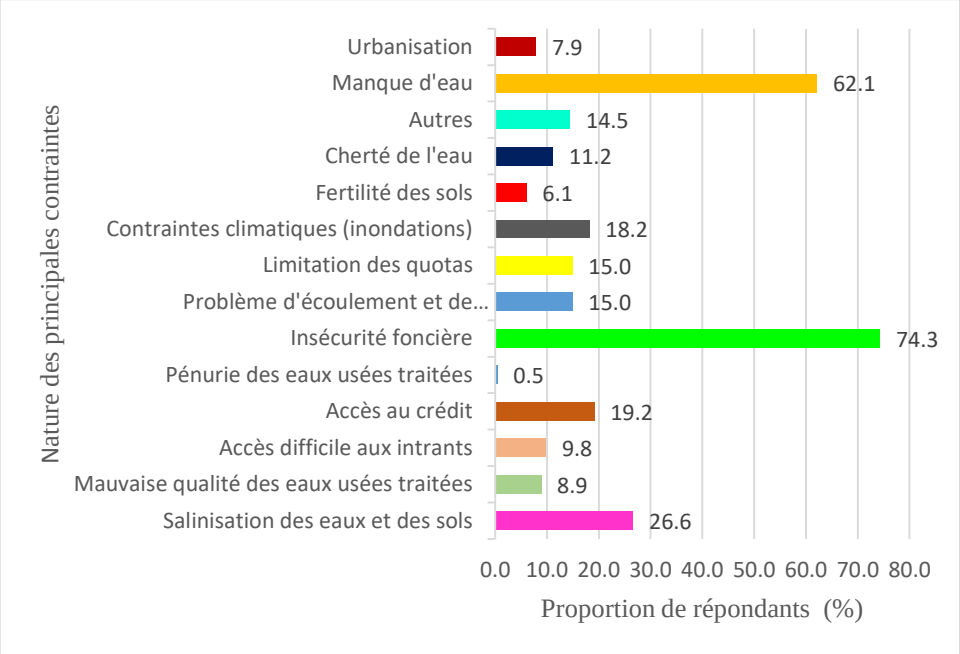

Fig.6: Nature et niveau d'importance des contraintes pour les agriculteurs

Les fonctions reconnues aux zones de production agricoles à Dakar

L'AUP est une activité remplissant diverses fonctions au niveau des territoires dans lesquels elle se développe. Cette multifonctionnalité, bien relatée dans la recherche (Duchemin, 2013, Ba, 2011, Duchemin & al, 2008) renseigne par contre très peu sur les perceptions ou les fonctions que les agriculteurs assignent à leurs zones de production. Or, il apparaît que les agriculteurs dakarois considèrent principalement les espaces qu'ils occupent en milieu ou en périphérie de la ville comme des zones de production agricoles à part entière (figure 7). Tandis que 70 % d'entre-eux mettent en avant la fonction de zone génératrice de revenus, l'importance de la fonction environnementale dans les choix des agriculteurs est également soulignée. En effet, 45 et 44% des agriculteurs voient leur espace agricole respectivement comme une zone de recyclage des déchets urbains et un espace vert pour la ville. La fonction éducative ressort également dans les résultats (25 % des agriculteurs) comme support pédagogique pour les élèves et étudiants de la région. Seuls 18 % des agriculteurs voient leur espace agricole comme une réserve foncière de la ville, qui sera appelée tout ou tard à disparaître au profit de projets immobiliers ou d'infrastructures majeures. En comparant ces résultats avec les travaux menés dans quelques zones agricoles de la région de Dakar (Ba, 2011 ; IAGU, 2011), on constate un profil tout à fait similaire en termes de réponses.

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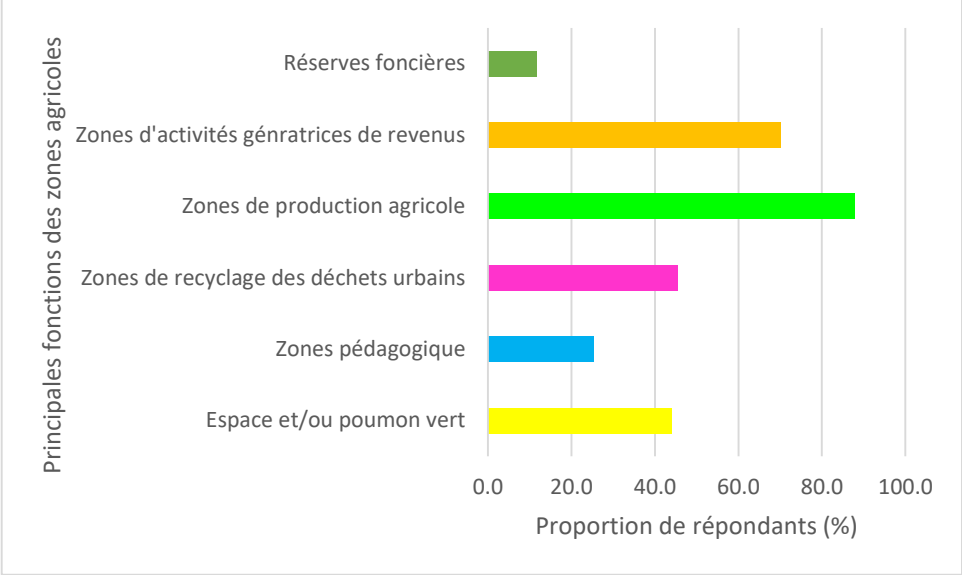

Fig.7: Principales fonctions reconnues aux zones agricoles par les producteurs

Bilan socioéconomique du maraîchage urbain

L'évaluation économique du maraîchage urbain à Dakar a été menée à travers des enquêtes quantitatives rétrospectives menées entre les mois de mars et juin 2016. Les résultats concernent donc la campagne agricole précédente à la période de déroulement de l'enquête. Cette méthode d'évaluation économique présente des inconvénients car elle n'est basée que sur les propos recueillis auprès des producteurs dans un contexte où la question des revenus est généralement taboue. Toutefois, elle a l'avantage de permettre de comprendre au mieux les performances économiques de cette activité, pratiquée très souvent par des acteurs qui ne tiennent aucun compte d'exploitation. La non-tenue d'un compte d'exploitation par les producteurs et la volatilité des prix des légumes d'une saison à l'autre, voire même au cours d'une semaine, rendent difficile tout calcul d´un résultat net de production. Pour pallier cela, l'étude s'est basée sur une estimation par les producteurs de leurs coûts de production et de leurs bénéfices nets par campagne. Les coûts de production sont l'ensemble des charges qui entrent directement dans le fonctionnement de l'exploitation maraîchère tandis que le bénéfice constitue le revenu gagné par le producteur après déduction de ces charges de production. Par ailleurs, la diversité des exploitations agricoles des zones étudiées explique le choix de présenter le profil économique du maraichage urbain à Dakar par zone de production. Cette diversité s'exprime également en termes de capacités d'investissement et de production. C'est ainsi que la zone de Lendeng bénéficiant des plus importantes superficies agricoles par rapport à celles de Malika ou de la grande Niayes de Pikine, enregistre les charges et les résultats nets de production les plus importants (tableau 3)[7]. Cela traduit également de plus grandes disponibilités financières pour réaliser les investissements requis.

Tableau 3 : Compte d'exploitation d'un maraîcher moyen par campagne (en euros)

Rubriques	Malika	Niayes de Pikine	Rufisque (Lendeng)	Moyenne des trois zones
Semences	64,80	109,70	208,40	125,30
Engrais et phyto	22.76	37,40	76,20	44,40
Fumure organique	54,11	82,30	106,30	95,60
Charges d'irrigation	12,43	26, 40	274,90	91,40
Salaires personnel	43,93	73,30	199,90	103,00
Préparation du sol	3,75	12,30	71,30	28,30
Total des charges	201,78	348,80	986,90	487,90
Résultat net/campagne	764,50	975,50	2 136,10	**1 242,20**
Revenu net annuel	3 076,80	4 044,00	8 544,40	**4 968,80**

[7] Les valeurs sont en euros et 1 euro= 655 FCFA

Des revenus agricoles au-dessus de la moyenne dakaroise

L'esquisse du compte d'exploitation des maraichers montre que le revenu net moyen par campagne obtenu à l'échelle des trois zones d'étude est de 1242 euros. Sachant que près de 98 % des producteurs interrogés mènent quatre campagnes par année, on obtient un revenu net annuel moyen pour un maraîcher de 4969 euros soit un revenu mensuel de 414 euros. En comparaison, le salaire minimum interprofessionnel garanti (SMIG) dans le pays et le salaire moyen d'un employé sont respectivement de 55 et 174 euros (ANSD, 2017) donc largement inférieurs au revenu net moyen d'un maraîcher. Ce revenu constitue aussi l'essentiel des ressources des ménages agri-urbains. Il représente en effet 82.5% des dépenses de consommation, la partie restante étant assurée par des revenus issus d'autres activités, qu'environ 50% des ménages agro-urbains déclarent pratiquer. Les disponibilités financières des ménages agri-urbains s'avèrent ainsi être très proches de celles des ménages de la région dakaroise dans son ensemble. Si l'on compare les dépenses de consommation des ménages (nous ne disposons pas de données sur les revenus des ménages dans la région de Dakar) le différentiel est minime, de l'ordre de 2% (tableau 4 ci-après)[8].

Tableau 4 : Structure des principaux postes de dépense des ménages agri-urbains et urbains de Dakar

	Ménages agri-urbains		**Ensemble ménages de Dakar**	
Types de dépenses	Montants (en euros)	Part (%)	Montants (en euros)	Part (%)
Alimentation	2677,20	44,4	2290,40	38,9
Éducation	324,20	5,4	270,80	4,6
Santé	156,30	2,6	194,30	3,3
Transport	234,60	3,9	370,90	6,3
Communication	78,30	1,3	29,40	0,5
Logement (électricité+eau +autres combustibles, meubles+articles de ménage)	2248,50	37,3	2019,60	34,3
Autres dépenses	307,40	5,1	712,40	12,1
Total	6026,60	100,0	5887,90	100,0
Revenu net annuel du maraîchage	4 968,80	82.5%		

Il se traduit tout de même par une capacité à investir davantage dans des postes de dépenses centraux pour le bien-être du ménage, avec 17% de dépenses en plus au niveau de l'alimentation, de 19.5% pour l'éducation et de 11.3% en plus pour le logement. Les dépenses de santé sont, étonnamment bien inférieures (-24%).

Finalement, l'importance des revenus issus du maraîchage peut également être appréciée en relation aux seuils de pauvreté appliquée dans la région de Dakar. Au plan général, le revenu agricole est 7% supérieur au seuil de pauvreté monétaire, fixé à 4630 euros annuels (ANSD, 2013). Au plan des dépenses alimentaires, même en considérant une somme légèrement inférieure à celle calculée de notre échantillon, du fait de l'existence de revenus extra-agricoles[9], le niveau moyen de dépenses serait 13% supérieur au seuil de pauvreté alimentaire (ANSD, 2013).

Ces différents résultats permettent d'apprécier toute la valeur commerciale du maraîchage urbain et son importance dans l'économie des ménages agri-urbains.

L'AUP : une activité menacée

Le diagnostic de l'AUP nous a permis d'illustrer toute la dynamique de cette activité, ses impacts sur l'économie des ménages agri-urbains et sa contribution à la sécurité alimentaire des ménages et de la région. Pour autant, l'avenir de ce secteur d'activité est menacé par des contraintes grandissantes, qui, pour partie, dépendent de sa faible reconnaissance par les acteurs en charge de la planification territoriale urbaine.

Des contraintes foncières tenaces

[8] Les données régionales sont issues du deuxième rapport de suivi de la pauvreté au Sénégal (ESPS). Le tableau de la structure des dépenses a été modifié dans l'optique de mener une comparaison avec la structure des dépenses issues de nos travaux. Ainsi, certains postes de l'ESPS ont été regroupés à cet effet.

[9] En l'occurrence, dans la mesure où les revenus agricoles représentent le 82.5% des dépenses totales, on appliquerait ce même ratio à la ligne budgétaire des dépenses alimentaires.

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La question foncière a toujours été l'un des problèmes majeurs auxquels les activités agricoles menées dans un contexte urbain et/ou périurbain a dû faire face (Diagne 2008 ; Guèye et al., 2009 ; Dauvergne et al., 2010 ; Sposito, 2010). Les contraintes foncières de l'AUP dakaroise sont doubles. En premier lieu elles renvoient à la taille des parcelles. La superficie moyenne (0.38 ha par producteur) calculée dans nos enquêtes et qui correspond aux valeurs relevées dans d'autres études (Gaye & Niang, 2010 ; IAGU, 2011 ; Niang, 2014), est relativement exiguë. A l'heure actuelle, comme démontré, cela est encore suffisant pour couvrir les besoins des ménages agri-urbains. La situation va vraisemblablement changer à l'avenir. D'un côté, les règles en vigueur dans la transmission du capital foncier est susceptible d'amener à son partage parmi les ayants droit. De l'autre côté, la forte dynamique d'urbanisation que connaît la région de Dakar, et qui se traduit par l'arrivée, chaque année, de 100'000 nouveaux habitants (Ba, 2008), induit une demande très importante de surfaces pour la construction de logements et cela se fait systématiquement au détriment des zones agricoles. Cette marginalisation foncière du maraîchage et des producteurs est encore plus accentuée par l'ambiguïté du régime foncier sénégalais (Mendret, 2006 ; Dahou & Ndiaye, 2009 ; Guèye et al., 2009) et la non-prise en compte de la pratique agricole dans les documents cadre de gestion du territoire (Kedowide et al., 2010). Pour ces différentes raisons, la question de l'insécurité foncière apparaît comme le principal sujet de préoccupation exprimé par les agriculteurs.

Des contraintes d'eau en perpétuelle croissance

Les contraintes liées à l'eau s'inscrivent dans un contexte de compétition accrue entre les différents usages. L'importante pression démographique qui caractérise la croissance urbaine dakaroise se traduit par une surexploitation de la nappe phréatique dans l'ensemble des Niayes, Dakar y compris, ce qui a conduit à une remontée du biseau salé entrainant un processus de salinisation des réserves d'eau (Guèye-Girardet, 2010) dans les zones jadis très propices au maraîchage. Pour cette raison, les producteurs cherchent des alternatives, en s'équipant en mini-forages permettant de puiser l'eau à une profondeur plus importante (jusqu'à 12 m) ou en se raccordant au réseau de distribution de l'eau de la SDE. Cette deuxième solution, malgré son coût plus important, serait de nature à répondre de manière plus adéquate aux besoins en eau des maraîchers urbains. Dans les faits, elle s'avère fragile, tant en raison de la priorité donnée par les instances publiques à la satisfaction des besoins domestiques qu'en raison du déficit dans la distribution d'eau potable (de 162.000 m^3 par jour en période de pointe (Sposito, 2010)), dont souffre la région de Dakar. C'est pourquoi, pendant les pics de chaleur de l'été, les zones agricoles comme Lendeng irriguant avec de l'eau fournie par la SDE subissent des coupures récurrentes pouvant durer toute une journée. Pour faire face à cette situation, certains producteurs n'hésitent pas à employer des ouvriers pour s'occuper de l'irrigation pendant la nuit.

III. CONCLUSION

Aujourd'hui, les impacts économiques de l'AUP d'une part en termes de productions et d'autre part en termes de revenus financiers particulièrement dans les pays en voie de développement comme le Sénégal ne sont plus à démontrer (Padilla, 2005 ; Aubry et al., 2010 ; De Bon et al., 2010 ; Chagomoka et al., 2015). Plusieurs travaux ont mis en exergue l'apport des activités agricoles urbaines dans l'approvisionnement alimentaire des ménages agri-urbains sous le prisme d'une activité d'autoconsommation (Casale, 2006, Mfoukou-Ntsakala et al., 2006, Olahan, 2010). Si cette réalité n'est pas à sous-estimer, force est de constater que l'AUP revêt de plus en plus un caractère marchand dans les pays en voie de développement et récemment dans certains pays développés (Aubry, 2013 ; Toullalan, 2012), ce qui lui permet de mieux jouer son rôle en matière de sécurité alimentaire des ménages agri-urbains. Notre recherche démontre que la vocation purement commerciale du maraîchage dakarois, du fait des revenus qu'il génère (cinq à six fois supérieurs au SMIG sénégalais), fait de cette agriculture si particulière une activité urbaine à part entière, à même de faire face à l'ensemble des dépenses des ménages : alimentation, logement, éducation, santé. Et ceci de manière légèrement meilleure que la moyenne des ménages urbains dakarois. A la lumière de ces constats, son maintien, voire son extension, dans le tissu urbain ferait entièrement sens. Paradoxalement, ce qui relève d'une évidence socio-économique doit de plus en plus composer avec des contraintes environnementales (disponibilité d'eau) et surtout avec une pression foncière constante, qui résulte d'une croissance urbaine chaotique et mal maîtrisée, menaçant, à terme la survie même de l'AUP à Dakar.

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The Impact of Forests in Climate Change

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Abstract— *As parallel to industrialization and increasing population, pressures on natural resources have rised, soil, water and air have been polluted. These problems threaten the human. So, environmental protection or in awider meaning nature conservation became a very important duty for the human in this century. The duty is aspecial action against eradication of living base for the organisms Forest ecosystems are of course economicalnatural resources. The ecosystem is a monotonous forest area by compositions, characteristics andrelationsihips of the main elements within the forest. In the article, firstly, forest ecosystems and their functionswere examined, then, importance of forest as natural resources, their roles for preventing risk of climaticalchanges, and contribution of forests for sustainable development within Turkiye were studied.*

Keywords— Climate change, Forest resource, Forest Ecosystems.

I. INTRODUCTION

At the beginning of the environmental problems threatening the world in recent years is the problem of "global warming and climate change". The importance of global warming and climate change, economic, ecological and sociological problems that are effective in almost every part of the world is increasing and it is noteworthy.

Since global issues can only be solved by global cooperation, the Framework Convention on Climate Change, which was adopted in 1992 at the Rio de Jenario Environment and Development Conference and entered into force on 21 March 1994. This contract was strengthened by the Kyoto Protocol in 1997.

The region in which Turkey is located faces water shortages, drought and soil erosion problems, Turkey places the harmful and violent effects of global warming among the countries that will live first. In this respect, Turkey participated as the 189th party to the Framework Convention on Climate Change as of 24 May 2004. Turkey's per capita responsibility for producing carbon dioxide emissions, which cause global warming, is less than in other OECD and European Union countries. However, since 1980, Turkey's energy-related gas emissions have increased and can be avoided, necessitating the change of existing technology. In order to control energy-related environmental problems in the name of sustainable development and to minimize the negative environmental impacts of energy activities, Turkey should set energy-related policies well and set targets closer to "renewable energy" in particular

"Global warming and climate change" emerged as a result of industrialization, energy production, the destruction of forests and other human activities, especially in the use of fossil fuels, is one of the biggest environmental problems that threaten the world. This problem is growing more and more with economic growth and population growth. Global warming is the process of artificially raising the temperature of the earth's atmosphere with the atmospheric layers near the earth as a result of the intensive increase of the greenhouse gases in the atmosphere as a result of various activities of the people. Global climate change is a change in other climate elements (precipitation, humidity, air movement, drought, etc.) due to global warming.

It has been estimated that human beings have not undergone very large changes in the world climate and temperatures. However, the findings of climatologists show that this situation does not remain the same throughout the history of the world, and that the world climate system is far from being a stagnant structure. Cold periods that last for tens of millions of years after warm periods that last for hundreds of millions of years, warmths that last ten thousand years in these periods, and relatively light, cold, hot periods that last for tens or hundreds of years.

According to some scientists, in the past 250 thousand years, the world was warming 1 degree. According to recent researches, the climate of the world, which should be in the cooling period nowadays, is not a cold turn; on the contrary, it shows that it has entered a hot round to the extent of danger. According to some researches, the world has warmed up to 1 degree from 1850 to 2000, while some other studies have shown that mean global temperature increases from 0.5 to 0.8 ° C from 1860 to sun. It is stated that the Industrial Revolution, which started in the year 1790, had a great effect. Dangerous aspect of this situation is that the speed of heating is doubled. Since 1979, the temperature has increased by 0.12 degrees every 10 years. The Intergovernmental Panel on Climate Change (IPCC), established by the UN, with over 150 countries, shows some evidence that climate change is alive now (Godrej, 2003).

The main reasons for global warming and climate change in the world are listed as follows:

World Movements: In the 1930's, the elliptical orbital orbits around the Sun's Earth have been proven by

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scientists who have been extruded every 95,000 years. This period brings to mind a hundred thousand years of ice age.

Earth Movements: Some climatologists suggest that continental drifts, mountain occurrences, changes in the magnetic field of the Sun, and sunspots may have an impact on climate change.

Increase in Greenhouse Gas Emissions: The most important cause of global warming is the atmospheric release of greenhouse gases. In particular, the increase of carbon dioxide (CO2) and methane (CH4) gases in the atmosphere increases the temperature of the earth's surface (CNN Türk, 2007).

Greenhouse gases inhibit the energy balance of the planet and cause the surface temperature to rise, preventing the infrared radiation reflected from the earth's surface from escaping to the far side. This effect of greenhouse gases is called "greenhouse effect of the atmosphere", which is called "global warming under the influence of greenhouse gases"

Other Reasons: The most important changes human beings make on earth with their own hands, apart from greenhouse gas emissions, are the rapid expansion of agricultural areas, the rapid destruction of forested areas, the desertification of semi-arid areas and the urbanization

For example, while the reduction of forest areas increases the amount of carbon dioxide and thus the greenhouse effect, desertification slows down global warming by reducing solar heat by increasing the amount of dust in the atmosphere. Urbanization is important not only because of the direct impact of global climates, but also because it creates heat islands that are warmer than their surroundings.

II. GLOBAL WARMING AND EFFECTS OF CLIMATE CHANGE

The effect of greenhouse gases on the emergence of earth's climate and climate has undeniable precaution. Greenhouse gases, by keeping some of the sun's rays reflected from the earth, allow the earth to stay at a temperature that people and other living things can live. If there were no greenhouse gases in the atmosphere, it is estimated that the average temperature of the earth would be 33 ° C colder than the daylight. Particularly during the last 30 years, the effects of global warming have been increasing, mainly due to technological developments, increased fuel consumption and population

growth, increased emissions of atmospheric greenhouse gases, and ozone depletion. Global warming is not just a phenomenon of increasing temperatures in every part of the world. According to the scientists; global warming will lead to extreme temperatures and fires in another region, while intense drought in a certain region of the world, and in another region, severe tornados and storms following the storms. The sea level rise will be seen. Along with the change of ecosystems, biodiversity will face the danger of extinction. As a result of global problems in the production of food, more poverty and illness will emerge. As a result of global warming, high summer temperatures, forest fires, reduced rainfall and water resources, drought and desertification, and so on. it is inevitable that Turkey will be influenced by negative changes as well as some countries.

Especially since the second half of the 20th century, natural disasters threatening the life of plants and animal species, especially humans, have begun to emerge with the rapid increase of greenhouse gases In other researches carried out on the same subject, the effect of human energy use on global warming was found to be highest (Kadıoğlu, 2007)

Sudden and unstable climatic conditions may make it impossible to find food because it can cause erosion, landslides, flood disasters, forest fires and desertification in arable areas, leading to the rapid disappearance of agricultural areas. Since the burning of forests, desertification, erosion events will cause more greenhouse gases such as CO2 to be released into the atmosphere, the effects of global warming will begin to be less visible.

III. CLIMATE CHANGE AND FORESTS

There is a two-way relationship between climate change and forest ecosystem. On the one hand, forests, ecosystems, lands, trees, trees and herbaceous plants; carbon dioxide in the atmosphere. In this regard, they play an important role in combating climate change by creating important carbon sink areas. On the other hand, however, during the forest fires is the release of organic carbon in the forests, forest soil and dead and alive cover to the atmosphere. In this way, if forests are not properly protected, they can play a role in accelerating climate change.

On a global scale, forest ecosystems hold about 3 billion tons of carbon dioxide released every year as a result of human activities. This means 35% of CO2 emissions from fossil fuels when calculated based on 2007 base of Carbon Dioxide Information Analysis Center. There is no less costly way to prevent deforestation to reduce 1 ton of carbon dioxide emissions. Therefore, it is of great importance to protect forest areas and to increase carbon capture capacity of forests to combat climate change (OGM; 2011).

Forests also play an active role in adaptation, reducing the impact of climate change. For example, forest ecosystems can mitigate the effects of climate change by reducing the effects of floods and erosion, and in the case of reduced agricultural production due to drought, they can benefit as additional income for the people of the region through forest fuels (OGM, 2011).

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On the other hand forest ecosystems, are sensitive areas. Forest fires are expected to be more frequent and more intense in forest ecosystems, especially due to climate change, due to increased disease and harmfulness in the trees and high temperature and hot weather fluctuations (IPCC, 2007). Each year, on average 1% of world forest areas are burned with various causes. The fires cause both the destruction of forests, which are important carbon sink sites, and the release of organic carbon held in these sinks into the atmosphere as carbon dioxide during combustion.
Therefore, forests play a crucial role in combating climate change in their right corridors, and they accelerate climate change if they are misused.
In this respect, forests should be less influenced by climate change, methods should be defined to help forest ecosystems to adapt to the inevitable effects of climate change, and strategies should be developed on the rational use of forests to combat climate change.
Tropical forests are the largest carbon deposits on Earth. 80% of the total carbon stock is in the tropical forests. Tropical forests are temperate zone with% i7, boreal with 3% (forest zone follows cold) (BROWN 1997).
According to the evaluations made in the 1980s, it is reported that all the forests in the earth have stored 830 Pg carbon (petagram = 1015 g = 1 gigaton = 1 billion tons) in total and that the amount stored in the soil is 1.5 times more than the storage in the vegetation (Brown, 1997). In this total budget, young temperate and boreal forests serve as a net repository, while tropical forests, which are constantly destroyed, emerge as a clear CO2 source (emissions). Globally, forests are a clear source of carbon, and the reasons for this include deforestation, particularly in tropical regions. However, proper management of forests will ensure that the clear CO2 emissions from the forests are stopped and serve as a clear repository. In this way, 11-15% of fossil fuel emissions can be stored in CO2 forests (Brown, 1997). Globally meaning forests with quantities of C stored in terrestrial ecosystems It is estimated that in 2005, 572 billion tons of stems (280 billion tons of Carbon equivalent) were carried; 33% in South America, 21% in Africa, 11% in Asia and 4% in Oceania. In 2005, it is estimated that the total forest carbon is 633 billion tons, which is equivalent to 160 tons of carbon per hectare. The total carbon in the forest biomass in Europe is 16% of the global total, while the carbon in the earth in Europe is more than 40% of the global total. Greenhouse gas emission rate (especially CO2) is calculated on the basis of biomass loss based on land use change and deforestation estimates. Globally, the rate of decline of forest carbon is estimated to be 1.6 billion tons per year, with 0.25% of total forest carbon. Tropical forests have an important influence both on input and output in global carbon budget. For example, forest vegetation in the Amazon region has 70 billion tonnes of carbon deposits, and deforestation between 1970 and 1998 resulted in atmospheric release of approximately 7 billion tonnes of carbon dioxide, equivalent to an average of 0.4 billion tonnes of carbon per year. Despite the uncertainties in estimating forest-related carbon emissions, there is no doubt about the significant role of forests in carbon sequestration and forest-wide emissions to the global carbon cycle.
This issue is discussed under specific headings during the international climate negotiations process, due to the special importance of climate change and fighting forests. The concept of REDD has been on the agenda for the first time in 2005, reducing the emissions from deforestation and deforestation in developing countries and protecting forests, the role of sustainable management of forests and increasing forest carbon stocks. Especially in developing countries with significant forest reserves of the world, such as rain forests, the importance of reducing greenhouse gas emissions resulting from deforestation and degradation of forests and protecting forests is emerging.

IV. CLIMATE CHANGE AND TURKISH FORESTS

In the 2007 IPCC Intergovernmental Panel on Climate Change (IPCC), it is stated that climate change, which includes Turkey, may destroy forest ecosystems due to climate change and thus may lead to socio-economic changes (OGM, 2011). Climate change is shown in scientific studies which will increase forest fires and tree-damaging insect population in Turkey and thereby increase deforestation (OGM, 2011).
The 12 million hectares of forests in Turkey correspond to approximately 60% of the forest area is located in the sensitive Mediterranean climate zone.
In addition to forest fires, harmful insects and diseases also damage forest areas due to the increase in temperature. Due to these insects that damage forests, the ability, quality and quantity of regeneration of forests is decreasing. Approximately 2 million hectares of pests (insects, fungi, etc.) are affected in the forest area every year in Turkey, causing an average of 1 million m3 of wood product loss (OGM, 2011).
With 78 million hectares of land, Turkey has a rich diversity in ecological care. In this richness, forests also play an important role as species and composition. According to the estimations made by the year 2015, forest areas occupy 28.6% of the country area. Trees without forests are not included in these areas.
With the Five-Year Development Plans, the forest inventory was started in 1963, and the forest inventory records of the whole country were made in 1980 and the forest inventory data obtained in 1980 was published.

According to the inventory evaluation results of this period; the overall forest area was 20.2 million hectares, the total tree wealth was 935 million m3, and the annual current increase was 28 million m3. An annual average of 23 million m3 of this increase is planned as a benefit for wood production. So; 5 million m3 increase every year, left in the forest for wealth accumulation (OGM, 2006).

After 1973, the information in the renewed plans was updated and the amount of forest area of the country in 1999 was determined as 20.8 million hectares. The forest area, which reached 21.2 million hectares in 2004, constituted 27.2% of the country's general area. The forest area, which was determined as 21.7 million hectares between the years 2005-2012, reached 27.7% of the country's general area and 22.3 million hectares between 2013 and 2015, reaching 28.6% of the country's total area

The various effects of forest ecosystem on the climate are listed as follows: Forests increase rainfall. Considering the forested and non-forested areas with the same conditions, according to the results of the research done in the former Soviet Union; the amount of rainfall in the forested area was found to be 50% higher than the forested steppe zone (Anonim, 1998 and Dağdaş, 2003). The forests increase the air humidity! For example, an oak tree raises the air humidity by an average of 570 liters of water per day, with an average of 20 tons of water per year roots of water per year, giving atmospheres to the atmosphere (Anonim, 1998). As the relative humidity of air increases, the vaporization power of the atmosphere decreases. For example, when the relative humidity is 20%, the evaporation power is 2000 bar, the relative humidity is 70%, and the evaporation power is 500 bar. In this respect, the water economy of the growing environment remains dormant and moist (Dağdaş, 2003).

The forests cleans the air we breathe! The findings of a research carried out are as follows: One hectare pine forest cleans and cleans 30 to 40 tons of spruce, 32 tons of spruce forest and 68 tons of beech forest (Anonim, 1998). The beech tree strains out, absorbing 700 Kg of dust and 300 Kg of venom in a year. Excessive contamination will alarm with deterioration of the torso. City forests like natural forests also prevent air pollution! Swallow the dust! Cleans the air! The results reached in the findings of a research are as follows: 420-850 in the city center of Hamburg. The amount of dust in mgr / m^3 was measured as 100 mgr / m^3 in the city park. Forest air contains 90-99% less tonnes of air in the city (Anonim, 1998). In another research, one liter of trees in the air without trees; 3-4 times more than trees and 10 times more dust than parks (Ürgenç, 1990).

Forests also compensate for temperature changes! A coniferous forest absorbs most of the solar energy from the "roof top" (1.3 g Kal / cm^2 / min). 61% of these are given again as atmospheres (Çepel, 1983 and Dağdaş, 2003). From here it can be predicted what the negative temperature effect will be when the forest cover is raised from the center. The

It has been determined that the fatty acid content of tea seeds of Artvin region is similar to the seeds grown in different countries in the literature. In the study, tea seed oil was compared with sunflower oil and linoleic acid content was found to be lower. This feature increases the stability of cooking oil.As a result, the tea seed oil from Artvin Turkey was defined as high quality cooking oil, like olive oil.

In addition, the composition of the outer and inner shell of tea seeds was also determined. These results indicate that these waste products are a serious holocellulose (62.1%) source. Mixture of outer and inner shell is including 33.1% and 41.4% α-cellulose and lignin respectively. Tea seed shells can be utilized at pyrolysis, alcohol production, fibreboard production and wood plastic composite production like wood and annual plants.

V. CONCLUSIONS AND SUGGESTIONS

At the front line of environmental problems that threaten the world are "global warming and climate change" and the ecological transformations that accompany it. The release of greenhouse gases (especially CO2, CH4 and NOX), naturally found in the atmosphere and preventing our Earth from over cooling, has shown a post-human increase in activity. As natural backwashing processes are challenged, the density of greenhouse gases has constantly risen and, as a result, a process is beginning to take place, with the average temperature rising on the surface of the earth. Since the mid-1990s, global warming and climate change have become an irrefutable fact of mankind

The forests that make up an important ecosystem of our country are climate sensitive. Because; Forests Prevent overheating of the atmosphere as it absorbs most of the short-wave solar radiation from the sun itself. It contributes significantly to the formation of rainfall. It also absorbs CO2 and produces Oxygen. It forms a strong micro-airconditioning area between the bottom of the leaves and the ground. It provides the protection of soil and bottom creatures against direct solar radiation, which is effective during the day, and adverse effects caused by extreme colds during nights and cold winter days (Kayhan, 2006).

Depending on the effects of climate change, degradation and shrinkage of forest areas is extremely important for our country. In particular, strategies and plans for adaptation of forests in Turkey to climate change need to be urgently developed when it is considered that 50% of Turkey's existing forest areas are degraded forests.

It is important to remember that forests play a key role in combating climate change with carbon sequestration, as well as by reducing the impact of climate change. From

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this point of view, methods should be developed to ensure that forest ecosystems are less affected first by climate change, methods to help forest ecosystems should be identified while adapting to the inevitable effects of climate change, and strategies should be developed on rational use of forests to combat climate change.

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A Case Study on Agro-based E-Commerce Portal

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Abstract— This paper has investigated the practice of E-Commerce portal named Metrotarkari for marketing vegetables and fruit items in Kathmandu valley. A case study approach underpinned the study so as to identify current issues and practice of E-Commerce portal for vegetable and fruit items thereby adopt appropriate strategies for its sustainability in this sector. The study used explanatory form of analysis on the issues of business model, payment system, distribution system, overall challenges and marketing strategies based on the face to face interview with chief operating officer of Metrotarkari. The result shows that their B2B feature is serving more customers than B2C feature does in daily basis. The cash on delivery has been the preferable option of payment system although they have facility of Paypal, E-Sewa and Sctmoco. The main reason behind the problem in maintaining and delivering quality items is the lack of their own inventory and their dependency on others vendors. Establishing their own cold store or inventory and appending the C2C feature in their existing portal are major suggestions made to provide benefit to farmers and customers thereby sustain in this sector.

Keywords— E-Commerce, Metrotarkari.com, business model, payment gateway.

I. INTRODUCTION

E-Commerce is the shortened term for Electronic Commerce [1]. It is doing business transactions and communications through computer networks and networks of personal linked computers via the World Wide Web [2]. E-Commerce has established itself sophisticatedly in the developed countries; however it is yet to make roots in most of the developing countries [3].

In Nepal, The vegetable crops occupy 7.3 percent of the total cultivated agricultural land [4] which indicates the increasing value of vegetable sector in Nepalese economy. Kathmandu is a valley situated in hilly area of central Nepal. However, It has high population density and ever increasing food demand land capabilities and cultivation potential seems diminishing. The valley encloses the entire area of Bhaktapur district, 85% of Kathmandu district and 50% of Lalitpur district. Its three districts, Kathmandu, Lalitpur, and Bhaktapur, cover an area of 899 square km [5].

Besides, the traditional agriculture markets, there are few online markets for foods and groceries in Kathmandu valley named as MetroTarkari, Bhatbhateni, Muncha, Meroshopping, Kaymu and Foodmandu which might be some solutions in agricultural market to deliver the fresh products with reasonable prices to the digital consumers but there should be proper governing body to ensure about their reasonable product price, product quality and customer satisfaction.

II. LITERATURE REVIEW

In a study entitled "E-Commerce in Nepal: a case study of an underdeveloped country", Ngudup concluded that even in countries with poor infrastructure and access to information technology, evidence exists that dynamic enterprises and governments have taken advantages of the possibilities offered by E-Commerce [6]. Countries with poor communication and internet infrastructures should therefore act now in order to develop a strong E-Commerce market to prevent landing on the wrong side of the digital divide.

Similarly, In a study "Barriers to E-Commerce and competitive business models in developing countries: A case study", Kshetri indicated that economic factors (high ICT access charge, low penetration rate of credit cards), sociopolitical factor (Nepal at level 0 in adoption of digital and electronic signature (DES)) and cognitive factors (related to knowledge, skill and confidence related to E-Commerce usage) play important roles in the adaptation of business models in the context of the developing world [7]. This paper illustrated influence of Thamel.com on its business partners' ICT adoption. It provided an overview on Thamel.com's strategy to overcome some E-Commerce barriers and to overcome cognitive barriers, the company provided delivery services as well as delivery confirmation via digital pictures of gift delivery [7].

Another study entitled "A Case Study of Electronic Commerce in Nepal" recommended three projects: a business-consumer (B2C) site for marketing Buddhist Thanka paintings via the internet, a series of vertically focused workshops bringing together members of the Nepalese IT community and members in industries which may be likely E-Commerce candidates, and the establishment of a village-connectivity pilot project [8].

In the global scenario, for marketing the farm and dairy products, Walmart at US has a section named as Walmart Grocery. 'Walmart Pickup' is the new service of Walmart Grocery [9]. In pickup service, customers can simply shop their grocery lists online, choose a time to pick up their orders and

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then pull in to a designated parking area at their local stores, where associates will load the items into their cars. There are challenges to bring an entire grocery store full of products to an online market place. The vast disconnect between supplier and retailer is one of the reasons why online grocery has been so slow to take off [10].

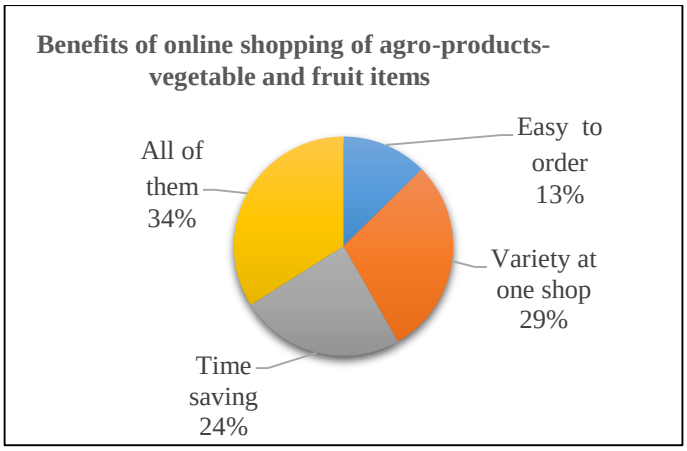

Fig.1: Benefits of online shopping of agro-products-vegetable and fruit items [11]

A study entitled "Consumer Attitudes Towards Online Grocery Shopping in Kathmandu Valley", K.C. and Timalsina mentioned that the major advantages of the online shopping of agro-products are easy to order, variety at one shop and time saving.

III. RESEARCH METHODOLOGY

This study used a single-case research design and primary data sources. Researcher selected an E-Commerce portal named Metrotarkari among various online portals in Kathmandu valley because it was only portal that was serving vegetables and fruit items along with other groceries to their customers in Kathmandu valley. The researcher visited its Kathmandu based office and interviewed the company's chief operating officer (COO). Subsequently, several rounds of email exchanges took place with him in Kathmandu.

Basically, a case study is an in depth study of a particular situation rather than a sweeping statistical survey. It is a method used to narrow down a very broad field of research into one easily researchable topic. A case study is based on opinion and is very much designed to provoke reasoned debate. There really is no right or wrong answer in a case study [12]. There are two different approaches to case studies; the analytical approach and problem oriented method [13]. In this study, researcher followed both approaches.

IV. RESULTS AND DISCUSSION

The result consists of the business model of Metrotarkari, payment systems, distribution system and marketing strategies and current problems it is facing. The result is presented as follows:

A. Business Models

Regarding business models, Metrotarkari follows B2B and B2C transaction model. In B2B model, Metrotarkari is serving several restaurants, schools, canteens, INGOs, NGOs etc. every day as per the requirement. Especially in the morning time their van delivers the grocery items from vegetables to dry items to other small business units. They make offline contact with these units. Mostly phone call is used rather than internet in this transaction model. In B2C model they are serving their customer directly who approach them either through website or phone call. Through this model farmers are not getting the reasonable amount where customers are charged high due to the intermediaries between them. Intermediaries between them are dealers, Kalimati vegetables and fruit center/wholesaler, sub dealers and retailers. Analyzing this distribution system it is found that farmers are not benefited by Metrotarkari, only few customers are benefitted in terms of saving time and easiness regarding shopping style. Apart from this model, another popular model is C2C where a customer can directly sale their products to another customer. In case of agricultural products the real producers, the farmers, can directly approach their fresh product to the customers. The benefit of this model is that the farmers get reasonable amount of their effort where customers get fresh product. COO of Metrotarkari seems positive regarding the integration of C2C features with the existing model. But there are few problems in current agricultural market of Nepal. First of all, the intervention of intermediaries is high. There are three to five levels of intermediaries before the product reaches from farmer to customer. The agro-products come from India, China and different parts of the nation to Kalimati via different intermediaries. One of the barriers regarding the implementation of this model is low literacy rate of farmers and lack of awareness regarding the use of IT and E-Commerce [14].

B. Payment System

The major headache for online business is payment system. There is no such payment gateway system to purchase the product directly from out of country. Nepal Rastra bank hasn't set up such provision yet for user to use their national account to pay directly from abroad for the goods they purchase online. Recently, Metrotarkari has used 'paypal payment gateway system' for those who want to purchase the goods from abroad to their relatives inside country. There is still a problem on it. They don't have their Paypal account institutionally but a personal account of a shareholder from abroad. Inside country they are trying to integrate the local payment gateway system such as E-Sewa, Sctmoco, I-Pay. However, there are problems regarding the integration of these systems. Metrotarakri found that these local payment systems service charges are high for them and the banking systems are not flexible as per their requirement.

Thus, the cash on delivery is only the best option for payment from customers. However, it is also not free from drawbacks.

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In other business there is provision of taking advance from customers as they are confirmed to buy the product or service. But in case of online business there is no such provision. The only basis of payment is the trust and faith between these two parties. So there is always risk on revenue generation from cash on delivery payment option. The fraud customers and mistrust of public on online business is another risk on this option.

C. Logistic Management

Metrotarkari has own inventory for dry grocery items but not for vegetables, fruits and dairy items. They are using another vendor's store for it. Whenever customer orders vegetable items, first of all they confirm the order with exact location. Then they decide on the vendors for shipping the ordered items. Currently Metrotarkari has three main vendors in Kalimati, Baneswor and Nakkhu who provide the ordered items to them. The distribution team of Metrotarkari consists of three staffs along with a delivery van. The job of this team is to receive the confirmed order, contact the vendors, check the quality, and package the items, location tracking and deliver the right items to the right customer at right time. They also receive the cash and the item in case of return from the customers.

D. Marketing Strategies Based on Marketing Mix

a) Product/Service: Metrotarkari is providing service regarding vegetables, fruit items and more than 30 new seasonal items. They are providing free home delivery service with return if customers are not satisfied. They are offering the particular items those are highly consumable during festivals and cultural events such as ghee and chakku in the first of Magh, valentine special gifts during valentines week, Kwaati during Janaipurnima and so on.

b) Place: Mainly, Metrotarkari delivers its service inside ring road of Kathmandu Valley and 3 km periphery of the ring road. They also serve in the main areas outside the ring road such as Bhaisepati, Hattiban, Budhanilkanta, Dhapasi and many other core areas.

c) Price: The price of the goods is similar to the current market price. It additionally provides assurance on quality and return in case of damage. Regarding gift voucher and incentives to customer Metrotarkari found reward point system that they have been providing to their customer is ineffective on their business. Thus, they are seeking other vendors to reward repeated customers.

d) Promotion: Metrotarkari is in growing stage. Promotional activities will certainly help on their business. They think that they are not ready for aggressive promotional activities yet. They advertise their portals by using social media such as facebook page called Metrotarkari. They have been participating in various social and fund raising events. Last time they participated in Idea studio program hosted by Nepal television.

E. Current Problems

The major problem that Metrotarkari is facing regarding the quality and variety of products because for vegetable items they are dependent on different vendors. If vendors do not select and package the ordered items properly then there is always a risk on items to be returned by the customer and also the bad impression to them. Ultimately, they can lose the customers' trust and chance to lose them as well. The another problem is in home delivery. Due to the traffic congestion and difficulties in finding exact location of the destination in Kathmandu there is always some delay on delivering the ordered items. Moreover, the vegetables items are very sensitive in case of their freshness. Maintaining the correct temperature during delivery which poses a threat regarding the maintenance of freshness. There is always a chance of damage or degrading on freshness and quality of such items which may lead to the customers' dissatisfaction.

F. SWOT analysis of Metrotarkari

a) Strengths:

- Ordered item distribution system
- Free delivery strategy
- Product varieties-more than 30 seasonal items which is not available in local market easily
- Increasing number of customer in B2B model

b) Weaknesses:

- Inadequate research on market and customer satisfaction
- Lack of adequate facilities
- No precise delivery timing
- Compromise in product quality

c) Opportunities:

- Growing internet and Smartphone users
- increasing Publics' literacy rate and awareness is increasing
- Advertisement on Social media such as Facebook and Twitter
- increasing number of customers

d) Threats:

- New competitors based on online groceries such as Sastodeal, Chizbiz etc.
- Traffic congestion and difficulties in location finding
- Unmanaged urbanization
- Payment system complexities

The finding of the present study is different in the Latin American background where it is found closer in Indian background. As per the report by Statista, in Latin America the majority (65%) of online shoppers preferred to pay via credit card [15] . A total of 36 % of shoppers opted for digital payment systems where 35% shoppers preferred cash on delivery option [15]. On the same context, preferred payment method of online shoppers in India was to pay via cash on delivery. Cash on delivery forms an important aspect of the online shopping website in Indian online shopping market [16]. In a study based on Nepalese context, K.C. and Timalsina found that 62 % People have chosen for very important option

while shopping online groceries [11] . The present study, to some extent, supports the finding of Zwass . He found that B2B transactions are of larger volume and value, higher risks, less buyers, and different way of making purchasing decisions in comparison to B2C transactions [17].

V. CONCLUSION AND RECOMMENDATION

The result showed that though there is lack of inventory and dependence on other vendors B2B feature of Metrotarkari.com is serving more customers than in B2C feature in daily basis. The cash on delivery is the preferable option for payment although they have facility of Paypal, E-sewa and Sct-Moco. Traffic congestion and difficulties in finding exact location of the destination in Kathmandu is major reason for delayed delivery.

After analyzing the business model; payment and distribution system, the researcher has found that the current business model and distribution system seems not so beneficial for producers of agro-products. In terms of intermediation they are like other retailers. The price of Metrotarkari is similar to other retailers but Metrotarkari is providing home delivery with assurance of quality. Adaptation of another model is necessary to provide benefits to the genuine producer. Hence, the researcher suggests Metrotarkari to be both farmer and customer friendly helping the farmers sell their product in bulk amount with getting reasonable price where customers can get fresh items directly from farmer in 15-20% less price than before implying the win-win situation. For this, Metrotarkari team is suggested to establish the cold storage and a good distribution team to carry fresh items from farmers in bulk amount. In order to facilitate the professional farmers, the research suggests to integrate C2C feature in their existing portal especially in local product section.

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Assessment of local pollution influence on the weather and impact on the air quality over subtropical southern Africa

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***Abstract*—** *This study discusses the possible role of air pollution and influence on air quality (AQ) and weather over southern Africa. Although it has long been thought that a lotof pollution occurs in the industrialized regions of the Northern Hemisphere, it is quite clear that a vast amount is also generated in the south, and southern Africa contributes significantly. Since industrial revolution, the sub-continent has become one of the major sources of atmospheric emissions mainly from human-induced activities to meet demand for energy supply and other lifelong needs. The main sources include biomass burning (BB), Aeolian dust and industrial emissions. In the process, trace components generated can negatively impact the atmosphere through aerosol influence on cloud properties and radiation budget, and also affect human health. While anthropogenic emissions are enhanced during winter, natural emissions occur and fluctuate throughout the year. In the case of dust aerosols, they are common over the Kalahari-Namib Desert where dust devils frequently develop overland, keeping the air filled with haze.Commongas pollutants include carbon monoxide (CO), sulphur-and nitrogen oxides($SO_x$and NO_x) from the copper belt(Zambia),Highveld(South Africa), BB-dominated areas and other isolated locations. In particular, a high degree of correlation between weather and pollution in urban source centers and the effect of weather on human health are of special interest. In most cases, humans are exposed to high pollutant levels likely exceeding AQ standards. We propose that more attention should be paid to the rapidly increasing regional pollution levels, as experience from other regions suggest that this can alter climate and AQ composition.*

Keywords*— *air pollution, weather, air quality, anthropogenic, human health.

I. INTRODUCTION

1.1 Climate and atmospheric chemistry

Southern Africa is characterized by tropical and subtropical climate conditions, and is influenced by the long coastline from the Indian to the Atlantic Oceans. The climate is dominated by a hot-wet (summer) season running from October to March and a cool-dry (winter) season from May to August .Consequently, the sub-continent is a large source of atmospheric pollutants, both natural and anthropogenic (Piketh et al., 1999), with a complex mixture of aerosols ranging from combustion products such as biomass burning (BB), domestic fires, fossil fuels (automobiles and industries/manufacturing) and dust particulates. These emissions can have a direct impact both on the local and global atmospheric pollution, providing large sources of chemical gas- and particle phase into the atmosphere.The negative atmospheric impact due to trace components–referred to as 'air pollution', occurs when harmful substances are introduced into the atmosphere, making the air not comfortable for living. Example can be photochemical smog or dust storms at the earth's surface. Pollution is influenced by species concentration and increases when the rate of emission production is higher than removal processes. Some of the gaseous pollutants -referred to as greenhouse gases (GHGs) can be very harmful in the atmosphere and influence the solar energy budget.

1.2 Pollution, weather and air quality

Pollution can have direct and indirect effects (e.g. acidification, eutrophication, stratospheric ozone depletion) with a wide range of impacts on human health, ecosystems, agriculture and air composition. It can be influenced by several factors: weather (or meteorological) components such as wind over/near an emitting source region, chemical transformations in the air, polluting sources (some sources are stationary while others are mobile) and transport mechanisms. To date, industrialization has impacted on the air quality (AQ) through emission of gases and particles and is also attributed to climate change. The weather and AQ are linked through human health as people can be exposed to higher concentrations of air pollutants and suffer disproportionately from effects of deteriorating AQ.For example, people in large populated cities are often exposed to high levels of pollution and can develop respiratory ailments that hinder their comfortable lives (Schwela, 2012).Aerosol pollution may aggravate heart and lung disease and is associated with heart attacks and

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cardiac arrhythmias, difficulty in breathing (Jhun et al., 2015, and references therein), and makes people more susceptible to respiratory infections.

Just like weather, pollution can change hourly or even daily (USEPA, 2003) from one place to another. AQ pollutants may also have climatic effects; depending on the pollutant, which may be warming or cooling, or a combination of the two (AQEG, 2007). The main cause of air pollution by humans isuse of fossil fuels, power generation, industrial and other domestic operations (e.g. burning of firewood, agricultural activities and waste). Becauseof wide-ranging air pollution problems, governments and other organizations such asthe World Health Organization (WHO) put in placesome governing standards and regulations to monitor the amount of emissions in the atmosphere.AQ guidelines provide an assessment on health effects due to air pollution; threshold limits are set to ensure that the total average concentrations of pollutants do not exceed certain range or levels within which human can be exposed to.

The purpose of this study is to highlight the role of regional pollution, and possible influence on the weather and AQ over southern Africa. We are motivated by the fact that the sub-continent experiences a significant amount of pollution in addition to its varying climatic conditions. The rest of the paper is arranged as follows: section 2 givesan overview of the climate and pollution over southern Africa; section 3 is the methodology and data acquisition; section 4 focuses on results analysis. Lastly, section 5 is the conclusion on the state of AQ and pollution.

II. CLIMATE OUTLOOK AND POTENTIAL DRIVERS

2.1 Climate pattern

Southern Africa is prone to frequent droughts and uneven rainfall distribution withtwo distinct seasons (a hot-wet summer and cool dry winter) that significantly determine and control its climate. The most influential factors include the Inter Tropical Convergence Zone (ITCZ), ocean currents, and quasi-stationary high-pressure systems (St. Helena High in the South Atlantic and Mascarene High in the Indian Ocean).Much of the rainfall isreceived between November and February –the period corresponding to the southward displacement of the ITCZ in the Southern Hemisphere (SH) (maximum displacement occurs in January), whereas the dry season occurs when the ITCZ retreats northward (maximum displacement in July).ITCZ annually changes its position on either side of the equator, moving between the Tropics of Cancer and Capricorn (Fig. 1).Its position shifts the belts of planetary winds and pressure systems to the north and to the south annually.

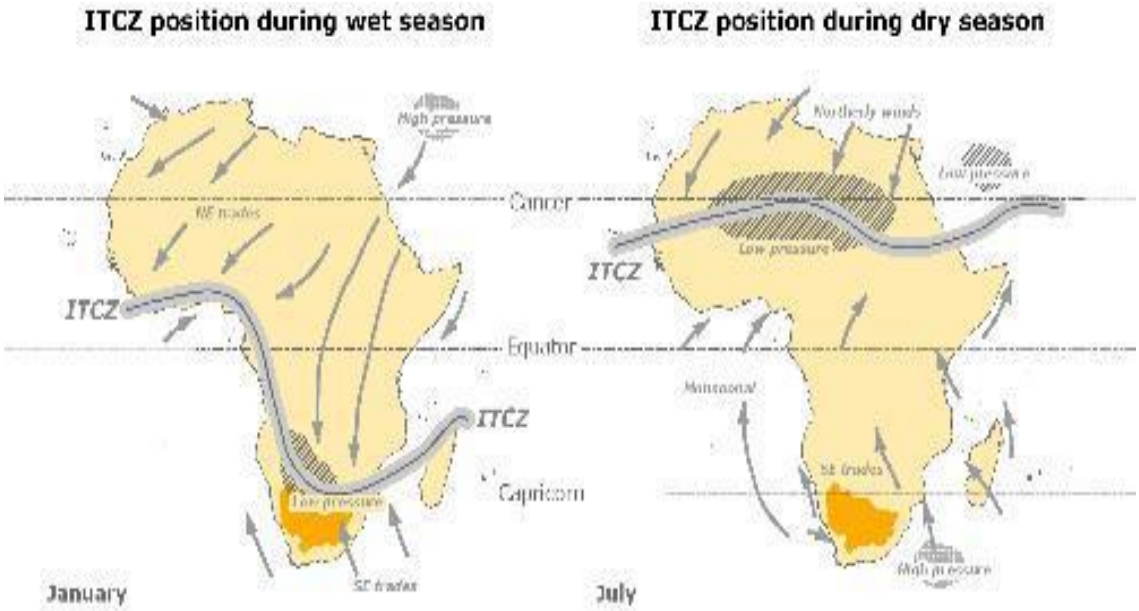

Fig. 1: Southward and northward displacement of the ITCZ between the wet (summer) and dry (winter) seasons in Africa. Source: (http://www.orangesenqurak.com/river/climate/basin/regional.aspx?print=1

The South Atlantic and Indian Oceans also play an important roles in the climate. For example, the east coast is influenced by the southward-flowing Mozambique Current(bringing warm water and humid air from the equator, creating a humid warm climate). Contrastingly, the west coast is influenced by the cold Benguela Current from the Atlantic Ocean–which produces a drier climate. There is a strong rainfall gradient over the interior, running from east to west, mainly occurring during summer in the form of thunderstorms. High daily and

seasonal temperature ranges are also observed as a result of altitude and interior "continental" location (i.e. lack of ocean influences).Total rainfall gradually decreases westward; much of the central and western regions are semi-desert with low and unreliable rainfall. An exception is the southern and western Cape regions of South Africa (this is influenced by maritime conditions and receive winter rainfall as part of a more temperate climate). These parts receive their winter rainfall due to cold fronts rolling from the Atlantic Ocean (Hudson and Jones, 2002) when they are embedded in the westerly wind regimes (Obasi, 2001).

2.2 Transport climatology

Air transport climatology over subtropical southern Africa is classified as daily synoptic situations into predominant circulation types. Percentage zonal transport in easterly and westerly directions;total transport is a function of circulation type and frequency, as well asplume dimensions (Tyson et al., 1996a,b).Two major pathways–the Natalplume (eastwards) and the Angolanplume (westwards)are the main carriageways of pollution overland.A typical example is the so-called "river of smoke"outflow from the mainland into the Indian Ocean (Swap et al., 2003). The semi-permanent subtropical continental anticyclones, transient mid-latitude ridging anticyclones and mid latitude westerly disturbances produce major transport into the south-western Indian Ocean along the Natal plume, whereas the quasi-stationary tropical easterly waves result in appreciable transport into the tropical South Atlantic Ocean through the Angolan plume.

Detailed circulation patterns over southern Africa is given by Tyson et al. (1996a)and Garstang et al. (1996), who described for example, four major circulation types occurring with different frequencies:semi-permanent subtropical anticyclones, transient mid-latitude ridging anticyclones, westerly baroclinic disturbances and barotropic quasi-stationary tropical easterly waves. According to the authors, transport in ridging highs and westerly perturbations are much less and occur throughout the year, with a slight tendency peak in spring.Monthly, seasonal and annual mass fluxes over and out of southern Africa form transport fields with substantial aerosol concentrations.

2.3 Potential climate drivers

2.3.1 El Nino southern oscillation

El Niño–Southern Oscillation (ENSO) is an irregularly periodical variation in winds and sea surface temperatures (SSTs) over the tropical eastern Pacific Ocean, affecting much of the tropics and subtropics. The Southern Oscillation is an accompanying atmospheric component, coupled withSST change. The warming phase(accompanied with high surface pressure) is known as El Niño, while the cooling phase (accompanied with low air surface pressure in the tropical western Pacific) is known as La Niña. The two phasescan each last for several months and their effects vary in intensity.El Niño events often begin in the middle of the year with large-scale warming of surface water in the central and eastern equatorial Pacific Ocean and changes in the tropical atmospheric circulation (WMO, 2014).

Generally, El Niñoevents occur every two to seven years and can last up to 18 months; they peak during November–January and decay in the first half of the following year. Strong and moderate El Niño events have a warming effect on the average global surface temperatures (WMO, 2014).Over southern Africa, El Niño usually results in less rain, and normally drought soccur particularly during the critical agricultural period of October to December, and isa major driver to climate variability affecting rainfall (Nicholson and Entekhabi, 1987).For example, taking into account the magnitude of negative impacts in agricultural areas world wide,El Niño of 1992 was classified as themost severe event in 30 years (FAO, 2014). As a result, the economic sector is likely to be affected noting that more than half of the region's population depends on agriculture.

2.3.2Sea surface temperatures

SSTsdefine the predictability of climate in sub-Saharan Africa. This is warm (cool) in the southwest Indian Ocean and cool (warm) in the southeast Indian Ocean; increased (decreased) summer rains may occur over large areas of southeastern Africa. For example, El Niñois the dominant pattern of variability at global scale associated with droughts in the northern and southern hemispheres (Giannini, 2010).SST poles are reversed in sign, decreased precipitation occurs over southeastern Africa as a result of increased low-level divergence or low-level flow and this flow being drier than average.

2.3.3Monsoon effects

Monsoon is traditionally defined as the seasonal reversing wind accompanied with corresponding changes in precipitation. Two main components of the African monsoons are the West African monsoon (prevail during the Northern Hemisphere (NH) summer (June–September)), and the East African monsoon(common during spring (March–May) and autumn (October–December)).A combined influence of the Indo-Pacific and the Atlantic Oceans drive the inter-annual and the decadal monsoon variability over these regions.Key features of the West African monsoon are the low level southwesterly flow from the Atlantic Ocean and the ITCZ north of the equator. For example, the West African Sahel is well known for its severe droughts that ravaged the region during the 1970s and 1980s (Nicholson, 2013). East African monsoon is associated with the ITCZ

http://dx.doi.org/10.22161/ijeab/3.1.28 *ISSN: 2456-1878*

moving south of the equator. The land-sea contrast forces the summer northern movement of the ITCZ to be displaced further than it would otherwise be (Bigg, 2003). Long-term precipitation changes from both northern and southern regions of Africa are linked to the monsoon circulations and controlled by precessional variation in summer insolation (Schefub et al., 2005, and references therein). The so-called long rains prevail during spring and the short rains during autumn. The transition (equinox) between seasons brings most rainfall to East Africa.

2.4Sources of air pollution

There are three major air pollution sources defined over Africa:eolian dust, biomass burningand industrial (anthropogenic) emissions (Fig. 2), contributing to the overall global emissions (Piketh and Walton). However, several other common sources also contribute to the overall air pollution over southern Africa, including urbanization, motorization, economic activity, fossil fuel usageand open burning (including vegetative fires and waste burning).

2.4.1 Biomass burning

While domestic fuel (e.g. wood and coal) is widely used in most parts of Africa, the largest source of BB comes from vegetation (Swap et al., 2002; 2003).Human-induced BB is the main source of aerosols in the SH (Eck et al., 2003; Torres et al., 2010) contributing over 86% of the total global emissions of black carbon (soot) -the largest source being the African savannah (Skaeda et al., 2011, and reference therein). BB dominates the chemical atmospheric burden of emissions over most parts of Africa (Crutzen and Andreae, 1990; Liousse et al., 2010; Scholes, et al., 2011), especially during the dry season (Matsueda et al., 2002; Li et al., 2003; Chédin et al., 2005; Magi, 2009). This contributes about 30% of global burning emissions (Smith et al., 2001; Pikethand Walton, 2004), followed by wind-blown dust and industrialemissions respectively. Savannah fires are the global largest source of BB mostly in the tropics (Andreae et al., 1996; Seinfeld and Pandis, 2006), contributing about 75% of all related fire emissions (Piketh and Walton, 2004) with more than 60% of the earth savannah coming from Africa (Li et al., 2003).

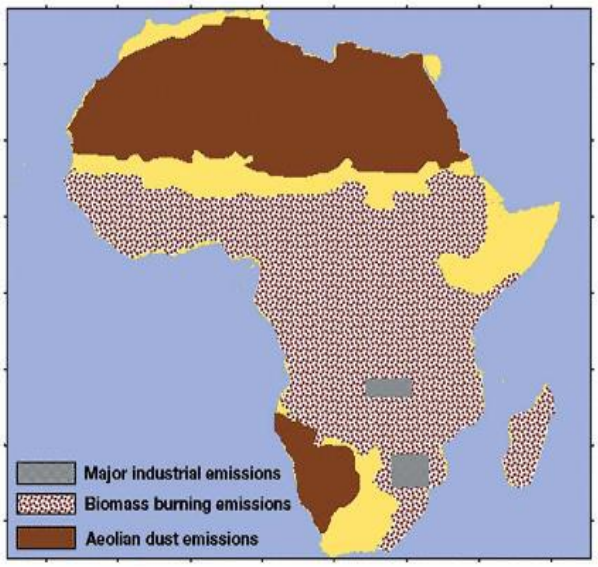

Fig. 2: Major emission sources over Africa, showing dominant source categories. To a lesser extent, other sources do contribute significantly, although not as large as these. (Source: Piketh and Walton, 2004).

The bulk of burning occurs in tropical central Africa (Fig. 3), compared to the much drier north and south extremities. Intense burning reaches its peak around May–Augustand drops towards summer (September–October) -marking the beginning of the wet season (Piketh et al., 2002; Tummon et al., 2010, and reference therein).Figure 3 showsBB intensity over Africa in 2005, indicating the seasonal fire patterns. The images are based on fires detected by the Moderate Resolution Imaging Spectroradiometer (MODIS) from the National Aeronautics and Space Administration (NASA)'s Terra and Aqua satellites. Each image is a 10-day composite of fire detections (marked in red and yellow); the series include images from every other 10-day period from 1 January through June to 18 August 2005. Red colour indicates locations with few fires detectedduring the 10-day period whereas yellow indicates many fires detected over an area.

2.4.2 Industrial/Anthropogenic emissions

The most significant industrial trace gases emitted over southern Africa include sulphur- and nitrogen oxides (SO_x and NO_x), carbon monoxide (CO)and other gas compounds. Most of the gas emissions are concentrated over the Mpumalanga highveld (South Africa), the copper-belt (Zambia) and other isolated points such as theBamangwato Concessions Limited (BCL) mine in Botswana.However, BCL is currently not operating, after being placed under liquidation in 2016. Also, there is a large amount of aerosol pollution generated from various source sectors such as cement and timber processing, construction/manufacturing sites, mining and other operations across the sub-continent.

Some studies(e.g. Benkovitz et al., 1996; Meter et al., 1999) had proposed that emissions from industrial emissions contributed up to 2,24 Mt per annum.For example, it was estimated that the spatial distribution contributed a total of 1.1 Mt of sulphur emitted into the regional atmosphere annually (Sivertsen et al. (1995). Out of this, 66% was said to come from South Africa alone (Piketh et al., 1999), from which about 90% was from the Mpumalanga's highveld area (Sivertsen et al., 1995; Wells et al., 1996). South Africais the most industrialized country in southern Africa (Piketh and Walton, 2004), and one of the world's most carbon intensive countries with per capita emissions higher than most European countries (DEA, 2014).The Cu-Ni and smelter plantin Botswana has been one significant source of sulphur emissions (before its closure), accounting for 0.19 Mty^{-1}. During winter(when anthropogenic emissions increase) it would be expected that industrial and BB emissions become well mixed over land.

2.4.3 Aeolian dust

Desert dust is particularly the main aerosol component in many arid and semi-arid regions (e.g. Sahara and sub-Saharan Africa: Zakey et al., 2006). These emissions (emitted through suspension, saltation and creeping processes) occur mostly in episodic events determined by threshold near-surface wind conditions. Naturally, dust aerosols originate from the local bare soils (devoid of vegetation or grass). Dust can be a regional scale climatic forcing agent when vast dust plumes are suspended into the atmosphere.This is particularly true for regions wheredust plumes are suspended into the atmosphere andtransported over hundreds to thousands of kilometers. Large amounts of mineral dust can be transported across, which in combination with other emissions lead to persistent haze because of lack of wet removal (Knippertz et al., 2015). The fine dust particles can be lifted up to higher altitudes and transported over long distances away from their source regions. Their effects can be felt not only locally, but also at places/regions far awayfrom their source regions (Zakey et al., 2006).

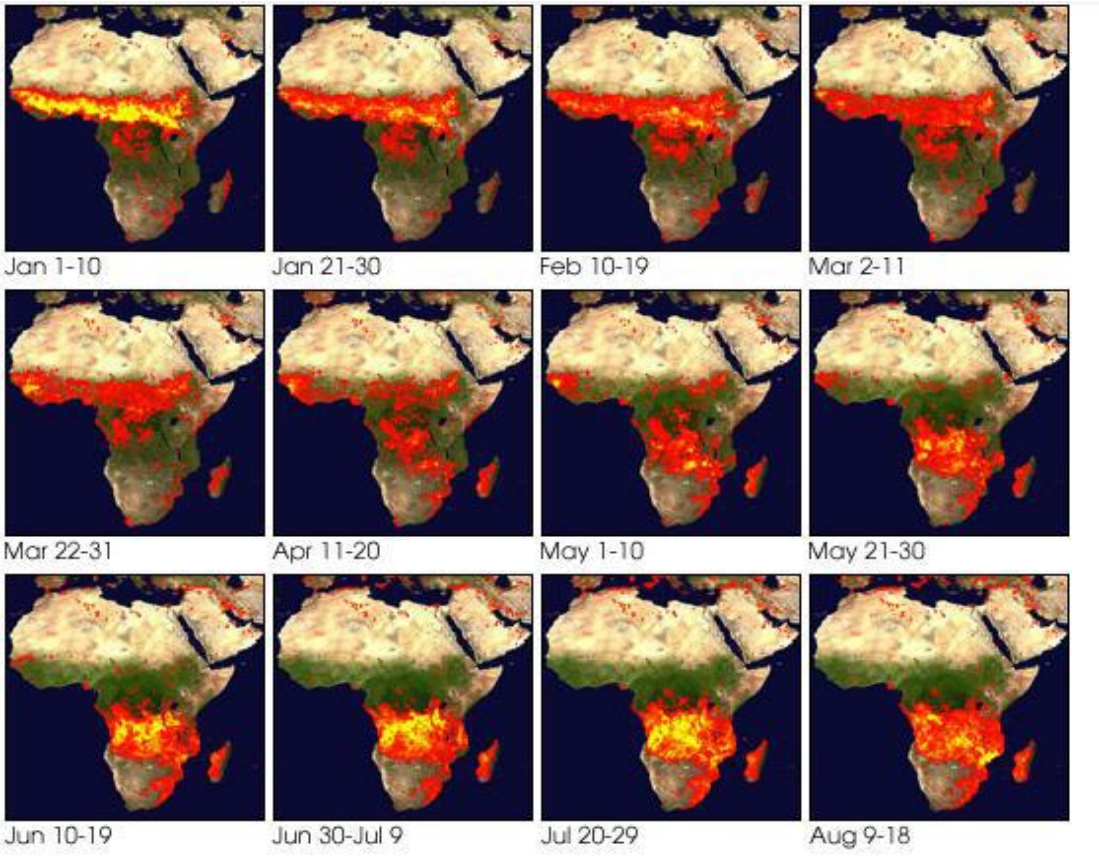

Fig. 3: Biomass burning intensities between 1 January and 18 August 2005;the highest burning intensities occur from May to August (dry season). Source: (https://earthobservatory.nasa.gov/IOTD//view.php?id=5800).

Africa has vast tracks of desert dust aerosol emissions, mainly from the Sahara Desert (North Africa) and the southwest coast of Namib Desert (southern Africa).Strong winds known as "berg winds" (mountain winds) can loft plumes of dust directly out into the Atlantic Ocean depending on the wind speed and direction (Piketh and Walton, 2004).

2.4.4 Automobiles and othersources

The rapid, unplanned and uncoordinated town/city growth has negatively impacted population centers in different regions, resulting in many people moving between the

city centers to urban area outskirts. High population increases together with pollution levels seriously compromise existing transportation systems and significantly increase the challenge of creating future transportation systems (Road management, 1998).Populationgrowth naturallyinfluences car ownership (privately owned).For example, motor vehicle fleets have recently increased in countries such as Botswana and Zimbabwe (Simukanga et al., 2003), due to imported second-hand vehicles no longer meetingcertain standards intheir countries of origin (Wiston, 2017). These imported vehicles are not properly maintained or checked on how they emit pollutants.This increase in number of vehicles results in an increase in fuel consumption.For example, in city centers where traffic congestion can be responsible for 90–95% of CO and 60–70% of NO_x(Schwela, 2004). NO_x(where NO_x = NO + NO_2) and hydrocarbons pose a major threat to human health and natural resources (Chanda, 2014). These emissions can contribute to photochemical smog, especially in areas experiencing high traffic density such as central business districts (CBDs). The escalating emission rates are also exacerbated by road congestions, poor vehicle maintenance and high average age of the vehicle fleet.

2.5 Weather, pollution and air quality

Air pollution has a significant impact on climate and air composition in many ways. For example, GHGs in the atmosphere can trap heat and contribute to global warming. These gases(e.g. carbon dioxide (CO_2), ozone (O_3), methane (CH_4))once emitted can remain in the atmosphere for decades to centuries. They absorb and emit radiation within the thermal infrared range. Their concentrations can become well-mixed throughout the global atmosphere depending on the prevailing meteorology, regardless of location of emission source and their effects on climate could be long lasting (USEPA, 2009). Also, particles such as black carbon (BC) and nitrates absorb and scatter radiation, therefore affecting the radiative balance. Similarly, the weather can affect air pollution; it can either raise the development of pollution or stop its development depending on the environmental conditions such as in tropical areas where precipitation often occurs. It is more likely that pollutants would be washed out by precipitation (Pachauri, 2007). Changes in atmospheric gas- and aerosol concentrations, land cover and solar radiation alter the energy balance of the climate system (IPCC, 2007) and are drivers of climate change. The changes in energy balance due to these factors are expressed as radiative forcing–a process used to compare the warming or cooling influence on global climate.

In principle, any pollutant that contributes to local/regional pollution and act as a radiative forcing agent or changes solar distribution may potentially produce a linkage between AQ and climate change (AQEG, 2007). Changes in weather and climate can affect AQ as different areas experiencevarying climatic conditions and different pollutant concentrations. For example sunshine, rain, precipitation and wind can affect the amount of air pollution in an area, leading pollution washout, heat absorption or pressure variation in the atmosphere. Photo-energy makes some pollutants undergo chemical reactions in the atmosphere; higher temperatures and strong winds can also speed up chemical reactions in the atmosphere, rain washes out water-soluble pollutants. The wind can carry air contaminants away from their source,anddistribute themto other areas. Higher (strong) winds resultin fastdispersal of contaminants and increasetheirmixing in the atmosphere; the boundary layer may also change due to mixing.

III. METHODOLOGY

3.1 Data acquisition and sources

Meteorological and chemical data is obtained from several databases:National Oceanic and Atmospheric Administration (NOAA) (www.esrl.noaa.gov/psd/data/composites/hour./), the geostationary Dundee Satellite (http://www.sat.dundee.ac.uk/satellites.html) and the NASA's archived Giovanni (www.giovanni.gsfc.nasa.gov/Giovanni/service)forwind field, temperature, precipitation, clouds and chemical emissions over the study domain. NASA's Giovanni (Geospatial Interactive Online Visualization and Analysis Infrastructure) is a web-based application developed by the Goddard Earth Sciences Data and Information Services Center (GES-DISC)to provide simple and intuitive way to visualize, analyze, and access vast amounts of Earth science remote sensing data. Satellites provide quantitative data, images (maps) showing spatial distribution and pollutant concentration over an area.

We focus on the summer (Nov–Jan) and winter (May–July) seasons over the 10-year period (2000–2010) looking at the trend of pollutants and climatology over the sub-continent. Specifically, we look at emission scenarios in 2000, 2005 and 2010 at 5-day intervals -both surface and temporal distributions. Pollution patterns are then discussed in relation to the meteorological parameters over the same period and locations in order to understand how the weather interacts with the chemistry. For example, the period 2000–2010experienced more constructions and industrial activities over southern Africa. This period also falls within the time during which many African countries were preparing for the world

http://dx.doi.org/10.22161/ijeab/3.1.28 *ISSN: 2456-1878*

soccer tournament (2010 World Cup), which took place between June and July 2010 in South Africa. Most activities were aimed at attracting tourists and investors to the African continent.

IV. RESULTS AND DISCUSSION

We present and discuss outputs for meteorological and chemical variables over the domain as highlighted above. First we start with the meteorology before the chemistry and later look at their interactions. Because the chemistry is driven by the meteorology one needs to first understand and appreciate the dynamics in the meteorology.

4.1 Meteorology (weather pattern)

(a) Wind speed and direction

Winds are particularly important in the chemistry because of their direct influence on transport, atmospheric stability and mixing of pollution. For example, wind speed determines how quickly pollutants mix with the surrounding air and how fast they move away from their sources. This is important for any weather-pollution studies because, by knowing the wind field,one can describe transport patterns and possible influence on/by pollution. Figure 4 shows near-surface (1000-mb) mean wind field, from the NOAA's Earth Systems Research Laboratory (ESRL) database between 05–10 December and 05–10 June in 2000, 2005and 2010 respectively.The left panels represent summer while the right panels represent winter.

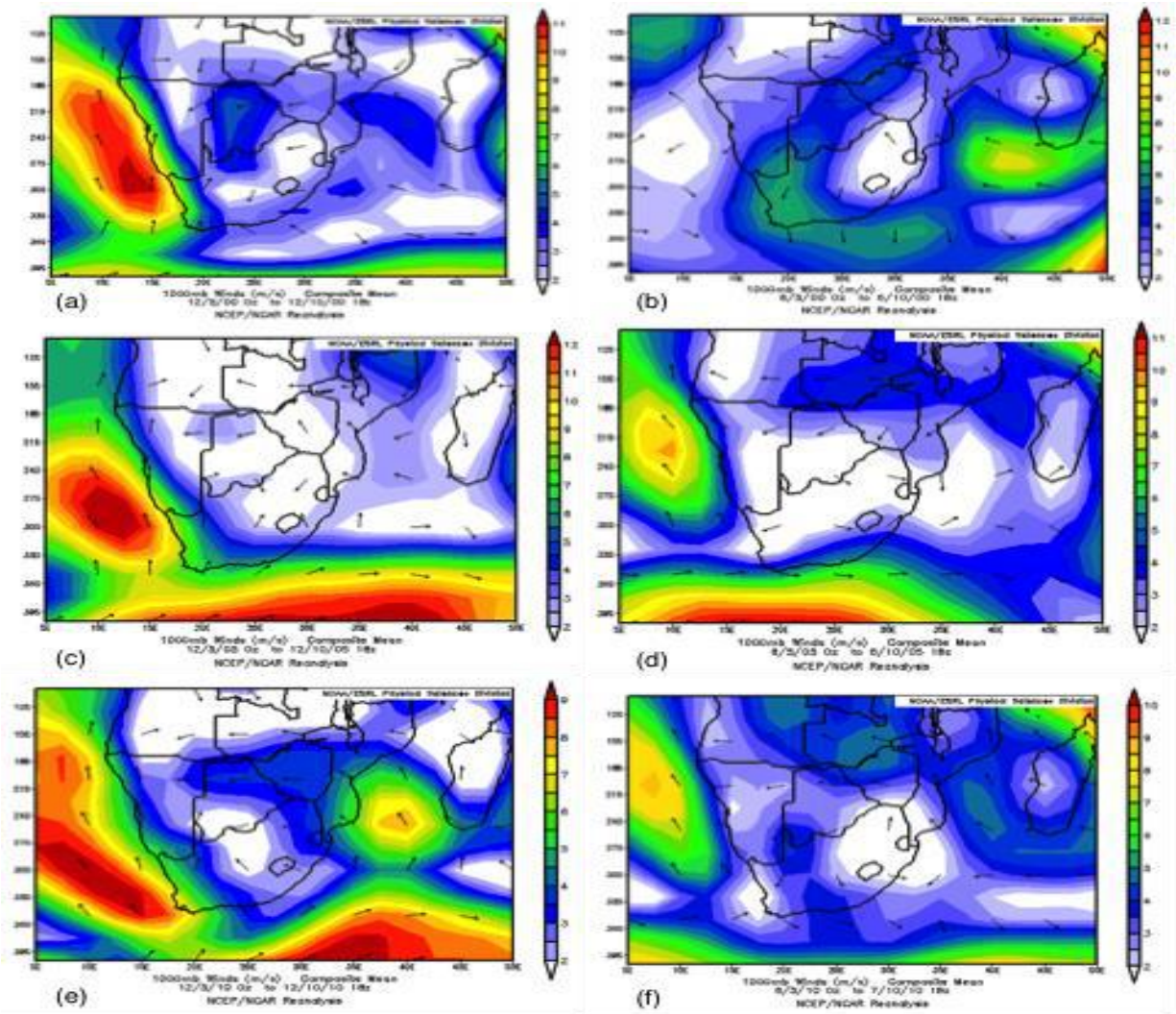

Fig 4: 1000-mb wind field during summer and winter in 2000 ((a), (b)); 2005 ((c), (d)); 2010((e), (f)) respectively. Output between 05–10 December and June. Arrows indicate wind direction and scales are colour-coded in terms of wind speed.Source: NCEP/NCAR reanalysis observation.

High wind speeds occur in summer especially over the Atlantic and southern part of the domain. For example, summer speed reach over 11 m/s in 2000 and 9 m/s in winter, south of Madagascar (Fig. 4(a) and (b)). The winds were mostly northeasterly coming from the Indian Ocean during summer and strong southerly over the west coast of Namibia, whereas in winter they are mostly easterly and northeasterly in the south. The winter of 2005 had relatively low winds than in summer. Summer maxima reach over 12m/s while winter maxima was

http://dx.doi.org/10.22161/ijeab/3.1.28 *ISSN: 2456-1878*

below 11m/s. The pattern is almost similar but more clearly defined in 2010 and the summer of 2005 (Fig. 4(c–f)). This indicates the possible direction of pollution transport, especially in the south where winds are westerly. The pattern also shows that the center of strong winds over the Atlantic Ocean slightly moved away from the sub-continent (southeasterly) between 2000 and 2010 during summer, and a new center developed from the east.

(b) Temperature

Next we show mean temperature distribution (Fig. 5). Again there are similar patterns in temperature as observed for wind. Summer temperatures were higher over the Kalahari, extending to the northeastern part of Namibia and central Zambia-Malawi, reaching 309K while most of the landmass experienced temperatures between 290K and 306K. Winter had the temperature maximum of just over 300K over central Angola while the rest of the sub-continent remained cooler, especially over the oceans. Higher temperatures were observed in the summer of 2005 (Fig. 5(c)) reaching over 307K over Zambia and the southern part of Kalahari, while winter temperatures (Fig 5(d))had a maximum of just above 300Kover Angola and average of 250K over much of the southern part. The summer of 2010 (Fig. 5(e)) had its temperature maxima over Botswana-Namibia, while winter had maximum temperature over northern Angola/southern Democratic Republic of Congo (DRC).

c) Precipitation and clouds

We also show accumulated precipitation (Fig.6), where we observe higher precipitation during summer (maximum of 40 kg/m^2) over southern and northeastern Mozambique and west of Angola. Lowest rainfall (below 15 kg/m^2) was experienced over the western part of South Africa and southwestern tip of Namibia. The highest rainfall in winter was 32 kg/m^2 over eastern Mozambique coast with low rainfall across Lesotho and southern parts of South Africa (Fig. 6 (b)). More precipitation occurred over the Mozambique coast (Fig. 6(c)), reachingwell over 45kg/m^2 whereas winter was mostly dry, with the highest rainfall around 17 kg/m^2 over the Indian Ocean and northern part of Mozambique coast.

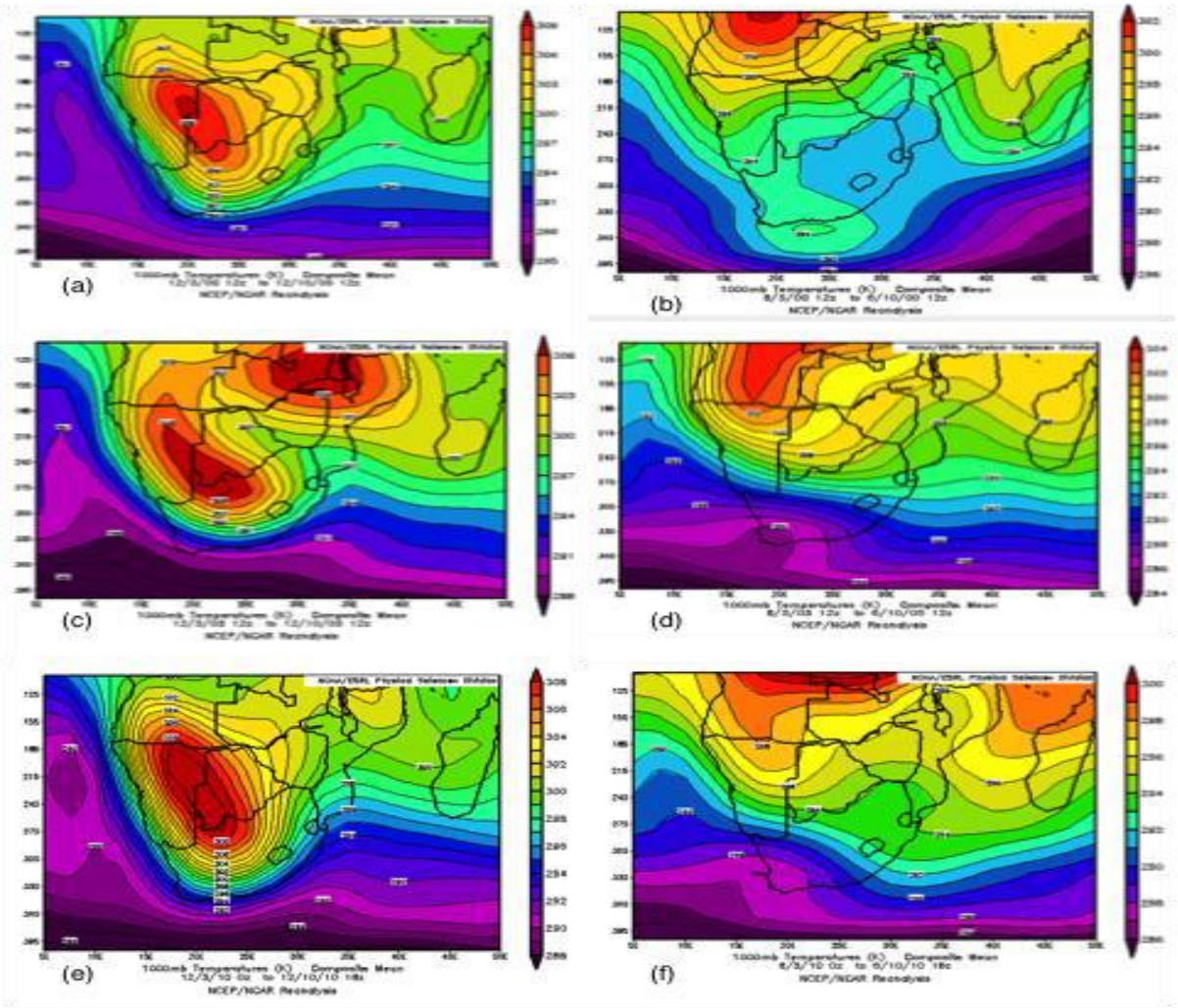

Fig 5: 1000-mb temperature during summer and winter in 2000 ((a), (b); 2005 ((c), (d)); 2010 ((e), (f)) respectively. Contours indicate temperature variation and scales are colour-coded in terms of temperature range.Output between 05–10 December and June. Source: NCEP/NCAR reanalysis observation.

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The summer of 2010 also shows precipitation almost over the same location as in 2005(about 40 kg/m^2 between Mozambique-Madagascar), while the winter was drier over much of the landmass. Overall, it appears that summers accumulate more precipitation over the Indian Ocean and west of Angola.

Clouds are one of the important factors in weather, because they are directly linked to pollution through aerosols. To better understand aerosol–cloud effects, one needs to know whether there are clouds over the domain and where they are located. Figure 7 shows satellite imagery (from the Dundee satellite station) in 2005 and 2010.

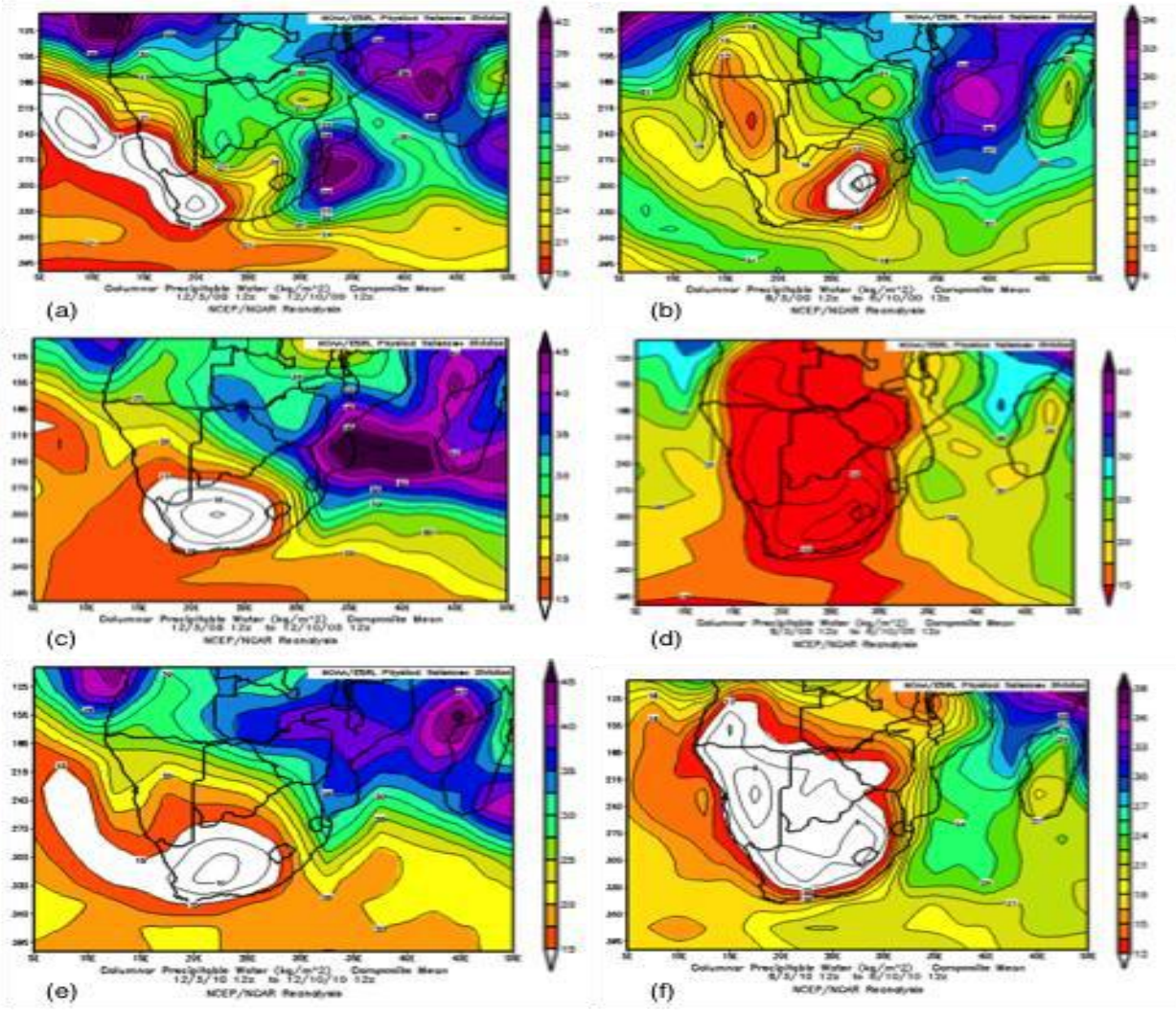

Fig 6: 1000-mb accumulated precipitation between summer and winter in 2000 ((a), (b); 2005 ((c), (d)); 2010 ((e), (f)) respectively. Contours indicate precipitation variability, scales are colour-coded in terms of precipitation maximum. Output between 05–10 December and June. Source: NCEP/NCAR reanalysis observation.

Satellites provide estimates of area coverage of clouds by the cloud-top temperature and the column-integrated optical thickness.They maintain an up-to-date archive of images covering the globe. The summer of 2005 (Fig.7(a)) had substantial cloud cover, mostly over the central-towards-east and some cloud patches over the Atlantic Ocean. Much of the domain was clear during winter, with few cloud bands over the oceans. Similarly, the summer of 2010 (Fig.7(c)) had more clouds concentrated mostly over the northern part of southern Africa and marine environments. While the winter was similar to that of 2005, few cloud patches appear over the South Atlantic Ocean.

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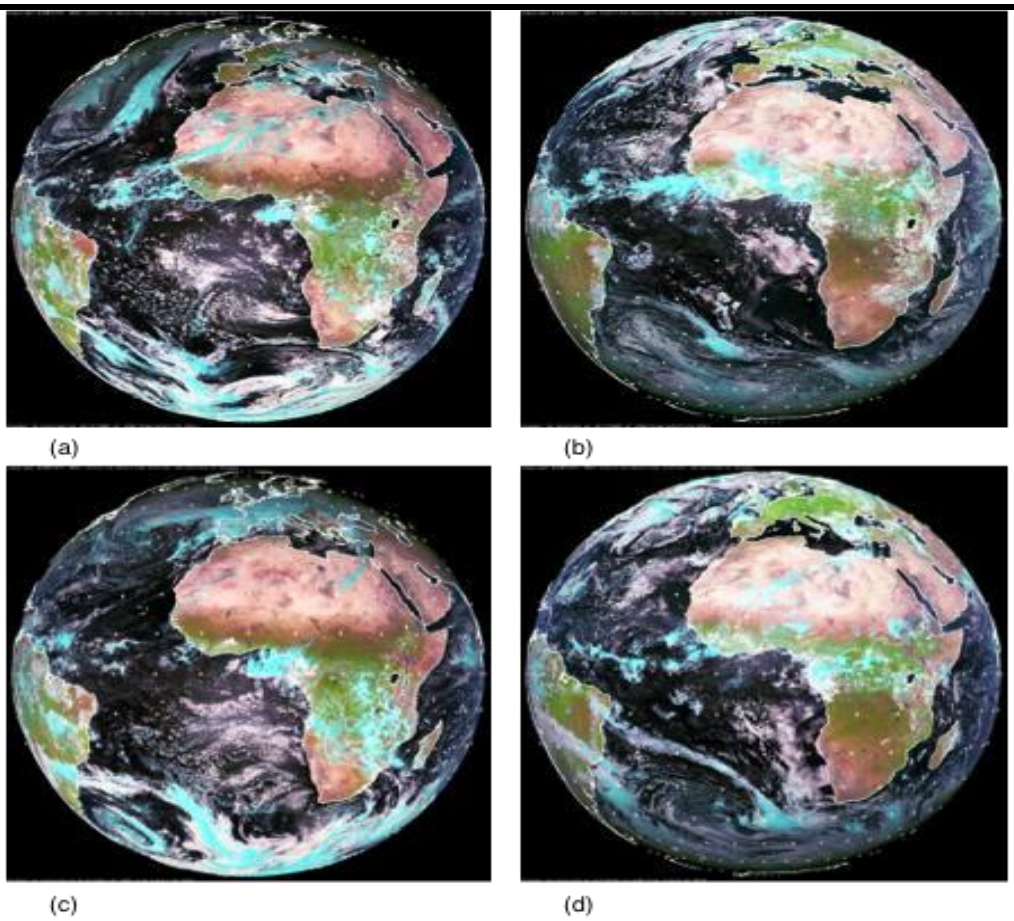

Fig 7: Accumulated column integrated clouds over Africa between summer and winter in 2005 ((a), (b) and 2010 ((c), (d)) respectively.Output between 05–10 December and June.Source: Meteosat infrared satellite images (http://www.sat.dundee.ac.uk/).

4.2 Emissions (pollution)

(a) Dust

Now we look atchemical emissions, focusing ondust (aerosols), NO_x, SO_2 and CO (gases). These are primary pollutants(i.e. directly from the sources) and are shown for the same time periods as the meteorology discussed above for consistency.Figure 8 shows dust concentration obtained from the NASA's Giovanni database.More dustappearsduring summer along the southwest coast (Namib Desert) throughout the years, and a significant amount over DRC in 2000.One also notices an increase in the east along the Kenyan coast during the winter of 2005 (Fig. 8(d)). The winter of 2010 shows dust over the Atlantic, along Namibia-Angola coasts. Dust emissions are generally common along the west (Kalahari-Namib Desert) than other geographical locations. However, that is so expected for this part of the globe because region is drier than its eastern counterpart –the so-called Kalahari Transect (Shughart et al., 2004).

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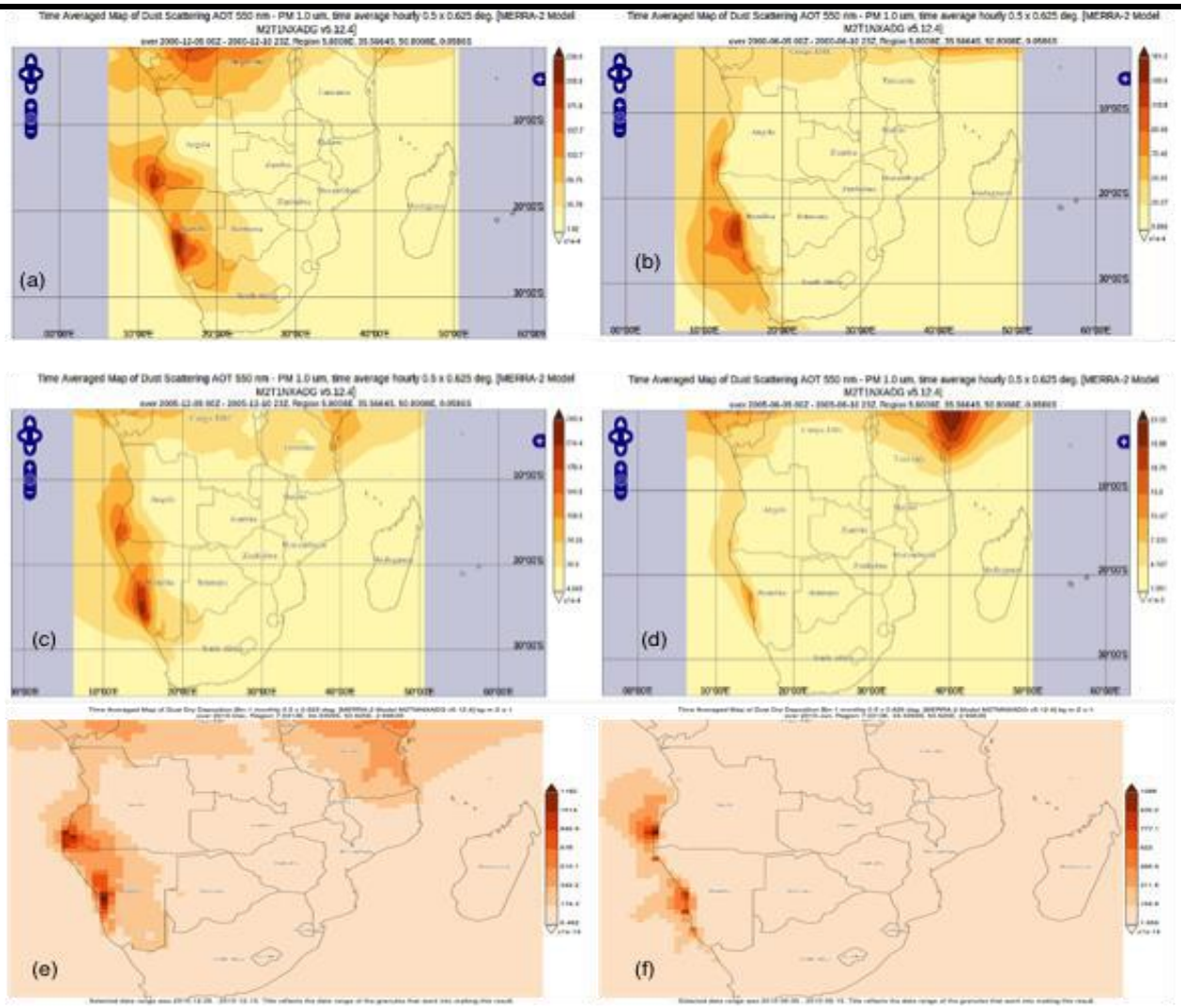

Fig. 8: Dust concentrations between (a) summer and (b) winter respectively. Contours indicate increasing particle concentration and scales colour-coded in terms of concentration maxima.Source: MODIS onboard the Aqua satellite measurement (http://giovanni.gsfc.nasa.gov/giovanni/).

(b) Sulphur and nitrogen oxides

Next we show the spatial distribution for SO_2 (Fig. 9), from the same database. As expected, more SO_2 is observed over/around industrial locations highlighted earlier (see Sect. 2.4). SO_2 is an air pollutant often associated with combustion in power generation, fossil fuel refining and ore smelting (Zunckel, et al., 2000) such as the mines. About 1.8 million tons or more of SO_2 is emitted from electrical generation annually. For example, the annual ambient SO_2 concentration from South Africa, on average, is said to approach 20 ppb guideline (WHO; Kgabi, 2012). These are quite clear in the southern part– with more industrial operations.Typical locations include the copper-belt, highveld and other isolated small plants.

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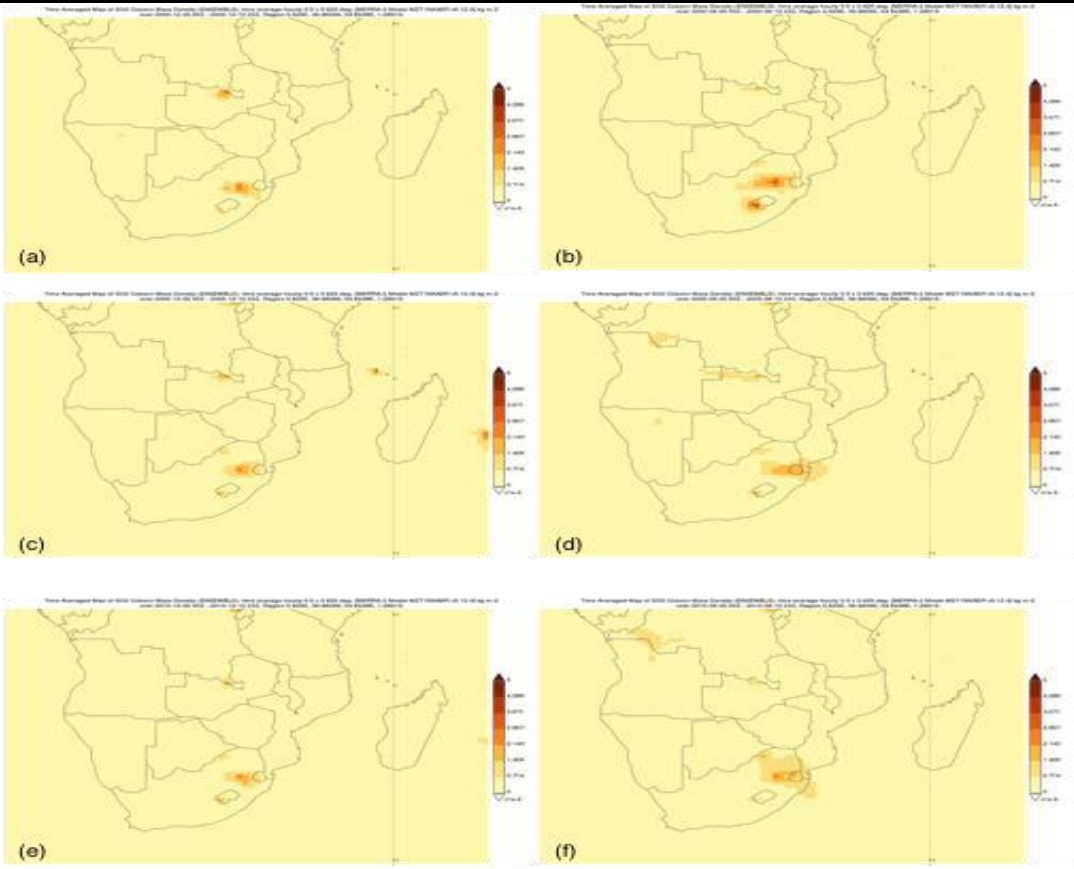

Fig. 9: SO_2 concentrations between (a) summer and (b) winter respectively. Contours indicate increasing gas concentration and scales colour-coded in terms of concentration maxima. Source: MODIS onboard the Aqua satellite measurement (http://giovanni.gsfc.nasa.gov/giovanni/).

Similarly, we can show another common gas pollutant NO_x (herein NO_2) to compare with the SO_2 (Fig. 10). $NO_x$is an important primary pollutant produced from anthropogenic and natural sources (e.g. fossil fuel combustion, BB, soil bacteria, lightning, oxidation of ammonia, mobile transport and other heavy machinery).While SO_2 is from the MODIS-on-board the Aqua satellite measurement, $NO_x$is a snap shot from satellite imagery in 2006.Again hot spots (column density order of 10^{16} mol/cm^2) for NO_2 are observed over the Highveld and Angola-DRC; concentration patterns show how emissions spread to other areas from the source locations.

(c) Carbon monoxide

Next we show the spatial distribution of CO (Fig. 11) also from the NASA's Giovanni database. The concentration increases (more reddish) in the top-left corner (west) into the South Atlantic Ocean, but declines towards the east. Again concentration decreases towards the south. While the pattern is similar throughout, the summer of 2005 (Fig. 11(a)) has low CO concentration than other panels. However, the summer of 2010 (Fig. 11(c)) shows more wide emission coverage and some isolated hotspots over Madagascar.

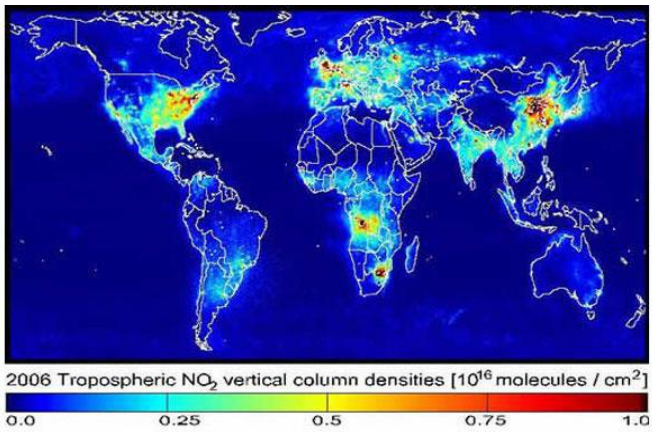

Fig. 10: Satellite images of tropospheric nitrogen dioxide (NO_2), 2006. There are high possible chances of trans-boundary pollution from South Africa and Angola into other countries. (Source: http://www.learner.org/courses/envsci/unit/text.php?).

It is also worth noting that concentrations increase mostly over the BB areas (tropical central) than anthropogenic-dominated regions. One distinct difference between the spatial distribution of the emissions in dust and COis that gases are more spread than aerosols.

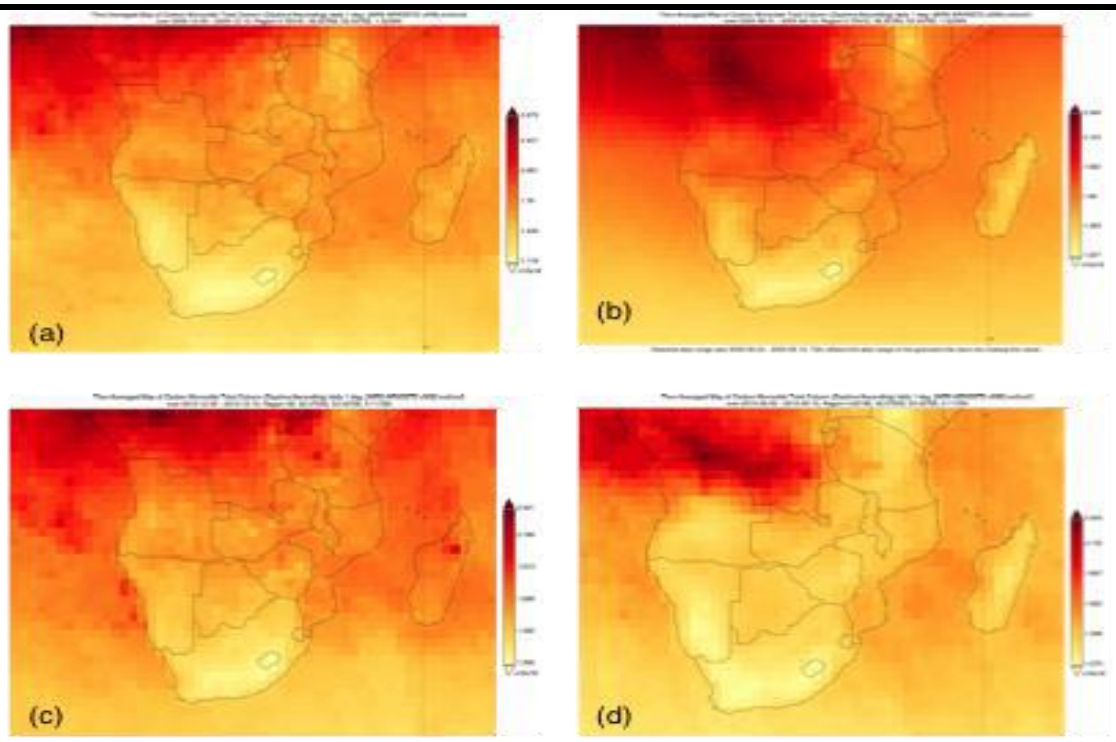

Fig. 11: COconcentrations between (a) summer and (b) winter respectively. Contours indicate increasing gas concentration and scales colour-coded in terms of concentration maxima. Source: MODIS onboard the Aqua satellite measurement (http://giovanni.gsfc.nasa.gov/giovanni/).

4.3 Meteorology-chemistry interactions and influences

The atmosphere over southern Africa has unique meteorological and chemical characteristics that influence pollution transport and climate forcing (Garstang et al., 1996). Emission distribution is strongly influenced by the transport dynamics and air masses that prevail over most of the time (Kirkman et al., 2000). These circulation systems and transport pathways play vital roles in the local meteorology. For example, the frequent high-pressure systems act as 'accumulation' mechanisms for trace gas and aerosols associated with haze layers and the westerly wave passages are responsible for the 'clean out' signatures (Stein et al., 2003). There is an indication that emissions have increased over the years, more pronounced during summer, although sometimes during winter (e.g. in 2005 there was a substantial increase in wind speeds).

Consequently there is a decline in precipitation with very little or no rainfall over the coastal areas. Temperatures along the west coast (between 2005 and 2010) appear to be cool in summer and become cooler in winters. It would of interest to try to understand all potential climate controls as highlighted earlier. Satellite images indicate more cloud coverage in summer, correlating with the rise in dust aerosols (more aerosols enhance the chance of cloud formation because they act as sites for developments). However, more clouds (precipitation) may result in pollution washout from the atmosphere, leading to pollution removal (wet deposition), whereas strong winds can also carry out pollution (dry deposition) from the atmosphere or away from the source. Urban pollution can enhance down welling radiation during clear nights and lead to increase in night-time minimum temperatures when warm air is mixed from aloft because of radiative destabilization (Knippertz et al., 2015). However, these kinds of interactions between weather parameters and chemistry are cyclic between the seasons.

We also show temporal emission distribution for dust, SO_2 and CO_2 concentrations from 2000 through 2010 (Fig. 12).The trends show fluctuating patterns in pollutant concentrations across all the species. As expected, CO has the highest concentration than SO_2 and dust. Global pollutants' emissions increase mainly driven by the demand for energy and transportation. As highlighted earlier, the changing meteorological patterns and seasonal human activities play vital roles in pollution levels. For instance the summer winds in 2000 show wind divergence over the Mpumalanga Highveld while the winter SO_2 concentration appear to be spread in all directions but mostly directed towards the Mozambique coastal areas.

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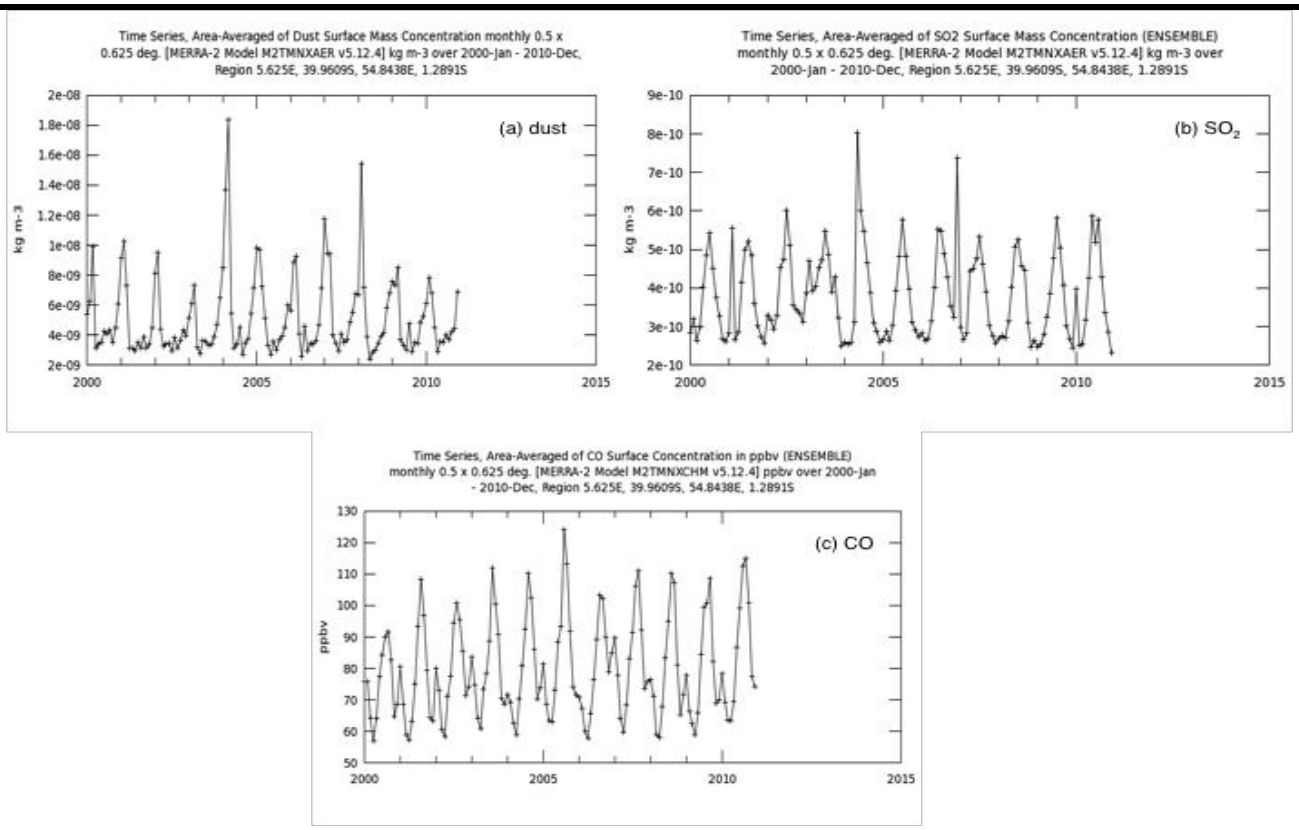

Fig. 12: Temporal distribution of (a) dust, (b) SO_2 and (c) CO_2 concentrations between 2000 until 2010 over southern Africa. Source: MODIS-on-board the Aqua satellite measurement (http://giovanni.gsfc.nasa.gov/giovanni/).

Common air circulations during winter (mostly westerly) also have the potential to carry the SO_2 emission observed over the highland and further distribute it across. Between 2005 and 2010, wind patterns become more intense in summer, this could possibly scatter emissions further. Sulphate aerosols can also act as sites for cloud formation (cloud condensation nuclei [CCN]), althoughclouds formed around these compounds have varying radiative properties than other CCN. Increases in sulphate abundance may lead to more clouds or increase cloud albedo, and cause a net reduction in solar radiation reaching the earth surface (AQRS, 2001).An increase in the rainfall intensity in summer would imply more pollution washout from the atmosphere, especially in the proximity of polluting areas. On the other hand, frequent winds in winter increase likelihood of pollution transport and/or result in the build up of pollution (haze) above. This can alter radiation budget (depending on the intensity of absorbing or scattering aerosols present), or impact on cloud microphysical properties.

V. CONCLUSION

Southern Africa is characterized by the tropical and subtropical climatic conditions with great influence from the adjacent oceans, as well as the common air circulations. Since the late 20thCentury, the sub-continent has transformed from a rural to a complex society and has made great strides towards industrialisation, urbanisation, and economic development (Simukanga et al., 2003). These changes, compounded with population growth, brought about environmental problems virtually non-existent in the past. Owing to different major pollutant sources, the region has experienced a considerable change recently and is recognised as a major source of pollutants (Wiston, 2016). Emissions exhibit a mixture of large quantities of aerosols and gases from BB, domestic fires, fossil fuel sources [automobiles and industries], construction, manufacturing and Aeolian dust.

While the SH summer is often characterized with more cloud coverage and wetness, winter is characterized with common air masses blowing over land. It is quite clear that pollution can influence the AQ composition over a given location. Consequently, some of the areas experiencing high levels of air pollution are the most populous and/or capital cities. Although there might not be national AQ problems on record, a number of air pollution conditions exist where severe AQ problems are likely to occur; examples being locations within active BB and high anthropogenic emissions. Vast amount of pollutants can be transported over long distances, impacting other areas downwind or those initially not in the vicinity of emitting sources. Under these conditions, humans are often exposed to high levels of pollution, which is not always monitored and sometimes exceed

threshold limits (Wiston, 2017). Bad AQ cannegatively impact human health and may lead to respiratory problems such as heart failure, asthma, cardiac arrests, congestive heart failure and eye irritations. Anthropogenic and human-induced BB emissions increase mostly in winter when industrial activities are in operation and more vegetation burnt for various purposes. On the other hand, natural emissions are generated and fluctuate throughout the year.It is worth noting that southern African pollution plays a significant role on the weather and regional air quality composition.

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Estimated carbon stored on some landscape forests in South East Sulawesi

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Abstract*—Carbon stored in several forest landscapes in Southeast Sulawesi such as the Jati stand on the People's Forest, Pine stands in Protected Forest, Mangrove Forest vegetation, Natural vegetation of urban forest, and Campus Forest show the weight of carbon stored per hectare different from one vegetation to another. The objective of this research is to know the biomass and the amount of carbon stored up (levels of the tree, pole, stakes), the lower plants, litter and nekromassa in various forest landscape in Southeast Sulawesi. Biomass is obtained through the use of allometric equations (plants on the surface), and measures the wet weight and dry weight (bottom plants, woody necromassa and non-woody). Furthermore, the estimated amount of carbon stored. The results showed Pinus stand (Pinus mercusii) carbon stored 65,992 tons ha^{-1}, stand Teak (Tectona grandis) 36.213 tons ha^{-1}, Mangrove vegetation (Bruguiera gymnorrhiza, Lumnitzera racemosa, Rhizophora sp, Sonneratia alba, and Avicennia alba) 68.12 tons ha^{-1}, natural vegetation Forest City 50.01 tons ha^{-1}, natural vegetation Forest Campus 98.18 tons ha^{-1}. The availability of carbon estimation information in various forest landscapes can be used as supporting data for REDD + programs aimed at addressing climate issues.*

***Keywords*— Biomass, Carbon estimation, Forest landscape, Vegetation.**

I. INTRODUCTION

Forest landscape is a description of land cover both within forest area and outside forest area in the form of forest and non forest. This can be described in terms of the area of land cover. Area of land cover in and outside the forest area of Southeast Sulawesi Province 3,638,000,7 Ha. Where land cover area in forest area is 1,943,000,8 Ha and not forest area 1,694,000,9 Ha [1].

REDD + activities are one of the mitigation or mitigation measures caused by climate change in the forestry sector by reducing emissions from deforestation, degradation and conservation, SFM and increased carbon stocks.

[2], [3], [4] that, there are 5 carbon sources to be measured through field measurements; above ground biomass, below ground biomass, dead wood, litter, and soil. While the source of carbon to 6 that is harvested wood products (harvested wood products) has not been taken into account. Carbon needs to be measured because basically carbon stock is the amount of carbon stored in vegetation, other biomass in the soil.Efforts to reduce GHG concentrations in the atmosphere (emissions) is to reduce the release of CO_2 into the air. Therefore, the amount of CO_2 in the air must be controlled by increasing the amount of CO_2 uptake by the plants as much as possible and suppressing the emission release as low as possible. [2]Thus maintaining the integrity of natural forests, planting trees on agricultural lands and protecting peatlands is essential to reduce excessive amounts of CO_2 in the air.

II. MATERIALS AND METHODS

2.1 Materials

The results of the research took place in the teak plantation forest (*Tectona grandis* Lf)) in North Buton District, Mangrove Forest in Latompa village, Maligano sub-district, Muna district, Pinus stands (*Pinus mercusii*) in Nanga-Nanga Protection Forest Kendari City, Natural Forest Vegetation in Kecamatan Baruga Kendari City, and natural vegetation Forest Campus in District Baruga Kendari City.

2.2 Methods

This research method using Desk Study approach by summarizing the results of existing carbon estimation research that took place in 2012 until 2015, and also collects materials and other information from literature related to climate issues. The results are then studied in the form of qualitative and quantitative descriptive.

III. STATISTICAL ANALYSIS

Biomass calculations use some allometric equations (Table 1), and estimates of carbon stored in several forest landscapes in Southeast Sulawesi use the equation ie C = BK (ton / ha) x 0.46 [2]

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Calculations Biomass used in the results of research, can be seen in Table 1 below:

Table.1: Biomass Calculation Methods of several forest landscapes in Southeast Sulawesi

Forest Landscape	Type of Vegetation	Biomass Calculation			
		Top plants (allometric equations)	Lower plants	Nekromassa	
				Woody	Not woody
Protected forest	Pine stands (*Pinus merkusii*)	$Y=0{,}0936 \int D^{2{,}432}$ [5]	$BK=(BK_{sc}/BB_{sc}) \times BB_{tot}$ [6]	$Y=\pi.\int.H.D^2/40$ [6]	$BK=(BK_{sc}/BB_{sc}) \times BB_{tot}$ [6]
Forest City	Natural vegetation	Tree branched: [5] $Y=0{,}11 \int D^{2{,}62}$ Not Branching:[6] $Y=\pi.\int.H.D^2/40$ Pillars and Stakes:[7] $Y=10^{0{,}535+\log 10(BA)}$	$BK=(BK_{sc}/BB_{sc}) \times BB_{tot}$ [6]	$Y=\pi.\int.H.D^2/40$ [6]	$BK=(BK_{sc}/BB_{sc}) \times BB_{tot}$ [6]
Campus Forest	Natural vegetation	Tree branched:[5] $Y=0{,}11 \int D^{2{,}62}$ Not Branching: [6] $Y=\pi.\int.H.D^2/40$ Pillars and Stakes:[7] $Y=10^{-0{,}535+\log 10(BA)}$	$BK=(BK_{sc}/BB_{sc}) \times BB_{tot}$ [6]	$Y=\pi.\int.H.D^2/40$ [6]	$BK=(BK_{sc}/BB_{sc}) \times BB_{tot}$ [6]
Forest People	Teak Plant (Tectona grandis Lf)	[8]$Y=0{,}08842D^{2{,}6014}$	-	-	-
Mangrove forest	Standby Mangrove	Trunk: [9] $Y=0{,}079211DBH^{2{,}470895}$ Branch:[9] $Y=0{,}481575x1{,}24628^{DBH}$ Leaf: [9] $Y=0{,}171711x1{,}96367^{DBH}$	-	-	-

IV. RESULTS AND DISCUSSION

4.1 Pine stands in Protected Forest

The Protected Forest has an area of 307.54 ha. [10] Study on Pine stands (*Pinus mercusii*) in Nanga-Nanga Protected Forest, from 5 point sampling plots can be explained that the number of individual Pinus (*Pinus mercusii*) averages 1045 individuals ha^{-1}, and diameter averages 21 cm. Mean of biomassa on Pine Plant Various Levels of vegetation 159.286 ton ha^{-1} and carbon deposit 65.992 ton ha^{-1}.

4.2 Natural Vegetation in City Forest

[11] Forest City Baruga is one of the city forest in Kendari City is established with an area of 3 ha with natural environmental conditions so that the management must be able to meet one of the ecological functions as a carbon sink. [12] The results study show that the number of individual vegetation in City Forest, from 8 point sampling that the number of individual of vegetation (tree, poles, stakes, seedling and puppies) averages 4781.25 individuals ha^{-1}. Mean of biomassa natural vegetation in city forest 108.719 ton ha^{-1} , and carbon deposit 50.01 ton ha^{-1}. Upper plants, plants and necromassas only have a total stored carbon of 50.01 tons ha^{-1}. This is possible because the city forest with natural vegetation is located within the city of Kendari, making it very susceptible to disruptions that could lead to forest degradation.

4.3 Natural Vegetation in Campus Forests

Haluoleo University Campus Forest is a natural vegetation located within the campus in Kecamatan Kambu Kendari with an area of 58.96 Ha. The research results [13] explain that the campus forest, there are 81 species of plants, 65 families, from 4975 specimens. The family has the largest number of species of Myrtaceae, Euphorbiaceae and Fabaceae.

The tabulation of the results of biomass and carbon (C) calculations on each plot of forest in Halu Oleo University Campus can be seen in Table 2 [14].

4.4 Teak stands in the People's Forest

The observed teak stands are located in other areas of use (APL) with the status of land owned by some communities in the sub-district of Labuan, Wakorumba Sub-district, North Buton Regency, Southeast Sulawesi. The width of the Teak stand (*Tectona grandis Lf*) is 20.42 ha and the age of Teak ranges from 5 years to 16 years. [15] The stand of Teak in Labuan Sub-district People's Forest has an average

density of 1025 trees ha^{-1} at the age of 5 years and age 6 to 16 years. Based on the table 3 below shows that, the more the age of the teak stand (*Tectona grandis* Lf), the more carbon deposits are stored in the stands.

Carbon stored in Teak (*Tectona grandis* Lf) shows the increasing age of teak, increasing the amount of carbon stored, where at the age of 16 years there are 78.96 tons ha^{-1} of stored carbon, while the age of 5 years 16.89 tons ha^{-1}. If the average is from the age of 5 to 16 years, then it is assumed that every age has 36.213 tons of carbon stored ha^{-1}.

4.5 Carbon Stored in Mangrove Forest

The area of mangrove forest in Latompa Village, Maligano Sub-district, Muna Regency, Southeast Sulawesi Province 160 Ha. The types of compilers Mangrove Forest consists of *Bruguiera gymnorrhiza, Lumnitzera racemosa, Rhizophora sp., Sonneratia alba,* and *Avicennia alba* [16]. Based on the results [16] study, the amount of biomass and carbon storage in each mangrove species in Latompa Village, Biomassa 148.13 ton ha^{-1} and Carbon storage 68.12 ton ha^{-1}. The total amount of carbon stored is only about 68,12 ton per hectare. . [17] Land use system consisting of trees with species with low wood density values, the tree biomass will be lower when compared to land with species with high wood density values.

4.6 Recapitulation of Estimated Saved Carbon

Carbon stored in several landscapes of forests in Southeast Sulawesi are protected forests, urban forests, campus forests, community forests and mangrove forests show different amounts of stored carbon. This can be seen from the structure of the constituent vegetation, the vegetation type, the age of the plant, the stand site, and the degree of damage to the forest landscape. The more layered growth rate in a stretch (the level of trees, poles, stakes, seedlings), the higher the carbon content stored in the vegetation. Vegetation consisting of various types has a higher stored carbon than a one species vegetation. This can be seen in Table 4 below.

V. CONCLUSIONS AND RECOMMENDATIONS

Forest landscapes in Southeast Sulawesi have different amounts of stored carbon. The largest amounts of stored carbon among forest landscape observed in upper plants, under plants and necromassas, respectively were in the campus forests of 98.181 tons ha^{-1}, then in protected forests (Pinus stands), and urban forest (natural vegetation) of 50, 01 tons ha^{-1}. Forest landscapes in Southeast Sulawesi have different amounts of stored carbon. The largest amount of stored carbonaceous forests, under plants and necromassas, respectively were in the campus forests of 98.181 tons / ha, then in protected forests (Pinus stands), and urban forest (natural vegetation) of 50, 01 ton / ha. While the forest landscape that only observes carbon stored in the upper plants, the highest in the mangrove forest (68.12 tons ha^{-1}), and the lowest in the forest (Teak plant) with 36.213 tons ha^{-1} of stored carbon. The amount of carbon stored in each forest landscape is affected by the structure of the stand, the number of vegetation constituents, the age of the plant, the site where the vegetation develops, and the extent of damage. The natural vegetation composed of complete stand structures (trees, masts, saplings and seedlings) tends to have higher amounts of carbon stored, compared to one standing layer alone. Natural vegetation of mixed species also tends to have higher amounts of stored carbon than vegetation consisting of only one type of constituent. Carbon stored in plants will be higher, as the age of the plant increases. In addition, mangrove vegetation with tidal habitats (tidal vegetation), although a natural vegetation but has a lower amount of carbon stored than in the natural vegetation habitat on land.

Carbon stored in plants will be higher, as the age of the plant increases. In addition, mangrove vegetation with tidal habitats (tidal vegetation), although a natural vegetation but has a lower amount of carbon stored than in the natural vegetation habitat on land. The People's Forest Program is very good at reducing carbon emissions, but it is best to use multi-species cropping patterns with layered structures.

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Table.2: Calculation of biomass and carbon (C) in each plot of forest in Haluoleo University campus

Observation Grid	Average Total Biomass (tons ha^{-1})	Average Total Carbon (tons ha^{-1})
Tree (d>30cm)	151.3385	69.615710
Tree (d 5-30cm)	50.4429	23.203734
Lower Plants	2.9401	1.352446
Nekromassa Wood	3.77065	1.734499
Nekromassa Not Woody or Rough litter	4.9444	2.274424
Amount	213.4365	98.180813

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Table.3: Biomass and carbon stored at each age of teak stand (Tectona grandis) in Labuan Sub-district People's Forest

Age Teak (Year)	Stand Teak		Litter under teak stands		Saved Carbon Amount (tons ha^{-1})
	Biomassa (tons ha^{-1})	Carbon Saved (tons ha^{-1})	Biomassa (tons ha^{-1})	Carbon Saved (tons ha^{-1})	
5	26,34	13,17	7,43	3,72	16,89
6	32,26	16,13	7,66	3,83	19,96
7	36,21	18,11	8,22	4,11	22,22
8	43,13	21,57	8,15	4,08	25,65
9	49,40	24,70	8,35	4,17	28,87
10	56,47	28,23	10,44	5,22	33,45
11	73,08	36,54	11,03	5,51	42,05
13	102,84	51,42	12,91	6.45	57.87
16	140,56	70,28	17,36	8.68	78.96
	Average				**36.213**

Table.4: Recapitulation of stored carbon estimates in various forest landscapes in Southeast Sulawesi.

Forest Landscape	Type Vegetation	Estimated Saved Carbon **(**tons ha^{-1}**)**				
		Plant on	Lower plants	**Nekromassa**		amount **(**tons ha^{-1}**)**
				Woody	Not woody	
Protected forest	Pine stands (*Pinus merkusii*)	64,047	0,181	1,515	0,249	65,992
Forest City	Natural vegetation	47,962	0,259	0,541	1,248	50,010
Campus Forest	Natural vegetation	92,819	1,352	1,734	2,274	98,181
Forest People	Teak Plant (*Tectona grandis Lf)*	36,213	-	-	-	36,213
Mangrove forest	Standby Mangrove	68,12	-	-	-	68,12

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Morphometric characterization of three Tsetse Fly Species - *Glossina M. Morsitans, G. P. Palpalis* and *G. Tachinoides* (Diptera: Glossinidae) from Ghana

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***Abstract*—** *Tsetse flies (Diptera: Glossinidae) are the main vectors of Human African Trypanosomiasis (HAT), or sleeping sickness and Animal African Trypanosomiasis, (AAT) or Nagana in Sub Saharan Africa. In Ghana, whilst HAT is no longer a major public health issue, AAT is still widely reported and causes considerable losses in the livestock sector resulting in major impacts on agricultural production, livelihoods and food security in the country.*

Application of morphometric techniques can reveal the existing level of population differentiation in tsetse flies, providing guidance on the distribution of genetically defined subpopulations. Morphometric techniques were used to compare size and shape of three tsetse fly species- G. m. morsitans, G. p. palpalis and G. tachinoides of Ghana, and also compare populations of G. p. palpalis collected from three geographical regions (Northern, Eastern and Western) of Ghana.

Flies were sampled from four sites in the Western, one site in the Eastern and three sites in the Northern Region using standard un-baited biconical traps. Right wings and right hind legs of selected flies from different collection sites were removed and mounted on microscope slides using glycerin as the mounting medium. Images of the prepared slides were captured under a Leica EZ4 D microscope with an inbuilt camera connected to a laptop.

Linear and proportions of wing and hind tibia measurements were arcsine-root transformed before analyzing with a general linear model in analysis of variance (ANOVA). Multivariate statistical analyses were used to detect any possible variations.

Results of the GLM analyses of linear and ratio data revealed that different linear combinations can be used to characterize tsetse species of different populations. The ratio value hind tibia/wing length (th/at) significantly distinguished fly populations into four groups, Northern, Eastern, Western and the lab colony; this is an indication that hind tibia/wing length is a good morphometric feature which can be used to discriminate flies from different regions of Ghana.

The principal components and canonical variates as well as Mahalanobis squared distances confirmed linear and ratio separations. Therefore based on these differences in morphometric characters observed, the three tsetse species were distinguished from each other.

Similar work on morphometrics needs to be done to include more regions and many other body parts such as proboscis length, antennal length, thorax and abdomen length and width in order to establish stronger morphometric tools for discriminating different tsetse fly species.

***Keywords*— Characterization, Ghana, Morphometric, Tsetse Fly, Wing length.**

I. INTRODUCTION

1.1 Background

Tsetse flies (Diptera: Glossinidae) are the main vectors of Human African Trypanosomiasis (HAT) or sleeping sickness and Animal African Trypanosomiasis (AAT) or nagana in Sub-Saharan Africa (SSA) [1,2]. AAT is widely reported in Ghana and causes considerable losses in the livestock sector resulting in major impacts on agricultural production, livelihoods and food security in the country [3, 4].

A variety of vector control techniques may be used in order to reduce tsetse fly populations, including the use of insecticide-impregnated traps and targets, live-bait application, sequential aerial spraying with pyrethriod, and sterile insect release (SIT) [5,6,7].

Morphometric techniques aim at measuring size, shape, and the relationship between size and shape (allometry) [8], and have become one of the major tools for the study of population structures of insect vectors [9].

Besides its evident contribution to insect systematics, the main epidemiological contribution of morphometrics to medical entomology has been to help decision making in the development of vector control strategies [10, 11, 12].

In the current study, we use a morphometric approach to examine the possible differences that may exist between different populations of tsetse fly species of Ghana.

The objective of this research was to apply morphometric analyses to check for size and shape differences among three species of tsetse flies- *G. p. palpalis*, *G. m. morsitans* and *G. tachinoides* in Ghana.

II. MATERIALS AND METHODS

2.1 Study area

The study areas comprised of four sites in the Western Region, one site in the Eastern Region and three sites in the Northern Region (Figure 1). These sites were geo-referenced (Table 1) using a handheld Global Positioning System (eTrex® LEGEND C GARMIN).

Fig.1: Map of Ghana showing various sampling sites in the three regions.

Table.1: Collection sites, geographic coordinates and number of slides prepared per site.

Region	District	Site	Site Code	GPS Coordinates			Slides Prepared	
				Lat-N	Long-W	Altitude (m)	M	F
Eastern	Suhum	Tomem	TMM	05.97661º	000.36671º	140	20	25
Northern	West Mamprusi	Bogdoo	BOG	10.23878º	00.71902º	119	06	09
	North Gonja	Daboya	DAB	09.29785º	01.51569º	108	08	18
	Savelugu/Nanton	Kuldanali	KUL	09.68993º	00.97743º	103	04	14
Western	Jomoro	Alawule	ALW	05.03849º	002.70271º	17	25	25
		Ezinlibo	EZN	05.01449º	002.72064º	20	25	25
		Nawule	NAL	05.03798º	002.72401º	16	25	25
		Tikobo	TKB	05.05396º	002.70091º	19	25	25

2.3 Entomological survey

Tsetse flies were sampled in September, October and November, 2013 in the Western and Eastern Regions, and in April, 2014 in the Northern Region, using standard un-baited biconical traps with blue-black material. Traps were randomly set in tsetse fly favourable biotopes in four localities in the Jomoro District of the Western Region, one site in Suhum District of the Eastern Region, one site in Savelugu/Nanton District, another site in the West Mamprusi District and a third site in the North Gonji District (all of the Northern Region). Traps were set for periods of 1-3days per field visit, and flies collected twice daily.

In addition to the field collected samples, one other population, *Glossina morsitans morsitans* was kindly provided by the Ghana Atomic Energy Commission (GAEC) from their laboratory colony for comparative purposes. The laboratory colony was reared under optimal conditions and adult flies maintained at a temperature of 24ºC and 70-80% relative humidity.

2.4 Slide preparation, image capture and measurement

Based on the number of specimens available, 50 flies (25 females and 25 males) each from Tikobo (TKB), Nawule (NAL), Ezinlibo (EZN) and Alawule (ALW) in the Western

Region; 45 flies (25 females and 20 males) from Tomem (TMM) in the Eastern Region; 25 flies (17 females and 8 males) from Bogdoo (BOG), 15 flies (9 females and 6 males) from Kuldanah (KUL), 18 flies (14 females and 4 males) from Daboya (DAB) in the Northern Region, and 100 flies (50 females and 50 males) from the laboratory colony were randomly selected for slide preparation. The right forewings and right hind legs of selected flies were carefully removed from the thorax, using two pairs of fine curved Swiss forceps, and temporary slide mounts were prepared of the detached wings and legs of the flies.

Images of the prepared slides were captured under a Leica EZ4 D microscope with an inbuilt camera connected to a laptop. From the digital pictures, sixteen landmarks on the wing were selected in Leica (vision 8.0) to represent unambiguous homologous locations on all specimens [13], and distances between the landmarks were computed to characterize the wing as estimations of size and shape differentiation in the specimens.

2.5 Data analysis

Linear and proportions of wing and hind tibia measurements were arcsine-root transformed before analyzing with a general linear model (PROC GLM, SAS Institute Inc., 2001) in analysis of variance (ANOVA). When ANOVAs were significant (P = 0.05), the means were separated using the Student-Newman-Keuls (SNK) test [14, 15, 16, 17]. All data metrices were $\log_{10}$ transformed first before the statistical treatment. Multivariate statistical analysis i.e., analysis of variance, principal components analysis and canonical analysis were used to detect any possible variations. All morphometric analyses were performed using Statistical Analysis System software version 8.2 (SAS Institute Inc., 2001). Wing and hind tibia measurements were analyzed separately to allow covariances between distances, sexes and species to interact in a more meaningful way [14].

III. RESULTS

3.1 Size comparison of three tsetse fly species of Ghana

Many of the linear measurements showed significant differences, and separated the flies into various populations. Wing length (*at*) showed significant difference (P<0.0001) and partially separated the flies into four clusters, in which the lab colony (*G. m. morsitans*) stood out to be the largest, followed by the *G. p. palpalis* population from the Western region and the other two *palpalis* populations (*G. p. palpalis* from the Eastern and *G. p. palpalis* from the Northern Regions), being similar in size and smaller than *G. p. palpalis* from the Western but larger than *G. tachinoides* from the Northern Region (Table 2). The costa vein (*ab*) was important and separated the lab colony (*G. m. morsitans*) from *G. tachinoides* population as well as the three *palpalis* populations (*G. p. palpalis* populations from the Eastern and Western, and Northern Regions respectively), making it one of the distinguishing features that characterizes the three tsetse fly species (Table 2).

Linear combinations as ratios were important in separation of the fly populations into various species. There was significant difference (P<0.0001) among *G. p. palpalis* population from the Eastern, *G. tachinoides* population from the Northern and lab colony (*G. m. morsitans*) based on *gh/hi* (hatchet blade line to outside length of hatchet cell) indicating that the three tsetse species, *G. m. morsitans*, *G. p. palpalis* and *G. tachinoides* can be morphologically characterized by this ratio value *gh/hi* (Table 3).

Table.2: Mean linear measurements of three tsetse species (G. m. morsitans, G. p. palpalis and G. tachinoides) of Ghana

Population	Mean Linear measurements (mm) (±SE)							
	at**	ww	ab	ac	ad	ae	af	fg
G. p. palpalis (Eastern)	7.405 ± 0.094c* (65)	2.856 ± 0.028a (65)	3.128 ± 0.032b (65)	5.431 ± 0.072a (65)	6.408 ±0.067b (65)	7.030 ±0.073a (65)	7.246 ±0.072a (65)	2.198 ±0.031a (65)
G. m. morsitans (Lab colony)	7.959 ± 0.051a (100)	2.874 ±0.018a (100)	3.275 ±0.028a (100)	5.511 ±0.046a (100)	6.558 ±0.065a (100)	7.220 ±0.052a (100)	8.183 ±0.717a (100)	2.170 ±0.018a (100)
G. p. palpalis (Northern)	7.436 ±0.117c (18)	2.639 ±0.047b (18)	2.918 ±0.073c (18)	5.342 ±0.148a (18)	6.227 ± 0.138b (18)	6.648 ±0.117b (18)	7.061 ±0.126a (18)	2.125 ±0.029a (18)
G. tachinoides (Northern)	6.915 ±0.092d (40)	2.532 ± 0.036c (40)	2.783 ± 0.040d (40)	4.832 ±0.071b (40)	5.853 ±0.079c (40)	6.372 ±0.074c (40)	6.592 ±0.086a (40)	2.011 ±0.028b (40)
G. p. palpalis	7.715 ±0.033b	2.874 ±0.011a	3.052 ±0.018b	5.384 ±0.030a	6.514 ±0.038a	7.128 ±0.032a	7.364 ±0.032a	2.210 ±0.013a

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(Western)	(200)	(200)	(200)	(200)	(200)	(200)	(200)	
F	33.85	37.70	30.88	16.64	13.68	27.51	1.72	10.23
df	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1448	<0.0001

Population	Mean Linear measurements (mm) (±SE)							
	gh**	hi	ij	jk	kg	kl	ho	th
G. p. palpalis	0.860±0.011b*	2.560 ±0.028a	0.165±0.003ab	1.638 ± 0.016b	1.452 ±0.016a	0.327 ±0.005b	2.806 ±0.089a	2.370 ±0.018a
(Eastern)	(65)	(65)	(65)	(65)	(65)	(65)	(65)	(65)
G. m. morsitans	0.923 ±0.008a	2.526±0.019ab	0.181 ±0.006a	1.669±0.015ab	1.472 ±0.012a	0.318 ±0.003b	2.810 ±0.035a	2.263 ±0.018b
(Lab colony)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
G. p. palpalis	0.842 ±0.018b	2.453 ±0.037b	0.161±0.003ab	1.749 ±0.175a	0.337 ±0.009c	1.378 ±0.028a	2.593 ±0.068b	2.222 ±0.051b
(Northern)	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(18)
G. tachinoides	0.793 ±0.013c	2.255 ±0.034c	0.140 ±0.003b	1.485 ± 0.019c	1.254 ±0.023b	0.308 ±0.005b	2.499 ±0.038b	2.079 ±0.025c
(Northern)	(40)	(40)	(40)	(40)	(40)	(40)	(40)	(40)
G. p. palpalis	0.896 ±0.005a	2.608 ±0.013a	0.183 ±0.004a	1.650 ±0.007b	1.478 ±0.008a	0.331 ±0.003b	2.836 ±0.013a	2.408 ±0.008a
(Western)	(200)	(200)	(200)	(200)	(200)	(200)	(200)	(200)
F	27.65	30.02	6.59	8.66	428.69	2736.14	11.87	54.25
df	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	<0.0001	<0.0001

* Means in the same column followed by same letters are not significantly different (P=0.05), using Student-Newman-Keuls (SNK) test. ANOVA on arcsine-transformed proportion values

**Measurement code: at= wing length, ab=length of costal vein, ac= length of vein 1, ad= length of vein 2, ae= length of vein 3, af= length of vein4, fg=hatchet cell to wing tip, gh= basal height of hatchet cell, hi= outside length of hatchet cell, ij= apical height of hatchet cell, jk= hatchet handle, kg= hatchet blade line, kl= cross vein (r-m), at = wing length, ww= wing width, ho= hatchet cell to lower end of the wing, th= hind tibia, fg= hatchet cell to posterior end of the wing

Table.3: Mean ratio values of three tsetse species (G. m. morsitans, G. p. palpalis and G. tachinoides) from Ghana.

Population	Meanproportion (mm) (±SE)						
	gh/kg**	kg/hi	gh/hi	gh/jk	jk/hi	ho/hi	kl/ho
G. p. palpalis	1.689 ± 0.005a*	1.757 ± 0.003c	2.976± 0.003a	1.905± 0.004a	1.560 ± 0.004a	2.513 ± 0.003b	3.106 ± 0.004b
(Eastern)	(45)	(45)	(45)	(65)	(65)	(65)	(65)
G. m. morsitans	1.595 ± 0.003b	1.715 ± 0.003d	2.740 ± 0.002c	1.808 ± 0.002c	1.513 ± 0.003a	2.398 ± 0.003b	3.289 ± 0.003a
(Lab colony)	(100)	(100)	(100)	(100)	(100)	(100)	(100)
G. p. palpalis	0.398 ± 0.059c	7.246 ± 0.003a	2.915 ± 0.006ab	1.934± 0.024a	1.395 ± 0.076b	2.506 ± 0.007a	0.709± 0.020c
(Northern)	(18)	(18)	(18)	(18)	(18)	(18)	(18)
G. tachinoides	1.577 ± 0.005b	1.799 ± 0.003b	2.841 ± 0.003b	1.876 ± 0.004b	1.515 ± 0.004a	2.433 ± 0.005b	2.985± 0.005b
(Northern)	(40)	(40)	(40)	(40)	(40)	(40)	(40)
G. p. palpalis	1.650 ± 0.002ab	1.764 ± 0.002c	2.907 ± 0.001ab	1.841 ± 0.002bc	1.577 ± 0.002a	2.538 ± 0.002a	3.096 ± 0.003b
(Western)	(200)	(200)	(200)	(200)	(200)	(200)	(200)

F	4433.66	1421.53	30.45	9.35	7.36	14.59	3629.23
df	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Population	Mean proportion (mm) (±SE)						
	kl/kg**	kl/hi	kl/jk	ho/fg	kg/fg	gh/fg	ww/at
G. p. palpalis (Eastern)	4.425 ± 0.003a* (45)	7.813 ± 0.002a (45)	5.00 ± 0.003a (45)	2.151 ± 0.005a (45)	1.506 ± 0.008b (45)	2.545 ± 0.005a (45)	2.584 ± 0.003d (45)
G. m. morsitans (Lab colony)	4.608 ± 0.002a (100)	7.937 ± 0.001a (100)	5.236 ± 0.002a (100)	2.062 ± 0.002b (100)	1.472 ± 0.003b (100)	2.353 ± 0.002b (100)	2.770 ± 0.002b (100)
G. p. palpalis (Northern)	0.244± 0.076b (18)	7.779 ± 0.008b (18)	1.181 ± 0.039b (18)	2.169 ± 0.007a (18)	1.289 ± 0.003c (18)	2.525 ± 0.007a (18)	2.817 ± 0.004a (18)
G. tachinoides (Northern)	4.049 ± 0.004a (40)	7.299 ± 0.002c (40)	4.808 ± 0.003a (40)	2.174 ± 0.004a (40)	1.605 ± 0.005a (40)	2.538 ± 0.003a (40)	2.732 ± 0.003c (40)
G. p. palpalis (Western)	4.444± 0.002a (200)	7.874 ± 0.001a (200)	4.950 ± 0.002a (200)	2.146 ± 0.002a (200)	1.492 ± 0.003a (200)	2.463 ± 0.002a (200)	2.681 ± 0.001c (200)
F	12799.4	3569.04	1160.31	12.11	779.54	22.01	25.94
df	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398	4, 398
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

3.2 Principal components and canonical variates analyses of three tsetse species of Ghana

All the 16 wing variables were subjected to principal component analysis (PCA) to reduce the number of dimensions and to find out major sources of variation. Projection of these wing variables on the first two principal axes showed a partial or fuzzy separation of the populations of different tsetse fly species (Figure 2a). The first two components accounted for 77.98% (PC1 + PC2 = 52.05% + 25.93%) of the total size variation and provided a reasonable approximation of the total amount of variation among the populations of the tsetse species. The third, fourth, and fifth components contributed 7.30%, 3.47% and 2.69% respectively, and did not improve in the separation of the species.

Projection of the data on first two canonical axes however, showed a similar separation trend among the five tsetse populations, with the five populations separated into two clusters; the two *palpalis* populations (*G. p. palpalis* from the Eastern region and the Western region), lab colony (*G. m. morsitans*) population, *G. tachinoides* population from the Northern clustered together, while the population of *G. p. palpalis* from the Northern was widely separated from the others and stood out alone along the negative PC1 axis (Figure 2b). The first two canonical variates contributed a total of 98.97% (CV1+ CV2=97.65% +1.32%) to the total variance with the third and fourth variates contributing 0.74% and 0.29%, respectively.

The Mahalanobis squared distances (D^2) of population was calculated to see the cohesiveness of members in a group and the closeness of the populations to each other. The biggest distance value (D^2=1567) was between *G. p. palpalis* population from the Eastern and *G. p. palpalis* from the Northern region, whereas the smallest distance was between *G. p. palpalis* (Eastern) and *G. p. palpalis* (Western) (D^2=3.1873).

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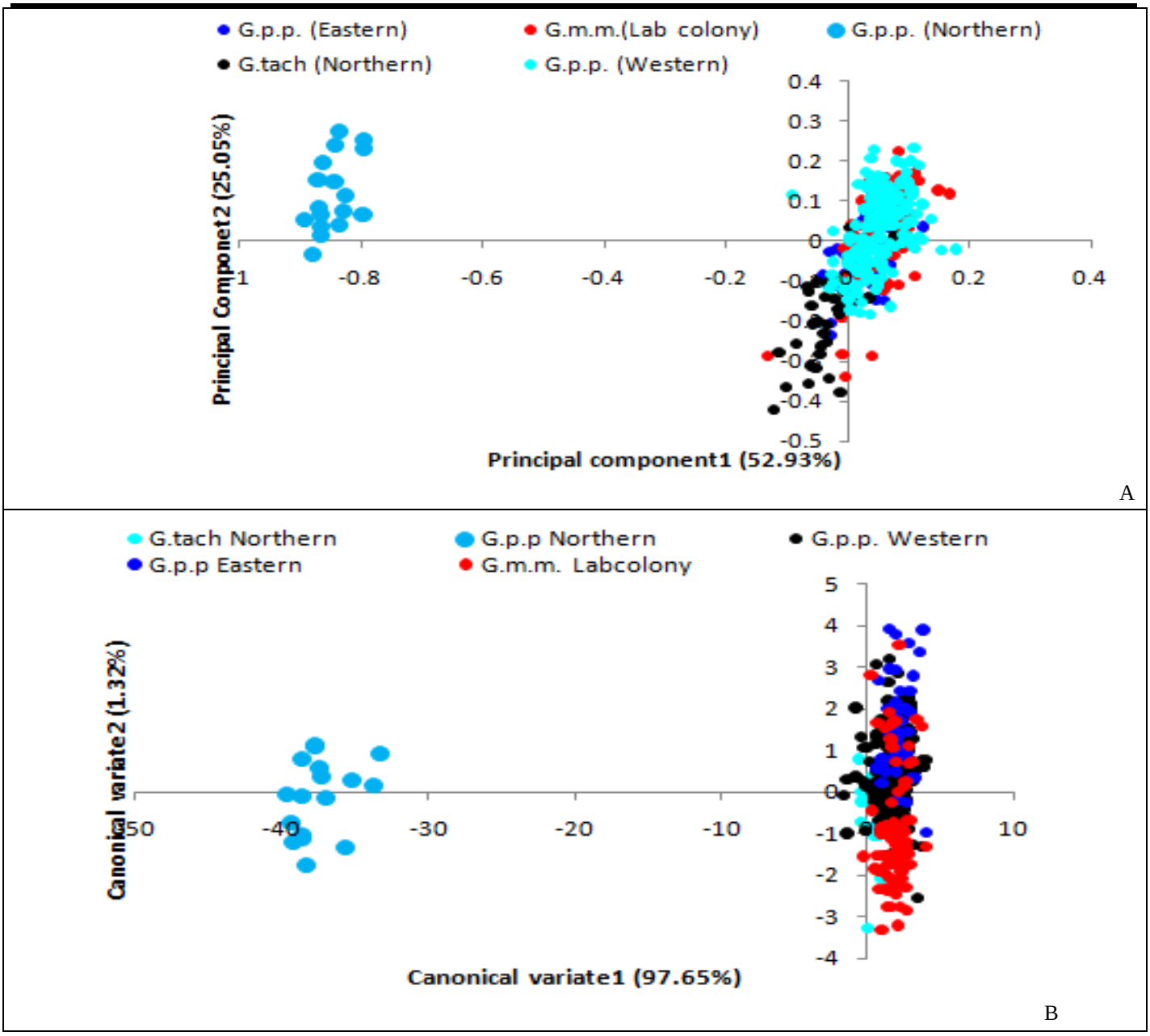

Fig.2: Projection of wing data of three species of tsetse flies of Ghana. (A) First two principal components (B) First two canonical variates

3.3 Comparison of *G. p. palpalis* populations from three Regions of Ghana

Wing length (*at*) was significantly different (P<0.0001) and separated Eastern, Northern and Western populations from lab colony, whereas wing width (*ww*) and hatchet blade line, (*kg*) were important and separated *G. p. palpalis* of Eastern and Western populations together with laboratory colony from those of Northern Region (Table 2).

The ratio value, *th/at* (hind tibia/wing length) significantly (P<0.0001) separated fly populations into four groups, Northern, Eastern, Western and the lab colony, an indication that hind tibia/wing length is a good discriminating tool (Table 3).

Wing data projected on the first five principal components showed a partial separation of *G. p. palpalis* collected from three regions in Ghana (Northern, Eastern and Western) and *G. m. morsitans* from laboratory colony (Figure 3a). The first two components contributed 76.63% (PC1 + PC2 = 56/47% + 19.16%), the third to fifth components respectively (PC3 + PC4 + PC5= 7.72% +

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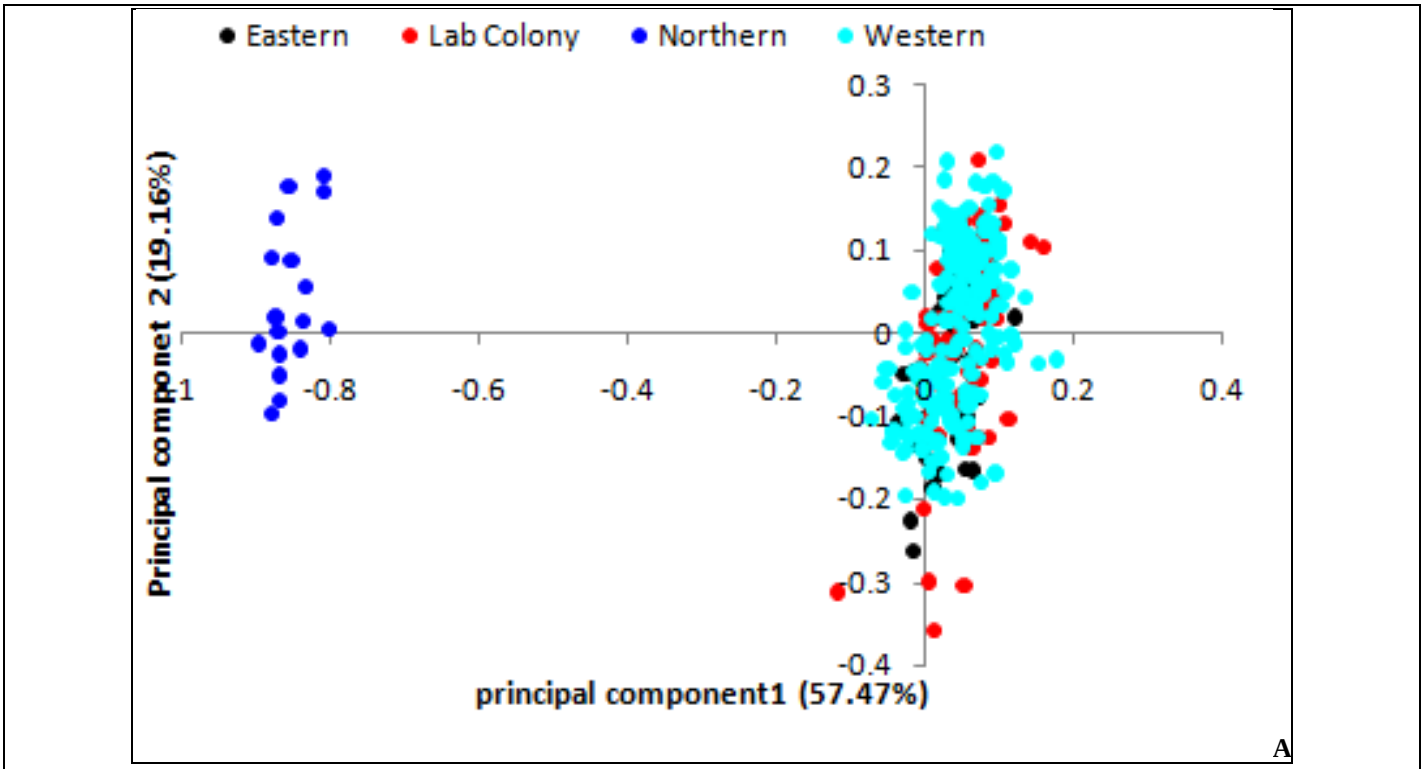
3.78% + 2.94%) contributed little in the separation of the flies from the different populations. Likewise projection of the data on the first two canonical variate axes showed a similar pattern of separation with four populations forming two clusters (Figure 3b), *G. p. palpalis* populations from the Eastern, Western regions and laboratory colony clustered together while *G. p. palpalis* from the Northern population distinctly separated from the other three populations. The first two canonical variates contributed 99.7% (CV1 + CV2 = 98.38 + 1.32%).

Table.4: Mahalanobis squared distances (D^2) between clusters representing flies, from different regions in Ghana.

Region	Eastern	Lab colony	Northern	Western
Eastern	0			
Lab colony	9.323	0		
Northern	1540	1519	0	
Western	3.13	3.93	1512	0

The largest Mahalanobis squared distance (D^2=1540) was between Eastern and Northern, followed by Northern and Western (D^2 = 1512), Eastern and laboratory colony (D^2 = 9.32) and the lowest distance (D^2 = 3.92) being between laboratory colony and the Western (Table 4), an indication that flies from Western Region are more closely related to those of the laboratory colony than flies from the Northern and Eastern Regions.



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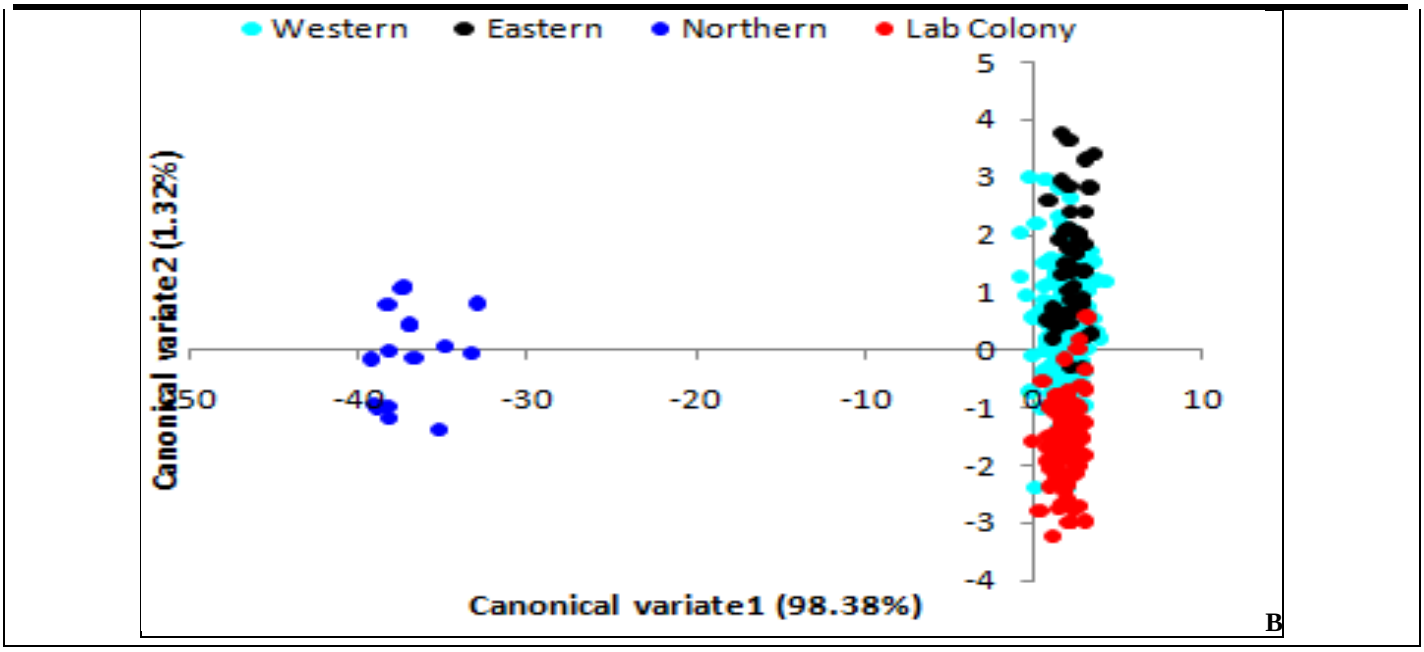

Fig.3*: Projection of wing data of 263 G. p. palpalis sampled from Northern, Eastern and Western Regions and 100 G. m. morsitans from lab colony (A) First two principal components (B) First two canonical variates of the flies*

IV. DISCUSSION

In the absence of allometry, large specimens tend to have larger dimensions and have a greater deal of variance associated with overall size [15, 16, 18]. In order to minimize the effects of allometry, wing-tibia measurement data were $\log_{10}$ transformed [19], to equalize standard deviations across the differently sized variables and ensures multivariate normality of the data [15, 17, 18].

Results of the GLM analyses of linear and ratio data revealed that different linear combinations can be used to characterize tsetse species populations. Most of the linear variables showed significant differences ($P<0.0001$) in size and shape among the populations. For example, the population from the lab colony (*G. m. morsitans*) always stood out as the largest in most of the measured linear variables. The costa vein (*ab*) and wing length (*at*) are the two main features that morphologically characterized the three tsetse fly species studied. The larger size observed in *G. m. morsitans* (lab colony) may be related to laboratory conditions, where optimal conditions like temperature (24 - 29ºC), relative humidity of 60% are provided to the flies. In tsetse flies, colder environmental conditions increase metabolic rate as observed under typical forested regions in Africa [20]. This condition brings about larger body size possibly as a consequence of increased feeding requirements or liquid storage in the body of the flies. Variation observed among the different tsetse species populations was in line with work by Adeleke *et al.* [21] on populations of *Culex quinquefasciatus* and *Mansonia africana* in south- west Nigeria. Their results based on morphometric analyses on antennal length, proboscis length, fore-, mid and hind leg length, revealed variations in the two mosquito species, and concluded that wing length could be a good discriminating variable in characterizing members of complexes.

Separations along the first principal axes are usually attributed to overall size, while those along the second principal axes are attributed to shape [15, 16, 17]. The Principal components and canonical variate plots have demonstrated their worth as important taxonomic tools through evolutions of the role played by the overall body size in separating insect pests as well as vectors that are of medical importance [17].

The smallest Mahalanobis squared distance observed between *G. p. palpalis* (Eastern) and *G. p. palpalis* (Western) means that probably the two populations are more closely related morphologically compared to the other populations. The *G. p. palpalis* (Northern) skewing towards the negative axis on the PC1 and CV1 suggest that they were the smallest among the five populations, while *G. m. morsitans* having larger scatter points might also be the largest among the groups.

The lab population of *G. m. morsitans* was expected to be larger since it was maintained under optimal conditions with minimal stress as compared to *G. p. palpalis* populations in the wild. The *G. p. palpalis* were collected

from three different regions in different agro-ecological zones, and they are expected to adapt to the zones. The Western and Eastern regions are considered to be the forested regions of the country where rainfall is high most times of the year. Climatic conditions in these two regions probably favour growth of the flies and hence size increase compared to the Northern population, where the climate is hot throughout the year. Similar results were obtained by Solano *et al*. [22], where significant differences were observed in *G. p gambiensis* populations collected from regions in Burkina Faso and others from Senegal. The difference observed in *G. p gambiensis* populations from their findings was as a result of geographical distances between the sampling regions. Kandemir *et al*. [23] also carried out morphometric analyses on different population structures of dwarf honey bees (*Apis florea* Fabricius, 1876) in Iran. According to their findings, populations from different regions were significantly different based on characters observed in the fore- and hind wings of the insects. Several factors may be involved in morphological differences, such as geographical distances, ecological differences or even trapping methods. The variation in morphometric traits might also be an adaptation to the various Agro-Ecological Zones of the flies.

The results of the current study on principal components and canonical variates analyses suggest that the examined regional populations of *G. p. palpalis* can be categorized into different groups. As shown in figures 3a and b, the Northern fly population was distinct from the other populations along the first two canonical variate axes. However the other populations, Eastern, lab colony and Western were not quite distinguishable from each other. It can be suggested that the Northern population had the least gene flow compared to the other populations because of geographical distances between them. This could also suggest that the amount of morphological differences could be predicted by the level of geographic isolation [24].

The *G. m. morsitans* (lab colony) was characterized morphometrically by the wing variables, *kg, jk, ad, gh, ij* and *hi* and stood out to be the largest in most cases. Wing length (*at*) and costa vein (*ab*) morphometrically characterized the three tsetse species into separate group each. The principal components and canonical variates as well as Mahalanobis squared distances showed size differences among the different populations, which act to confirm linear and ratio separations. Therefore based on these differences in morphometric characters observed, the three tsetse species were distinguished from each other.

Similar work on morphometrics needs to be done to include many other body parts such as proboscis length, antennal length, thorax and abdomen length and width in order to establish stronger morphometric tools for discriminating different tsetse fly species.

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Identification of Arabica Coffee Production in Altitudes Place in Lintong Ni Huta of Humbang Hasundutan

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Abstract— *Coffee is one of mainstay plantation commodities in Humbang Hasundutan Regency and as a source of countries income, providers of employment, and encourages the development of agribusiness and agroindustry. Lintong Ni Huta is one of coffee production center in Humbang Hasundutan Regency that famous with Coffee Lintong. The purpose of this study was to determine the suitability of the land and to identify the production of coffee types at various altitudes in Lintong Ni Huta. The results of agriculture coffee plantation and production In Lintongnihuta is the largest and most located in the villages in the altitude of 1400-1500 meters mean abaut sea level and the altitude of coffee planting in Lintongnihuta regency does not affect coffee production.*

Keywords— Lintong Coffee Production, Altitudes Place.

I. INTRODUCTION

Coffee is a plantation crop that has long been cultivated. as well as a source of income not less than one and a half million peasants in Indonesia, coffee is also a commodity mainstay of exports and sources of foreign exchange income of the country. Indonesia's coffee now judging by the results, the fourth rank largest in the world. Coffee has a long history and has an important role for economic growth in Indonesia. Coffee (Coffea sp) Can contribute 11% of the total export of Indonesian plantation crops. Estimated area of coffee plantation exploitation in Indonesia about 1,227,787 ha with production of 637,539 tons. North Sumatera as one of the largest coffee plantation area after South Sumatera, Lampung, Aceh and East Java with coffee plantation area of 81,474 ha with production of 60,307 tons (Directorate General of Plantation, 2017). Coffee is one of the mainstay of plantation commodities in Humbang Hasundutan Regency and has a role as a source of income of foreign countries,and sources of income, providers of employment, and encourage the development of agribusiness and agro-industry. Lintong Ni Huta is a coffee production center in Humbang Hasundutan regency famous for its Lintong Coffee. Coffee plantation area in this regency with 9.246 Ha harvested area and 6,461 tons production. Coffee plantations comprise 48.45% of the area of agricultural land and plantations. (Humbahas In Figures, 2011) the development of coffee cultivation requires shade plants as a protective against direct sunlight to reduce the evapotranspiration process. Rainfall and temperature are climate factors that affect coffee production. In this case the volume and distribution of rainfall throughout the year, and the altitude of the place determines the suitability of growing coffee plants (Rothfos, 1980). The annual rainfall required for Arabica coffee growth ranges from 1,100-1,300 mm and for Robusta coffee is 1,550-2,000 mm. Distribution of rainfall is very effected in determining the pattern of plant fertilization because it is associated with the process of breaking the dormancy of interest. While the height of the appropriate place for the growth of Arabica coffee is 1000-2000m above sea level. whose temperature conditions range from 15 0C to 24 OC, and for the Robusta coffee <700 MASL. whose temperature conditions range from 24 OC-30 OC (Wilson, 1985).Several factors affecting production and quality, especially on organoleptic characteristics, have not been scientifically identified, need further research to identify those prioritized factors. Organoleptic characteristic is an advantage for coffee type. The height of place, the type of soil, the climatic conditions such as temperature, humidity, until processing, storage, and marketing techniques need to be tested in relation to the quantity and quality of coffee production.

II. LITERATURE REVIEW

Cultivation of Coffee Crops

Economically, the growth and production of coffee plants is highly dependent on or affected by climate and soil conditions. Another basic requirement that can not be ignored is looking for superior seeds whose production is high and resistant to pests and diseases. After these requirements can be met, an important thing is maintenance, such as: fertilization, pruning, shade trees, and the eradication of pests and diseases

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Varieties Coffee Sigarar Utang

This coffee is a type of arabica coffee that thrives in mountainous areas with an altitude of 700-1700m above sea level. This type of coffee is very suitable to be grown in cold climates such as in Tapanuli. Sigarar coffee plants have semi-dyed stature, short branch segments, a lush canopy covering the entire surface of the tree so that the trunk is not visible from the outside. The nature of the secondary branching is very active even the primary branch above the ground surface forming a dangling fan touching the ground. The old leaves are dark green, the young leaves (flush) are brown. When planted without leafless leaf shelves and leaf blades panting, when viewed at a glance the shape of long tapered leaves and edges of wavy leaves. Young fruit is green while the ripe fruit is bright red, round long elongated round fruit shape and 100 ripe fruit (red) on average - 196 gr. Production potential ranges from 800 to 2300 kg of seed / ha. Sigarar type coffee from debt is somewhat susceptible to leaf rust disease, especially grows at an altitude of less than 1000 MASL, is also susceptible to nematode parasites (Panggabean E, 2011)

Soil and Climate suitable for coffee growth

Coffee will grow if plant growth requirement can be fulfilled that is soil with effective deep enough (> 100 cm), slack, well drained, and enough water, enough nutrition, especially potassium (K), enough organic. material 3%). The ideal soil acidity (pH) for coffee plant growth ranges from 5.3 to 6.5. As long as this coffee is commonly grown in Indonesia there are two types of Robusta and arabica. Both types of coffee physiologically require requirements of different climatic conditions. Arabica coffee requires higher land than Robusta coffee, because it is grown in wetlands besides growth and productivity it is more susceptible to leaf rust disease. Arabica coffee with some criteria of planting place: High 700-1400 MASL, daily air temperature 15-24 celcius, average rainfall 2.000-4.000 mm / years, dry month 1-3 month / year, acidity level (pH) soil 5.3 - 6, organic matter content> 3%, soil effective depth> 100 cm, maximum slope of 40%. Arabica coffee cultivation close to sea level is infected with leaf rust disease. Medium at an altitude of more than 2,000 m is often disturbed with dew upas. (Panggabean E, 2011). Coffee is affected by extreme season conditions, where prolonged dry seasons or excessive rain disturb flowering and fertilization. Conditions of high rainfall will disrupt the process of pollination of coffee flowers assisted by wind pollinators and beetles. Before the bloom, the physiological flowers of coffee will have a period of dormancy. At that time the flower buds stopped its development for several months (1-4 months). Factor is development of interest after the dormancy period is the availability of ground water. Although the dormancy period has passed but if the groundwater is not sufficient to the needs of division process lowers then the flowers will not bloom. The flowering process is usually triggered by the arrival of the rainy season or through the watering process (Cannell, 1985).

Growing Terms

Coffee plants have special properties because each species needs a slightly different environment. Environmental factors that greatly affect the growth of coffee plants, among others, the height of the place, rainfall, sunlight, wind, and soil (Najiyati, 2004).

A. Place Height

The actual height of the place does not directly affect the growth of the coffee plant. Air temperature factors have a direct effect on the growth of coffee plants, especially the formation of flowers and fruits and sensitivity to disease attacks. In general, high temperatures are affected by the altitude of the sea surface.

b. Rainfall

Rain is the most important climate factor after the height of the place. This factor can be seen from rainfall and rainfall time. Rainfall will affect the availability of water needed by plants.

c. Sun

In general, coffee does not like a lot of direct sunlight, but it requires sunlight that spreads / spreads. Large direct sunlight increases evaporation of soil and leaves, thus disrupting the balance of photosynthesis, especially in the dry season. In addition to its effect on photosynthesis, sunlight also affects the process of flower bud formation. A lot of sunlight will stimulate the formation of flower buds. Thus, if coffee plants throughout the year produce continuous direct sunlight then the plants will form flowers throughout the year.

As a result the flowering becomes irregular and the plant produces flowers beyond its ability so that the number of successful flowers becomes a bit of fruit.

To arrange the arrival of sunlight, usually among coffee plants grow shade plants. This shade factory is set so that coffee plants can grow in the shade and get enough sunlight.

d. Wind

The role of the wind is to help move the pollen from one plant to another with different clones. Thus, pollination occurs that can produce fruit. In addition to the positive effects on coffee plants, sometimes the wind also has a

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negative effect, especially during high winds. Strong winds will directly damage the plant canopy or abort the flowers. Strong winds that come in the dry season will also accelerate the occurrence of evapotranspiration (evaporation of water from plants and soil) resulting in drought.

e. Soil

In general, coffee plants require loose soil, fertile, and rich in organic matter. Therefore, the soil around the plant should often be given organic fertilizer to be fertile and loose so that the root system grows well. In addition to loose soil and rich in organic matter, coffee also requires a bit of acid soil, which is between pH 4.5 to 6.5 for robusta coffee and pH 5-6,5 for arabica coffee. If the soil pH is less than that amount then the coffee plant can still grow, but less able to absorb some nutrients so the land needs to be chalked. On the other hand, the coffee plant does not want a slightly alkaline soil (pH above 6.5) so that lime is not excessive (Panggabean E, 2011)

III. METHODOLOGY

The research was conducted in Lintong Ni Huta in various altitudes. The altitudes of the study sites ranged from 1200 MASL to altitude> 1500 MASL divided into several groups of places as follows: 1200 - 1300 MASL, 1300 - 1400 MASL, 1400 - 1500 MASL and > 1500 MASL. The research method is used field research that is descriptive analysis both quantitative and qualitative, the research based on solving factual problem that exist at this time. The collected data is arranged, explained, and then analyzed. Data used in this research are climate data (rainfall), area and productivity of coffee plant. This data was obtained by citing data and reports from related offices (Office of Statistics and Dishutbun Kabupaten Humbang) and taken directly from farmers as samples.

Interview

Structured interviews were conducted using questionnaires with coffee farmers. The contents of the questionnaire on research related issues are plant age, area, type of coffee grown, plant height, number of stems of tree, number of branches of tree, number of coffee beans tree branch, coffee production.

IV. RESULT AND DISCUSSION

Growth and Production The average coffee at an altitude of 1200 - 1500 meters above sea level in Kecamatan Lintongnihuta shows not significant difference where the height of the growing place is still in accordance with the need to grow arabica coffee plants. From the results of interviews with farmers (figure 1) shows that at an altitude of 1400 -1500 MASL obtained growth and better coffee production, this is possible because at the sunlight received by plants enough for photosynthesis activity and cloudy air conditions serve as a shade that prevents excessive sunlight from that received by coffee plants due to the coffee plant will produce large amounts of continuous interest in full sun exposure is also required to balance carbohydrate production to keep the fruit load as presented by DaMatta (2004) the coffee plant can become regarded as a species with widespread plasticity in response to various radiations

Table.1: Observation of Growth Conditions of Coffee Crops in each Land

Identifying Growth and Production of Coffee	**Altitudes (MASL)**			
	1200-1300	**1300-1400**	**1400-1500**	**>1500**
Average Plant Height (m)	1.54	1.83	1.67	1.58
Number of stems / trees	1.33	1.00	1.50	1.21
Number of Branches / Trees	19.58	19.58	22.50	16.67
Number of Coffee Beans / Branches	45.83	50.83	85.00	63.33
Total Coffee Beans / Trees	854.17	929.17	2066.67	1247.92

Table.2: Characteristics and ownership of Arabica coffee plantation in some altitudes places in Kecamatan Lintong ni Huta

Description	**Altitudes (MASL)**			
	1200-1300	**1300-1400**	**1400-1500**	**> 1500**
a. The average age of Plants (years) : * 2 – 4 * 5 – 6 * > 6	√	√	√	√
b. Plant Coffee Covered other plants: * Yes * No	√	√	√	√

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c. Average plant height:: * 1 – 1.5 * 1.5 – 2 * 2 – 2.5 * 2.5 – 3	 √	√	√	√
d. Number Branches of tree: * 1 – 5 * 5 – 10 * 15 – 25 * > 25	√	√	√	√
e. Number of Coffee Beans / Branches: * 10 * 20 * 50 * 100 * > 100	 √	 √	√	√
f. Mean weight of seed / tree (mg): * < 250 mg * 250 - 500 mg * 500 - 750 mg * > 750 mg	 √	√	√	√
g. There are other plants besides coffee: *only coffee * Mixing	√	√	√	√
h.Tree Density / acre: * 50 * 80 * > 80	 √	 √	 √	 √
i. Coffee Condition: * Pruning * fertile	 √	 √	 √	 √
j. Is there a flower on the fruit stalk: * Yes * No	√	√	√	 √
k. Pests that attack: * No * Mushroom * Insect	 √	 √	 √	 √
l. Types of Countermeasures: * Mechanical * Chemistry * Biological	√	√	√	√
m. Fertilization : * once a years * twice a years	 √	 √	 √	√
n. Type of Fertilizer: * Organic * inorganic	 √	 √	 √	 √
o. Harvest forecast of the year : * Faster * Lower	 √	 √	 √	√
p. % harvest compared to last year: * 30 %	√	√	 √	

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* - 20% * - 10% * > 10%				√
q. Rainfall compared to last year: * - 30% * - 20% * - 10% * > 10%	√	√	√	√

Results of Farmer Questionnaire

Farmers are still conventional and farming traditional coffee by letting coffee grow as it is. Pruning the coffee plant is still a strange thing, which is done only on plants that are not productive growth. It is necessary to change the habit and understand the usefulness of pruning as one part of care to maintain the balance of plant development in order to produce optimal fruit production. To get high quality results, the coffee picked after cooking is the time of red fruit skin. For arabica coffee the time required from flower bud formation until ready to be harvested is 6 – 8 month. The average harvest data was obtained in June 2016, at the time of flowering around November-December 2015 where the number of rainy days is quite high (Table. 4) thus affecting the formation of the yielded interest slightly affecting the crop average% compared to last year. which decreased by 20% to 30%. The resulting flower remains on the fruit stalk although at the time of fruit enlargement, except at altitude> 1500 MASL there is a new flower on the fruit stalk. This happens because the sunlight that continues to stimulate flowering. Higher fruit loads reduce seed size due to competition between carbohydrates during filling fruits. These results can be offset by agricultural management such as fertilization, tree pruning to help farmers improve the sustainability of coffee plantations, produce higher quality seeds and higher and ultimately increase their income. (Vaast, P, et al 2006) The altitude of the place correlates with the temperature, precipitation and soil organic matter. In relation to this condition, the higher the cultivation area, the lower the temperature and the higher the organic matter. The taste of Arabica coffee is getting better as the place grows. This condition is related to the temperature at the flowering period, the filling of the fruit, and the ripening of the fruit. This is expected to affect the taste of Arabica coffee. The higher the place also looks the better the physical quality of coffee beans indicated by the low percentage of black seeds, pests and disabilities. (Karim, A, Hifnalisa, 2012).Climate also has a big effect on coffee plant productivity. Climate effects begin to emerge from the main branch before flowering. And this continues to be felt at the opening of flowers until the path of pollination, the growth of young fruit grow old and ripe fruit, in coffee plants. Toward the dry season the weather generally starts to clear, the air is never cloudy. Because the rain has begun to fall, it means the solar radiation will be more and more, then the temperature will also increase. Primary branches (plagiotropes) of growing flowers begin to prepare for growth. Therefore, the more radiation, the preparation of the formation of flowers on coffee trees will be faster. Conversely, if irradiation decreases, preparation is slow and the amount of interest in the preparation of coffee trees is also low (Beer, J, et al., 1998) From previous research, it was found that the production to taste of coffee is determined by the way of processing, varieties, and height of the planting place (Mawardi, et al., 2008; Karim and Hifnalisa, 2011).

Coffee Cultivation in Kecamatan Lintongnihuta

The condition of coffee planting in several villages in Lintongnihuta with different height levels in Table 3 is seen at an altitude of 1200-1300 meters above sea level, only Sitio II village with planting area around 104 ha with coffee production of 60.48 tons. Area and production is lower than coffee plant in other villages such as 1,300-1400 bowls with the best coffee production of 117.48 tons from planting area of 202 ha, Siponjot village, the altitudes 1400-1500 masl is Tapian Nauli village with Production at most 131.43 ton is generated from 226 ha of coffee planting area and> 1500 masl altitude there is 112.82 tons of coffee production from 194 ha planting area of Dolok Margu village. The average coffee production for Kecamatan Lintongnihuta was 0.58 ton / ha. From 22 villages in Kecamatan Lintongnihuta 12 villages are located at an altitude of 1400-1500 masl with coffee cultivation area of 1570 ha, 8 villages are located at an altitude of 1300-1400 masl with an area of 885 ha of coffee and each 1 village is at an altitude of 1200 - 1300 masl with coffee planting area of 104 ha and in altitude> 1500 masl there are 104 ha. The largest coffee cultivation area compared to the villages in Siponjot and Sibuntuon Partur, about 30% of the total area is coffee cultivation. Arabica Coffee Types of Sigararutang from Kecamatan Lintongnihuta have a very prominent role as a source of community income, employment and foreign exchange. Revenue of farmers / village / year average higner located in the village at an altitude of 1400-1500 mdpl is

Rp.4.945.683.200, Besides the height of the place, the combination of factors of plant diversity, soil type, soil fertility level where the coffee grows until the manage of the harvest crop produces a difference of production to the quality of the coffee plant. The combination of these factors is complex even from a single plantation site that finds variety in production and quality.Climate change such as temperature rise, rainfall changes affect land management and fertile soil both physically, chemically and biologically (Singh.B.P, et al, 2011)

Table.3: Characteristics of Coffee Planting in Kecamatan Lintongnihuta in various altitudes site

No	Village	Area (Ha)	Coffee Area (Ha)	Altitudes	Production (ton)	Population (btg)	Types of coffee	Ph	Type of soil	Soil Fertility Rate	Revenue Farmers everyVillage / Year
1	Sitio II	541,13	104	1200-1300	60,48	145.600	Arabica	3.5 - 5	Latosol, Lithosol, Podzolik	Medium / fertile	3.931.345.600
2	Nagasaribu I	689,13	100	1300-1400	58,16	140.000	Arabica	3.5 - 5	Histosol, Podzolik, Latosol	Medium / fertile	3.780.140.000
3	Nagasaribu II	725,4	108	1300-1400	62,81	151.200	Arabica	3.5 - 5	Latosol, Lithosol, Podzolik	Medium / fertile	4.082.551.200
4	Siharjulu	1.235,03	84	1300-1400	48,85	117.600	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	3.175.317.600
5	Siponjot	632,88	202	1300-1400	117,48	282.800	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	7.635.882.800
6	Nagasaribu IV	688,58	94	1300-1400	54,67	131.600	Arabica	3.5 - 5	Latosol, Lithosol, Podzolik	Medium / fertile	3.553.331.600
7	Nagasaribu V	617,15	113	1300-1400	65,72	158.200	Arabica	3.5 - 5	Latosol, Lithosol, Podzolik	Medium / fertile	4.271.558.200
8	Nagasaribu III	906,75	106	1300-1400	61,65	148.400	Arabica	3.5 - 5	Latosol, Lithosol, Podzolik	Medium / fertile	4.006.948.400
9	Sigumpar	972,72	78	1300-1400	45,36	109.200	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	2.948.509.200
10	Hutasoit I	940,08	137	1400-1500	79,67	191.800	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	5.178.791.800
11	Lobutua	867,32	106	1400-1500	61,65	148.400	Arabica	4 - 5.8	Latosol, Lithosol ,Podzolik	Medium / fertile	4.006.948.400
12	Pargaulan	780,59	125	1400-1500	72,70	175.000	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	4.725.175.000
13	Sibuntuon Parpea	630,78	41	1400-1500	23,84	57.400	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	1.549.857.400
14	Sibuntuon Partur	502,25	174	1400-1500	101,19	243.600	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	6.577.443.600
15	Sitolu Bahal	1.031	119	1400-1500	69,21	166.600	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	4.498.366.600
16	Tapian Nauli	1.576,96	226	1400-1500	131,43	316.400	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	8.543.116.400
17	Hutasoit II	729,54	125	1400-1500	72,70	175.000	Arabica	4 - 5.5	Latosol, Lithosol, Podzolik	Medium / fertile	4.725.175.000

Note: Seeds of osas / seeds without horn / ready for frying
Average gross income of farmers / years = 838 kg x Rp. 65,000 (export) = 54,470,000
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V. CONCLUSION

Identification of coffee production In Lintongnihuta District is the largest and most located in the villages in altitude of 1,400-1500 meters above sea level. And the high coffee plantation in Kecamatan Lintongnihuta does not affect the production of coffee.

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Effect of Packaging Materials on Retention of Quality Characteristics of Dehydrated Green Leafy Vegetables during Storage

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Abstract*— *The objective of this study was to investigate the influence of blanching, dehydration and packaging on nutrient composition of Amaranthus gangeticus and Spinach oleracea. There was a loss of sugars, Proteins, vitamin- c and carotenoids were noticed due to blanching. But the colour of green leafy vegetables (GLV) is retained due to blanching. Leafy vegetables were dehydrated in cabinet dryer at 60^{o}C and packed in three packaging materials (Metalized polypropylene (MPP) 300 gauge, high density polyethylene (HDPE) 300 gauge, low density polyethylene (LDPE) 200 gauge) and stored at room temperature for 45 days to evaluate the best package for maximum retention of nutrients in leafy vegetables during storage. PP followed by HDPE was found to be good for retention of nutrients in dehydrated leafy vegetables during 45 days of storage. Irrespective of the losses of nutrients that take place during dehydrated packaging, GLV can be preserved by dehydration which is eco-friendly and easily adoptable.

Keywords*— *Blanching, dehydration, packaging, Spinach, Amaranthus, LDPE, HDPE, MPP.

I. INTRODUCTION

Green leafy vegetables are rich sources of micronutrients and antioxidants. These leafy vegetables are seasonal and perishable, owing to high moisture content. Green leafy vegetables have a unique place among vegetables because of their colour, flavour and health benefits. They are inexpensive, easy to cook serve as rich sources of carotene, ascorbic acid, folic acid, chlorophyll, calcium, iron, phosphorous, zinc and dietary fibre (1-3). Green leafy vegetables are abundant in supply during the peak season and results in spoilage of large quantities. The consumption of green leafy vegetables could improve the nutritional status of

poor rural and urban households because these plants are rich sources of minerals, vitamins carotenoids and phenolic agents (4,5).

Dehydration is a suitable alternative for post-harvest management to increase shelf-life and promote food security (6). Products with low moisture content can be stored at ambient temperatures for long period of time due to a reduced microbiological activity and minimized physical and chemical changes (**7**). Dried leafy vegetables are more concentrated tasty, nutritious, light weight and easy to prepare, store and use (8). Blanching is a primary step in processing of vegetables. It inactivate enzymes, retains colour and modification of product texture (9,10).

Among all the green leafy vegetables Amaranths plants were caped under the super food segment due to their health benefiting properties (11). Amaranths leaves are packed with carbohydrates, proteins, iron minerals and vitamins, and regular consumption helps in easing digestion, and weight management. Since it is high in iron content and dietary fibre, it is good for anaemic patients, and reduces cholesterol and risks of cardiovascular diseases.

Spinach is a super food loaded with protein, iron, vitamins, minerals and dietary fibres (12). The possible health benefits of consuming spinach include improving blood glucose control in people with diabetes, lowering the risk of cancer, reducing blood pressure, improving bone health, lowering the risk of developing asthma etc. Despite the importance of traditional vegetables in the diet, understanding of postharvest processing of traditional vegetables is limited. Considering the above mentioned importance the study was conducted to determine the characteristics of packed dried spinach and Amaranths leaf powder so as to use directly in the development of various food formulations.

II. MATERIALS AND METHODS

Plant materials

The leafy vegetables were procured directly from the field in Kanpur, Hyderabad. The leaves were separated from inedible portions and washed under running water to remove the adhering mud particles and drained completely.

Blanching

Washed leaves were Blanched at 100°C for 1 min in water and cooled immediately by dipping in cool water at a temperature of 20°C for few seconds. Blanched leaves were spread on trays in single layer and dried in a cabinet dryer at 60°C to a moisture content of 5-6% in the finished product.

Packing and Storage

The dried green leafy vegetable samples were ground to fine powder by using a mixer grinder and sieved through a 100 mesh size sieve and packed separately in MPP 300 gauge, HDPE 300 gauge, LDPE 200 gauge bags and kept at room temperature conditions (Temperature 32-38°C) for a period of two months for storage studies and product was drawn in 15 days interval for physico- chemical analysis.

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Analysis

The fresh GLV and blanched GLV were analyzed for the following components to study the effect of blanching. Dehydrated GLV powder was analysed to study the effect of packaging. Moisture content was determined by standard method of (13). Protein was estimated by (14) , Total chlorophyll, Total carotenoids, β-carotene, ascorbic acid content, ash content was determined by method of (15). Rehydration ratio of dehydrated GLVs was estimated as per (16).

Statistical Analysis

All measurements were performed in triplicate for each sample. Data were analyzed using statistical software (SPSS for Windows Version 16.0). Significant differences between the means were estimated using Duncan's multiple range tests. Differences were considered significant at $p < 0.05$.

III. RESULTS AND DISCUSSION

Dehydration is an excellent way to preserve food and is appropriate food preservation technology for sustainable development. GLVs are dehydrated to enhance storage stability, minimize packaging requirement and reduce transport weight. In the present study we have analyzed the effect of dehydration and packaging material on the nutritive value of spinach and Amaranthus leaves. The results of the study are presented in Tables 1, 2, 3.

Reducing sugars

Reducing sugars of blanched portion are higher than the dried portion. **(**17) also reported that carbohydrate content of blanched pumpkin leaves was more than air dried leaves. During the storage period, the spinach and amaranths showed decreased pattern of reducing sugar content. Packaging affected the reducing sugar content over the storage period of 45 days. MPP packaging material maintained higher value ($p<0.05$) of reducing sugar content than in HDPE and LDPE packaging. The reason for the decrease could be due to utilization of sugars for metabolic activities (18)

Total Sugars

GLVs showed a decrease in total sugars after blanching from 498.8µg/mg and 965.6µg/mg to 390µg/mg and 699.6µg/mg. Amaranths has more total sugars compared to spinach. PP, HDPE and LDPE film packaging showed maximum value of total sugars on day 15 and decreased thereafter. Spinach and amaranths leaves powder packed in MPP packaging have significantly higher level ($p<0.05$) of total sugars when compared to samples packed in HDPE and PP after 45 days of storage.

Moisture

Moisture content of food is very important on nutrient density and shelf-life of agricultural produce The moisture content of the unblanched and blanched GLV is ranged between 6.56 - 7.6% in spinach and 6.57- 7.5 in amaranths (Table 1). (19) Also observed more moisture retention capacity of blanched spinach leaves. Dried GLV packed in different packaging material showed a decrease in moisture content during the storage period. The rate of loss of moisture was low, there is a significant difference between the packing material at 5% level of significance after 45 days of storage, PP showed low moisture content than LDPE and HDPE for spinach, and PP showed low moisture content in Amaranthus powder than samples packed in HDPE and LDPE.

Table.1: The affect of packaging material on the dehydrated leafy vegetable (spinach).

		Packaging materials		
Parameter	storage period (days)	MPP	HDPE	LDPE
Reducing sugar	15	275.4±0.21	253.8±0.043	237.6±0.303
(µg/mg)	30	255.6±0.063	241.1±1.043	210.7±0.46
	45	243.2±0.003	235.1±0.97	201.2±0.04
Total sugar	15	249±1	224±1	212±1
(µg/mg)	30	220.3±0.333	197.6±0.333	175.3±1.33
	45	216.3±1.333	176.6±1.33	142±1
Protein	15	639±1	592.6±4.33	588.3±4.33
(µg/mg)	30	631±1	590.33±0.33	580.3±0.33
	45	630.3±1	585±1	573.6±2.33
chlorophyll	15	201.8±1.343	193.3±0.13	192.3±0.083
(µg/mg)	30	200.2±0.143	191.1±2.71	189.2±0.01
	45	198.3±0.13	187.2±0.063	185.2±0.01
Carotenoids	15	39.23±0.023	37.73±0.093	36.23±0.063
(µg/mg)	30	38.6±0.13	36.96±0.103	35.13±0.003
	45	36.52±0.129	34.36±0.093	32.4±0.173
Vitamin c	15	5.12±0.204	5.806±0.222	4.59±0.063
(mg/10g)	30	4.45±0.097	4.88±0.164	4.52±0.078
	45	4.33±0.093	4.82±0.129	4.44±0.207
Moisture	15	6.786±0.220	7.20±0.792	6.85±0.406
(%)	30	6.346±0.132	7.59±0.0007	6.29±0.016
	45	6.686±0.220	7.55±0.012	6.74±0.434
Rehydration	15	1.776±0.002	1.47±0.003	1.52±0.006
Ratio	30	1.85±0.009	1.96±0.004	2.21±0.01
	45	1.346±0.003	1.516±0.009	1.66±0.018
Ash content	15	1.79±4.9E-05	0.84±0.0004	1.15±0.001
(g)	30	1.78±3.6E-04	1.8±2.8E-05	1.82±9.33E-06
	45	1.8±0.0007	1.9±0.004	2.19±0.001

Table.2: The effect of packaging material on dehydrated leafy vegetable (amaranths).

		Packaging materials		
Parameter	storage period (days)	MPP	HDPE	LDPE
Reducing sugar	15	169.5±0.023	166.3±0.023	164.9±0.19
(µg/mg)	30	168.6±0.02	163.6±0.07	161.9±0.01
	45	166.4±0.043	159.4±0.01	156.1±0.043
Total sugar	15	509±1	496.3±2.33	379.3±1.33
(µg/mg)	30	485.5±2.33	462.6±2.33	371.3±1
	45	460.6±2.33	421.6±1.33	352±1
Protein	15	701±1	690±1	678.6±1.33
(µg/mg)	30	692.3±6.33	685.3±2.33	669±1
	45	689±1	678.6±2.33	659.6±2.33
chlorophyll	15	638.16±0.023	632.03±0.023	630.1±0.01
(µg/mg)	30	636.2±0.063	630.33±0.01	629.26±0.143

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	45	635.4+0.043	629.63+0.143	625.43+0.063
Carotenoids	15	79+0.01	76.1+0.02	75.5+3.103
(µg/mg)	30	78.2+0.003	75.4+0.09	71.1+0.04
	45	77.06+0.02	74.2+0.01	60+0.01
Vitamin c	15	6.57+0.035	6.12+0.06	5.13+0.19
(mg)	30	6.61+0.078	5.51+0.129	4.43+0.023
	45	6.43+0.095	5.37+0.073	4.3+0.138
Moisture	15	5.413+1.654	6.93+0.059	6.826+0.920
(%)	30	5.34+1.57	6.25+0.780	6.506+0.924
	45	5.28+1.54	6.18+0.768	6.353+0.740
Rehydration	15	2.2+0.09	2.55+0.02	1.833+0.005
Ratio	30	2.523+0.03	2.52+0.01	2.472+0.09
	45	2.51+0.01	2.107+0.008	2.33+0.01
Ash content	15	0.677+0.0008	0.541+2.03	0.451+0.001
(g)	30	1.532+0.0001	1.29+0.0009	1.133+0.002
	45	1.88+0.004	1.873+0.002	2.03+0.007

The above values are means+_ s.d of triplicates of each sample.

Ascorbic acid

Singh and associates (20) studied the blanched fenugreek, mustard leaves, bathu and spinach showed higher ascorbic acid content than the unblanched samples. Spinach and amaranths showed decrease in vitamin c after blanching from 6.833mg/g and 7.143mg/g to 5.674mg/g and 6.207mg/g. Dried GLV packed in different packaging material showed loss of ascorbic acid. Loss of ascorbic acid may be due to oxidation of ascorbic acid. There was no significant difference between packaging materials at 5% level of significance after 45 days of storage. PP had more ascorbic acid in Amaranthus powder than samples in HDPE and LDPE packaging material.

Chlorophyll

In the recent years, there has been increasing interest in plant phytochemicals because of reduced risk of chronic diseases such as cancer and cardiovascular (21). Spinach and amaranths showed decrease in chlorophyll content after blanching from 599.66µg/mg and 994.433µg/mg to 218.5µg/mg and 6414.33µg/mg. Lower chlorophyll content of cabinet drier was due to an inactivation of chlorophyllase enzyme which may be responsible for degradation of chlorophyll (22). There was no significant difference in chlorophyll content between packaging materials at 5% level of significance after 45 days of storage. Premavalli and K.S. Majumdar (23) also reported that total chlorophylls decreased during blanching and de-hydration.

Carotenoids

Seshadri S and Jain M and Dhabhai D (24) reported that, total and beta carotene retention in blanched + sulphated leaves was 73 and 72 per cent respectively compared to 62 and 59 per cent in blanched leaves. Reference (25) reported that, on dehydration the retention of β-carotene in savoy beet and fenugreek leaves on drying was 40.9 and 38.1 mg/100g. Both the leafy vegetables spinach and amaranths showed a decrease in carotenoids after blanching, from 46.77µg/mg and 136.5µg/mg to 37 and 79.933µg/mg, amaranths had more carotenoids than spinach. Both spinach and amaranths packed indifferent packing material had no significant difference at 5% level of significance after 45 days of storage in both spinach and amaranths MPP had more carotenoids followed by HDPE.

Protein

Both leafy vegetables spinach and amaranths showed decrease in protein level after blanching from 743µg/mg and 778 µg/mg to 695µg/mg and 728µg/mg. For amaranth protein content was lower than reported by (26). The dried leafy vegetables spinach and amaranths packed in different packing material had low rate of loss of protein during the storage period. There was no significant difference between different packing materials at 5% level of significance. For both leafy vegetable spinach and amaranths MPP had more protein content followed by HDPE and LDPE.

Ash content

Both spinach and amaranths leaves showed a decrease in ash content after blanching. The lower ash content in boiled leaves compared to raw leaves could be due to transfer of minerals from leaves to the boiling water (27).Spinach leaves had more ash content than amaranths leaves. This suggests that the amaranths leaves in the study are lower in mineral composition than spinach leaves. The mineral composition of the pumpkin leaf extract is also low (28,29). Leafy vegetables low in mineral composition may be beneficial to renal patients. The dried leafy vegetables packed in different packing material had no significant difference at 5% level of significance after 45 days of storage. Both spinach and amaranths powder packed in LDPE had more ash content than HDPE and MPP.

Rehydration ratio

Leafy vegetables spinach and amaranths had no significant rehydration ratio difference after blanching. The different packing material used for packing of spinach and amaranths powder had no significant rehydration ratio difference at 5% level of significance. LDPE pouch showed more rehydration ratio than spinach powder packed in HDPE and MPP. Amaranths powder packed in MPP showed more rehydration ratio than LDPE and HDPE packaging material. Degree of rehydration is dependent on sample preparation, sample composition and extent of the structural and chemical disruption induced by drying (30).

IV. CONCLUSIONS

Blanching is one of the most possible strategies for preservation of GLV, which are highly seasonal and perishable too. Blanching pre-treatment was used to improve nutrition properties of spinach and Amaranthus leaves. The abundantly available inexpensive GLV can serve as a pool house of nutrients and can be used in the developing countries to combat micronutrient deficiencies. Dehydrated LV has great potential to use throughout the year for preparation of food after rehydration. Dehydrated LV are rich in nutrients and could be used to develop commercial products. Developing new packaging and storage techniques are essential to extend GLV shelf life.

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Yield gaps and nutrients use efficiency of apple tree (golden delicious/MM106) in the middle Atlas Mountains of Morocco

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Abstract**—** *The main objective of this work was to evaluate use efficiency of nitrogen, phosphorus, potassium, calcium and magnesium in adult apple orchards in the Middle Atlas of Morocco and to establish preliminary reference norms for fertilizing this crop under local conditions. The study was based on soil and leaf analysis and data with regard to farming practices and yield on forty apple orchards (cv. Golden delecious/MM106) where nineteen are growing on silty-clay soil and twenty-one on sandy-loam soil. The results showed significant correlations between leaf content for each nutrient and yield level following polynomial equations, thereby indicating local reference norms for apple leaf analysis. Moreover, correlations were significant between leaf and soil contents that permitted to determine apple needs in nitrogen fertilizer and references norms for soil richness in phosphorus, potassium, calcium and magnesium to obtain yield potential in the study region. However, the found norms are less than international standards because of feebleness of yield level in the study region, largely related to deficient cultural practices adopted by farmers. In addition, investigation of leaf nutrients ratios N/K, N/Ca, K/Ca, K/Mg and Ca/Mg showed that there was disharmony in uptake of these nutrients originating particularly from high soil richness in Ca and Mg. Taking into account these considerations, the found references norms can be applied only under the adopted farming practices. Nevertheless, by improving local practices, reference values may change.*

Keywords**— apple tree, leaf analysis, macronutrients, Morocco, soil analysis.**

I. INTRODUCTION

In order to be more competitive and ensure the sustainability of quality of their soils, farmers should constantly improve fertilization efficiency of their crops [1]. This objective would be achieved through determination of critical level for each nutrient that designates its concentration in soil above which crops does not respond to a supply of this nutrient [2]. Determining this level usually based on trials, with a single factor, of escalating doses of a nutrient on crop yield at different levels of soil richness [3, 4].

In fruit trees, precise determination of the nutrient requirements is particularly difficult. Indeed, nutrients and metabolites may be stored in wood for use them at the next year by growing shoots [5]. To these difficulties are added those related to nutrients migration in soil and to particularity of tree root system [6]. Soil analysis permits to quantify soil richness in nutrients and to estimate fertilizer requirements but they provide no indication as their use by trees. However, leaf analysis is an effective tool for assessing nutritional status of trees and for readjustment of fertilizer requirements taking into account factors that may affect nutrients availability and their uptake by roots [7]. Thus, a deficiency in phosphorus would be explained by a low concentration of this nutrient in soil, an inhibition of its uptake caused by a high concentration in active limestone, or by combined effect of these two conditions [8]. Leaf analysis reveals also deficiencies induced by some fertilization practices. For example, a deficiency in potassium would be induced by nitrogen fertilization in soil containing a low concentration in potassium because the vegetative growth resulting from nitrogen generates important requirements in potassium [9]. Leaf analysis provides information about fertilizers assimilation and for this reason it complete soil analysis [10].

In Morocco, works on this topic, in particular for apple tree, are limited. Until now, interpretation of soil and leaf analyses for the Moroccan apple orchards based on reference norms established on others countries such USA, France, Netherlands, United Kingdom and South Africa. Establishment of norms under local conditions is therefore necessary for an efficient management of fertilization in Moroccan orchards taking into account local practices and production performances. The present work aims to establish recommendations for an efficient fertilization of adult apple tree in the region of Imouzzer Kandar in the Middle Atlas of Morocco, which constitutes a concentration area for apple production in

northern Morocco. It is also a contribution to identify the Moroccan norms of soil and leaf analysis for apple tree concerning nitrogen, phosphorus, potassium, calcium and magnesium.

II. MATERIALS AND METHODS

2.1 Cultural conditions

Study was carried out in farmer's fields in the region of Imouzzer Kandar in northern Morocco. The climate of this region is characterized by cold winters with a minimum temperature of -10 °C and hot summers with a maximum temperature of 40 °C. The annual average of precipitations is 500 mm concentrated in autumn and spring. However, summer is characterized by a rainfall deficit (fig.1).

The experiment consisted in characterization of nutritional status and production of forty adult apple tree orchards (cultivar *Golden delicious* in association with *Starking delicious* as pollinator and grafted on *MM 106* rootstock) 12-15 years old and planted at a density of 667 trees/ha (5x3m) among farmers orchards in relation to their usual farming practices. The forty orchards were selected from all parts of the study area in which nineteen orchards were planted on silty-clay soils and twenty-one orchards on a sandy-loam soil. The orchards were irrigated by submersion every week from flowering to fruit harvest (April – October) and practically pruned following the same manner, but differently fertilized. For all orchards, the intakes of nutrients concerned only nitrogen, phosphorus and potassium with amounts ranged from 6 to 165 kg/ha for nitrogen, from 0 to 135 kg/ha for phosphorus and from 0 to 112 for potassium. Weighed yield ranged from 8 to 26 t/ha.

2.2 Measurements

2.2.1 Soil analysis

Soil analysis was realized during dormancy period of apple tree in November on two soil horizons: 0-35 cm and 35-70 cm. Analysis were performed by the following methods: particle size by pipette method [11], total and active limestone by Drouineau method [12], pH by titration [13], organic matter by Walkley and Black method [14], total nitrogen by kjeldhal method [15], available phosphorus by Joret and Hebert method [16], exchangeable potassium using ammonium acetate [17] and exchangeable calcium and magnesium by complexometry [18].

Physical and chemical characteristics of soil, both silty-clay and sandy-loam, are indicated in Table 1 showing that the soil for all experimental orchards was alkaline, moderately calcareous, rich in organic matter and little charged in rocks.

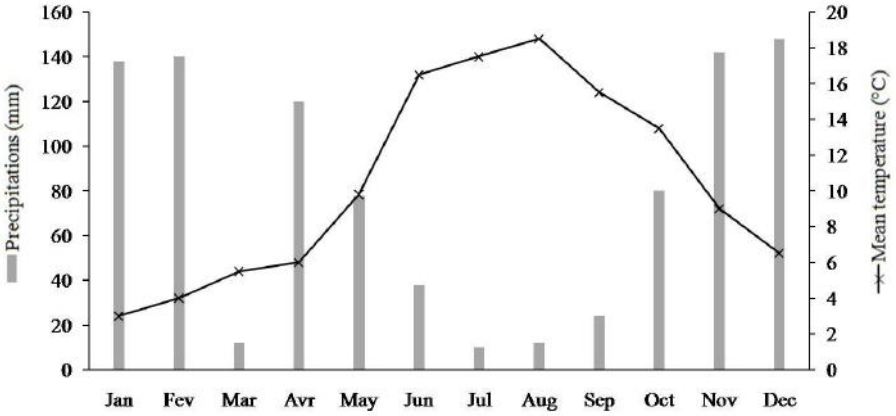

Fig.1: Monthly values of precipitation and mean temperature in the study region

Table 1. Physical and chemical characteristics of soil in the experimental orchards

	Orchards on silty-clay soil		Orchards on sandy-loam soil	
	0-35 cm	35-70 cm	0-35 cm	35-70 cm
Fine soil (%)	88.42	85.75	88.36	85.35
Total limestone (%)	33.45	31.71	35.30	33.04
Active limestone (%)	11.58	10.50	11.53	10.98
pH	7.81	8.02	7.83	8.04
Organic matter (%)	2.93	1.47	2.91	1.48

2.2.2 Leaf analysis

Leaves were taken with their petioles during the last decade of June 65 to 75 days after full flowering stage, from middle portion of growing shoots. At this period, nutrients content of apple leaves are relatively stable and for which references norms have been previously established [19]. Leaves samples were conveyed immediately to the laboratory where they were placed in a hydrochloric acid solution 0.1%, washed thoroughly with distilled water, dried at 70 °C for 48 hours and finely grinded.

Analysis concerned leaf content in nitrogen, phosphorus, potassium, calcium and magnesium following methods described by Rayan *et al*. [15]: nitrogen by kjeldhal method, phosphorus by spectrophotometer, potassium by flam photometer, calcium and magnesium by complexometry.

2.3 Statistical analysis

Data was used to evaluate significance of correlations established between soil richness in nutrients and nutritional status of apple tree based on leaf analysis and the obtained fruit yields. The significance of correlations was evaluated following Pearson test using SPSS software (version 17.0).

III. RESULTS AND DISCUSSION

3.1 Reference norms for leaf analysis

Apple yield varied with leaf nutrients contents following significant polynomial equations. According to determination coefficients of the found equations, apple yield was more determined by leaf potassium and nitrogen better than phosphorus, calcium and magnesium. This result is in line with the findings of Cheng and Raba [19] on Gala apple tree, of Raina *et al*. [20] on pear tree and of Kumar *et al*. [21] on kiwi tree. The curves of these equations indicated that reference norms of leaf analysis for high apple yield in the study site were 2.80-3.10 for nitrogen, 0.20-0.22 for phosphorus, 1.90-2.10 for potassium, 1.30-1.45 for calcium and 0.25-0.28 for magnesium (figure 2). These norms are higher than those recommended by Mahhou [22] in Sais plain in Morocco on *Golden delicious* grafted on rootstock *MM 106* for nitrogen and phosphorus that are respectively 2.35 and 0.14, but they are similar for potassium. These differences may be related to a variation in agricultural situations, especially to a difference in apple yield potential which is lesser in Sais plain compared to the mountainous region of Imouzzer Kandar because mainly of an insufficiency in chilling availability [23]. In comparison to other countries, these norms are similar to those adopted in France regarding all the analyzed macronutrients except nitrogen [5]. For this nutrient, the found norms are similar to the United Kingdom norms [24]. While in comparison with the American norms, the concordances were found only for phosphorus and calcium [25].

The found norms imply that the optimal ratios between leaf nutrient concentrations should be ranged from 1.38 to 1.63 for the ratio of N/K, 2 to 2.38 for N/Ca, 1.31 to 1.61 for K/Ca, 6.55 to 8.40 for K/Mg and 4.48 to 5.80 for Ca/Mg. These ranges values of ratios are in line with the France norms for N/K, K/Mg and Ca/Mg and with United Kingdom norms for N/Ca, K/Ca and Ca/Mg [5, 24].

Our results showed that the optimal values of ratios of N/K and Ca/Mg were effectively recorded in the most productive orchards where yields are higher than 22 t/ha, thereby indicating that there was a harmony in uptake of these nutrients in soil [26]. However, ratios values of N/Ca, K/Ca and K/Mg in these orchards were generally lesser than the optimal ranges (table 2). This disharmony between these nutrients is originating from an excessive uptake of Ca and Mg since there concentrations in leaf are higher, exceeding the optimal values by an average of 0.14% for Ca and 0.02% for Mg. The origins of excesses in Ca and Mg are related primarily to their high concentration in soil [27]. However, other factors may induce excess in Ca and Mg uptake such as the use of nitrogen as nitrate (NO_3), high contents in phosphorus and low amounts of potassium [28].

Nevertheless, the recorded excess in leaf Ca and Mg did not induce a reduction in apple yield. In fact, these tow nutrients are not toxic for plants even at high concentrations, but they induce indirect effects [29]. The high soil contents in Ca and Mg generate an increase in pH, which reduces the uptake of certain nutrients such as boron, iron, manganese and zinc. Because they are cations, they compete therefore with the uptake of other cations such potassium (K^+) and ammonium (NH_4^+) and may cause a deficiency of these nutrients [30].

3.2 Reference norms for soil analysis

The relationships between soil and leaf nutrient content were tested by logarithmic regression analysis regarding phosphorus, potassium, calcium and magnesium taking into account both the initial soil richness and the applied amount of fertilizers. However, for nitrogen, relationship was tested for nitrogen fertilizer only because of a lack of indications concerning mineralization of organic nitrogen (table 3).

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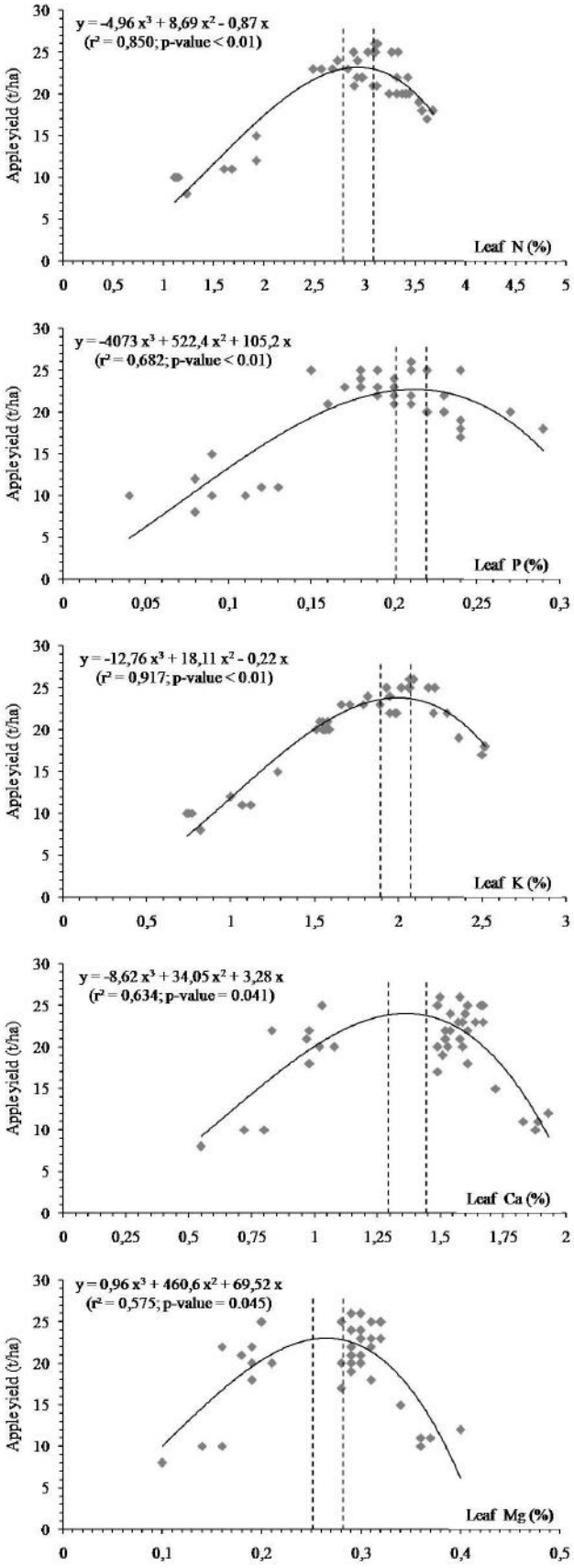

Fig. 2. Relationship between leaf nutrients content and apple yield

Table.2. Ratios of different leaf nutrients in the productive apple orchards compared to the optimal values

Ratios	N/K	N/Ca	K/Ca	K/Mg	Ca/Mg
Min	1.49	1.56	1.04	5.51	5.21
Max	1.50	1.69	1.13	5.96	5.30
Mean	1.50	1.63	1.08	5.73	5.26
Optimal values	1.38 - 1.63	2 - 2.38	1.31 - 1.61	6.55 - 8.40	4.48 - 5.80
French norms	1.13 - 1.57	1.13 - 1.68	0.75 - 1.43	4.29 - 9.09	4.00 - 9.09
United Kingdom norms	1.50 - 2.15	1.50 - 2.80	0.81 - 1.60	4.33 - 6.40	3.33 - 6.40

Table.3: Relationship between nutrients content in soil and apple leaf following soil texture

	Correlated factors	Regression equation	r^2	Optimal level in soil
Silty-clay soil	Fertilizer N (kg/ha) vs. Leaf N (%)	y=0.526ln(x)+0.53	0.58**	74 - 130
	Soil P_2O_5 (ppm) vs. Leaf P (%)	y=0.064ln(x)-0.09	0.71**	92 - 126
	Soil K_2O (ppm) vs. Leaf K (%)	y=1.187ln(x)-5.06	0.77**	350 - 415
	Soil Ca (meq/100g) vs. Leaf Ca (%)	y=0.552ln(x)-0.15	0.83**	14 - 18
	Soil Mg (meq/100g) vs. Leaf Mg (%)	y=0.109ln(x)+0.19	0.71**	1.7 - 2.2
Sandy-loam soil	Fertilizer N (kg/ha) vs. Leaf N (%)	y=0.634ln(x)-0.07	0.76**	92 - 146
	Soil P_2O_5 (ppm) vs. Leaf P (%)	y=0.074ln(x)-0.13	0.87**	82 - 106
	Soil K_2O (ppm) vs. Leaf K (%)	y=0.809ln(x)-2.78	0.57*	320 - 410
	Soil Ca (meq/100g) vs. Leaf Ca (%)	y=0.481ln(x)-0.05	0.86**	13 - 18
	Soil Mg (meq/100g) vs. Leaf Mg (%)	y=0.107ln(x)+0.19	0.85**	1.7 - 2.2

The results show that leaf contents in N, P, K, Ca and Mg are significantly correlated with their corresponding soil contents both in silty-clay and sandy-loam soils. Such correlations were reported in several previous works [31-33]. However, other trials reported that correlation is particularly low for phosphorus because of its low mobility in soil, thereby making its uptake limited at few millimeters from the hairy root [22, 34]. The significance of the found correlation for this nutrient under the cultural conditions of the present study may be related to the submersion irrigation, which was able to increase the mobility of phosphorus in soil and making it more available for roots.

Furthermore, data show that the correlation coefficients in sandy-loam soil are higher than those found in silty-clay soil except for potassium. This result is in agreement with those of Fan and Yang [35] who indicated that uptake ability is higher in sandy soil for majority of nutrients owing to their increased mobility. Particularly, the high value of correlation coefficient found for nitrogen in sandy-loam soil indicates that there was no considerable leaching of this nutrient that is known to be important in sandy soil [36]. In clay soil, the uptake ability is particularly higher for potassium as result of its high fixation on clay particles and clay-humic complexes that ensure exchange of this nutrient in soil [37].

For each nutrient, the corresponding soil contents to ensure the leaf reference norms are considered as optimal soil contents to get high apple yield (table 2). The found optimal soil content for P, K, Ca and Mg may be considered as reference norms for soil analysis for apple orchards in the study region. While for nitrogen, the found values constitute the optimal amounts of nitrogen fertilizer for a high apple production with an average of 88 kg/ha for apple orchards growing in silty-clay soil and of 130 kg/ha for orchards in sandy-loam soil. These nitrogen doses are near of those recommended in Morocco by Mahhou [22] for *Golden Delicious* 30 years

old and producing 40 t/ha. They are also near amounts generally recommended in Western USA and Eastern Canada [38, 39]. However, they are higher than France recommendations averaged at 50 kg/ha for an apple yield of 26 t/ha [40]. In fact, nitrogen dose vary following orchard situations. There is a significant disagreement in data on nitrogen amount used and removed by apple tree that is related to the differences in location, requirements of varieties and cultural practices [41]. Taking into account these considerations, the found optimal nitrogen doses concerns only the year of study and should be regarded as approximate for fertilizing apple tree in the region study for the next years.

Compared to the interpretation norms for soil test of Soltner [42], the found optimal nutrient contents in silty-clay soils are arranged in low level for P, sufficient level for K and high level for Ca and Mg. In sandy-loam soils, the optimal values are moderate for P and high for K, Ca and Mg. This disagreement may be related in large part to differences in yield level that is low compared to the production potential of the used variety that can reach 40 t/ha under optimal conditions [43]. This result imply that there were other factors that affect apple yield in the region study such as spring frosts, hail falls and inadequate cultural practices (irrigation, pruning, pests and diseases, etc.). Indeed, in previous diagnostic works carried out in the study region, it has been noted the existence of such factors that affect severely apple production [44, 45]. Nevertheless, for an apple yield of 26 t/ha, the found optimal nutrient contents may be used as soil reference norms for the region study. By comparing these norms to soil analysis data, it appears that the soil richness in K, Ca and Mg is able to satisfy the apple needs in these nutrients for all the tested orchards, thereby indicating that no intake in these nutrients is required. However, the intake of P is required since soil P content is low compared to found reference norms both in the silty-clay and sandy-loam soils (table 4).

Table.4: Recommended amounts of fertilizers (kg/ha) for an apple yield of 26 t/ha

	N	P_2O_5	K_2O	Ca	Mg
Orchards in silty-clay soil	88	75	0	0	0
Orchards in sandy-loam soil	130	84	0	0	0

IV. CONCLUSION

In adult apple orchards growing in the middle Atlas of Morocco, there is an amply variation in yield level, applied amounts of fertilizers, soil richness in nutrient and their concentration in leaf. These variations originate from differences in cultural practices, especially fertilization since relationships between apple yield and nutrient content in soil and leaf were significant. The relationship between yield level and leaf nutrient content served to determine the reference norms for leaf analysis with regard to nitrogen, phosphorus, potassium, calcium and magnesium required to ensure a high apple yield under local climate, soil and cultural practices. Furthermore, relationship between leaf and soil nutrient content brought out local apple nitrogen needs and reference norms of soil analysis for P, K, Ca and Mg. However, it should be highlighted that the found results are in preliminary order since the study was conducted during one year. The results are also in elementary order for establishment of the Moroccan reference norms for leaf and soil analysis in apple orchards.

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Effect of Temperature on the Shelf life of *Nono* (Locally Fermented Milk) and Yoghurt

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Abstract**—** *Effect of temperature on the shelf life ofnono(locally fermented milk) and yoghurt was carried out for 7 days and 3 months respectively. Freshly made nonowas kept under room and refrigerated temperature for 7 days. Chemical parameters such as protein, fats, carbohydrate, moisture and ash were analyzed within one hour of collection and on the 7th day. Some physical parameters such as texture and flavour were measured using visual appraisal just before the preservation and then on daily basis. Freshly made yoghurt was treated alike and kept for the period of 3 months (which is the claimed shelf life of yoghurt by most manufacturers). The physical, chemical parameters and microbial load were also measured at weekly intervals. The result of the physical and chemical parameters explains deterioration before the end of the experiment in both samples. It was also concluded that freshly made yoghurt kept at room temperature be consumed only on the first day of production and fermented milk is advised to be pasteurized before consumption due to the high microbial load.*

Keywords*— *Nono, Locally Fermented Milk, Yoghurt.

I. INTRODUCTION

Yoghurt is made by the control thermoduric fermentation of pasteurized non-fat or low-fat milk, carried around 45°C (Prescott *et al., 2005*).It is probably the most popular fermented milk product in the United states and in Nigeria too. (Prescott *et al.,2005*). It is produced both commercially and by individuals, using yoghurt-making kits. Apart from the microbiological quality of raw milk been of great importance in regard to product and food safety, raw milk should be unadulterated and free from taints, antibiotics, blood and visible segments. Since*nono*and fresh cow milk are produced by illiterate fulani's in village with poor knowledge of shelf life, product safety, sanitation and aseptic milking techniques, handlers of this product may unknowingly introduce pathogenic microorganisms into the product. Since these products do not undergo further processing before being sold for consumption, this food may become potential source of illness to their consumers (Uzeh*et al., 2006*).

Nono is a general name used for locally fermented cow milk and it is widely consumed in many African countries including Nigeria (Uzeh*et al., 2006*). It is an opaque white to milky colour liquid food gotten from fermented raw milk. It is a healthy food which consumption transverses the Sahara tribes of West African sub-region extended to the inhabitant of the Mediterranean region and also the Middle East. In the Middle East it is called "Dahi" or "Lassi" (Nahar*et al.*, 2007). Predominantly, *nono* is prepared and hawked by the nomadic Hausa Fulani cattle herders who control over 80% of Nigeria's cattle production.

Dairy product is essential part of the Nigerian food; therefore attention ought to be paid in to hygienic aspect of handling and distributing of such foods. This study was under taken to ascertain the shelf life of *nono* and yoghurt under room and refrigeratedtemperatures.

II. MATERIALS AND METHODS

The study was carried out at the Microbiology Laboratory of University of Agriculture Veterinary Teaching Hospital Makurdi, Benue state of Nigeria located in the Southern Guinea Savanna zone (Latitude 7°43′N and longitude 8°3′E). The area is warm with a minimum temperature of 24.20ºC and maximum temperature of 36.33ºC. The rain fall is between 508 and 1016mm and the relative humidity is between 39.50±2.20 and 64.00±4.8% (TAC,2009)

Sample Collection/Processing

Fresh milk collected in the evening was poured in to a calabash containing the starter culture and left to ferment over night.The leftover of *nono* made on the previous day served as the starter culture. The locally fermented milk popularly known as *nono*was then stored at room and refrigerated temperatures (4°C) for analyses.

Yoghurt was produced from powdered milk as described by Food Science and Technology Laboratory, University of Agriculture Makurdi using the Food Safety Standards. 1000ml of freshly prepared reconstituted milk was pre-heated to about 45°C and filtered. 15g of sugar was added, mixed and pasteurized at 95°C and held for 30 minutes. It was cooled to an inoculation temperature of 45°C in a closed container placed in a bath of iced water. 2.5g of starter culture *(Lactobacillus bulgarius and Lactobacillus acidophilus)* and 2ml of food flavor was added, mixed and incubated at 45°C for 3hours.

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Experimental Treatment/ Procedures

Table.1: Effect of Storage Time and Temperature on The Microbial load of Nono

TNC= Too numerous to count, Tem=Temperature, REF =Refrigerated, a,b,c means with different subscript on the same row are significantly different (p<0.05),

Days	Room temp.	Ref temp(4°C)
At collection	3.8± 0.46	3.8± 0.46C
Day 1	6.0± 051bc	3.0± 046
Day2	8.8± 0.51a	3.0± 0.46
Day3	TNC	3.1± 046
Day4	TNC	3.0± 0.46
Day5	TNC	3.0± 0.46
Day6	TNC	3.9± 0.46
Day7	8.3± 0.54b	5.2± 0.46a

Nono sample was collected and divided in to two. The first part was stored at room temperature and the 2nd part was kept under refrigerated condition (4°C). The microbial load and the pH at both temperatures were studied for the period of seven (7) days.

Freshly made Yoghurt was equally treated alike, but the parameters were studied on a weekly basis for a period of 3 months.

Microbial load

The determination of microbial load was done using 1ml yoghurt and*nono* samples serially diluted (10^1 to 10^5) in sterile water and 200µl of samples were plated unto nutrient agar plates and incubated at 37°C for 24hrs. The numbers of colonies were counted afterwards using a Standard Counter. Sampling was carried out for daily for *nono* and weekly for yoghurt.

Chemical Parameters

In all the experiments**,** the samples were analyzed for protein, fat, carbohydrate, moisture and ash. The fat was estimated by the Roese-Gottlieb Method following the procedures of Supplee and Bellis (2014), milk protein (N x 6.38) was determined using the semi-micro Kjeldahl and Markhams Distillation Apparatus and the ash content was obtained by drying and ashing a weighed milk sample (10ml) to a constant weight as 550 °C for 48 hours, while the moisture content was determined based on the principle of drying to constant weight has described by Osborne and Voogt (1978). The pH was determined by the use of a pH meter (WPA CD6). Determinations were done at the onset of Treatments and at the end of 30 hours, 7days and 3 month for Experiment 1, 2 and 3, respectively.

Physical Parameters

Sensory qualities of milk were evaluated by a jury of 5 panelists to determine the texture and flavour at 6 hourly intervals following the procedure of Meilgaard*et al.* (1999). The panelists tasted the samples and were asked to keep the milk products in their mouth for 12 seconds before scoring. The milk product samples were presented in random order. Water was used for rinsing mouth between samples (International Dairy Federation, 2002).

Statistical Analysis

Microsoft Excel spread sheet (2006) was employed for raw data entry. Transformation of microbial count was done using average dilution x 1/dilution factor x $^1/_{0.1}$ before the analysis, the data obtained were subjected to statistical analysis. Means that were significantly different were separated using least significant difference (LSD) as contained in SPSS (2010) for Windows (version 16). For all analysis, 95 % CF (confident factor) and P (probability)-value<0.05 was set for statistical significance of an estimate.

Table.2: Effect of Storage Time and Temperature on The pH readings of Nono

Days	Room temp.	Ref. temp.(4°C)
At collection	5.0± 0.46	3.8± 0.46C
Day 1	4.13± 051bc	3.0± 046
Day2	3.80± 0.51a	3.0± 0.46
Day3	3.73	3.1± 046
Day4	3.03	3.0± 0.46
Day5	2.43	3.9± 0.46
Day6	1.73	5.2± 0.46
Day7	1.13	

a,b,c means with different subscript on the same row are significantly different (p<0.05), TEMP= Temperature, Ref=Refrigerated

~~TABLE 3: Effect of Storage Time and Temperature on The Proximate Composition Of Nono~~

~~PARAMETERS~~	~~DAYS~~	
	~~1~~	~~7~~
~~Crude protein~~	~~2.82~~	~~2.64~~
~~Moisture~~	~~80.79~~	~~79.14~~
~~Carbohydrate~~	~~3.12~~	~~1.17~~
~~Fat~~	~~0.80~~	~~0.70~~
~~Ash~~	~~0.31~~	~~0.03~~

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~~TABLE 4: Effect of Storage Time and Temperature on Flavour Quality and Score Of *Nono*~~

~~Days of Storage~~	~~FLAVOUR QUALITY~~		~~FLAVOUR SCORE~~	
	~~Room .TEMP~~	~~REF. TEMP~~	~~Room .TEMP~~	~~REF .TEMP~~
~~1~~	~~PL~~	~~PL~~	~~100.00~~	~~100.0~~
~~2~~	~~SR~~	~~PL~~	~~68.0~~	~~100.0~~
~~3~~	~~SR~~	~~PL~~	~~52.0~~	~~100.0~~
~~4~~	~~OF~~	~~PL~~	~~28.0~~	~~100.0~~
~~5~~	~~OF~~	~~PL~~	~~20.0~~	~~100.0~~
~~6~~	~~OF~~	~~SR~~	~~20.0~~	~~64.0~~
~~7~~	~~OF~~	~~SR~~	~~20.0~~	~~60.0~~
~~Mean SD~~	~~-~~	~~-~~	~~24.0 ±73.3~~	~~18.5 ± 93~~

PL=Pleasing, SR= Sour, OF=Off flavour, TEMP= Temperature, REF=Refrigerated

Table.5: Effect of Storage Time and Temperature on The Proximate Composition of Yoghurt on the 1st and 90 Days (3months)

Parameters	1ST day	3 months(Refrigerated4°C)
Crude Protein	3.49	1.21
Moisture	90.49	84.61
Carbohydrate	3.45	2.80
Fat	2.31	1.86
Ash	0.45	0.10

Table.6: Effect of Storage Time and Temperature on The pH Readings of Yoghurt

Week	Room temp.	Ref. temp.(4°C)
At collection	4.6± 0.05	4.6± 0.05
Week 1	3.1± 0.01[a]	4.5± 0.05[a]
Week 2	2.96± 0.05[b]	4.53± 0.05[a]
Week 3	2.70±0.17[b]	4.13± 4.11[a]
Week 4	2.43±0.01	4.13± 4.11[a]
Week 5	2.20±0.17[b]	3.96± 0.05[a]
Week 6	1.96±0.11[c]	3.83± 0.11[a]
Week 7	1.50±0.06	3.43±0.11[a]
Week8	1.20±0.00[b]	3.20±0.00[a]
Week9	1.40±0.17[b]	3.06±0.01[a]
Week10	1.23±0.25[b]	2.83±0.05[a]
Week11	1.00±0.00[b]	2.70±0.17
Week12	1.00±0.00	2.56±0.11[a]

Table.7: Effect of Storage Time and Temperature on The Microbial load of Yoghurt (103 Cfu/ml)

Week	**Room temperature**	**Refrigerated temperature(4°C)**	**Week**
At collection	TS	TS	At collection
Week 1	3.7 ±0.006a	1.8 ± 0.007c	Week 1
Week 2	TNC	1.8 ±0.001 c	Week 2

Week 3	TNC	1.8 ± 0.002c	Week 3
Week 4	TNC	2.7 ± 0.004bc	Week 4
Week 5	TNC	2.3 ± 0.005 b	Week 5
Week 6	TNC	2.3 ± 0.001 b	Week 6
Week 7	3.7 ± 0.006a	2.3 ± 0.001 b	Week 7
Week 8	2.9 ± 0.004b	2.3 ± 0.001 b	Week 8
Week 9	1.7 ± 0.005b	2.3 ± 0.003 b	Week 9
Week 10	TS	3.0 ± 0.003 b	Week 10
Week 11	No growth	3.0 ± 0.003b	Week 11
Week 12	No growth	3.6 ± 0.003a	Week 12

TNC= Too numerous to count, TS= Too scanty, Tem=Temperature, REF =Refrigerated, a,b,c means with different subscript on the same row are significantly different ($p<0.05$),

Table.8: Effect of Storage Time and Temperature on Flavour Quality and Score control of Yoghurt

	Flavour Quality		*Flavour Score (%)*	
week 0	PL	PL	100.00	100.00
week 1	SS	PL	100.00	100.00
week 2	BT	PL	60.00	100.00
week 3	OF	PL	44.00	100.00
week 4	OF	PL	20.00	100.00
week 5	OF	PL	20.00	92.00
week 6	OF	PL	20.00	92.00
week 7	OF	PL	20.00	92.00
week 8	OF	PL	20.00	92.00
week 9	OF	PL	20.00	92.00
week 10	OF	PL	20.00	80.00
week 11	OF	PL	20.00	80.00
week 12	OF	PL	20.00	76.00

III. RESULTS AND DISCUSSION

The microbial load of fermented milk (*nono*) ranged between $3.8x10^3$ - $5.2x10^3$. The microbial load of *nono* on the first day of production was 3.8 $x10^3$and did not agree with the findings obtained by Savadogo*et al*.,2004. The reason for the contradiction could be linked to the different fermentation process practiced by many local producer of same product as documented by EL-Bakri and EL-Zubeir (2009). The microbial load multiplied rapidly at room temperature compared to refrigerated temperature (4°C). It stands to reason that storage of products at such temperature could increase its shelf life. The microbial load of yoghurt kept at room temperature multiplied rapidly to its peak and started declining until there was no visible colonies seen in the culture. This explains the four stages of bacterial growth. The lag phase which is delay in growth following inoculation of bacteria in to new medium during which time bacteria adapt to its medium. The log phase which is when they adapt to its medium and they reproduced rapidly, it is said here that the cells are in its highest activity during this phase. It is in this phase that the bacteria dominate the growth medium, deplete available nutrients and toxic waste accumulation slowing the state of production. The third phase is the stationary phase were the state of equilibrium is reached between the death of old cells and formation of new cells resulting in non-change in cell number. Afterwards the formation of new cells cease and the existing cells gradually die off. This is called the death phase.The microbial load in yoghurt kept under refrigeration (4°C) maintained the same number of microbial count from week 1 to week 3 as 1.8 x 10^3CFU/ml but increased in the 4^{th} week to 2.7 x 10^3. This might be due to the failure in power supply which leads to the rise in temperature giving room to microorganism to multiply. It is a well-known fact that microorganism multiply rapidly under normal temperature than at lower temperatures. There was a slight decrease to 2.3 x 10^3 on the 6^{th} and 7^{th} week this might possibly be as a result of the growth of psychotropic bacteria which grows in lower temperatures even below 5°C.

The pH of *nono* was 5.0 on the first day of production falls within the range of pH reported by EL-Bakri and EL-Zubeir (2009) but contradicts the 5.51- 6.29 (Adesokan*et al., (*2011), and 5.7 (Obi and Ikenebomeh, 2007). The

difference in pH could be as a result of some factors including the length of fermentation and the starter culture used. If the keeping time of *nono* is increased prior to the consumption, the acidity determines the number and kind of contaminating organism. It is assumed that at lower pH pathogenic organisms should be destroyed making *nono* safe for consumption. Jawetz et al.(1995) noted the presence of *S. cerevisiae* which is a pathogenic organism present in *nono* even at the pH below 5.47 and suggested that *nono* should be pasteurized before consumption.

Interestingly, the slight acidic nature of *nono* is of good medicinal value to human health as the implantation of the lactic acid in the intestines reportedly replaces the putrefying micro-flora there in whose metabolites have been considered to be responsible for various ailments may lead to premature death.

The difference in pH could be as a result of some factors including the length of fermentation and the starter culture used.

Proximate composition of *nono* at 7 days maintained under refrigeration showed a reduction in all the compositions as compared with the composition on the first day of production this could mean that microorganisms were present and active below 4°C. There was much decrease in all the nutrients of yoghurt samples stored under refrigeration (4°C).Most yoghurt producers claim 3 months as the shelf life of Yoghurt stored at less than 5°C, this seems not to be true from this study because of the changes in the nutritional values at this temperature when stored for this long.

Theflavour quality control as shown below showed *nono* to be pleasing on the first day of production and sour subsequent days if stored under room temperature. This explains the activity of microorganism (lactic acid bacteria) which turns it sour. *Nono*preserved under refrigeration was pleasing for 5 days and got sour on the 6th and 7th day. This may probably be the activities of psychrophiles that grows below 5°C or a fluctuation in the temperature due to failure in power supply that encourage the growth of microorganism which leads to souring of the product.

The flavor score maintained 100% up to 5 days under refrigeration and decreased due to deterioration. The sample stored under room temperature showed a rapid decrease in the score from day 1 due to the effect of microorganism that probably caused the deterioration.

The microbial load of yoghurt kept at room temperature multiplied rapidly to its peak and started declining until there was no visible colonies seen in the culture. This explains the four stages of bacterial growth. The lag phase which is delay in growth following inoculation of bacteria in to new medium during which time bacteria adapt to its medium. The log phase which is when they adapt to its medium and they reproduced rapidly, it is said here that the cells are in its highest activity during this phase. It is in this phase that the bacteria dominate the growth medium, deplete available nutrients and toxic waste accumulation slowing the state of production. The third phase is the stationary phase were the state of equilibrium is reached between the death of old cells and formation of new cells resulting in non-change in cell number. Afterwards the formation of new cells cease and the existing cells gradually die off. This is called the death phase.

The microbial load in yoghurt kept under refrigeration (4°C) maintained the same number of microbial count from week 1 to week 3 as 1.8 x 103CFU/ml but increased in the 4th week to 2.7 x 103. This might be due to the failure in power supply which leads to the rise in temperature giving room to microorganism to multiply. It is a well-known fact that microorganism multiply rapidly under normal temperature than at lower temperatures. There was a slight decrease to 2.3 x 103 on the 6th and 7th week this might possibly be as a result of the growth of psychotropic bacteria which grows in lower temperatures even below 5°C. Rodríguez-Alcalá*et al*., (2009) suggested that longer refrigeration time allows increased growth of psychotropic microorganisms and concomitant production of heat-stable enzymes, especially proteinases and lipases. The result of this present study contradicts the findings of Rodríguez-Alcalá*et al*., 2009 which states that Cooling to a temperature of 4ºC makes the bacteria inactive and prevents them to grow and produce the lactic acid.

Li and Li (1998) suggested that the tolerable limit of microbial load of yoghurt should be equal or less than $1.0x10^5$cfu/ml comparing this to the present studies, it is advisable to take refrigerated yoghurt from the very first day of production to the seventh day of production while it is suggested to take yoghurt kept under room temperature only on the first day of production. Taking it later than this may be detrimental to human health.

The pH of the yoghurt kept under room temperature dropped quickly compared to that kept under refrigeration. The pH of the yoghurt kept under refrigeration dropped and this agrees with the findings of Stamer (1976) who stated that Lactic bacteria are Mesophilic and can grow below 5^0C and some as high as 45^0C, with respect to growth pH some can grow as low as 3.2 some as high as 9.6 and most grow in the pH range 4.0-4.5. However, Henning(1999) also stated that the Coccus of produce 0.05% lactic acid and the rod about 0.6-0.8% (pH of 4.2-4.8). If incubation extend, the pH can decrease to about 3.5 with lactic acid increasing to about 2%.

The pH range 1.0-4.7 in the current study was lower than those reported by Omola*et al*, (2014). The minimum acceptable standard for pH is 4.4 in yoghurt (FAO, 1979)

therefore, the pH of the yoghurt kept under refrigeration and room temperature met the requirement of FAO,(1997) at 2 weeks and less than one week respectively. The pH of yoghurt decreased during the manufacturing process from the time it was inoculated with bacterial culture to the time it was manufactured from 6.7 – 4.7 this is because the lactic strains have the ability to ferment lactose to lactic acid which increases acidity and decrease pH. This result is comparable to the findings of Sokolinska et al, 2004.

The present study correlated pH directly with the number of colony forming unit which is in agreement with the findings of Micheal *et al.*(2013) who also observed a direct correlation of pH with number of bacterial cell.

Theproximate composition of yoghurt on the 1st day of production showed 3.49 , 90.49 , 3.45 , 2.31, and 0.45 for crude protein, moisture carbohydrate, fat, and ash respectively. At refrigerated temperature of 4°C , the values were 1.21, 84.61, 2.80, 1.86, and 0.10 of crude protein, moisture, carbohydrate, fat, and ash respectively was recorded. Microorganisms use the carbon present in the carbohydrate to produce energy for their survival. Lactic acid bacteria has the capacity to break down complex carbohydrate for their own use, this explains the decrease in the carbohydrate noted in this study at both temperatures. There was much decrease in all the nutrients of samples stored under refrigeration (4°C). Most yoghurt producers claim 3 months as the shelf life of Yoghurt stored at less than 5°C, this seems not to be true from this study because of the changes in the nutritional values at this temperature when stored for this long.

IV. CONCLUSION AND RECOMENDATIONS

From this study, it was concluded that *nono* prepared locally might not be safe for consumption due to the high microbial load however, it is advice that *nono* be pasteurized before consumption to reduce the microbial load.

The result obtained from this study suggests that refrigerated yoghurt is safe for consumption within seven days after production in contrast to one day only for yoghurt kept under room temperature. Taking it later than this may be detrimental to human health.

pH is also used for measuring spoilage in milk because of the correlation observed between the pH and the number of coliform forming unit(CFU).

pH should be considered for commercial and individual use for spoilage detection because of its low cost and ease of use.

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Morpho-physiological and Yield Responses Associated with Plant Density Variation in Soybean (*Glycine max* L. (Merrill))

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***Abstract*—** *Understanding morpho-physiological factors associated with yield decline at high density in soybean (Glycine max L.) can assist in optimizing productivity and seed quality. The objective of this study was to determine effects of different spacing on development and seed quality. The study tested the concept of yield plasticity. Five varieties that included determinate (SC Safari, Dina and Magoye) and indeterminate (Kaleya and Pan 1867) and three densities (300,000, 400,000 and 550,000 plants/ha) were used. A randomized complete block design arranged in 2 factor- factorial with variety and plant density and 4 replications was used. The experiment was done at Seed Control and Certification Institute in Chilanga, Zambia in 2015. Parameters assessed included: height, branches/plant, chlorophyll, nitrogen, 50 % flowering, pod-fill time, maturity duration, biomass, seed quality, yield and yield components. Significant effects for variety were present for all parameters while plant density effects were highly significant for number of branches/plant, biomass yield, pods/plant, seeds/pod and yield. Interaction effects were observed for pods/plant and seeds/plant. Traits positively and significantly correlated to yield were height, canopy biomass yield, pods/plant and seeds/plant. Biomass, pods/plant, seeds/plant and 100 seed weight contributed significantly to total variation of grain yield. Plant height, biomass yield, number of pods/plant, number of seeds/plant and hundred seed weight were critical parameters determining yield elasticity. Kaleya, Pan 1867 and Dina appeared more tolerant of planting at high density.*

Keywords*— *Soybean, density, yield decline, morpho-physiological, determinate and indeterminate.

I. INTRODUCTION

Soybean (*Glycine max* L. Merrill) is one of the important sources of food and feed. It is one of nature's most versatile plants, and produces an abundant supply of protein and oil in both temperate and tropical environments (Harold and Fudi, 1992). In addition to being a profitable cash crop, the high protein content (about 40 %) in soybean means it could also contribute to improved nutritional status of rural households. Soybean also has agronomic benefit of rejuvenating soils by fixing atmospheric nitrogen into the soil and improving the organic matter content when plant root residues decay (Lubungu et al. 2013).

Soybeans serve a variety of functions in the global food chain, ranging from use as edible oil to a source of protein for humans to use in livestock feed. Globally, approximately 87 per cent of all soybeans are crushed into soy meal and soy oil, with the remaining 13 per cent used for direct human consumption. The products derived from the soybean crushing process, consist approximately 80 % soy meal for use in animal feed; 1 and 20 % vegetable oil for human consumption and as a biofuel feedstock, respectively. Soybean cultivation is concentrated within four countries-USA, Brazil, Argentina and China-accounting for almost 90 % of world output while Asia (excluding China) and Africa, the two regions where most of the food insecure countries are located, together account for only 5 % of production of soybean. Among countries classified as undernourished, only India and Bolivia are significant producers of soybeans (IISD, 2014).

Low yields (less than 1 ton/ha in tropical Africa) and a shortage of fertilizer constrain the ability of some countries to increase production (IITA, 2009). Soya by-products provide low cost, high quality protein to feed rations. With a livestock sector projected to increase, soybean demand is anticipated to increase which offers significant opportunity for smallholder farmers to improve their cash base (Lubungu et al., 2013). New varieties of soybean are continuously being developed but production recommendations largely remain unchanged. It is postulated that further improvement in yield and higher resource use efficiencies (land, water and nutrients) are possible with improved agronomic/management practices (Wallace and Wallace, 1993). Accelerated population increase and emergence of

extreme climate- situation described as perfect storm by Godfray et al. (2010) have combined to reduce access to food and other agricultural products. This situation calls for adoption of strategies such as Sustainable Intensification which seeks to increase yields on less amount of land (Robinson et al., 2010). Increasing planting densities has been used on some cereals. Plant spacing and population reduction at critical growth stages has effects on plant physiological and morphological development and grain quality. Although yield decline at high population densities is known and soybean is known to have significant plant plasticity in terms of yield, the morphological and physiological changes that underlie this decline are not clearly understood.

Therefore, the objective of this study was to determine the effects of different plant spacing on whole plant development, yield and seed quality on selected Zambian soybean varieties. Specifically the study determined:

The effect of different plant spacing on soybean whole plant development, the effect of different plant spacing on soybean yield and the plant spacing effect on post-harvest seed quality by assessing germination and vigour. It is anticipated that the findings of this study will contribute to the understanding of the effect of different plant densities on plant development and seed quality in soybean.

II. MATERIALS AND METHODS

2.1 Location of experimental site

The study was conducted at the Seed Control and Certification Institute situated 15° 32.772' S and 28° 15.796' E and 1,246 m above sea level in Chilanga district of Lusaka Province of Zambia from December 2014 to April 2015. The length of the growing period for the plants ranged from 117 to 152 days. Planting was done on the 21st of December, 2014 and harvest was done by 22nd April, 2015. Some intra-seasonal dry spells were experienced. The USDA Soil classification terms the soils as being *Ultic Haplustalf*.

2.2 Soil Chemical Analysis

Soil chemical analysis for the experimental site were determined as pH ($CaCl_2$) being 6.4 which was considered slightly acid, Organic C (%) was 0.56 %, considered to be low while Nitrogen content (N %) was 0.03 and was considered to be very low. Exchangeable bases concentrations (cmol/kg soil) were found to be Phosphorus (P^{3-} 0.129 considered low), Potassium (K^+ determined as 2.483 considered to be moderate) and Calcium (Ca^{2+} found as 70 and considered to be high) (Bray et al, 1945).

2.3 Plant materials used

Five soybean genotypes were planted in the field obtained from seed companies. Selection of the genotypes was mainly based on differences in growth habits (determinant and indeterminate). Details of these varieties are presented in Table 1. Planting was done by drilling and ensuring that seeds were evenly spaced. Upon germination, stands were thinned to maintain the stated plant populations of 300,000 (D1), 400,000 (D2) and 550,000 (D3) plants Ha^{-1}).

Basal dressing fertilizer was applied soon after germination at the rate of 20 kg N, 40kg P_2O_5 and 20 kg K_2O (D Compound) per ha following the recommendations (Miti, 1995). Normal agronomical practices for growing soybean were followed.

Table.1: Soybean (Glycine max) materials used in the experimental trial

Variety	*Growth Type	*Nodulation	Origin	Year released
SC Safari	Determinant	Non promiscuous	SeedCo International (Z) Ltd	2004
Dina	Determinate	Non promiscuous	Maize Research Institute	2003
Magoye	Determinant	Promiscuous	Zambia Seed Company Ltd	1981
Kaleya	Indeterminate	Non promiscuous	Zambia Seed Company Ltd	1981
Pan 1867	Indeterminate	Non promiscuous	Pannar Seeds (Z) Ltd	2010

Source: SCCI 2013 Variety Register

2.4 Data collection

Data was collected on morphological and physiological traits as well as on yield and yield components. Data on vegetative and reproductive parameters was collected during the different development phases of the crop.

2.4.1 Plant height

Plant height was measured with use of a ruler at R6-R7 growth stage. This is because at this point the plant had attained its full height and root growth had ceased. Delaying to collect plant height data could lead to obtaining inaccurate results because at this point lodging and leaf fall associated with senescence would have set in (Casteel, 2011; Mc Williams, 1999).

2.4.2 Number of branches

The number of branches was measured by counting five plants at random and averaging the result at between R5 and R7 stage. The number of branches has a bearing on final yield obtained as pods tend to be borne on the branches. Many researchers have a positive correlation between the number of branches, pods and yield.

2.4.3 Chlorophyll content

Chlorophyll content (between V4 and V6) was obtained by use of Chlorophyll meter (Konica Minolta Spad 502Plus). SPAD chlorophyll meter reading (SCMR) is a quick, non- destructive measurement of the chlorophyll content of plant leaves (Moe, 2012).

2.4.4 N-Content

Nitrogen content assessment was done at the R3 growth stage when nitrogen fixation and nodulation are expected to be occurring. Leaves were sampled, dried at 65 ^{o}C in an open air circulating oven and crushed (Peoples et al, 1989; UNZA, 2014) before laboratory analysis for Nitrogen content using the Kjeldahl method.

2.4.5 Biomass weight at R3 and at harvest (Canopy biomass)

Biomass was determined by sampling 5 plants per replication at the R3 growth stage and drying them at 65 ^{0}C for 48 hours (Peoples et al, 1989) before weighing them. This was done to compare the weights of the three population densities. The biomass weight at harvest was obtained by sampling five plants at harvest time (R8) per replication and weighing them. At this stage, most roots had senesced and could not be harvested as part of total biomass so the canopy biomass instead was what was determined. The biomass weight was expressed as kg/m^2.

2.4.6 Yield and 100 seed weight

Yield was calculated as a function of base population, pod number, seeds per pod and seed weight (Casteel, 2011) at the harvestable moisture content of 15%. Seed weights were obtained by counting 100 seeds in three replicates, weighing them and obtaining an average to come up with an accurate 100 seed weight.

2.4.7 Days to 50 % flowering

The days to 50 % flowering occurs at the time a plant begins its reproductive growth phase. At this stage, about 50 % of flowers are fully open (UPOV, 1998). The number of days were calculated from the time of plant emergence to when the plants reach 50 % flowering and data was collected at the R1-R2 growth stage.

2.4.8 Days to pod filling

The total number of days from emergence to this stage was calculated as the days to pod filling. Maturity of genotypes differed on time taken to fill the pods. Full seed occurs at R6 growth stage and this stage is also known as the "green bean stage" (Mc Williams et al. 1999).

2.4.9 Days to maturity

The number of days was calculated from emergence to R8. This was the plant's whole growth period and determined varietal maturity differences and effects of plant density.

2.4.10 Number of seeds per plant and number of pods per plant

The number of seeds per plant was calculated by multiplying averages of locules per pod and pods per plant. Like the number of pods per plant, the number of seeds per plant contributes to the determination of the final yield (Casteel, 2011). The number of pods per plant was determined by counting pods of five sampled plants and finding the mean number of pods per plant. This was done at the R7-R8 growth stage when all the pods had fully formed and matured. The number of pods per plant is a significant factor in determining the plant yield (Casteel, 2011).

2.4.11 Field design

The field trial was laid out as a randomized complete block design arranged in 2 factor- factorial with variety and plant density and 4 replications used (Gomez and Gomez, 1984). The genotypes Kaleya, Magoye, Pan 1869, Sc Safari and Dina were the varieties assigned. The three plant population densities used were 300,000 plants/ha (D1), 400,000 plants/ha (D2) and 550,000 plants/ha (D3). The 400,000 plants/ha is the recommended plant population in Zambia (Miti, 1995).

2.4.12 Data analysis

Data was analyzed using the statistical package GenStat Version 12. Means were subjected to analysis of variance (ANOVA) where significant treatment effects were detected, mean separation was done using the least significant difference (LSD) and Bonferroni test for multiple comparisons. Relationships between selected parameters were determined using the Pearson's simple correlation test.

III. RESULTS

Results in Table 2 show that there were significant differences in treatment responses among the five varieties. The Population density used was significant for the parameters measured for yield ($P = 0.02$), number of seeds per pod ($P=0.005$), pods per plant ($P=0.004$), biomass weight at R3 growth stage ($P<0.001$), biomass weight at harvest ($P<0.001$) and number of branches per

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plant (P<0.001). Significant interaction between genotype and population density for number of seeds per pod and number of pods per plant were observed.

Table.2: Summary of ANOVA of treatment effects on 5 genotypes of soybeans (Glycine max) subjected to three levels of plant densities

Source of variation	D. F.	PH	NB	Chl T1	Chl T2	NC	50 % DF	DPF	DFM	B M_1	B M_2	NPD	NSD	SW	Yd	G	SV
Rep stratum	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rep.*Units* stratum																	
Variety	4	**	**	**	**	*	*	**	**	**	*	**	**	**	**	**	**
Density	2	ns	**	ns	ns	ns	ns	ns	ns	**	**	**	**	ns	*	ns	ns
Density x Variety	8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns
Residual	42																
Total	59																
CV %		4.4	4.3	2.8	2.4	17.2	0.1	0.1	6.7	8.8	8.5	11.2	11.3	3.0	13.7	3.6	3.5

Level of significance: ns : non- significant, * : significant at P = 0.05, **: significant at P ≤ 0.001.

3.1 Effect of genotype and plant population density for the morpho-physiological traits

Results in Table 3 for number of branches indicated that the effect of variety (p<0.001) and of density (p<0.001) was highly significant but the effect of the interaction was not. The highest number of branches were recorded from genotype Kaleya (5 branches/plant) followed by Pan 1867 (4 branches/plant) while the least number of branches per plant were recorded from Dina (3 branches/plant). A trend showed reduced numbers of branches per plant as plant density was increased from D1 (4.3 branches) to D2 (4.1 branches) and to D3 (3.2 branches).

The results show that the main effect of variety on biomass yield at both R3 (p<0.001) and at R8 (p<0.018) growth stages was significant as was the main effect of density (Table 3) while the interaction of the two factors was non-significant. The variety Dina had the highest biomass (23.95 kg/m^2) at R3 growth stage followed by Magoye (18.67 kg/m^2) while SC Safari (7.23 kg/m^2) had the least biomass. The effect of plant density for biomass yield at both R3 and R8 growth stages was significant; (p<0.001) and (p<0.001), respectively.

At R8 growth stage biomass yield for variety Kaleya (23.96 kg/m^2) and Magoye (23.11 kg/m^2) were non-significantly different but the two varieties were significantly different from the other three varieties (Table 3). Variety SC Safari (16.25 kg/m^2), Dina (18.46 kg/m^2), and Pan 1867(17.42 kg/m^2) did not show significant differences from each other. The results show a strong positive increasing trend in the amount of biomass with the rise in population density from D1 (10.67 kg/m^2) to D3 (20.24 kg/m^2) at R3 growth stage and D1 (10.40 kg/m^2) to D3 (30.88 kg/m^2) with regression constants of (R^2 = 0.975 at R3 and R^2 = 0.982 at R8), respectively.

Significant differences (p<0.001) were obtained in the means for the number of pods per plant (Table 4) between genotypes Magoye (41 pods/plant) and Kaleya (31 pods/plant) and the rest of the genotypes. However, there was non-significant difference among genotypes Sc Safari (19 pods/plant), Dina (23 pods/plant) and Pan 1867 (18 pods/plant) following an LSD of 5.3. The highest number of pods per plant were obtained by genotype Magoye followed by Kaleya with the least being Pan 1867 (41 pods/plant, 31 pods/plant, 18 pods/plant) respectively. The density effects (p=0.004) were observed with D1 (29.60 pods/plant) having most than D2 (26.94 pods/plant) and D3 (22.32 pods/plant) as shown in Table 4. Interaction effects (p=0.032) between variety and density were also observed Fig. 1.

Results for number of seeds per plant for variety and density are presented in Table 4 and they show that significant differences (p<0.001) were obtained in the means for the number of seeds per plant for the varieties Dina (51 seeds/plant), Magoye (90 seeds/plant) and Kaleya (67 seeds/plant). However, non-significant differences were observed between Sc Safari (37 seeds/plant) and Pan 1867 (32 seeds/plant). The effect of density was also significant (p=0.005) with D1, D2 and D3 having (63.2, 56.6 and 46.5 seeds/plant), respectively.

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This result showed a reduced trend in the number of seeds/plant as density was increased. Significant differences were also observed for the variety and density effects as presented in Fig. 2.

The results obtained for yield are presented in Table 4 and indicate that the main effect of variety was significant ($p<0.001$), as was the main effect of plant density ($p=0.018$) but the interaction of these two factors was non-significant. The highest yield was obtained by variety Magoye (3.64 ton/ha) followed by Kaleya (3.08 ton/ha) and the least was SC Safari in D1 (1.99 ton/ha). For the density, the highest yield was obtained in D3 (3.19 ton/ha), followed by D2 (2.81 ton/ha) with the least density being D1 (2.29 ton/ha), respectively. Significant differences were observed between D1 and D3 but there was no significant difference between D1 and D2 and between D2 and D3.

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Table.3: Main effects of variety and plant density on the number of branches per plant and biomass yield of soybean (Glycine max)

Treatment		Number of branches per plant	Biomass at R3	Biomass at harvest
Variety				
	Sc Safari	3.8 b	7.23 a	16.25 a
	Dina	2.983 a	23.95 c	18.46 ab
	Magoye	3.367 ab	18.67 bc	23.11 bc
	Kaleya	5.35 c	14.21 ab	23.96 c
	Pan 1867	4.067 b	10.98 ab	17.42 a
Density				
	D1	4.3 b	10.67 a	10.4 a
	D2	4.18 b	14.12 a	18.24 b
	D3	3.26 a	20.24 b	30.88 c
Factor Effects		P-values		
	Variety	<0.001	<0.001	0.018
	Density	<0.001	<0.001	<0.001
	Variety * Density	0.218	0.833	0.466

Table.4: Main effects of varieties and plant density on number of pods and seeds per plant and yield of soybean (Glycine max)

Treatment		Number of pods/plant	Number of seeds/plant	Yield (ton/ha)
Variety				
	Sc Safari	19.15 a	37.3 a	1.99 a
	Dina	22.5 a	51 ab	2.93 ab
	Magoye	40.72 c	89.6 c	3.64 b
	Kaleya	30.88 b	67 b	3.08 ab
	Pan 1867	18.18 a	32.3 a	2.18 a
Density				
	D1	29.6 b	63.2 b	2.29 a
	D2	26.94 ab	56.6 ab	2.81 ab
	D3	22.32 a	46.5 a	3.19 b
Factor Effects		P-values		
	Variety	<0.001	<0.001	<0.001
	Density	0.004	0.005	0.018
	Variety * Density	0.016	0.032	0.433

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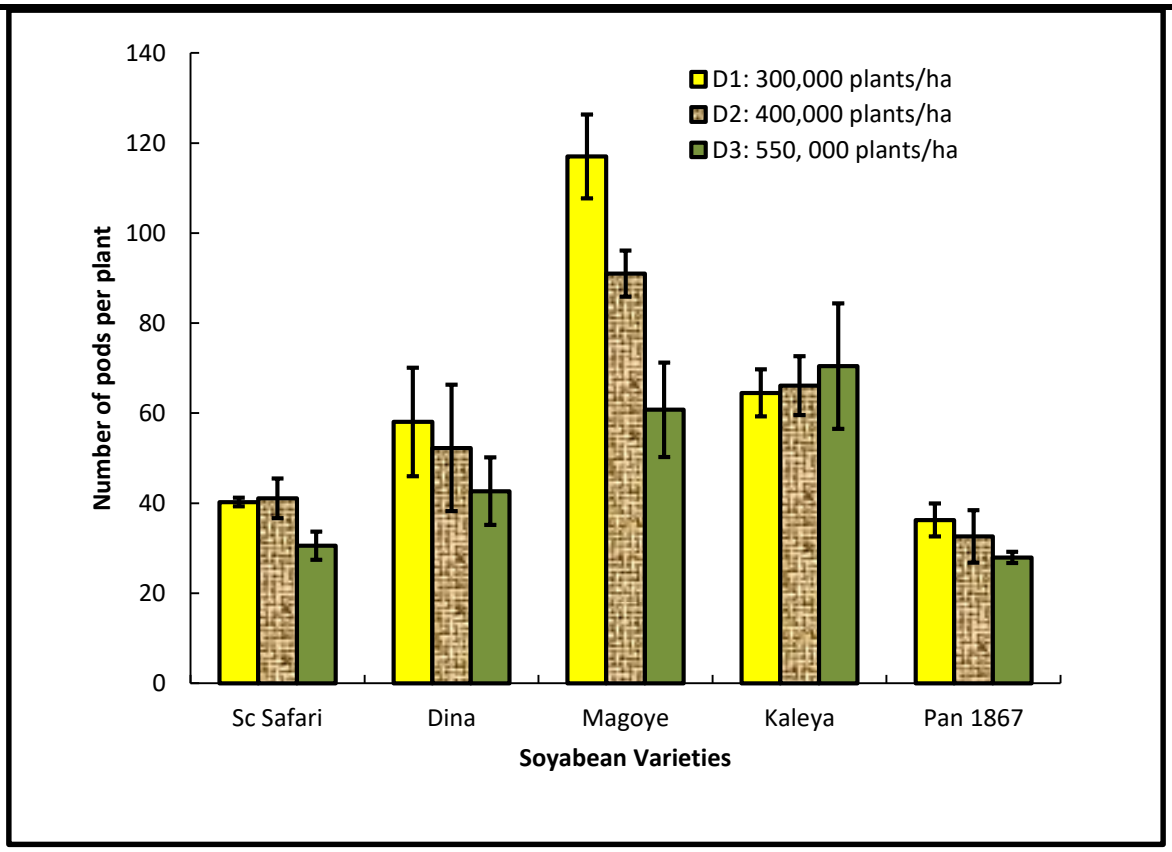

Fig.1: Interaction effect of soybean (Glycine max) variety and population density on number of pods per plant. Bars indicate standard errors of means.

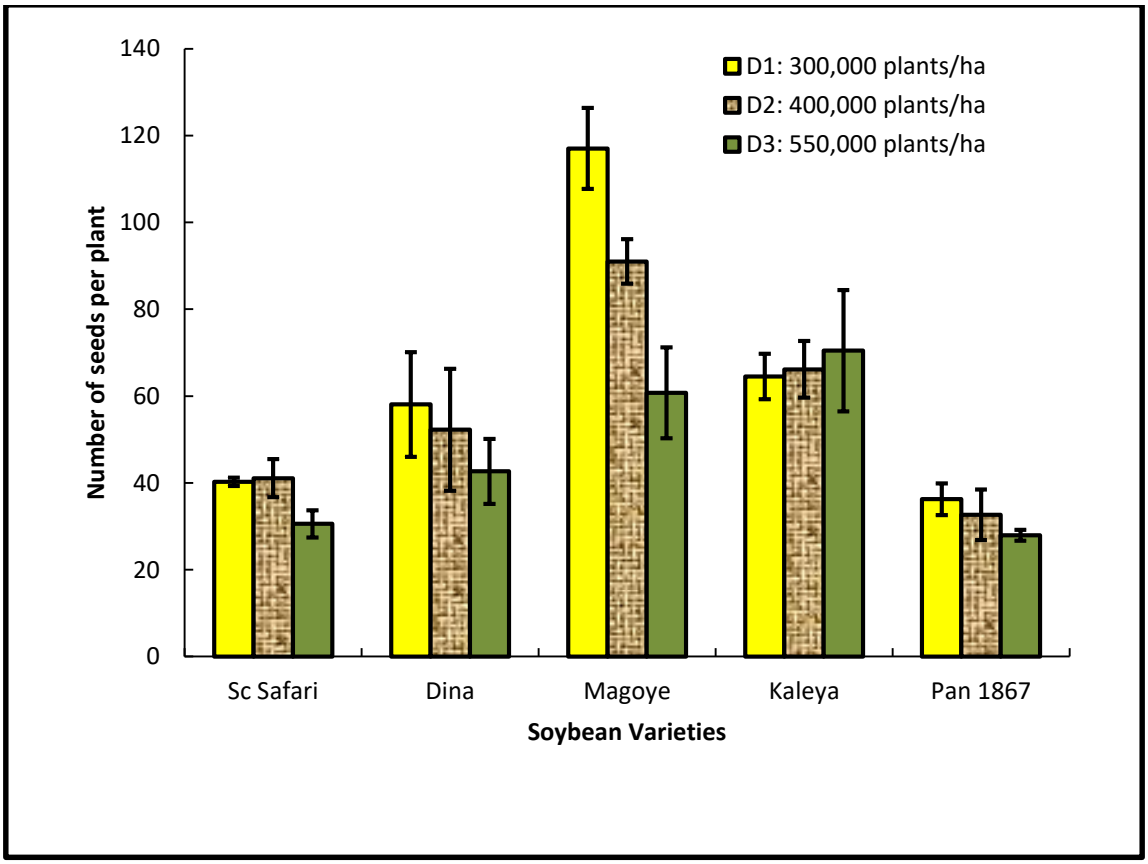

Fig.2: Interaction effect of soybean (Glycine max) variety and population density on number of seeds per plant. Bars indicate standard errors of means.

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3.2 Relationship among morpho-physiological traits, grain yield and yield components of five soyabean (*Glycine max*) genotypes

The strength of association for traits measured with yield as well as the inter component correlation amongst the components are here presented (Table 5). The results showed that plant height (r=0.58*), number of pods per plant (r=0.70*), number of seeds per plant (r=0.73*) and biomass weight at harvest (r=0.60*), were positively and significantly correlated respectively while other traits showed little positive and negative correlation.

The results recorded in Table 5 also show a strong positive and significant inter component correlation between components. Strong positive correlations were observed between plant height and 50 % days to flowering (r=0.79*), plant height and days to pod filling (r=0.74*), number of pods and number of seeds (r=0.98*), number of pods and days to maturity (r=0.54*), number of seeds and plant height (r=0.59*). A strong negative correlation was observed between hundred seed weight and 50% days to flower (r=-0.54*), hundred seed weight and number of pods per plant (r=-0.51*) and hundred seed weight and number of seeds per plant (r = -0.55*). Other correlations not reported were either weak positive or weak negative hence not well correlated.

3.3 Stepwise multiple regression

The seed yield was used as the dependent variable while the morpho- physiological traits were used as independent variables (Table 6). Significant and small contribution to total variations was observed among the independent variables in the study. Biomass yield at harvest (R8) had a significant influence on grain yield having the highest Wald statistic of 99.99 %. Other variables; plant height, number of pods per plant, number of seeds per plant and hundred seed weight showed significant contributions to total variation with an average R^2 of 85.3%. Further additions of other variables to the model did not show significant differences, thus not included in the model. The prediction model for yield was generated as in: (1).

(1) Yd = -1.934 + 0.006191 BM_2 + 0.1205 SW + 0.054 NSD - 0.0478 NPD + 0.00279 PH

Table.5: Results of correlation between yield and each pair of variables for soybean (Glycine max)

100SW	-													
50%DF	-0.538*	-												
BM_1	-0.130	0.562 *	-											
NC	-0.166	- 0.120	-0.304	-										
NB	-0.077	- 0.349	-0.413	0.326	-									
BM_2	-0.107	0.134	0.424	-0.086	-0.156	-								
ChlT1	0.380	-0.518 *	-0.356	0.042	0.145	-0.213	-							
ChlT2	0.301	- 0.433	-0.081	-0.092	0.094	-0.101	0.587 *	-						
DFM	-0.257	0.452	0.106	0.067	-0.081	-0.076	-0.385	-0.417	-					
DPF	-0.205	0.885*	0.632*	-0.239	-0.313	0.089	-0.409	-0.333	0.365	-				
NSD	-0.550 *	0.523*	0.095	0.175	0.279	0.109	-0.401	-0.379	0.490	0.335	-			
NPD	-0.511*	0.438	0.033	0.204	0.316	0.092	-0.401	-0.384	0.538 *	0.247	0.979*	-		
PH	-0.358	0.792*	0.609*	-0.055	-0.265	0.280	-0.336	-0.180	0.327	0.738*	0.591*	0.529*	-	
Yd	-0.249	0.415	0.330	0.053	0.065	0.598 *	-0.276	-0.242	0.271	0.336	0.733*	0.697*	0.584*	-
	100SW	50%DF	BM_1	NC	NB	BM_2	ChlT1	ChlT2	DFM	DPF	NSD	NPD	PH	Yd

* Correlation is significant at P≤0.05.

Table.6: Multiple regression of yield on morphological and physiological traits in soybeans (Glycine max) subjected to varying population densities

Response variate:	Yd
Fitted terms:	Constant, 100 SW, BM_2, NSD, NPD, PH

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	5	70.66	14.1311	69.6	<.001
Residual	54	10.96	0.203		
Total	59	81.62	1.3834		

Estimates of parameters

Parameter	estimate	s.e.	t(54)	t pr.	
Constant	-1.934	0.526	-3.68	<.001	Wald statistic
BM_2	0.006191	0.000619	10	<.001	99.99
100SW	0.1205	0.0264	4.57	<.001	20.85
NSD	0.054	0.012	4.48	<.001	20.07
NPD	-0.0478	0.0266	-1.8	0.078	3.22
PH	0.00279	0.00513	0.54	0.588	0.3

Percentage variance accounted for 85.3

Standard error of observations is estimated to be 0.451.

Key:
Yd: Yield
100 SW: 100 seed weight
BM_2: Biomass weight at harvest
NSD: Number of Seeds per pod
NPD: Number of Pods per plant
PH: Plant height

IV. DISCUSSION

The present study focused on determining the effect of different plant spacing on whole plant development, yield and seed quality on selected Zambian soybean varieties and in particular, plant spacing on soybean whole plant development performance as well as seed quality parameters with particular reference to germination and vigour. The effects of density stress, like all other stresses depend on the plant development stage, the stress applied, the degree and the duration of the stress. In this study, plants were subjected to three levels of population density during the whole growth duration which resulted into a wide variation in the responses of the five genotypes to morpho-physiological traits, grain yield and yield components. A marked genotypic variability in traits measured was observed among the different genotypes. Varying plant density showed some impact on important morpho-physiological traits and grain yield and yield components in all the genotypes tested.

Differences among the genotypes as well as the plant density used in this study were significant for the number of branches. Non-significant interactions were observed. The number of branches per plant was significantly influenced by the plant density in this study. There was marked reduction in number of branches as the plant density was raised from D1 to D3. These findings are supported by several researchers who found similar results. Mehmet (2008), Bullock et al. (1998) and Ball et al. (2000) all report finding the number of branches to significantly vary among plant densities. Ayub (2011) found out that increasing the seed rates decreased the number of branches. The reason for having less number of branches at higher seed rates may be due to more competition among plants for light, space and nutrients at higher seed rates. These results are supported by the findings of Biswas et al. (1997) who observed inverse relationship between seed rate and number of branches per plant. Shamsi and Kobraee (2011) noted that the effect of cultivar on number of branches per plant was significant. In the study carried out by Çalifikan et al. (2007), branch number per plant significantly varied among the row widths which led to the conclusion that

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significant variation resulted from density differences among row widths. Plants grown in low plant density conditions received higher solar radiation compared to denser populations, which caused a greater portion of vegetative dry matter to be allocated into the branches. Therefore, plants in wider rows were capable of partitioning more resources to increase branch number in response to plant density. Consequently, the ability of soybean to branch was greater in wide rows.

Significant differences among the varieties were observed for the biomass weight at both R3 and at harvest (R8). Subjecting the genotypes to the different plant densities also had a significant effect on biomass. As the population density was lowered from the optimal (D2 to D1), biomass weight reduced, while increasing population density from optimal to higher density (D2 to D3) resulted in increased biomass weight with all varieties showing marked increment ranging from 54 % to 101 %, with Kaleya having the highest biomass rate increment. The results are in conformity with research conducted by Squire (1990), who states that the rate at which a stand produces dry matter and the amount produced by the time it is harvested; both depend on many environmental and physiological factors. The main factors, other than solar radiation, that cause differences among the (C4 and C3) plants are plant population density, the composition of the stand and temperature. All these affect the three main attributes of a stand in different ways; these attributes being the leaf area, its conversion ratio for solar radiation and duration. The population density has a moderate effect on the conversion of intercepted radiation to dry matter, but its influence on production is mainly through leaf area index. Production therefore increases as population rises, and effectively reaches a plateau when further increase in population results in only slightly more intercepted radiation. Ayub et al. (2011) and Amissah-Arthur et al. (1999) found that dry matter was significantly increased with increase in seeding rates. This increase can be attributed to more plant population at given seed rates. It is also true then than biomass of an individual plant tends to reduce in higher population stands, a fact observed in this study. Sekimura et al. (2000), states that plants exhibit great morphological plasticity in their response to the environment such as the number of neighbouring plants (i.e. population density). Plant height, for instance, increases relative to [individual plant] biomass, stem diameter and leaf area as population density increases (Sekimura et al. 2000).

Significant differences among the five varieties for number of seeds per plant and pods per plant were observed. Also, the number of seeds per plant and pods per plant were significantly influenced by the three population densities in this study. The reduction in plant population from normal plant density to lower plant density (D2 to D1) resulted in the increase in the number of seeds per plant and pod number per plant while the increase from normal density (D2 to D3) resulted in lowered number of pods and seeds per plant. Similar results were obtained by Çalifikan et al. (2007), Shamsi and Kobraee (2011) and Bing et al. (2010) who reported that grain yield and number of pods per plant were declined with increasing density while Shamsi and Kobraee (2011) recorded more number of pods per plant at lower density. According to Mc Williams et al. (1999), temperature or moisture stress at (R3) can affect yield through total pod number, bean number per pod or seed size. Partial compensation with only temporary stress can occur in soybeans, but as the plant matures from R1 to R5.5 this ability to compensate will decrease. Very favourable conditions will result in greater pod number per plant at this time.

The high mean yields exhibited by genotype Magoye for all the environments could be attributed to its high number of pods per plant and number of seeds per plant which remained consistently high compared to the other genotypes across the three environments. The low yield exhibited by genotypes Sc Safari and Pan 1867 could be attributed to their shorter stature, shorter growing period and having lower number of pods and seeds per plant. The findings in this study are in agreement with Ball et al. (2000), Mehmet (2008) and Shamsi and Kobraee (2011) who all report that increasing the population reduces yield per plant but increases yield per unit area. The decreased yield per plant is more than offset by population, resulting in yield per square meter increasing to an asymptote as population increases. Variety Kaleya was the most plastic in terms of yield at 46.80 % followed by Pan 1867 at 17.67 % and Dina at 13.51 %. Despite Magoye having the highest yield overall, it did not respond plastically as density was raised from D2 to D3, Sc Safari also showed reduced yields as density was raised from D2 to D3 preferring to yield better in the optimal environment. Martin (1998) reports that large plants tend to bear a large number of seeds. Thus, seed yield potential per plant is closely related to the day length requirement of the variety and to the season of planting. It can therefore be said that the higher average yields obtained from Magoye, Kaleya and Dina could be attributed partly to their higher biomass yields. The duration of the plant growth also had an effect on the yields obtained. The average days to maturity for the genotypes (Sc Safari 120; Dina 152; Magoye165; Kaleya132; Pan 1867 117days), could explain the reason for the genotypes Magoye, Kaleya and Dina yielding more than the rest. The aspect of days to maturity is closely related to days to seed filling. Egli (1998) reports that longer seed filling periods are

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frequently associated with higher yields in many crops due to longer seed filling duration, (SFD) and resulting in a higher harvest index (HI), unless there is a proportionate increase in vegetative matter (VM).

There were significant differences among the genotypes for post-harvest seed germination as well as vigour. However, non-significant differences were observed for the plant density for the two parameters despite results showing slight reduction in germination and vigour with increase in plant density. Similar results were found in an experiment conducted by Shena et al. (2011) where increasing plant population resulted in reduced vigour, but, the differences were not significant at any densities. These results differ from those found by Castillo (1992), where in his experiment with garden peas (*Pisum sativum* L.), seeds from a population of 200 plants m^{-2} and 10 cm row width harvested at 15 % seed moisture content had lower vigour than less dense plantings, a fact attributed to high temperature and relative humidity within the crop canopy.

The strength of association for traits measured with yield as well as the inter component correlation amongst the components showed that plant height (r = 0.584*), number of pods per plant (r = 0.697*), number of seed per plant (r = 0.733*) and biomass yield at harvest (r = 0.598*), were positively and significantly correlated. To assess the cause and effect of yield in regression analysis, yield was used as the dependent variable while the morpho- physiological traits were used as independent variables. Significant and small contribution to total variations was observed among the independent variables in the study. Biomass yield at harvest had a significant influence on yield having the highest Wald statistic of 99.99 %. Other variables; plant height, number of pod per plant, number of seeds per plant and hundred seed weight showed significant contributions to total variation. Therefore biomass yield had the most influence on the observed yield as reported by Duncan (1986).

V. CONCLUSION

Use of different soybean varieties showed significant differences in all parameters studied. Varying plant density during the whole growth period showed different effects. An increase in plant density showed a reduction in most parameters under assessment except for yield and biomass. Number of branches, number of pods per plant and number of seeds per plant were reduced with increase in plant density. Varieties with greater potential to perform in elevated plant densities were identified as Kaleya, Pan 1867 and Dina and were seen to be elastic while Magoye and SC Safari were inelastic. A correlation analysis indicated a strong relationship between yield and plant height, biomass yield, number of pods per plant and number of seeds per plant. A stepwise multiple regression indicated that plant height, biomass yield, number of pods per plant, number of seeds per plant and hundred seed weight contributed significantly to the total variation in grain yield. Kaleya, Pan 1867 and Dina can be recommended for production under increased plant population. It is, however, with caution that this recommendation is advanced because these results are coming from a single study conducted in one location and for one season. Validation of the findings through multi-location and seasons trials is recommended.

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Effect of Soil Amendment on the Functional and Pasting Properties of False Horn Plantain Flour

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***Abstract*— *There is growing public perception that fruits from fertilized plantain plants have their organoleptic qualities reduced during food preparations. Such perception has the potential consequence of lowering production levels of the commodity in Ghana. To ascertain the veracity of this perception, a study was conducted between July 2012 and March 2015 to determine the effects of different rates of poultry manure, cocoa pod husk and NPK as soil amendments on nutritional composition, physical characteristics and functional properties of plantain pulp flour. The experimental design was a Randomized Complete Block with three replications. Flours from plantain with amended soils recorded moisture contents (8.41 % to 12.08%) which were within the acceptable levels for flours. The flour with the lowest moisture content was produced from plantain with CPH amendment (8.41%). The protein content of false horn plantain flour was however low (3.39 % to 5.27%).The plantain flour starch was not influenced by any of the soil amendments. On the other hand, flour produced from plantain with NPK+PM amendment had low bulk density and low water absorption capacity. Similarly, the false horn plantain flour had lower swelling power values compared to other flours.Flours from plantain with CPH amendment had lower oil absorption capacity. Flour from plantain with PM amendment was more likely to cook faster than the flour from the plantain with the other amendments. Flours from plantain with NPK+CPH amendment would form a more stable paste because of its lower breakdown value. In conclusion, the plantain flours were comparable to known food flours and therefore could be applicable as thickening agents and also find usefulness in fufu powder preparation and baking.*

Keywords*— *plantain, soil amendment, flour, pasting, functional properties and manure.

I. INTRODUCTION

The consumption of plantain is all year round whilst its production is seasonal and hence the need to reduce post-harvest losses through the processing of fruits into forms with reduced moisture content. Plantain production has, for some time now, experiencing an increasing production with a correspondent increase in surplus since 2001 (Dankye*et al*., 2007). It is assumed that in 2015, there will be about 912,000 Mt surpluses (MoFA SRID, 2014). These surpluses would have to be processed, exported or it will end up at the refuse dump (Dankye*et al*., 2007). This is due to the highly perishable nature of plantains as result of high metabolic activity leading to fast deterioration mostly after harvest (Demirel and Turhan, 2003).

For the efficient use of plantain flours as an ingredient for the food industry it is important to investigate the effect of soil amendments to determine its impact on their chemical, nutritional composition, shelf life as well as their functional properties. Therefore, it is plausible to characterize the nutritional and chemical composition, as well as their physicochemical, physical and functional properties of plantain flours in order to make any recommendation for its usage in the food industry. It is also important to note that some companies in the manufacturing of instant plantain fufu flour import flours to Ghana in spite of the surpluses recorded in plantain production over the years (Dankye*et al*., 2007).The conversion of plantain into flour which has the desired functional properties could contribute to reducing if not totally eliminating the import of flour into the country. Nonetheless, the preparation of flour from cooking banana is a surest means of value addition as well as extending the shelf life and enhancing transportation (Adeniji and Empere 2001).

Functional properties are the characteristics of food which dictate their behaviour, quality and acceptability during processing, storage, and preparation (Ishmeal*et al*.,2011).

Plantains are high in carbohydrate (31 g/100 g) and have relatively low fat content (0.4 g/100g). Notwithstanding, they are important sources of vitamins and minerals (Adenij*et al*., 2006), notably iron (24 mg/kg), potassium (9.5 mg/ kg), calcium (715 mg/kg), vitamin A, ascorbic acid, thiamin, riboflavin and niacin. Unripe plantain flours are rich in dietary fiber (8.82%) and resistant starch (16.2%), micronutrients which enhance the reduction of blood sugar level. They are, however, low in fat and oil as well as protein (Ayodele and Erema, 2011). The flour is used in confectionery and bakery industries in the treatment of intestinal disorders. They are also used in the preparation of infant diets (Adenij*et al*., 2006)

Plantains require high amounts of nutrients for optimum growth and fruit production but these nutrients are often supplied in part by the soil (Lahav, 1995). This is one of the reasons why in the West and Central African regions, the crop is predominantly cultivated in the home gardens where it receives continuous supply of organic matter and nutrients from household refuse (Baiyeri*et al.,* 2007). Animal manure is a source of nutrients and organic matter, which could improve soil bio-physical conditions (Munoz *et al*., 2004) for sustainable food production.

Improvement in soil fertility greatly influences the physical characteristics of both the plant and the fruit. Soil amendments strategy which seek to increase the nutritional status of the soil will therefore trigger a corresponding increase in the yields of crops grown on such soils (Ndukwe*et al.,* 2014).

Although it is known that organic and inorganic fertilizer levels affect nutrient composition of plantain fruit pulp (Ndukwe*et al*., 2014), little is known about how they influence functional properties of plantain pulp flour which is widely used in production of fufu flour and other food products. However, the effects of a particular soil amendment strategy on the flour quality of fruits of false horn plantain (Apantu pa) ought to be determined, hence the need for this investigation.

II. LITERATURE REVIEW

Effect of fertilizer on the quality characteristics of Produce

In the experiment conducted by Premsekhar and Rajashree (2009) to determine the influence of different organic manures on the growth, yield and quality of okra.It came out that, organic manures performed better by producing quality fruits with less fibre content. Application of farm yard manure at 20 t /ha recorded fruits with less crude fibre content and less moisture content. It was noted that, application of farm yard manure might have caused accumulation of nutrients and dry matter in fruits than synthetic fertilization which resulted in better quality of fruits grown on soils amended with farm yard manure.

In other experiment, Kipkosgei*et al.* (2003) researched on the effect of farmyard manure and nitrogen fertilizer on vegetative growth, leaf yield and quality attributes of black nightshade (*Solanumvillosum*). It was revealed that, the ß-carotene content in the edible portions increased with increasing levels of fertilizers. This was attributed to Nitrogen facilitating the formation of chloroplasts, which are rich in ß-carotene. Results also showed that farm yard manure increased the vitamin C content of edible leafy portions of *Solanumvillosum*whereas inorganic nitrogen fertilizers decreased vitamin C content.

Quality Indicators of Flour

In the cooking sense, flour is in a form of powder which is derived from cereal, grains, roots and tuber and other seeds. It is the major ingredient used in the baking industries for the preparation of varieties of food products (Appiah *et al.,* 2011). In the Middle East, North Africa, Europe and the Americas, wheat flours form integral part of their diet and are widely used in the preparations of breads and pastries. Good quality flours have higher gluten content which are able to produce lighter and softer baked products by embedding small gas bubbles. The amount of protein (gluten) in flour predicts its bread-baking quality for plain white flour Appiah *et al.* (2011).

FUNCTIONAL PROPERTIES

Matil (1971) defined functional property as the properties that affect the quality and acceptability of food as well as the characteristics that governs the behaviour of nutrients its composition when altered during storage, processing and preparation into various forms. Important functional properties that influences the suitability and usage of starchy stables such as plantain include; swelling power, water and oil absorption capacity, bulk density, solubility etc.

PASTING PROPERTIES

Otegbayo et al., (2006) stated that, during the heating and cooling of starch series of processes follow gelatinization which include granule rupture and subsequently polymer alignment due to mechanical sheer which finally result in pasting.

Flours are normally cooked into paste before eating, therefore the determination of pasting characteristics of flours are very essential in predicting quality index and the behavior of paste during and after cooking (Etudaiye*et al*., 2009).

III. METHODOLOGY

The experiment was conducted on the research field of Council for Scientific and Industrial Research Institute located (Horticultural Department Kwadaso Kumasi) at Kenyasi in the BrongAhafo Region of Ghana. The experiment was established as a demonstration field for farmers within the catchment area. The experiment which lasted for a period of two years commenced in 2012 and ended in 2014. This was done to demonstrate the influence of soil amendment on the yield and performance of two plantain groups that is, the False Horn and French Horn which are locally known as Apantu pa and Apem pa respectively. The area falls within latitude 7^0 $03^{'}.631''$North and longitude 2^0 29'.424'' west.

The flour obtained from the plantain was subjected to laboratory analysis**.** The laboratory work investigated the influence of soil amendments on the flour that was prepared from the false horn plantain.

The experiment was set up in the Randomized Complete Block Design (RCBD) with three replications.

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Data were collected on samples at three (3) different laboratories. The laboratory of CSIR (Abuakwa), Department of Horticulture laboratory (KNUST) and the Food Research laboratory (Accra).

Preparation and Application of Treatments

The poultry manure was kept for period of three (3) months to allow decomposition and elimination of heat. The manure was applied (top dressing) to each stand with a planting distance of 2m within rows and 3m between rows. Each plot had a total plant population of fifteen (15) and occupied an area of 6 x8m^2.

Cocoa Pod Husk was subjected to open air drying for a period of three (3) months and it was further crushed in a sack using wooden pistils into smaller particles of about 2cm. Cocoa Pod Husk was also applied (top dressing) to each stand.

The land was divided into three blocks, with each block made up of six plots. Each plot received different soil amendment including the control (no treatment). The treatments administered on the various plots comprise 10kg/stand (16660kg/h) of Poultry Manure(PM), 5kg Poultry Manure and NPK (0.065kg+0.045kg,0.18kg)/stand (108kgN+75kgP+300kgK/h and 8330kg/h PM), 10kg Cocoa Pod Husk/stand (16660kg/h), 5kg cocoa pod husk and NPK (0.065kg+0.045kg,0.18kg)/stand (108kgN+75kgP+300kgK/h and 8330kg/h CPH), NPK (0.13kg+0.09kg,0.36kg)/stand (216kg/h N,150kg/h P, 600kg/h K).

SAMPLE COLLECTION AND PREPARATION

For the purpose of this study, the false horn variety (Apantu pa) was used due to its popularity in flour preparation**.** Fresh firm and matured but green plantain fruits were harvested from the various plots for use in the flour preparation. Following the recommendations of Baiyeri and Ortiz (2000). Finger samples were collected from the second hand of the proximal end of the bunch. The fruits were sliced to 5mm diameter using a grater after they have been washed and peeled.

Preparation of False Horn Plantain Flour

Sliced plantain pulp were placed in an oven (Wagtech-Model GP120SSE300HYD) and dried at 60oC for 24 hours till it turned crispy. This was done by spreading out slices in layers of 1cm thick on stainless-steel shelves in a tray-drying accessory of the oven. Dried slices were then cooled and milled in a hammer miller for three (3) times till a fine powder was obtained. Flour samples were packaged in high density polyethylene for laboratory analysis. At the laboratory, flour samples were screened through a sieve with 0.25 mm diameter of hole (Model BS 410) before the various analyses were carried out.

Moisture

Two grams (2 g) each of the samples were weighed in triplicates and dried for 24hours at 60^{o}C in an oven (Wagtech-Model GP120SSE300HYD) to a constant weight. Samples were removed, allowed to cool and weighed. The moisture content calculated and expressed as a percentage of the mass of sample taken by repeating the procedure of weighing and drying of each sample (AOAC 1990).

Protein

The Micro-Kjeldahl method of protein determination was used in the determination of the percentage of protein in the various sample (AOAC, 1990). Methods involved in the protein determination are two (2).

Digestion

Two grams (2 g) of sample was weighed unto a filter paper pre-folded like an envelope and introduced in to the kjeldhal flask in triplicates. Anti-bumping agents and half spoonful of selenium base catalyst were added. The flask was shaken to get the samples thoroughly wet immediately about 25ml of concentrated H_2SO_4 was added. The heater turned on when the flask was placed on the distillation rack and the sample digested until the solution became clear. Cooling of the flask was allowed at room temperature to obtain a clear solution. The digested sample was transferred into 100 ml volumetric flasks and top up with distilled water to the 100ml mark.

Distillation and Titration

Two drops mixed indicator was added, when twenty-five of 2% boric acid was pipetted into a 250ml Erlenmeyer flask followed by 18 ml of 40% NaOH. The conical flask and its contents was placed under the condenser in a position that left the tip of the condenser completely submerged in the solution. The digested sample of ten millilitres (10ml) was poured into the steam jacket. The stopcock was closed to drive the liberated ammonia gas directly in to the collection flask and steam forced from the decomposition chamber by shutting the stop cock on the steam tap outlet. Distillation continued until the boric acid turned bluish green, before distillation was stop. The distillate titrated against 0.1N H_2SO_4 to a faint pink endpoint and the set up was then disconnected afterwards. Similar method was used to prepare a blank in which a similar piece of filter paper minus sample was used. The percentage nitrogen was multiply by the appropriate conversion factor of 6.25 to obtain the percentage protein.

Starch Determination

The Starch content was obtained by adopting the method of Lintner but with slight modification. Triturate 2.5g of flour with 20ml of water, and 40ml hydrochloric acid (sp.gr.1.15) added in small portions. Wash the mixture into a 200ml flask with hydrocholic acid (12% w/w HCL) add 10ml of 5% phosphotungstic acid to precipitate proteins and make the volume up to 200ml with 12% hydrochloric acid. Shake, filter and measure the optical

rotation of the filtrate in a 200-mm tube. Multiply the reading by 1.912 to get the percentage of starch directly.

Pasting Characteristics

American Association of Cereal Chemist Approved Method 22.10 (AACC, 1983) with slight modifications was used to determine the pasting characteristics of plantain flour samples which were in triplicates (moisture content ranging between 7.5 and 13.65 and corrected to 14%). A Brabenderviscoamylograph (Viscograph PT-100) from Food Research Institute, Accra was used to study all the pasting properties of the flour at 75 rpm and a torque of 700 g equivalent to 100 Brabender units (BU) (Demiate*et al.,* 2001). Under a constant stirring speed, the slurry was heated from 25–95^0 C at a uniform rate of 1.5^{0}C/min. Monitoring was continuously done on the torgue. Cooling took place under a controlled rate of 1.5C/min (Damardjati and Luh 1987) to 50^{0}C after torgue was formed. The cooked paste viscosity of 14% slurries in 420 ml water was measured. Pasting parameters including beginning of gelatinization, maximum/peak viscosity, end of final holding, break down and set back were recorded and expressed as Brabender Units (BU) (Demiate*et al.,* 2001).

Swelling Power and Solubility

Based on a slight modification of the method of Appiah *et al.* (2011) solubility and swelling power were determined. One (1 g) of flour was transferred into a 50ml capacity graduated centrifuge tube. A total volume of 40ml was obtained by adding deionized water. In other to avoid fragmentation of starch granules, the suspension was stirred just sufficiently and uniformly avoiding excessive speed. The sample in the centrifuge tube was heated at 85^{o}C in a thermostatically controlled temperature water bath (Grant type) for 30 min with constant stirring. The tube was cooled to room temperature after it had been removed and wiped dry. It was centrifuged for 15 min at 2200 rpm. The supernatant was evaporated and the residue weighed to ascertain the solubility. The sediment paste was weighed and the percentages of swelling power and solubility were then calculated. Determinations were done in triplicate.

Water and Oil Absorption Capacities

With slight modification of the method of Appiah *et al.* (2011), water absorption capacity was ascertained. 1.0 g of the sample in a beaker was top up with 10mL of distilled. A Rota mixer 7023 was used for missing the suspension for 3 min. The supernatant obtained was measured in a 10 mL graduated cylinder by centrifuging the suspension at 5000 rpm for 30min. The density of water was taken as 1.0 g/ml. The difference between the initial volume of water added to the sample and the volume of the supernatant was used to calculate the water absorbed.

The same methodology was adopted for oil absorption except that oil was used instead of water. The density of the oil used was 0.9095g/ml.

The volume of water or oil on the sediment was measured. Oil and Water absorption capacities were calculated in millilitres of oil or water absorbed per gram of flour respectively.

Bulk Density

Water absorption capacity was determined using the method of Appiah *et al*. (2011). 50.0g of the flour sample was weighed into a 100ml graduated measuring cylinder. The cylinder was then gently tapped repeatedly on the laboratory bench till a constant volume was obtained. The volume was then noted.

The bulk density (g/cm^3) was calculated as weight of flour (g) divided by flour volume (cm^3).

That is Bulk density (g/ cm^3) = weight of sample (g) /volume of sample after tapping cm^3.

IV. RESULTS AND DISCUSSION

QUALITY CHARACTERISTICS OF PLANTAIN FLOUR

Moisture Content

There were significant differences (P≤0.01) in the moisture content of the plantain flour among the various amended soils. Flour produced from plantain without any soil amendment (control) recorded the highest moisture content (12.08 %), significantly different from the other amendments except NPK+CPH amendment soil. Flour produced from plantain with CPH amended soil recorded the lowest moisture content (8.41%).

Ishmael *et al.,* (2011) and Onwuka and Onwuka, (2005) however, reported moisture contents of 61.10% and 61.6%, respectively which were comparatively higher than the findings in the present study. The lower pulp moisture content found in this study is an indication of the higher dry matter content of the pulp which is known to have an influence on the energy content due to the high carbohydrate content associated with high dry matter (Gowen, 1995). The moisture content of the flour was within the recommended range of 10% to 14% for flours (Akubor and Badifu, 2004**)**. Appiah *et al.* (2011) reported moisture content of 8.53% to 9.11% for flours obtained from breadfruit cultivars which were slightly lower than what pertained in the current study.

Higher moisture content in flours have been reported to enhance spoilage through creating favourable condition for microbial proliferation as well as enhance enzymatic deterioration (Oduro*et al*., 2009). Since the flours in the present study had acceptable moisture content they are expected to have longer shelf life.

Protein content

There were also significant differences (P≤0.01) in the plantain flour protein between the various amended soils.

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Flour produced from plantain with NPK amendment recorded the highest protein content (5.27 %), significantly different from the flours from NPK+PM amendment and the control which recorded the lowest protein contents of 3.39 and 3.44, respectively (Table 1). All the other amendments produced flours with protein content similar to that from the NPK amended soil.

This could be due to the readily availability of nitrogen and phosphorus needed for protein synthesis from the NPK as against the slow release of these same elements by the organic amendments. In the present study, the protein in the flour recorded from soils amended with NPK was higher than those quoted by previous researchers such as Ishmael (2011) (3.06%) and Onwuka and Onwuka (2005) (2.8%). The differences could be due to the type of fertilizer applied and the condition of the soils used for the plantain cultivation. In spite of these differences, the protein content of false horn plantain flour was generally low and as such required fortification with other high protein foods to boost the nutritional content of the flour as indicated by Zhao *et al.* (2004).

Starch Content

For starch content, flour produced from plantain without any soil amendment recorded the highest (51.33 %), significantly different from all the other amendments (Table 1). Flour produced from plantain with NPK+PM amended soil recorded the lowest starch content (30.67 %) although similar to those from NPK+CPH and PM amended soils.

From the present study, the findings are indicative that the flour starch is not influenced by any soil amendments. Starch characteristics such as swelling power, solubility pattern, pasting behaviour are important for improved quality of food products and could be useful for the development of composite blends from small scale industrial level as value-added products (Ikegwu*et al.*, 2010).

Table.1: Effect of soil amendments on protein and starch content of plantain flour

Treatment	Protein (%)	Starch (%)
NPK	5.27	40.33
PM	4.41	36.67
CPH	3.53	41.33
NPK+PM	3.39	30.67
NPK+CPH	3.76	32.00
CONTROL	3.44	51.33
LSD(0.01)	1.779	8.857
CV	17.99	9.17

FUNCTIONAL PROPERTIES OF FALSE HORN PLANTAIN FLOUR

There were no significant differences (P≥0.01) between the soil amendments with regards to the functional properties of the plantain flour. The functional properties assessed were bulk density, oil absorption capacity, water absorption capacity, solubility and swelling power. Bulk density ranged from 0.79g/cm^3 to 0.84g/cm^3. Oil absorption capacity ranged from 1.82ml/g to 10.73ml/g whereas water absorption capacity ranged from 1.37ml/g to 2.05ml/g. Solubility power ranged from 7.77% to 9.96% whiles swelling power ranged from 9.49% to 10.45%.

Bulk Density of Plantain Flours

There were no significant differences (P>0.01) between the various soil amendments with regards to the bulk density of the flour. The bulk densities ranged from 0.79g/cm^3 to0.84g/cm^3 with soil amended with PM recording 0.84g/cm^3 and NPK+PM recording 0.79g/cm^3. Ismael *et al.*, (2011) recorded a bulk density of 0.63g/cm^3 for false horn. The disparities in the values could be due to an interplay of several factors, such as environmental conditions during production, the fertility status of the soil and the method of processing the plantain into flour. Comparatively, the bulk density of fermented maize flour was reported to be 0.55g/cm^3 (Akubor and Badifu, 2004 and Mbata*et al.*, 2009). According to Bhattacharya and Prakash (1994), the bulk density of foods increases with increasing starch content. Furthermore, Okezie and Bello (1988)

stressed that high bulk density of food material is important in relation to its packaging. Ijarotimi and Ashipa (2005) documented that, higher bulk density is essential in the since that it offers greater packaging advantage as greater quantity of flour may be packed within a constant volume. Oladele and Aina (2007) also describe bulk density as the measure of the heaviness of a flour sample. Mbata*et al.*, (2009) indicated that weaning food should have low water absorption capacity and low bulk density in order to produce a more suitable and nutritious weaning food. Consequently, the flour produced from plantain with NPK+PM amendment might be suitable for preparing weaning food formulations.

Water Absorption Capacity of Plantain Flours

Water absorption capacity (WAC) for false horn plantain flour varied from 1.37ml/g to 2.05ml/g. Flours obtained from plantain grown on soils without any amendment

(control) recorded 2.05ml/g for WAC whiles flour from CPH amendment recorded the lowest WAC value of 1.37. Elmoneim*et al*. (2005) indicated that water absorption capacity gave an indication of the amount of water available for gelatinization. Low water absorption capacity is desirable for making thinner gruels. Desikachar (1980) indicated that a high water absorption capacity of flours increases its viscosity (consistency) when mixed with water, resulting in a thick paste but does not allow free-flow of the meal (Mosha and Lorri 1987).

Oil Absorption Capacity of False Horn Plantain Flour

False horn plantain flours had oil absorption capacity (OAC) ranging from 10.72g/g to 1.82g/g. Flour produced from plantain on soil amended with NPK+CPH recorded the oil absorption capacity of 10.72g whereas flour from plantain with CPH amendment recorded oil absorption capacity of 1.82g. The oil absorption capacity values recorded in the present study were higher than the 1.30g/g recorded by Mepba*et al.*, (2007). The variation could be due to the varietal differences of plantain used as well as the stage of maturity of the fruits harvested. In a study on breadfruit flour, an oil absorption capacity range of 0.38g/g to 1.13g/g was obtained (Appiah *et al*., 2011). Also, Udensi and Okoronkwo (2006) reported oil absorption capacity value of 2.2g/g for mucunabean. Generally, flours with lower oil absorption capacity have higher flavor retention abilities (Oladele and Aina, 2007). Consequently, flours from plantain with CPH amendment could have a high potential of retaining flavour and therefore could be desirable in food product formulation. Adejuyitan*et al.*, (2009) explained that the lower oil absorption capacity could be due to low hydrophobic proteins which show superior binding of lipids. For flours with high oil absorption capacities, such as that observed in the flour from plantain with NPK+CPH amendment, they could be useful in food formulation where oil holding capacity is needed for instance in the sausage making, soups and cakes (Aremu*et al.*, 2006).

Swelling Power of False Horn Plantain

Swelling power of false horn plantain flour ranges from 10.45 to 9.49. Ishmeal*et al.*, (2011) documented similar swelling power values (7.14 to 10.28) for false horn plantain flour. The swelling power values of plantain flour are comparable to those for soybean-fortified yam flour (6.8-9.6) (Jimo and Olatidoye, 2009). However, plantain flour exhibited a better swelling capacity than cassava and this could be due to the small particle sized plantain starch which is highly digestible in nature (Ojinnaka*et al*., 2009).

In comparison to the flour of other crops, there appeared to be no consistent trend as in some cases the swelling power values are lower (tigernut - 2.47, Oladele and Aina 2007); breadfruits cultivars - 4.84 to 6.23 (Appiah *et al.*, 2011) whiles in other cases the values are higher (cereal starches - 24 to 42 (Tester and Morrison, 1990). The swelling power test provided a suitable predictive method for identifying flours capable of producing high quality noodles(McComick*et al.*, 1991). The false horn plantain flours in the present study had lower swelling power values and as such might not be suitable for noodle production.

Also in the present study, solubility percentage strongly affected the swelling power of the plantain flour, an indication that as the solubility of the flour increased the swelling power also increased leading to an improved water absorption capacity of the flour (Etudaiye*et al.*, 2009). Johnson *et al*. (2001) indicated that higher solubility also permitted better digestibility.

EFFECTS OF SOIL AMENDMENTS ON THE PASTING PROPERTIES OF DERIVED PLANTAIN FLOUR

Gelatinization Temperature

There were significant differences ($P \leq 0.01$) between flours produced from the various amended soils with regards to temperature at the beginning of gelatinization (Table 2). Flours produced from plantain with NPK+PM amendment and the Control recorded the highest temperature at the beginning of gelatinization (77.47^0C and 77.45^0C respectively), which differed significantly from flours produced from PM amended soils which recorded the least temperature (75.45^0C). However, the temperature figure obtained from flours produced by PM amended soils was not significantly different from NPK, CPH, and NPK+CPH which recorded temperatures of 77.22^0C, 76.73^0C and 77.10^0C respectively.

Time Taken to Reach Peak Viscosity

There were significant differences ($P \leq 0.01$) between the flours produced from the various amended soils with regards to the time taken to reach peak viscosity (Table 2). Flours produced from soils amended with NPK+CPH and the control recorded the highest time taken to reach peak viscosity (1912.50 sec. and 1881.70 sec. respectively), which was significantly different from flours obtained from NPK amended soils which recorded the least time (1065.00 sec.).

Beginning of Gelatinization Time

There were significant differences ($P \leq 0.01$) between the flours produced from plantain grown on the various amended soils with regards to the time taken for the beginning of gelatinization. Flours produced from plantain grown on NPK+PM amended soil (1150.00 sec) and the control (1150.80 sec.) recorded the highest time for 'Beginning of gelatinization' which were significantly different from PM which recorded the least time (1065.00 sec.) (Table 2). The time recorded by flour produced from PM amended soils was not significantly different from NPK, NPK+CPH, and CPH which recorded time of 1141.70 sec., 1137.50 sec. and 1125 sec., respectively.

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Table.2: Effect of soil amendment on beginning of gelatinization temperature, timeand time taken to reach peak viscosity offalse horn plantain flour

Treatment	Beginning of gelatinization temperature (^{0}C)	Beginning of gelatinization time (sec.)	Time taken to Reach Peak Viscosity (sec.)
NPK	77.22	1141.70	1675.80
PM	75.45	1065.00	1811.70
CPH	76.73	1125.00	1791.70
NPK+PM	77.47	1150.00	1859.20
NPK+CPH	77.10	1137.50	1912.50
CONT.	77.45	1150.80	1881.70
LSD(0.01)	1.829	77.072	77.072
CV	1.50	4.30	0.85

Higher gelatinization temperatures (81.87^{0}C; 94.6^{0}C) have been reported for breadfruit flours by Appiah *et al.,* (2011) and by Oladele and Aina (2007) from other studies. Mira *et al.* (2005), explained that such higher gelatinization temperatures could be the result of delayed or restricted swelling and amylose leaching. Lower gelatinization temperature, on the other hand, is indicative of lower cooking temperature and shorter cooking time (Otegbayo*et al.,* 2006). Appiah *et al.,* (2011) reported that, flours with shorter cooking time is advantageous as it might reduce energy consumption (fuel) as well as reduce the cost and time of processing. From the present study therefore, flour from plantain with PM amendment is more likely to cook faster than the flour from the plantain with NPK+PM amendment.

Viscosities (Maximum, Final, Breakdown and Setback)

Flours from plantain with CPH amendment recorded the highest maximum viscosity (401.33BU) whereas flours from plantain with NPK amendment recorded the least viscosity of 354.33BU. Zakpaa*et al.,* (2010) reported a maximum viscosity of 677 BU for false horn plantain flour which is higher than the value in the present study. Also in an earlier study, maximum viscosities have been reported for Potato (3000 BU), Maize (77.5 BU), Wheat (300 BU) and Cassava (966 BU) (Ciacco*et al.,* 1997). The differences in values could be attributed to pasting characteristics which depend on granule size and amylose content. Larger granules have a lower surface area to volume ratio and therefore the association between hydrogen bond and granules are very weak, hence increased swelling (Zakpaa*et al.,* 2010). Maximum viscosity is an important feature of starch flour since it gives an indication of the ability of the flour to form a thick paste during cooking which is the result of the swelling power of the starch (Adebowale*et al.,* 2005; Opata*et al*., 2007). Kim *et al.,* (1995) indicated that starch with high viscosity is desirable as thickening agents in industry and in food systems.

Breakdown Viscosity

The breakdown values for false horn plantain flour ranged between 51.00 BU (NPK+CPH) to 93.00 BU (NPK) in the present study (table 3). The breakdown values generally indicate the difference between peak viscosity and minimum viscosity and show the degree of drop during heating and the extent of starch granule disintegration (Adebowale*et al.,* 2005). Higher breakdown in viscosity suggests lower ability of sample to withstand heating and shear stress during cooking. Therefore, starch samples with lower breakdown will have better ability to withstand heating and shear stress giving more stable cooked paste (Zobel, 1984). From the present study therefore, flours from plantain with NPK+CPH amendment would form a more stable paste.

Table.3: Effect of soil amendment on breakdown and setback of false horn plantain flour

Treatment	Breakdown viscosity (BU)	Setback viscosity (BU)
NPK	93.00	7.50
PM	79.00	-93.17
CPH	90.33	-14.83
NPK+PM	82.67	-70.67
NPK+CPH	51.00	-86.83
CONT.	71.17	35.83
LSD(0.01)	18.646	76.249
CV	15.08	129.70

Start of Cooling and End of Cooling

There were significant differences (P≤0.01) in start of cooling viscosity between the flours produced from plantain under the various soil amendments (Table 4). All the amendments and the control recorded significantly higher start of cooling viscosity values than that from the NPK amendment which recorded the least start of cooling viscosity value (Table 4). The end of cooling viscosity values also differed significantly (P≤0.01) between the flours produced from plantain under the various soil amendments (Table 4). Flour produced from plantain without amendment (control) recorded the highest viscosity at the end of cooling, significantly different from flour from NPK+PM and PM amendments.

Table.4: Effect of soil amendment on start of cooling and end of cooling on false horn plantain flour

Treatment	Start of cooling viscosity (BU)	End of cooling viscosity (BU)
NPK	261.33	268.67
PM	303.00	210.00
CPH	311.00	295.83
NPK+PM	309.50	238.83
NPK+CPH	317.67	255.00
CONT.	303.00	331.00
LSD(0.01)	23.083	87.808
CV	4.83	20.75

Start of Holding and End of holding

There were significant differences (P≤0.01) between the flours from the various amended soils with regards to the viscosity at the start of holding (Table 5). Flour produced from plantain amended with CPH recorded significantly higher start of holding viscosity values than the other amendments except that from the NPK + PM amended soil (Table 5). The least start of holding viscosity was recorded by NPK amended soil.

The end of holding viscosity values also differed significantly (P≤0.01) between the flours produced from plantain under the various soil amendments (Table 5). Flour produced from plantain without soil amendment (control) recorded the highest viscosity at the end of holding, which was not significantly different from flour from CPH amended soils.

Table.5: Effect of soil amendment on start of holding and end of final holding on false horn plantain flour

Treatment	Start of holding viscosity (BU)	End of final holding viscosity (BU)
NPK	349.50	227.50
PM	378.00	182.33
CPH	399.67	263.33
NPK+PM	387.00	213.17
NPK+CPH	359.00	198.00
CONT.	360.00	303.83
LSD(0.01)	14.777	67.626
CV	2.50	18.41

REGRESSION RELATIONSHIPS BETWEEN SOME AGRONOMIC PARAMETERS, FUNCTIONAL AND PROXIMATE PROPERTIES OF PLANTAIN FLOUR

There was a significant relationship between the swelling power of plantain flour and its solubility. The swelling power of the flour was positively affected by the flour's solubility. Solubility of the flour accounted for 88 % of the variation in the observed swelling power (Eqn 1).

Swelling Power = 7.08157 + 0.29512 (solubility %); R^2 = 0.88; p = 0.018; n =10 (Eqn 1).

There was also a significant relationship between the oil absorption capacity of the flour and the pulp weight of the plantain fruit. The oil absorption capacity of the flour was positively and significantly affected by the plantain fruit pulp weight such that 91 % of the variation in the oil absorption capacity of the flour was explained by the pulp weight of the plantain fruit (Eqn 2).

Oil Absorption Cap. = 0.02732 (Pulp Weight) – 3.23967; R^2=0.91; P=0.012; n=10 (Eqn 2).

Similarly, there was a significant relationship between the starch content of the flour and the pulp weight. The starch content of the flour was positively and significantly

affected by the fruit pulp weight such that 70 % of the variation in the starch content of the flour was explained by the fruit pulp weight (Eqn 3).

Starch Content = 27.8127 + 3.76498 (pulp weight); R^2 = 0.70; p = 0.046; n = 10 (Eqn 3).

There was a significant relationship between the maximum viscosity of the flour and the oil absorption capacity of the flour. The maximum viscosity of the flour was positively and significantly affected by the oil absorption capacity of the flour. However, only 25 % of the variation in the maximum viscosity of the flour was explained by the oil absorption capacity of the flour (Eqn 4).

Y(max. viscosity) = 372.501 + 1.33796(oil absorption); R^2 = 0.25; p = 0.035; n = 10. (Eqn 4).

There was also a significant relationship between the maximum viscosity of the flour and the starch content of the flour. The maximum viscosity of the flour was positively and significantly affected by the starch content of the flour. However, only 26 % of the variation in the maximum viscosity of the flour was explained by the starch content of the flour. (Eqn 5).

Y(max. viscosity) = 423.169 – 1.19374(starch); R^2 = 0.26; p = 0.030 ; n = 10. (Eqn 5).

V. CONCLUSION

Flours from plantain obtained from amended soils recorded moisture contents which were within the acceptable levels for flours. The flour with the lowest moisture content was produced from plantain with CPH amendment. The protein content of false horn plantain flour was, however, lower and as such required fortification with other high protein foods to boost the nutritional content of the flour.

The starch from plantain flour was not influenced by any of the soil amendments. On the other hand, flour produced from plantain onsoil amended with NPK+PM had low bulk density and low water absorption capacity and therefore might be suitable for preparing weaning food formulations. Contrarily, the false horn plantain flours had lower swelling power values and as such might not be suitable for noodle production.

Flours from plantain with CPH amended soil had lower oil absorption capacity which suggested that it would have a high potential of retaining flavour and therefore could be desirable in food product formulations. Similarly, flour from plantain with PM amendment was more likely to cook faster than the flour from the plantain with the other soil amendments. Flours from plantain with NPK+CPH soil amendment would form a more stable paste because of its lower breakdown value which will ensure its ability to withstand heating and shear stress and give more stable cooked paste.

Generally, the Plantain flour compared favourably with known food flours and therefore could be applicable as thickening agents and also find usefulness in fufu powder preparation and baking.

VI. RECOMMENDATIONS

1. Further studies could be done with other combinations of organic and inorganic fertilizers.
2. Soils amended with cocoa pod husk, poultry manure, NPK fertilizer and their combinations could be adopted as it is effective in enhancing the quality of plantain flour.
3. Sensory studies (texture, colour, and aroma) need to be conducted on the plantain flours emanating from the amended soils.

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Magnetically treated water on phytochemical compounds of *Rosmarinus officinalis* L.

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Abstract*— *Irrigation using water treated with static magnetic field (SMF) has recently been used as a strategy to stimulate the growth and development of different plant species. The aim of this study was to characterize the bioactive compounds and evaluate the anatomical structure of Rosmarinus officinalis L. irrigated with SMF-treated water. Results demonstrate that the treatment promoted plant growth, the number of trichomes and increased concentrations of secondary metabolites. Methanol-extracted leaves revealed that rosmarinic acid was detected in both experimental groups, without a difference in the level. Camphor, α-terpineol and verbenone were determined as the most abundant compounds present in these leaf extracts and were strongly increased in plants irrigated with SMF-treated water. Similar results were also observed for endo-borneol, bornyl acetate and β-amyrin concentrations.Taken together, these results indicate that irrigation with SMF-treated water can be used to improve the production of rosemary to obtain pharmaceutical products with an increased antioxidative activity.

Keywords*— *rosemary, leaves, static magnetic field, thin layer chromatography, gas chromatography.

I. INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is an aromatic plant of the Lamiaceae family that is frequently used in medicine. It is an important source of polyphenols and is known for its high antioxidative activity(Suong et al., 2011).The most abundant bioactive compounds in rosemary leaves are phenols, monoterpenes, diterpenes and their derivatives, including carnosic acid and related stable compounds such as carnosol, rosmanol, epirosmanol and 7-methylepirosmanol(Al-Sereiti et al., 1999). Rosemary isoften used for the production of natural antioxidant extracts andis reported to have a strong therapeutic potential in the treatment and prevention of many diseases including asthma,spasmogenicdisorders, liver disordersand hepatotoxicity, peptic ulcers, inflammatory diseases, ischemic heart disease, arteriosclerosis, Alzheimer and poor sperm motility(Suong et al., 2011; Fernández L.F. et al., 2014).

Rosemary naturally grows throughout Cuba and is frequently found in home gardens. Recently, it has been removed from the Cuban National Formulations of Phytopharmaceuticals due toproblems associated with its cultivation. Indeed, rosemary has a poor vegetative propagation and low seed production. However, it is included inthe priority list of plants for the development of Natural and Traditional Medicine in Cuba(Report to the National Commission for the Development of Traditional and Natural Medicine, 2008), emphasizing the need to develop new rosemary cultivation strategies.

Irrigation with magnetically treated water is an interesting strategy to improve rosemary production, as magnetic fields are known to strongly affect shoot growth and seed germination. Indeed, many reports have described the use of magnetically treated water and magnetic fields in agriculture. Its use has been associated with an increased

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plant metabolism (photosynthesis and water uptake) and improved plant growth and production. Irrigation using water treated with a static magnetic field (SMF)has been used for the cultivation of several plant species including tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativum* L.), rice (*Oriza sativum* L.), faba bean (*Vicia faba* L.), snow pea (*Pisum sativum* L var. macrocarpon) and chickpea (*Cicer arietinum* L.) (Gesterberger P. et al., 1978; Gilart F. et al., 2013; Grewal H.S. et al., 2011a).

This study aimed to characterize the bioactive compounds present in *R. officinalis* L. irrigated with SMF-treated water(100-150 mT)and to evaluate resulting changes in leaf histology underfield conditions.

II. MATERIAL AND METHODS

Plant material

Rosmarinus officinalis L. plants were cultivated onan experimental plot in Santiago (Cuba) and the leaves were used to preparemethanol extracts. Voucher specimen isdeposited at the Herbarium of Biodiversity and Ecology Center (BIOECO) under accession number RB 21324.

The experiment used an external magnetizer with permanent magnets designed, built and calibrated at the National Center of Applied Electromagnetism (NCAE).Magnetic induction ranged between 100 and 150 mT(Gilart F. et al., 2013). Plants were either irrigated with SMF-treated water(hereafter referred to as SMF plants) orwater not treated with SMF (referred to as control plants). Sixty plants were included in each treatment andgrown underthese conditionsfor 180 days. Irrigation was performed twice a day for 30 minutes through an air microjet system, consistingof aKSB ITUR pump and a valve-controlled system distributor. Irrigation was carried out using jets, which were set at aflow rate between 2.54 and 2.91 m^3h^{-1}. Thewater velocityrangedbetween 1.4 and 1.6 ms^{-1}.

Scanning Electron Microscopy

The analysis of trichome morphology and numberwas performed in leaf samples fixed in formalin-acetic acid-alcohol (FAA) solution (70%)(Johansen, 1940). For scanning electron microscopy (SEM), leaves were dehydrated in a graded ethanol series, submitted to critical point drying with CO_2 (Leica EM CPD-030) and coated with a thin layer of gold (Denton Vacuum Desk IV, LLC). The samples were analyzed using a JEOL-JSM 6390 LV scanning electron microscope (Jeol USA Inc) as described byGesterberger P. et al. (1978).

Preparation of rosemary extracts

In order to prepare methanol leaf extracts, *R. officinalis* leaves were dried in an oven at 40°C for 72 h. Subsequently, 3 g of dried leaf sample was macerated in100 mL of methanol for 4 to 6 h in a Soxhlet device. The extract volume was then reduced to 10 mL in a Büchi rotoevaporator and centrifuged for 3 min at 3000 x *g*.The supernatant was filtered through a Whatman paper (GF/A, 110 mm)and stored at 4°C until further analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

Methanol extracts were analysed usinga GC-MS system (Agilent7890A/5975C GC-MS System) equipped with a JWMS5capillary column (Agilent Technologies;30mx 25mm x 0.25µm). The chromatographic conditions used is shown in Table 1.

Table.1: Chromatographic conditions used for GC-MS analysis of methanol extracts ofRosmarinus officinalis L. leaves

Parameters	**Conditions**
Helium carrier gas flux	1 mL min^{-1}
Injection volume	1 µL
Injector temperature	270°C
Source temperature	290ºC
Interface temperature	230ºC
Column temperature	60-290ºC (5°C/min)
Injector	automatic
Flux range	0,95 $mLmin^{-1}$
Massspectra	70 eV
Splitratio	1:40

Chromatograms were analyzed usingthe Automated Mass Spectral Deconvolution and Identification System(AMDIS)(MSChem) and the resulting spectra were compared tothe NIST/EPA/NIH Mass Spectral Library 2011 (National Institute of Standards and Technology, Standard Reference Mass Spectra Database, USA).

Thin Layer Chromatography/High Performance ThinLayer Chromatography (TLC/ HPTLC)

Methanol extracts were also analysedusing a CAMAG TLC/HPTLC system equipped with a Linomat V applicator, a TLC scanner 3 and a 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software.A sample volume of 10µL was spotted in 5 mm bands on a precoated silica gel glass plate (20 cm x10 cm)usinga CAMAG microliter syringe. For the development of the plate, the mobile phase solvent system consisted of toluene,

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ethyl acetate and formic acid (70:20:10). To visualize the components, plates were first sprayed with vanillin in ethanol (1%) and then with a solution of sulfuric acid in ethanol (10%). The plate was heated to110°C for 5 min and then analyzedunder white light for the evaluation of terpenoids and phenylpropanoids. Alternatively, plates were analyzedusing natural product reagent. In this case, they were first sprayed with 1% methanolic diphenyl boric acid-b-ethylamine ester, followed by 5% ethanolic polyethylene glycol-4000 and then evaluated under UV light(365nm) for the detection of rosmarinic acid.For each of the bands observed, the retention factor (Rf) was calculatedas the ratio between the migration distance of the band and the migration distance of the solvent.

III. RESULTS AND DISCUSSION

Trichomes in SMF plants

Glandular structures are known as primary sites of secondary metabolite biosynthesis, secretion and storage, and generally consist of either simple subcutaneous glands or trichomes.Plants of the *Lamiaceae*family presentboth capitate and peltate glandular trichomes. Both have the same basicmorphology, consisting of a basal region, a stalk anda head. Whereas capitate glandular trichomesare formed bya head with one secretory cell and a stalk containingtwo cells, the peltate type consists of a headwith eight secretory cells, one basal epidermal cell,and a wide unicellular stalk (Boix et al., 2011; Fahn, 1979). In *R. officinalis*, non-glandular trichomes arepresent on the veins and leaf margins andare diverse in morphology,anatomy and microstructure. Basically, they are classified according to their morphology. They canbe either unicellular ormulticellular, and unbranched or branched(Marin et al., 2006; Werker et al., 1985).

Results of this study show that glandular as well as non-glandular trichomes were observed in plants subjected to both irrigation treatments, although non-glandular trichomes were more numerous (Figure 1). The number of both trichome types on the abaxial leaf surface was higher in SMF plants (Figure 1C-D) as compared to control plants (Figure 1A-B). Leaves of control plants contained approximately six peltate trichomes per mm^2, whereas those of SMF plants contained 16 per mm^2 on average. The trichomes are primary sites for biosynthesis, secretion and storage of secondary metabolites (Taiz et al., 2015). Greater efficiency could then be expected in the production of bioactive compounds for the treated plants, with respect to the control in the relation structure.

However, irrigation with SMF-treated water did not affect trichome structure, which is characterized by a prominent expandable cuticular layer (Boix et al., 2011).

Metabolites in SMF plants

The metabolites identifiedin methanol extractsof *R. officinalis* control plantsand SMF plants are listed in Table 2. Results show that irrigation with SMF-treated water strongly increased the levels of terpenoids including the bicyclic monoterpenes camphor, endo-borneol and bornyl acetate and the triterpene β-amyrin. Furthermore, terpenoids were the major class of bioactive compounds present in SMF plants (Table 2). Monoterpenes are produced in glandular trichomes, found on both leaf sides of *R. officinalis*(Boix et al., 2011).Therefore, the increased monoterpene levels in leaves of SMF plants are possibly related to their higher number of glandular trichomes (Figure 1).

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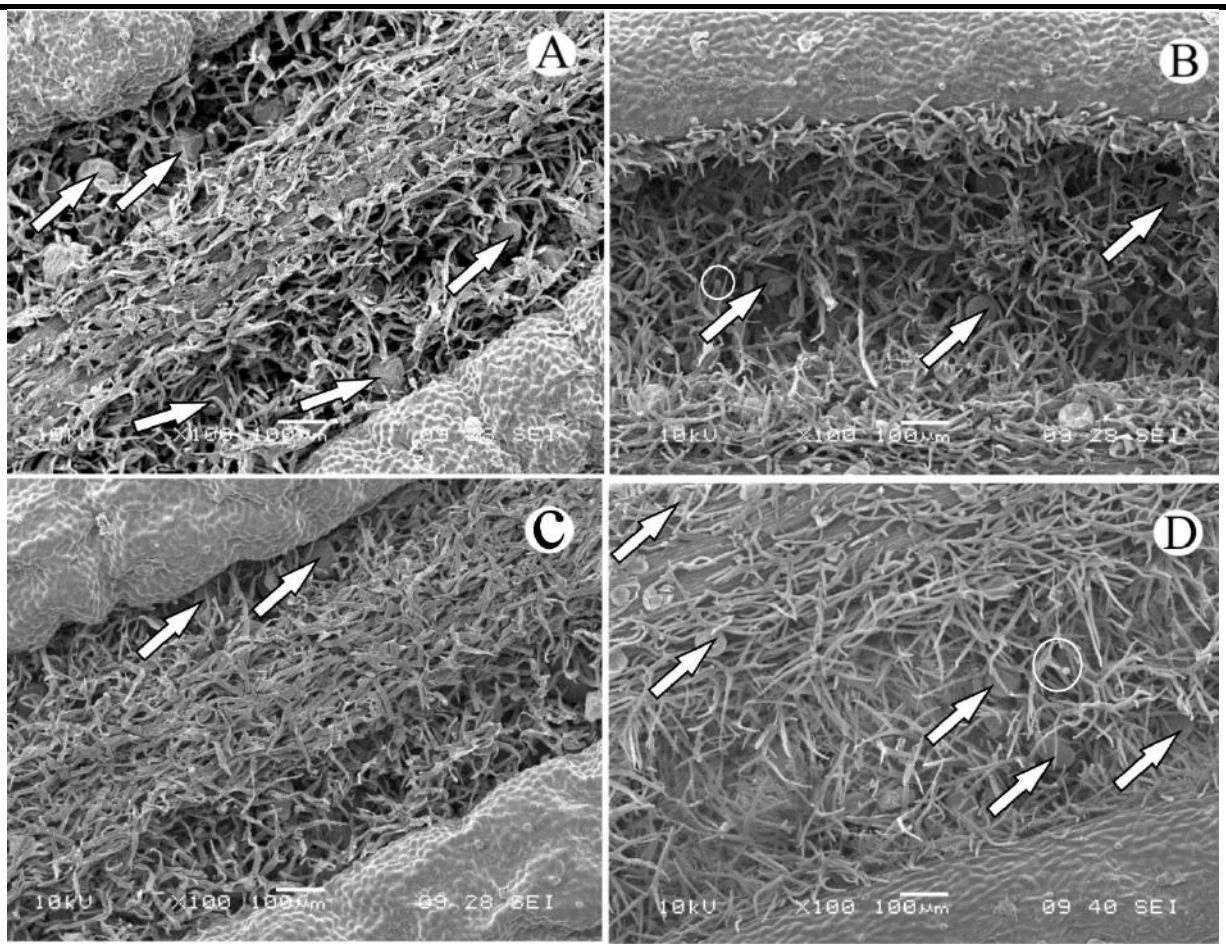

Fig.1: Scanning Electron Microscopic images of the abaxial epidermis of Rosmarinus officinalis L. leaves from control plants (A-B) and SMF plants (C-D). Circle: capitate glandular trichome. Arrow: peltate glandular trichome. Scale bars: 100 µm.

Table.2: Metabolites detected using GC-MS in methanol leaf extracts of Rosmarinus officinalis L. control and SMF plants.

Metabolites		Control	SMF
	RT	Relative area %	
camphor	6.37	16.44	23.40
endo-borneol	6.64	1.91	7.00
L-alfa-terpineol	6.80	14.00	10.3
verbenone	7.00	21.84	9.00
camphene	7.55	2.10	n.d.
bornyl acetate	8.07	2.55	14.40
2-methoxy-4-vinylphenol	8.26	0.44	n.d.
caryophyllene	10.92	3.41	3.40
phytol	26.40	11.21	9.00
β-amyrin	31.01	n.d.	18.10
squalene	41.32	12.05	n.d.
α-tocopherol	51.30	13.21	5.00
Total		99.15	99.60

Control: extract obtained from plants irrigated with water not treated with a static magnetic field; SMF: extract obtained from plants irrigated with a static magnetic field; RT: Retention time, n.d.: not detectable.

The related triterpene α-amyrin was also isolated from stems and leaves of *R. officinalis* by an extraction procedure using petroleum (60~90ºC) in a study byZhou et al. (2000). The α- and β-amyrins are pentacyclic triterpenes of natural origin, isolated from various plant sources such as resin, bark, stems, leaves, roots and rhizomes. Studies have demonstrated the pharmacological effects of these compounds against inflammation,microbial, fungal and viral infections and cancer. Furthermore, amyrins are also involved in the biosynthesis of other biologically active compounds such as avenacin, centelloside, glycyrrhizineand ginsenoside (Vázquez et al., 2012). Therefore, thehigher β-amyrin levels inleaves of SMF plants as compared to control plantsstrongly suggest a positive effect of irrigation with SMF-treated water on the therapeutic potentialof *R. officinalis.*

Furthermore, camphor concentrations were also increased in methanol leaf extracts of SMF plants as compared to those of control plants (Table 2). This oxygenated monoterpene was detected as the maincompound(23.2%)in essential oils of*R. Officinalis* leaves, followed by 1,8 cineol (13.4%), pinene (19.7%) and verbenone (8.2%)(Boix Y. F. et al., 2011; Boix Y.F et al., 2014).Like amyrins, camphorhasmany therapeutic properties.Recently, (Rašković et al., 2014) verified the high free radical scavenging activity and hepatoprotective effects of essential oils of aerial plant parts. Interestingly, one of the most abundant compounds present in these oils was camphor.

Furthermore, results of this study showed that bornyl acetate levels were strongly increased in SMF plants as compared to control plants (Table 2). Recent studies showed that bornyl acetate lowered the production of lysophosphatidylcholine (LPS)-induced proinflammatory cytokines suchas TNF-□,IL-1b, and IL-6. Therefore, irrigation of rosemary with SMF-treated water could possibly increase its anti-inflammatory potential for the treatment of inflammatory processes such as rheumatoid arthritis and osteoarthritis(Yang et al., 2014).

In addition, a decreasedlevel of vitamin E was found in SMF plants as compared to control plants (Table 2). Vitamin E is a fat-soluble compound that is mainly localized in membranes, protecting phospholipids against oxidative degeneration by reactive oxygen species (ROS).Itis involved in plantprotection against oxidative damage under differentstress conditions including drought, atmospheric pollutants, photosensitizing fungal toxinsand chilling(Fryer, 1992).The lower concentration of this vitamin in SMF plantspossibly indicates that irrigation with SMF-treated water diminishesoxidative stress.

Using TLC/HPTLC, the presence of rosmarinic acid was confirmedin methanol extracts of control plants andSMF plants(Figure2), The presence of this compound in *R. officinalis*extracts has already been reported by(Wagner H. et al., 1996). Rosmarinic acid is an ester of caffeic acid and has antioxidant, anti-inflammatory, antibacterial, antiangiogenic, antimutagenic and antiallergenic activities(Nunes et al., 2015).In methanolicextract obtained of control and with SMFplants therosmarinic acid level it was no difference between both conditions.Whereas rosmarinic acid was detected under both irrigation regimes, no differences were observed in the levels of this compound in plants of either group.

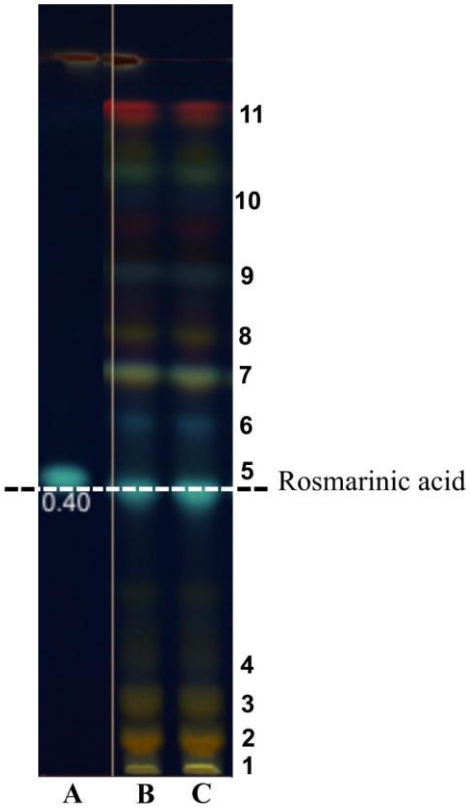

Fig.2: Thin Layer Chromatography/High Resolution Layer Chromatography (TLC/HPTLC) results showing standard rosmarinic acid (Rf = 0.40) (A), methanolic extracts from leaves of control (B) and SMF Rosmarinus officinalis L. plants (C).

Taken together, results of this study indicate that the levels of several secondary metabolites are increased in SMF plants as compared to control plants. This finding possibly explains the fact that irrigation of plants with SMF-treated

water improves plant growth. The results obtained in this study are in agreement with data available in literature. Indeed, previous studies using wheat plants demonstrated a higher total phenol content in plants irrigated with SMF-treated water as compared to control plants(Amira et al., 2010).Furthermore, it wasdemonstrated that irrigation with SMF-treated water leads to an increase in plant productivity and changes in water and mineral absorption(Hozayn et al., 2013; Mohamed et al., 2013).The amount ofwater assimilated by the plant also affectsrespiration and photosynthesis andthe concentrations of photosynthetic pigments, phenols and indoles.Similarly, research has shown that methanolextracts of*Solanum lycopersicum* irrigated with SMF-treated water (150-300mT) had a higher phenolic content as well as a higher antioxidant activity as compared to those of control plants(Dubois et al., 2013). Interestingly, irrigation with SMF-treated water was also shown to increase the fresh and dry weight of tomato plants, implying a positive effect on plant growth (Ahmed A.M., 2013). In addition, Qados (2011)reported that irrigation with SMF-treated water significantly improved the growth and yield, as well as protein content and photosynthetic pigment levels of *Vicia faba* L. plants.

We hypothesize that the increased levels of secondary metabolites in SMF plants could be related to an effect of SMF-treated water on cell membrane characteristics, resulting in an altered cell metabolism. Indeed, (Formicheva et al. (1992))reported that SMF-treated water significantly induced cell metabolism and mitosis in meristematic cells of pea, lentil and flax. Furthermore, gene transcription – playing an important role in regulating cellular processes – also seems to be affected by irrigation with SMF-treated water in *Pisum sativum* and *Cicer arietinum*(Grewal and Maheshwari, 2011). The effects of irrigation with magnetically treated water on plant secondary metabolite levels could also be a consequence of an influence on hormone levels, as increases in gibberellin (GA_3) and kinetin levels were observed in broad bean plants irrigated with SMF-treated water (Mohamed et al., 2013).

The effects of irrigation with SMF-treated water on these processes could be caused by changes occurring in the physical and chemical properties of water after application of a static magnetic field(Grewal et al., 2011b).

IV. CONCLUSION

Overall, results of this study indicate that irrigation with SMF-treated water could be used as a strategy to increase secondary metabolite levels in *Rosmarinus officinalis*, thereby promoting its therapeutic potential.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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Effect of Enzyme Treated Cassava Peel Meal Based Diets on Growth Performance and Nutrient Digestibility of Weaner Pigs

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***Abstract*—** *The experiment was conducted to evaluate the growth performance and nutrient digestibility of weaner-grower pigs fed diets containing 0 %, 50 %, 75 % and 100 % levels of cassava peel meal treated with 0.035g Natuzyme®/100g CPM. Sixteen (16) pure bred male Landrace weaner-grower pigs, averaging 13.33kg were allotted to four dietary treatments in a completely randomized design such that each pig was housed and fed individually as a replicate. Four experimental diets T_1, T_2, T_3 and T_4 were formulated and 0 %, 50 %, 75 % and 100 % maize was replaced with Natuzyme® treated cassava peel meal and fed for a period of 42days. At the end of the feeding trial, two pigs from each dietary treatment were randomly selected and starved for 24hours; faecal samples were collected for seven days, oven dried, weighed and sampled for digestibility analysis. Final weight, feed intake, weight gain and feed cost per kg live weight gain of pigs fed test diets decreased ($p<0.05$) while, feed conversion ratio increased ($p<0.05$) compared with the control. Nutrient digestibility of dry matter, crude fibre, crude protein, ash and nitrogen free extract decreased ($p<0.05$) while ether extract digestibility increased ($p>0.05$). 100% maize replacement with CPM treated with 0.035g of Natuzyme® in 100g of feed for weaner-grower pigs proved cheaper though with a slow growth rate.*

***Keywords*— cassava peel meal, Natuzyme®, growth performance, nutrient digestibility, pigs.**

Abbreviations: CPM- cassava peel meal

I. INTRODUCTION

The pig has been noted to compete with human beings for available cereal and grains (Adesehinwa *et al*., 1998). In view of this development animal researchers have shifted their attention to materials that are available but underutilized as feed ingredients for livestock. One of such materials is the cassava peel, which is underutilized in Nigeria because it is often burnt or left to rot away on farms and homesteads after harvesting and processing of the tubers (Akinfala and Tewe, 2001). Cassava peel meal contains up to 5% crude protein, 20% crude fibre depending on the variety (Aro *et al*., 2010). The fibrous content of cassava peel meal has limited its use in monogastric nutrition. Hydrocyanic acid, an anti-nutritional factor is also present in cassava peel. However, sun drying appreciably reduces its level in the material (Aletor *et al*., 1997). Dietary addition of exogenous enzyme like Natuzyme® has been reported to enhance the breaking down of fibre encapsulating the more soluble constituents so that digestion can be effective. Effects on performance of weaner-grower pigs fed varying levels of cassava peel meal without exogenous enzyme have been investigated (Ikurior *et.al.*, 1996). This study was conducted to investigate the effects of varying levels of cassava peel meal diets supplemented with Natuzyme® on growth performance and nutrient digestibility of weaner-grower pigs.

II. MATERIALS AND METHODS

The experiment was carried out at the Pig production unit on the Livestock Teaching and Research Farm, University of Agriculture, Makurdi, Benue State of Nigeria. Cassava peels were obtained from garri processing agro-allied small-scale industries in Makurdi metropolis. The peels were washed and sun dried for seven (7) days to reduce the moisture content to about 10%. The peels were then crushed using a hammer mill to obtain cassava peel meal (CPM), sampled for analysis and stored in bags until included in the diets.

Experimental diets

Four experimental diets T1, T2, T3, and T4 were formulated as presented in Table 1. T1 contained 0% cassava peel meal (CPM) without Natuzyme® and diets T2, T3 and T4 contained CPM treated with 0.035g

Natuzyme®/100g at 50%, 75% and 100%, respectively as replacement for dietary maize.

Table.1: Ingredient Composition of Weaner-Grower Pigs Diets (g/100g)

	Dietary Treatments			
	T1	**T2**	**T3**	**T4**
	Levels of Cassava Peel Meal Replacement			
Ingredients	**0 %**	**50 %**	**75 %**	**100 %**
Maize	50.00	25.00	12.50	0.00
Cassava Peel Meal	0.00	25.00	37.50	50.00
Full fat soya beans	32.00	32.00	32.00	32.00
Dried brewers grains	10.00	10.00	10.00	10.00
Rice offal	4.75	4.75	4.75	4.75
Bone meal	2.50	2.50	2.50	2.50
Common salt	0.50	0.50	0.50	0.50
Vitamins/Minerals premix[a]	0.25	0.25	0.25	0.25
Natuzyme® [+ +]	-	++	++	++
Zinc oxide[b]	+	+	+	+
Total	100.00	100.00	100.00	100.00
Calculated Nutrients:				
Metabolizable energy (Kcal/Kg)	3135.45	2786.45	2611.45	2437.45
Crude protein (%)	19.55	18.55	18.05	17.55
Dietary cost (₦/kg)	76.65	61.38	53.50	45.63

[a]Biomix premix supplied the following per kg of diet: vitamin A 12,000,000I.U ,vitamin D33,000,000 I.U, vitamin E 30,000 mg, vitamin K3 2,500 mg, folic acid 1,000 mg, niacin 40,000 mg, calpan 10,000 mg, vitamin B 25,000 mg,vitamin B12 20 mg, vitamin B1 2,000 mg, vitamin B6 3,500 mg, biotin 80 mg, antioxidant 125,000 mg, cobalt 250 mg, selenium 250 mg, iodine 1,200 mg, iron 40,000 mg, manganese 70,000 mg, copper 8,000 mg, zinc 80,000 mg, choline chloride 200,000 mg.

[b] zinc oxide 0.0125 g/100 g, Natuzyme- 0.035 g/100 g.

+ = zinc oxide

++ = Natuzyme

Experimental design and management

Sixteen (16) male weaner pigs were randomly allotted to four dietary treatments each of which had four replicates. Each pig was served drinking water *ad libitum.* Daily routine management activities were cleaning of pens, provision of experimental diets and drinking water, observation of each animal to know their health status. Each experimental animal was housed in a 183x75x106cm welded iron pipe, wire mesh, individual concrete floored pens while, each pen housed four individual crates provided with concrete feeding and watering troughs measuring 52x29x21cm and 47x37x26cm, respectively. The experiment was a completely randomized design.

Data collection

The mean weekly body weights and feed intake were recorded throughout the experimental period of 42days. Feed conversion ratio was calculated from feed intake and body weight gain. Feed cost/kg gain and feed cost/kg diet were calculated from prevailing local market price of feed materials.

Nutrient digestibility was determined by the use of two (2) pigs from each dietary treatment which were randomly selected and starved for 24 hours. A weighed amount of feed was offered daily and feacal samples collected for seven days, oven dried, milled and analyzed for dry matter, crude fibre, crude protein, ether extract, ash and nitrogen free extract using standard methods (AOAC, 1995) The proximate analysis of the experimental diets was also carried out using the same standard methods.

All data collected were subjected to analysis of variance using the procedure of Steel and Torrie (1980) and where significant differences were observed treatment means were separated using Duncan multiple range test (Duncan, 1955)

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III. RESULTS AND DISCUSSION

The experimental diets contained between 18-20 % crude protein (Table 1) in order to meet the protein requirement of weaner pigs recommended by NRC (1997). Similarly, the metabolizable energy of the diets (2,437.45 - 3,135.45 kcal/kg) though reducing as the level of treated CPM increased in diet, were also within the energy requirement of weaner pigs.

Table.2: Effect of Diets containing CPM treated with Natuzyme® on performance of Weaner-Grower Pig.

	Dietary Treatments					
	T1	T2	T3	T4		
	Levels of cassava peel meal replacement					
Performance indices	**0 %**	**50 %**	**75 %**	**100 %**	**SEM**	**LOS**
Number of pigs	4	4	4	4		
Average initial live weight (kg)	13.33	13.43	13.28	13.25	0.45	NS
Average final live weight (kg)	32.60^{a}	27.55^{a}	24.73^{b}	24.15^{b}	1.13	*
Average daily feed consumption (kg)	0.98^{a}	0.84^{ab}	0.78^{b}	0.78^{b}	0.03	*
Average daily weight gain (kg)	0.46^{a}	0.34^{b}	0.28^{bc}	0.26^{c}	0.02	*
Feed conversion ratio	2.16^{a}	2.51^{b}	2.85^{c}	3.06^{c}	0.10	*
Feed cost/kg live weight gain (₦)	165.56^{a}	154.06^{ab}	152.48^{ab}	138.63^{b}	3.63	*
Average number of days fed	42	42	42	42		

^{a,b,c} Means within same row with different superscripts differ (P<0.05) NS= Not Significant (P>0.05)
*Significant differences between means in rows (P<0.05) LOS = Level of significance SEM = Standard error of mean

The effect of the experimental diets on the growth response of weaner-grower pigs is presented in Table 2. It was observed that the diets had significant effect (p<0.05) on the live body weight, weight gain, feed intake and feed conversion ratio. Significant effect (p<0.05) was also observed for the feed cost/ kg live weight gain. These performance indices decreased as percent dietary maize replaced by CPM increased. This probably was due to CPM effect which increased the bulk of the feed thereby lowering the energy density of the diets and causing decrease in feed intake, weight gain and increase in feed conversion ratio. This observed performance can be attributed to the inability of the weaner pigs to digest the high fibre diets despite the supplementation with Natuzyme® and the low or poor quality amino acids in CPM. This is in agreement with the findings of Medel *et al*. (2000) who reported that the physical nature of a diet or of its ingredients has a large influence on feed intake and on its nutritional value which may have beneficial or adverse effect on the use and efficacy of enzymes. This is also in agreement with findings of Ikurior *et al*. (1996), who reported that as animals grow older they tend to handle fibre more efficiently due to their developed digestive system. The feed cost/kg live weight gain decreased (p<0.05) at higher levels of CPM in the diets. Therefore, it was cheaper to feed pigs on Natuzyme® treated CPM diets than the control diet. This agrees with the findings of Adesehinwa *et al*. (2008) who reported significant reduction in feed cost per kilogram live weight gain as a result of replacing maize in control diet with cassava peel supplemented with exogenous enzyme.

Table 3 presents the digestibility coefficient of weaner-grower pigs. Decrease (p<0.05) in nutrient digestibility occurred as CPM replacement of maize increased in diets of weaner pigs. This was observed particularly in crude fiber and ash digestibility and diet (T4) was mostly affected. Digestibility value ranged between 30-73 %. However, digestibility of ether extract increased (p>0.05).This decreased nutrient digestibility of weaner pigs could be attributed to the inability of the weaner pigs to handle fibre efficiently, among other inherent factors associated with CPM based diets. This is in agreement with findings of Thacker (2001) who reported that the extent and consistency of response of enzyme supplementation in pigs has been related to age of the animal, enzyme activity and dietary fibre level. CPM may also contain certain compounds that act as antioxidants and anti carcinogens which may interfere with nutrient absorption and utilization. Such compounds may also bind proteins preventing their complete enzymatic digestion (Montagac *et al*., 2009). In line with this report Van de Mierop (2001) reported that although enzymes are already in use for over two decades, a lot still has to be explained on why, how and to what extent an enzyme influences the digestibility of nutrients.

Table.3: Effect of Diets containing CPM treated with Natuzyme ® on Apparent Nutrients Digestibility Coefficients of Weaner-Grower Pigs

	Dietary Treatments					
	T1	T2	T3	T4		
	Levels of cassava peels replacement					
Nutrients	0 %	50 %	75 %	100 %	**SEM**	**LOS**
Dry Matter (%)	85.20^{a}	78.05^{b}	77.06^{b}	70.27^{c}	1.00	*
Crude Fibre (%)	74.45^{a}	70.57^{a}	69.40^{a}	52.92^{b}	3.45	*
Crude Protein (%)	89.12^{a}	87.77^{ab}	86.87^{ab}	82.59^{b}	1.08	*
Ash (%)	57.89^{a}	52.55^{a}	55.80^{a}	32.74^{b}	3.85	*
Ether Extract (%)	86.24	86.56	83.78	89.61	1.30	NS
NFE (%)	89.46^{a}	79.85^{b}	77.94^{bc}	73.22^{c}	2.28	*

$^{a.b.c}$ Means on the same row with different superscripts are statistically different ($p<0.05$), NS = Not significant
* = Significant ($p<0.05$), NFE= Nitrogen free extract, LOS= Level of Significance, SEM=Standard error of mean

IV. CONCLUSION AND RECOMMENDATION

In conclusion, the study revealed that treated CPM can be used to replace maize to lower production cost but should be used preferably in older pigs (grower-finisher) to enhance the utilization of the exogenous enzyme. Further study is hereby recommended on the utilization of CPM based diets treated with Natuzyme® in weaner pigs (weaner-grower) to evaluate the effectiveness or efficacy of Natuzyme®.

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