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FOREWORD

I am pleased to put into the hands of readers Volume-3; Issue-4: Jul-Aug 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: Sept, 2018

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Productivity of Soybean on Different Agroecosystems

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Abstract**—** *This study aims to see the growth and productivity of soybeans in different agroecosystems. The study was conducted on paddy field located in Bumi Setia village, Seputih Mataram sub-district, Central Lampung district, and on dryland located in Mandah village, Natar sub-district, South Lampung district, Lampung Province, Indonesia, from April to July 2015. Soybean varieties grown at each location were Grobogan varieties. The variables observed were crop emergence, plant height at harvest, number of plant harvested, number of pods per plant, empty pods, weight of 100 grains, pod pests and productivity. Data were analyzed by t test. The results showed that soybean productivity in dryland was 64.25% lower than productivity in paddy fields. The low yield of soybean varieties of Grobogan in dryland was caused due to drought factor when forming and filling pods. This can be seen from the decrease of weight of 100 grains of soybean seed in dryland up to 51.82% than in paddy field. The status of Grobogan varieties soybean vigor may change from large seed to medium seed if the water requirement is not optimum during the growing season.*

Keywords**— Soybean, agroecosystem, productivity, pod pest.**

I. INTRODUCTION

Soybean is a food commodity source of vegetable protein is very important, especially for the population of Indonesia. In Indonesia, soybeans are consumed in the form of tofu and tempeh. Therefore, the biggest consumers are from industry of tofu and tempeh, then the next rank is feed industry, so the market potential of soybean in Indonesia is very wide and will continue to grow (Zakaria *et al*., 2010). Soybean has a wide use because it is highly nutritious and produces antioxidant substances (Krisdiana, 2007).

Grobogan varieties of soybeans released in 2008 with Decree of the Minister of Agriculture 238 / Kpts / SR.120 / 3/2008. Grobogan varieties derived from the purification of the local population of Malabar Grobogan, with a yield potential of 3.40 tons/ha and the average yield of 2.77 tons/ha, the age of plants ± 76 days, and seed weight ± 18 g/100 grains (Kementerian Pertanian, 2013). This indicates that Grobogan varieties are large seeds.

The use of superior varieties is one of the important technological components in an effort to increase the production and productivity of soybeans. But efforts to increase production and productivity in soybean plant many obstacles, namely the existence of pests and diseases. Pod pests are one of the factors that can decrease soybean productivity. Loss of results due to this pod pests are the highest reaching 80-90%, even puso if no control measures (Baliadi *et al*., 2008; Bedjo, 2011). The soybean pod pests are grouped into three types namely pod borer (*Etiella sp*.), Pod suckers (*Nezara viridula*), *Piezodorus hybneri*, *Riptortus linearis*), and pod eater (*Helicoverpa armigera*) (Baliadi *et al*., 2008; Marwoto & Indiati, 2009; Naseri *et al*., 2010; Bae *et al*., 2014).

Symptoms of pest attack are varied, symptoms of pod borer attack if there are a hole in the skin of the former pod, the larvae into the seeds and damage the seeds by leaving the dirt from the borer (Tohamy & El-Hafez, 2005), symptoms pod sucker attacks if the skin is wrinkled and there are a brown to black spots on the skin of the seeds (Bayu & Tengkano, 2014), whereas the pod eater symptom seen to be a large hole in the pod where the seed is located or the pods are eaten, soybean pod pests leave no dirt in the pod (Malik, 2013), this distinguishes between the symptoms of *Helicoverpa armigera* attack with symptoms pod borer attack (*Etiella sp*.). One effort to anticipate the explosion of pest populations on soybean cultivation by cultivation technique can be done by planting soybean short age such as Grobogan varieties (Marwoto & Indiati, 2009). The results showed that Grobogan varieties had secondary metabolite compounds that could inhibit or reduce the development of larvae and imago of *H. armigera* (Siahaan & Redsway, 2014).

Soybean cultivation is cultivated on diverse agro-ecosystem conditions that affect the diversity of planting time (Zakaria *et al*., 2010). In Indonesia, the largest soybean area in paddy fields are about 60%, which are planted after rice, the rest is grown on dryland. This condition shows that the area of soybean cultivation is

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mostly found in areas where infrastructure is relatively well established and relatively fertile than on dryland (Subandi, 2007). According to Atman (2006), paddy fields after paddy and dryland have the greatest potential for the development of soybean crops. However, according to Han (2006), soybean production in dryland agro-ecosystem is not maximal yet, increasing productivity tends to move slowly. This study aims to see the growth and productivity of Grobogan varieties of soybean in two different agroecosystems in Lampung, Indonesia.

II. METHODOLOGY

The study was conducted on two agroecosystems, namely paddy field and dryland. The paddy field was located in the village of Bumi Setia, Seputih Mataram Subdistrict, Central Lampung district, while the dryland was located in Mandah village, Natar subdistrict, South Lampung district, Lampung Province, Indonesia. The study starts from April to July 2015. On dryland the plant was fertilized with 75 kg of urea + 100 kg SP36 + 100 kg KCl + 1 ton of organic fertilizer + 500 kg of dolomite per hectare, and in paddy field the plant was fertilized with 25 kg urea + 50 kg SP36 + 50 kg KCl + 1 ton organic fertilizer per hectare. Aplication of organic fertilizers and dolomite when processing the soil 2 weeks before planting. Urea, Sp36 and KCl fertilizers were given when the plants were 7 days old. Plant spacing was 40cm x 15cm, 2 seeds per planting hole. The plots size of observation were 5m x 2m randomly assigned, each location with 18 plots as replicates. Plants were sprayed insecticide biweekly until the plant was formed pods, and the treatment was the same between in paddy field with in dryland. The variables observed were crop emergence, plant height at harvest, number of harvested plants, number of pods per plant, empty pods, weight of 100 grains, pod pests by observing the attack symptoms on pods and seeds (each of 5 sample plants) and yield (conversion from tiles 5m x 2m). Data were analyzed by t test. Intensity of pod pests attack were calculated by using formula:

$$I = \frac{a}{a+b} \times 100\%$$

I = Intensity of attack (%)
a = Number of pods attacked
b = Number of healthy pods

III. RESULTS AND DISCUSSION

3.1. Plant growth and productivity.

The average of crop emergence, plant height and number of harvested plants in paddy fields and in dryland were not significantly different (Table 1). This shows the growth of homogeneous plants in the two agroecosystems. Crop emergence ranged from 82.6 to 85.08%. Crop emergence indicates the state of seed quality before planting. If the seed quality is good then the crop emergence will be high. Factors affecting seed quality include genetic factors, environmental factors and seed status factors (physical and physiological condition of seeds). Genetic factors are innate factors associated with genetic composition of seeds. Environmental factors that affect the quality of seeds are related to conditions and treatments during preharvest, postharvest, and when marketing seeds. Physiological factors of seed are related to seed performance such as maturity level, degree of mechanical damage, level of obsolescence (relationship between initial vigor and duration stored), health level, size and density, chemical composition, structure and moisture content (Supriyadi, 2009; Admin, 2012). The seeds of soybeans in this study were good because of their growing >80% (Harnowo *et al*., 2013).

The observation of the average height of the plants ranged from 46.79 to 47.52 cm (Table 1), which was slightly lower than that of the Grobogan varieties listed in the descriptions of 50-60 cm (Kementerian Pertanian, 2013). Grobogan varieties of crops grown in Limpok Regency of Aceh Besar were lower by 42.33 cm (Bakhtiar *et al*., 2014). Plant height of soybean are influenced by genetic factors, but may also be influenced by environmental factors (Han, 2006; Bakhtiar *et al*., 2014).

Table.1: Average component of plant growth in two agroecosystems.

Agroecosystem	Crop emergence (%)	Plant height (cm)	Number of harvested plants
Paddy land	85.08	46.79	171.14
Dryland	82.60	47.52	181.14

The numbers in the same column were not significantly different based on the 5% t test.

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The yield components such as number of pods, empty pods, productivity and weight of 100 soybean grains planted in paddy field were significantly different from those grown in dryland. Productivity of soybean of Grobogan varieties were higher in paddy field than in dryland (Table 2). The number of pods per plant, the number of seeds per pod, and the size of the seeds directly affect the outcome (Hakim, 2012).

Table.2: Average yield components on two agroecosystems.

Agroecosystem	Number of pods per plant	Empty pods (%)	Productivity (tons/ha)	Weight of 100 grains (grams)
Paddy land	39.42 *	4.08 *	2.07 *	22.77 *
Dry land	33.90	8.50	0.74	10.97

*The numbers in the same column followed by * are significantly different based on the 5% t test*

Planting soybeans at the right time will avoid from the constraints of drought or floods and interference of pests and diseases (Atman, 2006; Zakaria *et al*., 2010). In this activity soybeans were grown both in paddy field and in dryland in the late rain season. But in the paddy field due to planting on time so still get the optimum rainfall during its growth. While in the dryland due to planting rather late so that drought when flowering and filling pod, it can be seen from rainfall data in May-June at soybean location in dryland no rain at all (Table 3), whereas at that time plant of soybean at flowering phase and pod formation until filling of pods, so that this take effect on soybean yield components grown on dryland.

Drought or lack of water during the flowering phase can cause a decrease in the number of pods and seed size (Kari & Nuralini, 1993 *in* Harsono *et al*., 2013; Suhartina *et al*., 2014). Drought stress inhibits the distribution of carbohydrates from the leaves to the pods so that the number and size of seeds decreases (Liu *et al*, 2004).

It was possible that the weight of 100 grains of Grobogan soybeans grown in dryland changed its status from large seeds to be medium seeds due to weight of 100 grains to 10.97 grams, while soybeans grown in paddy field have weight of 100 grains about 22.77 grams (Table 2), and this were higher than those listed in the description (Kementerian Pertanian, 2013). Soybean seeds are small if they weigh 8-10 grams/100 grains, classified as medium size if they weigh >10-13 grams/100 grains, and are considered large seeds if they weigh >13 grams/100 grains (Suharno & Didik, 2008; Direktorat Jenderal Tanaman Pangan, 2013; Ginting & Tastra, 2013). This indicates that the effect of drought during pod formation and filling of pods can decrease the weight of 51.82% soybean seeds. Farmer preference for seed size of soybean varies, some prefer large-seeded soybeans, some also want small or medium seeded soybeans. With the results of this study is expected farmers can consider about size seed of soybean that preferenced, because soybeans initially large seeds could have turned into a small seeds. The genetic properties of a variety may change due to environmental factors. Some components of environmental factors that are important in determining the growth and production of plants include solar radiation, temperature, soil, and water.

Table.3: The rainfall data in 2015.

Months	Paddy land		Dryland	
	Rainfall (mm)	Rainy day (day)	Rainfall (mm)	Rainy day (day)
March	189	14	232.8	21
April	220	14	121	12
May	57	10	0	0
June	93	8	0	0
Total	559	46	353.8	33

Source: BPTP Lampung ; BPP Seputih Mataram,Central Lampung, Indonesia

Soybeans grown in paddy field experience optimum growth so that their productivity were also better than soybeans grown on dryland. Rainfall in paddy field was always present during soybean growth (Table 3).

The need water for soybean that harvested at 80-90 days ranges from 360-405 mm during its growth period, equivalent to 120-135 mm per month. Stadia soybean plants that are critical of water shortage are in the stadia start flowering until the end of flowering, then stadia of forming and filling pod, until pod maturation (Sumarno & Ahmad, 2013).

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The productivity of soybean of Grobogan varieties that were grown on dryland due to drought was lower by 64.25% compared to Grobogan varieties grown in paddy field (Table 2). This was in line with the opinion of Adisarwanto (2010), the decrease of yield due to abiotic stress such as lack of water in soybean crops can reach 40-80%. In addition, percentage of empty pods in soybeans grown on dryland were higher than soybeans grown in paddy field. The amount of empty pods can also decrease of yield.

3.2. The attack of pod pests

Identified pod pests seen from the symptoms of the attack were pod borer, pod suckers and pod eaters both in paddy field and in dryland. The pod sucking attacks in paddy field were not significantly different from those in dryland, whereas pod borer and pod-eaters in paddy field were significantly different from those in dryland and the intensity of their attacks were higher in soybean crops grown in paddy field. This indicates that the low productivity of soybean in dryland was not caused by pod pests attack, because pod pest incidence in paddy field such as pod borer and pod eaters were higher and significantly different than in dryland (Table 4). Low of soybean productivity in dryland was more caused by drought factor.

Table.4: Average of pod pests attack on soybean crops in two agroecosystems

Agroecosystem	Pod borer (%)	Pod suckers (%)	Pod eater (%)
Paddy land	6,37 *	2,55	2,64 *
Dry land	3,33	3,32	0,95

*The numbers in the same column followed by * are significantly different based on the 5% t test*

The intensity of pest attacks on both agroecosystems were relatively low, ranging from 0.95-6.37% (Table 4), but they were included above the control threshold, except for pod-eaters in dryland below the control threshold. The control threshold of pod pests are if the damage of pods are > 2.50% (Baliadi *et al*., 2008). The extent of damage caused by pod pests on soybean crops are determined by various factors including high population, plant growth phase, plant response to pests, planted varieties and control measures (Bayu, 2015).

Increased pod pests incidence in the field are thought to be related to the extent of soybean cultivation and the availability of host plants continuously (Baliadi *et al*., 2008; Samosir *et al*., 2015). According to Marwoto and Indiati (2009), pest attacks will increase as water shortages and temperatures increase. However, in this study, pod borer attack and pod eaters attack were higher in paddy land than in dryland, except for pod sucking pests did look higher on dryland than in paddy field, but both were not significantly different (Table 4) . The pest population dynamics in the field may be influenced by climatic and weather conditions, and the presence of host plants and natural enemies. Although in this study low soybean productivity in dryland was caused by drought factor, but the third pod pests both in paddy field and dryland have correlation to productivity with correlation

coefficient r = 1, which means the attack of he third types of pod pests were very strong can decrease soybean productivity (Munir, 2008).

IV. CONCLUSION

Productivity of soybean in dryland was lower 64,25% than productivity in paddy field. However, the low yield of soybean varieties Grobogan in dryland was caused more due to drought factor when forming and filling pods. This can be seen from the decrease of weight of 100 grains of soybean seed in dry land up to 51,82%. The status of Grobogan varieties soybean vigor may change from large seed to medium seed if the water requirement is not optimum during the growing season. But pod pests too have a very strong correlation to decrease of yield with correlation coefficient r = 1 both in paddy land as well in dryland.

REFERENCES

[1] Adisarwanto T. (2010). Strategi Peningkatan Produksi Kedelai Sebagai Upaya Untuk Memenuhi Kebutuhan di Dalam Negeri dan Mengurangi Impor. Pengembangan Inovasi Pertanian. Vol. 3 (4): 319-331.

[2] Admin. (2012). Faktor Yang Mempengaruhi Mutu Benih. http://smakita.net/faktor-yang-mempengaruhi-mutu-benih/

[3] Atman. (2006). Pengelolaan Tanaman Kedelai di Lahan Kering Masam. Jurnal Ilmiah Tambua. Vol. V (3). September-Desember: 281-287.

[4] Bae SD, Kim HJ, & Mainali BP. (2014). Infestation of *Riptortus pedestris* (Fabricius) Decreases The Nutritional Quality and Germination Potensial of Soybean Seeds. J Asia-Pac Entomol 17: 477-481.

[5] Bakhtiar, Taufan H, Yadi J., & Suwayda S. (2014). Keragaan Pertumbuhan dan Komponen Hasil Beberapa Varietas Unggul Kedelai di Aceh Besar. J. Floratek 9: 46-52.

[6] Baliadi Y, W Tengkano, & Marwoto. (2008). Penggerek Polong Kedelai, *Etiella Zinckenella*

Treitschke (Lepidoptera: Pyralidae) Dan Strategi Pengendaliannya di Indonesia. Jurnal Litbang Pertanian. 27 (4): 113-123.

[7] Bayu MSYI. (2015). Tingkat Serangan Berbagai Hama Polong Pada Plasma Nutfah Kedelai. PROS SEM NAS MASY BIODIV INDON. Vol. 1 (4) Juli: 878-883.

[8] Bayu MSYI & W Tengkano. (2014). Endemik Kepik Hijau Pucat, Piezodorus hybneri Gmelin (Hemiptera: Pentatomidae) dan Pengendaliannya. Buletin Palawija 28: 73-83.

[9] Bedjo. (2011). Pengaruh konsentrasi HaNPV Terhadap Penekanan Populasi Hama Pemakan Polong Kedelai Helicoverpa armigera. Suara Perlindungan Tanaman 2 (2): p.10

[10] Direktorat Jenderal Tanaman Pangan. (2013). Pedoman Teknis Pengelolaan Produksi Kedelai. Kementerian Pertanian. 134p.

[11] Ginting E & IK Tastra. (2013). Standar Mutu Biji Kedelai. Pp. 444-463 *dalam* Kedelai. Teknik Produksi dan Pengembangan. Badan Litbang Pertanian. Pusat Penelitian dan Pengembangan Tanaman Pangan.

[12] Hakim, L. (2012). Komponen Hasil dan Karakter Morfologi Penentu Hasil Kedelai. Penelitian Pertanian Tanaman Pangan. Vol. 31(3): 173-179.

[13] Han B. (2006). Uji Adaptif Beberapa Varietas Kedelai (Glycine max (L) (Merril) Pada Agroekosistem Lahan Kering Spesifik Lokasi Pada Musim Tanam Juni di Kebun Percobaan Lampineung Nanggroe Aceh Darussalam. J. Floratek Vol. 2: 78-85.

[14] Harnowo D, J Rachman H, & Suyamto. (2013). Kebutuhan dan Teknologi Produksi Benih Kedelai. Pp. 383-415 *dalam* Kedelai. Teknik Produksi dan Pengembangan. Badan Litbang Pertanian. Pusat Penelitian dan Pengembangan Tanaman Pangan.

[15] Harsono A, RD Purwaningrahayu, & A Taufiq. (2013). Pengelolaan Air dan Drainase pada Budi Daya Kedelai. Pp. 253-280 *in* Kedelai. Teknik Produksi dan Pengembangan. Badan Litbang Pertanian. Pusat Penelitian dan Pengembangan Tanaman Pangan.

[16] Kementerian Pertanian. (2013). Deskripsi Varietas Unggul Kedelai. Badan Litbang Pertanian. Balai Penelitian Tanaman Aneka Kacang dan Umbi. 79p.

[17] Krisdiana R. (2007). Preferensi Industri Tahu dan Tempe Terhadap Ukuran dan Warna Biji Kedelai. Iptek Tanaman Pangan. Vol. 2 (1): 123-130.

[18] Liu F, CR Jensen, & MN Anderson. (2004). Drought Stress Effect on Carbohydrate Concentration in Soybean Leaves and Pods During Early Reproductive Development: its implication in altering pod set. Fild Crops Research 86: 1-13.

[19] Malik H. (2013). Hama Yang Sering Menyerang Tanaman Kedelai. http://simple420. blogspot. co.id/2013/01/

[20] Marwoto & SW Indiati. (2009). Strategi Pengendalian Hama Kedelai Dalam Era Perubahan Iklim Global. Iptek Tanaman Pangan. Vol. 4 (1): 94-103.

[21] Munir S. (2008). Statistik Deskriptif (1). Analisis Korelasi Product Moment. Fakultas Ekonomi Program Kelas Karyawan. Universitas Mercu Buana. 8p.

[22] Naseri B, Fathipour Y, Moharramipour S, & Hosseininaveh V. (2010). Nutritional Indices of The Cotton Bollworm, Helicoverpa armigera, on 13 Soybean Varieties. J. Insect Sci 10 (5): 1-14.

[23] Samosir S, Marheni, & Syahrial O. (2015). Uji Preferensi Hama Kepik Hijau Nezara viridula L. (Hemiptera: Pentatomidae) Pada Tanaman Kacang Kedelai dan Kacang Panjang di Laboratorium. Jurnal Online Agroekoteaknologi Vol. 3 (2), Maret: 772-778.

[24] Siahaan P & Redsway DTM. (2014). Perkembangan Ulat Buah *Heliotis armigera* (Lepidoptera: Noctuidae) Pada Beberapa Varietas Kedelai (*Glycine max* L.). Jurnal Ilmiah Sains. Vol. 14 (1), April: 46-51.

[25] Subandi. (2007). Kesiapan Teknologi Mendukung Peningkatan Produksi Menuju Swasembada Kedelai. Makalah Pada Simposium Tanaman Pangan V. Bogor, 28-29 Agustus 2007. Pusat Penelitian dan Pengembangan Tanaman Pangan. Bogor.

[26] Suharno & Didik H. (2008). Karakteristik Biji Kedelai Untuk Produksi Tahu dan Tempe di Kendari, Sulawesi Tenggara. Buletin Teknologi dan Informasi Pertanian: 6-13.

[27] Suhartina, Purwantoro, Novita N, & Abdullah T. (2014). Stabilitas Hasil Galur Kedelai Toleran Cekaman Kekeringan. Penelitian Pertanian Tanaman Pangan. Vol. 33 (1): 54-60.

[28] Sumarno & Ahmad GM. (2013). Persyaratan Tumbuh dan Wilayah Produksi Kedelai di Indonesia. Pp. 74-103 *dalam* Kedelai. Teknik Produksi dan Pengembangan. Badan Litbang Pertanian. Pusat Penelitian dan Pengembangan Tanaman Pangan.

[29] Supriyadi H. (2009). Petunjuk Teknis Pengelolaan Tanaman dan Sumberdaya Terpadu (PTT) Kedelai. BPTP Jawa Barat. BBP2TP. Badan Litbang Pertanian. Departemen Pertanian. 14p.

[30] Tohamy HT & El-Hafez GA. (2005). Integrated Crop Management System For Controlling Cowpea Pod Worm, *Etiella zinckenella* (Treit) in Relation To

http://dx.doi.org/10.22161/ijeab/3.4.1 *ISSN: 2456-1878*

Soybean Yield at Minia and New Valley Regions. Egyptian J Agric Res 83: 1079-1098.

[31] Zakaria AK, Wahyuning KS, & Reni K. (2010). Analisis Daya Saing Komoditas Kedelai Menurut Agro Ekosistem: Kasus Di Tiga Provinsi Di Indonesia. Jurnal Agro Ekonomi. Vol. 28 (1). Mei: 21-37.

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Soil - Plant Nutrient Correlation Analysis of Maize Varieties at the Guinea Savannah

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Abstract*—Field trials were conducted during the rainy season of 2008 and 2009 at the Institute for Agricultural Research farm in Samaru (11^0 $11^{'}$ N, 7^0 $38^{'}$E) within the northern Guinea savanna ecological zone of Nigeria to evaluate correlation relationships among soil, yield and yield quality of maize varieties. The objectives of the study are to correlate among soil, grain yield and grain composition. The treatments consisted of four rates of nitrogen fertilizer (0, 50, 100 and 150kgNha^{-1}), two rates of micronutrients (0, cocktail mixtures) Cu, Fe, Zn, B and Mo and four maize varieties SAMMAZ 14, SUSUMA (QPM), SAMMAZ 11 and SAMMAZ 12 (normal maize) which gave a total of thirty-two (32) treatments. There was basal application of 60kgha^{-1}P and 60kgha^{-1}K. These treatments were tested in a randomized complete block design with three replications with a total of 96 plots respectively. The fertilizer treatments were factorially combined. Significant correlations were obtained between grain parameters and other yield parameters such as Stover (r= 0.669, P < 0.05); 1000grain weight (r= 0.617, P < 0.05); crude proteins (r= 0.364, P< 0.05) and total nitrogen in grain (r =0.993, P < 0.05). Grain yield also increased as soil pH (r =0.26, P < 0.01); TN (r =0.19, P < 0.01); Calcium (r =0.17, P <0.05); Zn (r =0.24, P < 0.01); Cu (r =0.31, P < 0.01) and B (r =0.49, P <0.05) increased while it decreased as crude protein (-0.39, P<0.05) of the grain decreased.*

***Keywords*—correlation, maize, Northern Guinea Savannah, quality protein, soil nutrient.**

I. INTRODUCTION

The soil of the Northern Guinea Savanna which stretches from Latitude 7º – 12ºN is characterized by the sub-humid climate covering well over 50% of the land area. The Savanna soils are highly weathered, coarse textured, low in organic matter content (2.0-10.0gkg^{-1}) and cation exchange capacity (6.0-10.0cmolkg^{-1}). They are generally acidic and poorly buffered with respect to most nutrients (Jones and Wild, 1975; Balasubramanian and Nnadi 1980; Kang and Wilson, 1987). The annual rainfall ranges from 800mm-1900 mm (Uyovbisere and Lombin, 1991). They are generally low in total nitrogen (N), values range from 0.8 to 2.9 gkg^{-1}, with a mean of 0.5 gkg^{-1}(Jones and Wild, 1975). This low value is closely linked with low organic matter content of the soils. Total phosphorus (P) is also generally low too with values ranging from 13 to 630 ppm, but a range of about 100 to 400ppm have been reported in the savannah soils (Mokwunye, 1974).

Improving nutritional quality of agricultural crops is a noble goal, which is important in cereal crops where plants have poor nutritional quality (Vassal, 2006). The nutritional well-being and health of all people are known to be vital prerequisites for the development of societies (Prasanna *et al*., 2001). Maize is gaining popularity in the Northern Guinea Savanna zone of Nigeria. In fact, it is replacing the traditional cereals, millet and sorghum (Onwueme and Sinha, 1991). Whatever the type of maize, they all require heavy fertilizer application for optimum yield (Awotundun, 2005). For mineral fertilizer, a rate of 100-150 kg N, 40-50 P_2O_5 and 80-100 kgK_2O ha^{-1}has been recommended for maize in the savanna zone (Onyinbe*et al*., 2006) while, FPDD (2002) recommended 120 kg N, 60 kg P_2O_5, and 60 kg K_2O.

Maize is progressively assuming the position as the major crop of the sub-humid and semi-arid savanna with respect to economic prospects for the farmers. It is a staple food crop in the ecological zone. A study was carried out to evaluate correlation relationships among soil, yield and yield quality of four varieties of maize in a northern Guinea savanna of Nigeria.

Objectives of the Study are:

- Evaluate relationship between the grain yield and other yield parameters.
- Correlate the soil nutrients with plant composition

II. MATERIALS AND METHODS

The field trials were carried out during the cropping season of 2008 and 2009 in Samaru, Zaria at the Northern Guinea Savanna ecological zone of Nigeria. Samaru is located at longitude 11º 11' N, latitude 7 º 38' E at 686m above sea level. The region has an annual rainfall average of about 1060mm (Owonubi *et al*.,1991). The soil is classified as Alfisol in the USDA Soil classification system (www.nrcs.usda.gov).

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The site was divided into three blocks each, consisting 32 plots, giving a total of 96 plots and each plot measuring 12 m^2. There were 4 ridges in a plot, 3m long at 0.75m x 0.25m spacing. The experiment was laid out in a randomized complete block design with three replications and treatment was factorially combined. The maize planted were two quality protein maize (QPM) – Sammaz 14 and Susoma and two normal maize varieties – Sammaz 12 and Sammaz 11. Three maize seeds were sown in drills and thinned to one per stand. Weeding was done in each year with the use of hand-hoe.

Nitrogen was applied in 2 split doses at two weeks after planting (2WAP) and four weeks after planting (4WAP) at the rate of (0, 50, 100, 150 kg ha^{-1}) with Urea (46 %). Basal application of phosphorus and potassium were applied as 60 kg P_2O_5 ha^{-1} as single super phosphate (SSP), and 60 kg K_2O ha^{-1}potash (MOP), (60%) respectively. The cocktail micronutrient mixtures of Fe, Zn, B, Mo, and Cu were applied at the rate of 22.85gha^{-1}). The P, K, and micronutrients were all applied 2 weeks after planting immediately after thinning to one plant per stand.

Field observations were made in each plot. The response of maize varieties to the various treatments were evaluated, evaluation between grain yield and other yield parameters, grain composition and soil nutrients were studied.

Statistical Analysis

All data collected was subjected to statistical analysis using SAS statistical computer software (SAS, 2005). The correlation between grain yield, grain parameters and some soil chemical properties were established.

III. RESULTS AND DISCUSSION

The first paragraph under each heading or subheading should be flush left, and subsequent paragraphs should have a five-space indentation. A colon is inserted before an equation is presented, but there is no punctuation following the equation. All equations are numbered and referred to in the text solely by a number enclosed in a round bracket (i.e., (3) reads as "equation 3"). Ensure that any miscellaneous numbering system you use in your paper cannot be confused with a reference [4] or an equation (3) designation.

Characterization of the soils used for the study

The soils used for the field trials were characterized for their physical and chemical properties as shown in Table 1.

Table.1: Physico-chemical properties of the soil used for the study

Parameters	Field Study (2008)	Field Study (2009)
	0-20 (cm)	0-20 (cm)
Sand (gkg^{-1})	540	530
Silt (gkg^{-1})	330	350
Clay (gkg^{-1})	130	120
Textural class	Sandy-loam	Sandy-loam
pH_{H20} 1:2.5	5.8	5.7
pH_{CaCl2} 1:2.5	5.3	5.4
Organic carbon (gkg^{-1})	5.4	5.2
Total nitrogen (gkg^{-1})	0.1	0.1
Available P ($mgkg^{-1}$)	8.9	7.6
Exchangeable acidity ($cmolkg^{-1}$)	0.4	0.6
Exchangeable bases ($cmolkg^{-1}$)		
Calcium	3.6	3.1
Magnesium	1.3	1.4
Sodium	0.5	0.4
Potassium	0.3	0.3
Effective CEC ($cmolkg^{-1}$)	5.7	5.1
Micronutrients ($mgkg^{-1}$)		
Extractable Zinc	18	10
Extractable Iron	55	52
Extractable Copper	0.6	0.6
Extractable Molybdenum	12	11
Extractable Boron	0.2	0.1

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Soil characteristics and geology

Soils of the experimental sites have been classified as Typic Haplustalf an Alfisol in the USDA Soil Classification system and it is developed in deeply weathered pre-Cambrian, basement complex rock overlain by aeolian drift materials of varying thickness (; Ogunwole, 2000). The soils were sandy loam in texture and low in clay contents (125gkg^{-1}) in the combinedfield soils respectively. Organic carbon contents of the soils were 5.4gkg^{-1} and 5.2gkg^{-1} which were low for the soils respectively. Some other workers have observed similar level of organic carbon in savanna soils, which implied low fertility status for the cultivated soil (Moberg and Esu, 1989).

The total nitrogen content of the soils is 0.1gkg^{-1}. The low level of total nitrogen in the soil could be attributed to low organic matter contents of these typical savanna soils (Jones and Wild, 1975). The available P content of the soil was moderate with values of 8.9mgkg^{-1} and 7.6mgkg^{-1} for the field soils. The exchangeable site was dominated by calcium and magnesium as characteristic of savanna soils. These cations are the most abundant in the exchange complex of savanna soils. The K saturation of field soils was 0.3%respectively. The sodium content was generally low 0.5cmolkg^{-1}and 0.4 cmolkg^{-1}as may be expected for good arable soil although Na contents were higher than K in both soils. The higher Na content in the cultivated soils relative to K must have been introduced in fertilizer materials or other amendments employed over time for crop production. The effective CEC values for the soils were 5.7 and 5.1molkg^{-1} respectively. The micronutrient values were found to be low to moderate in the soils and have been recorded to be deficient in most savanna soils (Lombin, 1985; Mulima*et al*., 2015). These soils were therefore low in natural fertility and their productivity will decline quite rapidly under continuous cultivation, which by implication requires to be fertilized in order to sustain good crop yields (Lombin, 1987).

The combined relationships between grain yield and other yield parameters were derived by simple correlation as presented in Table 2. Grain yield was significantly related with Stover yield and 1000 grain weight with r values of 0.67** and 0.62** respectively. The grain yield showed a significant but negative (P< 0.05) correlation with protein contents of the grain (r= -0.36**). There was a positive relationship between the grain yield and Stover yield, 1000grain- weight and plant height indicating that all these growth parameters increase or affects the grain yield of the maize. This is expected as a vigorous plant would invariably yield good harvest. The grain yield was negatively correlated with the protein contents of the grain which means the protein concentration in the grain decreased as grain yield increases. This is in accordance with Orit- Monasterio (2001) who reported same in his work. The protein content of the grain was positively influenced by the grain nitrogen. The lysine and tryptophan contents of the maize were not significantly affected by the grain yield which suggests that there was no particular pattern of relationship established between yield and quality. Lysine had a positive influence on the tryptophan content of the grain which means that increase in lysine content increases the tryptophan content of the maize.

Table.2: Correlation coefficient (r) between agronomic parameters and some grain parameters

	Grain yield	Stover yield	1000 grain weight	Plant Height	Total Nitrogen in grain	Crude protein	Lysine	Tryptophana
Grain yield	1.000							
Stover yield	0.669**	1.000						
1000 grain weight	0.617**	0.627**	1.000					
Plant Height	0.308	0.077	0.049	1.000				
Total Nitrogen in grain	-0.363**	0.017	0.004	0.032	1.000			
Crude protein	-0.364**	0.011	0.003	0.025	0.993**	1.000		
Lysine	0.083	0.027	-0.009	-0.025	0.022	0.021	1.000	
Tryptophan	-0.131	0.034	-0.095	-0.056	0.081	0.088	0.480**	1.000

** = Significant at 5%

* = Significant at 1%

The correlation matrix between grain/plant nutrients and soil parameters was shown in Table 3. The pH (r= 0.26*), soil N (r=0.19*), zinc (r=0.24*), and copper (r=0.31*) were positively correlated (P< 0.01) with the grain yield while the grain yield was positively and highly significantly correlated (P< 0.05) with boron (0.49**) and calcium (0.17**) contents of the soil respectively. Crude protein exhibited positive and significant correlation with

organic carbon (r= 0.24**) content, exchangeable acidity (r= 0.14*) and pH (r= 0.02*) of the soil while it was highly significant but negatively correlated with boron (r= -0.40**), copper (r= -0.45**) and sodium (r= -0.16*) contents of the soil.

The crude protein was positively and significantly correlated with exchangeable acidity (r=0.14*), soil pH (r= 0.19*) and organic carbon (r=0.24*) and negatively correlated (P< 0.05) with sodium (r= -0.16*), boron (r= -0.40**) and copper (r= -0.45**) contents of the soil. Lysine content of the grain increased as tryptophan contents and soil N increased with r values of 0.19** and 0.07* and decreased with exchangeable acidity (r= -0.16**), sodium (-r=0.16**) and copper (r= -0.17**) contents of the soil while tryptophan increased with soil N (r= 0.15*) and decreased with exchangeable acidity (r= -0.05*) and exchangeable copper (r= -0.23**).

The pH of the soil was highly and positively correlated (P< 0.05) with exchangeable acidity (r =0.30**) and available phosphorus (r =0.30**) while it was positively correlated (P< 0.01) with exchangeable sodium (r =0.17*), extractable zinc (r=0.04*) and extractable boron (r =0.16*).

It was also correlated negatively with organic carbon (r =-0.14*) and exchangeable magnesium (r =-0.15*). The exchangeable acidity was positively correlated with available phosphorus (r =0.34**) and negatively correlated with soil N (r =-0.22*), extractable copper (-0.15*) and boron (r =-0.17*). Organic carbon content of the soil was significantly (P<0.05) and positively correlated with available phosphorus (r =0.20**) and significantly correlated (P<0.01) with exchangeable potassium (r =0.19*) but negatively correlated with extractable zinc (r =-0.20**), copper (-0.14*) and boron (r =-0.17*). Soil N was positively and significantly correlated with lysine (r =0.07*) and tryptophan content (r =0.15*) of the soil with a negative correlation with exchangeable acidity (r =-0.22*) and extractable boron (r =-0.15*) content of the soil. Available phosphorus of the soil was positively correlated with exchangeable potassium (r=0.27*) and negatively correlated with exchangeable calcium (r =-0.25*). The exchangeable calcium was highly and positively correlated with exchangeable magnesium (r =0.80**). Magnesium was highly and significantly correlated with boron (0.22**) and copper (0.14**) while zinc was positively and highly significantly correlated (P< 0.05) with boron (0.29**) and copper (0.23**) as presented on Table 3.

The grain yield increases as nitrogen content of the soil increased and soil pH was favorable to support the growth and yield of the maize since the pH of the soil was moderately acidic. Micronutrients such as zinc and boron supply from the soil also increased grain production since they are constituent of protein synthesis. This is in accordance with Osiname*et al* (1973) who reported that low zinc in the soil have been found to reduce maize yield in several parts of Africa. Anonymous (2009) inferred that Zn fertilization in maize significantly improved plant height, 100 grain weight and protein content of the maize. The grain yield was negatively correlated with exchangeable calcium. The soil pH increases with exchangeable acidity, available phosphorus and boron while it was negatively correlated with organic carbon, exchangeable magnesium and extractable zinc. The availability of zinc decreases as soil pH increased which implies that at low pH (moderately acidic), there was availability of micronutrients and macronutrients such as Zn, B, Cu, Ca and N contents in the soil and this also implies that within allowable limits for conducive crop performance, increase in soil pH, soil N, Ca, Zn, B and Cu would increase grain yield. There was a negative and significant relationship between the grain yield and the protein content of the maize in that as grain yield increases the protein content of the grain decreased. This infers that the quantity of grain produced do not determine the quality of the maize. Increased crude protein, exchangeable acidity, pH and organic carbon contents of the soil and uptake in sodium, magnesium, zinc, boron and copper contents of the soil increased the content of grain N. The crude protein content of the maize increases as the organic carbon and pH contents of the soil increased with negative correlation with Na, B and Cu. This shows that increase in uptake of these nutrients from the soil will increase the crude protein contents of the maize. Lysine and tryptophan contents of the grain maize varieties are positively affected by N, Na, Cu contents of the soil and exchangeable acidity. This infers that the amino acids increase with soil N and shows that all protein fractions in the grain are reduced when N in the soil is limiting (Pixley and Bjamason, 1993).

The increase in soil pH demonstrates a strong association with phosphorus, sodium, zinc and boron contents of the soil while availability of zinc decreases as soil pH increases. Organic carbon and magnesium contents of the soil increased as soil pH decreases. This infers that pH of the soil was favorable to support the growth and yield of the maize since the pH of the soil was moderately acid while increase in acidity of the soil increase phosphorus contents of the soil. Increase in nitrogen, boron and copper contents of the soil takes place at decrease soil acidity. Phosphorus is positively and significantly correlated with potassium and negatively correlated with calcium. This indicated that increase in phosphorus increases the potassium content and decreased the calcium content of the soil. This is called calcium induced P. K interacts with P and together they can interact with other nutrients in soil.

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Table.3: Coefficient (r) between grain yield, other yield parameters and some chemical properties of the soil

	G. yld	TNg	CP	Lys	Tryp	pH	Exacidity	OC	TNsoil	AvP	K	Na	Ca	Mg	Zn	B	Cu
G. yld	1.00																
TNg	-0.36**	1.00															
CP	-0.36**	0.99**	1.00														
Lys	-0.06	0.02	0.21	1.00													
Tryp	0.11	0.08	0.09	0.19**	1.00												
Ph	0.26*	0.20*	0.19*	-0.07	-0.08	1.00											
Exacidity	-0.11	0.14*	0.14*	-.16**	-.05*	0.30**	1.00										
OC	-0.04	0.24**	0.24**	0.02	-0.02	-0.14**	-0.16	1.00									
TNsoil	0.19*	0.05	0.07	0.07*	0.15*	-0.05	-0.22*	0.12	1.00								
AvP	0.05	0.03	0.03	0.09	-0.01	0.30**	0.34**	0.20**	-0.01	1.00							
K	-0.11	-0.05	-0.04	-0.04	0.13	-0.02	0.12	0.19*	--0.05	0.27*	1.00						
Na	0.18	-0.16*	-0.16*	-0.16**	-0.08	0.17*	-0.21	0.03	0.04	0.05	0.15*	1.00					
Ca	0.17**	0.01	0.01	-0.08	-0.00	-0.19	-0.06	-0.04	0.05	-0.25*	0.14	0.05	1.00				
Mg	0.04	-0.14*	-0.15	-0.08	-0.03	-0.15*	-0.12	-0.04	0.10	-0.24	0.10	0.03	0.80**	1.00			
Zn	0.24*	-0.38**	-0.37	0.17	0.25	0.04*	-0.04	-0.20*	0.04	-0.05	0.10	0.15	0.04	0.10	1.00		
B	0.49**	-0.41**	-0.40**	-0.02	-0.10	0.16*	-0.17*	-0.17*	-0.15*	-0.09	0.03	-0.01	0.11	0.22**	0.29**	1.00	
Cu	0.31*	-0.44**	-0.45**	-0.16*	-0.23*	0.09	-0.15*	-0.14*	-0.07	-0.10	-0.03	0.10	0.03	0.14*	0.23**	0.38	1.00

**** = Significant at 5%**

*** = Significant at 1%**

KEY G. yld–Grain yield TNg—Total nitrogen in grain CP—Crude protein Lys--Lysine Tryp—Tryptophan pH—pH soil Exacidity OC—Organic Carbon TNsoilAvP—Available phosphorus Exch K Exch Na Exch.Ca Exch Mg Extrac Zn Extrac B Extrac Cu

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IV. CONCLUSION

The correlation analysis showed that all the yield parameters influenced grain yield positively and the grain yield increased as soil pH, total nitrogen, calcium, zinc, copper and boron contents of the soil increased. However, crude protein contents decreased with increase in grain yield indicating some elements of dilution of nutrients taken up as yield increased. Crude protein contents increased as totals soil N, pH, and organic carbon contents of the soil increased while lysine and tryptophan contents of the maize increased with N and K contents of the soil and was negative and significantly correlated with B and exchangeable acidity of the soil.

REFERENCES

[1] Awotundun, J. S. (2005). Comparative Effects of organic and inorganic fertilizer on the yield of pop-corn. In: Proceedings of the 29th Annual Conference of the Soil Science Society of Nigeria. December, 6th-10th, 2004. University of Agriculture Abeokuta, Nigeria:175-179.

[2] Balasubramanian, V. and Nnadi, L.A (1980). Crop residue management and soil productivity in savannah areas of Africa. FAO Soils Bulletin. Pp 106-120.

[3] FPDD (2002): Fertilizer use and management Practices for Crops in Nigeria. Pp 1-20.

[4] Jones, M. J. and Wild, A. (1975). Soils of West African Savannah Tech Comm No. 55 Commonwealth Bureau of soils Harpenden.Pp 246.

[5] Kang, B. T. and Wilson, G. E. (1987). The development of alley cropping as a promising agroforestry technology. In Agroforestry: A decade of development. Steppler, H. A. and Nail P. K. R. (eds) ICRAF, Nairobi, pp 227-243.

[6] Lombin, L. G. (1987). Fertilizer requirements of the major cereal crops in the Nigerian Savanna. *Proceedings of the National Fertilizer Seminar held at Port Harcourt*, 28-30, Oct. pp 106-110.

[7] Lombin, L. G (1985). Evaluation of the Micronutrient Fertility of Nigeria's Semi-Arid Savannah Soils: Boron and Molybdenum, *Soil Science and Plant Nutrition*, 31:1, 13-25

[8] Moberg, J.P. and Esu, I. E. (1989). Characteristic and composition of soils of Northern Nigeria. Report submitted to the IAR, ABU, Zaria.

[9] Mokwunye, A. U. (1974). Some reflections on the problems of available phosphorus in soils of tropical Alfisol.

[10] Mulima I. M., Shafiu, M., IsmailaM. and Benisheikh, K. M (2015). Status and Distribution of Some Available Micronutrients in Sudan and Sahel Savanna Agro-Ecological Zones of Yobe State, Nigeria. *Journal of Environmental Issues and Agriculture in Developing Countries, Volume 7, Num*ber 1, pp-18-29

[11] Ogunwole, J.O. (2000). The macro- environment of cereal based intercrop at Samaru, northern Nigeria. Ph.D Thesis Ahmadu Bello University, Samaru-Zaria.

[12] Onwueme, I.C and Sinha T.D. (1991). Field crop production in tropical Africa. Technical center for Agriculture and Rural Cooperation pp. 159-175.

[13] Onyinbe, J. E., Kamara, A.Y., and Omoigui, L. O. (2006). Guide to soybean production in Borno State, Nigeria: Promoting Sustainable *Agriculture* in Borno State (PROSAB). Ibadan, Nigeria. Pp 1-13.

[14] Ortiz- Monasterio, Ivan (2001). Improving human nutrition by enhancing bio-available protein and micronutrient concentrations in maize, wheat and triticale.

[15] Osiname, O. A. Schulte, E. E. and Corey R. B. (1973). Soil tests for available copper and zinc in soils of Western Nigeria. Journal of Science Food Agriculture 24: 1241-1249.

[16] Owonubi, J.J., Abdulmumin, S., Malgwi, W.B. and Mua'zu S. (1991). Review of soil water balance studies of the Sudano-Sahelian zone of Niger. In: Soil water balance in the Sudano –Sahelian zone proceeding of Niamey workshop. Feb.1991. M.V.K. Siva Kumar, J. S. Wallace C. Renord and C. Grivoux 329-388. JAHS Publ. No. 199.

[17] Pixley, K.V. and Bjarnason, M.S. (1993). Combining ability for yield and protein qualityamong modified endosperm opaque-2 tropical maize inbreds. *Crop Science* 33: 1229-1234. Africa. *Samaru Conference Paper No 3*

[18] Prasanna, R.M.; Vassal, S.K., Kassahum, B. and Sin.N. (2001). Review Article onQuality Protein Maize. *Current Science* 81:0:1308-1319.

[19] SAS (2005). Statistical Analytical system. SAS Users guide. Statistics. Version 5.*SAS Institute Cary*, NC.

[20] Attia, T., Martin, T., Forbuzie, T., Angwafo, T., & Chuo, M. (2018). Human Wildlife Conflict: Causes, Consequences and Management Strategies in Mount Cameroon National Park South West Region, Cameroon. *International Journal Of Forest, Animal And Fisheries Research*, *2*(2), 34-49. doi: 10.22161/ijfaf.2.2.1

[21] Uyovbisere, E.O. and Lombin, G. (1991). Response of early maturing maize (*Zea mays* L.) variety to potassium and zinc fertilization in the Nigerian Savannah.

[22] Vassal, S.K. (2006): The quality protein maize story. *Food and nutritional Bulletin*, 21 (4): 45-50.

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Effect of Compost Made from Decomposing Cocoa Pod and Animal Dung on the Yield of Maize Crop

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Abstract— *Ondo State has the largest production of cocoa in Nigeria, cocoa pod on most cocoa plantation in the state are usually left as an environmental nuisance on the cocoa plantation. Effect of compost made from decomposing cocoa pod and animal dung on the yield of maize crop were investigated for nine weeks. Cocoa pods were decomposed using animal dung (poultry droppings, pig and cow dung) in ratio 3:1 of cocoa pod to each of the animal dung. Cocoa pod and poultry droppings mixture has the highest bacterial, fungal and yeast population of 2.6 x10^6 – 2.9 x 10^6 (cfu/ml), 8.4 x10^5 – 9.2 x 10^5 (sfu/ml) and 4.0 x10^4 – 12.0 x 10^4 (cfu/ml). It also had the highest moisture content (88.81%) and temperature (35^{0}C) among the composting materials. Fifteen bacteria and thirteen fungi were isolated during the decomposition; common microorganisms that occur throughout decomposition were Bacillus sp, Pseudomonas sp, Escherichia coli, Staphylococus aureus, Serratia Sp, Fusarium sp, Mucor sp, Tricoderma sp, Aspergillus sp, Cladosporum sp and Neurospora sp. The pH of decomposing samples ranged from 6.10 to 7.81 at the initial stage of decomposition and pH of 7.79-9.07 at maturity of the compost. Poultry cocoa compost has the highest NPK (nitrogen, phosphorus and potassium) content of 1.345, 7.955 and 23.016 (mg/kg) respectively. Experimental setup was carried out in the field and in the screen house which the cocoa pod compost and NPK fertilizer was use to plant yellow and white maize. The compost fertilizer had a better better output than the NPK fertilizer. Height-350cm, grithy-8cm, cob-15 (big and strong) colour of maize plant (deep green leaves) while height-320cm, grith -4cm, cob-10 (small and colour of maize plant (greenish yellow leaves) for the cocoa compost and NPK fertilizer respectively were at the field Cocoa pod and poultry droppings mixture has the highest of the yellow and white maize (136cm and 126cm) respectively while the NPK sample has the lowest height of 55cm and 50 cm respectively of yellow and white maize.*

Keywords— Microorganisms, Composting, Decomposition, Cocoa pod wastes, Occurrence, screen house and NPK fertilizer.

I. INTRODUCTION

Composting occurs through the activity of microorganisms naturally found in soils. Under natural conditions, earthworms, nematodes and soil insects such as mites, sowbugs, ants, and beetles do most of the initial mechanical breakdown of organic materials into smaller particles. Under controlled conditions, composters break down large particles through grinding or chopping. Once optimal physical conditions are established, soil bacteria, fungi, actinomycetes and protozoa colonize the organic material and initiate the composting process. These mesophilic organisms function best at warm temperatures (10-45^{0}C) [1]. Poultry droppings are the feces of chickens used as an organic fertilizer, especially for soil low in nitrogen. Of all animal manures, it has the highest amount of nitrogen, phosphorus, and potassium [2].Cacao (*Theobroma cacao.L*) belongs to the genus *Theobroma* classified under the subfamily Sterculioidea of the mallow family Malvaceae [3] A cocoa pod (fruit) has a rough and leathery rind about 3 cm thick (this varies with the origin and variety of pod). It is filled with sweet, mucilaginous pulp enclosing 30 to 50 large seeds that are fairly soft and white to a pale lavender color. While seeds are usually white, they become violet or reddish brown during the drying process [4]. The animal dung added to cocoa pod is to serve as a booster which hastens the rate of decomposition of the pod waste, both serves as carbon and nitrogen sources decomposing microorganisms in the production of the compost [5]. The aims of this research therefore are determination of microbial population, microbial identification, and

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frequency of occurrence of microorganisms that decomposed the wastes. Moisture content, pH and temperature of the composing materials during decomposition are also examined.

II. METHODS

2.1 Sources and collection of materials

The organic waste used for the production of the biofertilizer was cocoa pod waste and animal dung which were poultry droppings, cow and pig dung. The cocoa pod waste was obtained from Ajipowo Farm at Idanre in Ondo State and the animal dungs were collected from an agricultural farm at Oba-Ile, Akure. The decomposition of the organic wastes was carried out at the screen house using the windrow method.

2.2 Compost preparations: The pod wastes was cut to smaller pieces of 5cm breadth and length and five samples were made of the decomposing materials formations which were mixed in 3:1 according to the research of [6]. Each sample contains 25kg of cocoa pod waste and 8.3kg of animal dung. The samples were mixed as follows; cocoa pod waste and poultry droppings, cocoa pod waste and pig dung, cocoa pod waste and cow dung and cocoa pod waste and combination of each of the animal dung. The pod waste and the animal dung of each of the samples were mixed thoroughly together and watered, turning of the compost was done every five days using shovel for nine weeks.

2.3 Microbial and physiochemical analyses

Microbial identification was determined on each organic waste to be decomposed. Microbial load, pH, temperature and moisture content were carried out at the first week of decomposition and throughout the period of decomposition (nine weeks) on a weekly basis [6]. The temperature of the decomposing materials was measured using Mercury thermometer graduated in degree centigrade. The pH of the decomposing materials was determined at the first week of decomposition and at the subsequent weeks. The pH was determined using a glass electrode pH meter. The microorganisms which were mainly bacteria, fungi and yeast were isolated using nutrient agar, marconkey agar, eosin methyl blue and potato dextrose agar. Samples were collected from decomposing materials using sterile spatula into a sterile beaker and sealed tightly prior to microbial analyses. Bacteria were isolated and identified according to [7].

2.4 Experimental sites: The experimental sites was used for these research to observe the effects of the cocoa compost samples produced White and Yellow maize grains was planted on the field and the Screen house**.** At the Field Yellow (SWAN 1) and white (DMRLSR WHITE) maize grains was planted. The treatments applied were Yellow and white maize grains planted with each of the various treatments. At the Screen house**,** Soil was taken from the field and were sterilized at 180^0C for 3hrs in an Hot air oven and when cooled, Mineral analysis of the sterilized soil was determined before using it to plant, 1kg of the sterilized soil was poured into buckets and labeled according to the treatments to be applied. Five grams of each treatment were applied into each labeled buckets and the Yellow (SWAN 1) and White (DMRLSR WHITE) maize grains were planted for four weeks.

2.5 Field management and planting: Three seeds of the Yellow and White maize grains were planted per hole to a depth of 3-4cm at 60cm intra space and 90cm inter space; each samples of the cocoa compost were incorporated in to the soil as labeled on the rows. Nitrogen, Phosphorous and Potassium (NPK) fertilizer was applied at the fourth week of planting for planting on the field and at the second week of planting in the screen house NPK fertilizer was also applied. Height of each plant on the field and the screen house were measured weekly and recorded using measuring rule.

III. RESULT/ DISCUSSION

The bacterial, fungal and yeast population of the composting materials as illustrated in Figures 1, 2 and 3 show the population of the various samples, control (cocoa pod waste only), cocoa pod and pig dung, cocoa pod and poultry droppings, cocoa pod and cow dung and cocoa pod and combination of all the animal dung. In all the samples there was a general rapid increase in the microbial population from the first week of decomposition to the third, from fourth till maturity, there was a decrease in the microbial population.

Bacterial population of the various samples (Figure 1) ranged from 2.2×10^6 to 1.5×10^6 (cfu/ml) from the Al sample to the Po sample. Fungal population (Figure 2) of ranged from 7.8×10^5 sfu/ml to 3.8×10^5 sfu/ml from the Al sample to the Po sample. Yeast population (Figure 3) ranged from 12×10^4 to 3×10^4 (cfu/ml) from the Al sample to the Po sample. Yeast growth was observed only at first three weeks, at the later weeks of decomposition there was no growth. Cocoa pod and poultry droppings mixture has the highest bacterial, fungal and yeast population of 2.6 $x10^6$ – 2.9×10^6 (cfu/ml), 8.4 $x10^5$ – 9.2×10^5 (sfu/ml) and 4.0 $x10^4$ – 12.0×10^4 (cfu/ml). Percentage frequency of occurrence of the bacteria, fungi and yeast isolated during decomposition as represented in Tables 1 and 2. Among the bacterial population, *Bacillus* sp and *Echerichia coli* has the highest percentage frequency of occurrence of 11.7% while

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Micrococcus sp and Lactobacillus sp has the lowest of 2.6% and among the fungal population *Aspergillus flavus* and *Fusarium sporotrichoides* has the highest percentage frequency of occurrence of 11.1% while *Pennicillum notatum* has the lowest of 2.5%.

Weekly moisture content of the decomposing materials during decomposition is shown in Figure 4. The moisture content of the samples was considerably high in the first four weeks of decomposition after which it decreased gradually in the later weeks. The cocoa pod and combination of all the animal dung (Al sample) had the highest moisture content of $86.06^{c}\pm0.01$ to $82.75^{d}\pm0.11$ at the first to third week of decomposition. The control (cocoa pod only) had the lowest moisture content of $74.36^{a}\pm0.02$ to $63.40^{b}\pm0.10$ from the first to ninth week of decomposition. The cocoa pod and poultry dropping sample had the lowest moisture content of $61.99^{a}\pm1.14$ at the ninth week of decomposition. The pH of the various decomposing materials (Figure 5) was relatively low at the early weeks of decomposition (week 1 to 6) and was alkali at latter weeks. The control (cocoa pod only) had highest acidic pH of 6.10-6.08 at the first and second week of decomposition. The cocoa pod and poultry dropping (Po sample) had a neutral of 7.26-7.08 at the first and second week of decomposition. At the maturity of the biofertilizer the pH of the various samples are alkali and control has the highest alkali pH of 9.07.

There was a general rise in temperature of the sample (Figure 6) in the first three weeks of decomposition and decreased gradually until maturity. Cocoa pod and poultry droppings had the highest temperature of 35°C on first weeks of decomposition to 27°C at maturity while control which is only cocoa pod waste had the lowest temperature of 29°C at the first week of decomposition to 27°C at maturity. Mineral composition of the matured biofertilizer as presented in Table 3 shows the mineral composition of each of the biortilizers samples. Poultry biofertilizer has the highest NPK (nitrogen, phosphorus and potassium) content of 1.345, 7.955 and 23.016 (mg/kg) respectively.

Result and Discussion

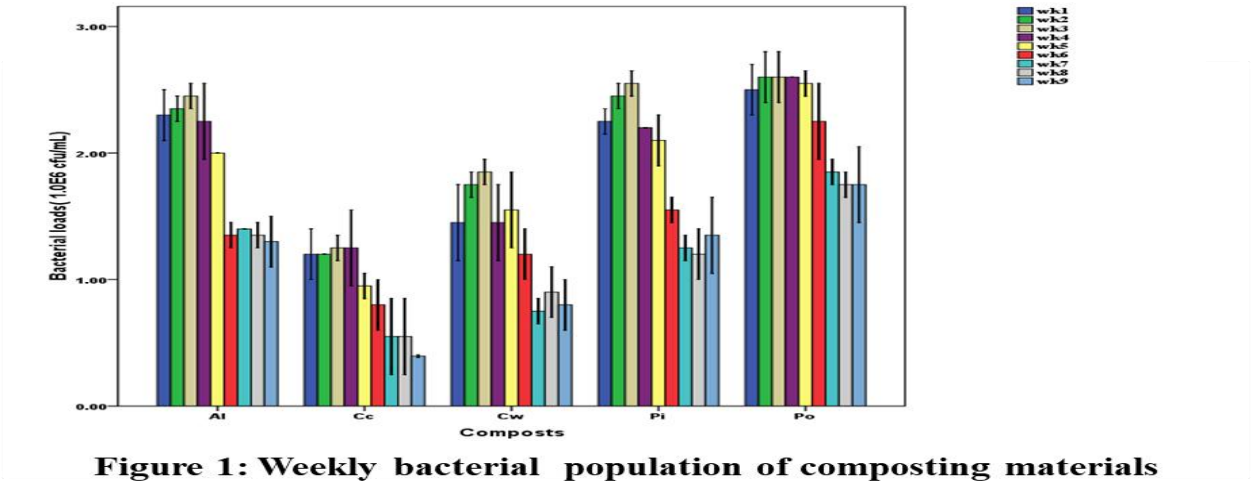

Figure 1: Weekly bacterial population of composting materials during decomposition (cfu/ml)

KEYS: Cc-Control
Pi- Cocoa pod and Pig dung
Po- Cocoa pod and Poultry droppings
Cw- Cocoa pod and Cow dung
Al-Cocoa pod, Poultry droppings, Pig and Cow dung

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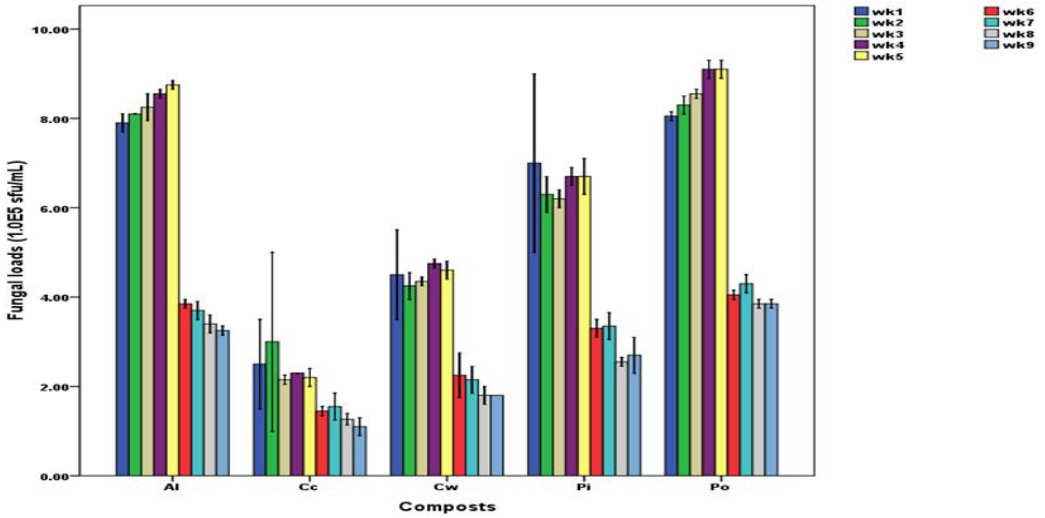

Figure 2: Weekly fungal population of composting materials during decomposition (sfu/ml)

KEYS: Cc-Control
Pi- Cocoa pod and Pig dung
Po- Cocoa pod and Poultry droppings
Cw- Cocoa pod and Cow dung
Al-Cocoa pod, Poultry droppings, Pig and Cow dung

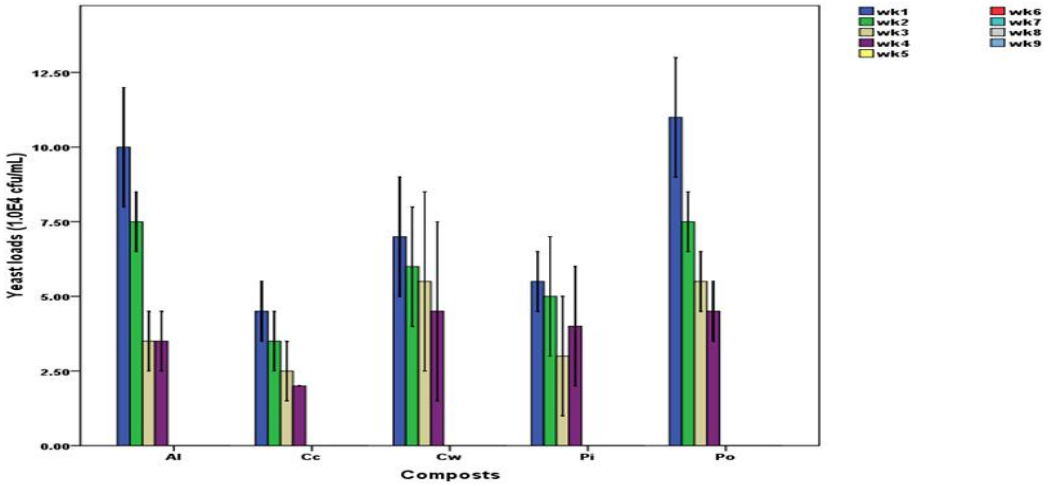

Figure 3:Weekly yeast population of composting materials during decomposition (cfu/ml)

KEYS: Cc-Control
Pi- Cocoa pod and Pig dung
Po- Cocoa pod and Poultry droppings
Cw- Cocoa pod and Cow dung
Al-Cocoa pod, Poultry droppings, Pig and Cow dung

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Table 1: percentage frequency of occurrence of bacteria during decomposition

IS	WK 1	WK2	WK3	WK4	WK5	WK6	WK7	WK 8	WK9	TN	% of O
A	+	+	+	+	+	+	+	+	+	9	11.7
B	+	+	-	-	-	-	-	-	-	2	2.6
C	-	-	+	+	+	+	+	+	+	7	9.1
D	-	-	-	+	+	+	+	+	+	6	7.8
E	+	+	-	-	-	-	-	-	-	2	2.6
F	-	-	-	-	+	+	+	+	+	5	6.5
G	+	+	+	+	+	+	+	+	+	9	11.7
H	-	+	+	+	-	-	-	-	-	3	3.9
I	-	+	+	+	+	+	+	-	-	6	7.8
J	+	+	+	-	-	-	-	-	-	3	3.9
K	+	+	+	+	-	-	-	-	-	4	5.2
L	+	+	+	+	+	+	+	-	-	7	9.1
M	-	+	+	+	-	-	-	-	-	3	3.9
N	-	+	+	+	+	+	+	+	+	8	10.4
O	+	+	+	-	-	-	-	-	-	3	3.9
										77	100

Keys:+-Present , --Not detected, TN-Total number of isolate (77), IS-Probable organisms, A- *Bacillus* sp, B- *Micrococcus* sp, C- *Pseudomonas* sp, D- *Bacillus* sp, E- *Lactobacillus* sp, F-*Proteus* sp G- *Escherichia coli*, H- *Salmonella* sp, I- *Bacillus* sp J- *Actinomyces* sp, K- *Enterobacter* sp, L- *Staphylococcus* sp, M- *Pseudomonas* sp, *N- Serratia* sp *and O- Klebisella* sp.

Table 2: percentage frequency of occurrence[1] of fungi and yeast during decomposition

IS	WK 1	WK2	WK3	WK4	WK5	WK6	WK7	WK8	WK9	TN	% of O
A	+	+	+	+	+	+	+	+	+	9	11.1
B	-	+	+	+	+	+	+	+	+	8	9.9
C	+	+	+	+	+	+	+	-	-	7	8.6
D	+	+	+	+	+	+	+	+	+	9	11.1
E	-	+	+	+	-	-	-	-	-	3	3.7
F	-	-	-	+	+	+	+	+	+	6	7.4
G	+	+	+	+	-	-	-	-	-	4	4.9
H	+	+	+	+	-	-	-	-	-	4	4.9
I	-	-	-	-	+	+	+	+	-	5	6.2
J	-	+	+	-	-	-	-	-	-	2	2.5
K	-	+	+	+	+	+	+	+	+	8	9.9
L	-	-	-	-	-	-	+	+	-	3	3.7
M	-	-	-	-	-	-	+	+	-	5	6.2
N	-	-	-	-	+	+	+	+	+	4	4.9
O	+	+	+	+	-	-	-	-	-	4	4.9
										(81)	100

Keys: +-Present , --Not detected, TN-Total number of isolate present(81), IS-Probable organisms. A- *Fusarium sporotrichoides*, B- *Mucor mucedo, C-Tricoderma viride*, D- *Aspergillus flavus*, E-*Articulospora inflata*, F- *Cladosporium* species,G- *Rhizopus nigiricans*, H-*Aspergillus niger*, I- *Aspergillus fumigatus* J- *Penicillum notatum*, K- *Neurospora crassa*, L- *Geonabotobotryum apiculatum*, M- *Penicillum italicum*, N- *Sacchromyces cerevisae, O-Schizosacchromyces pombe*

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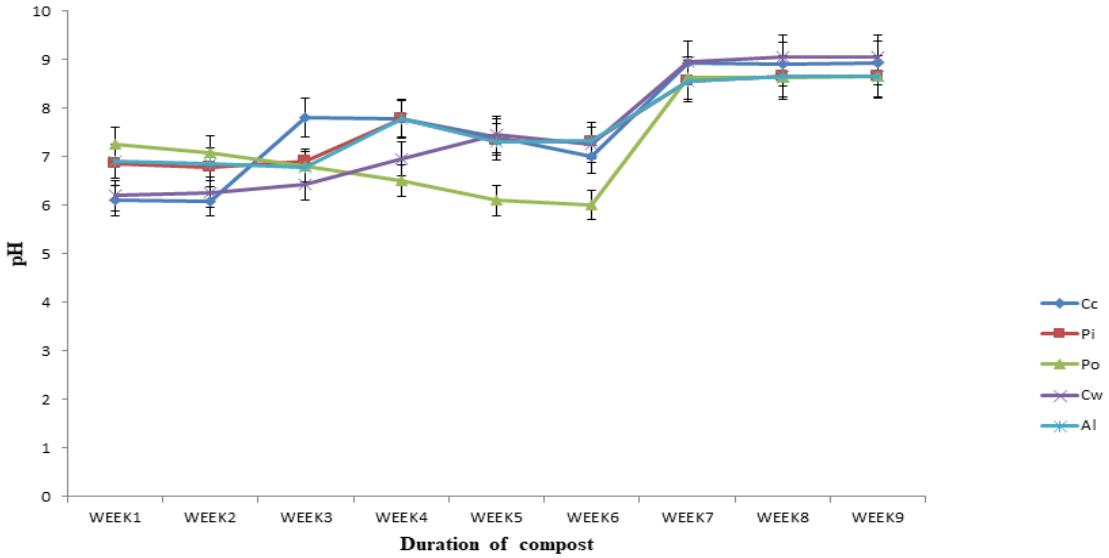

Figure 4: Weekly pH of the decomposing materials

KEYS: CC-CONTROL, PI-Cocoa pod and pig dung, PO- Cocoa pod and Poultry dung, Cw-Cocoa pod and Cow dung, ALL-Cocoa pod, Poultry droppings, Pig and Cow dung.

Table 3: Proximate composition of the matured biofertilizer

SAMPLE	MOISTURE %	FAT %	ASH %	CRUDE FIBRE %	PROTEIN %	CHO %
Cc	$56.34^{a}\pm0.04$	$5.05^{a}\pm0.01$	$5.38^{a}\pm0.06$	$7.74^{b}\pm0.09$	$6.75^{a}\pm0.03$	$13.63^{d}\pm0.05$
Pi	$62.33^{e}\pm0.10$	$6.40^{b}\pm0.52$	$6.43^{b}\pm0.12$	$8.28^{c}\pm0.03$	$7.69^{b}\pm0.06$	$10.38^{b}\pm0.05$
Po	$62.19^{d}\pm0.07$	$7.01^{b}\pm0.08$	$6.39^{b}\pm0.15$	$9.32^{a}\pm0.05$	$9.26^{e}\pm0.01$	$10.85^{c}\pm0.03$
Cw	$57.26^{b}\pm0.11$	$5.22^{a}\pm0.10$	$7.86^{c}\pm0.10$	$9.32^{d}\pm0.02$	$7.40^{c}\pm0.00$	$7.21^{a}\pm0.02$
Al	$59.67^{c}\pm0.15$	$6.33^{c}\pm0.17$	$5.50^{a}\pm0.03$	$8.50^{a}\pm0.03$	$8.24^{d}\pm0.03$	$13.63^{e}\pm0.05$

KEYS: Cc-Control, Pi- Cocoa pod and Pig dung, Po-Cocoa pod and Poultry droppings, Cw-Cocoa pod and Cow dung, Al- Cocoa pod, Poultry droppings, Pig and Cow dung.

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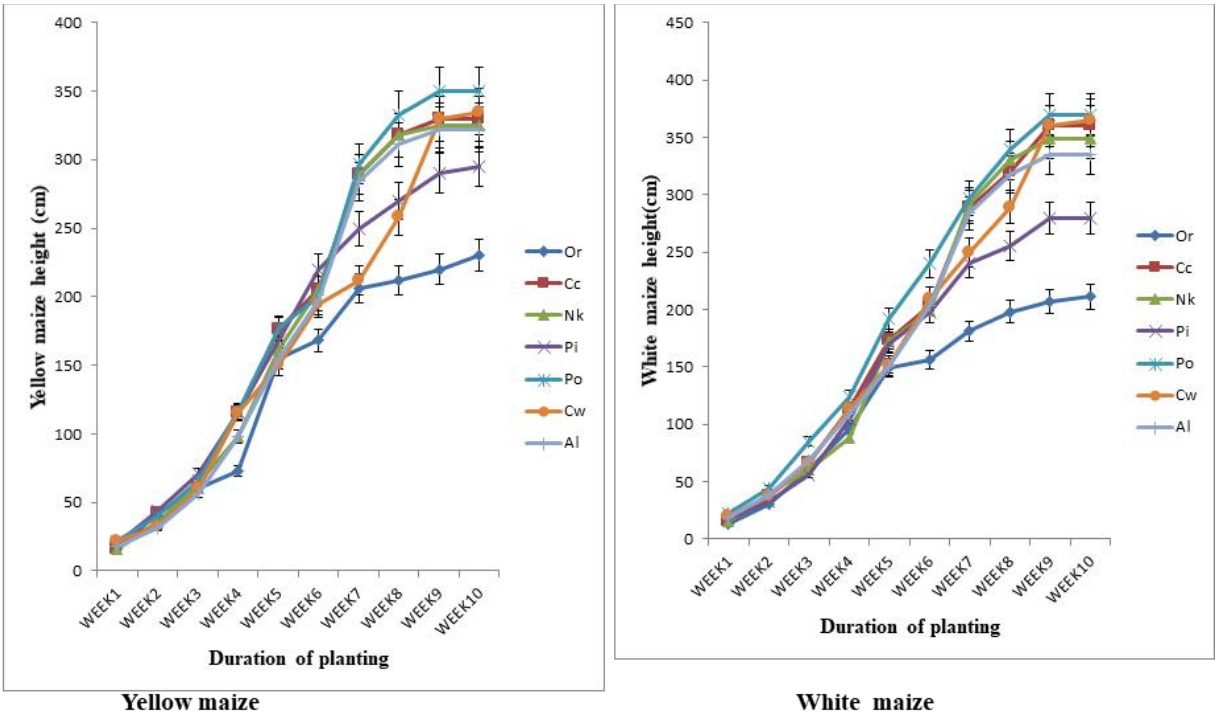
Table 4: Mineral composition of the

Treatments	Na(mg/kg)	Mg(mg/kg)	Ca (mg/kg)	K (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	P (mg/kg)	N (%)
Cc	0.836^{a}±0.01	1.507^{c}±0.00	0.327^{d}±0.00	10.526^{a}±0.04	1.430^{d}±0.01	0.705^{a}±0.51	6.143^{c}±0.02	1.030^{a}±0.01
Pi	1.002^{c}±0.00	0.927^{a}±0.00	0.322^{c}±0.00	16.294^{c}±0.00	0.660^{b}±0.01	0.105^{a}±0.01	3.670^{b}±0.01	1.203^{b}±0.01
Po	0.967^{b}±0.01	5.089^{a}±0.01	0.329^{d}±0.00	23.016^{a}±0.00	1.620^{a}±0.01	0.135^{a}±0.01	7.955^{d}±0.04	1.345^{d}±0.01
Cw	1.077^{d}±0.00	1.320^{b}±0.00	0.309^{a}±0.00	13.065^{b}±0.01	0.435^{a}±0.01	0.080^{a}±0.00	3.744^{b}±0.00	1.245^{c}±0.01
Al	1.055^{d}±0.00	2.113^{d}±0.00	0.318^{b}±0.00	16.952^{d}±0.03	1.025^{c}±0.01	Nd	3.403^{a}±0.00	1.490^{a}±0.01

KEYS: Cc-Control, Pi- Cocoa pod and Pig dung, Po-Cocoa pod and Poultry droppings, Cw-Cocoa pod and Cow dung, Al- Cocoa pod, Poultry droppings, Pig and Cow dung.



Yellow maize White maize

Figure 5: Comparison between the heights of the Maize on the field

KEYS: Or-Control, CC-Cocoa pod only, Nk-NPK treatment, PO-Cocoa pod and Poultry droppings, PI- Cocoa pod and Pig dung, Cw- Cocoa pod and Cow dung, AL- Cocoa pod and Poultry droppings, Cow and Pig dung.

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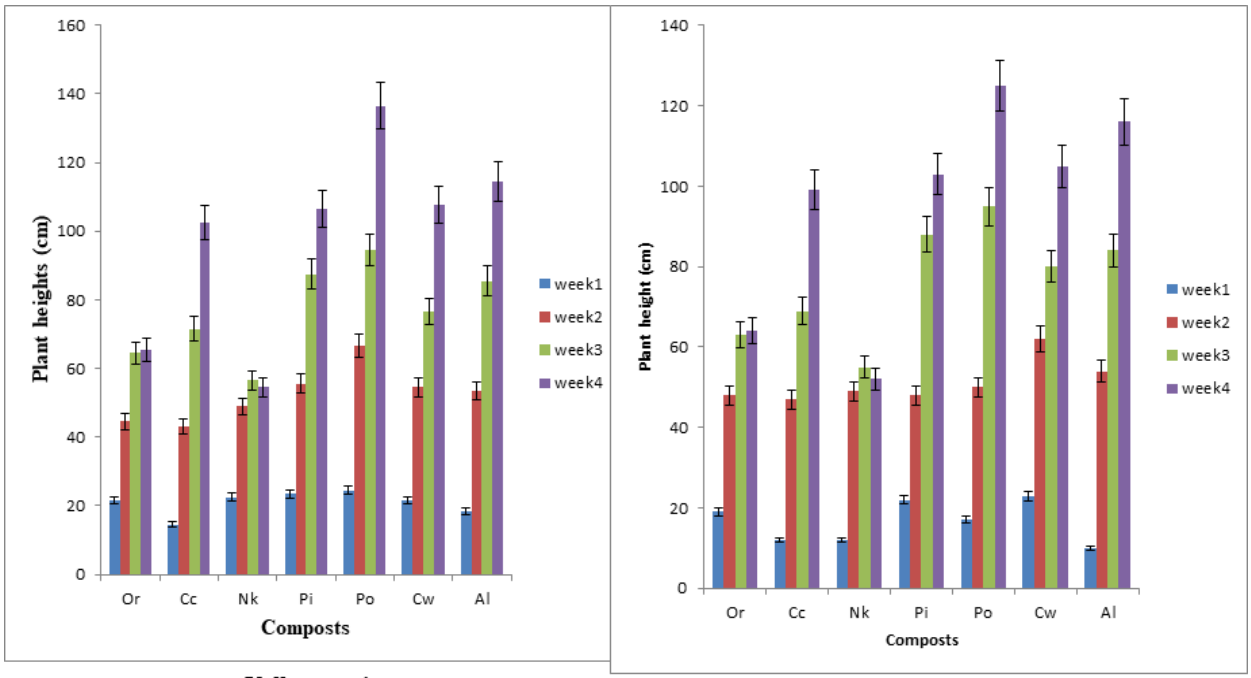
Table 5:Yeilds of Maize planted on the field at maturity

TREATMENT	YELLOW MAIZE	WHITE MAIZE
PO	300g	250g
PI	200g	170g
ALL	180g	200g
CW	200g	250g
CC	170g	160g
NPK	160g	150g
ORD	80g	90g

KEYS: ORD- Maize planted without any treatment, CC-Cocoa pod only, Nk-NPK treatment, PI-Cocoa pod and Pig dung, Po- Cocoa pod and Poultry dung, Cw-Cocoa pod and Cow dung, AL- Cocoa pod and Poultry droppings, Cow and Pig dung.



Yellow maize **white maize**

Figure 6: comparison between the heights of the Yellow and White maize in the screen house

Keys: Or-Control, CC-Cocoa pod only, Nk-Nitrogen Potassium Calcium treatment, PO-Cocoa pod and Poultry droppings, PI- Cocoa pod and Pig dung, Cw- Cocoa pod and Cow dung, AL- Cocoa pod and Poultry droppings, Cow and Pig dung.

In all the samples (Figures 1 - 3) there was general increase in the bacterial, fungal and yeast population at the early weeks of decomposition from the first week of decomposition (week 1-4) and at the later weeks there was a decrease in the microbial population. The initial increase might be due to the presence of high level of carbon and nitrogen present in the composting materials which favour the bacterial, fungal and yeast population. The decrease in the microbial population at the later weeks might be attributed to low level of nutrient present in the compost

due to the utilization of the nutrients by the microorganisms in the compost pile and the physicochemical parameters of the composing materials might not favour the increase in growth of these microorganisms at maturity (Hargerty *et al*. (1999). Among all the composing samples cocoa pod and poultry droppings sample has the highest bacterial, fungal and yeast population during decomposition, this might have resulted from high level of nutrient present in the poultry dropping which was higher than other nitrogen sources used.

The percentage frequency of occurrence (Tables 1 and 2) of the bacteria, fungi and yeast isolated during decomposition, showed the microorganisms with the highest frequency of occurrence were *Bacillus* sp *Echerichia coli*, *Aspergillus flavus* and *Fusarium sporotrichoides* of 11.7%, 11.7%, 11.1% and 11.1% occurrence respectively while *Micrococcus* sp, *Lactobacillus* sp and *Penicillium notatum* has the lowest percentage frequency of occurrence of 2.6%, 2.6% and 2.5% respectively. This probably could have been aided by the ability of these microbes to be able survive mesophilic range of temperature, the near fairly neutral to alkaline pH of the compost system and the moderately high moisture content of the compost system this agrees with Blanc *et al*., (1997). The pH of the various samples (Figure 4) was neutral at the first week to the sixth week of decomposition and at the later weeks was alkali. At the early stage of the decomposing materials the release of organic acid may temporarily lower the pH (acidity) and production of ammonia from nitrogenous compounds may raise the pH (alkalinity) (Haug and Roger, 1994). There was a general rise in temperature of the samples of composing materials (Figure 6) in the first three weeks of decomposition and decrease gradually until maturity. The mineral composition of the various samples of the matured cocoa compost (Tables 4) showed a high level of nitrogen, phosphorus and potassium content. Poultry cocoa compost has the highest NPK (nitrogen, phosphorus and potassium) content.

IV. CONCLUSION

Production of high quality humic substances from cocoa pod using various animal wastes as a booster was enhanced by activities of microorganisms, the physiochemical condition and chemical composition of the organic materials. Proper moisture, correct carbon to nitrogen ratio, frequent aeration ensures early maturity of the coca compsot. The organic materials used in the cocoa compost production process were converted to rich humic substances; hence an excellent waste recycling process and most importantly will help to substitute the use of chemical fertilizer which has altered most agricultural farmlands. The poultry droppings and cocoa pod has the best output when used for planting than the other animal dungs.

REFERENCES

[1] Lopez, R. J. and Baptista, M. (1996). A *preliminary comparative study of three manure composting systems and their influence on process parameters and methane emissions. Compost Science and Utilization* Vol. 4, No. 3: 71-82.

[2] Patterson, P.H., E.S. Lorenz, and W.D. Weaver, Jr. 1998. Litter Production and Nutrients from Commercial Broiler Chickens. Journal of Applied Poultry Research, 7:247-252.

[3] Juan, C. Motamayor, P. L., Jay, W, Rey, L., David, N., Kuhn, J. Steven, B. and Raymond, J. (2008). "Geographic and Genetic Population Differentiation of the Amazonian ". *PLoS ONE* 3 (10).

[4] Terry G. Powis, W., Jeffrey, H., María, C. R., Ponciano, O. C., Michael, B., David C., Michael D. C. and John G. H. (December 2007). "Oldest chocolate in the New World". *Antiquity* 81 (314). ISSN 0003-598X. Retrieved 2011-02-15.

[5] Lopez, R. J. and Baptista, M. (1996). A *preliminary comparative study of three manure composting systems and their influence on process parameters and methane emissions. Compost Science and Utilization* Vol. 4, No. 3: 71-82

[6] Adegunloye, D. V., Adetuyi, F. C., Akinyosoye F. A., and Doyeni, M.O. (2007). Microbial Analysis of compost Using Cowdung as Booster. *Pakistan Journal of Nutrition*. 6(5): 506-510.

[7] O.B., B., K.J, O., M.O, A., & Y.A., B. (2017). Impact of Rotten Cocoa POD on Soil Microorganisms from Ikeji-Arakeji Metropolis, Osun State, Nigeria. *International Journal Of Horticulture, Agriculture And Food Science*, *1*(4), 24-28. doi: 10.22161/ijhaf.1.4.4

[8] Holt, J. G., Krieg, N. R., Sneath, P. H., Stanley, J .J. and Williams, S. T. (1994). *Bergeys manual of determination bacteriology*. Wilkins Publishers, Baltimore, 5th Edition. Pg 34-95.

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Evaluation of the Effectiveness of Fungi (*Candida Tropicalis* and *Aspergillus Clavatus*) in Bioremediation of used Engine Oil Contaminated Soil using Bioaugmentation Technique

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***Abstract*—** *Used engine oil is a petroleum or synthetic oil that has been used and as a result of such use, is contaminated by physical and chemical pollutants. These pollutants are harmful to humans, animals and plants following exposure. Evaluation of the effectiveness of fungi in bioremediation of used engine oil (UEO) contaminated soil was investigated. Fungi were isolated from soil samples obtained from automobile workshops in Mgbuka-Nkpor, Nigeria. The isolates were screened for UEO biodegradation potentials in mineral salt broth. They were identified using the cultural and microscopic characteristics and confirmed using the 18SrRNA gene sequence. The effectiveness of the isolates in bioremediation of UEO contaminated soil was also investigated using bioaugmentation technique. A total of 8 fungal isolates were obtained from this study. Two that showed the highest extent of biodegradation of UEO in the screen flasks were identified and confirmed as Candida tropicalis and Aspergillus clavatus. At the end of the experimental period, oil contaminated soil inoculated with the mixed culture of the isolates (C. tropicalis and A. clavatus) showed the highest reduction in concentration of UEO (95.42%). Higher biodegradation rate and shorter half-life of total petroleum hydrocarbon (TPH) was observed in soil microcosm containing the isolates, when compared to the uninoculated control. Therefore fungi such as C. tropicalis and A. clavatus isolated from automobile workshops can facilitate the bioremediation of UEO contaminated soil.*

Keywords*— *Aspergillus clavatus, bioremediation, bioaugmentation, Candida tropicalis, used engine oil.

I. INTRODUCTION

The petroleum hydrocarbons are hazardous to various forms of terrestrial and aquatic life like fish, bird and human, and are also carcinogenic, mutagenic and potentially immunotoxigenic [1]. Indiscriminate disposal of used engine oil into gutters, water drains, open vacant plots and farmlands, has led to contamination of soil with hydrocarbons, resulting in serious hazardous effects to human health, animals and plants, as well as pollution of groundwater, which has limited its use. The traditional physical and chemical treatment approaches to clean up the petroleum hydrocarbons are expensive and appear ineffectual as they do not lead to complete mineralization, and awfully can produce toxic byproducts or residues. In contrast, as an innovative and eco-friendly strategy, bioremediation involving microbial agents, such as protozoa, bacteria, fungi, plants offers successful alternatives to clean-up the petroleum pollution [2]. Biological methods can have the edge over these treatments in removing oil spills. Bioremediation technology is a safe, economical, more efficient, reliable method that is harmless and ecofriendly [3, 4].

Many microorganisms such as bacteria, fungi, and yeast use their enzymatic activity to utilize hydrocarbons as a sole carbon source [4, 5, 6]. Among fungal bioremediating agents, mold species of *Aspergillus, Penicillium, Fusarium, Amorphoteca, Paecilomyces,* and *Talaromyces,* and yeast species of *Candida, Yarrowia,* and *Pichia* have been recognized in hydrocarbon degradation and its derivatives [7, 8]. Fungi have advantages over other microorganisms in that they produce classes of enzymes that can interact with several types of polycyclic aromatic hydrocarbons with a

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fairly high degree of non-specific activity. Fungi are also tolerant to high concentrations of recalcitrant compounds and are able to flourish in extreme conditions. Some recent studies have reported the use of a mixed population of fungal strains that could enhance biodegradation efficiency, especially on high concentrations of oil [6, 9].

Bioaugmentation is an approach that involves introduction of microorganisms that possessed biodegradation potentials into the contaminated environment to assist the indigenous microbes with biodegradative processes [10]. Bioaugmentation has several advantages over other techniques [11]. When a specific microbial population is injected, the degradation process can start immediately, while biostimulation, for instance, involves a delay after injection of nutrients as the microbial population propagates and also nutrient are not specific, so that all microbes will potentially propagate, diluting the effect of the nutrients [11]. The present study aims to evaluate the effectiveness of fungi *C. tropicalis* and *A.* clavatus in bioremediation of used engine oil contaminated soil using bioaugmentation technique.

II. MATERIALS AND METHODS

2.1 Collection of Samples

Soil samples were collected randomly using a pre-cleaned hand scoop at a depth of 0 – 3cm from 3 automobile workshops at Old Motor Spare Parts popularly called Mgbuka-Nkpor (6°9'N 6°50'E), Nigeria. Uncontaminated soil samples were also collected randomly from a fallow plot of land about 100m from contaminated sites, and placed in a sterile container. Used engine oil (UEO) used in this study was collected direct from the engine of 911 Lorry [12, 13] at Mgbuka-Nkpor. Samples were transported in cold storage container to the Microbiology Laboratory of the National Agency for Food and Drug Administration and Control (NAFDAC), Agulu, Nigeria, for analysis.

2.2 Isolation of fungi from used engine oil contaminated soil

The mold *Aspergillus* sp and yeast *Candida* sp were isolated from the soil samples obtained from automobile workshops (at Mgbuka-Nkpor, Nigeria) on mineral salt medium of Zajic and Supplission [14], with composition (g/L); K_2HPO_4, 1.8; KH_2PO_4, 1.2; NH_4Cl, 4.0; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $FeSO_4.7H_2O$, 0.01 and agar, 15 g. Fifty micrograms per millilitre (50µg mL^{-1}) of each of penicillin G and streptomycin was incorporated into the medium to inhibit interfering bacteria. The medium pH was adjusted to 5.5. The whole preparation was autoclaved, distributed into sterile petri dishes and allowed to solidify. One gramme of the homogenized soil sample was measured into 9 ml of sterile distilled water in a test tube and swirled gently. 1ml of the sample was pipetted and serially diluted up to 10^{-3} dilution. 0.1ml of the sample from the 10^{-2} and 10^{-3} dilutions were transferred onto the surface of a freshly prepared mineral salt agar using the spread plate technique [15]. A Whatman No. 1 filter paper saturated with sterilized used engine oil was placed inside the lid of the plates. The plates were incubated at 28°C for 7 days. Each distinct colony on oil degrading enumeration plates were purified by repeated sub culturing onto the surface of a freshly prepared Sabouraud Dextrose Agar (SDA) (Merck, Germany) plates to obtain pure cultures of the isolates. The pure cultures were maintained on SDA slants.

2.3 Screening test for UEO biodegradation potentials of the fungal isolates

The isolates were screened for used engine oil biodegradation potentials on mineral salt broth using the method of Olajide and Ogbeifun [16], with determination of pH and total viable count at time intervals as biodegradation indices. The residual hydrocarbon was also determined at time interval using the Spectrophotometric method [17].

2.4 Identification of the isolates

The cultural characteristics of the pure isolates on SDA were noted, and the microscopic features were observed using the wet mount and the microslide culture technique with reference to the Manual of Fungal Atlas [18, 19]. The isolates were also confirmed using 18S rRNA gene sequence.

2.5 Evaluation of the effectiveness of the isolates in bioremediation of UEO contaminated soil.

To evaluate the effectiveness of the isolates in bioremediation of UEO contaminated soil, soil microcosms were prepared in 250 ml Erlenmeyer flask using mineral salt broth. One hundred grammes (100g) of the uncontaminated soil samples were added into 60ml of mineral salt broth in 250 ml Erlenmeyer flask. The slurries obtained were spiked with 5g of sterile UEO simulating a soil contamination corresponding to 50,000 mg kg^{-1} soil.

Bioaugmentation was carried out by inoculating 10 ml of standard inocula (OD = 1.0) of each of the pure and mixed culture of the isolates (*C. tropicalis* and *A. clavatus*) into the flasks. Treatment with only soil and UEO served as control 1. Additional control 2 was also set up which contained autoclaved soil and sterile UEO to monitor abiotic loss of oil in the oil-contaminated soil. The flasks were

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incubated in triplicate in an Orbital Shaker at 120 rpm and 28°C for 56 days. The total petroleum hydrocarbon (TPH) content of the flasks was determined at 0, 14, 28, 42 and 56 days, using Spectrophotometric method [17]. TPH data were fitted to the first-order kinetic model [20]:

$C = Coe^{-kt}$, where C is the hydrocarbon content in soil (mg kg^{-1}) at time t, Co is the initial hydrocarbon content in soil (mg kg^{-1}), *k* is the biodegradation rate constant (day^{-1}), and *t* is time (day). The model estimated the biodegradation rate and half-life of hydrocarbons in soil relative to treatments applied. Half-life was then calculated from the model of Yeung *et al*. [21]. Half-life = ln (2)/k, where ln (2) is the natural logarithm of 2 (approximately 0.693).

The effectiveness of the isolates in remediation of used engine oil contaminated soil was also determined by calculating the net percentage loss of TPH in the oil contaminated soil, using the formula: Net % loss = % loss of TPH in soil microcosm inoculated with the isolates - % loss of TPH in uninoculated soil microcosm (ie. control 1).

2.6 Statistical Analysis

Data were analysed and presented as mean ± standard deviation (SD) of three replicates. The Student's t-test was used to test the significance of difference between the mean values. A statistical package for social sciences (SPSS) software was used for statistical analysis in this study and test for significance between means was implied at P = 0.05 level.

III. RESULTS

3.1 Isolation and screening of UEO degrading fungi

A total of eight hydrocarbon utilizing fungi (labeled A-H) were isolated from soil samples obtained from used engine oil contaminated soil. The relationship between pH, TVC and oil loss were shown in Figures 1 to 3. Generally, a decreasing trend in pH was observed in the experimental flasks within the incubation period, with a concomitant decrease in hydrocarbon levels, as growth (TVC) increases. However, the decreasing trend in pH was more evident in the experimental flasks containing isolate A and D (Fig. 1). Moreover, there was an increase in total viable count (TVC) from 0 to 12 days, with a slight decrease on the 16th day (Fig. 2), in all the experimental flasks. The hydrocarbon losses were higher (> 70 %) in the flasks containing isolate A and D while the hydrocarbon losses in the flasks containing isolates B, C, E, F, G and H, were lower (< 70 %) within the experimental period (Fig. 3). In the control flasks, no growth was observed within the experimental period, with no significant oil loss and pH change (Figs. 1 to 3). Based on these observations, isolates A and D were selected for further studies.

3.2 Identification of the isolates

Based on their cultural and microscopic characteristics, isolate A was identified as yeast belonging to the genus *Candida* sp while isolate D was identified as mold belonging to the genus *Aspergillus* sp. To confirm the results of the cultural and microscopic identification of the isolates, the 18S rRNA gene sequence of the isolates were determined. Database comparism using BLAST program revealed that the yeast isolate had a high similarity of 98 % with those of *Candida tropicalis*. However, the mold isolate had 100% similarity with those of *Aspergillus clavatus*. The expected values (E-value) for the isolates are zero.

3.3 Evaluation of the effectiveness of the isolates in bioremediation of UEO contaminated soil.

3.3.1 Biodegradation of used engine oil contaminated soil

The level of biodegradation of used engine oil throughout the experimental period is shown in Figure 4. There was a rapid reduction in the TPH within the first 14 days of the study in the soil microcosm inoculated with the pure and mixed culture of the isolates compared to the uninoculated control. At the end of the experimental period (56 days), oil contaminated soil inoculated with the mixed culture of the isolates (*C. tropicalis* and *A. clavatus*) showed the highest reduction in concentration of used engine oil (95.42 %). This was followed closely by 90.63 and 90.42 % reduction in soil microcosm inoculated with the pure cultures of *A. clavatus* and *C. tropicalis*, respectively. Uninoculated control 1 showed 54.17 % reduction at the end of the experimental period. Moreover, there was 4.0 % abiotic loss in the autoclaved soil containing sterile used engine oil (i.e., control 2) at the end of the experimental period.

3.3.2 The Net Percentage (%) Loss of TPH in Soil during Bioremediation

As shown in Table 1, the highest net percentage loss of TPH was observed at 14 day in the soil microcosm containing the pure and mixed culture of the isolates. However, it was observed that the net percentage loss of TPH in soil inoculated with both the pure and mixed culture of the isolates decreased from 14 day throughout the experimental period. Moreover, the net percentage loss in soil inoculated with the mixed culture of the isolates was higher throughout the experimental period (from 14 to 56 days), when compared to the single cultures.

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3.3.3 Biodegradation Rate Constant and Half-Life

Table 2 shows the biodegradation rate constant (k) and half-life ($t_{1/2}$) for the different soil microcosms within the experimental period. Soil inoculated with the mixed culture of the isolates showed the highest biodegradation rate (day^{-1}) and lowest half-life (days), while the uninoculated soil (control) showed the lowest biodegradation rate (day^{-1}) and highest half-life (days).

IV. DISCUSSION

The species of some of the isolates used in this study have earlier been reported as hydrocarbon degraders [22]. George-Okafor *et al*. [8] reported the isolation of *Aspergillus* spp., *Syncephalastrum* spp., *Trichoderma* spp., *Neurospora sitophila*, *Rhizopus arrhizus*, *Mucor* spp. and yeast species of *Candida, Yarrowia*, and *Pichia* from petroleum contaminated soil.

The decreasing trend in pH observed during the screening test in this study could be as a result of mineralization of hydrocarbons in used engine oil by the isolates. This was in agreement with the findings of Olajide and Ogbeifun [16], who reported a decreasing trend in pH during hydrocarbon degradation by *Proteus vulgaris*. Sepahi *et al*. [23] reported that microbial degradation of hydrocarbons often leads to production of organic acids, thus the organic acids probably caused the reduction in pH.

The increase in the growth (TVC) of the isolates in the media containing used engine oil could be attributed to the ability of the organisms to utilize UEO as a sole source of carbon. It could also be that the cultural condition was adequate for the growth of the organisms. This was in agreement with the findings of Vanishree *et al*. [24] who reported that several fungal isolates such as *Fusarium solani*, *Fusarium oxysporium*, *Trichoderma viride* and *Aspergillus niger* cultured in mineral salt medium (MSM) at pH 5.5 showed good growth. The decrease in the growth of the isolates on the 16^{th} day could be due to the decrease in pH level. It could be that the pH became too acidic for the organisms to thrive. It could also be as a result of decrease in substrate (UEO) used by the organisms as a sole source of carbon and energy. The findings of Obire and Nwaubeta [25] who reported an initial gradual increase in the bacterial population following the application of petroleum hydrocarbon but a decline as the biodegradation progressed supports this explanation. Similarly, Akpoveta *et al*. [17] reports that hydrocarbon degrading fungi increased within the first seven days from 2.16×10^4 cfu/g to 11.1×10^4 cfu/g and decreased progressively to 1.5×10^4 cfu/g within the next four weeks. A decrease in substrate will therefore result in a drop in the population of oil-degraders.

The higher net percentage (%) loss of TPH in UEO observed in the soil microcosm inoculated with the mixed culture suggested that the isolates could co-exist with no adverse effect and possibly have a synergy, which may be responsible for the higher net % loss of oil observed in this study. The advantages of employing mixed cultures have been reported [26, 27]. Ghazali *et al*. [28] reported that some species are able to remove the toxic metabolites that prohibit the activities of the other species. Then it is possible that the other species degrade complex compounds totally.

High biodegradation rate and low half-life observed in soil microcosm inoculated with the mixed culture of the isolates could be attributed to the high net loss (%) of TPH throughout the experimental period. However, the higher biodegradation rate and lower half-life observed in soil microcosm inoculated with both the single and mixed culture of the isolates compared to the uninoculated control could be due to the previous exposure of the isolates to soil contaminated with UEO. It was speculated that native strains of oil contaminated soil already shaped by selective pressure, could take advantage with respect to sensitive strains in accomplishing biodegradation of hydrocarbon polluted environment. This way, they could help to overcome an important limitation in bioremediation applications such as the toxic effect of petroleum hydrocarbons, which inhibit biodegradation process [29].

V. CONCLUSION

Bioaugmentation with the fungal isolates enhanced the remediation of used engine oil contaminated soil as reflected in the biodegradation constant and half life of the total petroleum hydrocarbon observed in the soil microcosm containing the isolates, when compared to the uninoculated soil microcosm. This bioaugmentation strategy may contribute to overcome a critical bottleneck of the bioremediation technology. Finally, fungi such as *C. tropicalis* and *A. clavatus*, isolated from automobile workshops can efficiently facilitate the bioremediation of used engine oil contaminated soil.

REFERENCES

[1] Dhar K., Dutta S. and Anwar M.N. (2014). Biodegradation of petroleum hydrocarbon by indigenous fungi isolated from Ship breaking yards of Bangladesh. *International Research Journal of Biological Sciences*, 3(9): 22-30. www.isca.in, www.isca.me.

[2] Bento F.M., Camargo F.A.O., Okeke B.C. and Frankenberger W.T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation, *Bioresource Technology*, 96, 1049-1055.

[3] Prakash B. and Irfan M. (2011). *Pseudomonas aeruginosa* is present in crude oil contaminated sites of Barmer Region, India. *Journal of Bioremediation & Biodegradation*, 2(5): 129. doi:10.4172/2155-6199.1000129.2.

[4] Hidayat A. and Tachibana S. (2012). Biodegradation of aliphatic hydrocarbon in three types of crude oil by *Fusarium* sp. F092 under stress with artifi cial sea water. Journal of Environmental Science and Technology, 5(1): 64.

[5] Khaled M. G, Saleh M.A. and Majid A.A. (2016). Bioremediation of diesel fuel by fungal consortium using statistical experimental designs. *Pollution Journal of Environmental studies*, 25(1): 97-106. DOI: 15244/pjoes/42493.

[6] Martin Y.B., Acosta S., Sanchez A., Toledo A., Gonzalez F. and Garcia R.M. (2012). Study and isolation of aerobic hydrocarbon-degrading bacteria from Cuban shorelines. *Biotechnologia Aplicada*. 29, 80.

[7] Chaillan, F., le Fleche, A., Bury, E. Phantavong, Y.H., Grimont, P., Saliot, A. and Oudot, J. (2004). Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. *Research in Microbiology*, 155: 587-595.

[8] George-Okafor, U., Tasie, F. and Muoteo-Okafor, F. (2009). Hydrocarbon degrading potentials of indigenous fungal isolates from petroleum contaminated soils. *Journal of Physical and Natural Sciences*, 3 (1): 1-6.

[9] Malik Z.A. and Ahmed S. (2012). Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium. *African Journal of Biotechnology*, 11(3): 650.

[10] Abioye, O.P. (2011). Biological remediation of hydrocarbon and heavy metals contaminated soil, *Soil Contamination*, 7:127-142.

[11] Weston, A. and Balba, T. (2003). Bioaugmentation. *Innovative Technology Group*, 3(4): 1-2.

[12] Mbachu AE, Chukwura EI and Mbachu NA. (2016). Isolation and characterization of hydrocarbon degrading fungi from used (spent) engine oil polluted soil and their use for polycyclic aromatic hydrocarbons (PAHs) degradation. *Universal Journal of Microbiology Research*,4(1): 31-37. DOI:10.13189/ujmr.2016.040105 http://www.hrpub.org/

[13] Mbachu AE, Chukwura EI and Mbachu NA. (2017). Waste engine oil degrading potentials of indigenous fungi isolated from auto-mechanic workshops: impacts of heavy metals (Zn and Pb) co-contamination and pH. *American Journal of Life Science Researches*, 5(1): 6-7.

[14] Zajic, E. and Supplission, B. (1972). Emulsification and degradation of "Banker C" fuel oil by microorganisms. *Biotechnology and Bioengineering*, 14, 331–343.

[15] APHA (1998). Standard methods for examination of water and wastewater, 20th Edn. Washington, D.C. American Public Health Association.

[16] Olajide, P.O. and Ogbeifun, L.B. (2010). Hydrocarbon biodegrading potentials of a *Proteus vulgaris* strain isolated from Fish samples. *American Journal of Applied Sciences*, 7 (7): 922-928.

[17] Akpoveta, O.V., Egharevba, F., Medjor, O.W., Osaro, K.I. and Enyemike, E.D. (2011). Microbial degradation and its kinetics on crude oil polluted soil. *Research Journal of Chemical Sciences*, 1(16): 8-14.

[18] Barnett, H.L. and Hunter, B.B. (2000). Illustrated genera of imperfect fungi. 4th edn. Laskin, A.I. and Lechevalier, H.A. (eds). CRC Press, West Palm Beach, Florida, pp 1- 197.

[19] Watanabe, T. (2002). Morphologies of cultured fungi and key to species. *In*: Pictorial atlas of soil and seed Fungi. 2nd edn. Haddad, S., Dery, E. Norwitz, B.E. and Lewis, R (eds). CRC Press LLC, 2000 N.W. Corporate Blvd., Boca Raton, Florida 33431. Pp 1- 486.

[20] Abioye, O. P., Agamuthu, P. and Abdul Aziz, A.R. (2012). Biodegradation of used motor oil in soil using organic wastes amendments. *Biotechnology Research International*, 2012: 1 – 8.

[21] Yeung, P. Y., Johnson, R. L. and Xu, J. G. (1997). Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration. *Journal of Environmental Quality*, 26, 1511–1576.

[22] April, T.M., Foght, J.M. and Currah, R.S. (2000). Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in Northern and Western Canada. *Candian Journal of Microbiology*, 46(1): 38-49.

[23] Sepahi, A.A., Dejban, G.I., Emami, M. and Nakhoda, A.M. (2008). Isolation and characterization of crude oil degrading *Bacillus* spp. *Iranian Journal of Environmental Health Science and Engineering*, 5: 149-154.

http://dx.doi.org/10.22161/ijeab/3.4.4 *ISSN: 2456-1878*

[24] Vanishree, M., Thatheyus, A.J. and Ramya, D. (2014). Biodegradation of petrol using the Fungus *Penicillium* sp. *Science International*, 2 (1): 26-31.

[25] Obire, O. and Nwaubeta, O. (2002). Effects of refined petroleum hydrocarbon on soil physicochemical and bacteriological characteristics. *Journal of Applied Sciences and Environmental Management*, 6 (1): 39-44.

[26] Akoachere,J.T.K., Ahenji, T.N., Yongabi, F.N., Nkwelong, G. and Ndip, R.N. (2008). Lubricating oil-degrading bacteria in soils from filling stations and auto-mechanic workshops in Bueau, Cameroon: occurrence and characteristics of isolates. *African Journal of Biotechnology*, 7(11): 1700-1706.

[27] Larik, I.A., Qazi, M.A., Kanhar, A.R., Mangi, S., Ahmed, S., Jamali, M.R. and Kanhar, N.A. (2016). Biodegradation of petrochemical hydrocarbons using an efficient bacterial consortium: A2457. *Arabian Journal of Science and Engineering*, 41: 2077-2086.

[28] Ghazali, F.M., Rahman, R.N.Z.A., Salleh, A.B. and Basri, M. (2004). Biodegradation of hydrocarbons in soil by microbial consortium. *Internati onal Biodeterioration and Biodegradation*, 54: 61-67.

[29] Alisi, C., Musella, R., Tasso, F., Ubaldi, C., Manzo, S., Cremisini, C. and Sprocati, A.R. (2009). Bioremediation of diesel oil in a co-contaminated soil by bioaugmentation with a microbial formula tailored with native strains selected for heavy metals resistance. *Science of the Total Environment*, 407: 3024-3032.

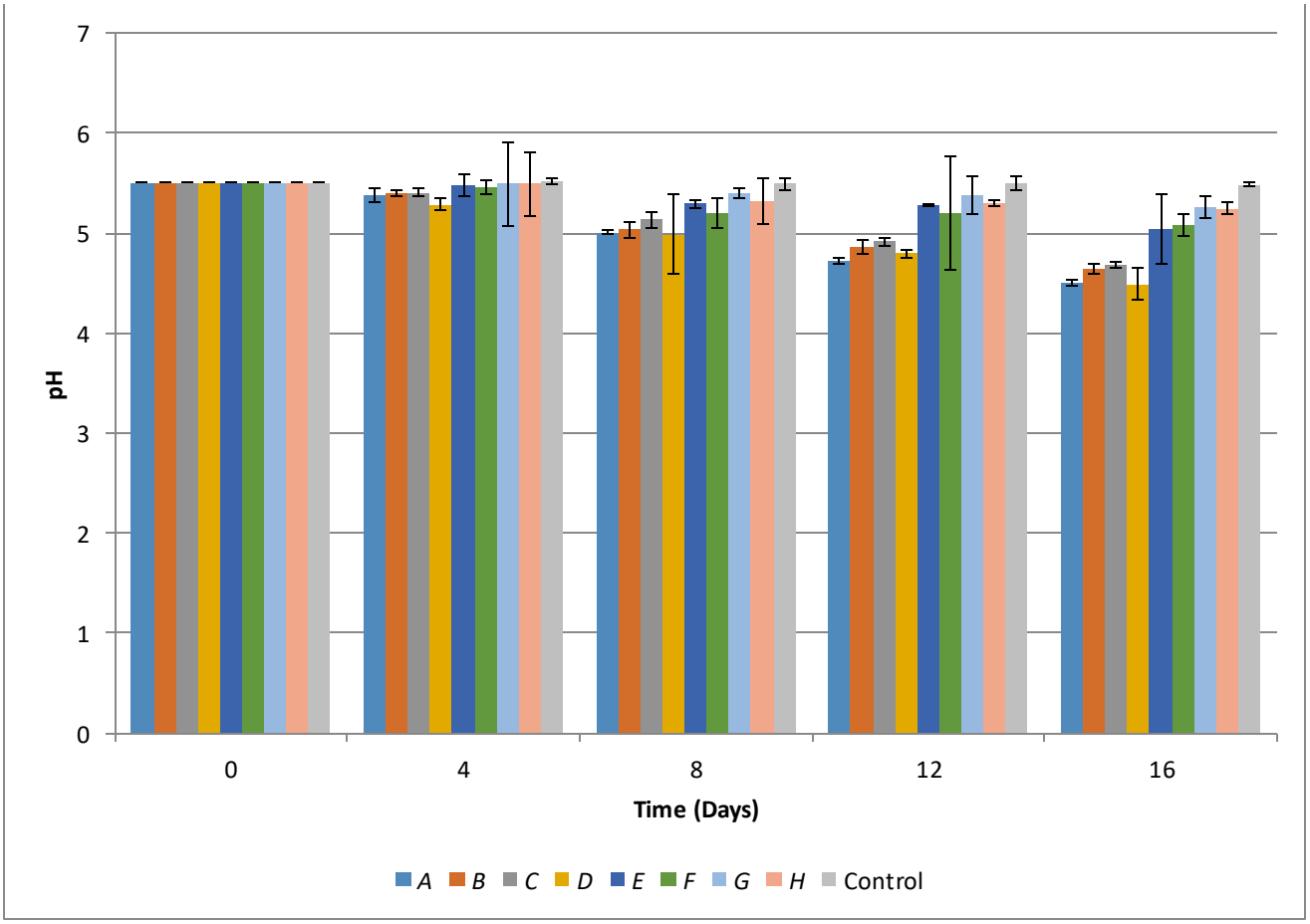

Fig..1: Changes in pH with time during utilization of UEO by the isolates. Bars indicate the average of triplicate samples while the error bars shows standard deviation (±SD).

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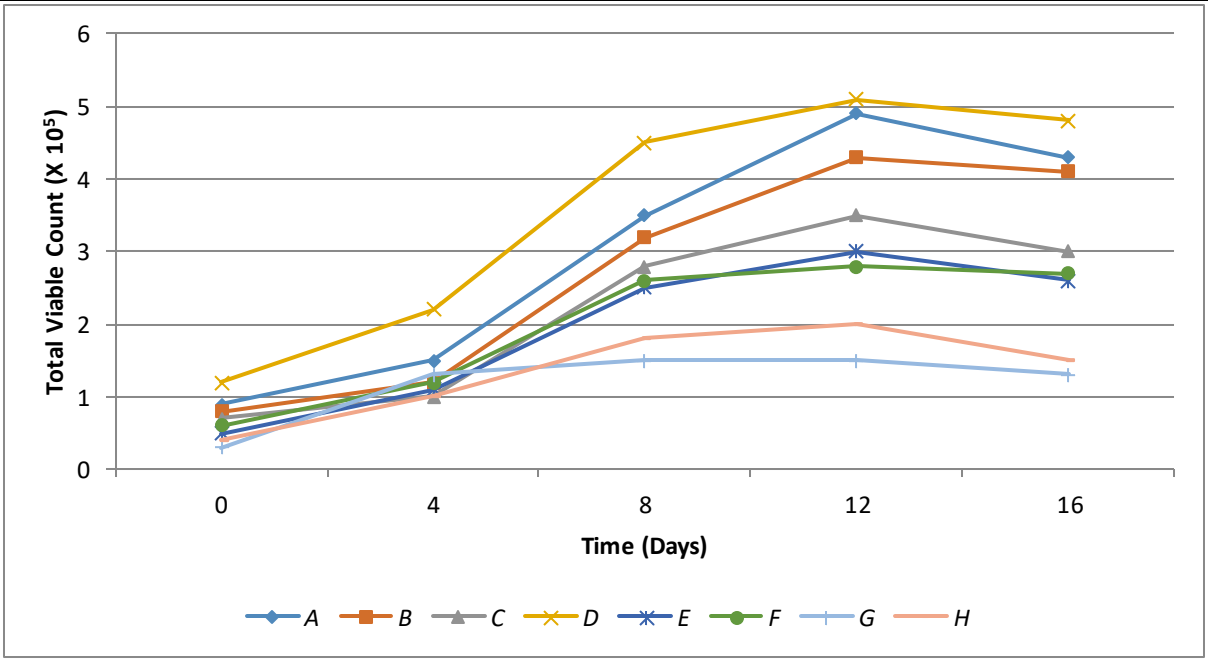

Fig.2: Changes in total viable count with time during utilization of UEO by the isolates.

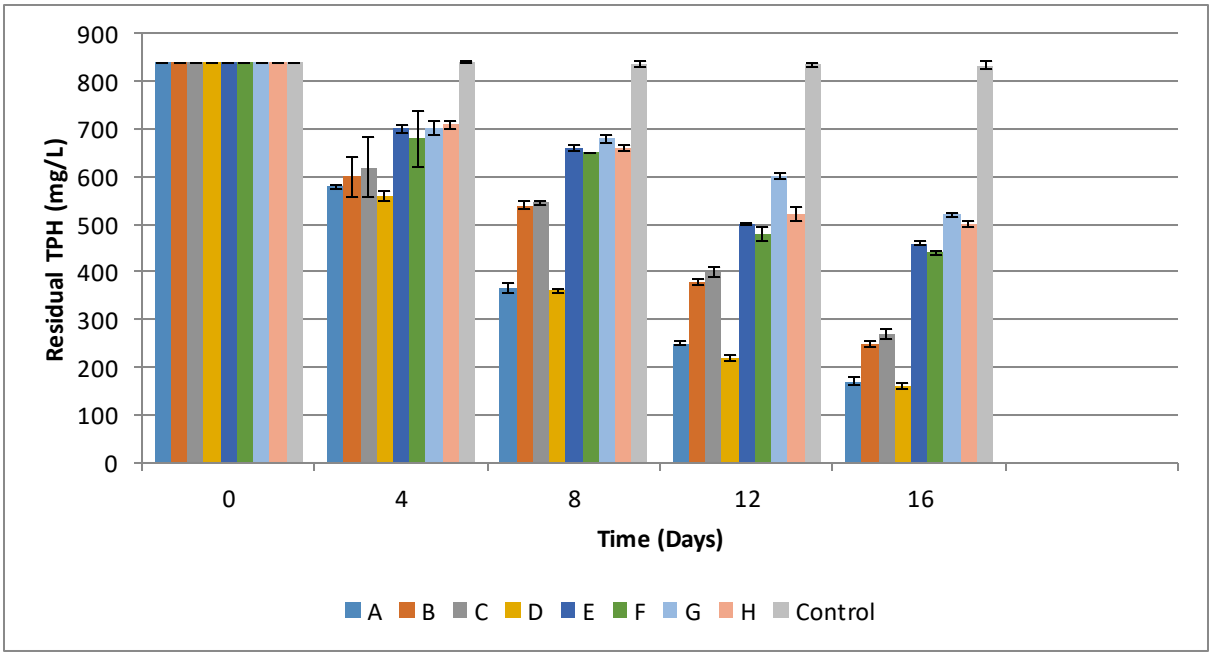

Fig.3: Changes in total petroleum hydrocarbon with time during utilization of UEO by the isolates.

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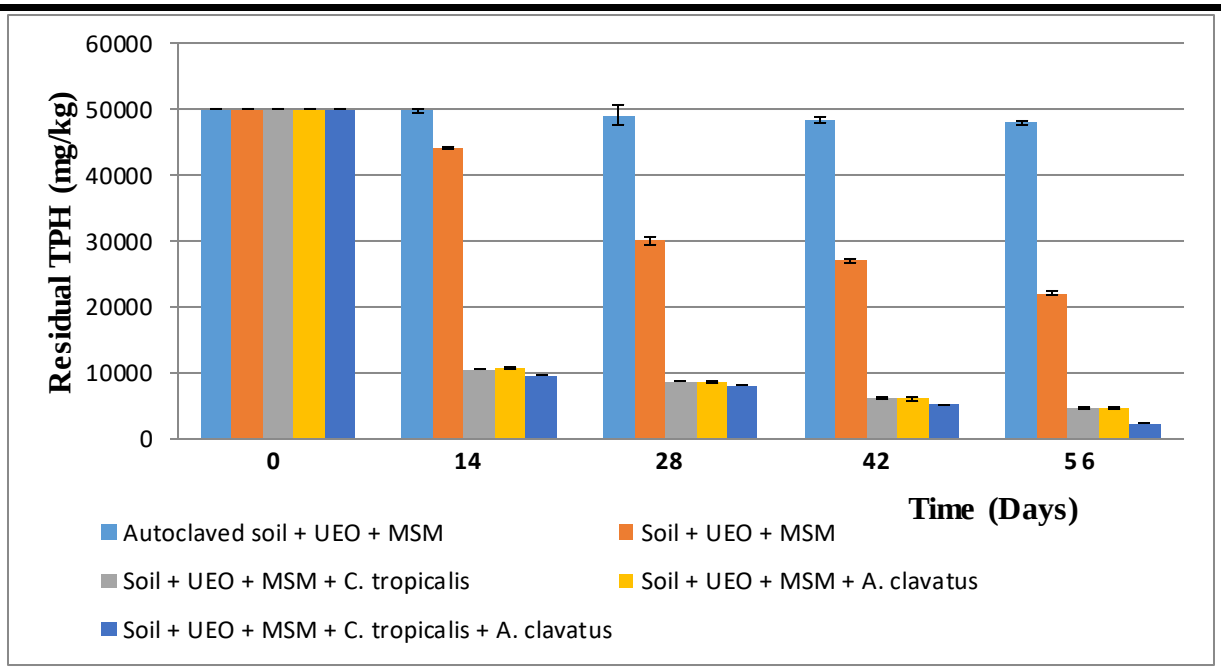

Fig.4: Residual total petroleum hydrocarbon in soil during bioremediation

Table.1: Net percentage (%) loss of TPH in soil during bioremediation

Microcosm set up	Time (days)				
	0	14	28	42	56
A	0	67.27±0.13	43.60±0.14	43.27±2.23	36.27±0.96
B	0	66.86±0.26	43.79±0.72	43.48±0.69	36.46±0.44
C	0	69.07±0.76	44.81±0.44	45.34±0.39	41.25±0.32

A = Soil + UEO + MSM + *C. tropicalis*, B = Soil + UEO + MSM + *A. clavatus*, C = Soil + UEO + MSM + *C. tropicalis* + *A. clavatus*. Net % loss = % loss of TPH in inoculated soil microcosm - % loss of TPH in uninoculated soil microcosm (control 1).

Table.2: Biodegradation rate and half-life of TPH in oil-polluted soil

Microcosm set up	Biodegradation constant (k) day^{-1}	Half-life ($t_{1/2}$) days
A	0.2295±0.013^{b}	3.02
B	0.2303±0.009^{b}	3.01
C	0.3013±0.029^{b}	2.30
D	0.0553±0.004^{b}	12.53

A = Soil + UEO + MSM + *C. tropicalis*, B = Soil + UEO + MSM + *A. clavatus*, C = Soil + UEO + MSM + *C. tropicalis* + *A. clavatus*, D = Soil + UEO + MSM. Values followed by letter b are different significantly at $P < 0.05$ level.

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Optimization of insecticidal potency of composites of aqueous, acetone, and ethanol extracts *of Piper guineense seed* on *Callosobruchus maculatus* by the simplex-lattice mixture experimental design.

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Abstract— *The objective of this study was to investigate the effect of composites of extracts of Piper guineense seed on Callosobruchus maculatus using the mixture experimental design of the response surface methodology (RSM).Callosobruchus maculatus damages stored legumes and grains resulting in huge agronomic and economic losses. Synthetic chemical insecticides is currently in useto check the wastage despite their known toxicity to man and the environment. Efforts to find alternatives to chemical synthetic insecticides has spotted Piper guineenseas a promising alternative candidate but less work has been done on its preparation and formulation for optimum activity. Fresh Piper guineenseberry was harvested from Essien Udim Local Government Area, of Akwa Ibom State, it was dried in the sun to moisture content of about 14% and ground to pass through 100 mesh sieve. The ground seed was extracted with ethanol and concentrated to obtain a slurry. Single blends of aqueous, ethanolic, acetone extracts of Piper guineense seed showed increasing insecticidal potency on the testinsect than binary blends.The model of dead insect was significant (R^2=0.9931, Mean=68.69), bean damage was significant (R^2=09786, Mean 63.46). Optimization analysis of experimental data revealed that 0.09, 0.437, 0.473 proportions of aqueous, ethanolic, and acetone extracts of Piper guineense seed respectively produced 95% and 31% dead insects and bean damage respectively at a desirability level of 76.80%.Result of the study shows that composites extracts of Piper guineense seeds could be a useful controller of stored maize.*

Keywords— Mixture experimental design, blends, Callosobruchus maculatus, PiperguineenseSchum. Et.Thonn.

I. INTRODUCTION

Callosobruchus maculatus infestation is a major contributor to qualities deterioration of stored cowpea, (*Vigna anguiculata*), 'black eye'. According to [1], the insect infestation causes considerable physical and nutritional and agronomic losses on the product, with negative impact on the economy and a threat to food security [2-3]. Cowpea is a tropical crop of the Fabaceae family, and is almost a staple food for both the poor and the rich households [4-6] reports that cowpea is rich in protein, fat, minerals and vitamins and affordable especially by the low income households and very easy to prepare with high taste profile[7]. About 12.5million hectares of cowpea is cultivated worldwide; yielding well over 3million tones of beans annually. The crop has been described as a 'wonder' crop of Nigeria agriculture [8-9].

At emergencies of insect infestation, farmers and food processors use synthetic chemical insecticides; aldrin/diedrin, chlordane, endrin and DDT to protect stored cowpea [10] Synthetic insecticides are chemicals that are purposely applied to suppress and protect agricultural and industrial products from the damaging action of insects and pests[11-12,10]. Although, synthetic insecticides exhibit high insecticidal potency and good prediction of insecticidal action against the weevil [13-15] they are toxic to the environment [16-19] because they have high persistence at

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the point of application, insecticidal residues have been implicated in food poisoning and related health effects [20-21]. In order to avert the problems of synthetic chemical insecticides are being investigated [22-24]. Bioinsecticides are eco-friendly and most of the plants used for their development have been used for food preparation and in folk medicine without reported adverse effect on human health[25,26,24,10].

Piper guineense is one of the outstanding potential candidate for use in bioinsecticides [27], the presence of phytochemicals like monoterpenic, sesquiterpenes nepetalactone confer the virtue on the plant seed. Bioinsecticides would be preferred to the synthetic counterpart because it is cheap, available, effective, environmentally friendly and renewable [27-29,19,24].

The study used the mixture experimental design to determine the optimum formulation of bioinsecticides fromaqueous, ethanol, and acetone extracts of *P. guineense*against *Callosabruchus maculatus*.

II. MATERIALS AND METHODS

Piper guineense seed was obtained from Utu Ikot Ukpong, Essien Udim Local Government Area of Akwa Ibom State, Nigeria. Young cowpea weevils were obtained from Akwa Ibom State Agricultural Development Programme (AKADEP) Office, Ikot Ekpene.Distilled water, ethanol, acetone were of analytical grade.

Preparation of plant product extracts

P. guineense was soaked and bruised between the palms to remove the berries, the seeds were separated from the fruit and dried, blended with Super-Master food blender (No.1, Japan) to pass through 300µm sieve. The granulated plant product was divided into 3 groups. Each group was extracted with distilled water, ethanol and acetone under refluxed with reflux system (Model No. 1220, Germany) for 4 hours named, the different extracts were labeled; A, B, and C and stored in different 100ml capacity bottles for use

Experimental design: background

The mixture experimental design is a flavour of response surface experiment in which the characteristics of the mixture is a function of the proportions of each component [30]. These proportional amounts of each ingredient may be measured by weight, volume, and mole ratio. The components in a mixture experimental design assume equations 1 and 2, where the components add up mostly to unity (1 or 100%).

$$\sum_{j=1}^{n} xj = 1 = 100\% \qquad 1$$

$$Xj = 1.0 - \sum_{i=1}^{j-1} xj - \sum_{i=j+1}^{n} xj \qquad 2$$

Thesimplex-lattice flavour of the design consists of equal proportions of 0, 1/3, 2/3, and 1 for common *aqueous extract* (A), *ethanol extract* (B) and *acetone extract* (C) of *Piper guineense* seed respectively designated by {3, 3} Design.The design determines the desired performance of pure, binary and centroidblendsin the experiment [31,32]. The simplex-lattice design [33] was used for the experiment, augmented with axial check blends and overall centroid with replication. A layout of the three blends of the design is shown in Table 1.

Table.1: Specification of primary components

	Units	Codes and real values of primary components	
		Lower (real)	Upper (real)
A	ml	0	1
B	ml	0	1
C	ml	0	1

A (aqueous extract), B (ethanol extract) C (acetone extract) of *P. guineense*

From Table 1 the constraints on the levels of the primary components in the designassume equations 1a, 1b, and 1c of the plant products.

0% (0ml) ≤ A)≤ 1%(ml) … (a)

0% (0ml) ≤ B) ≤ 1%(ml) … (b)

0% (0ml) ≤ C) ≤ 1%(ml) … (c)

Where

A + B + C = 1 or 100% … (d) of the mixture.

The mathematical function in equation 2 existed for each response, Y_k, in terms of the 3 components.

$Y_i = f(A, B, C)$ … (e)

Where Y_i is a dependent variable, β_i, β_{ii}, and β_{ij} are linear, quadratic, and interactive effects of the independent variables respectively.

Animal assay

Young weevils were introduced into 14 Petri dishes (totaling 72 in triplicates) containing 100 grains of disinfected cowpea and stored in a laboratory microclimate with average temperature of about 29°C and relative humidity of 70%. The plates were inoculated according to the experimental design in Table 1.

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Animal assay was carried out according the method of [27]. Ten (10) eggs of cowpea weevil were introduced into the Petri dishes which contained cowpea samples which were soaked in the different blends of the plant extracts, according to the experimental plan in Table 2. The plates were covered with porous material and set aside in the laboratory. After 49 days, three (3) days after the theoretical life-cycle of the weevil, the Petri dishes were opened, the number of dead insects determined by failure of probed insects to move (Y_1), and percentage of completely damaged grains determined by the number of holes on the beans (Y_2) were determined.

III. RESULTS AND DISCUSSION

Table 2, shows the standard and natural runs of the experiment, proportions of the extracts; aqueous, ethanol and acetone in each formula, experimental runs 1 to 14, percentage of dead insects, and cowpea bean damage based on number of holes and weight loss on the cowpea beans.

Table.2: Experimental layout and results from a mixture design

Std	Run	A	B	C	No. of dead insects	% grain damage by wt of beans
5	1	0.50	0.00	0.50	75	39
3	2	0.00	0.00	1.00	81	49
9	3	0.17	0.67	0.17	82	38
12	4	0.00	1.00	0.00	59	84
6	5	0.00	0.50	0.50	95	32
14	6	0.50	0.50	0.00	67	72
13	7	0.00	0.00	1.00	78	80
10	8	0.17	0.17	0.67	90	31
8	9	0.67	0.17	0.17	59	89
4	10	0.50	0.50	0.00	48	65
1	11	1.00	0.00	0.00	40	89
2	12	0.00	1.00	0.00	73	53
7	13	0.33	0.33	0.33	87	45
11	14	1.00	0.00	0.00	54	91

Std=standard run, Run=natural run, A=aqueous extract, B=ethanolic extract, C=acetone extract of *P. guineense*seed

The general linear model of data obtained from the experiment in Table 3 shows the ANOVA, regression and coefficients of the parameters. The table shows that the model of number of dead insects was significant ($p<0.05$) and that the linearity coefficient was significant (99.31% and mean of 68.69). the pure blends of the aqueous, ethanol, acetone extracts were significant on the parameter (dead insects) ($p<0.05$), the binary blends of aqueous/acetone and ethanolic/acetone extracts appeared to be significant ($p=0.0783$ and $p=0.0572$) respectively, while the tertiary blend was not significant.

Table.3: ANOVA, regression analysis, and coefficients of the parameters

Source/Effect	Estimate	p<value
Model		0.0006
A	46.34	0.0003
B	66.16	0.0001
C	79.35	0.0001
A*C	41.88	0.0783
B*C	137.61	0.0572
A*B*C	NS	NS
$R^2 = 0.9931$		
Mean =68.69		

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The predictive model of dead insects is as follows:
Number of dead insects = 46.34A + 66.16B +79.35C + 41.88AxC + 137.61BxC …(3)

The table further revealed that parameter estimate of acetone extract shows higher value of 79.55 than ethanol (66.16) and aqueous extract (46.34) respectively, it show that single blend of acetone extract may process higher insecticidal potency than ethanolic and aqueous blends. The cross product of aqueous/acetone (A*C) and ethanol/acetone (B*C) appeared to be significant ($p<0.0788$, and $p<0.0572$), indicated that the interaction of the blends was antagonistic on the model.Equation 3 shows the contribution of the estimates to the model which could be manipulated to produce bioinsecticides of a required insecticidal potency. Response surface plot (Figure 3) shows that higher insecticidal potency increased with the higher proportion of acetone extract, and lower proportion of aqueous and ethanolic extracts of *P. guineense* seed extracts.

Percentage damage on cowpea bean

Table 4 shows the following analysis of variance (ANOVA) results and reveals the predictive estimates for a quadratic polynomial fit to the data. The table shows that the model of parameter was significant ($p<0.05$) showing an adequate quadratic polynomial fit (R^2=97.86%) for the predictive model.

Table.3: ANOVA, regression analysis, and coefficients of the parameters for beans damage.

Source/Effect	Estimate	p<value
Model		0.0001
A	50.00	0.0001
B	66.60	0.0004
C	82.68	0.0017
A*B	-40.66	0.4448
A*C	-142.70	0.0649
B*C	-489.29	0.0375
R^2 = 0.9786		
Mean =63.46		

The predictive model of damage to the cowpea bean is as follows:
Percentage damage to cowpea bean = 50A + 66.60B + 82.68C – 40.66AB – 142.70AC – 489.29 … 4

The pure blends of aqueous, ethanolic and acetone were significant on the parameter ($p<0.05$). The cross product of ethanolic/acetone extracts was significant to the parameter ($p<0.05$) aqueous/ethanolic appeared to be significant. The table further reveals that the parameter estimates of acetone extract shows higher value of 82.68 than ethanol (66.60) and aqueous extract (50.00) respectively, it show that single blend of acetone extract may exhibited higher insecticidal potency than ethanolic and aqueous blends of the extracts. The cross product of aqueous/acetone (B*C) and ethanol/acetone (AC) appeared to be significant ($p<0.0375$, and $p<0.0649$) respectively, indicating that the interaction of the blends was antagonistic on the model.

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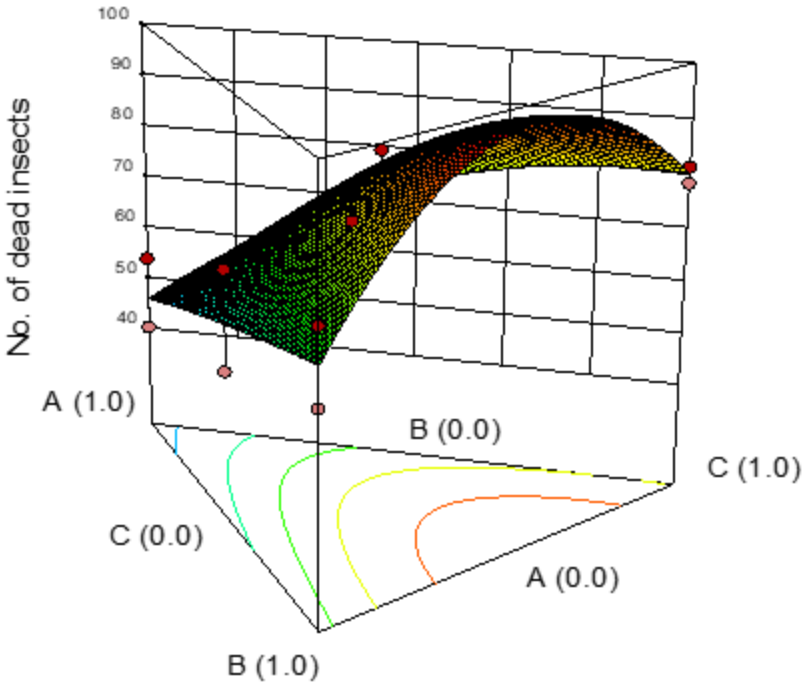

Equation 4 shows the weight of the estimates on the model which could be manipulated to protect cowpea from damage of *C. maculatus*. Response surface plot (Figure 4) shows that bean seed protection increased with the proportion of acetone extract in the formulation.

Optimization

Optimization analyses of data from the experiment showed that 0.09, 0.437, and 0.473 proportions of aqueous, ethanolic, acetone extracts of Piper guineense seed respectively produced 95% and 31.13% of dead insect and bean damage respectively at a desirability level of 76.90.

Biochemical mechanisms insect protection

All efforts to protect stored legumes from attach of bruchid seek to destroy the defensive mechanisms of insects by genetic engineering[34],anti-protease enzymes present in stored product to inhibit digestion [34] or inhibition of synthesis of neurotransmittersin particular, which regulates the central nervous system, muscular in all living things[35]. ACh is synthesized in certain neurons by the enzymecholine acetyltransferase from the compounds choline and acetyl-CoA. **Equation 5:**

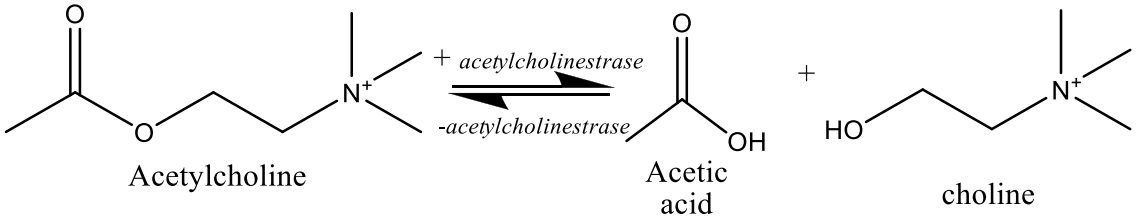

Fig.5: Degradation of acetylcholine molecule

The enzyme acetylcholinesterase converts acetylcholine into the inactive metabolitescholine and acetate, Equation 5. This enzyme is abundant in the synaptic cleft, and its role in rapidly clearing free acetylcholine from the synapse is essential for proper muscle function. Certain neurotoxins in insecticides work by inhibiting acetylcholinesterase, thus leading to excess acetylcholine at the neuromuscular junction, causing paralysis of the muscles needed for breathing and stopping the beating of the heart. Failure to synthesize the Ach leads to inactivity and dead of the organism, in man and other larger animals the break synthesis of the chemical is reversible [36].

Observations in our study suggest that aqueous, ethanol, and acetone extracts of *Piper guineense*could

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interrupt the function of acetylcholesterase in insect from normal living and reduce damage to stored product [37], it was noticed that the pure blends of the ethanolic and acetone were more potent than the binary and ternary blends. This observation was similar to the one reported by [38] and attributed to the relative extraction coefficient of the active components from *P. guineense*. For instance pure blends of acetone extract of *P. guineense*showed higher percentage of dead insects in all cases than the ethanolic extract, while the aqueaaous extract showed lower insecticidal potency on the animal modes. This could be attributed to the incomplete extraction of the active ingredients from the plant materials, also the combination of the extracts with the aqueous extract blends showed antagonistic effects on the parameter than those treated with the pure blends of ethanol and acetone alone. Therefore grain damage was low in units treated with ethanolic and acetone extracts of *P. guineense*, the observation was reported by [39,1].

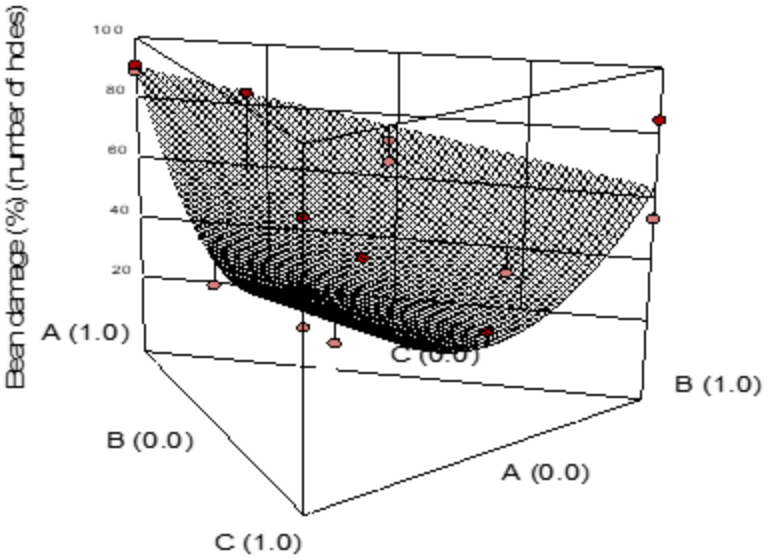

Fig.2: Response surface plot of plant extracts against bean damage by number of holes

IV. CONCLUSION

The result of the work showed that the aqueous, ethanol, and acetone extracts of *Piper guineense*seed could protectblack eye cowpea against attack of *C. maculatus*under the conditions of the experiment.In the study, pure blends of acetone and ethanol were more potent in that order than aqueous extract. The binary blend of ethanolic and acetone and aqueous showed antagonismin response. Formulation of blends of the extracts could be useful for short period of storage.Longer period of storage and replacement of some safe insecticides with the botanicals should be investigated.

REFERENCES

[1] Chomchalow N (2003) Protection of stored products with special reference to Thailand. Au J. T. 7(1): 31-47.

[2] Jacobson MDK, Reed MM, Crystal DSM and Sodastorm EL (1978). Chemistry and biological activity of insect feeding deterrents. Entomologia Exp. Appl. 24:248-257.

[3] Jimenez A, Romojaro F, Gomez JM, Lianos MR, Sevilla F (2003) Antioxidant systems and their relationship with the response of pepper fruits to storage at 20ºC. Agric. Food Chem. 51(21): 6293-9.

[4] Oyenuga VA (1968). Nigerian foods and feedstuffs. Ibadan University Press, p.99

[5] Olatunde AF (1989). Maize utilization and nutrition: In Food crop production, utilization and nutrition. (eds. B. N. Mbah, & D. O. Nnanyelugo) Department of Home Sciences and Nutrition of Nigeria, Nsukka. pp 96-110.

[6] Konan KC, Fofana I, Coulibaly A, Koffi NE, Chatigre O, Biego GHM (2016). Optimization of Storage Methods of Cowpea (Vigna Unguiculata L. Walp) bagged Pics containing biopesticide (Lippia Multiflora) by Central composite Experimental design in Cote D'ivoire. International Journal of Environmental & Agriculture Research (IJOEAR) 2(7): 1-11.

[7] Baidoo PK, Mochiah MB, Owusu-Akywa M. (2010) The effect of time of Harvest on the Damage Caused by the Cowpea Weevil Callosobruchus maculatus (Fab.)

http://dx.doi.org/10.22161/ijeab/3.3.50 *ISSN: 2456-1878*

(Callosobruchus: Bruchidae) Journal of Stored Products and Postharvest Research, 1(3), pp. 24-28.

[8] Ofuya TI (2003) Beans, insects and man. Inaugural Lecture Series 35. The Federal University of Technology, Akure, Nigeria. 45pp.

[9] Longe OO (2016) Evaluating the efficacy of some plant powders on the bean weevil [*Callosobruchus maculatus* (Fabricius) (Coleoptera: bruchidae)] affecting stored cowpea seeds (*Vigna unguiculata*). Asian Journal of Science and Technology 7(2): 2486-2490.

[10] Waghunde RR, Shelake RM, Sabalpara AN (2016) Trichoderma: A significant fungus for agriculture and environment: A Review. African Journal of Agricultural Research, 11(22): 1952-1965.

[11] Birch ANE, (2011) How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. J Exp Bot. 62:3251-3261.

[12] Srivastava KP, Dhaliwal GS (2010) A Textbook of Applied Entomology. Concepts in Pest Management. Kalyani Publishers, New Delhi, 1.

[13] Sarwar M (2015a). The Killer Chemicals as Controller of Agriculture Insect Pests: The Conventional Insecticides. International Journal of Chemical and Biomolecular Science, 1 (3): 141-147.

[14] Sarwar M (2015b). Microbial Insecticides-An Eco-friendly Effective Line of Attack for Insect Pests Management. International Journal of Engineering and Advanced Research Technology, 1 (2): 4-9.

[15] Sarwar, M. (2016) Families of Common Synthetic Agrochemicals Designed to Target Insect Pests or Vectors in Landscapes and Households Chemistry: a Review, Chemistry Research Journal, 1(3):7-13.

[16] FAO/WHO (1977). Pesticide residues food quality: FAO plant production and protection Paper 10 Sup.

[17] Essam Abdel-Salam Shaalan, Deon Vahid Canyon, Mohamed Wagdy, Faried Younes, Hoda Abdel-Wahab, and Abdel-Hamid Mansour (2005) Aedes aegypti and Culex annulirostris mosquitoes. Journal of Vector Ecology, 30 (2): 284-288.

[18] Coul O, Walia S (2009) Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. CAB Review: Perspectives in agriculture, veterinary Science, Nutrition and Natural Resources. 4. N. 049.

[19] Chaturvedi M, Sharma C, Chaturvedi M (2013) Effects of Pesticides on Human Beings and Farm Animals: A Case Study. Academy for Environment and Life Sciences.

[20] Sonchieu J, Ngassoum M B, Tchatchueng JB, Ashutosh KS Laxman PS (2013) Contamination of cowpea and by-products by organophosphorous pesticide residues in Ngaoundere markets: dietary risk estimation and degradation study. African Journal of Food Science, 7(5): 92-102.

[21] Zhu F, Lavine L, O'Neal S, Lavine M, Foss C, Walsh D (2016) Insecticide Resistance and Management Strategies in Urban Ecosystems: A Review. Insects, pp 1-26.

[22] Schmitterer H and Ascher KRS. (1987) Natural pesticides from the neem trees *A indica,A. Juss*. and other tropical plants. Proc. 2nd Int. Neem Conf; Rauischholzhausen, 25-28 May 1983. GT2, Eschborn; 587.

[23] Opender K, Suresh W, Dhaliwal GS (2008) Essential Oils as Green Pesticides: Potential and Constraints. Biopestic. Int. 4(1): 63-84 (2008).

[24] Nnamonu LA and Onekutu, A. (2015) Green pesticides in Nigeria: an overview. Journal of Biology, Agriculture and healthcare. Vol. 5.

[25] Baier AH, Webster BD, (1992). Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms. Evaluation of damage. J. Stored Prod Res 28: 289-293.

[26] Al-Zaidi AA, Elhag EA, Al-Otaibi SH, Baig MB, (2011) Negative effects of pesticides on the environment and the farmer's awareness in Saudi Arabia: a case study. J Anim Plant Sci 21:605-611.

[27] Ojimelukwe PC (2002). Potentials of *Xylopia aethopica* for short term protection of cowpea (var. IT-81D-975) seeds in Nigeria. Nigerian Agric. Journal. 3139-48

[28] Aktar MW, Sengupta D, Showdhury A (2009) Impact of insecticides use in agriculture: benefits and hazards. Journal of Interdiscip Toxicol. 2(1): 1-12.

[29] Echereobia CO, Okerere1 CS, and Emeaso KC (2010). Determination of repellence potentials of some aqueous plant extracts against okra flea beetles Podagrica uniforma. Journal of Biopesticides 3(2): 505 – 507.

[30] Bondari K. (1999). Interactions in entomology: multiple comparisons and statistical interactions in entomological experimentation. Journal of Entomological Science 34:57-71.

[31] Box GEP and Behnken DW (1960) Some New Three Level Design for the Study of Quantitative Variable, Technometrics, 2 (4): 445-475.

[32] Cornel JA (2002) Experiment with mixtures: Models, and the analysis of mixture data, 3rd Edition, 680 pages, John Wiley & Sons, Inc. USA.

http://dx.doi.org/10.22161/ijeab/3.3.50 *ISSN: 2456-1878*

[33] Scheffe H (1963), Simplex lattice design for experiment with mixture. Journal of Royal Statistical Society, Series B 28: 235-263.

[34] Zhu-Salzman K, Koiwa H, Salzman RA, Shade RE, Ahn JE (2003) Cowpea bruchid *Callosobruchus maculatus* uses a three-component strategy to overcome a plant defensive cysteine protease inhibitor Insect Molecular Biology, 12(2), 135–145.

[35] Shadashiva CT, Naidoo TR, Naido Y, Naido G (2013) Antioxidant and acetylcholinesterase activities of three species of the family Lamiaceae. Bangladesh Journal of Botany, 43(3):335-339.

[36] ICCBS (2008). International Centre for Chemical and Biological Sciences. Scientific Evaluation and Product Development Series. Pp 1-2.

[37] Dubey, S. C., Bhawana, B. and Ashok, K (2008). Current status of plants products as botanical pesticides in storage pest management. Journal of Biopesticides 1(2):182-186.

[38] Arong GA and Usua EJ, (2006) Comparative development of cowpea bruchid, (*Callosobruchus maculatus* (F.) (Coleptera: Bruchidae) on pods and seeds of some cowpea cultivars (*Vigna Unguiculata)* .Global Journal of Pure and Applied Sciences, Vol. 12, No. 1, pp 35-40.

[39] Asawalam, E. F. and Emosairue, S.O. (2006). Comparative efficacy of Piper quineense (Schum and Thonn) and Pirimphosmethyl on Sitophylus zeamais (Motsc) Tropical and Subtropical Agroecosystems 6(3):143-148.

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The Development of Rubber, Coffee and Palm Oil Commodity in South Sumatra, Indonesia using Swot Analysis

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Abstract**—** *The purpose of this research is to re-analyze the internal and external factors that become the strength, the weakness, the opportunities and the threat in implementing the strategy of Rubber, Coffee and Palm Oil commodity development in South Sumatera Province. Analysis tool used with SWOT approach. The results show that the districts/cities in the province of South Sumatra superior commodities rubber, coffee, and oil palm are in the area of aggressive strategy. Factors that become the main force for the land area with high soil fertility.*

Keywords**— *Leading Commodity, Rubber, Coffee, Oil Palm and SWOT Analysis.***

JEL Classifications:A10,B49,H19

I. INTRODUCTION

The agricultural sector, in particular, the plantation sub-sector has a role to improve the economy in a country or region. So the plantation sub-sector is one of the natural wealth owned by the Indonesian nation. The plantation business has an important and strategic role in national development, especially in increasing the prosperity and prosperity of the people, the state income of foreign exchange, the provision of employment, the acquisition of added value and competitiveness, the fulfillment of domestic consumption needs, even the raw materials of domestic industry (Hadiguna, 2012; Hafif, Ernawati, & Pujiarti, 2014).

According to the Law of the Republic of Indonesia Number 39 the Year 2014 that plantations are all activities of natural resources management, human resources, production facilities, tools and machinery, cultivation, harvesting, processing, and marketing related plantation crops. Plantation crops are a major supporter of the agricultural sector in generating foreign exchange resulting in the export of Indonesia's main agricultural commodities from plantations. Plantation products that are exported include palm oil, rubber, tea, coffee and tobacco (Dumairy, 1997).

The contribution of the agricultural sector cannot be doubted for the country's foreign exchange. According to Central Bureau of Statistics Central Jakarta (2017), the agricultural sector is the second most influential sector to growth, after processing industry, and still above the trade and construction sector. For the second quarter of 2017, the agricultural sector contributed 13.92 percent while in the quarter I 2017 contributed 13.59 percent increased by 0.33 percent. Although there is an increase in plantation sub-sector to economic growth, there are some problems faced, namely the area of plantation sub-sector is slowing down, productivity level tends to slow down, lack of infrastructure in plantation center (Ministry of Agriculture Directorate General of Plantation, 2016).

Establishment of plantations in South Sumatera Province can be reflected in the last three years with the area of smallholder plantations continue to increase with a total area of 2012 in the area of 2,429,132 Ha, 2013 to 2,542,801 Ha, and in 2014 of 2,620,992 Ha, most of the commodities cultivated by rubber, palm oil, coffee, coconut and other commodities of hope. Although plantation production has increased in the last three years with details of total plantation production in 2012 amounted to 3,562,990 Ton, in 2013 of 3,845,982 Ton, and in 2014 amounted to 4,114,840 Ton.

According to BPS of South Sumatera Province, the largest rubber producer in Indonesia is South Sumatera Province followed by North Sumatra and Riau. Of the total rubber production in Indonesia, 20% of production is produced by South Sumatera Province (Plantation Office of South Sumatra Province, 2016).

The existence of agriculture sub-sector problem above is needed the strategy so that superior product development

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in South Sumatera Province can compete in the international arena so that will increase exports which is superior from that province with an approach of Strengths, Weaknesses, Opportunities, and Threats. This analysis is based on the ability to find and the environment, so that the strategy can actually be realized from the strength it has and the opportunities it faces. The activities in the SWOT analysis process are to understand all information in a case, analyze the situation to find out what issues are going on and decide what action should be taken to solve the problem (Collett, 1999; Rangkuti, 2006; Valeriani & Wardhani, 2015; Wardhani & Valeriani, 2016).

This research refers to Nuga & Asimiea (2015) research in Negeria, Akhtar & Pirzada (2014) in Pakistan, Panca & Anhar (2013) and Wahyudy & Asrol (2015), so it is necessary to re-analyze internal and external factors of strength , opportunities, and threats in implementing the strategy of developing Rubber, Coffee and Palm Oil commodities in South Sumatera Province.

II. LITERATURE REVIEW

Commodities are the main commodities, commercial goods, crops and local handicrafts can be used as export commodities or raw materials which can be classified according to their quality in accordance with international trade standards (F.Rahardi, 2004; Marx, 1895). These commodities or commodities are said to be superior commodities according to Ely (2014) and Lahiani, Nguyen, & Vo, (2013) are potential commodities that are seen as winning a competition with similar products in other regions. Such advantages can be attained due to their high production efficiency due to the high bargaining position of both suppliers, buyers, and high competitiveness to competitors, new entrants, and substitutes.

According to the Directorate General for Regional Development of the Ministry of Home Affairs (1999) the following criteria of commodities are as follows: 1) having prominent and innovative local content in agriculture, industry and services, 2) having high competitiveness in the market, both in terms of quality, (3) Having the characteristic of the region because of the involvement of the local community (local labor), 4) having the guarantee and the raw material content which is sufficiently, stable, and sustainable, 5) Focused on high value-added products, both in packaging and processing, 6) Economically beneficial and beneficial to increase the income and capabilities of human resources and 7) Environmentally friendly, non-destructive to the environment, sustainable and non-destructive to local culture.

International trade is an act of exchange of goods and services made by a resident of a country with a resident of another country on the basis of mutual agreement. International trade is driven by the diversity of resources between countries (Nopirin, 1999). The main factor that is the reason for the country to trade internationally is the difference of resources between countries and each country aimed at achieving economies of scale in production (Krugman, Obstfeld, & Melitz, 2012). Differences between countries that encourage international trade are differences in natural resources, capital resources, labor and technology resulting in differentiation of production efficiency between countries (Halwani, 2002). Foreign trade has a very important role in economic growth and economic development of a country. The growth model developed by Keynes, international trade is one of the variables that affect a country income.

SWOT stands for strengths, weaknesses, opportunities, and threats. Understandings of strengths, weaknesses, opportunities, and threats in the SWOT analysis (Rangkuti, 2006). According to Jurevicius (2013) and Harrison (2010) the SWOT analysis component is: 1) Strenght (S) is strength analysis, situation or condition that is the strength of an organization or company at the moment, 2) Weaknesses (W) is weakness analysis, situations or conditions that are the weakness of an organization or company at this time, 3) Opportunity (O) is the analysis of opportunities, situations or conditions that are opportunities outside an organization or company and provide opportunities for the organization to grow in the future, 4) Threats (T) is threat analysis, how to analyze the challenges or threats that must be faced by a company or organization to deal with a variety of unfavorable environmental factors on a company or organization that causes decline. If not immediately overcome, the threat will be a barrier for a business concerned either in the present or in the future.

III. METHODOLOGY

In order to obtain internal and external factors in developing rubber, coffee and palm oil commodities in South Sumatera Province, descriptive research method is used. The identification of internal and external factors is done by using Internal analysis tool - External Strategic Factor Analysis Summary (IFAS - EFAS), Space Matrix and SWOT Matrix.

The analytical stages in the SWOT are utilizing all data and information in the quantitative models of strategy formulation (Rangkuti, 2006; Coman & Ronen, 2009). SWOT analysis is first done by scanning. The use of some analysis will be better so as to produce a strategy formula that can solve the problems and strategies that are

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formed in accordance with the objectives and the environment it faces.

IV. DATA AND RESULT

4.1 Analysis of Internal and External Environment

In analyzing the SWOT and strategy of superior product policy, the researcher analyzes each commodity by taking into account internal factors and external factors for internal factors are land availability, commodity quality, skill quality, labor quantity, production scale, product derivation while external factor price, the market, the number of traders, technology, institutions, and infrastructure. Based on the results of surveys, observations, interviews and FGDs, internal environmental analyzes consisting of strengths, weaknesses (Weakness) and external environmental analyzes consisting of opportunities (Opportunities) and threats from the development of superior rubber, coffee, and palm oil products can be seen in Table 1.

Tabel.1: Matriks Internal factory rubber, Coffee, Palm Oil Commodity

1. Rubber Commodity			
INTERNAL FACTOR			
STRENGTHS		***WEAKNESS***	
1	Has a large area of land, soil fertility is high and productive	1	Product quality is still low
2	Number of manpower available	2	Product derivatives are still a lot of work in other regencies or provinces.
3	Medium-scale production scale to large scale	3	Some work skills are still low
EXTERNAL FACTOR			
OPPORTUNITIES		***THREATS***	
1	The number of suppliers that supply relatively many	1	Rubber prices fluctuate
2	Only some districts/cities are using appropriate technology	2	Institutions are not yet supportive
3	Both local and domestic markets are available even in overseas exports	3	Infrastructure is not adequate
2. Coffee Commodity			
INTERNAL FACTOR			
STRENGTHS		***WEAKNESS***	
1	Has a large area of land, fertility is high and productive	1	Some work skills are still low
2	Derivative products of new instant coffee and powder, but will be developed other products, because of the more coffee with a variety of flavors.	2	Scale production in small-scale districts/cities
3	Number of manpower available	3	Some quality or quality of coffee is low
EXTERNAL FACTOR			
OPPORTUNITIES		***THREATS***	
1	Coffee market opportunities in Indonesia in the future is quite bright	1	Coffee prices fluctuate and compete with other countries
2	Institutional support	2	Technology is still traditional
3	The number of suppliers that supply relatively many	3	Infrastructure is not adequate
3. Palm Oil			
INTERNAL FACTOR			
STRENGTHS		***WEAKNESS***	
1	Has a large area of land, fertility is high and productive	1	The product derivatives are still small

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2	Scale production in districts/cities of medium and large scale	2	Product quality is still low
3	Number of manpower available	3	The number of merchants supplied is still small
EXTERNAL FACTOR			
OPPORTUNITIES		***THREATS***	
1	Both local and domestic markets are available even in overseas exports.	1	Palm oil prices fluctuate and compete with other countries
2	Institutional support	2	Product derivatives are still low
3	Technology can be done with appropriate	3	Infrastructure is not adequate

4.2 Analysis of IFAS Strategic Factors – EFAS

After analyzing the internal and external conditions of the leading commodities of rubber, the calculation of internal and external factors is weighted to determine the location of the strategic development quadrant that is considered urgent to do. The calculation of factor weight is done by tabulating the score of EAS - EFAS. The following is a calculation of the internal and external factor weights contained in the IFAS and EFAS analysis tables shown in the following table 2

Tabel.2: Calculation of Internal and External Factor Scoring Score of Rubber Commodity

No	**Factor-Factor**	Weight	Rating	Value
	Internal Factor			
	Strenght			
1	Has a large area of land, soil fertility is high and productive	0,34	4	1,36
2	Number of manpower available	0,33	3	0,99
3	Medium-scale production scale to large scale	0,33	2	0,66
	Amount	**1,00**		**3,01**
	Weakness			
1	Productivity quality is still low	0,33	2	0,66
2	Institutions are not yet supportive	0,34	2	0,68
3	Some work skills are still low	0,33	1	0,33
	Amount	**1,00**		**1,67**
	Opportunities			
1	The number of suppliers that supply relatively many	0,35	4	1,4
2	Only some districts/cities are using appropriate technology	0,30	3	0,6
3	Both local and domestic markets are available even in overseas exports	0,35	2	0,7
	Amount	**1,00**		**2,7**
	Threats			
1	Rubber prices fluctuate	0,4	2	0,8
2	Institutions are not yet supportive	0,2	2	0,4
3	Infrastructure is not adequate	0,4	3	1,2
	Amount	**1,00**		**2,4**
	S-W = 3,01-1,67 =1,34			
	O-T = 2,7 – 2,4 = 0,3			

Table.3: Calculation of Internal and External Factor Scoring Score of Coffee Commodity

No	**Factor-Factor**	Weight	Rating	Value
	Internal Factor			
	Strenght			
1	Has a large area of land, soil fertility is high and	0,3	3	0,9

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	productive			
2	Derivative products of new instant coffee and powder, but will be developed other products, because of the more coffee with a variety of flavors.	0,5	3	1,5
3	Number of manpower available	0,2	2	0,6
	Amount	**1,00**		**3,00**
	Weakness			
1	Some work skills are still low	0,3	2	0,6
2	Scale production in small-scale districts/cities	0,3	3	0,9
3	Some quality or quality of coffee is low	0,4	3	1,2
	Amount	**1,00**		**2,67**
	Opportunities			
1	Coffee market opportunities in Indonesia in the future is quite bright	0,4	3	1,2
2	Institutional support	0,3	3	0,9
3	The number of suppliers that supply relatively many	0,3	3	0,9
	Amount	**1,00**		**3,00**
	Threats			
1	Coffee prices fluctuate and compete with other countries	0,4	3	1,2
2	Technology is still traditional	0,3	1	0,3
3	Infrastructure is not adequate	0,3	3	0,9
	Amount	**1,00**		**2,4**
	S-W = 3-2,67 = 0,33			
	O-T = 3 – 2,4 = 0,56			

Table.4: Calculation of Internal and External Factor Score Oil Palm Commodity

No	**Factor-Factor**	Weight	Rating	Value
	Internal Factor			
	Strenght			
1	Has a large area of land, soil fertility is high and productive	0,3	4	1,2
2	Scale production in districts/cities of medium and large scale	0,4	3	1,2
3	Number of manpower available	0,3	3	0,9
	Amount	**1,00**		**3,3**
	Weakness			
1	The product derivatives are still small	0,3	3	0,9
2	Product quality is still low	0,4	2	0,8
3	The number of merchants supplied is still small	0,3	2	0,6
	Amount	**1,00**		**2,3**
	Opportunities			
1	Both local and domestic markets are available even in overseas exports	0,4	3	1,2
2	Institutional support	0,3	3	0,9
3	Technology can be done with appropriate	0,3	3	0,9
	Amount	**1,00**		**3,00**
	Threats			
1	Palm oil prices fluctuate and compete with other countries	0,4	2	0,8
2	Technology is still traditional	0,3	2	0,6

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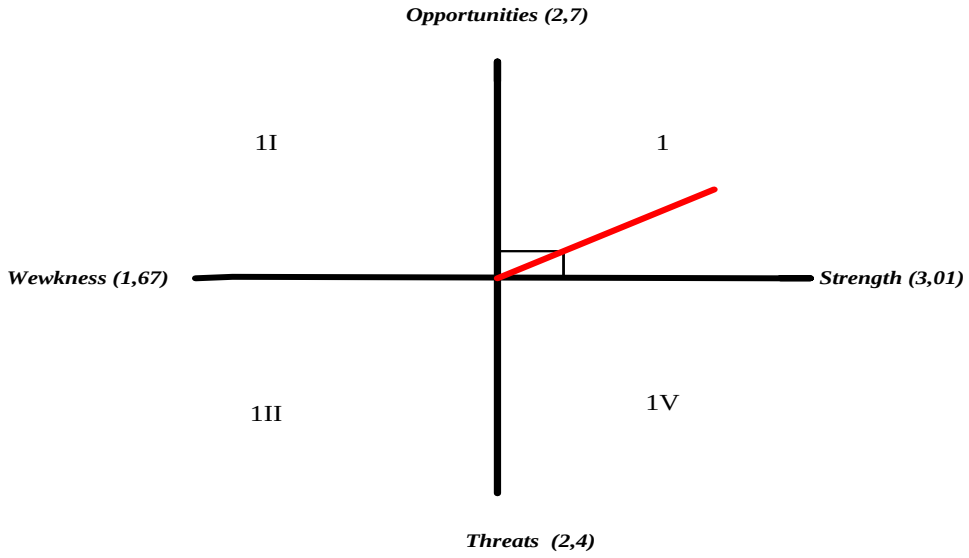
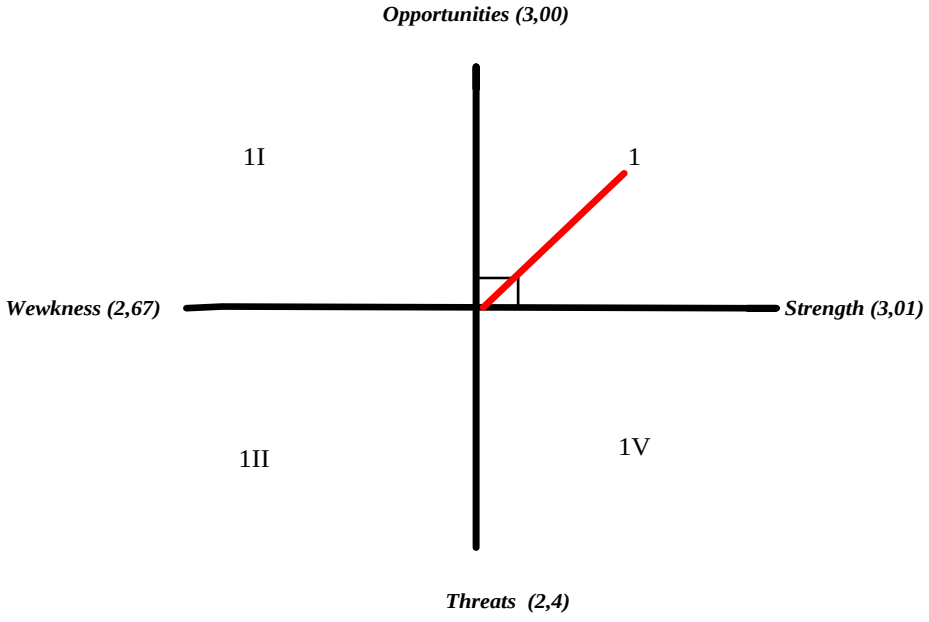
3	Infrastructure is not adequate	0,3	2	0,6
	Amount	**1,00**		**2,00**
	S-W = 3,3 – 2,3 = 1,0			
	O-T = 3,0 – 2,0 = 1,0			

Based on the above table and the strengths, weaknesses, opportunities, and threats of the three leading commodities that can be seen in Figures 1, 2 and 3



Picture: 1 Diagram of SWOT Analysis Rubber Commodity



Gambar: 2 Diagram Of SWOT Analysis Coffee Commodity

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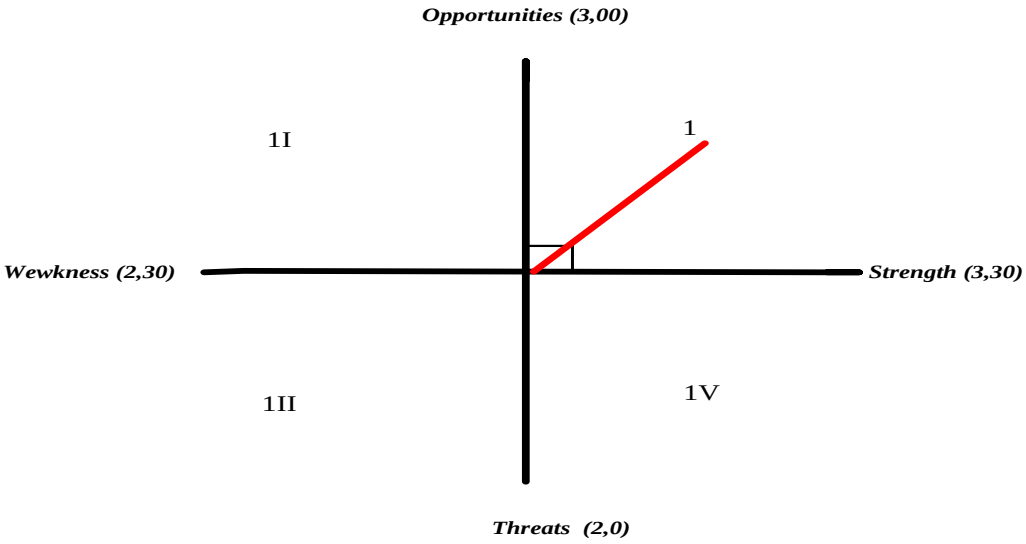

Gambar: 3 Diagram Of SWOT Analysis Palm Oil Commodity

Based on the quadrant's location formulation in the Drawing Strategy that is urgent to be implemented in the framework of developing rubber, coffee and palm oil commodities in South Sumatera province in quadrant I (due to its value +) or lies between external opportunities and internal strength and includes into areas of aggressive strategy for that formulation aggressive strategy to develop rubber, coffee and palm oil commodity Table:

Table.5: Aggressive Strategy Formulation of Rubber Commodity Development

Internal Factor / External Factor	**Strength** • Has a large area of land, fertility is high and productive • The number of manpower available. • Medium to large scale production scale	**Weakness** • Product quality is still low • Product derivatives are still a lot of work in other regencies or provinces • Some work skills are still low
Opportunity • The number of suppliers that supply relatively many • Only some districts/cities are using appropriate technology. • Local and domestic markets are available even in overseas exports	**Strategy S-O** • Optimizing the available land by replanting the crops in collaboration with the traders who supply the ingredients in the process of the cremation so that it has added value. • Optimizing Workforce available in the use of technology. • Increase larger production scale with better-standardized rubber export.	**Strategy W-O** • Improve quality and synergize with merchants • Increase product derivatives with good technology based so that the product is in accordance with national and international standards. • Increase labor skills for the rubber market to penetrate foreign markets
Threat • Rubber prices fluctuate • Institutions are not yet supportive • Infrastructure is not adequate	Strategy S-T • Make rules or policies related to land used and inflatable prices remain stable or tend to increase despite world market	**Strategy W-T** • The quality of the product is improved so that the price can go hand in hand with the others • Product derivatives are re-

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	conditions decline. • Strengthening of regional commodity institutions and their marketing and networking • Increased infrastructure such as roads to remote areas in the area, the plantation is still red so if it rains, it is difficult to enter the area.	created with distinctive features that exist in the region and synergize with related copper like banks, cooperatives, and others. • Improving the quality of the workforce is not only related to the productivity of rubber commodities but in the future, the product of marketing and marketing.

Table.6: Aggressive Strategy Formulation of Coffee Commodity Development

Faktor Internal / FaktorEksternal	**Strength**	**Weakness**
	• Has a large area of land, fertility is high and productive • Derivative products of new instant coffee and powder, but will be developed other products, because of the more coffee with a variety of flavors • Number of manpower available	• Some work skills are still low • Scale production in small-scale districts/cities • Some quality or quality of low coffee
Opportunity • Coffee market opportunities in Indonesia in the future is quite bright • Institutional Support • The number of suppliers that supply relatively many	**Strategy S-U** • Optimizing available land by replanting crops so that market opportunities increase • Institutional strengthening of farmers to create a derivative of coffee products with several different flavors. • Optimizing the number of available manpower as well as establishing cooperation with merchants who supply tools of production equipment etc.	**Strategy W-O** • Increase labor skills for the coffee market to penetrate foreign markets to increase. • Increase the scale of production by doing institutional development • Improve the quality of coffee beans with traders supplying related to the quality of quality seeds.
Threat • Coffee prices fluctuate and compete with other countries • Technology is still traditional • Infrastructure is adequate	**Strategi S-T** • Make rules or policies related to land used and coffee prices are not fluctuating remain stable or tend to increase despite world market conditions decline. • An innovation of processed products with good technology • Increased infrastructure	**Strategi W-T** • Increase the skill of labor in processed so that price can bargaining with others. • The scale of production is further enhanced through good technology • Increased quality of coffee with supporting facilities and infrastructure.

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Table.7: Aggressive Strategy Formulation of Palm Oil Commodity Development

Faktor Internal / FaktorEksternal	**Strength**	**Weakness**
	• Has a large area of land, fertility is high and productive. • Medium and large production scale • Number of manpower, widely available	• The product derivatives are still small • Product quality is still low • The number of merchants supplied is still small
Opportunity • Both local and domestic markets are available even in overseas exports • Institutional support • Technology can be done with appropriate	**Strategi S-O** • Optimizing available land by replanting crops so that market opportunities increase. • Increase the scale of production with greater institutional strengthening • The amount of manpower available is much more optimized and can use the right technology	**Strategi W-O** • Increase product derivatives so that exports are no longer raw materials • Improve product quality with institutional strengthening • Use of appropriate technology in synergy with merchants suppliers
Threat • Prices fluctuate • Institutions are not yet supportive • Infrastructure is not adequate	**Strategi S-T** • Creating rules or policies related to land used and fluctuating coffee prices remain stable or tend to increase despite world market conditions decline. • Strengthening of regional commodity institutions and their marketing and networking • Increased infrastructure such as roads to remote areas in the area, because the plantation is still red so if it rains, it is difficult to enter the area.	**Strategi W-T** • The product derives not only CPO or cooking oil but more innovative creates a product derivative at an affordable price society. • Product quality is enhanced by institutional strengthening. • Increased infrastructure

The South Sumatra government should coordinate with regencies and municipalities in relation to the area of rubber commodities despite replanting (rejuvenation) of rubber commodities with a rejuvenation area in 2017 of 14,750 ha, the expansion of this rejuvenation is lower than in 2016 of 19,600 (Ha). The rejuvenation fund is funded by the government such as seed provision, fertilizer, and poison grass. For the supply must be in coordination with the merchant supplying because government funds fall somewhat slowly so that suppliers can supply suppliers with the payment can be done if the fund has been liquid or in other words the debt first. Then the government issued funds on land rejuvenation must also take into account its workforce, from funds launched by the government of 5.6 million used for seeds, fertilizers, and poison grass. The number of available manpower must improve the quality or skill of continuing education in the hope of increasing rubber productivity and can compete with other countries.

Rubber market is not only the raw materials that are exported but the derivative products that have characteristic of South Sumatera province considering South Sumatra are often done an international event. Increasing the scale was small to large scale with support from the government and the community as well as capital support and cooperation with investors both from within and abroad. For large-scale companies still, maintain or increase production and innovate for

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expansion as well as create value-added derivative products and increase foreign exchange countries and especially South Sumatra province.

The area of coffee commodity in South Sumatera is the largest coffee area in Indonesia of 15.190 (Ha), but with commodity area is not equal to the increase of the population of Indonesia as well as South Sumatera Province so that every year the available land is limited, long ago, as farmers will use their land to grow crops that produce faster to meet their daily needs. Most of the coffee age in the regency or city in South Sumatra is getting older so it needs replanting gradually It is expected that by replanting the coffee market will increase especially the export and not the famous South Sumatra Province as the biggest coffee producer but the famous is Lampung, whereas the coffee in production by Lampung comes from South Sumatra, therefore there needs to be a policy to improve the management system, the promotion of development.

The institutional strengthening aspect is being improved with strong coordination so that it can assist the government in conducting supervision to run coffee development programs. Where institutional strengthening through the empowerment of farmer institutions to form partnerships by building awareness on farmers with a consciousness of community or group that grows on the basis of the need rather than forced from the encouragement of certain projects. Farmer groups that can run their activities independently become the criteria of advanced or business-oriented farmer groups. Government and private assistance in supporting and facilitating farmer partnership activities to increase cooperation partners in the business network. Through this form of partnership is expected to occur transfer of technology, knowledge, and expansion of information for business development.

Counseling on strengthening the effectiveness of institutional coordination of farmers, farmer institutions became an option to facilitate the government to oversee and coordinate. In Law No.16 of 2006, the institutional function is the container of the learning process, cooperation vehicle, the unit of facilities and production infrastructure, processing unit and marketing, and supporting service unit. Once the importance of institutional coordination to carry out its functions properly, so it takes effect in carrying out its services to each member. Optimizing the number of labor available and cooperating with merchants who supply seeds of production equipment quickly and cheaply.

Oil palm plantations consist of smallholder plantation, state plantation and private plantation with total area owned by South Sumatera Province of 1,064,373 (Ha) so that in Sumatera area of South Sumatera province the largest area of oil palm is third largest after Riau and North Sumatra. This condition has resulted in the fact that South Sumatera Province has wide potential in the development of oil palm commodity. It is also necessary to replant the oil palm plantations since some of the oil palm plantations have been aged 15 years and over, replanting is done gradually (underplating). Besides replanting the land area can be converted to oil palm plantation which is the factor of supply of CPO to the market, because Indonesia is a producer of CPO other than Brazil, Colombia, Cameroon, and others.

During this time the processing of CPO mostly dominated by large investors, because the investment required to build an MCC unit requires a lot of capital. After mobilizing hundreds of billions of rupiah for mega projects of tens of thousands of hectares of oil palm plantations, further integrating CPO processing into it. The result is that CPO processing technology is very capital-intensive, and it is hard to imagine that a palm oil mill can be made as small and as simple as a rice mill. Although the small-scale and even large scale for an oil palm plantation effort institutional role is very important like financial institution (Bank) and nonbank to reach the condition of palm plasma plantation sustainable. From the labor-intensive nature of these plantations, the availability of abundant labor with sufficient and inexpensive skills does not mean they are not obliged to improve science and technology. The era of globalization and the ASEAN Economic Community (MEA) is very important to learn a technology that progresses so rapidly because it will improve the competitiveness of palm oil commodity.

V. CONCLUSION

Results from the SWOT analysis of leading commodities rubber, coffee and palm oil in the districts/cities in South Sumatra are in areas of aggressive strategies that mean short-term or quick to make changes to these commodities. Factors that become the main force for the land area with high soil fertility. While the weakness factor is the quality of commodity productivity is still relatively low, skilled labor is low then the factor of the opportunity of the many traders who supply the commodity, the threat factor of rubber commodity inflated rubber price and inadequate infrastructure.

Strategies that are in the short term or rapidly done optimize the available land by replanting the plant back so that market opportunities increase. The scale of production is enhanced to a greater extent by institutional strengthening. Then the amount of manpower available is much improved and applying appropriate technology by training the workforce.

REFERENCES

[1] Akhtar, K., & Pirzada, S. S. (2014). SWOT Analysis of Agriculture Sector of Pakistan. *Journal of Economics and Sustainable Development*, *5*(11), 127–134.

[2] Badan Pusat Statistik Jakarta Pusat. (2017). *Statistik Indonesia Tahun 2017*. Jakarta Pusat: Badan Pusat Statistik.

[3] Collett, S. (1999). SWOT Analysis. *Computerworld*, (29), 58.

[4] Coman, A., & Ronen, B. (2009). Focused SWOT: Diagnosing critical strengths and weaknesses. *International Journal of Production Research*, *47*(20), 5677–5689.

[5] Dinas Perkebunan Provinsi Sumatera Selatan. (2016). *Statistik Perkebunan*.

[6] Dumairy. (1997). . *Perekonomian Indonesia*. Jakarta.: Erlangga.

[7] Ely, Kartikanidyah.(2014). Analisa Location Quatient dalam Penentuan Produk Unggulan pada Beberapa Sektor di Kabupaten Lingga Kepulauan Riau. *Jurnal Integritas Riau*.

[8] Hadiguna, R. A. (2012). Model Penilaian Risiko Berbasis Kinerja untuk Rantai Pasok Kelapa Sawit Berkelanjutan di Indonesia. *Jurnal Teknik Industri*, *14*(1), 13–24.

[9] Hafif, B., Ernawati, R., & Pujiarti, Y. (2014). Peluang peningkatan produktivitas kelapa sawit rakyat di Provinsi Lampung. *Jurnal Littri*, *20*(2), 100–108.

[10] Halwani, R. H. (2002). *Ekonomi Internasional dan Globalisasi Ekonomi*. Jakarta: Ghalia Indonesia.

[11] Harrison, J. P. (2010). *Strategic Planning and SWOT analysis*. *Essentials of Strategic Planning in Healthcare* (Vol. 1). Retrieved from

[12] Jurevicius, O. (2013). Apple SWOT analysis 2013 | Strategic Management Insight. *Strategic Management Insight*, *3*(3), 115–130.

[13] Kementerian Pertanian Direktorat Jenderal Perkebunan. (2016). *Statistik Perkebunan 2016*.

[14] Krugman, P. R., Obstfeld, M., & Melitz, M. J. (2012). *International economics : theory and policy*. *Policy* (Vol. New York,).

[15] Lahiani, A., Nguyen, D. K., & Vo, T. (2013). Understanding return and volatility spillovers among major agricultural commodities. *Journal of Applied Business Research*, *29*(6), 1781–1790.

[16] Marx, K. (1895). The Fetichism of Commodities and The Secret thereof. *Capitalist Production*, 71–83.

[17] Nopirin. (1999). *Ekonomi Internasional* (Ketiga). Yogyakarta: BPFE UGM.

[18] Nuga, B. O., & Asimiea, A. O. (2015). SWOT Analysis of the Nigerian Agricultural Sector. *IOSR Journal of Agriculture and Veterinary Science Ver. II*, *8*(4), 2319–2372.

[19] Panca, A., & Anhar, M. (2013). Strategi Pengembangan Agroindustri Kelapa Sebagai Upaya Percepatan Ekonomi Masyarakat di Kabupaten Ketapang. *Industria*, *3*(1), 13–26.

[20] Rangkuti, F. (2006). Reorientasi konsep perencanaan strategis untuk menghadapi abad 21. In *Analisis SWOT Teknik Membedah Kasus Bisnis* (p. 200).

[21] Rahardi,F.2004. *AgribisnisBuahIndonesia*.Jakarta: PenebarSwadaya.

[22] *Undang-Undang Republik Indonesia Nomor 39 Tahun 2014 tentang Perkebunan*.

[23] Valeriani, D., & Wardhani, R. S. (2015). Alternative Tourism in the Belitung Island with the Blue Tourism Concepts as the Effort to Achieve the Quality of Life, Quality of Opportunity and Quality of Experience. *Mediterranean Journal of Social Sciences*, *6*(5).

[24] Wahyudy, H. A., & Asrol, A. (2015). Analisis Strategi pengembangan agribisnis Karet Rakyat Di Kabupaten Singingi, Provinsi Riau. *Dinamika Pertanian*, *XXX*(3), 249–260.

[25] Wardhani, R. S., & Valeriani, D. (2016). Green Tourism Dalam Pengembangan Pariwisata Bangka Belitung.

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Perception of Household on Greening methods to Ameliorate Climate change in South-West of Oyo State

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***Abstract*—** *The project investigated the perception of household on greening methods to ameliorate climate change in south- west of Oyo state. The study was carried out in Oluyole local Government of Oyo state, Nigeria. Where two areas were randomly selected (Oluyole estate and Oluyole Extension). A total number of 91 structured questionnaires were retrieved out of 130 administered. Data collected were analyzed. Descriptive was used to analyzed socio economic characteristics, sources of information on greening, perception of respondent on greening, different greening method by the respondent and constraints, cross tabulation was used to explain association between socio characteristics and greening method and Pearson Product Moment Correlation (PPMC) was used to analyzed constraints and greening method by the respondents, relationship between the perception and greening method by the respondents. The result of the study revealed that majority of the respondents were male (65.9%),and between the ages of 31-40 (34.2%), majority also had tertiary education(58.2%) this showed that people within the study area were informed on the value of education, it was also revealed that majority of the respondent agreed that the human activities have led to climate change 3.51 mean value, Developing countries should take most of the blame for climate change (3.07) mean value and that laws governing the forestry management in Nigeria should be revisited with 2.03mean value. Respondents agreed that planting of trees, planting of tree crops, planting of flowers, planting of shrubs like Morida lucida, Moringa oleifera, Glyricidia sepium, management and development are the best method to ameliorate climate change, it was observed that deforestation, urbanization, industrialization, Lack of good policy by Federal Government are the major constraints. The use solar energy is the least that people use because majority are using generator which add carbon monoxide to the atmosphere. This study recommended that human activities should be controlled in order to modify our environment, planting of trees, planting of tree crops like mango, cashew, orange, coconut will serve as fruit for family as well as improvement on climate change, planting of flowers for beautification and planting of shrubs are the best method to ameliorate climate change. Laws should be enacted to avoid climate change based on deforestation and good policies should be put to practice by Federal Government base on industrialization in urban areas.*

***Keywords*—Climate change, greening, ameliorate.**

I. INTRODUCTION

Climate change and agriculture are interrelated processes, both of which take place on global scale. (Jeremy, 2008).Climate change is one of the most serious environmental threats facing mankind world-wide, it affects agriculture in several ways, including its direct impact on food production. Climate change, which is attributable to the natural climate cycle and human activities as adversely affected agricultural productivity in Nigeria (Ziervogel and Easterling, 2006). The weather pattern changes caused by climate change is also as a result pollution in the environment. Greening is the process of taking a greater interest in environmental issues and acting to protect the environment (MPL; 2009-2017) (https://www.macmilliandictionary.com)

Wisner (2004), report that the vulnerability of agriculture is not determined by the nature and magnitude of environmental stress life climate change parse, but by the combination of the societal capacity to cope with or/recover from environmental change. While the coping capacity and degree of exposure is related to environmental changes, they are both also related to changes in societal aspects such as land use and cultural practices. Agriculture in Nigeria is predominantly in the hands of rural small holder farmers, who have been generally described as poor and hungry. Some of the ways to improve living a green lifestyle and to

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ameliorate climate change include adopting the reuse recycle principle, energy saving practices. There are many energy efficient practices and appliances that you can use to turn your home into a green, energy efficient home. Reduction of pollution by reducing the use of harmful chemicals and substances in your home, and using eco friendly home cleaners and other eco-friendly products in your daily needs, Introduction of more plants and trees into our environment and encourage others to do the same. Growing of green garden using green gardening principles, or even plant trees in your name. Check out the tips you can adopt for daily green living for the home, including tips for green household cleaning and tips for recycling, and find out where you can find green household products and green gifts. (Onuoha,2009).

Energy conservation will show the earliest payback in terms of CO2 reductions in many cases an investment in energy conservation made this year will show CO2 reductions this year, and every year thereafter. Because we've been living in a world of artificially cheap energy for decades, there are huge opportunities for energy conservation (Onuoha, 2009).

A Forestation has a vital role to play in the fighting against global warming. Forests absorb and store carbon in their trees and soil. But if forests are cleared or disturbed, this carbon is released as carbon dioxide and other greenhouse gases. Up to a fifth of global greenhouse gas emissions come from deforestation and forest degradation, (Akinbami, 2003).

The general Objective is to determine the perception of household on greening methods to ameliorate climate change in South West Local Government Area of Oyo State Nigeria .

Specific Objectives

1. To examine the socio-economic characteristics of the respondents in the study area.
2. To determine the perception of respondents on greening
3. To ascertain source of information on greening by the respondent
4. To identify constraints faced by respondents in the study area.

Hypotheses of the Study

$H_0$1: There is no significant relationship between socio economic characteristics and th greening method practiced

$H_0$2: There is no significant relationship between the constraints and greening method by the respondent

$H_0$3: There is no significant relationship between perception and greening method

II. METHODOLOGY

The study was carried out in Ibadan south West is a Local Government area in Oyo State, Nigeria. Its headquarters are at Oluyole Estate in Ibadan. It has an area of 40km^2 and a population of 282,585 (census 2006)The target population of this study were people living in Oluyole Estate and Oluyole Extension area of Ibadan South –West of Oyo State.

The data collection of this project work was primary source of data collection from the people living in Oluyole Estate and Oluyole Extension area of Ibadan South –West of Oyo State, with the use of personal interview and questionnaires due to the practicing of greening on climate improvement. Random sampling technique was used to carry out this research work in Ibadan South- West of Oyo State, with total number of 130 well-structured questionnaires were distributed and 91 were retrieved.

Respondent were asked to respond to some of the greening method practice base on 3point scale Eleven greening method question were listed, hence, the highest mean value obtained was 2.59 while the lowest mean value was 2.25.

III. RESULT AND DISCUSSION

The result revealed that male with 65.9% are into greening practices more than female 34.1%. This can be attributed to the fact that male headed household are more than household headed by female this in line with (Arnold, 2009). This implies that respondents of younger generation are more enlightening about climate change more than older generation. The result also reveal that 20.9% of the respondents are within age of 20-30, 34.1% of the respondents are within the range of 31-40, and 28.6% within the range of 41-50, 16.5% are within the range of 51 above, this result indicate that more adult are involve in greening practice than the youth and this would increase the knowledge of the greening practice. This project supports the work of (Madhur, 2006) that adults are into greening practice than younger people in Africa. The table also indicates that 65.9% of the respondents are married, 8.8% are widow and 25.3% of the respondents are single in the study area which shows that majority of the respondents are adult..

According to the result 58.2% have tertiary education, and 30.8% had secondary education, while 6.6% had primary education and 4.4% had no formal education. This shows that people within the study area are informed on the value of education and this will surely add to the knowledge the residents have on climate change, the level of education could also be as a result of enabling people to make decision regarding production and managing their lives

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successfully to cope with everyday problems and to realize their opportunities (Swanson 2008).

Finally, 76.9% of the respondents have1-5, 19.8% and between 6-10children, while 3.3% has above 10 children. This shows that respondents in the study area are interested in their family size.

The result on sources of information on greening climate change revealed that majority of the respondents heard about climate change from the scientist with mean value (3.70) likewise Radio which is mass media source of information. This work is in line with Adekunle (1996). The use of media to spread information like Radio, local TV and projector are best tools in disseminating information.

Furthermore, the result shows the mean value 3.34 of the respondents heard of climate change through Government agency this is in support of Enger *et al*., 2012 that government are also good source of transforming information to the citizen through the agencies.

Lastly, less people heard of climate change through friends with the mean value of 3.13 and mean value of 2.53heard of climate change through local council. This also support the study of Boz and Ozcatalbas (2010) which revealed that family members, neighbor friends, input providers and mass media were key sources of information in Nigeria. Table 3 shows that (76.92%) of the respondents have access to information on climate change in the study area while (23.08%) of the respondents had lesser information on climate change. This implies that the high source of information in the study area contribute to practice greening to ameliorate climate change.

Table.1: The Socio Economic Characteristics of the Respondents

Variable	Frequency (n=91)	Percentage
Gender		
Male	60	65.9
Female	31	34.1
Age		
20-30	19	20.9
31-40	31	34.1
41-50	26	28.6
51above	15	16.5
Religion		
Christian	71	78.0
Islamic	20	22.0
Marital Status		
Single	23	25.3
Married	60	65.9
Widow	8	8.8
Educational level		
No formal education	4	4.4
Primary	6	6.6
Secondary	28	30.8
Tertiary	53	58.2
Household		
1-5	70	76.9
6-10	18	19.8
10above	3	3.3

Source: Field survey, 2016.

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Table.2: Sources of Information on Greening Climate Change

Source of information	No	Rarely	Occasionally	Regularly	Mean value
Scientist	1 (1.1)	6 (6.6)	12 (13.2)	72 (79.1)	3.70
Radio	1 (1.1)	4 (4.4)	27 (29.7)	59 (64.8)	3.58
Newspaper	6 (6.6)	8 (8.8)	17 (18.7)	60 (65.9)	3.43
Television	7 (7.7)	6 (6.6)	20 (22.0)	58 (63.7)	3.41
Government agency	1 (1.1)	12(13.2)	33 (36.3)	45 (49.5)	3.34
Pastor/cleric	8 (8.8)	9 (9.9)	25 (27.5)	49 (53.8)	3.26
Environmental organization	10(11.0)	10(11.0)	25 (27.5)	46 (50.5)	3.17
Family/	4 (4.4)	20(22.0)	26 (28.6)	41 (45.1)	3.14
Friends	6 (6.6)	15(16.5)	31 (34.1)	39 (42.9)	3.13
Local council	20(22.0)	27(29.7)	19 (20.9)	25 (27.5)	2.53

Field survey, 2016.Percentage in parenthesis:

Table.3: Categorization of Sources of Information on Greening Climate Change

Level of Information	Frequency	Percentage
HIGH	70	76.92
LOW	21	23.08
TOTAL	91	100

Perception of Respondent on Greening

The result on the level of perception of the respondent in the study area to amelioration of climate change in Oluyole Local Government Area. it was revealed that majority of the respondent agreed that the human activities have effect about climate change with mean value of (3.51), this is in support of Zievogel and Easterling, 2006, which says that either directly or indirectly, human has effect on climate change in Nigeria. The result also showed that developing countries should take most of the blame for climate change with mean value (3.07) this is in line with Nwafor and Ologunorisa, 2007, that the available research shows that climate change is global, likewise its impacts, but the most agent in term of countries are developing once, due to improved technologies introduce to Nigeria.

Government should re- visit law governing the forestry management in Nigeria which has a mean value of (2.03), this in line with Enger *et. al* 2012 that government should provide adequate incentive and management of environment in Nigeria to combat climate change.

Lastly, the respondents agreed that they can all do their best to reduce climate change with least mean value of (1.51), this is in line with Birner and Allison (2006), that both men and women are entitled to modify their environment through the use of flowers, lawns, trees etc. to improve climate change in Nigeria.

Table 5 shows that there is low perception on greening (53.85%) of the respondents and there is low perception on greening (46.15%) of the respondents.

Table.4: Perception of Respondent on Greening

Variables	SA	A	U	D	SD	Mean value
Human activities have effect about climate change.	18 (19.8)	5 (5.5)	10 (11.0)	28 (30.8)	30 (33.0)	3.51
Developing countries should take most of the blame for climate change.	20 (22.0)	15 (16.5)	13 (14.3)	24 (26.4)	19 (20.9)	3.07
Having a car is part of good lifestyle.	17 (18.7)	28 (30.8)	13 (14.3)	16 (17.6)	17 (18.7)	2.86
Climate change is just a natural fluctuation in earth's temperature.	27 (29.7)	30 (33.0)	8 (8.8)	9 (9.9)	17 (18.7)	2.54
National government should limit industrial activities in the country.	25 (27.5)	30 (33.0)	11 (12.1)	18 (19.8)	7 (7.7)	2.47
I will only do my bit to reduce climate	19 (20.9)	47 (51.5)	3 (3.3)	14 (15.4)	8 (8.8)	2.39

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change if everyone else did as well.						
It is inevitable because of modern society work.	33 (36.3)	28 (30.8)	10 (11.0)	9 (9.9)	11 (12.1)	2.30
Humans are severely abusing the planet.	42 (46.2)	17(18.7)	17 (18.7)	4 (4.4)	11 (12.1)	2.17
Should government provide incentives for people to look after the environment as to reduce effect of climate change?	38 (41.8)	33 (36.3)	7 (7.7)	5 (5.5)	8 (8.8)	2.03
Government should re- visit law governing the forestry management in Nigeria.	39 (42.9)	28 (30.8)	10 (11.0)	10 (11.0)	4 (4.4)	2.03
Humans have the right to modify the natural environment to suit their needs.	47 (51.6)	27 (29.7)	5 (5.5)	9 (9.9)	3 (3.3)	1.83
Greening will improve weather	45 (49.5)	29 (31.9)	7 (7.7)	7 (7.7)	3 (3.3)	1.83
Do you think anything can be done to tackle greenish effect	39 (42.9)	42 (46.2)	3 (3.3)	6 (6.6)	1 (1.1)	1.76
we can all do our best to reduce climate change	60 (65.9)	22 (24.2)		2 (2.2)	7 (7.7)	1.51

Percentage in parenthesis source: Field survey, 2016

Table.5: Categorization of Perception on Greening

Perception	Frequency	Percentage
HIGH	42	46.15
LOW	49	53.85
TOTAL	91	100

Source: Field Survey, 2016

The Different Greening Method Practice by Respondent was measured where majority of the respondent agreed that the planting of trees is the best method with mean value (2.59) and this is in line with Biner,2006, that both men and women are entitled to modify their environment through the use of flowers, lawns, trees etc. to improve climate change in Nigeria. Also the use of planting tree crops in environment with mean value (2.58) will ameliorate climate change and serve as a fruits for the family as well likewise the management and development of forestry can also be used to reduce climate change which has a mean value (2.45) and this statements is in support of Adams 2011, that forestry management is of good benefit through the releasing of (O_2) oxygen to air for consumption of human being in relating to climate change in Nigeria.

Lastly, the use of solar energy to generate power has mean value of (2.27) and this can also reduce climate change which will reduce the use of generator in the environments and will minimize climate change from the release of carbon monoxide to the atmosphere and, stopping the use of chemical that can cause depletion of ozone to environment has mean value of (2.25). This also is in line with, Ogboi 2012, wherehe stated that activities of man emitted some poisonous elements such as carbons (Co_2 and Co) sulphur, methane, nitrogen oxide, chlophlorocarbon (CFCs) etc.

The result on constraints to greening practices gives a mean value of (1.63) where the majority of the respondents strongly disagree that farming activities is not affecting climate change but contributed to improvement of climate in Nigeria. Also majority of the respondent agreed with mean value (1.23) that poverty is also constraint, this result is in line with (Wolfe *et al*, 2005) that wide-ranging effects of climate change on many facets of human societies such as poverty, and human activities. Deforestation with mean value of (1.16) is a constraint facing climate change where it was clearly showed that deforestation is a part of challenge facing climatic change in Nigeria due to increase in urbanization, Adams 2011.

The least constraints were discussed where Industrialization has mean value of (1.12) which is part of human activities and lastly lack of good policy by federal Government which is drastically affecting human in terms of improving climate change which has mean value of (1.10).

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Table.6: To Examine the Different Greening Method Practice by Respondent

Variables	Rarely	Occasionally	Regularly	Mean value
By planting of trees	6 (6.6)	25 (27.5)	60 (65.9)	2.59
By planting of tree crops	5 (5.5)	28 (30.8)	58 (63.7)	2.58
By planting of flowers for beautification	11 (12.1)	19 (20.9)	61 (67.0)	2.54
By management and development of forestry	15 (16.5)	20 (22.0)	56 (61.5)	2.45
By reducing cutting down of trees	15 (16.5)	24 (26.4)	52 (57.1)	2.40
By establishing of lawn	15 (16.5)	24 (26.4)	52 (57.1)	2.40
The use of trees for fencing	14 (15.4)	27 (29.7)	50 (54.9)	2.39
By planting of vegetable garden	13 (14.3)	31 (34.1)	47 (51.6)	2.37
By planting of shrubs	13 (14.3)	36 (39.6)	42 (46.2)	2.31
The use of solar energy to generate power	11(12.1)	44 (48.4)	36 (39.6)	2.27
By stopping the use of chemical that can cause depletion of ozone layer	23 (25.3)	22 (24.2)	46 (50.5)	2.25

Percentage in parenthesis source: Field survey, 2016.

Table.7: Constraints to Greening Practices.

Variables	Yes	No	Mean value
Farming activities	33 (36.3)	58 (63.7)	1.63
Poverty	61 (67.0)	30 (33.0)	1.32
Environmental dynamic	69 (75.6)	22 (24.1)	1.24
High energy supply	70 (76.9)	21 (23.1)	1.23
Lack of commitment by people	74 (81.3)	17 (18.7)	1.18
Deforestation	76 (83.5)	15 (16.5)	1.16
Urbanization	77 (84.6)	14 (15.4)	1.15
Increased temperature	77 (84.6)	14 (15.4)	1.15
Industrialization	80 (87.9)	11 (12.1)	1.12
Lack of good policy by federal Government	81 (89.0)	10 (11.0)	1.10

Source: Field survey, 2016. **Percentage in parenthesis**

HYPOTHESES TESTING

Table.8: Socio-economic characteristics of respondents and their attitudes toward sustainable urban vegetable farming

Variable	χ^2 – value	p-value	Decision
Age	8.630	0.259	Not significant
Sex	0.546	0.077	Not significant
Religion	3.880	0.206	Not significant
Marital status	4.743	0.042	Significant
Education	3.866	0.063	Not significant
Household size	1.726	0.135	Not significant

Table.9: PPMC Analysis of the Constraints and Greening Method by the Respondents

Variable	r-value	p-value
Constraints and greening method by the respondents	.003	-.310

Source: field survey, 2016

The table above shows that there is no significant relationship between the constraint and greening method by the respondents where the r-value is .003 and p-value is -.310 which is greater at 5% level.

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Table.10: PPMC Showing Relationship between the Perception and Greening Method by the Respondents

Variable	r- value	p-value	Decision
Perception and greening method by the respondents	-0.400	0.000	S

Source: field survey, 2016

Significant at 5%

The table showed that there was significant relationship between constraints and greening method by the respondents ($p<0.000$, $r=0.400$). This implies that Ho_3 rejected

IV. CONCLUSION AND RECOMMENDATION

It was observed that information on climate change was majorly heard by scientist in the study area. Human activities have effect on climate change and we can all do our best to reduce climate change because both men and women are entitled to modify our environment. Planting of trees and planting of trees and planting of tree crops are the best method to ameliorate climate change and the use of solar energy is the least that people use because majority are using generator which add carbon monoxide to atmosphere

Planting of trees will reduce climate change also planting of lawns, flowers etc. Planting of tree crops such as orange, coconut, cashew etc. which will serve as fruit for family as well as improvement on climate change. Law should be provided to avoid climate change based on deforestation. Good policies should be put to practice by Federal Government base on industrialization in urban areas.

REFERENCES

[1] Adams R. M. (2011): Agriculture, forestry and related benefits of air pollution control. *Am J Agric. Econ* 68:885–894.

[2] Adekunle, O. O. (1996): Radio Listenership pattern and knowledge of improved farm practices (Unpublished B.Sc. Thesis). Department of Agricultural Extension and Services; University of Ibadan.

[3] Akinbami, J. (2003): "An Integrated Strategy for Sustainable Forest–energy–environment Interactions in Nigeria." *Journal of Environmental Management* 69.2 (2003): 115- 28.

[4] Birner. A.E and Allison, F.E. (2006): Extension service in African. *Journal of agriculture and Extension Service*. Vol. 8P p.13. Revised 24th may, 2015.

[5] Boz, I., Ozcatalbas,O. and Babu I (2010): Determining information sources used by crop producers: A case study of Gaziantep province in Turkey. *African Journal of Agricultural Research*. 10(15): 271-272.

[6] Jeremy H, (2008): Tropical Deforestation, is one of the worst crisis, since we come out of the caves. Retrieved from (source) www. Monga ba u...com (2011).

[7] Madhur, H. (2006): Information and communication technologies for rural development and food security: Lesson from field experiences in Developing countries. J. African Sci. Society Vol.5, pp. 747-754.

[8] Nwafor S.O. and Ologunorisa T.E (2007): Global climate change: The driver of multiple causes of flood intensity in sub- Saharan Africa. Paper presented at the international conference on climate change and economic sustainability herd at Nnamdi Azikwe University Enugu, Nigeria 12 – 14 June 2007.

[9] Ogboi K.C (2012): *An overview of climate change: causes processes and manifestations*. Paper presented on 14th edition of NITP/ TOPREC

[10] Onuoha, C.M. (2009): Climate Change and Sustainable Development in Nigeria: The Mitigating Role of Green Wall Sahara Nigeria Programme, *African Institute for Applied Economics (AIAE)* Enugu forum policy paper 10.

[11] Swanson, B. (2008): Global Review of Good Agricultural Extension and Advisory Service Practices. Rome: FAO. Vol.5 (10), 980-987. Retrieved on July 1st 2015.

[12] Wolfe D.W, MD Schwartz, A. Lakso, Y Otsuki, RM Pool and NJ Shaulis. (2005): Climate change and shifts in spring phenology of three horticultural woody perennials in northeastern USA. *Internet J Biometeorology* 49:303-309. Meteorological Organization, Geneva

[13] Ziervogel M, and Easterling, C (2006*): Climate variability and change. Implication for household food security. Assessments of impacts and adaption to climate change* (AIACC) working paper NO 20. January 2006. The AIACC project office, international START secretariat Washington DC, USA

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Study on Genetic Variability, Heritability, Genetic Advance and Correlation among different characters in tomato (*Solanum lycopersicum* L.)

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Abstract*—The present investigation entitled "Studies on genetic variability in tomato (Solanum lycopersicum L.)" was carried out at the UCOA, vegetable research farm, Guru Kashi University, Talwandi Sabo, Bathinda during rabi 2015-16 to evaluate tomato genotypes. The experiment was laid out in CRD with three replications. Total 20 genotypes including check cultivar were evaluated for horticultural Traits contributing yield and quality (suitable for processing) .There is a wide variability in different genotypes in tomato. Traits i.e. Number of primary branches per plant, Days to first fruit harvest, Plant height (cm),number of fruits per cluster, number fruits per plant, average fruit weight (gm), equatorial diameter of fruit (cm), polar diameter of fruit (cm), number of locules per fruit, pericarp thickness (mm), fruit pH, Fruit TSS (^{0}brix), days to last fruit harvest and average yield per plant (kg) were studied during the investigation Analysis of variance showed significant differences among genotypes for all the characters under study during the investigation. High Phenotypic and Genotypic coefficient of variation were detected for characters like number of fruits per plant, number of locules per fruit, pericarp thickness and average yield per plant. High heritability coupled with genetic gain were recorded for number of fruits per plant, average fruit weight, number of locules per fruit and average yield per plant. Therefore these characters also show some scope for improvement through selection. A highly significant and positive phenotypic and genotypic correlation were found in number of fruits per cluster, plant height, number of fruits per plant and average fruit weight.*

***Keywords*—Tomato, Genotypes. Acc number, Traits, Heritability, Locules.**

I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the member of family solanaceae. Tomato is one of the most popular vegetable grown all over the world both for fresh markets and processing industry. It is grown practically in open fields, green houses and net houses. Among the vegetable production it ranks third after potato and sweet potato, however it ranks first in first in processed vegetable. China followed by India, USA, Spain and Egypt are leading tomato producing countries. In India during the year 2015-16 according to 3rd advanced estimates the area under tomato cultivation is 760.0 thousand hectare with the production and productivity of 18399.0 thousand million tonnes and 24.2 million tonnes per hectare respectively . Whereas during the year 2014-15 the area under tomato cultivation was 767 thousand hectare and the production 16985.0 million tonnnes with the productivity of 21.4 million tonnes per hectare. In Punjab the area under tomato cultivation was 7.6 thousand hectare with the production and productivity of 181.1 million tonnes and 24.5 million tonnes per hectare respectively (Ministry of agriculture and farmers welfare, Govt of India). This is low as comparative to the average productivity globally.

Tomato being a self pollinated crop, it has a tremendous potential for heterosis breeding and it is used in different breeding programmes. Variability in tomato is expected to be immense as the fruits vary greatly in shape and size (Bhardwaj and Sharma, 2005). To improve the productivity of tomato, the primary consideration should be to bring out genetic improvement of the crop and development of superior varieties by selection among and within the population through the use of available genetic variability. As yield is the main objective of a breeder, it is important to know the relationship between various characters those contribute to the yield. The degree of relationship or

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association of these characters with the yield can be known by correlation studies. Genetic parameters such as Genotypic and Phenotypic coefficient of variation (GCV and PCV) are useful in detection of variability present in genotypes available. Heritability and genetic advance help in determining the influence of environment in determining the influence of environment in expression of the characters and the extent to which improvement is possible after selection (H.F. Robinson *et. al*., 1949). Therefore the investigation was carried out in tomato with the objective to estimate phenotypic coefficient of variation, genotypic coefficient of variation, heritability, genetic advance, correlation coefficient.

II. MATERIAL AND METHOD

The investigation was carried out during the rabi season of 2015-16 at the vegetable research farm of Guru Kashi University, Talwandi Sabo (Bathinda). The experimental material consisted of 20 genotypes along with check cultivar i.e. Punjab Chhuhara. The experiment was laid out in completely randomizes design (CRD) with three replications in each treatment. Plants were transplanted on 3rd Dec, 2015 at the plant to plant spacing of 30 cm in plot having size of 3.0 $m^{2,}$ accommodating 10 plants per plot. During the experiment data was recorded for 14 different characters. The Genotypic and phenotypic coefficients of variation were calculated as per the method suggested by Burton and De Vane (1953). Heritability (in broad sense) and genetic gain was calculated as per suggested by Allard (1960). Whereas correlation coefficient values were calculated as per given by Fishers and Yates (1963).

III. RESULTS AND DISCUSSION

3.1 Genetic variability

The analysis of variance indicated significantly higher amount of variability among the genotypes for all the characters studied during the investigation. This showed that there is a great scope foe selection of breeding material to initiate any breeding programmme for crop improvement. But to know the absolute extent of variability the phenotypic coefficient of variance and genotypic coefficient of variance was calculated.

The Phenotypic coefficient of variance was found higher magnitude than genotypic coefficient of variance for all the character under the study, though the difference was very less under the majority of the cases. Phenotypic coefficient of variance was high for character like number of fruits per plant (40.92%), Number of locules per fruit (38.05%), Average fruit yield per plant (37.52%, Pericarp thickness (32.81%) and while moderate for No of fruits per cluster (28.59%), Average fruit weight (28.56%), No of primary branches per plant (23.09%), Polar diameter of fruit (19.07%), Plant height (18.58) and Fruit TSS (18.07). Low values of phenotypic coefficient of variation were observed in Equatorial fruit diameter (12.36%), Fruit pH (5.15), Days to first fruit harvest (3.62%), Days to last fruit harvest (3.34%). Whereas Genotypic coefficient of variance was high Genotypic coefficient of variation (Table 4.3) was high for characters like No of fruits per plant (35.88%), Average yield per plant (35.88%) and No of locules per Fruit (34.17%), while moderate for Average fruit weight (28.20%), Plant height (18.11%), Pericarp thickness (16.87%), Polar diameter of fruit (16.76%), No of fruits per cluster (15.62%) and Fruit TSS (15.37%). Low values of phenotypic coefficient of variation were observed in No of primary branches per plant (13.73%), Equatorial diameter of fruit (9.10%), Fruit pH (3.42%), Days to first fruit harvest (3.36%) and days to last fruit harvest (2.91%).

3.2 Heritability

The estimates of heritability varied from 26.43 to 97.52 % for different characters under study (Table 4.3). It was high for characters like Average fruit weight (97.52%), Plant height (95.06%), Number of fruits per plant (94.64%), Average yield per plant(91.46%), Days to first fruit harvest (85.80%) and No of locules per fruit (80.65%), while moderate for Polar diameter of fruit (77.26%), Days to last fruit harvest (75.80%), fruit TSS (72.28%) and Equatorial diameter of fruit (54.69%). Low values of Heritability were observed in Fruit pH (44.13%), Number of fruits per cluster (36.40%), No of primary branches per plant (35.37%) and Pericarp thickness (26.43%).

3.3 Genetic advance and genetic gain

The genetic gain (genetic advance expressed as percentage of population mean) was low to high in nature and ranged from 4.62 to 79.20 % (Table 4.3).High genetic gain was recorded for Number of fruits per plant (79.20%), Average yield per plant, Number of locules per fruit (63.21%) and average fruit weight (57.38%), while moderate for Plant height (36.38%), Polar diameter of fruit (30.36%) and Fruit TSS (26.91%). Low values of genetic gain were observed in number of fruits per cluster (19.41%), Pericarp thickness (17.86%), Number of primary branches per plant (16.82%), Equatorial diameter of fruit (13.86%), Days to first fruit harvest (6.40%), Days to last fruit harvest (5.21%) and Fruit pH (4.68%). Kumar *et al* (2013) also reported High phenotypic coefficient of variability (PCV), genotypic coefficient of variability (GCV) and heritability estimates

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coupled with high genetic gain were recorded for number of fruits per plant, yield per plant and fruit weight.

These results were found in accordance with Bangaru *et al.* (1983) Reported high GCV and PCV for number of fruits per plant. Mittal *et al.* (1996) who observed high heritability along with high genetic advance in number of fruits per plant. Aysh *et al.*(2012) observed Highest GCV and PCV for number of fruits per plant. High heritability for Fruit weight, number of locules per fruit and fruit yield was reported by Golani *et al.* (2007).

Table.1: Range, Mean and Genetic parameters for different characters under study in tomato

S.No	Characters	Range		Mean	PCV (%)	GCV (%)	Heritability (%)	Genetic advance	Genetic gain (%)
		Max	Min						
1.	Number of primary branches	3.0	6.0	4.62	23.09	13.73	35.37	0.78	16.82
2	Days of 1st fruit harvest	126.33	149.66	140.07	3.62	3.36	85.80	8.97	6.40
3	Plant height	51.00	95.33	74.44	18.58	18.11	95.06	27.08	36.38
4	Number of fruits per cluster	2.33	4.33	3.14	25.89	15.62	36.40	0.61	19.41
5	Number of fruits per plant	14.00	67.00	32.31	40.62	35.88	94.64	25.59	79.20
6	Average fruit weight (g)	28.0	68.38	42.88	28.56	28.20	97.52	24.61	57.38
7	Total yield per plant (kg)	0.58	2.14	1.31	37.52	35.88	91.46	0.94	70.69
8	Equatorial diameter of fruit (cm)	3.76	5.46	4.55	12.36	9.10	54.69	0.63	13.86
9	Polar diameter of fruit (cm)	3.50	6.23	5.00	19.07	16.76	77.26	1.52	30.36
10	No. of locules per fruit	2.00	5.66	3.04	38.05	34.17	80.65	1.93	63.21
11	Pericarp thickness (mm)	4.00	9.33	5.72	32.81	16.87	26.43	1.02	17.86
12	Fruit pH	3.56	4.16	3.87	5.15	3.42	44.13	0.18	4.68
13	Fruit TSS (Brix)	2.80	5.36	3.86	18.07	15.37	72.28	1.04	26.91
14	Days to last fruit Harvest	153.33	175.67	166.73	3.34	2.91	75.80	8.69	5.21

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3.4 Studies of correlation.

The correlation studies carried out during the investigation show that the in general the genotypic correlations were high than that of phenotypic correlation. In the investigation it was analysed that on the basis of phenotypic correlations among 14 characters (Table 2) showed that fruit yield per plant had positive and significant association with number of fruits per plant (0.7397), plant height (0.4215), number of fruits per cluster (0.4410), average fruit weight (0.3101) and polar diameter (0.2637). However, it showed significant negative correlation with number of day to first harvest (-0.2795) and pericarp thickness (-0.2688).

Number of fruits per cluster had significant positive correlation with number of fruits per plant (0.4477).Number of primary branches had significant positive correlation with fruit TSS (0.3207)and day to first harvest (0.2534), it showed significant negative correlation with fruit pH (-0.3033).Days to first fruit harvest had significant positive correlation with days to last fruit harvest (0.8538) and no. of locules per fruit (0.2621) and negative correlation with no. of fruits per plant (-0.4599).Plant height had significant positive correlation with equitorial diameter of fruit (0.3441), average fruit weight (0.3092), no. of locules per fruit (0.2893) and negative correlation with fruit pH (-0.2613).Number of fruit per plant had significant negative correlation with average fruit weight (-0.3599) and days to last fruit harvest (-0.2705).Average fruit weight had significant positive correlation with polar diameter (0.5991) and equatorial diameter (0.5778). Equitorial diameter had significant positive correlation with no. of locules per fruit (0.4568), polar diameter (0.2978), and fruit TSS (0.2952) and fruit pH (0.2652). Polar diameter had significant negative correlation with no. of locules per fruit (-0.5133).No. flocules per fruit had significant positive correlation with fruit TSS (0.3473).

Whereas the study of genotypic correlations among 14 characters under investigation show that fruit yield per plant had positive and significant association with number of fruits per cluster (0.6489), plant height (0.4390), average fruit weight (0.3148) and polar diameter (0.2736). However, it showed significant negative correlation with number of day to first harvest (-0.2956) and pericarp thickness (-0.5330).

Number of fruits per cluster had significant positive correlation with number of fruits per plant (0.7002), pericarp thickness (0.5379), fruit pH (0.3348), plant height (0.3271) and no. of locules per fruit (0.2571).Number of primary branches had significant positive correlation with fruit TSS (0.5322), day to first harvest (0.4785), plant height (0.3777), average fruit weight (0.3353) and days to last fruit harvest (0.3114), it showed significant negative correlation with fruit pH (-0.5793) and no. of fruits per plant (-0.3304).Days to first fruit harvest had significant positive correlation with days to last fruit harvest (0.9072), fruit TSS (0.3068), equatorial diameter (0.2745) and no. of locules per fruit (0.2626) and negative correlation with polar diameter (-0.2743).Plant height had significant positive correlation with equitorial diameter of fruit (0.4306), no. of locules per fruit (0.3394) and average fruit weight (0.3335) and negative correlation with fruit pH (-0.4334).Number of fruit per plant had significant negative correlation with average fruit weight (-0.3648) , equatorial diameter (-0.3283) and days to last fruit harvest (-0.3124).Average fruit weight had significant positive correlation with polar diameter (0.6501) and equatorial diameter (0.7342). Equitorial diameter had significant positive correlation with no. of locules per fruit (0.6150), fruit pH (0.4399) and fruit TSS (0.4022). Polar diameter had significant positive correlation with fruit pH (0.2705) and negative correlation with no. of locules per fruit (-0.6025), pericarp thickness (-0.5960) and days to last fruit harvest (-0.3092).Number of locules per fruit had significant positive correlation with fruit TSS (0.5333) and pericarp thickness (0.4258).Pericarp thickness had significant positive correlation with fruit TSS (0.3710). The estimates of genotypic and phenotypic correlation coefficients imparted that the genotypic correlation were higher magnitude than the corresponding phenotypic ones for most of the character combinations , thereby establishing predominant role of heritable factor.

The results those were carried out for correlation studies during the invest tigation were found to be in accordance with Singh and Cheema (2006) they observed that genotypic correlations were of higher magnitude than the corresponding phenotypic correlation values for most of the character combinations in tomato which were similar to results of correlation among different characters in the investigation carried out. The results also corroborated with the results carried out by Pradheep *et al* (2007) for correlation for fruits per plant, fruit weight and fruit yield. The results were also found to be in accordance with those of Shushay et *al* (2014) fruit yield and number of fruits per plant. The results for correlation studies were also in accordance the results carried out by Golani *et al*. (2007) for number of primary branches and number of locules per fruit.

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Table: 2 Phenotypic and Genotypic correlation of different characters of tomato

Characters		No fruits per cluster	No primary branches	Days to first harvest	Plant height	No of fruits per plant	Average fruit weight (g)	Equatorial diameter (cm)	Polar diameter (cm)	No of locules per fruit	Pericarp thickness (mm)	Fruit pH	Fruit TSS (Brix)	Days to Last fruit harvest	Average yield per plant (kg)
No fruits per cluster	P		-0.1501	-0.0921	.01728	0.4477**	-0.386	0.1086	0.0184	0.1239	0.1334	-0.0174	-0.0537	-0.0579	0.4410**
	G		-0.4429**	-0.1235	0.3271*	0.7002**	-0.0995	0.1833	-0.0110	0.2571*	0.5379**	0.3348**	-0.0276	-0.0420	0.6489**
No primary branches	P			0.2534*	0.2145	-0.2025	0.1810	-0.0276	-0.0481	0.0986	0.1158	-0.3033*	0.3207*	0.1711	-0.0277
	G			0.4785**	0.3777**	-0.3304**	0.3353**	0.1008	-0.1113	0.1843	0.1589	-0.5793**	0.5322**	0.3114*	-0.0525
Days to first harvest	P				0.0817	-0.4599**	0.1556	0.1421	-0.2116	0.2621*	0.0762	0.0375	0.2255	0.8538**	-0.2795*
	G				0.0920	-0.2120	0.1789	0.2745*	-0.2743*	0.2626*	0.1938	0.0204	0.3068*	0.9072**	-0.2956*
Plant height	P					0.1166	0.3092*	0.3441**	-0.0371	0.2893*	-0.0495	-0.2613*	0.0547	0.0462	0.4215**
	G					0.1083	0.3335**	0.4306**	-0.0734	0.3394**	-0.0758	-0.4334**	0.0640	0.0707	0.4390**
No of fruits per plant	P						-0.3599**	0.2383	-0.1331	0.0872	-0.1201	-0.1183	-0.0786	-0.2705*	0.7397**
	G						-0.3648**	-0.3283**	-0.1438	0.0794	-0.2300	-0.1315	-0.0728	-0.3124**	0.2487
Average fruit weight (gm)	P							0.5778**	0.5991**	0.0557	-0.1678	-0.0179	0.1117	0.0752	0.3101*
	G							0.7342**	0.6501**	0.0744	-0.4599**	-0.0551	0.1059	0.0794	0.3148*
Equatorial diameter (cm)	P								0.2978*	0.4568**	0.1183	0.2652*	0.2952*	0.0990	0.1809
	G								0.1424	0.6150**	-0.0317	0.4399**	0.4022**	0.1071	0.1840
Polar	P									-	-0.2148	0.1655	-0.0834	-0.2009	0.2637*

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diameter (cm)										0.5153**					
	G									-0.6025**	-0.5960**	0.2705*	-0.2075	-0.3092*	0.2736*
No of locules per fruit	P										0.0485	0.0140	0.3473**	0.1975	0.1327
	G										0.4258**	0.0890	0.5333**	0.1712	0.1497
Pericarp thickness (mm)	P											0.1301	0.2187	0.0386	-0.2688*
	G											-0.3612	0.3710**	0.1228	-0.5330**
Fruit pH	P												0.0107	0.0265	-0.1605
	G												0.0806	-0.0737	-0.1985
Fruit TSS (Brix)	P													0.1306	0.0029
	G													0.2214	0.0141
Days to Last fruit harvest	P														-0.1098
	G														-0.1235

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REFERENCES

[1] Allard, R.W., 1960. Principle of Plant Breeding. John Wiley and Sons Inc., New York, USA.

[2] Aysh, Al F, Serhan Al M, Shareef Al A, Nasser Al and Kutma M H. (2012). Study of genetic parameters and character interrelationship of yield and some yield components in tomato (*Solanum lycopersicum* L.). *International Journal of Genetics* **2**(2): 29-33.

[3] Bangaru, C., Muthukrishnan, C.R and Irulappan, I (1983) Genetic variation in F_2 generation of tomato. *Madras agric. J*. **70**:349-350.

[4] Bhardwaj, N.V., Sharma, M.K. 2005: Genetic parameters and character association in tomato. *Bangladesh Journal of Agricultural Research*, **30** (1) 49-56.

[5] Burton, G.W and De Vane, E.H (1953).Estimating heritability in tall fescue (*Festuca arundinancce*) from replicated clonal materials. *Agron. J*.**45**:515-518.

[6] Fisher, R. A., and Yates, Y., 1963. *Statistical tables for biological, agricultural and medicinal research. Edinberg*: Oliver and Boyd, Ltd.

[7] Golani, I.J., Mehta, D.R., Purohit, V.L., Pandya, H.M. and Kanzariya, M.V., 2007: Genetic Variability, Correlation and path coefficient studies in tomato. *Indian journal of agricultural research*, **41**(2): 146-149

[8] H. F. Robinson, R. E. Comstock and P. H. Harvey, 1949: Estimates of heritability and degree of dominance in corn, *Agronomy Journal*, **41**, 253-259

[9] Ministry of agriculture and farmers welfare, Govt. of India

[10] Mittal, Pankaj, Parkesh, Sant and Singh, A.k. (1966). Variability studies in tomato (*Lycopersicon esculentum* Mill) under sub humid conditions of Himachal Pradesh. *South Indian Horticulture*, **44**(5&6): 132-134.

[11] Pradheep, K., Veeragavathatham, D. and Auxcilia (2007). Correlation analysis in tomato (*Lycopersicon esculentum* Mill) with emphasis on virus resistance. *South Indian Horticulture,* **55** (1-6): 12-19

[12] Singh, H. and cheema, D.S (2006). Correlation and path coefficient studies in tomato. *Haryana J. Hortic. Sci*., **35**(1&2):126-129

[13] Shushay .C and Haile Z., 2014. Evaluation of tomato varieties for fruit yield and yield components Northern Ethiopia. *Int. J. of .Agri. Res.,* **10**:23-39.

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Evaluation of herbicides and their combinations for weed control in wheat (*Triticum aestivum* L.)

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Abstract— *The field experiment was conducted at the Student's Research Farm, P.G. Department of Agriculture, Khalsa College, Amritsar, Punjab, to study the evaluation of various herbicides and their combinations on wheat during 2016-17. The experiment was laid out in randomized block design with seven treatments such as weed free, weedy check, pendimethalin 2.5L/ha, pendimethalin 2.5L/ha + clodinofop 400 g/ha, pendimethalin 2.5L/ha + sulfosulfuron 32.5g/ha , pendimethalin 2.5L/ha + pinoxaden 1000 ml/ ha, pendimethalin 2.5L/ha + atlantis 400g/ha and replicated thrice. Results revealed that pendimethalin 2.5 L/ha + atlantis 400g/ha was found effective to control weed population and produced higher number of grains per ear and enhanced grain yield upto 62.3 per cent over weedy check.*

Keywords—Herbicides, Weed control, Wheat, Yield.

I. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important winter cereal crop of Punjab and staple food for millions of people in India and across the world. Regardless of all the other ways of crop yield enhancement, weed control is one of the important key factors in crop yield improvement particularly in Amritsar districts to cope with the annual weed population blast. Weeds compete with crop for available moisture, nutrients, space, light and provide shelter for harmful insect-pests which result in yield reduction. Weeds cause yield reduction upto 15-50 percent depending upon the weed density and weed flora (Jat *et al*.2003). Weeds not only reduce yield but also lower the quality of the produce and increases the cost of harvesting, threshing and cleaning. Apart from improved agronomic practices and preventive measures, chemical weed control is one of the important key factors to enhance the wheat production and productivity. Most of the farmers cultivating wheat crop in the region have a psychological competition among other farmers to eliminate weeds in their respective field. Therefore, adding over dose of chemical herbicides which develops resistance in various weed species associated with wheat crop. Moreover when they use combination of various herbicides it further aggravates economics of small farmers. Therefore, some suitable and judicious herbicidal combinations may check to prevent environmental pollution and to human health issues. Several combinations of herbicides are there that can provide good control of broad - and narrow- leaved weeds and cause significant reduction in their density and increases the yield as compared to weedy check (Chaudhary *et al.* 2008). Therefore, an experiment was carried out on various herbicides generally used in wheat to evaluate their impact on weed control in combinations and to assess the efficacy of the herbicides on grain yield of wheat.

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II. MATERIALS AND METHODS

The experiment was conducted at Students' Research Farm, Khalsa College, Amritsar during *rabi* season of 2016-17. Amritsar is located at 31° – 38' North latitude and 74° - 52' East longitude and at an altitude of 236 meters above mean sea level. Maximum temperature ranged between 14.9 ℃ and 41.2 ° C while minimum temperature ranged between 1.9 ^{0}C and 23.3^{0}C during this season. The soil of experimental site was sandy loam having pH 7.8, medium in organic carbon (0.49%), low available N (164.5%), high available P (31.7%) and high available K (347.5%). The wheat variety '*WH 1105'* was sown at 22.5cm spacing on 5th November 2016. The experiment was laid out in randomized block design with eight treatments and replicated thrice. The gross plot size was 4.5m x 4.5m. Herbicides were applied with knapsack sprayer. Pendimethalin was applied as pre-emergence at two days after sowing, while clodinofop, sulfosulfuron, pinoxaden and atlantis were applied as post-emergence at 32 days after sowing (DAS). The weed density and dry weight of narrow- leaved weeds and broad-leaved weeds were analyzed using transformation of square root *i.e* ($\sqrt{x+1}$), before carrying out analysis of variance and comparison were made on transformed values (Table 1).

III. RESULTS AND DISCUSSION

The data recorded were weed density (number/m^2), weed dry matter (g/m^2), grains /spike and grain yield (kg/ha). Increase in yield over weedy check was calculated for all the treatments.

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Effect on weeds

All the weed control treatments significantly reduced the weed density and dry matter of weeds (Table 2). Pre-emergence application of pendimethalin (30 EC) 2.5 L/ha along with the post-emergence application of atlantis 400 g resulted in the lowest weed density and weed dry matter. The better performance of this treatment might be attributed to the effective control of narrow-leaved weeds and broad-leaved weeds by pendimethalin along with atlantis. This was statistically similar to pendimethalin *fb* + sulfosulfuron, pendimethalin *fb* + clodinafop and pendimethalin *fb* + pinoxadin. The highest weed density and weed dry weight were recorded in weedy check. These results are in conformity with the findings of Kailkhura *et al*. (2015) that herbicidal combinations of pre-emergence application followed by post-emergence application were found most effective in controlling weed infestation.

Effect on crop

Among the yield components number of grains per spike is essential parameter for assessment of the impact of weed control treatments on yield. Increasing the number of grains per spike will increase the weight of the spike which in turn definitely improves the final yield (Hussain *et al*. 2013). Among the herbicidal treatments the highest number of grains per skike (43) were observed with combination of pre-emergence application of pendimethalin (30 EC) 2.5 L/ha along with post-emergence application of atlantis 400 g/ha. Whereas the lowest grains per spike was observed in weedy check (31.6). All the weed control measures resulted in significantly higher grain yield than weedy check. Weed free recorded the highest values of grain yield, may be due to least competition offered by weeds. Among herbicidal treatments combination, highest grain yield (5773 kg/ha) was recorded with pre-emergence application of pendimethalin 2.5 L/ha *fb* post-emergence application of atlantis 400 g/ha (Table 3). The higher grain yield may be due to the reduced weed competition and thereby increased crop growth. At the initial stage, pre-emergence application of pendimethalin controlled narrow and broad leaved weeds efficiently and succeeding weeds were control by atlantis, which offers broad range weed control. Similar results were reported by Khalil *et al*. (2013).

It was summarized that combination of pre-emergence application of pendimethalin 2.5L/ha with post-emergence application of atlantis 400 g/ha may be recommended for managing composite weed flora and obtaining higher yield in the wheat crop.

REFERENCES

[1] Chaudhary, S., Hussain, M. and Iqbal, J. 2011. Chemical weed control in wheat under irrigated conditions. *Journal of Agriculture Research* **49**(3) : 353-361.

[2] Hussian, Z., Marwat, K.H., Munsif, F., Samad, A. and Ali, K. 2013. Evaluation of various herbicides and their combinations for weed control in wheat crop. *Pakistan Journal of Botany* **45**(1): 55-59.

[3] Jat, R.S., Nepalia, V. and Chaudhary, P.D. 2003. Influence of herbicide and methods of sowing on weed dynamics in wheat (*Triticum aestivum*). *Indian Journal of Weed Science* **35**: 18-20.

[4] Kailkhura, S., Prataf, T., Singh, V.P., Guru, S.K. and Singh, S.P. 2015. Herbicide combinations for control of complex weed flora in transplanted rice. *Indian Journal of Weed Science* **47**(4): 414-416.

[5] Khalil, M.F., Hussan, G., Ahmad, G., Anwar, S. and Khan, S. 2013. Comparative efficacy of herbicides on yield and yield components of wheat (*Triticum aestivum*). *APRN Journal of Agricultural Biological Science* **8**(1): 76-80.

Table.1: Details of the herbicidal treatments of the experiment.

Treatments	Rate /ha	Time of application
Weed free	-	-
Weedy check	-	-
Pendimethalin (30EC)	2.5 L	Pre-em
Pendimethalin (30 EC) *fb*+ Clodinafop(15 WP)	2.5L *fb* 400 g	Pre *fb* Post-em
Pendimethalin(30EC) *fb* +Sulfosulfuron(75WG)	2.5 L *fb*32.5 g	Pre *fb* Post-em
Pendimethalin (30 EC) *fb* +Pinoxaden(5 EC)	2.5 L*f* 1000ml	Pre *fb* Post-em
Pendimethalin (30 EC) *fb* +Atlantis (3.6 WDG)	2.5 L*fb* 400 g	Pre *fb* Post-em

Pre-em= Pre-emergence, Post-em= Post-emergence, *fb*= followed by

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Table.2: Effect of different weed control treatments on weed density (number/m^2) and dry matter of weeds (g/m^2) in wheat.

Treatments	Weed density / m^2		Dry matter of weeds (g/m^2)	
	Narrow leaved weeds	Broad leaved weeds	Narrow leaved weeds	Broad leaved weeds
Weed free	1(0)	1(0)	1(0)	1(0)
Weedy check	7.6(58.2)	8.3(68.4)	14.8(221)	16.5(273)
Pendimethalin	2.3(4.44)	2.7(6.54)	6.7(44.5)	9.5(91.0)
Pendimethalin *fb* Clodinafop	1.5(1.42)	2.0(3.04)	3.9(14.7)	4.8(22.7)
Pendimethalin *fb* Sulfosulfuron	1.7(1.93)	1.8(2.26)	4.4(19.2)	3.7(13.1)
Pendimethalin *fb* Pinoxaden	1.6(1.75)	2.0(3.42)	4.0(15.4)	4.9(23.6)
Pendimethalin *fb* Atlantis	1.4(1.14)	1.7(2.01)	3.6(12.5)	3.5(11.6)
LSD (p=0.05)	0.34	0.44	1.28	1.56

Original data given in parenthesis was subjected to square root (+ 1) transformation before analysis. *fb*=followed by

Table.3: Effect of different weed control treatments on number of grains per spike, grain yield (kg/ha) and percent increase in yield of wheat.

Treatments	Grains/spike	Grain Yield (kg/ha)	Yield increase over weedy check (kg/ha)	Percent Increase
Weed free	43.1	5889	2259	38.35
Weedy check	31.6	3630	-	-
Pendimethalin	39.0	5107	1477	28.92
Pendimethalin *fb* Clodinafop	42.2	5663	2033	35.89
Pendimethalin *fb* Sulfosulfuron	42.9	5706	2076	36.38
Pendimethalin *fb* Pinoxaden	42.0	5591	1961	35.07
Pendimethalin *fb* Atlantis	43.0	5773	2143	37.12
LSD (p=0.05)	2.52	355		

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Effect of Compost Extract Fortified with Tempe on Chili Mosaic Virus Disease

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***Abstract*—** *Mosaic disease caused by multiple infections of viruses in one of the most devastating virus diseases of chili pepper (Capsicum annuum) in Indonesia. Improving plant resistance by treatment with exogenous bioactive compounds is promisingly developed for plant protection in organic chili production. We demonstrated the suppressive effects of a fermented water extract of compost fortified with over-fermented tempe (TCE) on mosaic disease and its aphid vector. TCE was applied weekly by foliar spraying at 0.2 and 2.0 % on potted Capsicum growing in field. The result showed that treated plant sprayed with TNF preparation exhibited a significantly slower disease progression as represented by a lower area under disease progress curve compared to control plant. Disease suppression was obtained at concentration as low as 0.2%. TCE-treated plants were significantly less colonized by Aphis gossypii than control plant.*

***Keywords*—Amino acid, Aphis gossypii, compost tea, cucumber mosaic virus, fermented water extract of compost.**

I. INTRODUCTION

Mosaic virus diseases are widely distributed and cause significant losses on chili pepper throughout the world. In Indonesia, mosaic virus diseases was mainly associated with multiple infection of *Cucumber mosaic virus* (*CMV*), *Chili veinal mottle virus* (*ChiVMV*), *Tobacco mosaic virus* (*TMV*), *Pepper yellow leaf curl virus* (*PYLCV*) and *Pepper vein yellowing virus* (*PeVYV*) (Putra *et al*., 2015). Infected plants show dwarf symptoms and produce curling leaf, mosaic and yellowing. Infection during early growth stage causes dwarfing and dropped flower, fail to produce fruit and can cause total losses. Infection during generative stage causes plant to produce small fruit, hard and have no market value. Yield loss due to viral infection is depending on growth stage and type of symptom. Chili plants with yellowing and mosaic symptom produced 80-84% less yield compared to those of healthy plant (Sukada *et al*., 2014).

The use of resistant plants is one of the most efficient, sustainable and frequently employed strategies to control virus infections in fields (Nicaise, 2014). One approach to improving plant resistance to virus disease is to use of resistance inducers of natural origin including living microorganisms, plant extracts, microbial cell-wall extracts, microbial metabolites, minerals, and ions (Aranega-Bou *et al*., 2014; Llorens *et al*., 2017; Siah *et al*., 2018). Numerous studies have demonstrated that plant disease resistance can be induced by treating plant surfaces with a variety of water-based compost preparations, referred to in the literature as compost extract or compost tea (Zhang *et al*., 1998; Al-Mughrabi *et al*., 2008; Siddiqui *et al*., 2009; St. Martin, 2014).

However, compost extract had been reported to have no or a minor control efficacy against viral disease (Kouyoumjian, 2007). Fortification of compost extract may increase its benefit to improve plant resistance against viral infection. Suppression against CMV infection on cigar tobacco was achieved after fortification of a compost extract from vegetable wastes with siderophores-producing *Pseudomonas aeruginosa* Ch1 as studied in a greenhouse test (Wahyuni *et al*., 2010). Fortification of compost with shrimp products resulted in an increase in amino acids content of compost extract (Suwandi, 2013). Soil drench, trunk and foliar spray using the compost extract preparation have known to improve plant tolerance against salt stress (Suwandi *et al*., 2014) and to recover plant from physiological stress (Suwandi *et al*., 2018). Over-fermented tempe is one of the inexpensive and abundant source of amino acids in Indonesia that contains more than 19% of total amino acids (Utami *et al*., 2016). This study examined the potential used of a fermented water extract of compost fortified with water extract of over-fermented tempe (TCE) to control mosaic disease and its vector *Aphis gosypii* on potted chili pepper under field condition.

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II. MATERIAL AND METHODS

2.1 Plant material

CMV free-certified chili cultivar F1 Lado was used throughout experiment. Seedling was prepared on insect-free growth room for 3 weeks. Seedlings were transplanted to a- 15L-black polyethylene bag (polybag) filled with top soil in mixture with fortified compost (N: 1.0 P: 6.1 K: 5.2 Mg: 8.6). Polybags were placed in 70-cm spacing in the experiment field of Faculty of Agriculture, Sriwijaya University. Plants were fertilized weekly with 250 mL/plant using 1% (w/v) a NPK 16-16-16 fertilizer. No pesticides were used during the experiment and weeds were cleaned manually.

2.2 Compost extract and treatment

Compost extract preparation was produced by fermentation of shrimp waste-enriched compost extract (SWCE) (Suwandi, 2013; Suwandi *et al*., 2018) in mixture with 20% (v/v) water extract of over-fermented tempe and 5% (w/v) sucrose. The entire brewer contents were vigorously shaking by hand and then left to ferment in a plastic bottle at ambient temperature for 7 days. Three types of preparations, TF, TNF and TFJK were used in the study. TF and TNF used tempe obtained from different production locations in South Sumatra. Juice of *Citrus amblycarpa* (Hassk.) Ochse (jeruk limau) at 5% (v/v) was mixed in the TF to produce TFJK preparation. The preparation was applied at concentration 0.2 and 2.0% by spraying at dosage 600 L/Ha started from one week after transplanting at one week interval.

2.3. Assessment of disease and aphid colonization

Both disease incidence and severity were measured in the study. Incidence of naturally diseased leaves were counted weekly and calculated as percentage of leaves showing typical mosaic symptom out of total leaves per plant. Severity of naturally occurring mosaic disease was evaluated weekly on a 0-to-5 scale as follows: 0 = no symptoms; 1 = mild deformation and mosaic of the youngest two leaves; 2 = pronounced leaf deformation and mosaic of the youngest two leaves, with progression of symptoms into sequentially older leaves; 3 = pronounced leaf deformation and mosaic progression beyond the two youngest leaves, with all leaves expressing some form of virus-induced symptoms; 4 = similar symptoms as described for a rating of 3, with plants also being stunted in growth (where stunting includes both reduced internode extension and smaller leaves); and 5 = severe stunting, with the majority of leaves being small, severely deformed, and tightly bunched together (Lee and Ryu, 2016). All plants in each treatment were scored; the ratings totaled and were divided by the number of plants multiplied by 5 to give a disease index in percent. Disease progression of each treatment was compared based on area under disease progress curve (AUDPC). The AUDPC of mosaic severity was calculated by using the formula suggested by Simko and Piepho (2012).

Aphid species colonizing the tested plant during experiment were identified according to Blackman and Eastop (2000). Number of aphids was recorded weekly starting from 7 days after transplanting.

2.4 Experimental design and data analysis

Experiment was arranged in a completely randomized block factorial design with 15 replications (3 preparations of compost extract × 2 concentrations + one control treatment). Data were analyzed using Proc Glimmix in SAS University Edition 2.7 9.4 M5 (SAS Institute Inc., Cary, NC, USA).

III. RESULTS

3.1 Disease development

Effect of compost extract treatment on development of mosaic disease was assessed based on incidence of diseased leaves and severity of diseased plants. Incidence of diseased leaves (Fig. 1) and mosaic severity (Fig. 2) were less progressive observed on compost extract treated plot compared to that of water treated plot. Higher disease suppression was exhibited by treatment with TNF preparation and the suppression was similar between a higher concentration (2.0%) and lower concentration (0.2%). Suppression of disease incidence and severity was starting to observe as early as one week after spraying and continuing to a same manner as increasing of plant ages. Regardless of treatments, disease was developed well after 5 weeks of transplanting. Disease protection by TNF and TFJK preparation was prominently observed until 5 weeks after transplanting as restricted disease was found on treated plants. TF preparation induced a similar level of disease progress compared to control, particularly until 5 weeks after transplanting, although a slight suppression of disease was later observed after 6 weeks transplanting. Overall development of diseased severity incidence was less progressive compared to that of disease incidence (Figs. 1 and 2).

Anova of AUDPC of mosaic severity showed that compost extract treated plants resulted in significantly (P<.0001) lower than water treated plant. There was a significant different (P main effect of preparation types =0.0005) between three preparations used. AUDPC was significantly lower on plants treated with TNF compared than TF and TFJK. Increased concentration from 0.2 to 2.0% did not significantly affect AUDPC (P main effect of concentration =0.0561) of mosaic severity. There was no significant interaction (P=0.6544) between concentration and types of preparation. Based on AUDPC, suppression of disease progress was ranged from 29.6 to 75.4%, respectively. Significant lower

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AUDPC or highest suppression of AUDPC was obtained after application with TNF preparation regardless of the concentration (Table 1).

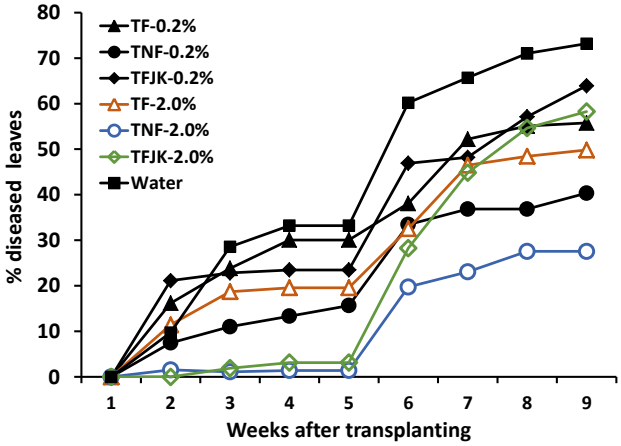

Fig.1: Incidence of diseased leaves on Capsicum annuum sprayed weekly with a variety of fortified-compost extracts

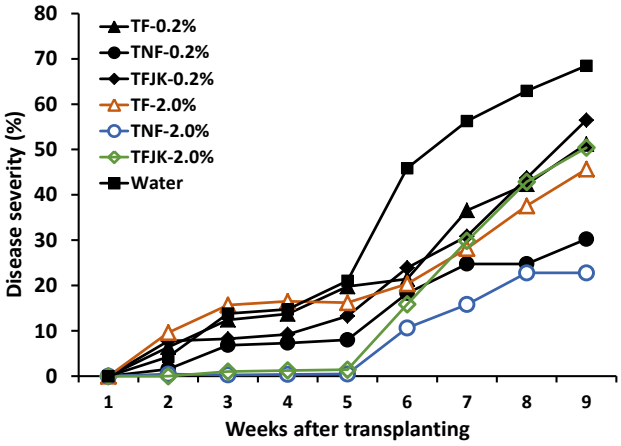

Fig.2: Progress curves of mosaic severity on Capsicum annuum sprayed weekly with a variety of fortified-compost extracts

Table.1: Area under disease progress curve (AUDPC) of mosaic severity after treatment with compost extract

Treatment of compost extracts	AUDPC[1)]	% disease suppression relative to control[2)]
TF-0.2%	12.48 ± 1.93 ab	29.6
TNF-0.2%	7.46 ± 1.79 bc	57.9
TFJK-0.2%	11.56 ± 1.33 ab	34.8
TF-2.0%	11.68 ± 2.07 ab	34.1
TNF-2.0%	4.35 ± 0.51 c	75.4
TFJK-2.0%	8.22 ± 1.09 bc	53.6
Water (control)	17.72 ± 1.41 a	-

*[1)]Mean ± SEM = Mean values ± standard error of means of fifteen replications. Means within the same column having a common letter(s) do not differ significantly (P=0.05) according to the Tukey HSD-test. [2)]% disease suppression = [(AUDPC of control - AUDPC of treatment)/ AUDPC of control]*100.*

3.2 Aphid colonization

Substantial aphid colonization started to observe at 8 and 9 weeks after transplanting. Aphid was found on 77 and 89 of 90 compost extract-treated plants at 8 and 9 weeks after transplanting, respectively. During those periods, colonization was found on all control plants (100% colonization). Anova of aphid number per plant showed that compost extract-treated plants resulted in significantly ($P<.0001$) lower colonization than water treated plant. Type of preparations significantly affected the aphid colonization (P main effect of preparation types ($P<.0001$) with lower aphid number on plant sprayed with TF and TNF compared to TFJK. Number of aphids colonizing plants sprayed with lower (0.2%) and higher (2.0%) concentration of compost extract were not significantly different ($P=0.1438$). Effect of compost extract spraying on aphid number was not significantly affected by its concentration (P interaction between preparation type and concentration = 0.1703). When combination of preparation types and concentration was compared, treatment with 0.2% TNF and 2.0% TF and TNF resulted in lowest aphid colonization (Fig. 3).

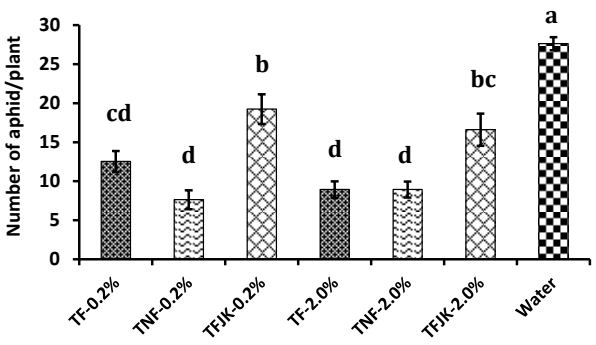

Fig.3: Colonization of aphid at 8 and 9 weeks after transplanting of Capsicum annuum sprayed weekly with a variety of fortified-compost extracts. Bars are means ± SEM of 15 replicate plants; bars without a letter in common are significantly different (P = 0.05) according to the Tukey HSD-test.

IV. DISCUSSION

In this study, we demonstrated the suppression effects of a compost extract preparation fortified with water extract of over-fermented tempe on mosaic disease and its aphid vector, *Aphis gossypii* on chili pepper. Plant treated with all three variants of the liquid preparation exhibited in significantly less progressive incidence and severity of mosaic disease and less colonization of *A. gossypii*

compared to control plant. Reduction in both disease and its vector population was obtained at concentration as low as 0.2%.

Plant treated with a lower concentration (0.2%) showed more delayed severity compared to the incidence progress curve. Even though infected, the severity progress had been delayed, suggesting a possible role of bioactive compounds containing in compost extract in plant resistance against viral infection. Compost extract used in the study contain at least 15 kinds of amino acid derived from water extract of over-fermented tempe. Utami *et al*. (2016) demonstrated that total amino acid contained in water extract of over-fermented tempe was 191 g/L and dominated by glutamic and aspartic acid. Amino acids and their metabolites are known to play essential roles during signaling processes as well as in enhancing plant immunity (Zeier, 2013), but few report available regarding induce resistance by exogenous application of amino acids. Exogenous treatment of rice roots with low dose glutamate induced systemic disease resistance against rice blast by regulating salicylic acid signaling pathway in rice leaves (Kadotani *et al*., 2016). Improved plant resistance against virus following application with amino-acid-based nutritional biostimulant at low concentration has been reported by Betti *et al*. (1992) demonstrated that. Foliar spray with 0.2-0.3% the amino acid preparation on chili pepper inoculated with *PepMV* reduced disease severity of mosaic disease. The viral disease suppression was suggested to be associated with correction of amino acid ratio (GLU+GLN/ASP) imbalance due to viral infection by amino acids contained in the biostimulant.

Disease severity at 8 and 9 weeks after transplanting was positively correlated with number of aphid colonization per plant suggesting that infestation level of the insect vector is responsible for severity of mosaic disease. It was likely that suppression of mosaic severity was associated with insecticidal activity of the fermented extract to *Aphis gossypii*. The fermentation liquid used in this study was derived from water extract of over-fermented tempe that reported to contain a non-proteinogenic amino acid, aminobutyric acid (Koh *et al*., 2012). β-aminobutyric acid (BABA), one of monomer of aminobutyric acid is known to have wide-ranging protection in a number of plant families against a variety of plant pathogens, nematodes and insect herbivores (Alexandersson *et al*., 2016). BABA has been demonstrated to reduce the performance of the pea aphid, *Acyrthosiphon pisum* on six legume plant species when applied as a root drench (Hodge *et al*., 2005). When applied on citrus, BABA was reported to citrus resistance to the Asian citrus psyllid, *Diaphorina citri* (Tiwari *et al*., 2013). Cao *et al*. (2014) suggested that mechanism of BABA-induced resistance in wheat to the grain aphid, *Sitobion avenae* is associated with direct toxicity of high BABA contents in plant phloem. Further study was needed to determine the role of amino acids contents in aphid suppression of the compost extract. Further study was needed to determine the toxicity of the compost extract on aphid and the role of amino acids contents on aphid performance.

Results from this study suggesting that disease suppression by the compost extract is possibly associated with either enhance host resistance or toxic activity of bioactive compounds containing in compost extract preparation. This findings point to the potential to develop a commercial biopreparation based on fortification of compost extract with amino acid-rich products to manage plant viral diseases. Further determination of bioactive compound and its mode of action need to be studied. Furthermore, the commercial formulation of compost extract preparation also led to successful protection of chili against CMV.

V. CONCLUSION

Compost extract preparations fortified with water extract of over-fermented tempe showed suppression effects on either mosaic disease or its aphid vector, *Aphis gossypii* on chili pepper. Reduction in both disease and its vector population was obtained at concentration as low as 0.2%.

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REFERENCES

[1] Alexandersson, E., Mulugeta, T., Lankinen, Å., Liljeroth, E., Andreasson, E. (2016). Plant resistance inducers against pathogens in solanaceae species—From molecular mechanisms to field application. International Journal of Molecular Sciences. 17(10):1673. doi:10.3390/ijms17101673

[2] Al-Mughrabi, K. I., Berthélémé, C., Livingston, T., Burgoyne, A., Poirier, R., and Vikram, A. (2008). Aerobic compost tea, compost and a combination of both reduce the severity of common scab (*Streptomyces scabies*) on potato tubers. Journal of Plant Sciences. 3:168-175.

[3] Aranega-Bou, P., Leyva, M. D. O, Finiti, I., and García-Agustín, P. (2014). Priming of plant resistance by natural compounds. Hexanoic acid as a model. Frontiers in Plant Science. 5:488. doi:10.3389/fpls.2014.00488

[4] Betti, L., Canova, A., Paolini, M., Merendino, A., and Maini, P. (1992). Effects of foliar application of an amino-acid-based biostimulant on the response of

pepper seedlings to *PepMV* infection. Advances in Horticultural Science. 6:97-103.

[5] Blackman, T.L., and Eastop, V. F. (2000). Aphids on the World's Crops: An Identification and Information Guide, 2nd Edition. Wiley, Chichester, UK, 466 pp.

[6] Cao, H. H, Zhang, M., Zhao, H., Zhang, Y., Wang, X. X., Guo, S. S., Zhang, Z. F., and Liu, T. X. (2014). Deciphering the mechanism of β-aminobutyric acid-induced resistance in wheat to the grain aphid, *Sitobion avenae*. PLoS ONE. 9(3):e91768. doi:10.1371/journal.pone.0091768

[7] Hodge, S., Thompson, G. A., and Powell, G. (2005). Application of DL-β-aminobutyric acid (BABA) as a root drench to legumes inhibits the growth and reproduction of the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae). Bulletin of Entomological Research. 95:449-455.

[8] Kadotani, N., Akagi, A., Takatsuji, H., Miwa, T., and Igarashi, D. (2016). Exogenous proteinogenic amino acids induce systemic resistance in rice. BMC Plant Biology. 16:60. doi:10.1186/s12870-016-0748-x

[9] Koh, S. P., Jamaluddin, A., Alitheen, N. B., Mohd-Ali, N., Mohd. Yusoff, H., and Long. K. (2012). Nutritional values of tempe inoculated with different strains of Rhizopus: its γ-aminobutyric acid content and antioxidant property. Journal of Tropical Agriculture and Food Science. 40(2):181-192.

[10] Kouyoumjian, R. (2007). Comparison of compost tea and biological fungicides for control of early blight in organic heirloom tomato production. PhD Thesis, Clemson University, South Carolina, USA.

[11] Lee, G. H., and Ryu, C-M. (2016). Spraying of leaf-colonizing *Bacillus amyloliquefaciens* protects pepper from cucumber mosaic virus. Plant Disease. 100(10):2099-2105.

[12] Llorens, E., García-Agustín, P., and Lapeña, L. (2017). Advances in induced resistance by natural compounds: towards new options for woody crop protection. Scientia Agricola. 74(1):90-100.

[13] Nicaise, V. (2014). Crop immunity against viruses: outcomes and future challenges. Frontiers in Plant Science. 25:660. doi:10.3389/fpls.2014.00660

[14] Putra, I. G. N. B. P., Puspawati, N. M., Nyana, I. D. N., Siadi, I. K., and Suastika, G. (2015). Identification of virus associated with mosaic, yellow, and chlorosis disease on chili pepper (*Capsicum frutescens* L.). E-Jurnal Agroekoteknologi Tropika (Journal of Tropical Agroecotechnology). 4:3. Retrieved from https://ojs.unud.ac.id/index.php/JAT/article/view/17292

[15] Siah, A., Magnin-Robert, M., Randoux, B., Choma, C., Rivière, C., Halama, P., and Reignault, P. (2018). "Natural agents inducing plant resistance against pests and diseases" in Natural Antimicrobial Agents. Sustainable Development and Biodiversity, vol 19, J. M. Mérillon and C. Riviere, Eds. Springer International Publishing AG, 2018, pp. 121-159.

[16] Siddiqui, Y., Meon, S., Ismail, R., and Rahmani, M. (2009). Bio-potential of compost tea from agro-waste to supress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. Biological Control. 49(1):38-44.

[17] Simko, I., and Piepho, H. (2012). The area under the disease progress stairs: calculation, advantage, and application. Phytopathology. 102:381-389.

[18] St. Martin, C. C. G. (2014). Potential of compost tea for suppressing plant diseases. CAB Reviews. 9(32):1-12.

[19] Sukada, I. W., Sudana, I. M., Nyana, I. D. N., Suastika, G., and Siadi, K. (2014). Effect of infection of some viruses to the decreasing yield on rawit pepper (*Capsicum frutescens* L.). E-Jurnal Agroekoteknologi Tropika (Journal of Tropical Agroecotechnology). 3:3. Retrieved from https://ojs.unud.ac.id/index.php/JAT/article/view/9610

[20] Suwandi, S. (2013). Shrimp waste-enriched compost extract, method for production thereof, and use thereof for control plant diseases and improving plant growth. Indonesia Patent ID P000035097.

[21] Suwandi, S., Hamidson, H., and Muslim, A. (2014). Biopriming treatment with enriched compost extract increased growth of rice seedling under salinity stress. Proceeding 2nd National Seminar on Suboptimal Lands: Inclusive agricultural technology for suboptimal lands. Research Center for Suboptimal Lands Sriwijaya University, Palembang, Indonesia. pp. 846-851.

[22] Suwandi, S., Junita, A., Suparman, S., Umayah, A., Hamidson, H., Muslim, A., and Irsan, C. (2018). Curative activity of watery fermented compost extract as a bark treatment against tapping panel dryness. Open Agriculture Journal. 12:74-83.

[23] Tiwari, S., Meyer, W. L, and Stelinski, L. L. (2013). Induced resistance against the Asian citrus psyllid, *Diaphorina citri*, by β-aminobutyric acid in citrus. Bulletin of Entomological Research 103:592-600.

[24] Utami, R., Wijaya, C. H., and Lioe, H. N. (2016) Taste of water-soluble extracts obtained from over-fermented tempe. International Journal of Food Properties. 19(9):2063-2073.

[25] Wahyuni, W. S., Mudjiharjati, A., Sulistyaningsih, N. (2010). Compost extracts of vegetable wastes as

http://dx.doi.org/10.22161/ijeab/3.4.10 *ISSN: 2456-1878*

biopesticide to control *Cucumber Mosaic Virus*. HAYATI Journal of Bioscience. 17:95-100.

[26] Zeier, J. (2013). New insights into the regulation of plant immunity by amino acid metabolic pathways. Plant, Cell & Environment. 36(12):2085-2103.

[27] Zhang, W., Han, D. Y., Dick, W. A., Davis, K. R., and Hoitink, H. A. J. (1998). Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Phytopathology. 88(5):450-455.

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A Survey on the Pteridophyte Flora of the 18 Selected Sacred Groves in Chalavara Grama Panchayath, Palakkad District, Kerala

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Abstract**—** *An exploratory survey conducted on Pteridophytic flora in the 18 selected sacred groves of Chalavara Grama panchayath, Ottapalamtaluk, Palakkad district, Kerala lead to the collection of 26 species of pteridophytes coming under 20 genera and 14 families. Among them, 02 families belongs to class Lycopsida, 01 family belongs to class Psilotopsida and remaining belongs to class Polypodiopsida. Out of 26 species 21 species are terrestrial, 3 species are epiphytes and 2 species are aquatic.*

Keywords**— Diversity, Sacred groves, Chalavara, Palakkad.**

I. INTRODUCTION

Sacred grooves are the conserved forest patches or protected areas. The sacred groves in Kerala are tightly bonded with religional backgrounds. The (Its) conservation is mainly based on cultural, aesthetical and religional aspects, In different areas this sacred places are devoted or dedicated for different Gods i.e. serpentine Gods, Nagadevatha, Nagayakshi .etc. Human activities are highly prohibited in these areas. Touching plants (and animals associated with them) in these sacred groves and gardens was forbidden to all except the temple priest, and his too is restricted to offerings to the Presiding temple Deity and curing the ailments of local people (the temple priest was in variably the village doctor). The study area includes one of the famous sacred groove and serpentine temple, Pathirakkunnathu mana. The present technocratic and scientifically oriented society mistakenly considers that religion is not interested in protecting and managing biodiversity. The truth, however, is that religious values very often help to protect biodiversity. The practice of protection of patches of forests with temples in their vicinity has long been in vogue in India and a few other parts of the World. In some instances, forest patches or gardens with local floristic elements (often called Nandavanas) have been specially created near established temples are declared sacred to ensure their protection and conservation. Such sacred groves and gardens dedicated to the worship of the Presiding Deity of each temple are mentioned in ancient Greek, Latin American and Indian literary works as well as in epigraphically records and copper plates of these countries. Data also come from folk traditions, history and traditional knowledge passed on through several generations.

Sacred grooves are biologically important places having maximum species diversity and richness. Besides this sacred grooves are the significant ecosystem in the terrestrial biome. Angiosperms are the abundant vegetation in here, mainly trees and climbers, herbaceous forms are relatively limited in number, however some pteridophytes, certain members of Asteraceae, Piperaceae, are substituting this purpose. In some areas large trees are dominant whereas in some other areas climbers are majority. The soil in these places is nutrient rich and water filled, rocky regions contain members *of Riccia, Funaria, Marsilia* .etc.

This study includes a Taxonomical approaches to the Pteridophytic flora in sacred groves. Pteridophytes are flourished in sacred groves. It's watery or moisture nature is suitable for pteridophytes, some are epiphytic forms on tree members some others are herbs and climber like *Lygodium*. Drastic variations among species are common. Sacred groves are the original replica of natural forests of the locality. It may comprise of a single tree to a very large forest tract. It is considered as sacred because of the deity associated with the grove. Hence it may call as temple forests, which are one of the oldest forms of conserving natural forests. This unique community linked forest conservation concept is followed in many tribal and agrarian regions of the world.

Pteridophytes constitute an important part of the world flora. Pteridophytes are of immense economic importance and there is a great need for their exploitation

towards the economic utility in our day to day life. Ferns show various economic values towards food and fodder, biological indicators, bio fertilizers, insect repellants, medicine and folk remedies. But still the question whether the full potential of these intriguous plants have been ever exploited by the humans remains. Quite a large number of them cultivated as ornamentals either indoors in the houses or outdoors in the botanical gardens due to their delicate beauty and grace. The hybrids of different species are now elegant, expensive, representative members in horticulture. Another chief economic importance of the pteridophytes is that their fossil remains contributed to the coal depositions of the world.Thus we can see that ferns are a group of unique plants and they have immersed potential to be studied and utilized in different areas of economic and academic interest. The current study is a humble attempt to enumerate and identify the pteridophytic flora of sacred grooves of the Chalavaragramapanchayath, Ottapalam, Palakkad, Kerala.

II. MATERIALS AND METHODS

The present study was carried out to assess and analyse the pteridophytes flora of sacred groves of Chalavara Grama Panchayath, Palakkad district, Kerala. The Materials for this study were collected from area of study during January 2016 to July 2016. Field trips to various habitats were made during the study period for plant collection. During the collection, field observations such as habit, habitat, and date of collection were noted in the field itself and the diagnostic features of all the specimens were studied and field notes were made on fresh plant materials.

From the available habitats, sporophytic habits were collected along with rhizome as far as possible. Plants collected were carefully handled and covered with moistened newspapers without causing any harm to the tender parts. In case of large plants, exact size was noted down and then the fronds were cut in to pieces, which were then used to prepare the herbarium. More than one specimens of each plant were collected for further identification, observation and herbarium preparation. About 36 species have been collected from the area. The colour photographs from the natural habitat were taken during the collection using digital camera (Sony-Cyber shot W810). Plants with sporophylls were collected as far as possible.

Fresh materials were used for the study in the laboratory. Morphological characters of the sporophytic plant body were studied. External characters of the spores and sporangia were also noted in selected plants. Enumerations of collected specimens were done by observing a morphological feature under stereozoom microscope (LABOMED D500) Identification of the specimens was done by using the Pteridophytes flora of the Western Ghats- South India (Manickam and Irudayaraj 1992). All the identified species of ferns have been classified and arranged according to Smith et al., 2006 with some modification as per Fraser-Jenkins (2009), regarding their correct nomenclature and classification of fern allies.

The collected specimens were properly processes as herbarium specimens, carefully spread on the newspaper. The large sized plants were bent into V or W shapes and these specimens were bound tightly in plant press. The newspaper sheets were changed regularly until the specimens got dried completely. The dried specimens were mounted on herbarium sheets by using synthetic gum. The herbarium sheets were neatly labeled by using the details from the field note book. All the herbaria prepared for the present study were deposited in Sree Krishna College herbarium.

III. RESULTS AND DISCUSSION

Fern and fern allies are almost neglected group of plants distributed all over the world. They are live in a wide variety of habitats from remote mountain elevations to dry desert rock faces, to bodies of water or in open fields. Some ferns are serious weed species. The present study revealed the occurrence of 26 species of pteridophytes in the sacred groves of Chalavara Grama Panchayath. This belongs to 20 genera under 14 families. Based on their habitats various members of fern and fern allies are classified in to following categories, out of 26 species21 species are (81%) terrestrial, 2 species are (8%) aquatic and 3 species are (11%) epiphytes. (Table 1).

The present study identified 2 ecologically significant species such as *Salvinia molesta (*African payal) and *Marsilea* minuta as their wide distribution as weeds in ponds and paddy fields. *Salvinia molesta*, a free floating aquatic fern, is one of the World's worst aquatic weeds (Madhusoodanan, 1989). As the plant die, organic debris accumulates at the bottom of the water column and can threaten fisheries by creating a shallow – water environment less suited to fish breeding (Sculthrope, 1985). *Pyrrosia heterophylla* growing as epiphyte in less polluted area, their absence indicates pollution (this species only obtained from). A detailed survey on the fern flora of a particular region becomes significant only if it can do any good to the conservation practices of these endangered plants. Lack of knowledge or rather interest among Botanists is one of the main reasons for negligence faced by ferns. So familiarizing ferns and including them with almost equal importance to

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the Angiosperms is a way out from the threatened status of these plants. The economic and medicinal use of them should be explored more and by this ferns can attain a significant position I plant kingdom. Prior to all this all the manipulations and misidentifications of these plants should be rectified for adopting better conservation strategies and for exploring the wide applications of these plants to be used for the betterment of mankind and life on earth as a whole.

Table.1: List of Pteridophytes collected from 18 sacred groves

Class	Family	Genus	Species	Habitat
I. Lycopsida	1. Lycopodiaceae 2. Selaginellaceae	1. *Lycopodiella* *Selaginella*	1. *Lycopodiella cernua* L. 2. *S. delicatula* (Desv.) Alston. 3. S. *wildenovi* (Desv. ex Poir.) Baker. 4. *S. ciliaris* (Retz.) Spring	T T T T
II. Psilotopsida	3. Ophioglossaceae 4. Gleicheniaceae	2. *Ophioglossum* 3. *Dicranopteris*	5. *Ophioglossum reticulatum* L. 6. *Dicranopteris linearis* (Burm.f.) Undrew.	T T
III. Polypodiopsida	5. Lygodiaceae 6. Marsileaceae. 7. Salviniaceae 8. Lindsaceae 9. Pteridaceae 10. Thelypteridaceae 11.Woodsiaceae 12.Blechnaceae 13.Lomariopsidaceae 14.Polypodiaceae	4. *Lygodium* 5. *Marsilea* 6. *Salvinia* 7. *Lindsaea* 8. *Pteris* 9. *Adiantum* 10. *Cheilanthes* 11. *Parahemionitis* 12. *Pityrogramma* 13. *Christella* 14. *Macrothelypteris* 15. *Athyrium* 16. *Stenochlaena* 17. *Nephrolepis* 18. *Drynaria* 19. *Pyrrosia*	7. *Lygodium flexuosum* (L.)Sw. 8. *Marsilea minuta* L. 9. *Salvinia molesta* Mitch. 10. *Lindsaea ensifolia* Sw. 11. *Pteris vittata* L. 12. *P. pellucida* Presl. 13. *P. confusa* T.G. Walker. 14. *Adiantum philippense* L. 15. *A. latifolium* Lam. 16. *Cheilanthes tenuifolia* (Burm. f.) Sw. 17. *Parahemionitis cordata* (Roxb. ex Hook. and Grev.) Fraser-Jenk. 18. *Pityrogramma calomelanos* (L.) Link 19. *Christella dentata* (Forssk.) Brownsey and Jermy. 20. *Macrothelypteris torresiana* (Gaudich.) Ching 21. *Athyrium hohenackeranum* (Kunze) T. Moore. 22. *Stenochlaena palustris* (Burm.) Bedd. 23. *Nephrolepis cordifolia* (L.) Presl. 24. *Drynaria quercifolia* (L.) J. Smith. 25. *Pyrrosia lanceolata* Farwell 26. *P. heterophylla* (L.) M. G. Price	T A A T T T T T T T T T T T T T E E E

T: Terrestrial; A: Aquatic E: Epiphyte

IV. CONCLUSION

A detailed survey on the fern flora of a particular region becomes significant only if it can do any good to the conservation practices of these endangered plants. Lack of knowledge or rather interest among Botanists is one of the main reasons for negligence faced by ferns. So familiarizing ferns and including them with almost equal importance to the Angiosperms is a way out from the threatened status of these plants.The present study identified 2 ecologically significant species such as *Salvinia molesta* (African payal) and *Marsilea minuta* as their wide distribution as weeds in ponds and paddy fields. Sacred groves as well as

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pteridophytes are needs an immediate attention in the modern sense.

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REFERENCES

[1] Fraser-Jenkins, C.R. 1997. New Species Syndrome in India Pteridology and the Ferns of Nepal. International Book Distribution. Dehra Dun.

[2] Fraser-Jenkins, C.R. 2008.Taxonomic Revisions of Three Hundred Indian Subcontinental Pteridophytes with a Revised Census- List. Bishen Singh Mahendra Pal Singh. Dehra Dun: 685.

[3] Fraser-Jenkins, C.R. 2009. A Brief Comparison of Modern Pteridophyte Classifications (Families and genera in India) *Indian Fern Jour*. 26: 107-126.

[4] Madhusoodanan, P.V. and Nampy, S.1998. Biodiversity of the Ferns and Fern allies of Kerala. *Indian Jour. Econ.Tax. Bot*. 22: 183-189.

[5] Madhusoodanan, P.V., Sijimoli, P.S. and Rajesh, K.P. 2001. Pteridology in South India- retrospection. *Indian Fern Jour*. 18: 18-34.

[6] Madhusoodanan, P.V., Sreerenjini, V.K., Smitha, A.B. and Prakashkumar, R. 2014. Invasion of *Salvinia molesta* D.S. Mitchell. (African Payal) in Kerala and its Management *Indian Fern Jour*. 31: 156- 166.

[7] Manickam, V.S. and Irudayaraj, V.1992. Pteridophyte Flora of the Western Ghats-South India. B. I. Publications, New Delhi. 3:48-49.

[8] Sculthorpe, C. D. 1985. The biology of aquatic vascular plants. Edward Arnold, London. pp. 124-151.

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Antioxidant activity, photosynthetic rate, and Spectral mass in bean Plants (*Phaseolus vulgaris* L.) in Response to Stress Defense Activators

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Abstract*—An increase in antioxidant activity is a common response in plants as a defense mechanism against biotic and abiotic stress factors, such response is also generated with the exogenous application of "defense activators", which have negative effects on plant metabolism. In this work, bean plants (Phaseolus vulgaris L.) cv. Pinto Nacional were treated with jasmonic acid (0.5 mM), salicylic acid (2 mM), Trichoderma asperellum (10^5 spores/ml), and Bacillus pumilus (10^5 CFU / mL), in order to determine the level of structural and metabolic response of the plants. On the seventh day after the application of the treatments, it was measured the enzymatic activity of catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD). In addition, leaf impressions were taken to measure the stomatal opening and conductance, photosynthetic rate, and the mass spectrum (mass/charge, m/z). The antioxidant activity increased in plants treated with jasmonic acid and T. asperellum, which in turn significantly increased the stomatal opening and conductance, and photosynthetic rate. The mass profile showed that the plants treated with T. asperellum have a greater quantity of masses/charge, of which some had statistically highly significant difference according to the means test Tukey ($p < 0.05$). It is concluded that some defense activators such as jasmonic acid and T. asperellum increase the antioxidant activity, defense response that concurs with the high photosynthetic and metabolic rate in bean plants.*

Keywords*— *Bacillus pumilus, Trichoderma asperellum, jasmonic and salicylic acid.

I. INTRODUCTION

Induced resistance is a "physiological state" in which there is an increase in the defensive capacity of plants as a natural response to different biotic and abiotic stimuli, it is also achieved with the exogenous application on the vegetable epidermis of some chemical compounds, microorganisms, as well as with metabolites produced by the same plants during tolerance to stress situations (Pieterse *et al*., 2014). In plant protection against phytopathogens, beneficial microorganisms are frequently used, such as the antagonists *Trichoderma* spp., and growth-promoting bacteria from the genus *Bacillus* spp. (Bisen et al., 2016; Niu et al., 2016). Similarly, chemical compounds, natural and synthetic, are used to increase the defensive capacity of economically important plants (Lin et al., 2009). The exogenous application of salicylic acid in order to induce " Systemic Acquired Resistance " and jasmonic acid for "Induced Systemic Resistance" have been valuable as experimental controls in tests on edible plant species as well as shrubby plants (Moreira *et al*., 2009; Hayat *et al*., 2010).

Enzymatic activity, as a biochemical defense mechanism, is a process that is presented to face the attack of pathogens, this occurs in response to the release of reactive oxygen species (ROS), a process known as "oxidative burst" (Bolwell and Daudi, 2009). ROS are toxic intermediates that result from the reduction of molecular oxygen in the plant-pathogen interaction, such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) (Helepciuc *et al*., 2014). Faced with this situation, the

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plants generate antioxidant enzymes that prevent the damage they can cause to themselves. Some of the enzymes that increase considerably, are catalase and peroxidase, which convert hydrogen peroxide into oxygen and water, and superoxide dismutase that transforms the superoxide anion to molecular oxygen and hydrogen peroxide (Helepciuc *et al*., 2014). These enzymes increase their synthesis considerably with the exogenous application of defense activators (Hafez and Seleiman, 2017).

The response of legumes to defense activators is very variable, and includes the formation of structural barriers as part of the first line of defense of plants (Oliveira *et al*., 2016). It has been proven that the induction of defense implies a metabolic cost for the plants. For example, there is evidence that the rupture of cells by pathogenic infections involves the formation of infection structures and have a strong impact on the water relation of plants (Grimmer *et al*., 2012); which is closely related to the stomatal behavior. The monitoring of the opening and stomatal conductance of the plants during the induction events turns out to be of great importance. A negative affectation by the defense inducers would cause the alteration of important physiological processes such as photosynthesis.

On the other hand, since it is known that plants modify their metabolic and physiological processes when they interact with various biotic and abiotic factors, the interest in elucidating such processes has been addressed through the use of "omic" sciences in plant species. Metabolomics is presented as a science that studies reactive metabolites, intermediates, or products of biochemical reactions mediated by enzymes. Ionization based mass spectrometry can be considered as a standard technique in metabolomics and is the ion source that offers the advantage that variables measured as mass charge, m/z can be directly linked to a metabolite by its atomic mass; and this in turn, with the presence of active metabolites related to plant defense (Massange-Sanchez *et al*., 2015). In order to facilitate this task, there are application software linked to databases that identify metabolites from a list of spectral masses with high precision (Winkler, 2015). The description of the metabolomic profile of plant species of economic importance under the effect of chemical and microbial defense activators constitutes an important tool for the detection of metabolites that participate in plant resistance. Bean (*Phaseolus vulgaris* L.) is a crop of great economic importance that can be attacked by different phytopathogens, among them bacterial diseases, therefore it represents a model for the studies that allow to detect the participation of metabolites in the resistance to the diseases within the family of the legumes.

This work was carried out in order to determine the antioxidant activity and the changes induced in the metabolism of bean plants (*Phaseolus vulgaris* L.) in response to the defense activators *Trichoderma asperellum*, *Bacillus pumilus*, jasmonic acid, and salicylic acid.

II. MATERIALS AND METHODS

2.1 Plant material

Seeds of bean cv. Pinto Nacional were sown in black polyethylene pots with peat as substrate, which was previously sterilized in an autoclave at 120 ° C during 15 minutes.

2.2 Preparation of treatments

The treatments applied were: salicylic acid at 2 mM (SA) (Sigma-Aldrich), jasmonic acid (JA) at 0.5 mM (Sigma-Aldrich), *Trichoderma asperellum* (Ta) (10^5 spores/ml), and *Bacillus pumilus* (B) (10^5 CFU/mL); previously identified strains (Castillo *et al*. 2011 and Guillén-Cruz *et al*. 2006). The treatments were applied to bean seedlings at 7 days after emergence and were sprayed on cotyledonary leaves. The pots with the plants were established in a greenhouse at a temperature of 28 ± 5 °C and 55 ± 10% of relative humidity.

2.3 Preparation of samples and extract for determination of total soluble protein content.

Four plants were collected per treatment, 7 days after starting the experiment, which were dehydrated for 24 hours in a *Labconco*® lyophilizer. The protein extract was obtained by placing 0.2 g of the lyophilized tissue in microcentrifuge tubes with 1.5 mL of phosphate buffer (Na_2HPO_4/NaH_2PO_4) 0.1 M at pH 7.0, subsequently, it was microcentrifuged at 12,000 rpm for 10 min at 4 °C. The determination of total soluble protein was carried out with the Bradford method (Bradford, 1976). Bovine serum albumin was used as standard in protein quantification.

2.4 Antioxidant activity.

In order to determine the catalase activity (CAT), the reaction mixture was made with 0.1 mL of protein extract. The reaction was initiated with the addition of 1 mL of peroxide at 100 mM. The reaction was stopped with the addition of 0.4 mL of 5% sulfuric acid. The enzymatic activity was estimated by calculating the decomposition of the peroxide per min/mg of protein. It was determined by measuring 2 reaction times, time 0, and 1 minute of reaction at a wavelength of 275 nm in a spectrophotometer (Thermo-Spectronic, Biomate, USA). The specific activity was expressed in units/mg of protein. Peroxidase activity (POX) was measured with the methodology of Baskaran et al. (2009). The reaction mixture (1.2 mL) was prepared with 0.1 mL of protein extract, 0.5 mL of 100 mM buffer, 0.2 mL of pyrogallol,

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and 0.2 mL of hydrogen peroxide. The reaction was stopped 1 minute later, with the addition of 5% sulfuric acid. The measurements were taken at a wavelength of 420 nm. In both determinations, the same enzyme extract was used. The Superoxide Dismutase (SOD) activity was measured with a 19160 measuring kit (Sigma-Aldrich). The procedure was carried out following the protocol of the kit. For this determination, 20 µL of each sample was deposited in the wells of the ELISA microplate, 200 µL of the working solution of the kit was added. The mixture was shaken slightly to mix and then incubated at 37 °C for 20 min. After this incubation time, 20 µL of the enzyme solution of the kit was added. The absorbance of the mixture was read at 450 nm in a microplate reader and the SOD activity was calculated using the following equation:

SOD activity (inhibition rate %) = (((blank 1 – blank 3) – (sample A – blank 2))/(blank 1 – blank 3)) x 100.

Where the blank 1 is a mixture of the working solution (200 µL) and the enzymatic solution of the kit (20 µL) containing 20 µL of double distilled water. Blank 2 contained the plant extract (20 µL) with the working solution (200 µL) and the dilution buffer (20 µL), whereas blank 3 was added with distilled water (20 µL) plus the working solution and the enzymatic solution in the same amount.

2.5 Measurement of stomatal conductance, photosynthetic rate, and stomatal opening.

The stomatal opening and conductance, and the photosynthetic rate, were measured on the seventh day of the experiment in 9 plants per treatment. To determine the stomatal opening, epidermal impressions of the leaves were taken. The leaf impressions were obtained with PVC glue applied with a brush on the adaxial and abaxial epidermis of the leaves, where a transparent adhesive tape was placed and fixed on a slide for observation under a compound microscope. The stomatal opening was determined by measuring the opening of the central stomata of 3 visual fields per leaf at 40x magnification with a compound microscope with integrated digital camera and the measurement was made with the AxioVision Rel software. 4.8. The stomatal conductance was recorded with a leaf porometer (SC-1, Decagon Devices, Pullman, WA) and the measurement of the photosynthetic rate was carried out with a foliar CO_2 assimilation measurement equipment (LI-6400).

2.6 Preparation of plant material for mass spectrometric profiling

The lyophilized plants were macerated in a porcelain mortar and sieved with a pore mesh of 297 microns (Mesh No. 50). The sieving obtained was placed in microcentrifuge tubes. To each tube was added 2 ml of 80% ethanol (prepared with absolute ethanol grade HPLC and milliQ water), subsequently they were sonicated for 20 minutes and centrifuged at 13000 rpm for 15 minutes to obtain 10 µl of supernatant. The supernatant was filtered in 0.2 µm Nylon before its injection to the single quadrupole mass spectrometer (Water SQ Detector). The measurements were made with an electrospray ionization detector (ESI) in positive and negative mode. The spectra were recorded in a range of 15 to 1000 m/z (mass/charge), with a run time of 1 minute and scans every 10 seconds. The masses were grouped in 0.1s intervals. The masses that showed greater intensity in the different treatments were recorded in box graphs with their standard deviation and a mean test was performed with Tukey at a 95% probability.

2.7 Statistical analysis

The statistical analysis of the masses was conducted with a randomized block design with 3 and 4 biological replicas. The means are shown with bars representing the standard deviation.

III. RESULTS AND DISCUSSION

3.1 Enzymatic activity

Enzymatic activity catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD), was measured with previous quantification of total soluble proteins. The analysis of the antioxidant activity was carried out in an attempt to elucidate the response mechanisms of the reactive oxygen species that are generated when the plants are in a state of stress. It was noted that the total soluble proteins remained at levels comparable to the activity of the enzymes (Fig. 1A). The statistical analysis of the enzymatic activity in the bean plants with the different treatments showed a significant difference with the application of jasmonic acid, which had a high catalase and peroxidase activity (Fig. 1B and 1C). *T. asperellum* promoted high superoxide dismutase activity (Fig. 1D). The lowest enzymatic activity resulted from the application of *B. pumilus* and the control. This highlights the fact that the high enzymatic activity concurs with the photosynthetic efficiency induced in plants treated with jasmonic acid.

The high antioxidant activity induced by jasmonic acid in bean plants has been observed in other species such as Arabidopsis thaliana, where it was observed that the application at low concentrations increases the antioxidant activity considerably, however, at high concentrations the response is inverse (Maksymiec and Krupa, 2002).

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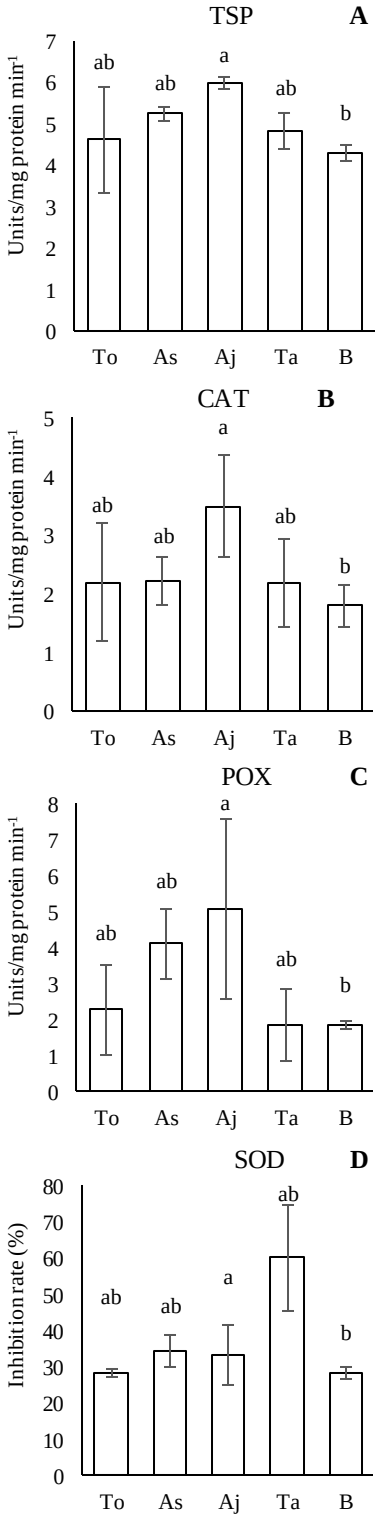

Fig.1: Total soluble proteins (TSP), catalse activity (CAT), peroxidase (POX), and superoxide dismutase (SOD) presented in bean plants (Phaseolus vulgaris cv. Pinto Nacional) with the different treatments. To: control plants, As: salycilic acid, Aj: jasmonic acid, Ta: Trichoderma asperellum, B: Bacillus pumilus. Bars followed by different letters show significant differences according to the test Tukey ($p \leq 0.05$).

On the other hand, there are several research works that demonstrate the ability of *Trichoderma* species as activators of antioxidant activity in plants. *T. virens* applied simultaneously with *Suillus luteus*, in plants of *Pinus sylvestris* var. *Mongolica*, increases the enzymatic activity superoxide dismutase in comparison to the enzymatic activity catalase and peroxidase even after thirty days after inoculation (Yin *et al*., 2014). In this work, *T. asperellum* applied to bean plants stimulated a high superoxide dismutase activity detectable on the seventh day after its application, not being statistically different in comparison to the rest of the treatments in later days (data not shown). On the other hand, *B. pumilus* applied to the plants does not induce a high enzymatic activity compared to the control. Previous research shows that the mixture of strains of *B. pumilus* with other species such as *B. amyloliquefaciens* consistently induces an increase in enzymatic activity in tomato plants (*Solanum lycopersicum* L.), which was more noticeable after a confrontation with phytopathogens (Jetiyanon, 2007). This suggests that a high expression of antioxidant activity in some plant species is only visible after the interaction of plants with pathogens.

3.2 Stomatal conductance, photosynthetic rate, and stomatal opening

In order to quantify the effect of the activators on the photosynthetic efficiency of the bean plants, at the same time the stomatal conductance and opening were measured. The results show that the plants treated with jasmonic acid, significantly have a greater stomatal conductance compared to the rest of the treatments (Fig. 2A). The plants treated with jasmonic acid, *T. asperellum*, *B. pumilus* and the control were not significantly different in stomatal conductance. Specifically, the greatest difference in conductance was observed in the plants treated with jasmonic acid and *B. pumilus*, in a range of 65 to 96 mmol m^{-2} s^{-1}. The photosynthetic rate was lower in plants treated with *B. pumilus* (9 µmol CO_2 m^{-2} s^{-1}) compared with those treated with jasmonic acid (13 µmol CO_2 m^{-2} s^{-1}) (Fig. 2B). The measurement of the stomatal opening showed that the plants treated with jasmonic acid and *T. asperellum* (1.8 and 1.7 microns) cause a statistically significant greater stomatal opening ($p < 0.05$) in comparison to the rest of the treatments, where the plants treated with *B. pumilus* were those with the least stomatal opening (1.0 microns) (Fig. 2C).

Previous studies show that jasmonic acid applied exogenously to plants induces a stomatal closure at concentrations higher than 10^{-06} M in monocots such as barley (Metodiev *et al*., 1996). In legumes such as broad bean, the same effect has been observed in the first minutes or hours of its application (Liu et al., 2005), however, there are no works that prove the effect after

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several days of its application. In this work, the jasmonic acid at 5 x 10^{-04} M produced an effect of higher stomatal opening in the bean plants 7 days later. The observed effect suggests that the response to stomatal closure in bean plants would be located at higher concentrations of JA.

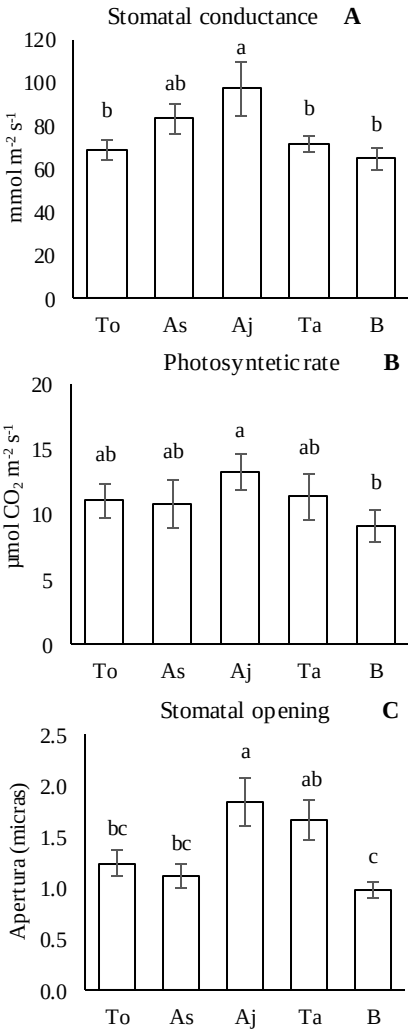
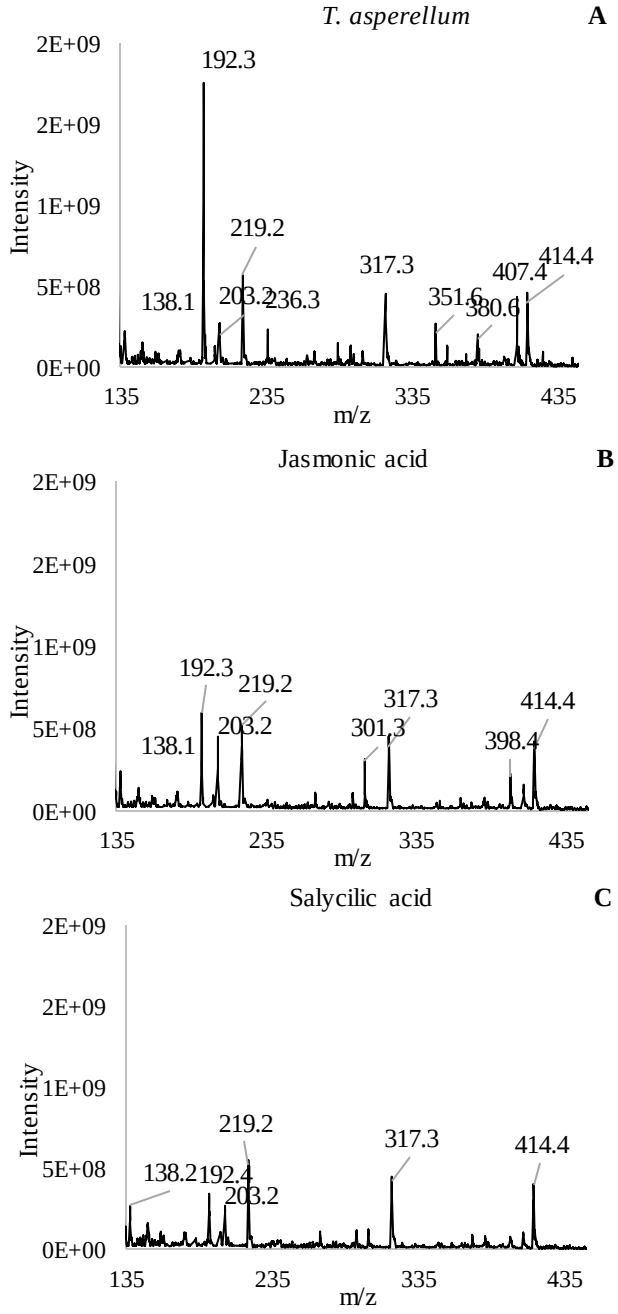


Fig.2: Stomatal opening and conductance, and photosyntetic rate presented in bean plants (Phaseolus vulgaris cv. Pinto Nacional) with the different treatments. To: control plants, As: salycilic acid, Aj: jasmonic acid, Ta: Trichoderma asperellum, B: Bacillus pumilus. Bars followed by different letters show significant differences according to the test Tukey ($p \leq 0.05$).

3.3 Mass spectral profile

In order to strengthen the evidences of the mechanisms used by the activators, the lyophilized samples of the treated plants were processed for an analysis of the spectral profile of the masses that are induced. The results showed that the most intense masses are located in the range of 135 to 435 mass/charge (m/z) (Fig. 3A-3E), being the plants treated with *T. asperellum* followed by jasmonic acid, those that visually more intense masses present and in which the m/z 192.3 stands out (Fig. 3A).



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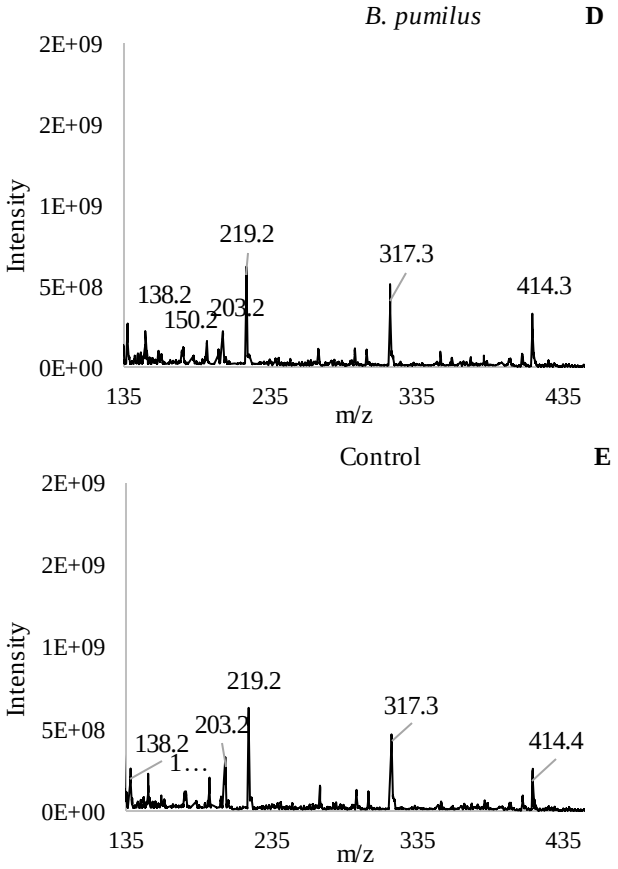


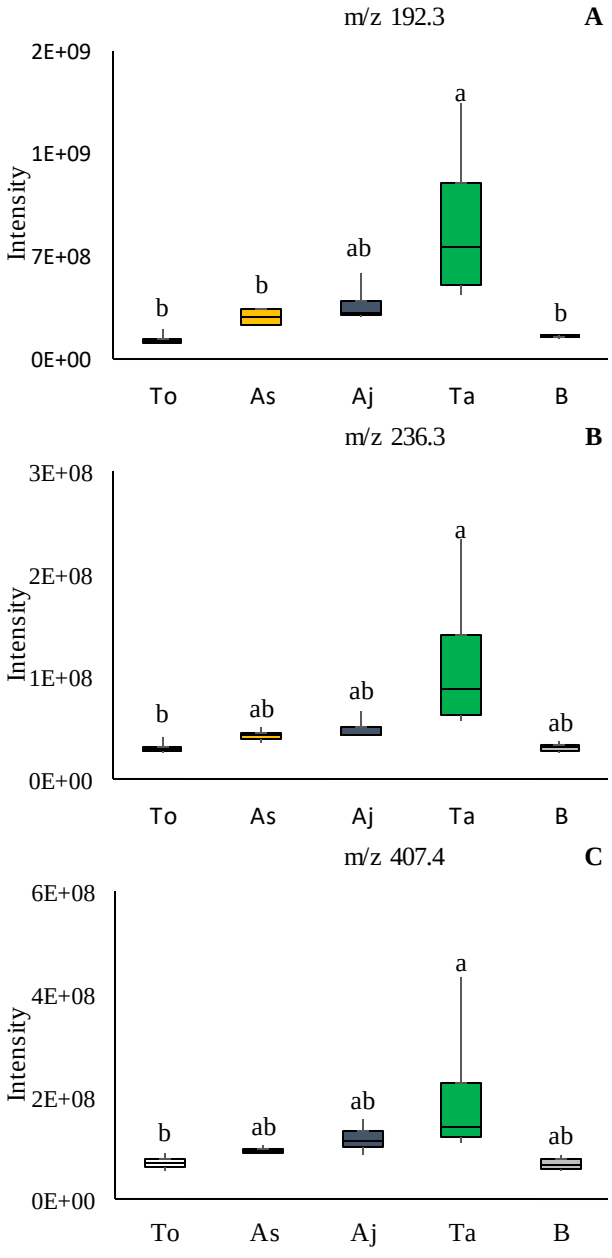
Fig.3: Spectral profile mass/charge (m/z) with higher intensity presented in bean plants (Phaseolus vulgaris cv. Pinto Nacional) with the different treatments.

The means test showed that the plants treated with *T. asperellum* present four highly significant masses (m/z 192.3, 236.4, 407.4, and 415.4) (Fig. 4A-4D). The mass 415.4 is statistically higher both in the plants treated with *T. asperellum* and jasmonic acid compared to the rest of the treatments (Fig. 4D). The means test of the most intense masses makes it possible to show that the pattern of the intensities in all the treatments is comparable to the pattern shown by the stomatal behavior and the antioxidant activity, in which it is observed that the plants treated with *B. pumilus* and the control plants result with statistical inferiority compared to the rest of the treatments.

The alteration of important metabolites for the metabolism of plants by root inoculation with *Trichoderma asperelloides* has been demonstrated in *Arabidopsis thaliana* (Brotman *et al*., 2012). Likewise, numerous studies report that the inoculation of plants with beneficial microorganisms elicits a systemic network that influences the primary metabolism, therefore the initial changes are observed in the levels of carbohydrates and secondarily in the levels of the compounds related to the plant defense (Weston *et al*., 2012). This suggests that the most intense masses found with statistically significant superiority in the plants treated with *T. asperellum* and jasmonic acid would be related to both primary and secondary metabolism, according to what was observed in the photosynthetic activity and antioxidant activity.

IV. CONCLUSION

The exogenous application of defense activators in bean plants produced substantial effects. Particularly, jasmonic acid and *T. asperellum* significantly increase antioxidant activity and stimulate a high photosynthetic rate. Similarly, there is a high expression of masses (putative metabolites) when using such activators, which suggests that the primary and secondary metabolism was not negatively affected.



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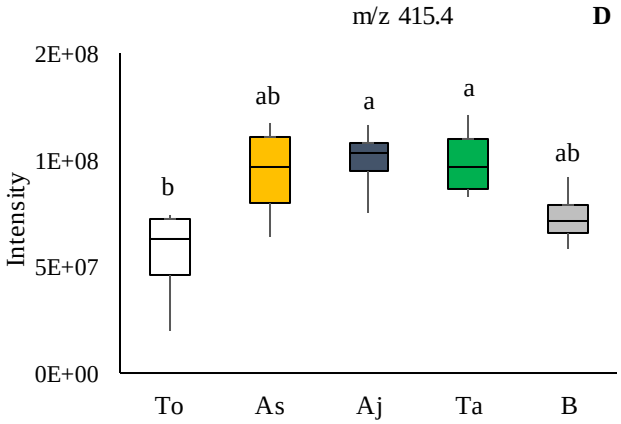

Fig.4: Behavior of mass charge (m/z) with higher intensity presented in bean plants (Phaseolus vulgaris cv. Pinto Nacional) with the different treatments. To: control plants, As: salycilic acid, Aj: jasmonic acid, Ta: Trichoderma asperellum, B: Bacillus pumilus. Bars followed by different letters show significant differences according to the test Tukey ($p \leq 0.05$).

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REFERENCES

[1] Bisen, K., Keswani, C., Patel, J. S., Sarma, B. K. and Singh, H. B. (2016). Trichoderma spp.: efficient inducers of systemic resistance in plants. In Microbial-mediated Induced Systemic Resistance in Plants, 185-195.

[2] Bolwell, G. P. and Daudi, A. (2009). Reactive oxygen species in plant–pathogen interactions. In Reactive oxygen species in plant signaling, 113-133.

[3] Brotman, Y., Lisec, J., Méret, M., Chet, I., Willmitzer, L. and Viterbo, A. (2012). Transcript y metabolite analysis of the Trichoderma-induced systemic resistance response to Pseudomonas syringae in Arabidopsis thaliana. Microbiology, 158(1), 139-146.

[4] Castillo, F. D., Padilla, A. M., Morales, G. G., Siller, M. C., Herrera, R. R., González, C. N. and Reyes, F.C. (2011). In vitro antagonist action of Trichoderma strains against Sclerotinia sclerotiorum y Sclerotium cepivorum.*American* Journal of Agricultural y Biological Science 6, 410-417.

[5] Grimmer, M. K., John Foulkes, M. and Paveley, N. D. (2012). Foliar pathogenesis y plant water relations: a review. Journal of experimental botany, 63(12), 4321-4331.

[6] Guillén-Cruz, R., Hernández-Castillo, F. D., Gallegos-Morales, G., Rodríguez-Herrera, R., Aguilar-González, C.N., Padron-Corral, E. and Reyes-Valdés, M. H. (2006). *Bacillus spp*. como biocontrol en un suelo Infestado con *Fusarium spp*, *Rhizoctonia solani* Kuhn y *Phytophthora capsici* Leonian y su efecto en el desarrollo y rendimiento del cultivo de chile (*Capsicum annum* L.). Revista Mexicana de Fitopatología, 24, 105-114.

[7] Hafez, E.M. and Seleiman, M.F. (2017). Response of barley quality traits, yield y antioxidant enzymes to water-stress y chemical inducers. International Journal of Plant Production, 11(4), 477-490.

[8] Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: a review. Environmental y experimental botany, 68(1), 14-25.

[9] Helepciuc, F. E., Mitoi, M.E., Manole-Paunescu, A., Aldea, F., Brezeanu, A. and Cornea, C. P. (2014). Induction of plant antioxidant system by interaction with beneficial y/or pathogenic microorganisms. Romanian Biotechnological Letters, 19(3), 9366-9375.

[10] Jetiyanon, K. (2007). Defensive-related enzyme response in plants treated with a mixture of Bacillus strains (IN937a y IN937b) against different pathogens. Biological control, 42(2), 178-185.

[11] Lin, T.C., Ishizaka, M. and Ishii, H. (2009). Acibenzolar-S-methyl-Induced Systemic Resistance against Anthracnose y Powdery Mildew Diseases on Cucumber Plants without Accumulation of Phytoalexins. Journal of phytopathology, 157(1), 40-50.

[12] Liu, X., Shi, W., Zhang, S. and Lou, C. (2005). Nitric oxide involved in signal transduction of Jasmonic acid-induced stomatal closure of Vicia faba L. Chinese Science Bulletin, 50(6), 520-525.

[13] Maksymiec, W. and Krupa, Z. (2002). The in vivo y in vitro influence of methyl jasmonate on oxidative processes in Arabidopsis thaliana leaves. Acta physiologiae plantarum, 24(4), 351-357.

[14] Massange-Sanchez, J. A., Palmeros-Suarez, P. A., Martinez-Gallardo, N. A., Castrillon-Arbelaez, P. A., Aviles-Arnaut, H., Alatorre-Cobo, F. and Délano-Frier, J. P. (2015). The novel y taxonomically restricted Ah24 gene from grain amaranth (Amaranthus hypochondriacus) has a dual role in development y defense. Frontiers in plant science, 6, 602.

[15] Metodiev, M. V., Tsonev, T. D. and Popova, L. P. (1996). Effect of jasmonic acid on the stomatal y nonstomatal limitation of leaf photosynthesis in barley leaves. Journal of plant growth regulation, 15(2), 75-80.

http://dx.doi.org/10.22161/ijeab/3.4.12 *ISSN: 2456-1878*

[16] Moreira, X., Sampedro, L. and Zas, R. (2009). Defensive responses of Pinus pinaster seedlings to exogenous application of methyl jasmonate: concentration effect systemic response. Environmental y Experimental Botany, 67(1), 94-100.

[17] Niu, D., Xia, J., Jiang, C., Qi, B., Ling, X., Lin, S. and Zhao, H. (2016). Bacillus cereus AR156 primes induced systemic resistance by suppressing miR825/825* y activating defense-related genes in Arabidopsis. Journal of integrative plant biology, 58(4), 426-439.

[18] Oliveira, M. D. M., Varya, C. M. R. and Félix, M. R. F. (2016). Induced resistance during the interaction pathogen x plant y the use of resistance inducers. Phytochemistry letters, 15, 152-158.

[19] Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. and Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. Annual review of phytopathology, 52, 347-375.

[20] Weston, D. J., Pelletier, D. A., Morrell-Falvey, J. L., Tschaplinski, T. J., Jawdy, S. S., Lu, T. Y. and Karve, A. A. (2012). Pseudomonas fluorescens induces strain-dependent y strain-independent host plant responses in defense networks, primary metabolism, photosynthesis, y fitness. Molecular Plant-Microbe Interactions, 25(6), 765-778.

[21] Winkler, R. (2015). SpiderMass: semantic database creation y tripartite metabolite identification strategy. Journal of Mass Spectrometry, 50(3), 538-541.

[22] Yin, D., Deng, X., Chet, I. and Song, R. (2014). Physiological responses of Pikus sylvestris var. mongolica seedlings to the interaction between Suillus luteus y Trichoderma virens. Current microbiology, 69 (3), 334-342.

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Effect of Spacing and Poultry Manure Rates on Growth, Yield and Quality of Cayenne Pepper (*Capsicum frutescens. L*) in Southern Rain Forest of Nigeria

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Abstract *– Field experiment was conducted at the Teaching and Research Farm of the Department of Agriculture, Ndele Campus, Ignatius Ajuru University of Education, Port Harcourt, Rivers State, to study the effects of poultry manure rates and crop spacing on growth, yield and quality of Cayenne pepper. The 3 x 3 factorial experiment with three replicates was arranged in a Completely Randomized Design. The main plots were three poultry manure rates (0, 10, 20tons/ha-1) and sub plots, three spacing (50cm x 50cm, 100cm x 50cm and 100cm x 100cm). Data collected were plant height, number of leaf per plant, leaf area; number of fruits per plot, fruit weight per plot, fruit yield per hectare, fruit lycopene and vitamin C contents. Results showed plant height increased with reducing planting distance and increasing Poultry manure rate; 50cm by 50cm fertilized at 20tons/ha produced the tallest plants with most number of leaves but least leaf area LA. Number of fruits, fruit weight and yield per plot, per hectare increased with increasing planting density and increasing Poultry manure rates lycopene and vitamin c contents increased with Poultry manure levels within the different spacing. Spacing of 50cm by 50cm fertilized with poultry manure at 20 tons per hectare is recommended.*

Keywords— Cayenne pepper, Poultry Manure, Rainforest, Spacing, Yield.

I. INTRODUCTION

Cayenne pepper, *Capsicum frutescens*, a member of hot pepper Solanaceous family, thought to be native of South America, is commonly used as ingredient in the preparation of soups, sauces, stew probably because it contains essential nutrients and vitamins such as A, E and C. It is called "sombo" and produced in Nigeria where it is consumed by the people fresh, dried or processed [1, 2, 3]. The land area under pepper production in Nigeria as at 1988 and increasing was estimated to be 100 – 200 hectares [4]. Factors accounting for this increasing land under *Capsicum* production on the one hand, is its consumption in Nigeria which accounts for 40 percent of the total vegetable consumed per day and on the other hand the lucrative export business. Nigerian pepper is in high demand abroad because of its pungency and good flavor and it can be readily dried, ground and packaged for export [5].

Although pepper can be grown all over Nigeria, the northern region between latitude 10° N and 12° N is the major area for production where an estimated 77,000 hectares of land under pepper cultivation yields about 695 000 metric tons [6]. The export attraction has encouraged pepper production in western Nigeria where production have been accompanied with a lot of research [1, 2, 7] and in the eastern region that even has special indigenous variety, "Nsukka yellow pepper" [8, 9]. Literature is scarce for *Capsicum* spp. production in the southern rainforest region of Nigeria.

Capsicum yield among peasant farmers are often very low [10]. In general *Capsicum* yield in the developing countries is comparatively lower than those of the developed countries in the region of about 10 – 30% less.

Reduced and inherent soil fertility and management practices, weeds infestation and diseases problems have been largely attributed to the lower yields [3, 5]. Crop intensification, higher planting densities, judicious use of fertilizers and organic matter maintenance have been proffered as panacea for improved production output of tropical crop production [11, 12]. The southern rainforest of

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Nigeria, a typical rainforest is characterized by high rainfall amounts and intensity that has been credited with high leaching that has resulted in impoverishing of the soil making it fragile [13]. Therefore, fertilization studies on pepper production in this region become imperative.

Organic manure application has been reported to be more beneficial over the use of chemical fertilization in tropical crop production, sustainability and soil fertility management. The addition of organic manure enhances crop yield because of improved soil productivity as a result of increased soil organic carbon content and improved soil physical, soil chemical and soil biological properties [6, 14]. Whereas the use of chemical fertilizers supply mainly one or a few macronutrients, in addition to their ruinous effect to the soil, organic fertilization conditions the soil, supplies several macro and micro nutrients, improves and maintain soil fertility status and also improves crop response to inorganic fertilization [15, 16]. The use of organic fertilizer has been reported to improve flavor and quality of vegetable crops as against the use of inorganic fertilizer. Inorganic fertilization dis-flavored the 'Nsukka yellow' pepper while organic manure enhanced flavor and opined the best option in the cultivation of the pepper [17]. Amaranth plants with organic manure treatment had higher nutritional values in the entire plant (leaf, stem, inflorescence and root) than those with inorganic fertilizer treatment [18]. The above informed the researchers on the study of organic fertilization instead of inorganic fertilization. Poultry manure among other sources of organic manure was selected because it is superior and has higher nutrient content than other sources at the farmer's disposal [19, 20, 21].

Increasing the number of crops per unit farmland is one of recommendations for increased yield or crop produce output. Yield increase as a result of closely spaced crops (i.e. higher density) in a crop like pepper is because more plants will produce more fruits, hence increase in number of fruits or yield [22]. Increasing the planting density of bell pepper resulted in higher yield (kg·ha-1) [23]. Southern Nigeria tropical rainforest is characterized by high rainfall amounts with wide monthly variation, intensification in terms of high planting density might favor high yield in cayenne pepper production.

The study was therefore undertaken to assess the effect of spacing and poultry manure rates on the growth, yield and fruit quality of *Capsicum frutescens* in the southern Rainforest of Nigeria.

II. MATERIALS AND METHODS

Study Site

The experiment was conducted at the Teaching and Research Farm of the department of Agriculture, Ignatius Ajuru University of Education, Ndele Campus, Port Harcourt, River state, between July and November, 2017. Ndele is located in the southern rainforest region with about 9.5 months of adequate rainfall, 2.5 dry season months, and cumulative insolation of 120 – 160 Kcal /cum per annum [24].

Nursery practice

Seeds of cayenne pepper variety of *Capsicum frutescens* were on enclosed 1 m x 1 m nursery beds made of dark top soil. The beds were watered before the seeds were broadcasted evenly and watered. After emergence, thinning was done, removing some seedlings so that strong healthy pepper seedling can be obtained for transplanting. Seedlings were watered daily and ready for transplant after 40 days.

Land Preparation

The total experimental area was manually cleared, grass stumps dug out. The soil was tilled with spade and 3 m by 3 m beds were constructed. The individual beds were then mapped out according to the respective spacing ready for seedling transplant.

Experimental Design/Treatment

The study adopted a 3 x 3 factorial experiment arranged in a randomized complete block design (RCBD) and replicated three times. The treatments were (i) Poultry manure rates (0, 10 and 20 tons per hectare) and (ii) Spacing (50 cm x 50 cm; 100 cm x 50 cm and 100cm x 100cm). Poultry manure rates were the main plots while spacing the sub plots, replicated 3 times to give a total of 9 main plots and 27 sub plots. Treatment combinations were randomly allotted to plots. Application of poultry manure was done 2 weeks after transplanting. Soil sample of the experimental sight were obtained using soil auger ad sent to the Laboratory for analysis.

Data collection and analysis

Vegetative and growth parameters measured at 2, 4 6, 8 and 10 weeks after transplanting were plant height (growth rate), number of leaves and leaf area.

Yield parameters determined at harvest were number of fruits per plot, fruit weight per plot and estimated fruit yield per hectare.

Fruit quality were accessed by determining fruit moisture and Vitamin C contents [25] and lycopene content [26].

Data collected were subjected to analysis of variance and means separated by Duncan Multiple Rang Test (DMRT) using PASW 18th Edition statistical software.

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III. RESULTS

Visual Observation

It was observed that application of poultry manure irrespective of the spacing resulted in darker green leaves of the pepper. Application of the manure also enhanced the establishment of the transplanted seedling.

Growth Response

Growth response (plant height) of cayenne pepper to varying rate of poultry manure and spacing is displayed in Table 1. Irrespective of treatments, plant height i.e. growth rate increased with age of the plants. Also, plant height increased with density i.e. spacing. The growth rate response to spacing was significant ($P \leq 0.05$), indicating that the variation in plant height was due to the different spacing. The trend was that plant height increased as the distance (spacing) between plants reduced. At 10 weeks after transplanting (WAT) cayenne plants spaced at 50 cm x 50 cm were 1.5 times taller than the most widely spaced plant at 100 cm x100 cm.

Application of poultry manure rates was also responsible for the variation in plant height (Significant Fcal $P < 0.05$). Increasing rates of poultry manure resulted in positive correlating increase in plant height of cayenne plants. While the difference in height between the control plants and those that received poultry manure was not significant at 2 WAT, it became significant by 10 WAT, the difference in height was 40% over the control plants.

Vegetative Growth

The influence of spacing and poultry manure on vegetative characteristics of cayenne pepper is highlighted in Table 2. Cayenne pepper spaced at 100 cm by 100 cm had plants with the least number of leaves, and significantly different from those spaced at 50 cm by 50 cm and 100 cm x 50 cm ($P < 0.05$). Though, pepper plant spaced at 50 cm by 50 cm had higher number of leaves than those spaced at 100 cm x 50 cm, the difference was not statistically or markedly different.

Poultry fertilizer rate had marked statistically significant effect on number of leaves of cayenne pepper. Number of leaves increased significantly with increase rates of poultry manure application. Plants that were fertilized with 20ton/ha PM rate recorded the highest number of leaves (14) compared to with the control plants.

There were marked variation in cayenne pepper leaf area due to the various spacing (Table 2). Pepper crops spaced 50 cm by 50 cm had the smallest size of leaves (less leaf area), while those spaced and 100 cm x 50 cm and 100 cm by 100 cm were not significantly different but produced leaves with higher leaf area than those spaced at 50 cm x 50 cm.

Poultry manure doses had marked leaf area variation in *Capsicum frutescens*. The Pepper crops that did not receive poultry manure had the smallest sized leaves (leaf area). Increasing levels of poultry manure resulted in increasing sizes of leaves. Cayenne pepper plant that received 20 ton/ha poultry manure application produced the highest leaf area of 56.8cm^2. This is followed by plants that received 10 ton/ha which produced leaf area of 53.9 cm ^{2}.

Fruit Yield

The effect of spacing and poultry manure rate on cayenne pepper fruit yield is shown on Table 3. Number of harvested fruit and fruit weight (yield) were markedly affected by spacing. Fruit yield and numbers increased with the closer high density spacing. Thus plants spaced 50 cm x 50 cm had doubled the yield of those spaced at 100 cm by 100 cm and was the yield per plot was 3 times over those spaced at 100 cm x 100 cm.

The response of *Capsicum frutescens* to poultry manure inclusion was such that fruit yield per plot and per hectare increased with increasing levels of the manure. The yield of the crop at application rates of 10 and 20 ton/ha was not significantly different. However there was marked difference in yield between poultry manure fertilized pepper crop and the control. The increase in yield of 10 and 20 ton/ha fertilized pepper plants over the control plants was 65% and 83% respectively.

Table.1: Effect of Spacing and Poultry Manure rates on Capsicum frutescens growth rate (Plant height cm)

Spacing	**Weeks after Transplanting**				
	2	4	6	8	10
50 cm x 50 cm	17.0^c	19.3^c	27.3^c	34.1^c	39.5^c
100 cm x 50 cm	16.3^b	17.7^b	24.3^b	28.4^b	32.1^b
100 cm x 100 cm	12.4^a	14.4^a	18.4^a	24.3^a	26.1^a
SE	.186	.173	.366	.529	1.020
Poultry Manure (PM) Rate					
0	14.5^a	16.5^a	21.4^a	25.5^a	26.2^a
10 ton/ha	15.2^a	16.9^a	23.8^b	29.9^b	34.4^b
20 ton/ha	16.0^a	18.0^b	24.9^c	31.4^c	36.9^b

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SE ±	.186	.173	.366	.529	1.020
Spacing X PM Rate	NS	*	*	*	*

*Means followed by same letter in each column are not significantly different at P < 05 by Duncan multiple rage test. * = Significant. NS = Not significant.*

Table.2: Influence of spacing and poultry manure rate on vegetative characteristics of Capsicum frutescens (10 WAT)

Spacing	**No. of Leaves**	**LA (cm^3)**
50 cm x 50 cm	13.8[b]	42.3[a]
100 cm x 50 cm	13.3[b]	47.4[b]
100 cm x 100 cm	12.0[a]	47.4[b]
SE	.203	.272
Poultry Manure (PM) Rate		
0 ton/ha	11.2[a]	24.5[a]
10 ton/ha	13.4[b]	53.9[b]
20 ton/ha	14.4[c]	56.8[c]
SE	.203	.272
Spacing & PM Rate	NS	NS

** Means followed by same letter in each column are not significantly different at P < 05 by Duncan multiple rage test *.*

Table.3: Effect of Spacing and Poultry manure levels on yield in Capsicum frutescens

Spacing	**Number of Fruit**	**Fruit weight/plant (g)**	**Fruit yield kg/ha**
50 cm x 50 cm	192.4[a]	456.7a	507.4
100 cm x 50 cm	111.2[b]	265.2b	294.6
100 cm x 50 cm	61.7[c]	148.9c	165.4
S.E.	1.478	4.147	
Poultry Manure (PM) Rate			
0 ton/ha	83.8[a]	194.2[a]	215.7
10 ton/ha	133.5[b]	321.5[b]	357.2
20 ton/ha	148.0[c]	355.0[b]	394.4
S.E.	1.478	4.147	

** Means followed by same letter in each column are not significantly different at P < 0.05 by Duncan multiple rage test *.*

Table 4 shows the quality effects to spacing and poultry manure levels by cayenne pepper. Per cent moisture content was not influence by spacing and poultry manure rates as no clear trend was observed. However the widely spaced crops at 100 cm by 100 cm had more moisture in the fruit. Within each spacing treatment vitamin contents in the fruits increased with manure rates, with plants spaced at 50 cm x 50 cm producing pepper plants with highest vitamin c as manure level increase.

Lycopene content in cayenne fruit were positively affected by poultry manure in all spacing treatment. Plants that did not receive manure had lowest levels of lycopene; however among the plants that were spread at 100 cm by 50 cm lycopene content of the control plants had higher lycopene levels than those fertilized with poultry manure.

In plant spaced at 100 cm by 100 cm, increasing poultry manure rate beyond 10 ton/ha resulted in reduction of lycopene content in the fruit. In plants spaced at 50 cm by 50 cm increasing poultry manure content resulted in increasing levels of lycopene in fruit.

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Table.4: Influence of spacing and poultry manure rates on fruits quality cayenne pepper

Spacing	Poultry manure rate Ton/ha	% Moisture Content	Vitamin C	Lycopene (mg/kg)
50 cm x 50 cm	0	77.77	0.26	28.50
	10	74.43	0.61	31.12
	20	75.74	0.77	34.45
100 cm x 50 cm	0	79.43	0.26	28.50
	10	69.1	0.3562	17.95
	20	73.27	0.4272	24.89
100 cm x 100 cm	0	79.87	0.26	28.51
	10	70.2	0.2637	60.19
	20	75.4	0.5148	49.65

IV. DISCUSSION

Effect of Spacing and Poultry Manure Rates on Growth and Yield of Cayenne Pepper

This study observed that plant height, that is, growth rate increased with spacing. This is in line with studies by other researchers who reported increase in plant height in closely spaced green pepper in Kenya [27]. It was also observed that increasing the rate of poultry manure led to increasing plant height of cayenne pepper. This observation corroborates other research findings on aromatic pepper, *Capsicum annum* L var (Nsukka yellow) in Nsukka, Enugu State, Nigeria [28]; that plant height increased with increasing poultry manure rates.

Reducing planting distance and increasing poultry manure rate resulted in increasing number of leaves. This is similar to the reports increasing vegetative growth with poultry manure application in Nsukka yellow anomatic pepper at Nsukka [29].

The fruit yield of cayenne pepper increased with spacing per plot and per hectare. This could be as a result of higher population of plants, with each individual plants producing high number of fruits. Similarly in a different study on green pepper, closely spaced pepper recorded higher yields than widely spaced pepper [27]; while higher yield was observe in tomatoes with increasing levels of poultry manure [30].

Effect of Spacing and Poultry Manure on fruit quality of Cayenne pepper

Increasing levels of poultry manure resulted on the increasing contents of lycopenes and Vitamin C in this study. This finding is similar to the findings of other research done on tomato; which indicated that lycopene content increased with poultry manure levels [30]. However their findings [30] that control tomatoes plant had higher vitamin content than those receiving poultry manure is contrary the observation in this study. It was observed that the control plants had lower levels of Vitamin C compared with cayenne pepper that received poultry manure.

V. CONCLUSION

Spacing cayenne pepper at 50 cm by 50 cm will result in higher growth rate and yield. Application of 20 ton/ha of poultry manure will result in higher growth rate and yield. Spacing has no significant difference on lycopene and vitamin content in cayenne pepper. Increasing rate of poultry manure results in higher Vitamin C content in cayenne pepper. Application of 20 ton/ha poultry manure and a spacing of 50 cm by 50 cm is recommended for cayenne pepper production in the southern rainforest zone of Nigeria.

REFERENCES

[1] Alegbejo, M.F., Orakwe, F.C. & Ado, S.G. (1999). Characteristics of chilli pepper cultivars released by the Institute for Agricultural Research, Samaru, Nigeria. Capsicum and eggplant Newsletter,18, 21-24.

[2] Denton O.A., Olufolaji A.O. (2000). Nigeria's most important vegetable crops. In: Akoroda, M.O. (Compiler). Agronomy in Nigeria: A Book in Support of Agronomy Re-Union Day on 4th October 2000. Department of Agronomy, University of Ibadan, Nigeria 85-93.

[3] Grubben, G.J.H., & El-Tahir, I.M. (2004). Capsicum annum L. In: (Grubben, G.J.H. and Denton, O.A, Editors). Plant Resources of Tropical Africa 2. Vegetables. (pp.154-163). PROTA Foundation, Wageningen, Netherlands/CTA Wageningen, Netherlands.

[4] Ado, S.G. (1988). Evaluation of Capsicum in Nigeria. PGR Capsicum Eggplant Newslett., 17: 16-17.

[5] Erinle, I.D. (1989). Present status and prospects for increased production of tomato and pepper in Northern Nigeria. Tomato and pepper in the tropics AVRDC Shauhua Taiwan. p 545.

[6] FAO. (2000). Fertilizers and their Use. 4th Edn., Food and Agriculture Organization, Rome, Pages: 34.

[7] Alabi, D.A. (2006). Effect of fertilizer phosphorus and poultry droppings treatments on growth and nutrient components of pepper Capsicum annum. African Journal of Biotechnology, 5 (8): 671-677.

[8] Ajayi AR and Eneje CN (1998). Preference of Nsukka Yellow Pepper Among Growers and Consumers in Nsukka agricultural zone of Enugu State,Nigeria. Proc. 15th HORTISON conf., Ibadan, 8th - 11th April. pp. 184-187.

[9] Echezona, B.C and Nganwuchu, O.G. (2006). Poultry Manure application and varietal effects of chilli-pepper (*Capsicum* spp.) on insect pest and disease in a humid –tropical environment. Journal of Agriculture, Food, Environment and Extension 5 (2): 49-58.

[10] Adigun, J.A. (2001). Influence of intra-row spacing and chemical weed control on the growth and yield of chilli pepper (*Capsicum frutescens* L.) in the Nigerian Northern Guinea Savannah. Nigerian Journal of Horticultural Science 5:67-73.

[11] Agboola, A.A., (1982). Organic manuring and green manuring in Tropical Agricultural systems. Proc. of 12 Inter. Cong. of Soil Science, New Delhi, India 8-16 February, pp: 198.

[12] Hamma, I.L. and Ibrahim, U. (2013). Management Practices for Improving Fertility Status of Soils in Nigeria. World Journal of Agricultural Sciences 9 (3): 214-219.

[13] Elliott, S.D., Blakesley, D. and Hardwick, K. (2013). Restoring Tropical Forests: a practical guide. Royal Botanic Gardens, Kew; 344 pp.

[14] Udoh, D. J., Ndon, B. A., Asuquo, P. E. and Ndaeyo, N. U. (2005). Crop Production Techniques for the Tropics. Concept Publication Lagos. Pp.446.

[15] Mahmoud A. M and Soliman A .Sh (2017). Comparative Study on the Influence of Organic Fertilizer and Soil Amendments on Evening Primrose (*Oenothera biennis* L.). International Journal of Agricultural Research, 12: 52-63.

[16] Akande, M. O., Kayode, C. O., Oluwatoyinbo, F. I. and Adediran, J. A. (2008). Efficiency of NEB-33 fortified fertilizers on growth and yield of pepper (*Capsicum frutescens*). African Journal of Biotechnology, 7 (7): 873-877.

[17] Onugha AI (1999). Growth and yield response of Nsukka yellow pepper, Capsicum annuum, to plant population and levels of organic manure application, B. Agric. Project report, Dept. of Crop Science, University of Nigeria, Nsukka, Nigeria 43 p.

[18] Mofunanya, A.A.J., Ebigwai, J.K., Bello O.S. and Egbe A.O.(2015). Comparative Study of the Effects of Organic and Inorganic Fertilizer on Nutritional Composition of *Amaranthus spinosus L*. Asian Journal of Plant Sciences 14 (1): 34-39.

[19] Ikeh A.O. Ndaeyo N.U, Uduak I.G. Iwo G.A. Ugbe L.A, Udoh E.I. Effiong G.S. (2012). Growth and yield responses of pepper (*Capsicum frutescens* L) to varied poultry manure rates in Uyo, Southeastern Nigeria, ARPN Journal of Agricultural and Biological Science; 7(9): 735-742.

[20] Asiegbu, J.E. (1987). Effect of organic matter substrate sources and time of photosynthetic-sink removal on Flower and Pod Production I Okra *Abelmoschus esculentus* (L) Moench. East Afr. Agric. Forest. J., 52, 293 -297.

[21] De Lannoy, G. and Romain, H. R. (2001). Crop Production in Tropical Africa. McGraw Hill Publishers Ltd. New York Pages 245

[22] Cavero, J., Ortega Gil, R., and Gutierrez, M. (2001). Plant density affects yield, yield components and color of direct-seeded paprika pepper. HortScience 36(1), 76-79.

[23] Nasto T. H., Balliu A. and Zeka N. (2009). The influence of planting density on growth characteristics and fruit yield of peppers (Capsicum annuum). Acta Hort. 830 906-912.

[24] Ansa, J. E. O. (2016). Effect groundnut spacing on yield and weed control in the rainforest agro-ecological zone of Nigeria. Direct Research Journal of Agriculture and Food Science. Vol. 4 (12): 49-52.

[25] AOAC (2005). Association of Official Analytical Chemist (AOAC), Official methods of analysis, 6th ed., Washing- ton DC, USA, 1980.

[26] Suwanaruang, T. (2016). Analyzing lycopene Content in Fruits. (International Conference on Inventions & Innovations for Sustainable Agriculture 2016, ICIISA), Agriculture and Agricultural Science Procedia 11: 46 – 48.

[27] Edgar, O. N., Gweyi-Onyango J. P. and Korir N.K. (2017). Plant Row Spacing Effect on Growth and Yield of Green Pepper (*Capsicum annuum L.*) in Western

http://dx.doi.org/10.22161/ijeab/3.4.13 *ISSN: 2456-1878*

Kenya. Archives of Current Research International 7(3): 1-9.

[28] Baiyeri, P. K., Otitoju, G. T., Abu, N. E., and Umeh, S. (2016). Poultry manure influenced growth, yield and nutritional quality of containerized aromatic pepper (*Capsicum annuum* L., var 'Nsukka Yellow'). African Journal of Agricultural Research. Vol. 11(23): 2013-2023.

[29] Abu, N. E., and Odo, C. V. (2017). The effect of plant density on growth and yield of 'Nsukka Yellow' aromatic pepper (*Capsicum annuum* L.). African Journal of Agricultural Research. Vol. 12(15), pp. 1269-1277.

[30] Adeniyi, H. and Ademoyegum, O. (2012). Effect of different rates and sources of fertilizers on yield and antioxidant components of Tomato. Agric J 7 : 135—138.

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Performance of EcoSan Toilets at Majumbasita in Dar Es Salaam – Tanzania

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Abstract**—** *The performance of Ecological Sanitation (ECOSAN) toilets at Majumbasita-Ukonga in Dar es Salaam, Tanzania was studied in order to assess their feasibility for low cost and effective environmental protection. The studied parameters for urine were pH, TKN, phosphorus , potassium and E-coli and for faecal sludge were temperature, pH, TS, VS, COD , TKN, ammonium, phosphorus, potassium and Ascaris eggs. Results indicated a high pH up to 10.3 in the faecal contents due to addition of ashes. The temperatures were between 27^0C – 31.7^0C during the whole study period. The total COD measurements varied from 33 - 74 gCOD/l, while TS and VS were respectively 57– 81 gTS/l and 21 – 46 gVS/l. The results for TKN, ammonium, phosphorus and potassium in faecal sludge were 5045 – 6080mg/L, 5207-5852 mg/L, 29-70mg/L and 105-176 mg/L, respectively. Ascaris eggs were efficiently removed from faecal sludge that were strictly dry and had a pH of more than 10. The results for TKN, ammonium, phosphorus and potassium in urine were 4285-5010 mg/L, 111-195 mg/L and 190-251 mg/L, respectively. E-Coli were present in urine with pH less than 11.5 and were efficiently removed from urine with pH more than 11.5. Presence of pathogens in urine implies the separated urine can be reused in tree growing and not for fertilising food crops consumed raw.*

Keywords**— *ECOSAN toilets, Faecal Sludge, Urine, Performance, Environmental protection.***

I. INTRODUCTION

Environmental sanitation problems have continued to grow in complexity despite receiving little attention in Tanzania. The hygienic disposal of excreta that does not endanger health and welfare of the community is important (Obeng *et al*, 2015; Hu *et al*, 2016)). There are many constraints in improving the existing sanitation situation that centre on political, economic, social and cultural contexts of health and disease. Serious constraints (Obeng *et al*, 2015), which are still prevalent to-date, are: funding limitations; insufficiency of trained personnel; operation and maintenance; logistics; inadequate cost-recovery framework; insufficient health education efforts; inappropriate institutional framework; intermittent water service and non-involvement of communities. Given the inherent unsustainability of large scale sewerage, on-site sanitation concepts, dealing with human excreta collection and treatment on-site, that is, the location where it is deposited, can provide a hygienic and satisfactory solution (Obeng *et al*, 2015; Taseli, 2016; Mahenge, 2013; Shen, 2013), because it is not an expensive and high technology. For reuse of human "waste" and turning it into something "useful and valuable" (Breslin, 2014; Esrey, 2001), ecological sanitation (ECOSAN) had been introduced as a pilot scale at Majumbasita-Ukonga in Dar-es-Salaam city, Tanzania by EEPCO (Environmental Engineering and Pollution Control Organization - Non Governmental Organisation) through UNICEF funding. These introduced toilet systems are in the category of improved pit-toilets and they are 'dry' accumulation systems or so-called ECOSAN toilets which receive only faeces. Moreover, after faeces deposit, ashes are added to increase the pH and reduce moisture. If the moisture content and ashes additions are balanced, it is believed that the faeces will decompose to form a soil conditioner in about four months (Esrey *et al*, 2001). This idea of decomposition was given without thorough exploration of the conditions needed for biodegradation to proceed and was further ascertained in this study. Pathogens are killed in the dry alkaline compost that can be used as soil fertilizer. The urine is collected separately and can also be used as fertilizer (Esrey *et al*, 2001). To get an insight in the performance of these structures the following study objectives were to: Get the baseline information related to composting toilet systems in the country; Check the performance of composting toilets in pre-treatment of human excreta; Verify the quality of separated human urine, as it is believed to be sterile, people are using it directly in agriculture without knowing the safety associated with its re–use.

II. MATERIALS AND METHODS

2.1 Description of the Research Area

Majumbasita, is one of the unplanned settlements at the peri-urban part of Dar-es-Salaam city in Tanzania. It is about 11 km from the city centre, in the western direction and closer to the Dar-es-Salaam International Airport (DIA) in Kipawa ward, Ilala Municipality; with a

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population of about 23,000 inhabitants. Houses are mostly occupied by owners, with few inhabited by tenants. The size of plots varied from about 170 - 400 square metres (EEPCO, 2007) during study. The piped water supply from the city network is inadequate for the inhabitants; 85% depend on well water (Mato, 2002) and are forced to use hand-dug wells although the quality is doubtful (Elisante and Muzuka, 2017; Addo, 2016). It was noted that there is intermittent supply of water per week; that is, the supply was for 2-4 hours per supply (Elisante and Muzuka, 2017). Only 5% of the residents get it once per week, 63.2% two days/week, 28% three days/week, 2% four days/week and 1.2% manage to get water for >4 days/week. Moreover, their laboratory results revealed that, *E-Coli* count for samples from boreholes with depths 1.8 metres and 6.75 metres were 3000 FC/100 ml. and 178 FC/100 ml., respectively (Mato, 2002; Addo, 2016). The intensity of *E-Coli* showed a decreasing trend with depth. The wells deeper than 70 metres due to increase in pressure were free from faecal contamination implying that, water from deeper soil layers was bacteriologically safe for drinking purposes at that moment. Similarly, for the piped water supply in that area, it was observed that, there was an increase in faecal pollution in the service pipes (values ranged from 3 - 76 FC/100 mL for 25 sampling points chosen). The area experiences high water table (HWT) evidenced by the raised pit toilets (75%) in the area, while some dwellers (4.8%) did not have any toilet facility; they use neighbours toilets (EEPCO, 2007, Elisante, Mato, 2002 and Muzuka, 2017).

2.2 Schematic Presentation and Functioning of ECOSAN Toilets

The schematic presentation of ECOSAN toilet at Majumbasita is as shown in Figure 1.

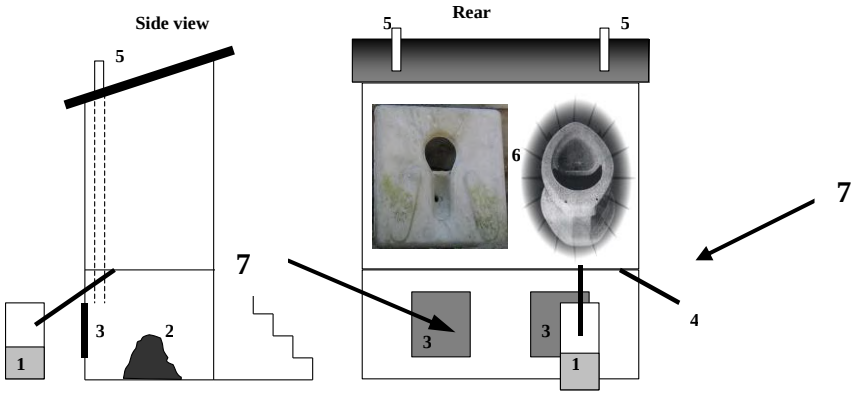

Fig.1: Photographs of caption of the ECOSAN toilet at Majumbasita

Symbol´s Explanation:
(1) Urine tank of 20 litres capacity (2) Accumulating faeces and ashes heap in the toilet vault (3) Doors at the back of the pit-chamber for desludging purposes(4) Wash water outlet (5) Vent pipe (6) Drop hole slabs (squatting and seating type) (7) Separated Urine hole.

The components of the ECOSAN include two pits, two openings for removal of dehydrated faecal material, two vent pipes (for each pit), two squatting pans or one squatting pan and one seat riser, or two seat risers, two urine drain pipes and one drain pipe for anal cleansing water, one plastic container for urine, superstructure, squatting slab and roof. The components are provided in duplicate because when one component is in use, the other one is on stand-by basis. If the pit in use is full, it is closed for a time and the other stand-by pit is opened for use. It is expected that, the dehydrated material in the closed pit decomposes for a period of not less than 6 months and then the system will be desludged and the sludge used in agriculture. So far none of the systems has been desludged. Normally, after defecation, a handful of ashes from charcoal stoves or burnt wood ashes are poured into the pit. Ashes (charcoal/plant ash) are added inside the faecal chamber to raise pH (for pathogen destruction), reduce odour and dehydrate the faecal material. The pH must be ≥ 10 because it is unfavourable pH for most pathogens especially *ascaris* (Taseli, 2016). Obeng remarked that, estimating the volume of ash is difficult (Obeng *et al*, 2015). Experiences indicates that approximately twice the volume of faeces has to be added (Vliet *et al*, 2013) while, Kujawa suggested five times the volume of faeces or 0.3 m^3 per person per year for all waste (Kujawa-Roelveld, 2016). At Majumbasita 0.1-0.33 litre of ashes/person/day/defecation is added without formal control of the amount. The charcoal ashes are kept inside the superstructure on the squatting slab in order to avoid inconveniences. No water or any liquid material is allowed to mix with faeces inside the chamber. However, due to the religious beliefs of some people, water must be used for anal cleansing and hence, a place in between the two squatting holes was modified to cater for cleaning purposes and the wash-water led to the outside through a pipe. For this purpose, a small plastic water container of 5 litres is provided for this need. The wash water is not treated but ends up in the surrounding area signifying a

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need for proper disposal. The 20-litre urine tank is kept outside and usually emptied when it is full.

2.3 Sampling and Analysis

Samples were collected from 10 ECOSAN toilets. A sampling device with specifications: Model – Eijcamp; Made is Agrisearch Equipment, was used to collect faeces. One kilogram of faecal sludge and one litre of urine were collected per toilet for laboratory analysis. Analyzed parameters in faeces were pH, ambient temperature of the samples, COD, TS, VS, TKN, NH_4^+-N, PO_4^{-3}-P, K and Ascaris eggs. Analyzed parameters in urine were pH, TKN, PO_4^{-3}-P, K and *E*-coli. The parameters were determined by using Standard Methods (2002).

III. RESULTS AND DISCUSSION

3.1 pH, Temperature and Ascaris Eggs Results in Faecal sludge

The pH ranged from 8.3 - 10.3 due to addition of ashes. The correlation by SPSS between pH and age of sludge is highly significant at P<0.001.It was furthermore evident that, the pH increases with increasing age of sludge in the toilet vault. Similarly, the ANOVA showed the same conclusions with R^2 of 63%. Tropical condition temperatures observed, 27 - 29.9^{0}C coupled with the pH suggests that, it is possible to have mesophilic digestion in the bottom part of the heap of feaces in ECOSAN pit only during the first months of operation. After that the pH goes up and it is logical to assume that no digestion goes on. The results in Ascaris eggs varied from 0 – 4000 counts/1000 mg. *Ascaris eggs* were efficiently removed from faecal sludge that were strictly dry and had a pH of more than 10.

3.2 COD, TS and VS Results in the Faecal Sludge for ECOSAN Toilets

The results in Table 1 indicate a TS range of 57 to 81 gTS/l, which is low range considering that the TS-content of fresh faeces is about 254.62 (±4.19) gTS/kg (Strande and Brdjanovic, 2014). Given the nature of toilet use, that is, with addition of ashes, it was anticipated to find higher TS values, and therefore, apparently, the urine was not completely separated. Even a small amount of urine going cumulatively into the faeces vault can lead to relatively low TS values. The total COD concentration in faecal material ranged from 33 to 74 g COD/l whereas the values for VS were between 21 - 46 gVS/l. The VS and COD concentration decreased with time due to the addition of the ashes (dilution). Table 1 results are within the literature reported values for faecal sludge from toilets and unsewered public toilets quality in different cities noted by Water Resources Research Institute (WRRI) in Accra, Ghana and SANDEC (Water and Sanitation in Developing Countries). They conducted numerous analyses of untreated septage and public toilet sludges and results reported by Semiyaga (2015), which varied from 49-97 gCOD/l. This also confirms that, the ECOSAN faeces accumulation chamber suffered from an intrusion of urine. The calculated average of COD:VS ratio was 1.57 gCOD/gVS, but it varied between 0.67-2.47 gCOD/gVS. Such a condition might be due to the fact that varying amount of ashes are added after defacation and therefore making the sludge in the vault to be inhomogeneous.

Table.1: The experimental results of human faecal sludge for ECOSAN toilets at Majumbasita

Age of Toilet (months)	4 months average	6 months javerage	8 months average
pH	8.3	9	10.3
Total COD (g/l)	74	52	33
TS (g/l)	57	71	81
VS (g/l)	46	34	21
VS/TS (%)	81	47	25
COD/VS	1.61	1.53	1.57
NH_4^+-N(mg/l)	5852	5207	5418
Total-N(mg/l)	6080	6077	5045
PO_4^{3-}-P(mg/l)	60	70	29
K(mg/l)	105	176	141
Ascaris eggs(/1000 mg)	667	273	0

3.3 Kjedahl Nitrogen, Total Nitrogen, Phosphorus and ammonium in faecal sludge

The ammonia-nitrogen concentrations for faecal sludge from undiluted samples from ECOSAN toilets ranged between 5207 - 5852 mg NH_4-N/L on the day of

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sampling, while the TKN values varied from 5045-6080 mg/L, values that are similar to those obtained by Strauss and Heinss (1995). Their result ranged between 2800 – 6000 mg/l. The phosphorus and potassium results found varied from 29-70 mg/L and 105 – 176 mg/L, respectively. These results are comparable with those found by Mashauri and Senzia (2002) for ECOSAN toilets at Majumbasita which were 28.5 mg/L and 166.43 mg/L for phosphorus and potassium, respectively.

3.4 Urine

The assessed pH of the collected urine from ten (10) ECOSAN toilets ranged between 6.27-11.80 and the temperature from 26.1-31.7 ^{0}C. From the fact that, the urine pH was 6–7 when excreted, but during its storage would raise to between 9-9.4 as a result of the degradation of urea (Hijikata *et al*, 2015), the higher values found in our measurements implies that, there is a certain amount of ashes which went into the urine tank. Furthermore, the results show that all of the toilets with pH less than 11.5 in urine were found with *E*-coli at a range of 50 -4500 counts. The detection of *E-Coli* counts in the urine storage tanks indicates there were faecal contamination and escape of ammonia gas from urine tanks (i.e the tanks were not airtight). With adequate separation, the "pure" urine is expected to be free of pathogens (Mahenge, 2013) but about 50% of the toilets were out of the maximum WHO Guidelines for unrestricted reuse in agriculture (1000 counts/100ml) especially for crops eaten raw; this could be risky. The result suggests that, direct use of urine separated in Majumbasita into the gardens could introduce pathogens into the soil and may infect vegetables, which then obviously represents a health hazard. The pH results compares with those found by Farzadkia (2014) for double vault-urine diverting (DVUD) toilet which where from 6.2–13.0 due to addition of additives. Nakagiri and Niwagaba (2016) found out that, addition of lime, ash and soil resulted in corresponding pH values of 11, 9.4 and 8.8. However, they found that, pH of 11–12 is reached in treatment methods using lime. The PO_4-P results in the urine were 111-195 mg PO_4-P/L and they are comparable to Kujawa-Roelveld, (2016). The TKN and potassium values were 4285 – 5010 mg-N/L and 190-251 mg K/L, respectively.

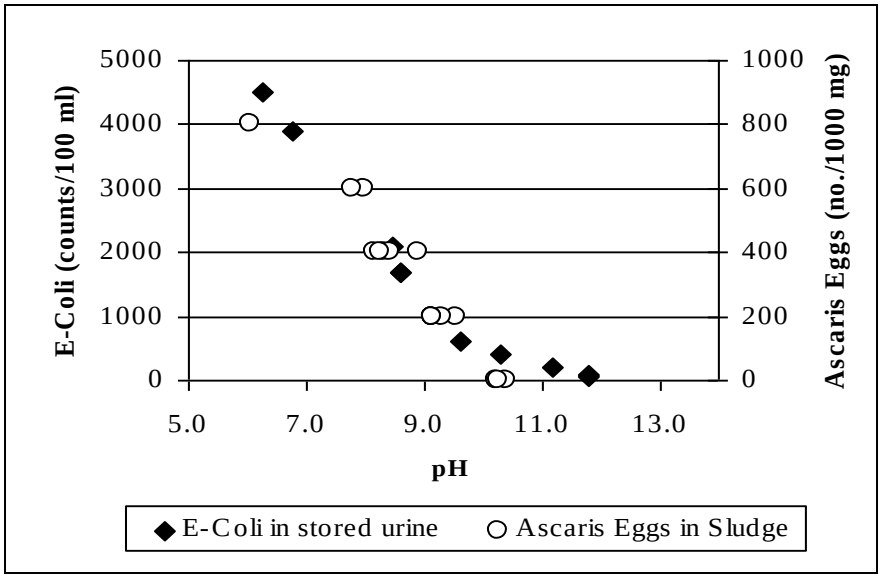

Fig.2: The E-Coli, Ascaris eggs and pH results in sludge and urine of ECOSAN toilets

IV. CONCLUSIONS

By using the ECOSAN toilets, such as those installed in Majumbasita, groundwater contamination can be avoided, since they are constructed above ground. More information needs to be collected as to the amount of ash(es) to be added to the toilet vault, the proposed 3 times the volume of faeces will occupy the main part of the volume of the chamber. A complete separation of pure urine seems to be difficult for users as evidenced by faecal coliforms observed in urine. The separated urine can be used directly as fertiliser in tree growing, but not for fertilising food crops that are consumed raw, due to presence of pathogens. The extent of biological sludge stabilization is likely to be small in view of the prevailing high pH values. A lot of urban small scale agriculture needs to be developed in order to enable the reuse of all the collected sludge and urine, otherwise, transport for reuse outside the city is necessary. *Ascaris eggs* and *E-Coli* are efficiently removed in ECOSAN toilets with high pH of more than 10 for faecal sludge and more than 11.5 for urine. Advocacy on the use of ECOSAN toilets improves the separated urine quality.

REFERENCES

[1] Addo N (2016). To Assess the Level of Heavy Metal Pollution in Groundwater in Ilala Dar es salaam.

Msc. Thesis, Open University of Tanzania. *OUT, http://repository.out.ac.tz/1537/*

[2] APHA, AWWA and WEF (2002). Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, DC

[3] Bethune DN; Chu A; Ryan MC (2015). Passive evaporation of source-separated urine from dry toilets: prototype design and field testing using municipal water, *Journal of Water, Sanitation and Hygiene for Development*. 5(3): 392-401.

[4] Breslin E (2014). Introducing ecological sanitation in northern Mozambique. WaterAid Mozambique.

[5] EEPCO (2007). Dry Ecological Sanitation Alternative in Tanzania. *http://www.eepco-tz.org/wp-content/uploads/2011/05/Dry-Ecological-Sanitation-Alternative-in-Tanzania-2007.pdf*

[6] Elisante E; Muzuka ANN (2002). Ecological Sanitation Toilets in Tanzania, *3rd International Conference on Integrated Environmental Management in Southern Africa, Johannesburg, August 27-30*.

[7] Elisante E; Muzuka ANN (2017). Occurrence of nitrate in Tanzanian groundwater aquifers: A review. *Applied Water Science-Springer*. P. 71-87.

[8] ENVIS Centre on Hygiene, Sanitation, Sewage Treatment Systems and Technology (2014). Low-cost Sanitation. *http://www.sulabhenvis.nic.in/Database/LowCostSanitation_5930.aspx*.

[9] Esrey, AS; Hillers, A; Sawyer, R (2001). Closing the Loop: Ecological Sanitation for food Security. UNDP and SIDA, Publications on Water Resources, Mexico

[10] Farzadkia M; Bazrafshan E (2014). Lime Stabilization of Waste Activated Sludge. *Health Scope International Journal*, 3:1-5.

[11] Hijikata N; Yacouba H; Bonzi M; Coulibaly L (2015). Ecological sanitation products reuse for agriculture in Sahel: effects on soil properties. *Soil Discuss*. 2: 291-322.

[12] Hu M; Fan B; Wang H; Qu B; Zhu S (2016). Constructing the ecological sanitation: a review on technology and methods. *Journal of Cleaner Production*. p.1 - 21.

[13] Kujawa-Roeleveld K; Zeeman G (2016). Anaerobic Treatment in Decentralised and Source-Separation. *Reviews in Environmental Science and Bio/Technology- Springer*. 5 (1):115–139

[14] Mahenge AS (2013). The Study of Ecological Sanitation Systems in Dar es Salaam, Tanzania. *International Journal of Development and Sustainability*. 2:2256-2265.

[15] Mashauri, DA; Senzia, MA (2002). Reuse of Nutrients from Ecological Sanitation Toilets as a Source of Fertiliser. *3rd International Conference on Integrated Environmental Management in Southern Africa, Johannesburg, August 27-30. http://www.cemsa.com*

[16] Nakagiri A; Niwagaba CB (2016). Are pit latrines in urban areas of Sub-Saharan Africa performing? A review of usage, filling, insects and odour nuisances. *BMC. Public Health*. 3(1): 1-16.

[17] Nansubuga I; Banadda N; Verstraete W (2016). A review of sustainable sanitation systems in Africa. *Bio/Technology - Springer*, 15:465 - 478

[18] Obeng PA; Oduro-Kwarteng S; Keraita B (2015). Impact of water pollution on human health and environmental: On-Site Sanitation Systems for Low-Income Countries. ed. McKeown, USA, p. 319-341.

[19] Semiyaga S; Okure M; Niwagaba C (2015). Decentralized options for faecal sludge management in urban slum areas of Sub-Saharan Africa: A review of technologies, practices and end-uses. *Resources, Conservation and Recycling-Elsevier*. 104:109-119.

[20] Shen KY (2013). Environmental Protection and Sustainable Development of Golf. *Trans Tech Publ*. p. 4069-4072.

[21] Strande L; Brdjanovic D (2014). Faecal sludge management: Systems approach for implementation and operation. IWA publishing, UK.

[22] Taseli BK; Kilkis B (2016). Ecological sanitation, organic animal farm, and cogeneration: Closing the loop in achieving sustainable development—A concept study with on-site biogas fueled trigeneration retrofit in a 900-bed university hospital. *Science Direct*. p. 129:102 - 119.

[23] Vliet B; Buuren J; Mgana S (2013). Urban Waste and Sanitation Services for Sustainable Development: Harnessing. Tanzania p. 1-153.

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First Report of ethnobotanical studies of tehsil Noorpur Thal, District Khushab, Punjab, Pakistan

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Abstract**—** *The study is based on gathering of information by interviewing villagers, herbalists, hakims and farmers, following a preset list of questions followed by analysis of the data collected. Plant samples were gathered and their morphological characteristics described. Their various uses including medicinal uses, where reported were gathered. The present work is a significant contribution to the existing knowledge because ethno botany as a interdisciplinary science understanding of local social dynamics, institutions and different values attributed to resources. These values may be symbolic, religious or political for a given society, while same plant resources may represent only an economic value for our social group. Fuel wood needs are also met by collecting dried fodder branches, by products of crops and dried animal dung. It is recommended that botanical ganders of medicinal plants should be established. Data was analyzed at P(0.05 -5%).*

Keywords— Interviewing Villagers, Medicinal Uses, Ethno botany, Plant Samples, Conservational Reserves, Botanical Garden, Products of Crops and Animal Dung.

I. INTRODUCTION

From ancient Time, plants are being used in various diseases. Many of today's drugs have been derived from plant sources. Pharmacognosy is the study of medicinal and toxic products from natural plant sources. More than six thousand species are used as medicines drives from medicinal plant are $43 billion. More than 75% of Pakistani population depended on tradition medicines for all are most of its medicinal needs. Ethnoparmacological study not only envisage the possibility of identifying new herbal drug, but also bring on record the hidden knowledge confied to traditional society all over the world (Leporatti 1990) bhattari, 1992 padhye at al 1992 , yang 1992, omino et al, 1993, Gils et al, 1994, bhanday et al, 1997, Verma et al, 1998).

Study Area

The very word Khushab, derived from two Persian words Khush" and "Aab" meaning good or peasant potable water. District Khushab is one of the four districts of Sargodha Division. The district lies between north latitude 31-33 to 32-43 degree and east longitude 71-35 to 72-37 degrees. The average length of the District from north to south is about 116 Kilometers: while its breadth from east to west is about 56 kilometer. The District comprises an area of 1,627,688 acres or 6,511 sq. Kilometer.

The few trees to be found in the dry and sandy that are chiefly Jund, (Prosopis cineraria (L) Druce), which is found in graves protected by the reputation of some departed scunt: stunted kikar, rarely found the round ponds and a grave of beri (ZizyphusnummulariaBurm.f.) trees found the town ofNurpur, which are specially protected by a clause in the village administration paper. The characteristic bushes of the region are the lana (salaola), akk (Calotropis pro cera R. Br.) and harmal (paganumharmala 1.) which have alteady been described and the phoy (Calligounmpolygonoidea Linn.) a good fodder plant, little found except in RakhNurpur, but (Pauderia pilosa) a low whitish plant with flower heads like catkins, khipp, (Crotalaria burhia) some time used for making ropes for temporary use and summa and karturnma (Citrulus colosynthis (L.) Shrad.) with its trailing stems and beautiful green and yellow orange likes fruit scattered in the profusion over the sandy hills. Their taste is very bitter, but goats eat them and medicine for horses is prepared from them to prevent indigestion.

In the past only cultivation consisted of small patches of cheap millets and pulses, or very inferior watermelons. But it has since been discovered that excellent grass of crops can be raised in an ordinary winter and year by year larger areas are devoted to raising them, the change from pasture to agriculture as the principal means of livelihood is going on apace. The resulting development of land is, of course, over-shadowed by the brilliant success of the lower Jhelum Canal, but is nonetheless remarkable.

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In the flooded lands along the rivers lei or pilchi (*Tamarix dioica* Roxb. Ex Royh) springs up considerable thickets and is used for wattling, baskets and roofs.The akk (*Calotropis procera* R. Br.) is very common in sandy soil. It is also useful for snakebite (Ajibade et al., 2005).

II. METHODOLOGY

The survey was conducted from March, 2003 to February 2006. The methodology was based on interviews using checklist and questionnaire of information (Martin, 1995). The interviewees in the villages were chosen at random. Total No of interviewees conducted are 750 consists of 400 males and 350 females. The interviewees were landholders (zamindars), Agriculturists, pansars, Hakims and Farmers, and most of them were mainly graduates and Government employee. In the first step, detailed knowledge about the local and indigenous people was collected. A regional study on the epidemiology tradition medicines and ecology of the people and their environment was prepared. In order to prioritize plant collectors, a number of international data basis were searched to obtain all the relevant ethno-medical, biological and chemical information on the plant known to be used in that region.

Following parameters were adopted for the study:

A. ETHNOMEDICINAL USES

- Uses of herbal medicinal
- Parts of the plants used
- Ailments treated
- Success of use
- Source of supply
- Average annual stock (quantity)
- Average annual sale (quantity)
- Types of people treated
- No. of people treated per day
- Trend in use of medicinal plants

B. FODDER USES

- Fodder priority
- Fodder effects
- Animal types
- Preferred pats

C. ETHNOBOTANICAL USES

- Vegetables and pot herbs
- Fruit yielding
- Poisonous plants
- Method of use
- Prices per KG.
- Plant grown/cultivated
- Plant material stored
- Quantity sold per year
- Sold in the form (dry/fresh)
- Used in the form (dry/fresh)
- Total number of species traded
- Harvesting season
- Method of preparation (infused/boiled/distilled/fresh juice)
- Details of preparation
- Method of internal application (infusion/decoction/syrup chewed)
- Method of external application (poultice, fixed oil, lotion cream)
- Age groups of people using the species.
- Health maintenance
- Types of livestock treated
  Livestock ailment treated
- Use of herbs in combination with other herbs .
- Period of storage of plants/herbs

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- Processing Additive used
- Domestic, community-wise and market value Species preferred for sale
- Average price per unit
- Source of fuel for domestic purpose _
- Average consumption of fuel per day for each household

D. FUEL SOURCES AND ITS CONSUMPTION

- Source of fuel for domestic purpose
- Average consumption of fuel day for each household
- Fuel types (i. e. fuel-wood, kerosene oil, LPG, crop residues, cow-dung, wood-waste, charcoal)
- Average monthly fuel requirement in summer and winter seasons
- ,Variation of fuel requirement in summer and winter seasons
- Dependency of people on wood fuel
- Prices of different types of fuels
- Availability of wood fuel
- Source of wood fuel
- Main cases of non-availability of fuel wood fuel
- Species used for wood fuel
- Preferred species of wood fuel
- Estimated percentage increase of trees III the surrounding
- Source of information about trees
- Suggestion to increase tree cover in the area

Table.1: Species used for different ailments

S.No	Name of species	Scientific Name	Part used	Illness	Success
1.	AK	*Calotropis procera* R.Br.	Stem	Joint Pain	Comforts
2.	Harmal	*Peganum harmala* L.	Seed	Abdominal Pain	Comforts
3.	Akashbel	*Cuscuta reflexa* Roxb.	Stem	Phorey	Comforts
4.	Tumma	*Citrullus colocynthus* (L.) Schard	Seed Fruit Root Oil of seed Root	1. Constipation 2. Stomach ailment 3. Immunity for Rani Khet Diseases 4. Sun stroke/Heat 5. Abdominal congestion 6. Ammnorrhea 7. Ascites 8. Asthma 9. Billousnes 10. Cerebral congestion 11. Elephantiasis 12. Epilepsy 13. Facial paralysis 14. Fever 15. Gout 16. Hepaticcongestion 17. Jaundice 18. Leprosy 19. Liver dibility 20. Neuralgic complication 21. Paralysis	1. Comforts 2. Very Effective 3. Very Effective 4. Cold effect

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			Root Poultice of root Juice Oil of Seed	22. Rheumatism 23. Sciatica 24. Visceral congestion 25. Inflamation of breast 26. Remedy of dropsy Snake bites scorpion stings and bowl complatints (dysentery, diarrhea) Epilepsy and for growth and blackening of hairl	
5.	Khoob Klan (Chuniakha)	*Sisymbrium irio* Crantz ex Steud	Seed	1. Typhoid 2. Small Pox (Chechak) 3. Chest debility, cholera,cough, fever, harassness vocal organ debility, vomiting	Removes Small Pox (chechak grains)
6.	Saunf	*Foeniculum vulgare* Miller	Whole Plant	1. Digestion problem 2. Gas Trouble 3. Female illness 4. Nervous disease	1. Increases Digestion 2. Gas trouble recovers. 3. Treatment. 4. Comforts.
7.	HarniKaKhaj	*Cistanchetabulosa* Wight	Whole Plant	Blood Purifier	Comforts
8.	Jawah	*Carum copticum* Benth	Whole Plant	Blood Purifier	Comforts
9.	Boophali	*Corchorusaestuans* Linn.	Whole Plant	Stomach and liver heat	Patient becomes healthy
10.	Lauhurian	*Tecomella undulate*	Whole Plant	Defect in Uterus	Patient becomes healthy
11.	Kahnu		Whole Plant	Defect in Uterus	Patient becomes healthy
12.	Bhakra	*Tribulus camaldulensis*. L	Seed	Gall Bladder illness, Kidney Allergy	Most successful
13.	Boophali	*Corchorus aestuans* Linn.	Whole Plant	1. Maleness in Man 2. Liqueria	Successful
14.	Hareer/Arhar	*Cajanuscajan* L.	Root	Spermatorrhoea	Successful
15.	Asgandh/IksinNeelwat	*Withania somnifera* L.	1. Root Decoction	1. Weakness of sexual organ. 2. Premature ejaculation 3. Leucorrhoea and frequent miscarriage (ladies) 4. Emaciation (women and children) 5. General debility 6. Glandular swelling 7. Leucoderrma	Successful

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			2. Root Paste and Cow Urine	8. Loss of memory 9. Nervus exhaustion 10. Rhumatica affection. 11. Snile debility 12. Syphilis 13. Skin diseases	
16.	Puthkanda	*Achyrandthes aspera* L.	Root	14. Impotency	Successful
17.	Bathu	*Chenopodium album* L.	1. Cooked leaves . 2. Leaf extract 3. Root powder	1. Urinary troubles and colic 1. Piles 2. Cought 3. Worms 1. Spermatorrhoea	Successful
18.	Drunk	*Polygonum plebijum* R.Br.	1. Plant Decoction 1. Plant ash + Oil	1. Colic complaints 1. Eczema	Successful
19.	Jau	*Hordeum vulgare* Linn.	Leaf Juice	Cataract	Successful
20.	Jund	*Prosopis cineraria* (Linn) Druce	Leaves	Leucorrhoea	Successful
21.	Mako/MirchBooti	*Solanum nigrum* L.	1. Leaf paste and branches 2. Whole plant Decoction	1. Jaundice 2. High fever 1. Spermatorrhoea	Successful
22.	Kashmiri Kiker	*Prosopis julflora* Swartz			
23.	Pilchi/Lei/Frash	*Tamarixdloca* Roxb. ex Roth	1. Bark (Bitter and Tonic)	1. Annal Fisher 2. Cough 3. Diarrhoea 4. Dysentry 5. Pectrol Affection 6. Piles 7. Uleers 8. Leucorrhoea 9. Spoleen Trouble 10. Leucoderrma	
24.	Chiraita	*Swertia chiraita*	Whole plant and Decoction	All kinds of fever particularly (i) Pneumonia (ii) Malaria (iii) Typhoid	

Table.2: Ethno-botanical uses of different plant species

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S. No	Local Name	Scientific Name	Part Used	Fuel Wood	Timber	Fodder
1.	Shrin	*Albizia lebbek* (L.) Willd		-		Fodder
2.	Kiker	*Acacia nilotica* L.	Leaves & Wood	Excellent Fuel	Agricultural implements	-do-
3.	Beri	*Zizyphus numularia* (Burm.f.)	Leaves	-	-	-do-
4.	Shisham	*Dalbergia sisoo* Roxb. Ex DC.	Leaves	-	-	-do-
5.	Khagal	*Tamarix dioica* Roxb. ex Roth	Stem	Fuel	Building	-
6.	Sufeda	*Eucalyptus blobulus*	-	-	Building	-
7.	Channa	*Cicer arietinum* L.	Seed & Stem	Fuel	-	Fodder
8.	KhoobKalan	*Sisymbrium irio* Cranz ex Stued	-	-	-	-do-
9.	Jund	*Prosopis cineraria* (L.) Druce)	Stem	Fuel		-do-
10.	Gowara	*Cyamopsis tetragonoloba* L.	Stem	Fuel	-	-do-
11.	Bursin	*Trifolium repens* L.	Except roots	-	-	-do-
12.	Jowar	*Sorghum bicolor* (Linn.) Moench	Except roots	-	-	-do-
13.	Bajra	*Pennisetum typhoideum* (Burm. F.) Staff & Hubbard	Except roots	-	-	-do-
14.	Loosen	*Trifolium alexandrianum* L.	Except roots	-	-	-do-
15	Jowadar	*Avena sativa* Linn.	Except roots	-	-	-do-
16	Kashmiri Kiker	*Prosopis julifora* (Sw.) DC.	Except roots	Fuel	Construction	Fodder paper and consmeticindustry industry
17	Wheat (Kanak)	*Triticum aestivum* Linn.	Hay/Stem	-	-	
18	Sarsoon	*Brassica comperis* L.	Stem/Leaves	-	-	-do-
19	Kallar grass	*Leptochola fusca*	Stem/Leaves	-	-	-do-
20	Juo	*Hordeum vulgare* Linn.	Upper part	-	-	-do-
21	Gana	*Saccharum spontaneum* Linn.	1. Stem 2. Thin end of the stock (Tili) 3. Shea thing petiole after being burnt at the lower end beaten with a mallet yield a fiber (Munj)		1. Thatching and making chairs 2. Making baskets and screens (sirki) 3. Munj is twisted into ropes	
22	Kah	*Saccharum spontaneum* Linn				1. Grazing for Buflalos 2. Making brushes 3. Use to strew on the floors of mosques
23	Dhub	*Desmostachya bipinnata* (L.) stapf				Making ropes

MARKET SURVEY

Table.3: Prices of different species

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S.No	Name of Species	Local Name	Qty (Kg)	Price (Rs.)
1.	*Peganum harmala* L.	Harmel	1	50
2.	*Cusuta reflexa* Roxb.	Ahashbel	1	500
3.	*Citrulluscolocynthus* (L.) Schrad	Tumma	1	50
4.	*Tribulus camaldulensis* L.	Bhakra	1	100
5.	*Calotropis procera* R.Br.	Ak	1	1000
6.	*Eucayptus globules*	Sufeda	40	110/(2.7/K.g)
7.	*Tamarix dioica* Roxb. ex Roth	Khagal	40	100 (2.5/kg)
8.	*Dalbergia sissoo* Roxb. ex DC.	Shishum	40	200 (5/kg)
9.	*Citrulus colocynthus* (L.) Schrad.	Tumma	1	15
10	*Trianthma portulacastrum* L.	Biskhapra	1	30
11.	*Acacia nilotica* L.	GondKiker	1	40
12.	*Sisymbriumirio*Crantz.ex Steud	KhoobKalan (Chaniakha)	1	50
13.	*Cicer arietinum* L.	Channa (Black)	1	20
14.	*Cicer arietinum* L.	Channa (White)	1	40
15.	*Foeniculum vulgare* Miller	Sounf	1	20
16.	*Zizyphus nummularia* (Burm.f.)	Beri	1	3
17.	-	Lahurian	1	1500-28000
18.	*Corchorus astuans* Linn.	Boophali	1	65
19.	*Peganum harmala* L.	Harmel	1	40
20.	*Tribulus calendulensis* L.	Bhakra	1	40
21.	*Plantago psillium*Forssk.	Isbaghol	1	80
22.	*Tribulus cameldulensis* L.	Bhakra	1	20
23.	*Corchorus astuans* Linn.	Boophali	1	150

Statistical Analysis

Table.1: Variation in the price of fuel yielding species by using ANOVA Statistical Analysis of the price of fuel yielding species of Thal

	Prices		Total	
REPLICATION	50	40	20	110
	50	50	65	165
	15	20	40	75
	30	40	40	110
TOTAL:	**145**	**150**	**165**	**460**

Source of variation	Sum of Squares	Degree of Freedom	Mean Squares	Computed Frequency
Row means	1383	3	461	F1=2.82
Column means	54	2	27	F2=.165
Errors	980	6	163	
Total	2417	11		

Non-significant

Table.2: Variation of average monthly wood-waste fuel in summer and winter

SUMMER		WINTER	
X1	X^2 1	X2	X^2 2
40	1600	80	6400

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40	1600	80	6400
40	1600	80	6400
80	6400	1600	25600
40	1600	80	6400
80	6400	1600	25600
80	6400	1600	25600
40	1600	80	6400

At 5% Significant

Table.3: Variation of Fuel Wood Consumption in summer and Winter Seasons

WINTER		**SUMMER**	
X1	X^2 1	X2	X^2 2
120	14400	60	6400
800	640000	600	360000
240	576000	200	40000
600	360000	400	160000
80	6400	40	1600
240	56700	200	40000
400	160000	40	1600
120	14400	80	6400
94	8836	80	6400
174	30276	120	14400
147	21609	94	8836
120	14400	80	6400
120	14400	80	6400
100	10000	100	10000
120	144000	80	6400

At 5% Significant

Table.4: Variations in the kerosene Oil consumption in the summer and Winter

SUMMER		**WINTER**	
X1	X^2	X2	X^2
20	**400**	**25**	**625**
20	**400**	**25**	**625**
10	**100**	**15**	**225**
10	**100**	**15**	**225**
2	**4**	**4**	**16**
1	**1**	**2**	**4**
1	**1**	**2**	**4**
2	**4**	**4**	**16**
10	**100**	**15**	**225**
10	**100**	**15**	**225**
10	**100**	**15**	**225**
10	**100**	**15**	**225**
60	**3600**	**15**	**225**

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60	**3600**	**15**	**225**
60	**3600**	**15**	**225**

At 5% Significant

Table.5: Variations in the Wood waste consumption in the summer and Winter

SUMMER		**WINTER**	
X1	X^2 1	X2	X^2 2
40	**1600**	**80**	**6400**
40	**1600**	**80**	**6400**
40	**1600**	**80**	**6400**
80	**6400**	**160**	**25600**
80	**6400**	**40**	**1600**
40	**1600**	**80**	**6400**
80	**6400**	**40**	**1600**
40	**1600**	**80**	**6400**
94	**8836**	**80**	**6400**
174	**30276**	**120**	**14400**
147	**21609**	**94**	**8836**
120	**14400**	**80**	**6400**
120	**14400**	**80**	**6400**
100	**10000**	**100**	**10000**
120	**14400**	**80**	**6400**

At 5% Significant

Table.6: Variations in LPG consumption in the summer and Winter

SUMMER		**WINTER**	
X1	X^2 1	X2	X^2 2
20	**400**	**800**	**6400**
20	**400**	**800**	**6400**
10	**100**	**2**	**4**
10	**100**	**2**	**4**
2	**4**	**2**	**4**
1	**1**	**4**	**16**
2	**4**	**15**	**225**
10	**100**	**15**	**225**
10	**100**	**15**	**225**
10	**100**	**15**	**225**
60	**3600**	**15**	**225**
60	**3600**	**15**	**225**
60	**3600**	**15**	**225**
60	**3600**	**15**	**225**

At 5% Significant

Table.7: Variations in the Crop-residues consumption in the summer and Winter

SUMMER		WINTER	
X1	X^2 1	X2	X^2 2
120	**14400**	**160**	**25600**
120	**14400**	**160**	**25600**
40	**1600**	**80**	**6400**
400	**160000**	**600**	**360000**
80	**6400**	**120**	**14400**
20	**400**	**2**	**4**
80	**6400**	**160**	**25600**
10	**100**	**20**	**400**

At 5% Significant

Table.8: Variations in the Cow-dung consumption in the summer and Winter

SUMMER		WINTER	
X1	X^2 1	X2	X^2 2
120	**14400**	**160**	**25600**
120	**14400**	**160**	**25600**
80	**6400**	**160**	**25600**
800	**640000**	**1000**	**100000**
40	**1600**	**80**	**6400**
10	**100**	**10**	**100**
320	**102400**	**640**	**409600**
40	**1600**	**80**	**6400**
40	**1600**	**80**	**6400**
60	**3600**	**80**	**6400**
30	**900**	**70**	**4900**
80	**6400**	**10**	**10000**
40	**1600**	**20**	**400**
40	**1600**	**80**	**6400**
40	**1600**	**80**	**6400**
60	**3600**	**80**	**6400**
60	**3600**	**80**	**6400**

III. DISCUSSION

Species Used For Different Aliments

There are about 24 plant species, which are used for different aliments. Ak (calatropis procera) R. Br. is used against skin diseases, eczema, toothache, abdominal pain and asthema (jadhev, 2008a). Harmal (pognum hermella) is used as narcotic, emetic anodyne, hypnotic, anti-lice and fumigated by ladies during small-pox. Dried pulp of bitter fruit of Tumma (citrullus colocynthus) (L.) Shrad. is effective in constipation(Usmanghani, et al., 1997). Fruit of Thumma (citrullus colocynthus) (L.) is useful for the stomach ailments and immunity for Rani Khet diseases and has cold effects against sun-stroke (Heat).

Seeds of Khoob Kalan or chanakhla (sysimbrium irio) Crantz ex steud are used as treatment against Typhoid, small pox. Whole plant of Sonuf (Foeniculum vulgare)

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Miller. is used for digestion problems, gas troubles, female illness and nervous diseases.

Harni Ka Khaj (cistanche tubolose) Wight is effective in diarrhoae and cures sores (Baquar, 1989) and Jawah (carum copticum) Benth. is used as blood purifier. Whole plant of Boophali (corchorus aestuens) Linn. is used for stomach and liver heat . Whole plant of harmal (Pognum hermela) L. is used for the defect in the uterus.

Seeds of Bhakra (Tribulus calendulenisis) L, are used for gallbladder illness and kidney allergy. Whole plant of Boophali (corchorus aestuens) Linn. is used for maleness in man and leucorrhoea.

Leaves of Sumblu or norgundani (vitex negundo) Linn. are used for wounds, oraktsus, and rheumatic pain. Its stem is used for fever. Its juice is useful for gall bladder problems. Its root powder is used for menstrual disorder and restores fertility. If it is roasted seeds powder and wheat flavour is useful for easy delivery.

Neelwat (Withania somnifera) L. is used as an antiinflamatory and sedatve agent (Williamson et. al., 2009). Cooked leaves of Bathu (chenopodium album) L. are used for coronary troubles. Its leaf extract is useful for piles, cough and worms.

Plant decoction of drunk (Polygonum plebijum) R. Br. is used against colic complaints. Plant ash and oil is useful for Eczema. The root of this plant is used in bowel complaints and powdered herb is given in pneumonia (Trivedi, 2002). Leaf juice of Jau (Hordeum vulgare) Linn. are useful for cataract. Leaves of Jund (Prosopic cineraria) (L.) Druce is useful for leucorrhoea.

Leaf paste and decoction of Mako or Mirchibooti (solanum nigrum) L. is used against jaundice and in case of high fever, cough and liver diseases (Tridevi, 2002).

Problems related to herbal medicines Business.

1 Pure things are not available.
2 Wild plants are expensive.
3 Hard work and labour is required.
4 Most of area is cultivated.
5 Forests are less, so wild plants have reduced.
6 Wild plants have high prices.
7 People discuss more, the prices of medicines.
8 Pure medicines are not available.
9 Information about plants is negligible.
10 Herbal medicines are shelter-classics Govt. is not paying any attention.
11 Trained people are not enough.
12 Area is being populated.
13 People do not collect plants due to low prices.
14 They insist on purchasing low prices.

People treated per day different places.

Mostly 10 people are treated per day at Adhi kot almost 80 persons are treated per day at Jamali Baluchan. Mostly 20 people are treated per day at Noor pur thal. About 50 people are treated per day at Peeluwance and 20 people are treated at Quluanwala. All classes are treated. 15 people are treated per day. Mostly poor and middle class are treated daily at Biland.

Suggestions to increase the cover of the area.

1. Government should give permission for forest plantation by giving free nurseries.
2. Government should make contact with ther farmers.
3. No of tubewell have to increase.
4. Farmers should be provided with fir nurrsries and plants from the Government nurseries.

Discussion regarding Statistical Analysis and Ethnobotany

A- Variation in the prices of different fuels yielding species of Khushab District.

Variation in the prices of different fuels yielding species of Khushab District was determined by analysis of variation (ANOVA). Prices vary from Rs.15 to Rs.165 treatments and replicates were made and then total was taken. Sum of square of treatment, sum of square of columns and sum of square of errors were calculated which were 2417, 1383, 54 and 980 respectively (table 15).

By using source of variation, sum of square, degree of freedom and mean square, row means, and errors was calculated. Frequency (f1+f2) was found to know the significance of data. f1 was 2.82 which was the more than actual value i.e. f2 0.165. So it was found that the variations in prices of different species at that area were significant.

REFERENCES

[1] Alcorn, J.B. 1984, Development policy forests and peasant farms: reflection on Haustec-managed forests contributions to commercial production and resource conservation, economic botany 38 (4): 3 89-406.

[2] Altieri, M.A. and Merick, L.C. 1987, In situ conservation of crop genetic resources through maintenance of traditional farming system, Economic Botany 4 (!) : 86:96.

[3] Aumeeruddy Y, 1994. Representations to gestion paysannes des agroforeis en kperipherie du pare National Kerinci Seblat a Sumatra, Indonesie, People and Plants working paper no. 3, UNESCO, Paris (English and Spanish version available).

http://dx.doi.org/10.22161/ijeab/3.4.15 *ISSN: 2456-1878*

[4] Balee, W, and Gely, A. 1989. Management of forest succession in Amazonia: the Ka'apor case. In: Posey, D.A. and Balee, W. (eds), "Resource Management in Amazonia: Indigenous and fold strategies", Advances in Economic Botany 7: 129-158.

[5] Baquar, S.R., 1989. Medicinal Plants of Pakistan PP 1-489.Cunningham, A.B. 2001. Applied ethnobotany: people, wild plant use and conservation. People and Plants Conservation Series, Earth-scan, London.

[6] Jadhav, D (2008a). Medicined peants of India vol.z. Scientific Publisher, Jodhpur, India. PP.1-269

[7] Johnson, A. 1980. Ethnoecology and planting practices in a swideen agricultural system in: Brokensha D., Warren, D.M. and Werner 0 (eds), Indigenous knowledge systems and development, pp. 49-66. University Press of America, Lanham, Maryland, USA.

[8] Martin, G. 1995, Ethnobotany. A Method Manual,Chapman and Hall, London. Moerman, D.E., R.AW Pemberton, D.Kiefer and Brlin, 1999. A comparative analysis of five medicinal floras. JOURNAL Ethnobotany 19:49-67.

[9] Pei, S.J. and Sajise, P. 1995) Regional study on biodiversity: concepts, frameworks and methods. Yunnan Univeristy Press, China.

[10] Trivedi, P.C. (2002) Ethnobotany. Avishkar Publishers, Distributors, Jaipur, 303003 India. PP. 455.

[11] Usmanghani, K.A. Saeed, A & Alam, M.T. 1997. Indesyunic Medicine Traditional Medicine Of Herbal, Animal and Mineral Origin in Pakistan. 1-577. University of Karachi.

[12] Williamson, E., Driver, S. and Baxter, K. (2009). Stockley's Herbal Medicines Interactions. Pharmaceutical Press, RPS Publishing, London.

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Effect of Carom Seed Oil on the Antimicrobial, Physicochemical and Mechanical Properties of Starch Based Edible Film

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***Abstract*—** *Packaging material is necessary in the preservation process. Edible films containing essential oils can be incorporated into the conventional food packaging systems with a dual purpose, edible and natural preservative, that can maintain quality, extend the shelf life and reduce the risk of pathogen growth specifically in unprocessed or minimally processed foods like fruits and vegetables. In present study, pumpkin-arrowroot starch based edible film incorporated with carom seed oil at 0.5%, 1% and 1.5% were prepared and studied for the antimicrobial properties. Film with 1.5% Carom seed oil showed exceedingly good antimicrobial activities against E. coli, Staphylococcus and Aspergillus. The films were further studied for physical, mechanical and water vapour transmission properties. The results indicated that the film with 1.5% carom seed oil did not alter the mechanical properties of the film significantly, compared to control film and is ideal for coating to extend the shelf life of food products.*

***Keywords*— Edible film, carom seed oil, antimicrobial activity, mechanical properties.**

I. INTRODUCTION

Edible films are the films made using edible material and can be used on various food products to achieve their role in preservation of products (Fakhouri*etal*. 2015). The function of edible films is to provide mechanical integrity or handling characteristics to the food. These films can also act as carriers of active ingredients, such as antioxidants, flavours, fortified nutrients, colorants, anti-microbial agents, or spices (Regalado *et al*. 2006). Novel edible materials have been derived from many natural sources that have conventionally been regarded as discarded materials or even the low-cost sources which are yet to be regarded as a potential base for edible films (Shit and Shah 2014). Pumpkin is one of the well-known edible plants that is grown all year round. India is the second largest producer of pumpkin producing 35,500,000 metric tonnes per year (FAO, 2008) after China. It contains several phyto-constituents belonging to the categories of alkaloids, flavonoids, and palmitic, oleic and linoleic acids (Yadav *et al*. 2010). It can be used as a low-cost raw material for edible film preparation. Plasticizer such as glycerol is added to edible film to prevent from becoming brittle while aiding in the extensive and flexible properties (Wiset*et al*. 2014). Pectin is often added to films as it provides the properties of gel formation and selectivity to gas permeation through the film along with providing stability and thickening.

Essential oils are volatile complex compounds synthesized during the secondary metabolism of plants and impart antimicrobial properties due to the presence of alkaloids, phenols, terpenes and other derivatives(Akthar*et al*. 2014). Extensive research has shown that essential oils of oregano (*Origanum vulgare*),cinnamon (*Cinnamon casia*) and clove (*Eugenia caryphyllata*) are among the most active against strains of *E. coli*, yeasts and moulds (Du *et al*. 2011). Whey protein films with 2% oregano oil was effective against several microbes including *E. coli* O157:H7, *Staphylococcus aureus*, *Salmonella enteritidis*, *Listeria monocytogenes*, and *Lactobacillus plantarum* (Ravishankar *et al*. 2009). Carom (*Trachyspermumammi*) or ajwain seed is an aromatic and medicinally important seed spice used as flavouring agent and as a digestive stimulant to cure liver disorders. The active compounds present in the carom seed oil are the phenols especially thymol and some carvacrol that provide the antimicrobial properties by obstructing the peroxidation of liposome phospholipids in a concentration dependent manner (Prashanth *et al*. 2012). Carom seed oil showed antimicrobial activity against *S. aureus* at 1-8 µL/ml, *P. aeruginosa* at 8-32 µL/mland *E.*

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coli at concentrations of 2-32 µL/ml (Zomorodian*et al*. 2011). The aim of this work was to develop an edible film using carom seed having anti-microbial properties. This prepared film can be used to increase the shelf life of food products such as fruits, fish, cheese etc.

II. MATERIALS AND METHODS

2.1 Materials: Pumpkin, Arrowroot starch were purchased from the local market in Delhi. Carom seed oil was obtained from Mohan Oil Mills, INA market, Delhi. Glycerol and Citric acid were obtained from Molychem, Delhi. Pectin and L-ascorbic acid were obtained from Central Drug House Private Ltd. (CDH), Delhi. Pure cultures of *E. coli*, *Aspergillus* and *Staphylococcus* were grown and maintained in the microbiology laboratory of Institute of Home Economics, University of Delhi, Hauz Khas, New Delhi, India.

2.2 Preparation of the film solution

2.2.1Pumpkin puree preparation: Pumpkin was washed and cut, seeds and peels were removed and then it was pressure cooked until soft. It was then pureed using a mixer grinder.

2.2.2 Film preparation: Thirty grams of prepared pumpkin puree was taken and diluted with 60 ml distilled water to form the desired concentration for the film formation.The flowsheet for preparation of film is depicted in fig.1.

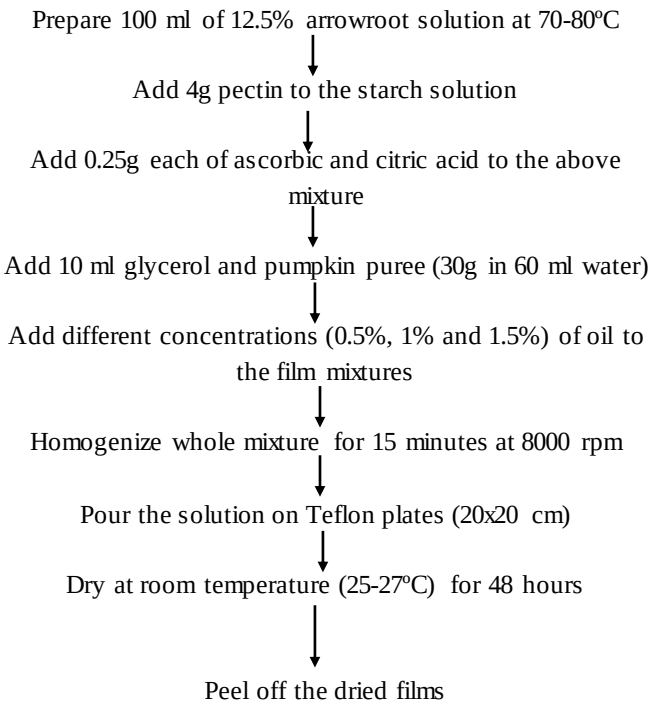

Fig.1: Flowchart for preparation of edible film

2.3 Microbiological Analysis: Microbiological analysis was done by agar well diffusion method given by Abbas *et al*. 2016. The test compound was introduced into the well and the plates were incubated at 37 ºC for 24 h for bacteria and for 5 to 7 days at 28ºC-30ºC for fungi. After incubation, the zone of inhibition was calculated and then subtracted from the diameter of the agar well. This difference was reported as the zone of inhibition of the film-forming solutions.

2.4 Physico-chemical and Mechanical Properties

2.4.1Thickness: Thickness of the prepared films was evaluated by the method given in ASTM D 6988 – 03, 2004. Measurements were done using manually operated thickness gauge. Samples measuring 10 x 10 cm were used. The sample was kept on an anvil. The press foot was raised and then gently lowered on to the sample. The reading on the dial gauge was recorded as the thickness of the sample. The above procedure was repeated to at 20 different locations on the sample to obtain the values of thickness. Readings were taken in triplicates for each sample and the results were expressed in mm.

2.4.2 Ash Content and Moisture content: Ash content (%) and moisture content (%) of the films were analyzed using the methodology given in AOAC (2000).

2.4.3 Film Solubility: Solubility of the films was determined according to the method mentioned by Romero-Bastida*et al*. 2005.

2.4.4 Tear strength and bursting strength: Tear strength and Bursting Strength of the films was evaluated using method given in Rangana (1999).

2.4.5 Tensile strength: It was conducted in accordance with IPC-TM-650 Test Methods Manual, using a Paramount digi strength tensile strength tester (capacity: 250 Kg, sensitivity: 100 grams). Triplicate readings were taken for each film and tensile strength was expressed as kg/cm^2.

2.4.6 Water vapor transmission rate (WVTR): It was evaluated using the dish method given in Rangana (1999).

2.5 Statistical Analysis: The obtained data was subjected to statistical analysis using one-way ANOVA (Post-hoc Duncan's test) to arrive at meaningful inferences at a significant level of ($p<0.05$) using the IBM SPSS Statistics 22 software.

III. RESULTS AND DISCUSSION

3.1 Antimicrobial activity: *E. coli*, *Aspergillus* and *Staphylococcus* are the most common food spoilage micro-organisms and thus were tested for executing the antimicrobial properties of the film by the agar well diffusion method. The control film solution without any carrom oil did not show any inhibitory effect against any of the microorganisms (Fig. 3) which confides with the results obtained by (Ravishankar *et al*. 2009) in apple puree edible films.

Film solution incorporated with 1.5% carom seed oil showed inhibitory effect against *E. coli*, *Staphylococcus* and *Aspergillus*. The film solution showed inhibition zone of 11.667 mm against *Staphylococcus* and 12.667 mm

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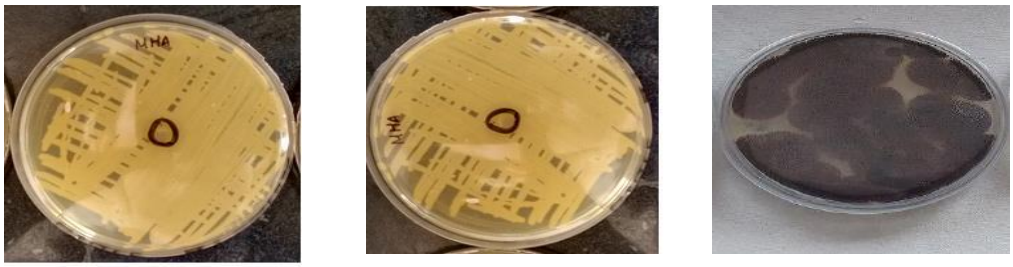
against *E. coli*. (Fig. 4). Film forming solution with 0.5% and 1% carom seed oil also showed inhibitory effect against *E. coli* and *Staphylococcus*, but the zone of inhibition was not as prominent. For *Aspergillus*, there was inhibitory action observed at 1.5% carom seed oil concentration (Fig. 4), but not for 0.5% and 1%. The activity can be explained by the fact that the addition of carom oil into the film solution resulted in diffusion of oil through the agar gel and provided a clear zone surrounding the film solutions.

Pure carom oil was also tested for its antimicrobial properties. The results showed that pure carom oil has inhibitory effect against *E. coli*, *Staphylococcus* and *Aspergillus* as no growth of microbial colonies were observed on the agar surface (Fig. 2).The strong antimicrobial potential of the carom oil is due to thymol and its precursors, cymene and terpinene. The results of the study are in accordance with the results obtained by Zomorodian*et al*. (2011) which showed the antimicrobial activity of carom seed oil (*Carumcopticum* oil) in concentration ranging from1-8 µL/ml for *S. aureus* and2-32 µL/ml for *E. coli*.As film with 1.5% carom seed oil showed better antimicrobial activity than film incorporated with 0.5% and 1% carom seed oil, therefore films prepared with 1.5% carom seed oil were further studied for physical, mechanical and water vapor transmission rate properties.

E. coli *Staphylococcus* *Aspergillus*

Fig.2: Inhibition zone exhibited by pure carom seed oil



E. coli *Staphylococcus* *Aspergillus*

Fig.3: Inhibition zone exhibited by control film solution

E. coli *Staphylococcus* *Aspergillus*

Fig.4: Inhibition zone exhibited by film solution containing 1.5 % carom seed oil

3.2 Film Thickness: Thickness is an important parameter in determining the workability of edible films as packaging materials for food products because the thickness of the films affects other characteristics of the films, such as tensile strength, elongation, and water vapor permeability and gas transmission rate (GTR) etc. GTR is inversely proportional to the thickness. The film thickness is dependent on both the composition of film and processing conditions (Arham*et al*. 2016). The film prepared without any carom seed oil (control) had

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thickness of 0.237 mm and the film incorporated with 1.5% carom seed oil had the thickness of 0.236 mm. The results depicted that there was no significant difference ($p<0.05$) in the films incorporated with carom seed oil in comparison to the control films. The plasticizer binds with starch to form starch-plasticizer polymer as a result, the starch-starch bond is replaced by the starch-glycerol-starch bond which leads to improvement in the thickness of film. Therefore, addition of glycerol in the manufacture of edible film resulted in increasing the film thickness (Arham*et al*. 2016).

3.3 Ash content and Moisture content: It was observed that bothcontrol film and film with 1.5% carom seed oil had similar ash content of 1%. Moisture content is important for the processing and handling of food. The moisture content of control film (15.96%) was found out to be higher than the film containing 1.5% carom oil (14.84%). Other studies also showed similar results as the incorporation of the hydrophobic essential oils can affect the ability of the film to retain water leading to the decrease in moisture content (Ghasemlou*et al*. 2013).

3.4 Film Solubility: Solubility is a physical property related to the ability of edible films to dissolve in water so that when ingested it can be digested properly, or if discharged into the environment it can decompose naturally. It is important that the film has low solubility, so that it cannot dissolve on the surface of product and retain high water resistance property. And on the other side, film with low solubility cannot protect the product from humidity and water loss (Arham*et al*. 2016). The film with 1.5% carom seed oil (6.420%) showed slightly higher solubility than the control film (6.324%). But there was no significant difference ($p>0.05$) between the control film and film incorporated with oil.

3.5 Tear Strength and Bursting Strength: Higher tear values may be needed for machine operations or for the package strength while low tear values are necessary and useful for the easy opening of some package types (Rangana 1999). Films containing 1.5% carom seed oil had higher tear strength i.e. 19904 g than the control film i.e. 18474.66 g. The results also indicated that there was a significant difference ($p<0.05$) between the films prepared with oils.

Bursting Strength is measure of resistance to rupture and primarily as an indication of the suitability of certain fiber material and the extent of processing (Rangana 1999). After the addition of carom seed oil, the bursting strength of the prepared edible film was reduced. The control film had the bursting strength of 1.733kg/cm^2 and the film with carom seed oil had 1.466 kg/cm^2.

3.6 Tensile Strength and % Elongation: Tensile Strength (TS) and Elongation at break (EB) are key indicators of a films strength and flexibility. The estimated values of tensile strength showed that control had higher value i.e. 0.139 kg/cm^2 and the film incorporated with 1.5% carom seed oil had lower value i.e. 0.008 kg/cm^2. The incorporation of essential oils into the film network caused a decrease in tensile strength because the stronger polymer-polymer interactions are replaced by weaker polymer oil interactions and hence the network structure gets weakened (Noshirvani*et al*. 2017). Other studies also showed similar results as film without oil had maximum tensile strength i.e. 38.238 kg/cm^2 and minimum for film containing cinnamon oil i.e. 10.706 kg/cm^2 (Souza *et al*. 2013). There was a significant difference ($p<0.05$) between the control sample and the film containing 1.5% carom seed oil. Elongation of the film decreases with addition of oil as control film showed higher Elongation at break value.

3.7 Water Vapor Transmission Rate (WVTR): Controlling moisture migration is crucial for maintaining the taste, texture and overall quality of packaged food products. By knowing the WVTR, the initial and critical moisture contents of food and the humidity gradient between the inside and the outside of the package, the shelf life of the product could be predicted to a fair degree (Rangana, 1999). The WVTR of packaging material is usually determined by the dish method. The film containing carom seed oil (148.59 g/m^2/24 hours) showed higher water vapour transmission than control film sample (117.89 g/m^2/24 hours).This phenomenon can be explained by the decrease in intermolecular forces of attraction between the polymer chains due to the increase in plasticizer concentration. The oil, being a plasticizer as well, along with glycerol and a firm gel structure producing pectin increased the hydrophilic character in the film, possibly because of more terpene than fats. The increase of hydrophilic to hydrophobic ratio in turn increased which promoted the dissemination of water molecules through the film. The unsaturated fatty acids in carom seed oil decreases the melting point of lipid compound, thus explaining the increase in water vapour transmission of the film as diffusion increases several folds in lipids than in saturated fatty acid or lipids (Adjouman*et al*. 2017). Moreover, due to double bonded linoleic acid (57-66%) being the major component present in the carom seed oil (agris.fao.org), as oleic acid aided in increasing melting of components, decreasing the density of the macromolecular network and thus effectiveness against the water transmission (Adjouman*et al*. 2017).

IV. CONCLUSION

Film with 1.5% Carom seed oil showed extremely good antimicrobial activities against *E. coli, Staphylococcus and Aspergillus.*Carom oil decreased the tensile strength by reducing the intermolecular interaction between the polymer chains. The film with 1.5% carom seed oil exhibited low moisture content which is ideal for a packaging material to extend the shelf life of food products. Other properties of the film were not significantly affected by the incorporation of carom seed oil. Our ultimate goal was to develop a commercially viable technology for the manufacture of edible films with cheaper raw materials incorporated with antimicrobial components in an efficient manner for protection of food products. Further studies could be conducted to assess the sensory characteristics and stability of the films incorporated with these essential oils.

REFERENCES

[1] AOAC. 17th Edition (2000). Official Methods of Analysis. Association of Official Analytical Chemists.

[2] Abbas, S.Z., Hussain, K., Hussain, Z., Ali, R., Abbas, T. (2016). Anti-Bacterial Activity of Different Soaps Available in Local Market of Rawalpindi (Pakistan) against Daily Encountered Bacteria.*Pharmaceutica Analytica Acta., 7(11),*522. DOI: 10.4172/2153-2435.1000522.

[3] Adjouman, Y.D., Nindjin, C., Tetchi, F.A., Dalcq, A.C., Amani, N.G., Sindic, M. (2017). Water Vapor Permeability of Edible Films Based on Improved Cassava (Manihot esculenta Crantz) Native Starches. *Journal of Food Processing & Technology*, *8*(3), 665. DOI: 10.4172/2157-7110.1000665

[4] Akthar, M.H., Degaga1, B.,Azam, T. (2014). Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms. A review in *Biological Sciences and Pharmaceutical Research, 2 (1),* 001-007.

[5] Arham, R., Mulyati, M.T., Metusalach, M., Salengke, S. (2016). Physical and mechanical properties of agar based edible film with glycerol plasticizer. *International Food Research Journal*, *23(4),* 1669-1675.

[6] ASTM d6988-03. (2004). Standard guide for determination of thickness of plastic film test specimens. *astm international*. DOI: 10.1520/d6988-03.

[7] Du, W.X., Bustillos, R.J.A., Hua, S.S.T., McHugh, T.H. (2011). Antimicrobial volatile essential oils in edible films for food safety, Science against microbial pathogens. *Communicating current research and technological advances.1,* 1124-1134.

[8] Fakhouri, M.F., Martelli, M.S., Caon, T., Velasco, I.J., Mei, I.H.L. (2015). Edible films and coatings based on starch/gelatin: Film properties and effect of coatings on quality of refrigerated Red Crimson grapes. *Postharvest Biology and Technology. 109*, 57–64.

[9] Food and Drug Administration. http://agris.fao.org/agris-search/search.do?recordID=PK2011000338, Accessed on 28 May 2017.

[10] Ghasemlou, M., Aliheidari, N., Fahmi, R., Shojaee-Aliabadi, S., Keshavar, B., Cran, M.J.,Khaksar, R. (2013). Physical, mechanical and barrier properties of corn starch films incorporated with plant essential oils. *Elsevier carbohydrates polymers, 98,* 1117-1126.

[11] In, Y.W., Kim, J.J., Kim, H.J., Oh, S.W. (2013). Antimicrobial Activities of Acetic Acid, Citric Acid and Lactic Acid against *Shigella* Species. *Journal of food science, 33,* 79–85. doi:10.1111/jfs.12025

[12] IPC-TM-650 test methods manual. The Institute for Interconnecting and Packaging Electronic Circuits, IL 60062-6135. Accessed on 14 February 2017

[13] Noshirvani, N., Ghanbarzadeh, B., Gardrat, C., Rezaei, R.M., Hashemi, M., Coz, L.C., Coma, V. (2017). Cinnamon and ginger essential oils to improve antifungal, physical and mechanical properties of chitosan- carboxymethyl cellulose films. *Food Hydrocolloids, 70,* 36-45.

[14] Prashanth, M.K., Revanasiddappa, H.D., Rai, K.M.L., Raveesha, K.A., Jayalakshmi, B. (2012). Antioxidant and antibacterial activity of ajwain seed extract against antibiotic resistant bacteria and activity enhancement by the addition of metal salts. *Journal of Pharmacy Research, 5(4),* 1952-1956.

[15] Rangana, S. (1999). Handbook of Analysis and Quality Control for Fruit and Vegetable Products. *Tata McGraw-Hill Education*,pp. 450-475.

[16] Ravishankar, A., Zhu, L., Olsen, C.W., McHugh, T.H., Friedman, A. (2009). Edible Apple Film Wraps Containing Plant Antimicrobials, Inactivate Foodborne Pathogens on Meat and Poultry Products. *Journal of food science, 74 (8),* 440-445.doi: 10.1111/j.1750 3841.2009.01320.x

[17] Regalado, C., Pérez-Pérez, C., Lara-Cortés, E., García-Almendarez, B. (2006). Whey protein based edible food packaging films and coatings. *Advances in Agricultural and Food Biotechnology*, 237-261.

[18] Romero-Bastida, C. A., Bello-Pérez, L. A., García, M. A., Martino, M. N., Solorza-Feria, J., Zaritzky, N. E. (2005). Physicochemical and microstructural characterization of films prepared by thermal and cold gelatinization from non-conventional sources of starches. *Carbohydrate Polymers, 60 (2),* 235-244.

http://dx.doi.org/10.22161/ijeab/3.4.16 *ISSN: 2456-1878*

[19] Shit, C.S., Shah, M.P. (2014). Edible polymers: Challenges and opportunities. *Journal of Polymers, 2014*,1-13.

[20] Souza, A.C., Benze, R., Ferrão, E. S., Ditchfield, C., Coelho, A.C.V.,Tadini, C.C. (2013). Cassava starch composite films incorporated with cinnamon essential oil: Antimicrobial activity, microstructure, mechanical and barrier properties. *LWT – Food Science and Technology*, *54*, 346-352.

[21] Wiset, L., Poomsa, N.,Jomlapeeratikul, P. (2014). Effects of Drying Temperatures and Glycerol Concentrations on Properties of Edible Film from Konjac Flour, *Journal of Medical and Bioengineering, 3 (3),* 171-174. DOI: 10.12720/jomb.3.3.171-174

[22] Yadav, M., Jain, S., Prasad, G.B.K.S., Yadav, H. (2010). Medicinal and biological potential of pumpkin. *Article in Nutrition Research Reviews*, *23*, 184–190.

[23] Zomorodian, K., Moein, R.M., Rahimi, J.M., Pakshir, K, Ghasemi, Y., Abedi, S., Sharbatfar, S. (2011). Possible Application and Chemical Compositions of *Carumcopticum*Essential Oils against Food borne and Nosocomial Pathogens. *Middle-East Journal of Scientific Research, 9 (2),* 239-245.

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Influence of Plant Growth Regulators on Somatic Embryogenesis Induction in *Seriphidium herba-album*

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Abstract*—Seriphidium herba-album (syn. Artemisia herba-alba) is a medicinal, aromatic, greenish-silver herb. It is used widely in folk medicine for treatment of diarrhea, abdominal cramps and in the healing of external wounds. It's also used for the treatment of diabetes mellitus, neurological disorders as epilepsy, Alzheimer's disease, depression and jaundice. In this study we assessed the protocol for callus induction, maturation of somatic embryogenesis, frequency of germination and conversion into plantlets for leaf explants of Seriphidium herba-album using different concentrations of PGRs. Highest induction frequencies of embryogenic calli occurred after 35 days on MS medium supplemented with 1.5 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BAP. Optimum MS medium for higher frequency of matured somatic embryos was recorded using 5.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA and somatic embryos also induced young in vitro grown plantlets when cultured in the medium containing GA3 and kinetin. Hence, attempts to induce direct somatic embryogenesis have been achieved up to embryo regeneration and maturation.*

Keywords*— *Seriphidium herba-album, callus induction, somatic embryos, plant growth regulators (PGRs).

Abbreviations*— *BAP− Benzyl Amino Purine; GA3-Gibberellic Acid; 2,4-D-2,4-di-chloro-phenoxy-acetic-acid; MS – Murashige and Skoog medium; NAA − α-Naphthalene Acetic Acid; Kn– Kinetin; PGRs- Plant Growth Regulators.

I. INTRODUCTION

Seriphidium herba-album (previously named *Artemisia herba-alba* Asso.) known also as desert wormwood (known in Arabic as shih, white wormwood, armoise herbe blanche). *Seriphidium herba-album* is a small perennial shrub native to northern Africa, western Asia, Southwestern Europe, and the Arabian Peninsula, where it grows on dry steppes. It grows from 8 to 16 inches tall, is strongly aromatic, and is covered with fine glandular hairs which give it a grayish aspect (Segal *et al*., 1987; Nahla *et al*., 2011). *Seriphidium herba-album* is one of the most important medicinal plants that have been used in folk medicine for the treatment of gastric disturbances and healing external wounds, remission of diabetic symptoms, activating the function of the liver, and healing rashes, joint pains, inflammations and rheumatoid arthritis, with no side-effects (Feuerstein *et al*., 1986; Essawi and Srour, 2000). Moreover, experimental evidence showed that *S. herba-album* decoctions have beneficial effects as antioxidants as they contain compounds like tannins (Ben Abid *et al*., 2007). *S. herba-album* was found to have antifungal activity against *Penicillium citrinum* and *Mucor rouxii* due to the presence of carvone and piperitone isolated from the fresh leaves of the plant (Mahmoud *et al*., 2007; Boutkhil *et al*., 2011). The tissue culture of various Artemisia species of various *Artemisia* species is largely focused on improving artemisinin production. In vitro cultures of this species have been the object of great amount of research (Gulati *et al*., 1996; Nin *et al*., 1996; Honda *et al*., 2001; Liu *et al*., 2004; Sujatha and Rajnitha Kumari, 2007a; Mannan *et al*., 2008; Rezvan *et al*., 2010; Gopinath *et al*., 2014). It is widely known that plants from a natural resource or plants propagated by conventional means generally produce small quantities of secondary metabolites which makes their price very high; hence the studies on plant tissues and plant micropropagation offer various advantages for obtaining massive amounts plant material. The plant micropropagation is an efficient method for propagating disease-free and genetically uniform plants. It also appears that both liquid and solid shoot cultures are a good choice for micropropagation in

laboratories because they reduce costs related to the automation of the procedure (Honda *et al*., 2001).The artemisinin production in shoot cultures of such species as *A. pontica*, *A. judaica*, *A. vulgaris*, *A. scoparia*, *A. absinthium* and *A. annua* was investigated by a number of researchers (Gulati *et al*., 1996; Pan *et al*., 2003; Sujatha and Rajnitha Kumari, 2007b; Singh and Sarin, 2010; Kour *et al*., 2014; Dangash *et al*., 2015). The present study is aimed to develop an appropriate and efficient regeneration protocol for the medicinal plant *Seriphidium herba-album* from leaf explants using different concentrations of PGRs for the first time, which has a high potential to be used in conservation and genetic transformation of this important medicinal plant.

II. MATERIALS AND METHODS

Plant material and sterilization.

Plant materials of *Seriphidium herba-album* were collected from North West coast of Egypt. The leaf explants were washed under running tap water for two hours. Surface sterilization was carried out under complete aseptic conditions in the Laminar Air Flow Hood by using aqueous mercuric chloride solution ($HgCl_2$) (0.1% w/v) for 5 minutes, then rinsed once with sterile distilled water and transferred to 2.5% aqueous solution of sodium hypochlorite for 20 minutes at the end of sterilization treatment; the explants were rinsed 3-4 times with antioxidant solution. Sterilized explants were cut into leaf segments (0.5-1.0 cm) and implanted into sterilized media containing various combinations of plant growth regulators.

Callus induction.

Leaf explants were cultured on solidified MS medium containing 2, 4-D (1.0-2.0 mg L^{-1}) alone or in combination with BAP (0.5-1.5 mg L^{-1}). Media were solidified with 2.5 g/L g L^{-1}, subjected to pH 5.8 before autoclaving (121°C). A total of 12 different hormonal combinations were tested. The effect of the hormonal composition was evaluated by counting the calli obtained after 35 days of culture in the dark at 25 ± 2°C. Percentage of induced callus in each culture was evaluated as follows: Number of callus/Total number of explants x100.

Somatic embryos induction.

Induced primary somatic embryonic calli were sub cultured for further production of embryonic callus and their subsequent germination. Medium for induction of somatic embryonic callus, their proliferation and germination was conducted by transferring the somatic embryos into concentration of BAP alone or in combination of NAA and different complex additives. In defining the media composition, 15 different media containing BAP (1.0, 2.0, 3.0, 4.0 and 5.0 mgL^{-1}) alone or in combination with NAA (0.5 and 1.5 mgL^{-1}) were tested separately for induction of somatic embryogenesis. Calli were incubated for eight weeks period in this medium. For germination of somatic embryos, MS medium supplemented with GA3 (1.0, 2.0 and 3.0 mgL^{-1}) in combination with Kn (0.5 and 1.0 mgL^{-1}) were tested. The culture was kept in growth room temperature at 25 ± 2°C with light intensity 3000 lux for 16 h photoperiod using cool white fluorescent lamps in the growth room for 8 weeks. Data were recorded as number and percentage of somatic embryogenesis formation.

Statistical analysis.

The experiment was carried out based on complete randomized design. Each of the experiments, excluding field performance study, was executed in five replicates with 20 samples per replication. For *in vitro* culture experiments, every single explant was treated as an experimental unit. Analysis of variance (ANOVA) was used to statistical analysis of experimental data using MSTAT Software (2009). Differences between individual means were estimated according to Snedecor and Cochran (1982). All values are reported as means ± standard deviation.

III. RESULTS AND DISCUSSION

Embryogenic callus induction.

Source of plant growth regulator is an important factor for impacting callus induction, somatic embryogenesis and plant regeneration. In most cases, successful plant regeneration needs a mixture of the different auxin and cytokinin. Callus were produced in all media containing 2,4-D alone or in combination with BAP. A considerable variation in callus induction percentage and biomass of *Seriphidium herba-album* was detected in parallel with a mixture of the different auxin and cytokinin. After 35 days, the highest Callus induction percentage (95.44%) was recorded with 1.5 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} BAP (**Table 1 and Fig. 1A**). Highest value of relative water content (83.72) was recorded under treatment with 2.0 mg L^{-1} 2, 4-D and 1.5 mg L^{-1} BAP under dark regimes compared with other treatments. The results stated that RWC is a contributing factor in nature of callus formation (friable and creamish green) data not presented. Similar results were reported by Anis *et al*. (2014) of *Artemisia annua*. They reported that embryonic callus induction was formed on MS medium supplemented with 2,4-D and TDZ. Dangash *et al*. (2015) found that callus induction from stem explant of *Artemisia annua* L. plant on MS medium containing BAP (0.5 mg L^{-1}) and NAA (1.5 mg L^{-1}). While, Nin *et al*. (1996) reported that no callogenic response from leaf explant on PGR-free medium and explants died after few days. 2, 4-D as callus inducing hormone produced light green, soft, friable and compact callus from leaf and stem explants. But at all concentration

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of 2, 4-D organogenic response was not observed within observation time. Etienne *et al*. (1991) stated that water status in the callus is apparently important for initiating somatic embryogenesis, and RWC appear to be good physiological marker of it embryogenic state.

Ganesan and Paulsamy (2011) observed 98.66% callogenic response from leaf discs with NAA at 0.9 mg L^{-1} in *A. annua* L. whereas Benjamin *et al*. (1990) observed callus induction from shoot buds using BAP plus IAA for *Artemisia pallens*. 2, 4-D at varying concentration (0.05-0.25 mg L^{-1}) in combination with BAP (0.5 mg/l) also produced light green and soft callus when supplemented in MS medium. When IBA and NAA were combined with Kin, the callogenic response was also low and callus was not good in texture. Xu and Jia (1996) observed best callus result in the presence of 2, 4-D with Kin for *Artemisia sphaerocephala*.

Table.1: *Influence of plant growth regulators concentrations on callus induction, relative water content (%) of the Seriphidium herba-album via leaf explants after 35 days.*

Growth regulators (mg/l)		Callus induction percentage	Relative water content (RWC)
2,4-D	BAP		
1.0	0.0	42.92±0.31^{j}	42.50±0.62^{h}
1.5	0.0	39.70±0.13^{k}	39.53±0.30^{i}
2.0	0.0	40.84±0.52^{j}	36.30±0.84^{j}
1.0	0.5	90.65±0.26^{c}	80.51±0.75^{c}
1.0	1.0	83.70±0.75^{e}	78.51±0.92^{e}
1.0	1.5	77.48±1.20^{i}	79.70±0.54^{d}
1.5	0.5	95.44±0.30^{a}	75.60±0.55^{e}
1.5	1.0	48.73±1.45^{h}	71.24±0.92^{g}
1.5	1.5	63.56±0.93^{g}	78.56±0.65^{e}
2.0	0.5	92.48±0.82^{b}	81.35±1.28^{b}
2.0	1.0	87.57±1.43^{d}	72.40±0.85^{f}
2.0	1.5	79.90±0.49^{f}	83.72±0.45^{a}

Values are presented by mean ± SE Same letters represent no significant differences between means at $P \leq 0.05$ level.

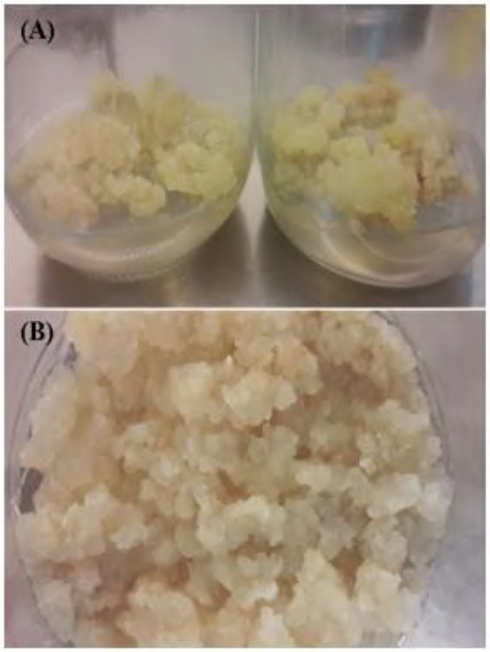

Fig.1: In vitro callus induction and Initiation of somatic embryogenesis from leaf explant of Seriphidium herba-album. (A): Induction of callus after 35 days on MS medium supplemented with 1.5 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} BAP under dark regimes. (B): Induction of embryogenic callus after eight weeks on the surface of primary callus that was induced from callus cultured on MS medium supplemented with 5.0 mg L^{-1} BAP and 0.5 mg L^{-1}

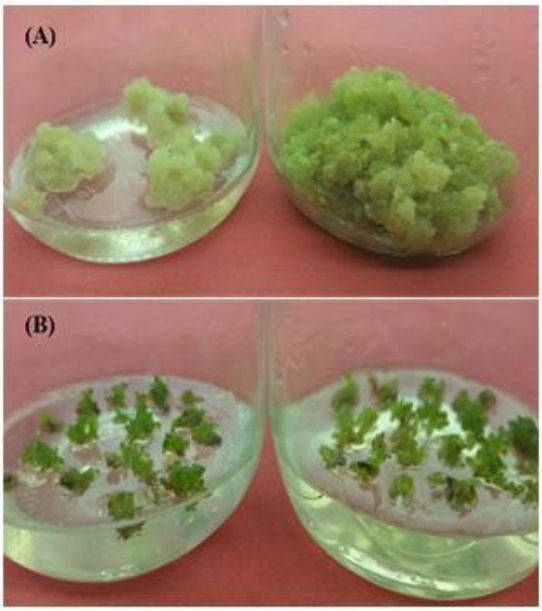

Fig. 2: Improved somatic embryo production and plant regeneration of Seriphidium herba-album. (A): Matured somatic embryos derived from callus cultured for eight weeks on MS containing with 5.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA under light. (B): small plant regeneration derived from callus cultured for four weeks on MS medium supplemented with 3.0 mg L^{-1} GA3 and 0.5 mg L^{-1} kinetin.

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Somatic embryos and development of plantlets.

The percentage of somatic embryos formation of embryogenic callus were 95.30% with the average number of somatic embryos per 1.0 g fresh weight of embryogenic callus were 86.38 of *Seriphidium herba-album*. These observations were recorded on MS medium supplied by 5.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (**Table 2 and Fig. 1B**). For further improvement and maturation of somatic embryogenesis, frequency of germination and conversion into plantlets, MS basal solid medium supported by GA3 at (1.0- 3.0 mg L^{-1}) in combination with kinetin (0.5-1.0 mg L^{-1}) were tested. The results in table 3 show that the somatic embryos germination percentage reached the highest value of 89.75% by using MS medium supplemented with 3.0 mg L^{-1} GA3 and 1.0 mg L^{-1} Kinetin under light (**Fig. 3A and B**) compared with other treatments. Results also show that the mean number of shoots on the explant (5.87) treated with MS containing 3.0 mg L^{-1} GA3 and 0.5 mg L^{-1} kinetin was significantly higher than the other treatments (**Fig 3A and B**). While, the best results of mean length of shoots formed per explant (14.28 mm) were produced on concentration of 3 mg L^{-1} GA3 and 1.0 mg L^{-1} kinetin compared to the other treatments (**Fig. 4A and B**).

Table.2: Influence of plant growth regulators concentrations on percentage of somatic embryos formation /explant and average number of somatic embryos/ 1.0 g FW of callus of the Seriphidium herba-album after eight weeks.

Growth regulators (mg/l)		Percentage of somatic embryos formation /explant	Average number of somatic embryos/ 1.0 g FW of callus
BAP	NAA		
1.0	0.0	40.75 ± 0.32^{k}	47.52 ± 0.24^{i}
2.0	0.0	48.56 ± 0.54^{j}	49.08 ± 0.72^{h}
3.0	0.0	72.30 ± 0.35^{g}	66.40 ± 0.56^{e}
4.0	0.0	83.82 ± 0.45^{d}	72.80 ± 0.25^{d}
5.0	0.0	85.32 ± 0.94^{c}	81.45 ± 0.84^{b}
1.0	0.5	76.46 ± 0.25^{f}	52.34 ± 0.33^{g}
1.0	1.0	65.41 ± 0.55^{i}	47.58 ± 0.62^{i}
2.0	0.5	79.75 ± 1.40^{e}	66.92 ± 0.48^{e}
2.0	1.0	68.50 ± 0.82^{h}	55.89 ± 0.37^{f}
3.0	0.5	80.40 ± 0.19^{e}	72.59 ± 0.65^{d}
3.0	1.0	68.50 ± 1.03^{h}	49.53 ± 0.72^{h}
4.0	0.5	87.80 ± 0.42^{b}	82.44 ± 0.55^{b}
4.0	1.0	79.50 ± 0.37^{e}	78.25 ± 0.22^{c}
5.0	0.5	95.30 ± 0.52^{a}	86.38 ± 0.98^{a}
5.0	1.0	80.09 ± 0.18^{e}	79.48 ± 0.09^{c}

Values are presented by mean ± SE Same letters represent no significant differences between means at $P \leq 0.05$ level.

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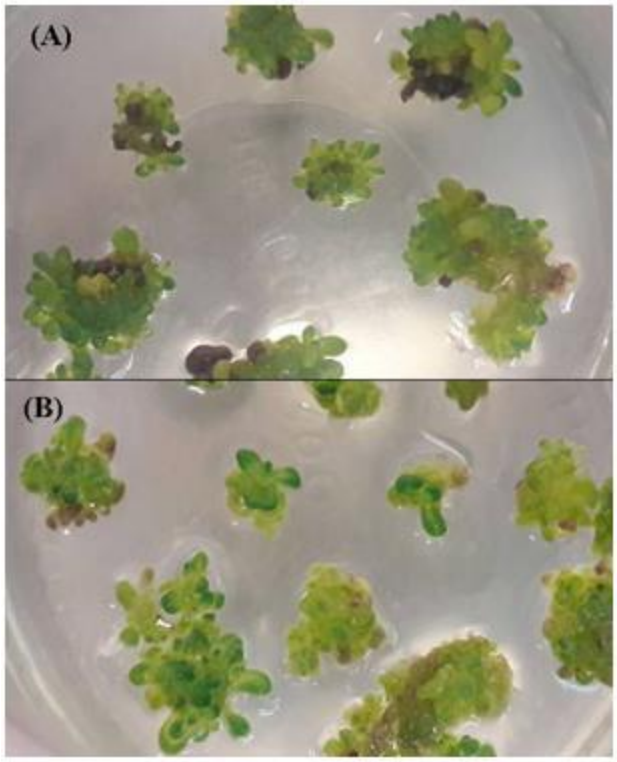

Fig. 3: In vitro plantlet obtained from a converted somatic embryo. (A): In vitro plantlet derived from matured somatic embryos cultured for eight weeks on MS containing with 3.0 mg L^{-1} GA3 and 0.5 mg L^{-1} kinetin under light. (B): MS containing with 2.0 mg L^{-1} GA3 and 1.0 mg L^{-1} kinetin under light.

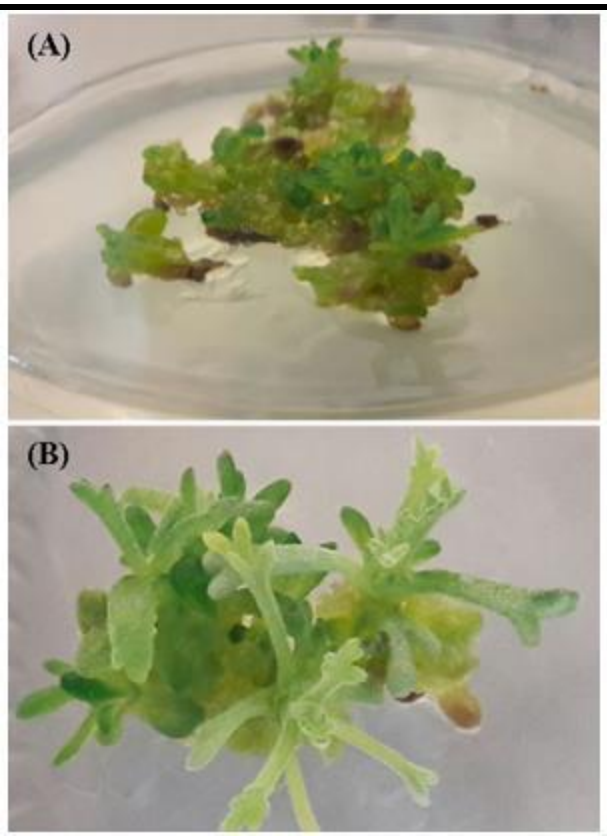

Fig. 4: Sub-culturing of somatic embryo on MS medium supplemented 5.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA under light (A) and differentiated into multiple shoots and shoot elongation on 3.0 mg L^{-1} GA3 and 1.0 mg L^{-1} kinetin (B).

Table.3: Influence of plant growth regulators concentrations on somatic embryos germination percentage, average number of shoots/explant and mean shoot length of the Seriphidium herba-album after eight weeks.

Growth regulators (mg/l)		Somatic embryos germination (%) (germinated/embryos tested)	Mean number of shoots/explant	Mean shoot length (mm)
GA3	Kn			
1.0	0.5	62.90 ± 0.77^{f}	3.54 ± 0.28^{c}	10.50 ± 0.12^{e}
1.0	1.0	68.83 ± 0.95^{e}	3.76 ± 0.09^{c}	12.59 ± 0.25^{c}
2.0	0.5	78.35 ± 0.65^{d}	4.59 ± 0.22^{b}	10.30 ± 0.16^{e}
2.0	1.0	80.60 ± 0.38^{c}	4.89 ± 0.34^{b}	13.80 ± 0.65^{b}
3.0	0.5	84.30 ± 0.59^{b}	5.87 ± 0.25^{a}	11.75 ± 1.02^{d}
3.0	1.0	89.75 ± 0.62^{a}	4.95 ± 0.17^{b}	14.28 ± 0.26^{a}

Values are presented by mean ± SE same letters represent no significant differences between means at $P \leq 0.05$ level.

Similar observations were also made by several investigators in *Artemisia annua* L. plants (Gonzalez *et al.*, 2013; Anis *et al.*, 2014). Also, Vergauwe *et al.* (1996) reported the shoot regeneration from leaf explants of *A. annua* L. on MS medium with 0.05 mg L^{-1} NAA and 0.05 mg L^{-1}BAP after 5 weeks of culture. In the present study, a result of shoot induction rate is in agreement with the report of Dangash *et al.* (2015) who showed that the best shoot induction 83.6% (2.83± 0.234) was observed on BAP (1.5 mg L^{-1}) in combination with NAA (0.05 mg L^{-1}). At different concentrations of Kin alone or in combination with NAA response of shoot induction was observed low. At 1.0 mg L^{-1} Kin, shoot induction was 56.2%. Also, Banyai *et al.* (2005) who considered 1mg/L

BAP with 0.1mg/L NAA as the best supplemented medium for leaf- explants-derived shoot regeneration. Almaarri and Yu Xie (2010) reported 100 and 66.6% shoot induction in different genotypes of *A. annua* on MS fortified with TDZ (1 mg L^{-1}) and BAP (1 mg L^{-1}), respectively. Similar results have also been reported by (Sharma *et al*., 2008; Hailu *et al*. 2013). *In vitro* micro propagation and organogenesis of various *Artemisia* species have been previously established by using several explants in order to produce large number of plants, such as *Artemisia vulgaris* L (Sujatha and Rajnitha Kumari, 2007b), *Artemisia annua* (Ganesan and Paulsamy, 2011; Gopinath *et al*., 2014).

IV. CONCLUSION

The different concentrations and combinations of PGRs, used in our study, were effective to induce calli, maturation of somatic embryogenesis, frequency of germination and conversion into plantlets. The combinations of 2,4-D and BAP supplemented medium induced friable calli in the leaf explants of *Seriphidium herba-album*. The creamish green calli with good growth were observed under dark regimes on MS medium supplemented with 2,4-D (2 mg L^{-1}) and BAP (1.5 mg L^{-1}). Somatic embryogenesis was induced from callus explant in presence of BAP and Kn as cytokinin. The regeneration system developed in this study will be useful for plant improvement through indirect somatic embryogenesis and genetic engineering of *Seriphidium herba-album* Moreover, this system can be available for the clonal propagation in order to obtain the strain containing a constant concentration of artemisinin in *Seriphidium herba-album*.

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REFERENCES

[1] Almaarri, K., Yu Xie 2010. *In vitro* direct organogenesis and micropropagation of Artemisia annua. *J Biotechnologie Vegetale*, 26: 327-337. http://damasuniv.edu.sy/mag/farm/images/stories/327-337_.pdf.

[2] Anis, M., Pravej, A., Malik, M. A., Athar, Ali., Javed, A., Abdin, M. Z. 2014. Impact of plant growth regulators (PGRs) on callogenesis and artemisinin content in Artemisia annua L. plants. *Indian Journal of Biotechnology*, Vol 13, January, pp 26-33. https://doi.org/10.1080/17429145.2014.893030.

[3] Banyai, W., Nakamura, I., Mii, M., Supaibulwatana, K. 2005. High regeneration frequency of transgenic plants in *Artemisia annua L.* by *Agrobacterium tumefaciens* mediated gene transformation. Proceedings of the 10th international congress of SABRAO. 22–24 August 2005. The University of Tsukuba, Japan 2005.

[4] Ben Abid, Z., Moncef, F., Abderrazek, H., Mohamed, H. 2007. *Artemisia herba-alba* Asso. (*Asteraceae*) has equivalent effects to green and black tea decoctions on antioxidant processes and some metabolic parameters in rats. *Annual Nutrition and Metabolism*, 51:216-222. https://doi.org/10.1159/000104140.

[5] Benjamin, B.D., Sipahimalani, A.T., Heble, M.R. 1990. Tissue culture of Artemisia pallens: organogenesis, terpenoid production. *Plant Cell Tissue Organ Cult*., 21: 159-164. https://doi.org/10.1007/BF00033436.

[6] Boutkhil, S., El Idrissi, M., Chakir, S., Derraz, M., Amechrouq, A., Chbicheb, A., El Badaoui, K. 2011. Antibacterial and antifungal activity of extracts and essential oils of *Seriphidium herba-alba* (Asso.) Soják and their combination effects with the essential oils of *Dysphania ambrosioides* (L) Mosyakin & Clemants, *Acta Botanica Gallica*, 158 (3): 425-433. https://doi.org/10.1080/12538078.2011.10516284.

[7] Dangash, A., Ram, M., Niranjan, R., Bharillya, A., Misra, H., Pandya,N., Jain, D.C. 2015. *In vitro* Selection and Hormonal Regulation in Cell Culture of *Artemisia annua* L. Plant. *JSM Cell Dev Biol & Developmental Biology*, 3(1): 1013:1-7. http://dx.doi.org/10.15835/nsb639109.

[8] Essawi, T., Srour, M. (2000). Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, 70:343–349. https://doi.org/10.1016/S0378-8741(99)00187-7.

[9] Etienne, H., Berger, A., Carron, M.P. 1991. Water status of callus from *Hevea brasiliensis* during induction of somatic embryogenesis. *Physiol. Plant.*, 82: 213-218.

[10] Feuerstein, I., Müller, D., Hubert, K., Danin, A., Segal, R. 1986.The constitution of essential oils from *Artemisia herba alba* populations of Israel and Sinai. *Phytochemistry*, 25 (10): 2343-2347. https://doi.org/10.1016/S0031-9422(00)81692-1.

[11] Ganesan, C. M., Paulsamy, S. 2011. Standardized protocol for the in vitro culture of Artemisia annua L.–A medicinal plant at high altitudes of Nilgiris, the Western Ghats. J Res Biol.; 1: 173-178. http://jresearchbiology.com/documents/RA0057.pdf.

[12] Gonzalez, F.A., Perkins, K., Winston, M.I., Xie, D. 2013. Efficient Somatic Embryogenesis

and Organogenesis of Self-Pollination Artemisia annua Progeny and Artemisinin Formation in Regenerated Plants. *American J Plant Sci*., 4: 2206-2217. http://dx.doi.org/10.4236/ajps.2013.411274.

[13] Gopinath, B., Gandhi, K., Saravanan, S. 2014. *In vitro* propagation of an important medicinal plant *Artemisia annua* L. from axillary bud explants. *Advances in Applied Science Research*, 5(1):254-258. http://dx.doi.org/10.3329/ptcb.v23i2.17518.

[14] Gulati, A., Bharel, S., Jain, S. K., Abdin, M. Z., Srivastawa, P. S. 1996. *In vitro* micropropagation and flowering in *Artemisia annua*. *Journal of Plant Biochemistry and Biotechnology*, 5: 31-35. https://doi.org/10.1007/BF03262976.

[15] Hailu, T., Abera, B., Mariam, E. G. 2013. *In vitro* mass propagation of Artemisia (*Artemisia annua* L.) CV: Anamed. *Plant Tissue Cul & Biotech*., 23: 165-176. http://dx.doi.org/10.3329/ptcb.v23i2.17518.

[16] Honda, H., Liu, C. Z., Kobayashi, T. 2001. Large-scale plant micropropagation. *Advances in Biochemical Engineering/Biotechnology*, 72: 157-182. https://doi.org/10.1007/3-540-45302-4_6.

[17] Kour, B., Kour, G., Kaul, S. Dhar, M. K. 2014. *In vitro* mass multiplication and assessment of genetic stability of in vitro raised *Artemisia absinthium* L. plants using ISSR and SSAP molecular markers. *Advances in Botany*, 13(8): 1-7 http://dx.doi.org/10.1155/2014/727020.

[18] Liu, C. Z., Murch S. J., El-Demerdash, M., Saxena, P. K. 2004. *Artemisia judaica* L. micropropagation and antioxidant activity. *J. Biotech*., 110: 63-71. https://doi.org/10.1016/j.jbiotec.2004.01.011.

[19] Mahmoud, H. S., Mohamed, H. B., El-Baroty, G. 2007. Fungicidal activity of *Artemisia herba-alba* Asso. (Asteraceae). *Journal of Environmental Science and Health*, Part B. 41:237-244. https://doi.org/10.1080/03601230500354774.

[20] Mannan, A., Shaheen, N., Arshad, W., Qureshi, R. A., Zia, M., Mirza, B. 2008. Hairy roots induction and artemisinin analysis in *Artemisia dubia* and *Artemisia indica*. *African. Journal of Biotechnology*. 18: 3288-3292.https://doi.org/10.5897/AJB08.516.

[21] Nahla S. Abdel-Azim, Khaled A. Shams, Abdel Aaty A. Shahat, Moustafa M. El-Missiry, Shams I. Ismail, Faiza M. Hammouda 2011. Egyptian herbal drug industry: Challenges and future prospects. *Research Journal of Medicinal Plant*, 5(2):136-144. https://doi.org/10.3923/rjmp.2011.136.144.

[22] Nin, S., Mosori, E., Schiff, S., Bennici, A. 1996. Callus cultures of *Artemisia absinthium* L. Initiation, growth optimization and organogenesis. *Plant Cell Tissue Organ Culture*. 45: 67-72. https://doi.org/10.1007/BF00043430.

[23] Pan, Z.G., Liu, C.Z., Murch S. J., El-Demerdash, M., Parveen, K. S. 2003. Plant regeneration from mesophyll protoplasts of the Egyptian medicinal plants *Artemisia judaica* L. and *Echinops spinosissimus* Turra. *Plant science*, 21(6): 525-530.https://doi.org/10.1016/S0168-9452(03)00220-6.

[24] Rezvan, K. B., Mysore S. S., Mallappa, H. N. 2010. *In vitro* Plant Regeneration from Leaf Explants of *Artemisia vulgaris* L. - A Medicinal Herb. *Modern Applied Science*, 4 (9):130-134. http://dx.doi.org/10.5539/mas.v4n9p130.

[25] Segal, R., Feuerstein, I., Danin, A. 1987. Chemotypes of *Artemisia herba-alba* in Israel based on their sesquiterpene lactone and essential oil constitution. *Biochemical Systematics and Ecology*, 15, 411-416. https://doi.org/10.1016/0305-1978(87)90054-8.

[26] Sharma, A., Yadav, A. S., Bajaj, A., Rai, A. 2008. Cost effective in vitro micropropagation protocol for conservation of plant resources with special reference to important medicinal plants. J Env Res Dev.;2: 357-364. http://www.sciencedomain.org/review-history/5518

[27] Singh, A., Sarin, R. 2010. *Artemisia scoparia* - A new source of artemisinin. *Bangladesh Pharmacological Society*. 5(1): 17-20. http://dx.doi.org/10.3329/bjp.v5i1.4901.

[28] Snedecor, G.W., Cochran, W.G. 1982. Statistical Methods. 7th Edition, Iowa State University Press, Towa, 511. https://doi.org/ 10.2307/2530332.

[29] Sujatha, G., Rajnitha Kumari, B. D. 2007a. High-frequency shoot multiplication in *Artemisia vulgaris* L. using thidiazuron. *Plant Biotechnology Reports*, 1: 149-154. https://doi.org/10.1007/s11816-007-0028-1.

[30] Sujatha, G., Rajnitha Kumari, B. D. 2007b. Effect of phyto-hormones on micropropagation of Artemisia vulgaris L. *Acta Physiol. Plant*. 29: 189-195. https://doi.org/10.1007/s11738-006-0023-0.

[31] Vergauwe, A., Cammaert, R., Vandenberghe, D., Genetello, C., Inze, D., Van Montagu, M. 1996. *Agrobacterium* tumefaciens-mediated transformation of Artemisia annua L. And regeneration of transgenic plants. *Plant Cell Rep*., 15: 929-933. http://dx.doi.org/10.1007/BF00231590.

[32] Xu, Z.Q., Jia, J. F. 1996. Callus formation from protoplasts of Artemisia sphaerocephala Krasch and some factors influencing protoplast division. *Plant Cell Tissue Org. Cult*., 44: 129-134. http://link.springer.com/article/10.1007%2FBF00048190.

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Determinant of Non-Organic Farming in Enrekang District of South Sulawesi

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Abstract**—** *Non-organik farming has a great impact on creasing agricultural production, but many negative impacts such a reduced soil fertility, environment damage and also negative impact on human health. The government has launched various programs to depelop organic farming to encourage farmers to swich to organic farming but has not been successful until now. The research aims to analyze the persistent determinant of non-organic farming by using Interpretative Structural Modeling (ISM) analysis. Result of research, there are three elements becoming persistent determinant of non-organic farming, that is: Facilities dan infrastructure of organic farming available, application of production facilities (fertilizers/chemical pesticides) easy and practical, easy market access for non organic products.*
Keywords**— Non-Organic Farming, ISM, fertilizers.**

I. INTRODUCTION

Agricultural development in Indonesia is an important part in the implementation of national development. Agriculture is placed as a leading sector in view of its role in food supply, employment provision, foreign exchange contributors through exports, encouraging business opportunities and provision of production factors.

Agricultural systems developed over several decades have contributed greatly to improving food procurement and improving living standards. The agricultural system is known for its green revolution technology with the use of superior varieties, the use of an organic fertilizer, chemical pesticides and the use of agricultural machinery for land processing and harvesting. This agricultural system has had a major impact on increasing agricultural production, but many negative impacts to the environment are reduced soil fertility and environmental degradation due to uncontrolled use of fertilizers and chemical pesticides, as well as negative impacts on human health which in the long run will accumulate in the body so that it becomes toxic to human health.

As a result of these negative impacts, the Government launched the organic agriculture development program through the commitment of "Go Organic 2010". In this commitment, it was proclaimed that in 2010 Indonesia will become the largest producer of organic agricultural products in the world. The "Go Organic 2010" program, which includes activities such as organic farming technology development, organic farming groups, rural development through organic farming, and developing organic food marketing strategies. But in fact, organic farming has not developed and is still very limited products produced. That is, not many farmers who apply organic farming business.

Other efforts by the government in encouraging farmers to shift from non-organic farming to organic farming in the form of counseling, training and assistance of organic farming equipment / materials have not fully received a good response from farmers. Therefore, a study is needed to find out the persistent determinant of non-organic farming, so that comprehensive information can be made as a basis for policy making for the development of organic farming in the future.

II. RESEARCH METHODS

A. Research Design

This research was designed using descriptive research design which was conducted in the form of field survey. This design seeks to reveal the things that occur descriptively, therefore the findings are deeper, broader and more detailed.

B. Location and Time of study

This research was conducted from August to September 2017 in Enrekang Regency. Selection of the location is done with the consideration that the area is the largest vegetable producing center in South Sulawesi so it is potentially in the development of organic vegetables. Another consideration is that in this area since a few years ago there have been some consistent farmer groups on organic vegetable farming.

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Types and Data Sources

This study uses primary data and secondary data, namely:

1. Primary data, ie data obtained from field research results, other than that obtained from the results of discussions and interviews with related parties. The interviews were structured on a pre-prepared list of questions.
2. Secondary Data, obtained from literature books, printed media, online media and from agencies or institutions related to the research, among others: the Office of Agriculture and Plantation, BPS, Extension Agency of Agriculture and Forestry, Food Resilience Department and respectively District Office of research location.

Data Collection Instruments

Taking / Collecting data with the following stages:

Sample Determination

To obtain data, a survey of experts / experts who have a level of understanding, mastery, and / or directly involved in the field of technical tasks of organic farming. sample is determined by purposive sampling consisting of experts / practitioners from various institutions / agencies concerned) as follows:

a. Regional Technical Agency in the form of Agency

1) Regional Development Planning Board 1 Person

b. Regional Technical Agency in the form of agency / Office:

1) Agriculture Agency 2 Persons
2) Department of Industry and Trade 1 Person
3) Food Security Service 1 Persons
4) Department of Industry and Trade 1 person
5) Department of Cooperatives, Small and medium business, labor and Transmigration 1 person
6) Environment Agency 1 person
7) Village Community Empowerment Department 1 person
8) Department of Animal Husbandry and Fisheries 1 person

c. Higher Education 1 person

d. UPT BPTTPH Prov. Sulsel 1 person

e. Agricultural Extension Worker (PPL) 3 Persons

f. Farmer Group 2 Persons

Interviewing

In order to obtain objective data, the implementation of the interview is conducted which is preceded by the socialization of the research objectives. This socialization is intended to provide understanding, importance and relevance of elements that have been established with the purpose of this study.

Preparation of Questionnaire

The questionnaire is prepared using all the elements as a grid and arranged in the form of a question by comparing the one element to the other in pairs. The questionnaire is intended to collect data to be analyzed with Interpretative Structural Modeling (ISM), using a comparison comparison of contextual relationships using the symbols V, A, X and O.

B. Data Analysis Method

The method of analysis used in this study is Interpretative Structural Modeling (ISM) to determine the persistent determinant of non-organic farming. Eriyatno in Marimin (2004) states that the methodology and techniques of ISM are divided into two parts, namely the preparation of hierarchy and the classification of sub elements. The basic principle is the identification of structures within a system that provide a high value of benefits in order to concoct the system effectively and for better decision making. Here's a brief description of ISM steps:

1. Identification of elements: The system elements are identified and listed. This can be obtained through research, brainstorming and others
2. Contextual relationships: A contextual relationship between elements is constructed, depending on the purpose of modeling
3. Structural Self Interaction Matrix (SSIM). This matrix represents the element of respondent's perception of the element of the intended relationship. There are four symbols used to represent the type of relationship that exists between the two elements of the system under consideration:

V ... the relation of Ei to Ej, not vice versa

A ... the relation of Ej's elemen to Ei, not vice versa

X ... the interrelation relationship between Ei and Ej (can be otherwise)

O ... shows that Ei and Ej are not related

4. Reachability Matrix (RM): A prepared RM then converts the SSIM symbols into a binary matrix The following conversion rules apply:

a. If the relationship Ei to Ej = V in the SSIM, then the elements Eij = 1 and Eji = 0 in RM

b. If Ei's relationship to Ej = A in the SSIM, then the elements Eij = 0 and Eji = 1 in RM

c. If Ei's relationship to Ej = X in the SSIM, then the elements Eij = 1 and Eji = 1 in RM

d. If the relationship Ei to Ej = O in the SSIM, then the elements Eij = 0 and Eji = 0 in RM

e. RM initial modified to show all direct and indirect reachability, ie Eij = 1 and Ejk = 1, then Eik = 1

5. The level of participation is undertaken to classify elements in different levels of the ISM structure
6. Canonnical matrix: grouping elements of the same level in developing this matrix. The resultant matrix has most of the higher triangular elements is 0 and the lowest is 1. This matrix is then used to prepare the digraph.

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7. Digraph is a concept derived from directional graph, a graph of interconnected elements directly and hierarchy level.

ISM is generated by moving the entire number of elements with the description of the actual elements. Therefore, ISM provides a very clear picture of the system elements and the flow of relationships.

The ISM output is divided into two according to Marimin (2004) ie the Power-Dependent Driver matrix and the structural model diagram. Power-Dependent Driver Matrix is a rank of each sub element and plot each sub element into four sectors along with its coordinates, hierarchy can be created every sub element manually. Determining the outline of the sub classification of the Power-Dependent Driver element is classified into four sectors:

Sector 1: Weak driver-weak dependent variable (autonomous) that contains variables that are generally unrelated to the system and may have small relationships although the relationship may be strong. Sub element of element entering sector 1 if, DP value <0.5 X and value D <0.5 X, X number of sub elements.

Sector 2: Weak driver-strongly dependent variable (dependent) which contains non-free variables. Sub element of element entering sector 2 if, DP value <0.5 X and value D> 0.5 X, X number of sub elements.

Sector 3: Strong driver-strongly dependent variables (linkage) that contain variables that must be carefully studied because of the unstable relationship between variables and each action in this variable can have an impact on other variables and influence feedback can magnify the impact. Sub element of element entering sector 3 if, DP value> 0.5 X and value D> 0.5 X, X number of sub elements.

Sector 4: Strong driver-weak dependent variable (independent) that contains the remaining parts of the system and called the free variable. Sub element of element entering sector 4 if, DP value> 0.5 X and value D <0.5 X, X number of sub elements.

The structural model diagram is the level level of each sub element determined by the level separation on the Reachability Matrix (RM). The determination of the levels of each sub element can be determined from the rankings of each sub element. The sub elements are interconnected directly and push each level at each level.

RESULTS AND DISCUSSION

The result of the study by experts and related parties, the persistent determinant of non organic farming in Enrekang Regency is translated into 6 elements as shown in table 1. The position and weight of each element is presented in table 2. The result of grouping into four sectors namely autonomous, dependent, linkage, and independent as presented in Figure 1. While the interpretation in the form of hierarchical structure is presented in Figure 2.

Table.1: Constant determinant element of non organic farming in Enrekang Regency

No.	Element
1.	Facilities available
2.	Application of saprodi (fertilizer, pesticide) is easy and practical
3.	Plant maintenance is easy
4.	Easy market access
5.	Low cost investment
6.	High crop productivity

The result of ISM analysis shows that from 6 (six) elements suspected to be persistent determinant of non organic farming in Enrekang Regency, there are 4 (four) elements which are strong determinant as seen in table 2 where DP> 0,50. The four determinants are: Facilities available, Easy and practical saprodi application, Easy market access and Low cost investment.

Table.2: Position and Weight of Determinant Element of Persistent Non-Organic Farming.

Posisi	**Determinan**	**Bobot**	
		DP	**D**
Independent	1. Facilities available	1,00	0,50
Its influence on the determinant is strong, but its relation to other activities is weak	2. The production input application is easy and practical	0,83	0,50
	4. Easy market access	1,00	0,50
	Average	0,94	0,50
Linkage	5. Low cost investment	0,67	0,67
(Its influence on the determinant			

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and its association with other activities is strong)			
	Average	0,67	0,67
Dependent (Its influence on the determinant is weak but its association with other activities is strong)	3. Plant maintenance is easy 6. High crop produktivity	0,33 0,33	1,00 1,00
	Average	0,33	1,00
Autonomous (Its influence on the determinant and its interrelationship with other activities is weak)	-	-	-
	Average	-	-

Determinant Element in Sector IV (Independent) Against Persistent Non-Organic Farming

The results of ISM analysis based on Driver Power (DP) - Dependent (D) as shown in Figure 4 show that there are three elements that enter into Independent sector (IV), namely: (1) Facilities available (2) Application of input (fertilizer, pesticides) easy and practical and (4) easy market access. This means that these three elements have a strong influence in the persistent determinants of non-organic farming and their association with other low factors.

Elements The available infrastructure is the main determinant of persistent non-organic farming. The availability of infrastructure facilities in addition to facilitate the farmers in terms of procurement of production facilities, cultivation of cultivation, harvest and post harvest and marketing of the results also have an impact on increasing production and productivity of agricultural products.

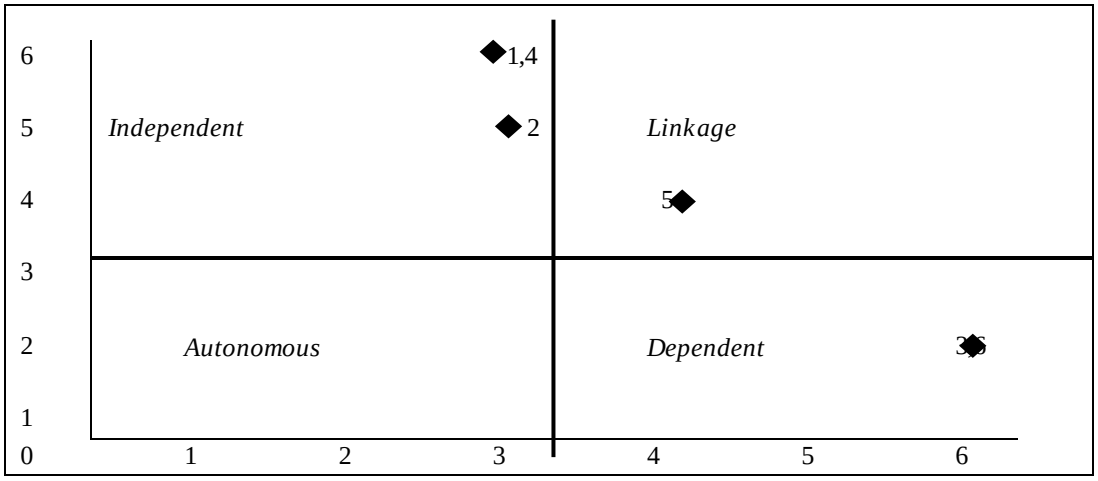

Fig.1: DP-P Matrix Element Determinant persistent non-organic farming

Easy market access is another major determinant factor in persistent organic farming. Along with the increasing availability of road infrastructure, the development of information technology and the opening of market access between islands of farmers more easily in marketing their products.

Application of saprodi (fertilizer, pesticide) is also a determinant factor in persistent non organic farming. The use of fertilizers and pesticides in supporting the increase of cultivated plant production is needed. Without the use of fertilizer, plant growth will be hampered which will impact on the low productivity of cultivated plants. Similarly with pesticides, is needed in overcoming the pest and disease diseases cultivation plants. Uncontrolled attacks of pests and diseases can lead to poor quality and quantity of farmers' products and can even lead to crop failure.

Determinant Element in Sector III (Linkage) Against Persistent Non-Organic Farming

The result of ISM analysis shows there is one determinant element in Linkage sector to persistent non organic farming that is cheap investment cost. This shows that the element is strong influence on persistent non

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organic farming and its dependence on other elements is strong.

Compared with organic farming, non-organic farming investment cost is much cheaper because it does not require any special treatment and requirement for farming land. Land for organic farming around the location of non-organic cultivation requires a barrier plant and this requires no small cost. Another thing that causes non-organic farming investment to be cheaper is easy transportation access; production facilities in the form of fertilizers, pesticides, seeds / seedlings are available and the price is cheaper.

Determinant Element in Sector II (Dependent) Against Persisten Non Organic Farming

The result of ISM analysis shows that there are two determinant elements in dependent sectors to persistent non organic usatani, that is easy crop maintenance and high crop productivity. This suggests that both elements have little effect on the persistent organic farming and its association with other large elements. Along with the increasingly advanced technology in the field of farming management in the form of land management, planting, plant maintenance, harvesting and post-harvest handling more easily and efficiently.

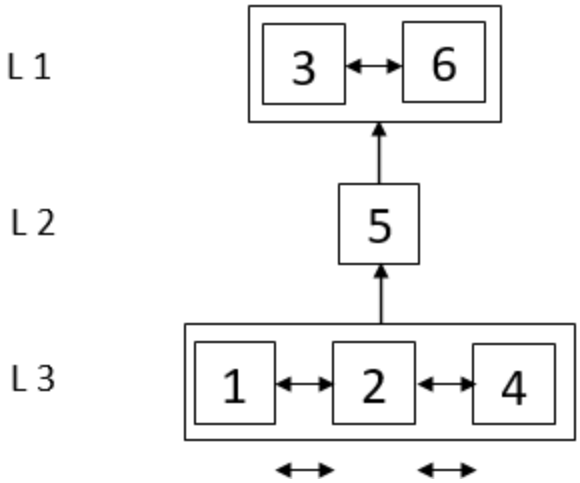

Fig.2: The hierarchical structure of persistent determinant elements of non-organic farming

The structure of the persistent determinant element of non-organic farming in Figure 5 shows that Elements of Infrastructure facilities are available; Application of saprodi (fertilizer, pesticides) is easy and practical; and easy market access at the highest level (level 3). Next on level 2 is the cost of cheap investment. While at level 1 (lowest) is easy plant maintenance and high crop productivity.

III. CONCLUSION

Elements that are strong determinants in which farmers do not switch from non organic farming to organic farming are: Facilities available, Application of production facilities (fertilizers, pesticides) easy and practical, and easy market access. These three elements are in the Independent position in the DP-P matrix which means that the three elements are very strong in the persistent Determinants of non organic farming and their interrelationship with other weak elements.

REFERENCES

[1] Arsyad, M., Nuddin, A., Yusuf, S. 2013. Strengthening Institutional Towards Smallholders Welfare, *Ryukoku Journal of Economic Studies*, Vol. 52, N0. 1, 2 Maret 2013.

[2] Didu MS. 2001. *Analisis Posisi dan Peran Lembaga serta Kebijakan dalam proses Pembentukan Lahan Kritis*, Jurnal Teknologi lingkungan. Volume 2. Nomor 1. Januari 2001. ISSN 1411-318X hlm. 93-105.

[3] IFOAM. 2008. *The World of Organik Agriculture – Statistics and Emerging Trend 2008*. http://www.soel.de/fachtheraaii

[4] Kementerian Pertanian. 2010. *Rencana Strategis Kementerian Pertanian* 2010-2014. Jakarta.

[5] Marimin. 2004. *Teknik dan Aplikasi Pengambilan Keputusan Kriteria Majemuk*. Grasindo. Jakarta.

[6] Mayrowani, H. 2012. *Pengembangan Pertanian Organik Indonesia* . Pusat Sosial Ekonomi dan Kebijakan Pertanian. Bogor.

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Influence of short- and long-term administration of Melengestrol acetate on estrus activity and reproductive performance of nulliparous Barki ewes

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Abstract**—***In Egypt, research focusing on estrous synchronization in small ruminants based on Melengestrol acetate (MGA) supplementation, particularly in nulliparous ewes, is still lacking. The present work aimed to evaluate effect of long-term and short-term administration of melengestrol acetate (MGA) treatments on estrus synchronization and reproductive performance of nulliparous Barki Ewes. This study was performed in Siwa Oasis Research Station (Tegzerty Experimental Farm for animal production), belonged to Desert Research Center, Egypt. Forty five nulliparous Barki ewes with age ranging from 15.5 to 16.5 months, and 38 ± 0.23 kg average live body weight were assigned to one of three groups: (1) control (C, n = 15); (2) long-term treatment with MGA (n = 15, 0.22 mg/ewe/d for 14 days) and (3) short-term treatment with MGA (n = 15, 0.22 mg/ewe/d for 7 days). At the end of MGA treatment (14 or 7 d) all treated ewes were injected by 600 IU PMSG intramuscularly. The results showed that, ewes treated with MGA exhibited highest (P<0.05) estrus response rate (100%) in short term-MGA, followed by long term-MGA (93.33%), whereas the lowest was observed in control group (80%). Conception rates after natural mating were 85.71% and 93.33% for long term and short term MGA treated ewes, respectively. However, it recorded 100% in the control group. Fertility rate was significantly the highest (P<0.05) in short term-MGA (93.33%) than other groups (73.33%). There were no significant differences (p>0.05) in terms of lambing rate and prolificacy among the control and treated ewes. In the meantime, mean values of serum estradiol 17-β were lower (P≤0.01) in long-term treated ewes compared to those of short-term treated ones (27.20±1.78, 32.67±1.27pg/ml), respectively, while the lowest (P<0.05) level was recorded in the control ewes (13.01±1.31 pg/ml). Furthermore, overall mean values of serum progesterone in the control group (1.32 ± 0.09 ng/ml) was higher (P<0.05) than those of long- and short-term MGA treated groups (1.01 ± 0.13, 0.92 ± 0.11, ng/ml), respectively. It is concluded that reproductive efficiency of nulliparous Barki ewes could be improved by short-term supplementation with MGA.*

Keywords**— *Melengestrol acetate (MGA), Estrus synchronization, Reproductive performance, Barki ewes.***

I. INTRODUCTION

Despite growing evidence of applicability of estrus synchronization approach in pluriparous ewes, limited information is available for exploiting this technique in nulliparous ewes. The reduction of age at first delivery is a major goal for modern small ruminant's production systems (Bandeira et al., 2004). However, it is necessary to understand reproductive behavior of nulliparous ewes. The first estrus in ewes varies according to several factors such as breed and period of the year born, while fertility of nulliparous ewes is associated with live weight, body condition score, sanitary management and nutritional status (Aktaset al., 2015). Hormonal treatment to control ovulation and reproduction is a prerequisite for successful breeding and increasing the number of pregnant females (Motlomelo et al., 2002).

In ewes, progestagens are widely used to synchronize estrus, and typically result in approximately 90% estrus exhibition within a 24-hour period and conception rate of 70–80% (Evans et al., 2001). Among the various methods or biotechnologies, which can be employed to control the reproductive cycle of the ewes is the use of synthetic progestins, like melengestrol acetate (MGA), which represents an ideal choice for this type of production in rural areas, where the farmers do not have the economic resource and sufficient technology (Álvarez

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and Ducoing, 2005). MGA is a low cost product, easy to administer (can be mixed with feed) and does not cause abortion (Salas et al., 2011).The percentage of ewes lambing after treatment with MGA has been reported to range from 25 to 85%, and appeared to depend on ewe breed (Safranski et al., 1992), length of treatment or length of breeding period (Powell et al., 1996), or use in combination with gonadotropins (Morrical et al., 1995).

Melengestrol acetate is attractive to sheep farmers, because the cost of treatment is much less than that associated with the use of intravaginal sponges. The most costly component of the traditional out-of-season breeding strategy is eCG/PMSG. Some research has called into question the requirement for stimulation of follicles with eCG. If the use of eCG proved to be unnecessary, it would markedly alter the economics of out-of-season breeding programs. Due to the limited understanding of nulliparous reproduction, the current work was conducted to evaluate effect of long-term and short-term Melengestrol acetate treatments on estrus synchronization response and reproductive performance of nulliparous Barki ewes.

II. MATERIALS AND METHODS

This study was carried out at Siwa Oasis Research Station (Tegzerty Experimental Farm for Animal Production), which belongs to Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Which lies between longitudes (Lat: 29 º 06" 29 º 24" N Long: 25º 16" 26 º 12" E)), and located 330 Km southwest of the Mediterranean shoreline and at 65 Km east of the Libyan borders. All animal were kept in semi-open pens roofed with wood throughout the experimental period (April 2016 – February 2017).

Animal's management

Forty five nulliparous Barki ewes, aged 15.5 – 16.5 months and average body weight of 38 ± 0.23 kg were used. Animals were fed a concentrate mixture according to their body weight requirements (NRC, 1985). Of the mixture contained crude protein not less than 14%, crude fiber not less than 15%, ash not more than 9%, crude fat not less than 2%, total digestible matter not less than 65%, and Egyptian clover (*Trifolium alexandrinum*) hay as a roughage ration *ad libitum*. Fresh water was available to all groups daily. All animals were clinically examined and were found to be free of any disease or reproductive disorders.

Experimental design

The ewes were equally divided into three groups: The first group: (n = 15) served as control. The second group: MGA long-term protocol (Long-Term. n = 15) received 0.22 mg/head/day Melengestrol acetate (MGA®200Premix NDC 0009-0952-01, Upjohn Company; Michigan, USA) for 14 days. The Third group: MGA short-term protocol (Short-Term. n = 15) received the same MGA concentration for 7 days. At the end of MGA treatment (14 or 7 d) all ewes were injected by 600 IU pregnant mare serum gonadotropin PMSG i.m (Gonaser, Laboratories HIPRA, S. A.-Avoda. Laselva, 135 17170 Amer (GIRONA), Spain.), and were exposed to intact Barki rams (1 ram per 15 ewes) fitted with a marking harness with rotation for a 17-day breeding period in the control group, and 4 days in the treated groups. The chemical composition of (MGA) is presented in Table (1).

Table.1: Chemical composition of Melengestrol acetate 200 Premix (MGA).

Ingredients	Concentration
Inactive	
Soybean hulls	96 %
Starch	2.98 %
Mineral Oil	1 %
Active	
Melengestrol Acetate	200 mg/kg

Blood samples

Blood samples were collected from the jugular veins of all animals into non-heparinized vacationer tubes at MGA withdrawal (day 7 or 14, 0 hr.) and at 24, 48, 72, and 96 h after injection of PMSG. Blood samples were centrifuged at 3000 rpm for 20 minutes, and serum was aspirated and kept at -20 ºC until analysis.

Hormonal and serum biochemical analysis

Serum progesterone (P_4) and estradiol 17- β (E_2) concentrations were analyzed using commercial ELISA kits (Monobind, USA) according to Abraham (1981). The intra -and inter-assay CV's are (9.3, 9.7 % and 8.2, 95 %), respectively. Concentrations of cholesterol (CHO), and Glucose (Glu) were determined using colorimetric kits (Diamond Diagnostic, Egypt).

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Statistical analysis

A General Linear Model procedure (SAS, 2004) was used for the statistical analyses of serum biochemical parameters and serum hormones concentrations at hours 0, 24, 48, 72 and 96 using the following model:

$Y_{ijk} = \mu + T_i + H_j + TH_{ij} + e_{ijk}$

Where,

Y_{ijk} = Any observation of k^{th} animal within i^{th} treatment within j^{th} hour
μ = Overall mean
T_i = Effect of i^{th} treatment (i = 1-3)
H_j = Effect of j^{th} hour (j = 1-5)
TH_{ij} = The interaction between treatment and hour
e_{ijk} = Experimental error

Another General Linear Model procedure (SAS, 2004) was used for the statistical analysis of birth and weaning weights and average daily gain for lambs using the following model:

$Y_{ij} = \mu + T_i + e_{ij}$

Where,

Y_{ij} = Any observation of j^{th} animal within i^{th} treatment
μ = Overall mean
T_i = Effect of i^{th} treatment (i = 1-3)
e_{ij} = Experimental error

Significant differences among means were detected using Duncan's multiple range test (Duncan, 1955). While, reproductive traits (estrus response), (conception, lambing, fertility, prolificacy, mortality and weaning rates) and sex ratio were analyzed using Chi-square test.

III. RESULTS AND DISCUSSION

Reproductive performance:

Little research has been focused on estrus synchronization in Egyptian sheep using MGA, and no results could be found on the feeding with MGA for synchronization purpose in nulliparous ewes. The results show that estrus synchronization can be done successfully in nulliparous Barki ewes with MGA (Table 2).

The short-term group (7 days) showed the highest percentage of ewes responded to estrus synchronization (100%) followed by long-term group (14 days) (93.33%) and control group (80%). Findings of estrus rates were in agreement with previous results reported by (Powell et., al 1996) who reported that estrus response of ewes treated with MGA for 8 days (short-term) was 90%, while was 92.3% in ewes treated with MGA for 14 days (long-term). In another study, Windorski et al. (2008) reported that treatment with MGA (0.3 mg/ewe/d) for 7 days increased the proportion of Rambouillet ewes displaying estrus by 5–20%. Later, Rojo and Salas (2015) evaluated the effect of long-term (17 days) MGA treatment on the induction and synchronization of estrus in ewes and observed that 95% of the females exhibited estrus. Likewise, Salas et al. (2011) obtained 100% ewes in estrus using MGA for 17 days, which was quite the opposite of what Emsen et al. (2011) reported (89% of ewes in estrus using 0.125 mg MGA for 12 days (long term) and 62% of ewes in estrus using 0.125 mg of MGA for 9 days (short term)), this difference of results in both studies may be due to the difference in dose of MGA which was half the dose used in this study.

Table.2: Reproductive efficiency of nulliparous Barki Ewes following long-term and short-term protocols of Melengestrol acetate treatment (LSM±SE).

Traits	Control	Long term-MGA	Short term-MGA
	N=15	N=15	N=15
Estrus response rate (%)	80^{b} (12/15)	93.33^{ab} (14/15)	100^{a} (15/15)
Conception rate (%)	100^{a} (12/12)	85.71^{a} (12/14)	93.33^{a} (14/15)
Lambing rate (%)	91.67^{a} (11/12)	91.67^{a} (11/12)	100^{a} (14/14)
Fertility rate (%)	73.33^{b}	73.33^{b}	93.33^{a}
Prolificacy rate (%)	100^{a}	100^{a}	100^{a}

$^{a-b}$ values within the same row with different letters differ (P< 0.05).

Estrus response = number of ewes showing signs of estrus/total ewes treated x100.

Conception rate = number of ewes conceived / number of ewes showing estrus and mated x100.

Lambing rate = number of ewes lambed / number of ewes mated x100.

Fertility rate =number of ewes lambed/total ewes treated x100.

Prolificacy rate =number of lambs born/number ewes lambed x100.

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The conception rate (Table 2) was higher (100%) in control group than groups treated with MGA (85.71 and 93.33%) in long and short term groups, respectively. The high pregnancy rate observed in the control group could be due to the percentage of ewes pregnant to the number of ewes which showed signs of estrus, which was further lower among groups. In this study conception rates in groups treated with MGA are much higher than those observed by Emsen et al. (2011), who reported that conception rate was 44% in ewes treated with MGA for 12 days (long-term) and 41% in ewes treated with MGA for 9 days (short-term). Findings of pregnancy rates were in disagreement with results reported by Keefe and Wichtel (2000) when naturally-mated ewes were treated with MGA with and without PMSG administration. The later authors obtained pregnancy rates of 43.3% and 31% for the aforementioned groups, respectively. (Salas et al., 2011) reported that the conception rate in the experimental group with 0.45 mg MGA/head/day for 17 days was 70% compared with 50% in control group. This result was higher than those obtained by Powell et al. (1996), who reported that lambing rate of ewes treated with MGA for 8 days (short-term) was 60%, while was 57.7% in ewes treated with MGA for 14 days (long-term).

Results of the present study demonstrated that lambing rate (Table 2) ranged between 91.67 to 100 % and was numerically higher in short-term group (100%) than other groups (91.67%), but with no significant differences. Our results of lambing rate were higher than those obtained in control, MGA and MGA+PG 600 groups (14.8, 40.5 and 41.2%, respectively) according to Safranski et al. (1992). But close to those obtained by (Windorski et al., 2008) who found that lambing rate in Rambouillet ewes was 80.4, 77.8 and 80.4% in control, MGA and MGA+PG-600, respectively. On the other side, Powell et al. (1996) compared 8, 11 and 14 days of MGA supplementation on lambing rates in out of season breeding programs and they concluded no difference. Based on these observations and the economics of using the product for a shorter period of time, they concluded that an 8-day course of treatment was best.

Fertility rate (Table 2) was found to be 73.33, 73.33 and 9.33% in the control, long and short terms groups, respectively ($P < 0.05$). This result was higher than those obtained by Emsen et al. (2011) who recorded fertility percentage of 45% when Morkaraman ewes were treated with 0.125mg MGA/ewe/day for 12 days, whereas Castonguay et al. (2002) used 0.4 mg MGA/ewe/day during 10 days and obtained fertility rate of 45%. In both studies, the low fertility rate may be due to when low doses are administered, MGA (≤ 0.12 mg) generates a high frequency of luteinize hormone (LH) pulses, triggering the development of persistent follicles (Colazo et al., 2007), while when high doses are administered (≥ 0.4 mg), LH suppression can be so intense to the extent which inhibits follicular development (Lopez et al 2007). It is recommended that the dose of 0.22 mg of MGA/ewe/day for 7 days (short-term) is administered, in order to get the best response in the lambing rate (100%) and fertility rate (93.33%) of nulliparous Barki ewes.

The percentage of prolificacy obtained was 1.0 lamb born by ewe for all groups and there was no significant difference among the groups. In this study, prolificacy rate (number of lambs born/number of ewes lambing) did not different among groups which were 1.0 in the three groups and therefore the use of PMSG had no effects on increasing lambs born. This opinion is consistent with what Safranski et al. (1992) reported, the number of lambs born per ewe lambing (prolificacy rate) was not different among treatments or genotypes. Consequently, the advantage that was obtained in ovulation rate through the use of PG-600 was not realized at lambing, although the number of luteal structures increased when PG- 600 was injected on the last day of feeding MGA, the number of lambs born per ewe was not significantly affected. In another work, Windorski et al (2008) reported that no significant differences of lambs born per ewe lambing between treated and untreated groups with MGA, where was 1.82, 1.91 and 1.89 for control, MGA and MGA+PG600, respectively. In that regard, Keefe and Wichtel (2000) reported that no significant differences of lambs born per ewe lambing between treated ewes with MGA with and without eCG and mated naturally, prolificacy rates were 1.46% and 1.66%, respectively.

Serum Estradiol 17-β (E_2) and progesterone (P_4) concentrations:

Changes in serum estradiol 17-β and progesterone concentrations in ewes are presented in Figure 1(A and B, respectively).

Overall mean of serum estradiol 17-β concentration were lower ($P \leq 0.01$) in long-term than in short-term groups (27.20±1.78, 32.67±1.27pg/ml, respectively) and compared with control (13.01±1.31 pg/ml). There was no effect or interaction between treatments and hours of synchronization (0, 24, 48, 72 and 96) after MGA withdrawal (figure 1). similar results were also obtained in camels (Abd El-Hamid et al., 2016), beef heifers (Funston et al., 2002), and ewes (Martínez et al., 2017; Rojo and Salas, 2015; Powell et al., 1996).The changes in serum estradiol 17-β concentration observed in the present study are clearly attributed to the changes in ovarian kinetics in both MGA-treated groups compared with control during the synchronization period.

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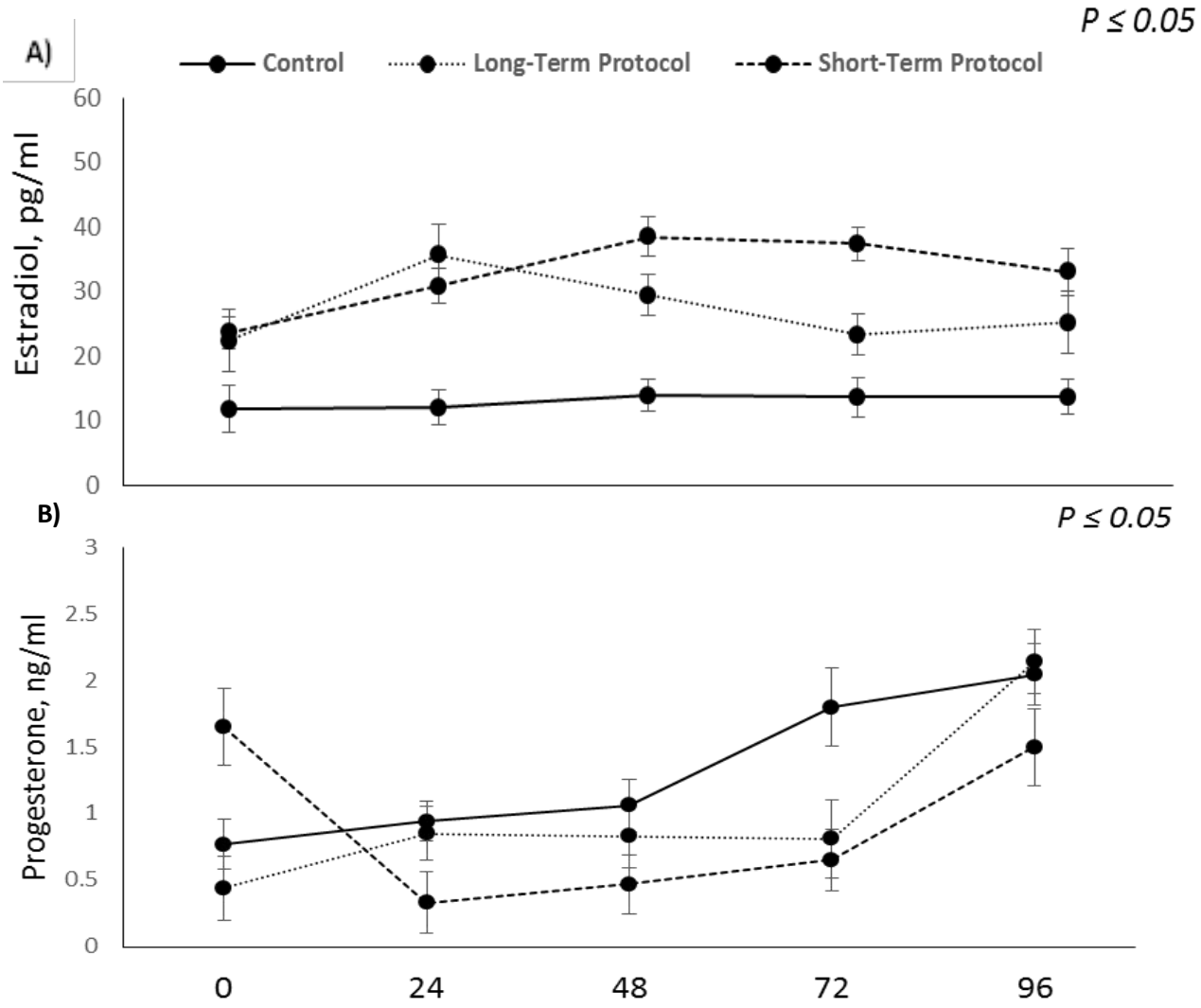

Fig.1: Concentrations of serum estradiol-17β (pg/ml) (A) and progesterone (ng/ml) (B) after MGA feeding at hours of 0, 24, 48, 72 and 96 of synchronization period in nulliparous Barki ewes.

Progestin treatments inhibited the ovarian growth of newer follicles and thereby slightly decreased estradiol concentration during the long or short-term (7 or 14) days of MGA feeding. Serum estradiol increased at after withdrawal of MGA feeding (Jabbar et al., 1994).

In the present study, overall means of serum progesterone concentrations in the control group (1.32 ± 0.09 ng/ml) was higher ($P < 0.05$) than those of long- and short-term MGA groups (1.01 ± 0.13, 0.92 ± 0.11, ng/ml) respectively. There were interactions ($P < 0.01$) between treatment and hours of synchronization. Serum P4 concentrations in the control and MGA-fed animals were affected by time during the experimental period. However, in progestin groups serum progesterone declined (0.47±0.22, 0.83±0.24 ng/ml) compared with control at 48 h of synchronization period, and increased at 96 hrs., while serum progesterone elevated in control group during synchronization period (Figure 1). It seems that MGA did not increase the natural progesterone in the peripheral circulation after withdrawal; however, it mimicked its function on synchronizing ovarian activities. This, in turn, should induce follicular growth, accelerate ovum development and therefore lower concentration of progesterone (Thompson et al., 1990; Scudamore et al., 1992), while the increased of serum progesterone in control group may be due to variations in ovarian activity.

Productive traits:

From Table (3), there were no significant differences among the experimental groups in birth weight, mortality rate, weaning rate, weaning weight and average daily gain of nulliparous ewes following both long-term and short-term MGA treatment. All groups in this study had almost the same birth weight being 2.94, 2.97 and 2.65 kg, for control, long and short term groups, respectively. These results were in agreement with those reported by Windorski et al. (2008) who concluded that no significant differences were observed between groups

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treated and untreated with MGA, where lambs birth weights were 4.6, 4.6 and 4.7 kg for control, MGA and MGA+PG600, respectively. Also, weaning weights at the three months of age were 16.7, 16.6 and 16.2 kg in the same groups, respectively. Average daily gain in control, long- and short-term groups was estimated to be 153, 151 and 150 g, respectively. Mortality rate from birth until weaning in control, long- and short-term groups were 9.09, 0.0 and 7.14%, respectively. The fit weaning rates were 90.91, 100 and 92.86% in control, long and short terms groups, respectively. There was no significant difference in sex ratio of lambs among the three groups.

Table.3: Productive performance of nulliparous Barki lambs following long-term and short-term protocols of Melengestrol acetate treatment (LSM±SE).

Traits	Control	Long-Term-MGA	Short-Term-MGA
Birth weight (kg.)	2.94[a]	2.97[a]	2.65[a]
(Lambs N)	(11)	(11)	(14)
Weaning weight (Kg)	16.70[a]	16.60[a]	16.20[a]
(lambs N)	(10)	(11)	(13)
Average daily gain (g)	0.153[a]	0.151[a]	0.150[a]
Mortality rate	9.09[a]	0.00[a]	7.14[a]
Weaning rate	90.91[a]	100[a]	92.86[a]
Sex ratio (M/F)	54.55[a]	45.45[a]	57.14[a]
	45.45[a]	54.55[a]	42.86[a]

[a-d] values within the same row with different letters differ (P< 0.05).
Weaning rate = number of lambs weaned / number of lambs born x100

Serum biochemical parameters:

Overall means of glucose (Glu) concentrations decreased (P ≤ 0.01) in both treated groups (69.86±2.12, 71.96±1.62 mg/dl, respectively) compared with control (79.79±1.82 mg/dl). There were interactions (P≤0.01) between treatment and hours. Serum glucose concentrations were not affected by time (0, 24, 48, 72 and 96) (Table 4). These data are in agreement with those reported by Abd El-Hamid et al. (2016) and El-Sherif and Assad (2001). In our results the decreased glucose concentrations with increased ovarian activity during synchronization period may be due to the uptake of glucose by ovarian tissues (Rabiee and Lean 2000).

Also, overall means of cholesterol (Cho) concentrations decreased (P ≤ 0.01) in MGA-treated groups (107.19±4.47and 108.96±3.92 mg/dl) compared with control (138.13±4.06 mg/dl). However, no interactions were detected between treatment and hours. Also serum cholesterol concentrations were not affected by time (0, 24, 48, 72 and 96) (Table 2). The same results were previously reported by Al-Bulushi et al. (2017) and Egbe-Nwiyi et al. (2015) in goats and sheep (Kandiel et al., 2016) during both synchronization and estrous period. Cholesterol is the precursor of the production of steroids hormone such as estrogens during ovarian activity (Arther et al., 1982).

IV. CONCLUSION

Our results of estrus response, fertility and lambing rates following melengestrol acetate (MGA) elucidate its efficiency when applied in nulliparous ewes. Additionally, MGA is a low-cost substance which makes it a convenient alternative to hormonal treatments when applying estrus synchronization protocols on a large scale. In conclusion, estrus can be synchronized and improving reproductive performance in nulliparous Barki ewes by using short-term MGA treatment (0.22 mg/ewe/day for 7 days).

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Table 4. Changes in serum glucose and cholesterol concentrations during synchronization period in nulliparous of Barki ewes (Mean ±SE).

Parameters	Treatment	Hours					Overall
		0	24	48	72	96	
Glucose (g/dl)	Control	64.05±3.71^{d}	81.09±3.71^{abc}	89.90±3.71^{a}	83.14±4.76^{ab}	80.26±4.18^{abc}	79.79±1.82^{A}
	Long-term	72.44±4.07^{bcd}	64.63±5.56^{d}	66.51±4.70^{d}	73.28±4.70^{bcd}	72.60±4.70^{bcd}	69.86±2.12^{B}
	Short-term	78.03±3.78^{abc}	66.06±3.44^{d}	69.31±3.78^{cd}	70.94±3.44^{bcd}	75.49±3.78^{bcd}	71.96±1.62^{B}
	Overall	71.51±2.22	70.59±2.50	75.24±2.22	75.79±2.50	76.11±2.44	
Cholesterol (g/dl)	Control	138.60±8.86	136.24±8.86	137.83±8.86	141.51±8.86	136.47±9.90	138.13±4.06^{A}
	Long-term	111.23±9.70	104.80±9.70	106.83±9.70	105.85±11.12	107.23±9.70	107.19±4.47^{B}
	Short-term	106.81±8.20	105.69±8.20	112.12±9.07	106.85±9.02	113.36±9.07	108.96±3.92^{B}
	Overall	118.88±5.16	115.58±5.16	118.93±5.32	118.07±5.61	119.02±5.52	

$^{a-d}$ values within the same row with different letters differ (P< 0.05).

^{A,B} values within the same column with different letters differ (P< 0.05).

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REFERENCES

[1] Abd El-Hamid, I.S., Nour El-Din, A.N.M., Zaghloul, A.A. El-Bahrawy, K.A., Elshahaw, I.I., Allam A.M., EL-Zarkouny, S.Z. and. Hassan, G.A. (2016). Effects of calcium salts of fatty acids rich in palmitic and oleic fatty acids on reproduction and serum biochemistry in Barki ewes. Small Rumin. Res., (144): 113-118.

[2] Abraham, G.E., 1981. The application of natural steroid radioimmunoassay to gynecologic endocrinology. In: Abraham GE, editor. Radioassay Systems in Clinical Endocrinology, Basel: Marcel Dekker, pp: 475-529.

[3] Aktas, A. H.; Dursun, S.; Dogan, S.; Kiyma, Z.; Demirci, U. and Halici, I. (2015). Effects of ewe live weight and age on reproductive performance, lamb growth, and survival in Central Anatolian Merino sheep. Arch. Anim. Breed., 58:451–459.

[4] Al-Bulushi, S., Shawaf, T. and Al-Hasani, A. (2017). Some hematological and biochemical parameters of different goat breeds in Sultanate of Oman "A preliminary study. J. Vet. World. (10): 461-466.

[5] Álvarez, R. L., and Ducoing, W. A. (2005). Reproductive Aspects of Sheep and Goats. Faculty of Medicine Veterinary and Zootechnics, National Autonomous University of Mexico. Accessed June, 2014. http://amaltea.fmvz.unam.mx/textos/escrito_Repro.pdf.

[6] Arther, G.H., Noakes, D.E. and Pearson, H. (1982). Veterinary Reproduction and Obstetrics. 5th (Ed.) Bailliere Tindall, London.

[7] Bandeira, D. A., Santos, M. H. B., Correia, Neto J. and Nunes, J. F. (2004). Aspectos gerais da caprino-ovinocultura no Brasil e seus reflexos produtivo e reprodutivo. In: Santos M H B, Oliveira M A L and Lima P F (Eds). Diagnóstico de gestação na cabra e na ovelha. São Paulo: Varela, pp.1-8.

[8] Castonguay, F., Leduc, G., and Goulet, G. (2002). Use of Melengestrol for Estrus Synchronization in an Artificial Insemination Program in Ewe. Journal Animal Science 80: 268-9.

[9] Colazo, M. G., Mapletoft, R. J., Martínez, M. F., and Kastelic, J. P. (2007). The Use of Treatments Hormonal (MGA) to Synchronize Oestrus and Ovulation in Heifers. Veterinary Science 9: 4-16.

[10] Duncan DB (1955). The multiple range and F- tests. Biometrics, 11: 1-42.

[11] Egbe-Nwiyi, T., Igwenagu, E. and Samson, M. (2015). The influence of sex on the haematological values of apparently healthy adult Nigerian Sahel goats. Sokoto J. Vet. Sci., (13): 54-58.

[12] El-Sherif, M.M.A. and F. Assad, (2001). Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi-arid condition. Small Rumin. Res., (40): 269-277.

[13] Emsen, E., Giménez, D. C., Kutuca, M., and Koycegiz, F. (2011). "Reproductive Response of Ewes Synchronized with Different Lengths of MGA Treatments in Intrauterine Insemination Program." Animal Reproduction Science 126 (1-2): 57-60.

[14] Evans, A.C.O.; Flynn, J.D.; Quinn, K.M.; Duffy, P.; Quinn, P.; Madgwick, S.; Crosby, T.F.; Boland, M.P. and Beard, A.P. (2001). Ovulation of aged follicles does not affect embryo quality or fertility after a 14-day progestagen estrus synchronization protocol in ewes. Theriogenology 56, 923–936 .

[15] Jabbar, G., Umberge, S. H. and Lewis G. S (1994). Melengestrol Acetate and Norgestomet for the Induction of Synchronized Estrus in Seasonally Anovular Ewes. J. Anim Sci.(72):3049-3054.

[16] Kandiel, M..M., El-Khaiat, H.M. and Karima, M.M. G. (2016). Changes in some hematobiochemical and hormonal profile in Barki sheep with various reproductive statuses. Small Rum. Res., (136): 87-95.

[17] Keefe, G.P. and Wichtel, J.J. (2000). Evaluation of melengestrol acetate and equine chorionic gonadotropin for out-of-season breeding in sheep on Prince Edward Island. Can. Vet. J. 41, 211–214.

[18] López, A. S., González, A. B., and Carrizosa, J. A. (2007). New Estrus Synchronization and Artificial Insemination Protocol for Goats Based on Male Exposure, Progesterone and Cloprostenol. Theriogenology 68: 1081-7.

[19] Martínez, J.A. R., Piña, R. G., Caratachea, A. J., Patiño, A. B., Villavicencio, J. L. E. and Razo, G., S. (2017). Estrus, Fertility and Prolificacy in Wool Sheep Treated With Melengestrol Acetate on the Reproductive and Non-Reproductive Period. Transylvanian Review. (22):5689-5695.

[20] Morrical, D.G., Youngs, C.R. and McClain, A. (1995). The influence of MGA and PG600 on the out of season reproductive performance of ewes. Anim Sci Leaflet;R1475.

[21] Motlomelo, K.C.; Greyling, J.P.C. and Schwalbach, L.M.J. (2002). Synchronization of oestrus in goats: The use of different progestagen treatments. Small Rumin Res. 45, 45–49 .

[22] NRC, 1985. Nutrition Requirements of Sheep, 6th rev. ed. National Academy Press, Washington, D.C., USA.

[23] Powell, M.R, Kips, M., Lumberton, W.R. and Keisler, D.H. (1996). Use of melengestrol acetate-

http://dx.doi.org/10.22161/ijeab/3.4.19 *ISSN: 2456-1878*

based treatments to induce and synchronize estrus in seasonally anestrous ewes. J Anim Sci;74:2292–302.

[24] Rabiee A. R. and I. J. Lean. (2000). Uptake of glucose and cholesterol by the ovary of sheep and cattle and the influence of arterial LH concentrations. Animal Reproduction Science. (64): 199 -209.

[25] Rojo-Martínez, J., A. and Salas-Razo G. (2015). The Use of Melengestrol Acetate as a Strategy to Improve Production Efficiency of Sheep in Rural Areas in Mexico. Journal of Agricultural Science and Technology. (5): 210-213.

[26] Safranski, T.J., Lamberson, W.R. and Keisler, D.H. (1992). Use of melengestrol acetate and gonadotropins to induce fertile estrus in seasonally anestrous ewes. J Anim Sci;70:2935–41.

[27] Salas, R. G., Mata, F., Perea, M., Garcidueñas, R., Gutiérrez, E., Caratachea, A., and Flores, P. (2011). "Estrus Grouping in Sheep Treated with Melengestrol Acetate (MGA)." Journal of Agricultural Science and Technology 1 (8): 1295-6.

[28] SAS (2004). Statistical analysis system User's Guide. Release 9.1. SAS institute, Cary, North Carolina.

[29] Scudamore C. L., Robinson. J. Aitken R. P. and Robertson, I. S. (1992). Comparison of priming with 2 dosages of fluorogestone acetate in pessaries on the quality of embryos recovered from superovulated ewes. Theriogenology. (37): 445 - 456.

[30] Thompson, J. G. E., Simpson, A. C., James, R. W. and. Tervit, H. R. (1990). The application of progesterone containing CIDR devices to superovulated ewes. Theriogenology. (33): 1297-1304.

[31] Windorski, E. J., Schauer, C. S. Wurst, A. K. Inskeep, E. K. and Luther, J. S. (2008). Effects of melengestrol acetate and P.G. 600 on fertility in Rambouillet ewes outside the natural breeding season, Theriogenology, vol. 70, pp. 227-232.

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Biofumigation: A Potential Aspect for Suppression of Plant-Parasitic Nematodes

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Abstract*—Plant-parasitic nematode cause economic loss to crops throughout the world. Biofumigation is the environmental friendly control option for the suppression of plant-parasitic as well as other pathogenic soil microbes. Glucosinolates are the main active compound present in some plants which are responsible for biofumigation process. To increase the efficiency of biofumigation selection of varieties containing more glucosinolates is highly desirable. Plant growth stage, soil temperature, soil texture, moisture, soil depth and soil microbes play important role in efficient biofumigation.*

***Keywords*— Biofumigation, glucosinolate compound, isothiocyanate compound, plant tissue, soil characteristics, soil microorganisms.**

I. INTRODUCTION

Agricultural crops are attacking by different insects, fungi, bacteria, viruses and nematodes. Plant-parasitic nematodes are the most common enemy to agricultural production. The plant parasitic nematodes cause about $157 billion annual losses of economic crops worldwide (Abad *et al*.,2008). Chemical nematicides are considered the most effective method in suppressing nematodes population. The chemical nematicides including fumigants such as Ethylene Dibromide, 1, 2-Dibromo-3-Chloro propane, Chloropicrin, Metam-sodium, Dazomet, Methyl Bromide and Methyl Iodide whereas non-fumigants nematicides viz., Aldoxycarb, Carbofuran, Oxamyl, Fenamiphos, Cadusafos and Fosthiazate are the widespread applied methods. These synthetic soil fumigants are highly toxic to pests as well as many beneficial soil organisms (Schreiner *et al*., 2001).Many of these soil fumigants exhibit vertebrate toxicity, high cost, resistance phenomena and other damaging environmental effects (Cox, 2006). Thus, all these negative impacts drive the scientists to find alternative methods of management that are sustainable, economically viable and non-polluting. For sustainable nematode management, it is important to have a holistic approach; taking into consideration cultural, biological and chemical options as part of an integrated management approach. Biofumigation and modified/innovative biofumigation are a sustainable approach to manage soil-borne pathogens, nematodes, insects and weeds. Biofumigation is defined as a process that occurs when volatile compounds with pesticidal properties are released during decomposition of plant materials or animal products(Angus *et al*. 1994;Halberendt 1996; Kirkegaard and Sarwar, 1998; Bello et al., 2000; Piedra Buena *et al*., 2007). Numerous studies in literature confirmed the ability of certain plants to suppress nematodes through the nematicidal activity of the secondary metabolites (Chitwood, 2002; Zasada & Ferris, 2004). Most research on biofumigation, however, has focused on using brassicaceous crops (Kirkegaard and Matthiessen, 2004). The suppressive effect of brassicaceous biofumigants on soil borne pathogens, weeds, and plant-parasitic nematodes has been demonstrated in numerous laboratory, greenhouse, and field studies (Ploeg and Stapleton, 2001; Ploeg, 2008; Zasada *et al*., 2010). The mechanism responsible for the biocidal effect of decomposing *Brassica* crops is thought to be based on a chain of chemical reactions ultimately resulting in the formation of biologically active products (Underhill, 1980). Cruciferous plants belonging to *Brassica* spp. contain glucosinolate compounds which are β-D-thioglucosides, sulphur containing stable and non-toxic compounds located in the cell vacuoles distinguished from one another by differences in their organic side chains (R groups) and classified as aliphatic, aromatic or indole forms, occur in all parts of the plant and degrade via enzymatic hydrolysis (Chew, 1988; Brown *et al*., 1991;Zasada and Ferris, 2004; Padilla *et al*., 2007). Glucosinolates, upon tissue disruption they come in contact with myrosinase (= thioglucosidase), an enzyme endogenously present in *Brassica* tissues, but stored in the cell walls or the cytoplasm, away from the glucosinolates (Poulton and Moller, 1993). The enzymatic hydrolysis of glucosinolates produces volatile isothiocyanates (ITCs), nitriles, SCN-, oxazolidinethione, epthionitriles and organic thiocyanates (Cole, 1976; Fenwick *et al*.,1983; Wathelet *et al*., 2004). The fumigant action of these volatile compounds that are released, suppresses plant pathogens soil-borne pathogens (Sarwar *et al*., 1998; Kirkegaard *et al*., 1993; Kirkegaard &Sarwar, 1998; Piedra Buena *et al*., 2007).

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Although ITCs are considered the most bioactive products, other compounds such as non-glucosinolate sulphur containing compounds, fatty acids, nitriles and ionic thiocyanates may also affect pest and pathogen populations (Matthiessen & Kirkegaard, 2006) .The first observations of the unique properties of GSLs and ITCs were recorded at the beginning of the 17th century (Challenger, 1959). The Family Brassicaceae contains more than 350 genera with 3000 species of which many are known to contain GSL. However, GSLs are not confined to brassicas alone. At least 120 structurally different glucosinolates have been identified in 16 different families of angiosperms. At least 500 species of non-brassica dicotyledonous angiosperms have also been reported to contain one or more of the over 120 known GSLs (Fahey *et al*., 2001). Each of the GSLs has its own chemical property and can be placed in one of three different classes, namely aliphatic, aromatic or indole forms (Zasada &Ferris, 2004; Padilla *et al*., 2007). There are over 100 different types of glucosinolates (Manici *et al*., 2000; Underhill, 1980). A single *Brassica* species can contain several different types of glucosinulates (Sang *et al*., 1984), and the types and quantities of glucosinolates are highly variable between species and even varieties (Rosa *et al*.,1997). As a result, the quantities and types of biocidal ITCs resulting from the breakdown of glucosinolates are higly variable. The nematicidal effect of the tested mustard may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock *et al*., 1989; Salem *et al*., 2015).

II. BIOFUMIGATION PROCESS

Incorporation the fresh mass of plant residues into the soil can be done directly if the mass is coming from grown crop or plant mass taken from elsewhere and brought into the plot or field. If the mass is transported to the field, the soil should be well prepared before the incorporation. During transportation of these organic materials in the field, care must be taken to retain the gases produced from biodegradation, by covering the piles of the bio-fumigant with plastic until the time of application. Generally a dose of 50 t to 100 t per ha is recommended depending on nematode population in the field. The bio-fumigant should be distributed uniformly and the field should be watered until the soil is saturated and cover the soil surface tightly with a transparent plastic film for at least 2 weeks. The film is removed 3-4 weeks after and the soil slightly removed in order to permit the gases to escape from soil. Planting of the desired crop can be done 24 hours later.

III. ASPECTS THAT INFLUENCE GSL RELEASE AND ITC ACTIVITY

3.1. Plants containing GSL

Most GSL-containing genera, are within the Brassicaceae, Capparaceae and Caricaceae families (Rodman, 1981). The Family Brassicacea (brassicas) contains more than 350 genera with 3000 species, of which many are known to contain GSL. However, GSLs are not limited to brassicas alone. At least 500 species of non-brassica dicotyledonous angiosperms have also been reported to contain one or more of the over 120 known GSLs (Fahey *et al*., 2001). The GSL concentration in the cells of the various plants in the families differs substantially. Therefore, it is important to identify species that will be effective in suppressing soil-borne pests and diseases, including nematodes. The plant species that generally are considered for biofumigation are found mostly in the family Brassicaceae, and include *Brassica oleracea* (broccoli, cabbage, cauliflower, kale), *Brassica rapa* (turnip), *Raphanus sativus* (radish), *Brassica napus* (canola, rapeseed), cv. AV Jade, *Eruca sativa* (salad rocket, arugula), cv. Nemat, , *B. juncea* (Indian mustard) cv. Caliente 199, and various mustards, such as *Sinapis alba* (white mustard) cv. Braco (Sarwar *et al*., 1998; Zasada and Ferris, 2004; Hartz *et al*., 2005; Everts *et al*. 2006; Melakeberhan *et al*., 2006; Roubtsova *et al*. 2007; Ploeg, 2007; Monfort *et al*., 2007; Lopez-Perez *et al*., 2010; Kago *et al*. 2013; Edwards and Ploeg, 2014).

Kwerepe and Labuschagne (2003) found that cruciferous residues at 60 kg/ha caused a higher reduction of *M.incognita*. Youssef and Lashein (2013) reported that crushed cabbage leaves (*Brassica oleracea*) incorporated into the soil at 5 g per pot, 10 days before transplanting of tomato cv. Super Strain B under greenhouse conditions reduce root-knot nematode population.

A thorough distribution of the plant tissue prior to soil incorporation and sufficient soil moisture at the time of tissue incorporation is important (Brown *et al*., 1991; Poulton & Moller, 1993; Morra & Kirkegaard, 2002; Matthiessen *et al*., 2004). This may be explained by quick decomposition of the tested residue in soil on the basis that nematicidal activity by nitrogenous by products depends on the C: N ratio of the amendment (Stirling, 1991). One way to ensure the effective release of ITC is to slash the leaves with a slasher and then to plough the slashed residues into the soil as soon as possible, using a rotavator or disc harrows. A flail chopper ensures the best maceration results

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and, consequently, a good GL-MYS interaction for the release of ITC. The latter technique remains applicable particularly for the *Brassica* spp. such as mustards, which have a high GSL concentration in the above-ground parts of the plant.

The growth stage of the crop (emergence, rosette, flowering, seed filling, ripening), the amount of biomass produced and the correct incorporation into the soil all contribute towards the success of biofumigation (Bellostas *et al*., 2004).The flowering stage of the plant maintains a higher GSL content than the vegetative plant parts. The GL-MYS interaction can be expected to take place more effectively later in the growing season, prior to seed set. In the root tissue, the concentration of GSL is higher in the earlier root growth stage, with decreasing concentrations during the root growth cycle. Different types of GSLs are present in the roots and shoots of different plant species (Van Dam *et al*., 2009). Studies that were conducted by Van Dam *et al*. (2009), in which the root and shoot GSL of 29 plant species were evaluated for their GSL concentration and profiles, showed that the roots had a higher GSL concentration, as well as more diversity than the shoots. The root and shoot concentration of specific GSLs was found to differ from one another, with the most prominent indole GSL in the shoots being 1H-indol-3-yl GSL, and with the roots having higher concentrations of aromatic 2-phenylethyl GSL.

The inclusion of sulphur fertilizers may improve the nutritional value of *Brassica* spp. Sulphur forms part of the process that takes place in the formation of secondary metabolites. The level of GSLs is dependent on the genetic factors of the plant, but can also vary according to environmental conditions and the availability of soil sulphur (De Pascale *et al*., 2007).

3.2. Soil temperature

Lopez- Perez *et al*. (2005) used some plant residues of broccoli, melon, and tomato with addition of chicken manure in pot experiments with *Meloidogyne incognita* infested soils and was observed that biofumigation to control *M. incognita* is unlikely to be effective under cool conditions but that at soil temperatures around 25ºC, broccoli is more effective than melon and tomato, and that the addition of chicken manure at this soil temperature may enhance the efficacy.This corresponds with earlier results by Ploeg and Stapleton (2001) and with recommendations by Bello *et al*.(2004). Low soil temperature slows down the enzymatic reaction during biofumigation, and therefore incorporation of green manure is not recommended at soil temperatures close to 0°C. The presence of organic matter seems to have an immobilizing effect on the degradation products, thus preventing them from reaching the target pests.

3.3. Soil depth

Roubtsova *et al*. (2007) studied the direct localized and indirect volatile effects of amending soil with broccoli tissue on *M. incognita* infested soil. Amending a 10cm layer lowered *M. incognita* than in the non-amended layers of the tubes by 31 to 71%, probably due to a nematicidal effect of released volatiles of broccoli. These results suggest that the fumigant nematicidal activity is limited and its effect requires a thorough and even distribution of the biofumigant material through the soil profile where the target nematodes occur.

Furthermore, the concentration of ITCs produced is also influenced by soil texture, pH, and microbial community (Bending & Lincoln, 1999; Price, 1999; Morra & Kirkegaard, 2002; Bellostas *et al*., 2004; Griffiths *et al*.,2011).

IV. BIOFUMIGATION IN INTEGRATED PEST MANAGEMENT (IPM)

Biofumigation is a definite choice as part of an integrated approach for nematode management and can be implemented as a biological alternative or in combination with certain chemical options. This will reduce the demands on chemical nematicide use. The positive biological activity of the GSL degradation products used for the suppression of some pathogenic fungi (Manici *et al*., 1997) and nematodes (Lazzeri *et al*., 1993) serves as an integral part of IPM (Lazzeri *et al*., 2004), because it has been proven to be effective against weeds, pathogenic fungi and nematodes (Van Dam *et al*., 2009). In addition to providing some disease control, growing and incorporating the biofumigant plant improves soil structure, assists in weed control, reduces soil erosion and provides organic matter to the organic producer for controlling diseases and pests (Griffiths *et al*., 2011).The potential for Brassicaceous amendment as part of an IPM approach consists of the role of the active compounds, in the direct suppression of nematodes, and also the secondary effect in the soil. The secondary effect plays a very significant part in promoting microbial and other microorganism diversity in the soil, and therefore can be expected to have a positive impact on the stimulation of competition among soil-borne diseases in the rhizosphere.

V. MANAGEMENT OF PLANT-PARASITIC NEMATODES

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Many *Brassica* spp. show nematicidal activity on plant-parasitic nematode species such as *M. incognita, M. javanica, Heterodera schachtii* and *Pratylenchus neglectus, C. xenoplax* and *Xiphinema* spp. (Thierfelder & Friedt, 1995; Potter *et al*., 1998; Riga & Collins, 2004;Monfort *et al*., 2007). A liquid formulation has also been developed from defatted *B. carinata* seed meal which has activity against *M. incognita* (De Nicola *et al*., 2013).

VI. CONCLUSION

Soil disinfestation is a major approach against soil borne micro-organisms. The practical value of using biofumigant crops to the farmers should be accessed through several factors which include extent of pesticide efficacy, effect on crop growth and yield as well as cost of production. The benefits of using biofumigant crops and agronomic practices in improving sustainable agricultural production require further exploitation of GSL and ITC to realize the goal of sustainable production with minimal environmental impacts.

REFERENCES

[1] Abad, P., Gouzy, J., Aury, M.J., Castagnone Sereno, P., and Danchin, E.G. 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology* **26(8)**: 909-915.

[2] Angus, J.F., Gardner, A., Kirkegaard, J.A.,and Desmarchelier, J.M. 1994. Biofumigation: Isothiocyanates released from brassica roots inhibit growth of the take-all fungus. *Plant and Soil* **162 (1)**: 107-112.

[3] Bello, A., Lopez Perez, J. A., Garcia Alvarez, A., Sanz, R., and Lacasa, A. 2004. Biofumigation and nematode control in the Mediterranean region. p. 133-149. In R. C. Cook and D. J. Hunt (Eds.). Nematology monographs and perspectives Vol. 2. Leiden and Boston: Brill.

[4] Bello, A., Lopez Perez, J.A., and Diaz Veruliche, L. 2000. Biofumigacion y solarizacion como alternativas al bromuro metilo. p. 25-50. In J. Z. Castellanos and F. Guerra O'Hart. (eds.) Memorias del Simposium Internacional de la Fresa. Zamora, Mexico.

[5] Bello, A., Lopez Perez, J.A., Sanz, R., Escuer, M., and Herrero, J. 2000. Biofumigation and organic amendments. p. 113-141. In Regional workshop on methyl bromide alternatives for North Africa and Southern European countries. Paris, France: United Nations Environment Program.

[6] Bellostas, N., Sorensen, J.C. and Sorensen, H. 2004. Qualitative and quantitative evaluation of glucosinolates in cruciferous plants during their life cycles. *Agroindustria* **4**: 267-272.

[7] Bending, G. D. and Lincoln, S. D. 1999. Characterisation of volatile sulphur-containing compounds produced during decomposition of *Brassica juncea* tissues in soil. *Soil Biology and Biochemistry* **31**:695-703

[8] Brown, P.D., Morra, M.J., McCaffrey, J.P., Auld, D.L., and Williams, L. 1991. Allelochemicals produced during glucosinolate degradation in soil. *Journal of Chemical Ecology* **17**:2021-2034.

[9] Challenger, F. 1959. Aspects of the organic chemistry of sulphur. Chapter V, p.162-206. Butterworths, London.

[10] Chew, F.S. 1988. Biological effects of 4 glucosinolates. p. 155-181. In H.G.Gutler, (ed.) Biologically active natural products: Potential use in agriculture Washington, DC: American Chemical Society.

[11] Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. *Annual Review of Phytopathology* **40**: 221-249.

[12] Cole, R. A. 1976. Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in Cruciferae. *Phytochemistry* **15**: 759-762.

[13] Cox, C. 2006. Fumigant factsheet: metam sodium. *Journal of Pesticide Reform* **26**: 12-16.

[14] De Pascale, S., Maggio, A., Pernice, R., Fogliano, V. and Barbieri, G. 2007. Sulphur fertilization may improve the nutritional value of *Brassica rapa* L. subsp. *sylvestris*. *European Journal of Agronomy* **26**: 418-424.

[15] DeNicola, G.R., D'Avino, L., Curto, G., Malaguti, L., Ugolini, L., Cinti, S., Patalano, G., Lazzeri, L.2013. A new biobased liquid formulation with biofumigant and fertilizing properties for drip irrigation distribution. *Industrial crops and products*.**42**:113-118.

[16] Edwards, S. and A. Ploeg. 2014. Evaluation of 31 Potential biofumigant Brassicaceous plants as hosts for three *Meloiodogyne* species. *Journal of Nematology* **46**: 287-295

[17] Everts, K.L., Sardanelli, S. , Kratochvil, R.J. , Armentrout, D.K. and Gallagher, L.E. 2006. Root-knot and root lesion nematode suppression by cover crops, poultry litter, and poultry litter compost. *Plant Disease* **90**: 487-492.

[18] Fahey, J.W., Zalcmann, A.T. and Talalay, P. 2001. The chemical diversity and distribution of glucosinolates

and isothiocyanates among plants. *Phytochemistry* 56: 5-51.

[19] Fenwick, G.R., Heaney, R.K., Mullin, W.J., 1983. Glucosinolates and their breakdown products in food and food plants. *Critical Reviews in Food Science and Nutrition* **18**: 123-201.

[20] Griffiths, H.M., Gies, D., Zitter, T.A. 2011. Brassicas as biofumigants for controlling soilborne organisms in potato production for upstate New York and northern Pennsylvania. Fact sheet (Online). (http :// vegetablemdonline. ppath. cornell. edu/ NewsArticles/ Brassicas% 20Factsheet%20Final%20August%20, 2011.pdf).

[21] Halberendt, J.M. 1996. Allelopathy in the management of plant-parasitic nematodes. *Journal of Nematology* **28 (1)**: 8-14.

[22] Hartz, T.K., Johnstone, P.R. , Miyao, E.M., and Davis, R.M. 2005. Mustard cover crops are ineffective in suppressing soil-borne disease or improving processing tomato yield. *Hortscience* **40**: 2016-2019

[23] Kago, E.K., Kinyua, Z.M., Okemo, P.O., and Maingi, J.M. 2013. Efficacy of *Brassica* Tissue and Chalim TM on Control of Plant Parasitic Nematodes. *Journal of Biology* **1**: 32-38

[24] Kirkegaard, J. A., and Sarwar, M. 1998. Biofumigation potential of Brassicas. I. Variation in glucosinolate profiles of diverse field grown Brassicas. *Plant and Soil* **201**:71-89.

[25] Kirkegaard, J., and Matthiessen, J. 2004. Developing and refining the biofumigation concept. *Agroindustria* **3**: 233-239.

[26] Kirkegaard, J.A., Gardner, P.A., Desmarchelier, J.M. and Angus, J.F. 1993. Biofumigation using Brassica species to control pests and diseases in horticulture and agriculture. p. 77-82. In Wratten, N. and Mailer, R.J. (eds.). Proc. 9th Australian Research Assembly on Brassicas. Agricultural Research Institute, Wagga.

[27] Knoblock, K., Weis, N. and Weigant, R. 1989. Mechanism of antimicrobial activity of essential oils. p. 5-9. 37th Annual Congress Medicinal Plant Research. Braunschweig.

[28] Kwerepe, B.C.,and Labuschagne, N. 2003. Biofumigation and soil solarization as integrated pest management (IPM) components for the control of root-knotnematode, *Meloidogyne incognita* (Kofoid and White) Chitwood on Bambara groundnut [Vigna subterranea (L.) Verdc]. University of Swaziland. *Journal of Agriculture* **11**: 56-63.

[29] Lazzeri, L., Curto, G., Leoni, O. and Dalla Valle, E. 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. *Journal of Agriculture and Food Chemistry* **52**: 6703-6707.

[30] Lazzeri, L., Tacconi, R. and Palmieri, S. 1993. *In vitro* activity of some glucosinolates and their reaction products toward a population of nematode *Heterodera schachtii*. *Journal of Agriculture and Food Chemistry* **41**: 825-829.

[31] Lopez Perez, J., Roubtsova, T. and Ploeg, A. 2005. Effect of three plant residues and chicken manure used as biofumigants at three temperatures on *Meloidogyne incognita* infestation of tomato in greenhouse experiments. *Journal of Nematology* **37 (4):** 489-494.

[32] Lopez Perez, J.A., Roubtsova, T., Garcia, M.D., and Ploeg, A. 2010. The Potential of five winter grown crops to reduce root-knot nematode damage and increase yield of tomato. *Journal of Nematology* **42**: 120-127.

[33] Manici, L. A., Lazzeri, L., Baruzzi, G., Leoni, O., Galletti, S., and Palmieri, S. 2000. Suppressive activity of some glucosinolate enzyme degradation products on *Pythium irregulare and Rhizoctonia solani* in sterile soil. *Pest Management Science* **56**: 921-926.

[34] Manici, L.M., Lazzeri, L. and Palmieri, S. 1997. *In vitro* fungitoxic activity of some glucosinolates and their enzyme derived products toward plant pathogenic fungi. *Journal of Agriculture and Food Chemistry* **45**: 2768-2773.

[35] Matthiessen, J.N., Warton, B. and Schackleton, M.A., 2004. The importance of plant maceration and water addition in achieving high brassica-derived isothiocyanate levels in soil. *Agroindustria* 3: 277-280.

[36] Matthiessen, J.N.,and Kirkegaard, J.A., 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Critical Reviews in Plant Sciences* **25**: 235-65.

[37] Melakeberhan, H., Xu, A, Kravchenko, A., Mennan, S. and Riga, E. 2006. Potential use of arugula (*Eruca sativa* L.) as a trap crop for *Meloidogyne hapla*. *Journal of Nematology* **8**: 793-799.

[38] Monfort, W.S., Csinos, A.S., Desaeger, J., Seebold, K., Webster, T.M. and Diaz-Perez, J.C. 2007. Evaluating brassica species as an alternative control measure for root-knot nematode (*M. incognita*) in Georgia

vegetable plasticulture. *Crop Protection* **26**: 1359-1368.

[39] Morra, M.J. and Kirkegaard, J.A. 2002. Isothiocyanate release from soil-incorporated Brassica tissues. *Soil Biology and Biochemistry* **34**: 1683-1690.

[40] Padilla, G., Cartea, M.E., Velasco, P., De Haro, A. and Ordas, A. 2007. Variation of glucosinolates in vegetable crops of *Brassica rapa*. *Phytochemistry* **68**: 536-545.

[41] Piedra Buena, A., Garcia Alvarez, A., Dies Rojo, M.A., Ros, C., Fernadez, P., Lacasa, A. and Bello, A. 2007. Use of pepper crop residues for the control of root-knot nematodes. *Bioresource Technology* **98**:2846-2851.

[42] Piedra Buena, A., Garcia Alvarez, A., Diez Rojo, M.A. and Bello, A. 2006. Use of cover crop residues for the control of *Meloidogyne incognita* under laboratory conditions. *Pest Management Science* **62**: 919-926.

[43] Ploeg, A.T., 2008. Biofumigation to manage plant-parasitic nematodes. p. 239-248. In Ciancio, A. and K.G. Mukerji (eds.).Integrated Management and Biocontrol of Vegetable and Grain Crops Nematodes. Springer-Verlag, Berlin.

[44] Ploeg, A.T., and Stapleton, J.J. 2001. Glasshouse studies on the effect of time, temperature and amendment of soil with broccoli plant residues on the infestation of melon plants by *Meloidogyne incognita* and *M. javanica*. *Nematology* 3: 855-861.

[45] Potter, M.J., Davies, K. and Rathjen, A.J. 1998. Supressive impact of glucosinolates in Brassica vegetative tissues on root lesion nematode, *Pratylenchus neglectus*. *Journal of Chemical Ecology* **24**: 67-80.

[46] Poulton, J.E., and Moller, B.L. 1993. Glucosinolates. p. 209-237. In Lea, P.J. (ed). Methods in plant biochemistry, vol. 9. PJ Academic Press, London.

[47] Price, A. 1999. Quantification of volatile compounds produced during simulated biofumigation utilizing Indian mustard degrading in soil under different environmental conditions, MS Thesis. University of Tennessee, Knoxville.

[48] Riga, E. 2011. The effects of *Brassica* green manures on plant parasitic and free living nematodes used in combination with reduced rates of synthetic nematicides. *Journal of Nematology* **43**: 119-121.

[49] Riga, E., and Collins, H. 2004. Green manure effects on *Meloidogyne chitwoodi* and *Paratrichodorus allius*, economically important nematodes in the Pacific Northwest of the USA. *Agroindustria* **3**: 321-322.

[50] Riga, E., Mojtahedi, H., Ingram, R., and McGuire, A.M. 2004. Green manure amendments and management of root knot nematodes on potato in the Pacific Northwest of USA. p. 151-158. In Cook, R.C. and Hunt, D.J. (eds). Nematology monographs and perspectives. Proceedings of 4th Congress of Nematology.

[51] Rodman, J.E., 1981. Divergence, convergence and parallelism in phytochemical characters: the glucosinolate-myrosinase system. p. 43-79. In Young, D.A. and Seigler, D.S. (eds). Phytochemistry and Angiosperm phylogeny. Praeger, New York.

[52] Rosa, E. A. S., Heaney, R. K., Fenwich, G. R., and Portas, C. A. M. 1997. Glucosinolates in crop plants. *Horticultural Reviews* **19**: 99-215

[53] Roubtsova, T., Lopez Perez, J., Edwards, S. and Ploeg, A. 2007. Effect of Broccoli (*Brassica oleracea*) tissue, incorporated at different depths in a soil column, on *Meloidogyne incognita*. *Journal of Nematology* **39**: 111-117.

[54] Salem, M. F. 2012. The Potential of biofumigation in solving air pollution in developing coutries. In D.G. Steyn and S. Trini Castelli (eds.). Air Pollution Modeling and its Application XXI, NATO Science for Peace and Security Series C: Environmental Security 4, Springer Science Business Media B.V.

[55] Salem, M. F.and Mahdy, M. E. 2015. Suppression of root-knot nematode through innovative mustard biofumigation. *Future of Food: Journal on Food, Agriculture and Society* **3(2)**: 41-50

[56] Sang, J. P., Minchinton, I. R., Johnstone, P. K., and Truscott, R. J. W. 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. *Canadian Journal of Plant Science* **64**: 77-93.

[57] Sarwar, M., Kirkegaard, J.A., Wong, P.T.W. and Desmarchelier, J.M. 1998. Biofumigation potential of brassicas. III. In vitro toxicity of isothiocyanates to soil-borne fungal pathogens. *Plant and Soil* **201**: 103-112.

[58] Schreiner, R.P., Ivors, K.L. and Pinkerton, J.N. 2001. Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression. *Mycorrhiza* **11**: 273-277.

[59] Stirling, G.R. 1991. Biological Control of Plant Parasitic Nematodes. Progress, Problems and Prospects. 282 p. Wallingford, UK: CAB International.

[60] Thierfelder, A. and Friedt, W. 1995. Development of novel rapeseed varieties (*Brassica napus*) resistant against beet cyst nematodes (*Heterodera schachtii*). Ninth Int. Rapeseed Cong. (GCIRC).Organising Committee of the Ninth Internatioal Rapeseed Conference Cambridge, UK, pp. 1208-1210.

[61] Underhill, E. W. 1980. Glucosinolates. p. 493-511. In E. A. Bell and B. V. Charlwood (Eds.) *Secondary plant products. Encyclopedia of Plant Physiology*, New Series .Vol. 8. Berlin: Springer-Verlag.

[62] Van Dam, N., Tytgat, T.G., and Kirkegaard, J. 2009. Root and shoot glucosinolates: a comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochemistry Reviews* **8**: 171-86.

[63] Wathelet, J., Lori, R., Leoni, O., Rollin, P., Quinsac, A. and Palmieri, S. 2004. Guidelines for glucosinolate analysis in green tissues used for biofumigation. *Agroindustria* **3**: 257-266.

[64] Youssef, M.M.A.,and Lashein, A.M.S. 2013. Effect of cabbage (*Brassica oleracea*) leaf residue as a biofumigant on root knot nematode, *Meloidogyne incognita* infecting tomato. *Journal of Plant Protection Research* **53**:271-274.

[65] Zasada, I.A., and H. Ferris, 2004. Nematode suppression with brassicaceous amendments: application based upon glucosinolate profiles. *Soil Biology and Biochemistry* **36**: 1017-1024

[66] Zasada, I.A., Halbrendt, J.M., Kokalis Burelle, N., LaMondia, J., McKenry, M.V., Noling, J.W. 2010. Managing nematodes without methyl bromide. *Annual Review of Phytopathology* **48**:311-328.

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Effect of Aloe Vera wastes on physico-chemical properties and microbiological activity in soils

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Abstract*—The aim of the present study was to explore the potential for using aloe vera wastes as amendment for soil to improve its fertility.*

Soil was exposed to four concentrations of aloin (rich in HAP) for 0, 7, 14 and 28 days. Physico-chemical parameters were analyzed: soil Ph, organic matter (OM), nitrogen, phosphorus, and cation exchange capacity (CEC). The activity of seven enzymes implicated in the C, N and S cycles were measured. Microbial Biomass was determined by the method of substrate induced respiration. BiologEcoplates (Biolog Inc., Hayward, CA) were used to estimate soil microbial functional diversity.

Our findings suggested a decrease on phosphorus and nitrogen content and an increase on CEC after aloin addition. Also, a decrease on microbial biomass and enzymes activities was observed, except for FDA. Ecoplates results demonstrate a decrease on microbial activities depending on the incubation time. Moreover, our results indicated that bacterial communities of the tested soils have more affinity to consume substrates as Amino acids and polymers.

Our results should be carefully considered in view of the agriculture waists reuse for a sustainable agriculture

Keywords*— *Aloe vera, aloin, microbial communities, enzymes activities, Ecoplates.

I. INTRODUCTION

The addition of residues as by-products of crop production is a common practice in agriculture. Indeed, previous studies have demonstrated that they support farm productivity, reduce soil degradation, and improve nutrient cycling in the agroecosystem. It has also been reported that crop residues improve soil structure and soil protection by reducing erosion [1,2], and increasing the stock of plant nutrients and soil organic matter content, thus enhancing soil fertility [3,4] and crop yields [5].

Aloe Vera belongs to Liliaceae family is considered as a perennial succulent plant. It produced secondary metabolites with numerous properties such as antibacterial, anti-inflammatory and antioxidant, [6,7]. Actually, the industry of aloe vera is in continuous expansion in Tunisia generating many wastes which are presenting a real problem. For this reason, aloe vera wastes management constitute an opportunity both to valorize these wastes and in the same time to improve soil fertility. However, information's about the magnitude and the effects caused by aloe vera wastes on soil microbial community, functionalities and diversity is still scarce.

Among toxic substances contained in aloin, polycyclic aromatic hydrocarbons (PAHs) are known by their harmful effect once in the ecosystem; they are one of the most common groups with known or potential toxic properties [8]. PAHs are a substantial threat to ecological function and soil biodiversity [9].The response of microbial communities to chronic inputs of PAHs [10,11,12] has received little attention compared to the effect of acute contaminations [13,14,15]. Indeed, previous studies demonstrated that PAH change bacterial communities structure and diversity [16,17,18].

Soil microorganisms are measured using the C and N content in the microbial biomass (MBC and MBN). It represents collectively the mass of all soil microorganisms, considered as a single soil organic matter fraction [19]. Among microbial indicators, community-level physiological profiles (CLPP), which are assessed using BiologEcoPlates™, allow for the detection of multiple microbial metabolic activities. The Biolog™ system has been adapted to the investigate the functional diversity of soil microbial communities [20,21].On the other hand, there is also growing interested in using soil enzymes as potential indicators of soil fertility, since enzyme activities a sensitive to numerous factors such as climate, type of amendment, agricultural techniques, crop type and edaphic properties [22,23,24].In addition, due to their importance for the soil andtheir rapid response to soil perturbations, soil enzymes areconsidered as indicators of soil quality [25,26,27,28].Indeed, soil enzyme activities such as arylsulphatase, β-glucosidase acid and alkaline

phosphatase, urease, anddeshydrogenase are sensitive to the presence of pollutant [29,30,31].

The fast expanding aloe vera industry in Tunisia urgently needs more information on the effect of industrial releases (containing the toxic substance aloin) and their repercussions on the ecosystem. Little is known about the impact of increased aloin reject on soil quality and fertility. The present work was conducted to assess the effect of Aloe vera wastes on microbiological and physico-chemical properties of soils in order to valorize them in sustainable agriculture.

II. MATERIAL AND METHODS

2-1- Experimental protocol

2-1-1 Soil samples

The soils used for this research were collected from an organic farming plot in the region of ChottMariem. The soils were sampled from the depth of 0-15 cm. The chemical and physical properties of these soils are presented in table 1. Before use, samples were air-dried and crushed to pass a (<2 mm) screen.

2-1-2 Extraction of aloin

Leaves were sampled from the mature plants of Aloe vera (var. barbadensis) (age between three and five years). The extraction of aloin was done in three steps (figure 1): First, the leaves were washed with water,then rinds were removed, and finally, yellow exudate (aloe latex) was collected from the leaves after cutting.

2-1-3 Earthworms Eisenia Andrei

E. Andrei earthworms [32] were cultured as described in the OECD guidelines [33]. Organisms were selected from a synchronized culture with a homogeneous age structure. Adult worms with clitellum of similar size and weight (400-500 mg) were utilized in the experiments.

2.1-4 Soil contamination and earthworm's exposure

Sampled soils were dried and sieved (<2 mm), then 500 g were placed in polyethylene pots. In this experience, we choose to work on five different concentrations of aloe exudates: C1: 1 %, C2: 5%, C3: 10% and C4: 20% in addition to the control. These concentrations are relative to the weight of the soil. For each concentration, three periods of exposition: 0, 7, 14, and 28 were realized.

At the end of the exposure period, the soil of each pot was homogenized. One part was conserved at 4°C for the determination of enzymatic activities and the functional diversity, and the other part was conserved at ambient temperature for the assessment of microbial biomass and the different physico-chemicals analyses .

2-2- Physico-chimical analyses

Soil pH was measured in soil suspension obtained by shaking 1 g of soil in water (soil/H2O ratio 1:2.5) for 1 hour and then by using a pH meter (MetrOhm 744). For organic carbon analysis, 25 mg of soil crushed at 250 lm were decarbonated with hydrochloric acid and then analyzed with a CHN analyzer according ISO 10694 procedure. Organic matter content was calculated by multiplying organic carbon concentration by 1.72 [34]. Mg was determined digestion using the Hossner method [35].

Nitrogen mineralization was determined by measuring the production of mineral N (NH4+and NO3-) during incubation. measurements were made according to the extraction protocol of Li et al., 2009 and with the use of Spectroquant® kit tests (Merck) according to the supplier's recommendations.

For the measurement of NH4+, 10 g soil sample (dry weight equivalent) was shaken with 50 ml of KCl (2.0 M) for 30 min. Filtration was performed with a PES polyethersulfone filter (0.45)after centrifugation for 15 min at 3500 g. Then the use of kit tests ((Spectroquant®, Merck) Well absorbance (690 nm) was measured with a BioTek EL800 Universal plate reader (Bio-Tek Instruments, Winooski, VT).

For measurement of NO3-, soil samples of 10 g weighed after the 7 day incubation were shaked with 50 ml of CuSO4 extraction solution (0.01 M) for 30 min. Filtration was performed with a PES polyethersulfone filter (0.45) after centrifugation at 3500 g for 15 min. Then we used the kit (Spectroquant®, Merck) and finally the NO3- was measured with a spectrophotometer at 493 nm.

The total organic N mineralization was estimated by the sum of the ammonification and nitrification rates.

Phosphorus mineralization was determined by an incubation procedure similar to nitrogen mineralization. The mineralisation of inorganic P was extracted with 0.5M NaHCO3. Then, we use the kit (Spectroquant®, Merck) and finally the Phosphate was measured with a spectrophotometer at 885 nm.

According to [36]'s method, the cation exchange capacity will be measured with 2.5 g of sol was shaked with 30 ml of Bacl2 solution (0.1M) for 1h then centrifuged at 3000g for 10min. The supernatant liquid was filtered at 0.45 µm and then used for the determination of the content of soduim, potasium, calcium and magnesium in ICP-AES.

2-3 Microbiological analyses

2-3-1 Microbial Biomass determination by the method of Substrate Induced Respiration (SIR)

Soil respiration was measured in samples at 50% water-holding capacity using the method of [37]. Fresh soil equivalent to 10 g dry soil was weighed intoa plastic beaker and supplemented with 10 mg of glucose which corresponded to the amount of glucose required for obtaining a maximum CO2 flush. The CO2 production rate was measured hourly during one day, using an automated IR gas analyzer system (490 MicroGC Agilent). Microbial biomass carbon was expressed as µg carbon per g soil [38].

2-3-2 Enzymes activities assay

Arylsulfatase, β-Glucosidase and alkaline-acid phosphatases activity assays were all based on p-nitrophenol release, after cleavage of a synthetic substrate (p-nitrophenyl sulfate and nitrophenylglucpyranosoide respectively). Arylsulfatase and β-glucosidase activities were assayed as described by [39]. Alkaline phosphatase and acid phosphatase activities were assayed as described by [40]. Microplate wells were loaded with 50 µl of a 1:10 soil distilled water solution, 25 µl phosphate buffer and 50 µl of the appropriate substrate (71.9 mmol L-1). Microplates were incubated for one hour at 37° C. At the end of the incubation, after added 125µl 2% Na2CO3 the microplates were centrifuged (14,000g for 5 min) and 50 µl of the supernatant transferred to a second microplate containing 250 ml 2% Na2CO3 to stop the enzymatic reaction. Well absorbance (410 nm) was measured with a BioTek EL800 Universal plate reader (Bio-Tek Instruments, Winooski, VT). The enzyme activity was expressed as the quantity of p-nitrophenolµg PNP released g−1 soil h−1.

Deshydrogenase activity was assayed using soil (6 g), incubated with triphenyl tetrazolium chloride (3%) for 96 h in the dark. Methanol was added to terminate the enzymatic reaction. The supernatant was filtered and the absorbance was taken at 485 nm [41]. The values were expressed as µg of triphenyl formazan (TPF) g−1soil h−1.

Urease (EC 3.5.1.5) activity was assayed as described by [42,43,44]. Soil (1.0 g) was weighted into screw-top test tube containing 0.5 mL of urea (0.02 M) and 4 mL of borate buffer (0.05 M, pH 10.0). After incubation at 37 °C for 4 h, the reaction was stopped by addition of 3 mL of KCl(2M) and the suspension was mixed for 30 min and centrifuged at 13000 rpm for 5 min.5 mL of solution containing sodium salicylate, nitroprussate, NaOH (0.3 M) and Na-dichloroisocyanide were added to the 1 ml of the surnageant. Finally, after agitation at 120 rpm in the dark for 30 min, the absorbance was measured at 660 nm and the enzyme activity was expressed as mg N-NH4+g−1 soil h−1.

The total enzymatic activity was measured using the fluorescein diacetate hydrolysis assay (FDA) [45]. Microplate wells were prepared with 100µl of simples and were incubated at 37 °C for 2 h with 50µl of phosphate buffer Mac Ilvain at PH (7,6 and 8,1) and 25 µl of 4.8 mM FDA solution. The suspension was centrifuged at 13,000 g at 4 °C for 3 min and 100 µl of supernatant was taken. The reaction was stopped by adding 100 µL of acetone. The absorbance was measured at 490 nm and the amount of FDA hydrolyzed was expressed as µg fluorescein g−1soil h−1.

All measurements were made at ambient soil PH, wells with soil and buffer but without substrate were used as blanks. The assays were conducted in triplicate thus ensuring the reproducibility of the laboratory analyses.

2-3-3- Functional diversity: BiologEcoplate assay

BiologEcoplates (Biolog Inc., Hayward, CA) were used to estimate soil microbial functional diversity based on utilization of 31 different substrates [46,47,48]

Wet soils (1g) were added in steril condition to 9 ml distilled water 0.85% NaCl and shaked one hour.Then the suspension was centrifuged for 5 min at 1300rpm to remove soil particles.Then the supernatant with bacteria was diluted ten fold in 0,85%NaCl distilled water, and used to inoculate BiologEcoplates with 150 µLper well. Three replicates per treatment were performed. The plates were incubated at 25 ◦C in darkness and the absorbance at 570 nm was measured every 24 h for seven days and was used to calculate three factor of the functional diversity indices. Absorbance values were blanked against the control and the first factor the average well color development AWCD=Σ (C−R)/N was calculated where C is color production with each well, R is the absorbance value of the plate's blank well, and N is the number of substrates (ECO plates, N=31), then the second factor is the substrate richness which represents the percentage of positive well (absorbance > 250 nm). The third factor is the substrate evenness where the functional diversity was calculated and classified by six different substrates family according to [49].

2-4 Statistical analyses

Resultsare presented as mean ± SD of 3 samples. R software was used for all the statistical analysis in this paper. The normality of the distribution was tested using the Shapiro– Wilk test. For multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data along with Tukey's test..

III. RESULTS

3-1-Effect of aloin on soil physico-chemical properties

Soil pH was significantly higher in soils amended with C4 in all tested conditions compared to control soil and the other aloin concentrations. Moreover soil pH increased after 7 and 14 days of incubation and decreased after 28 days for all tested concentrations, except for C 1 one (figure 2). No significant difference of organic matter (figure 3) between the different tested concentrations was observed. However, after 28 days, organic matter decreased in all soils, compared to the starting condition.

Nitrogen content (table 1) of soil following aloin addition was the highest with the application of C1 and C2 where means were respectively 15,41±3,34 and 13,51±1,91 mg/g. After 28 days, nitrogen content decreased in all soils. The most important decrease was observed for C1 (50 %).

Phosphorus content in soil in presence of aloin (table 1) was the highest when C2 was applied. After 28 days of

incubation, phosphorus content decrease in all conditions, except for C3. The most important decrease was noted in soils with C1 where means reached 0,09±0,03 mg/g.

Cation exchange capacity (table 1) was initially higher in soils with aloin, in comparison to control one. After 28 days, CEC increases in all soils. The most important increase was observed in the case of C3 and C4 where values reached respectively 18,97±0,24 ppm and 18,72±0,44 ppm.

3-2-Effect of aloin on soil microbiological activity

3-2-1-Substrate induced respiration

The incubation of soils with aloin resulted on a significant decrease along the incubation time in all the tested conditions, except for C3. The highest microbial biomass was found with the addition of the C4 aloin concentration where the value reached in the first day of incubation 5655,75 ± 1010,49 µg carbon g-1 soil (Table 2). Moreover, the most important decrease was observed in the case of soil incubated with C4 where microbial biomass reached 37384,1±544,479 µg carbon g-1 soil after 28 days of incubation (table 2).

3- 2-2 Soil enzymes activities

The response of soil enzymes under the effect of aloin incorporation is presented in figure 4. B-glucosidase activity was higher in soils amended with aloin, compared to control soil. The most significant value was observed in the case of C4 initially where the value reached 250,69±38,16PNP g−1 h−1. However, the activity decreased following the incubation time for all the aloin concentration.

The alkaline phosphatase was highest initially with the application of C4 concentration where value was 120,41 ±13,25PNP g-1 h-1. Along the incubation time, the enzyme activity had the same trend with the application of C1 and C2 where alkaline phosphatase decreased, contrary to C3 and C4 where enzyme activity increased after 28 days of aloin addition.

Acid phosphatase activity was higher initially (0 days) with the application of C4 where value was 48,08 ± 7,001PNP g-1 h-1. However, the enzyme activity decreased along the incubation time and reached 12,73±0,85PNP g-1 h-1 after 28 days of aloin incorporation. The same trend was observed for C3 despite an increase observed after 7 days. For C1 and C2, an increase was observed on acid phosphatase activity after 28 days of aloin addition.

The activity of arylsulphatase was highest initially with application of C4 where value was 148,97± 32,54 µmol PNP.g $_{dry\ soil}^{-1}$.h^{-1}. However, the enzymatic activity decrease with the incubation time. The same trend was observed in soils amended with all aloin concentrations.

Urease activity was the highest initially in the case of the application of C3 where value was 0,005 ± 0,0001µg NH4+g-1 h-1. However, the maximum of urease activity was observed with C4 after 7 days of incubation where value reached 0,006 ± 0,0003µg NH4+g-1 h-1. Moreover, urease activity increased along the incubation time and reached the maximum after 28 days of aloin addition for all the concentrations applicated except C4 one.

The dehydrogenase activity was the highest initially in the case of C2 and C3 concentration where values were respectively 2,179 ±0,05 TPF g−1 h−1 and 2,242 ±0,014 TPF g−1h−1 . However, the maximum of the enzyme activity was noted with C4 after 7 days of incubation where mean reached 3,124±0,39 TPF g−1h−1. Moreover, dehydrogenase activity decreased along the incubation for all the concentrations.

The FDA activity was more important with the increase of the aloin concentration. However, the enzymatic activity decreased within the incubation time. This decrease was noted especially with C1 where the enzymatic activity reached 7,84 ± 0,51µg de TPF/g sol/d.

3-3 Functional diversity: (Biolog)

The metabolic activity determined as AWCD (table 3) was the maximal at the starting point in soils amended with C3 and C4 aloin concentrations where values were respectively 1,52 ± 0,10 and 1,61 ±0,06. However, AWCD significantly decreased along the incubation time.

Regarding substrate consummation, ecoplates results (fig 5) suggested that there is no significant effect of the exposure period while the dose of aloin significantly affected microbial activity in soils. Moreover, amines and amides consumption increased as the dose of aloin increased, which is not the case for carboxilic acids. In general, the microbial communities of the tested soils have more affinity to consume Amino acids substrates than polymers and various other compounds.

IV. DISCUSSION

The aim of the present work was to assess firstly the impact of aloe vera wastes on physico-chemical properties of soils and second how these wastes affect soil microbiological activities. Indeed, Aloe vera wastes can be used as amendment in soil to improve its fertility. However, these waists are rich in natural PAHs such as the aloin A and B. Indeed, PAHs are resistant to be degraded tend to accumulate in the soil and potentially threaten soil ecology and more in general human health throw food chain [50]. So, environmental impact of aloe vera wastes must be assessed to avoid soil disturbance. Moreover, the impact of HAP pollution on both soil enzymes activities and bacterial functional diversity is poorly documented. It is of ecological interest to determine which functional and metabolic capabilities are selected for in a bacterial community under drastic selective pressure by different pollutants.

For this purpose, soils were exposed to different aloin concentrations. Firstly, their effects on physic-chemical properties of soil was determined and secondary, their impact on soil microbiological activities was assessed.The physicochemical properties were obviously modified after 28 d of exposure to Aloe vera waste. Soil pH had significantly increased with aloin concentration and this was probably due to the basic character of this industrial waste.

Furthermore, a slight decrease in OM amount after 28 d was recorded but there is no significant effect between treatments. As reported by several studies OM is one of the most important factor affecting hydrocarbons distribution in soils [51,52]. Thus, this decrease must be a result of the higher metabolization of those organic wastes which can be absorbed by organic matter along experimentation as proved by [53].

On the other hand, the CEC values increased with exposure period in all the experiments, and this could be related to the adsorption of the organic molecules to the clay particles of experimented soils which can increase the exchange capacity of soils and this was proved by [54,55]. Moreover, a slight decrease was also observed for nitrogen and phosphorus mineralization. This was in concordance with the work of [56].

Our findings suggested that aloin increases microbial biomass in soils in a dose dependant manner. This demonstrates that the addition of aloin to soils can act as significant sources of carbon for microbial growth and activity. Our results are consistent with previous studies indicating that the PAH induce microbial biomass in soils [57]. Besides being a pollutant with potential toxicity, HAPs are also a carbon source that could support bacterial growth [58]. The number of benzene rings determines a PAH's ability to stimulate enzymatic activity. Organic compounds containing three or four rings constitute a rich source of energy and carbon for microorganisms, whereas compounds containing a higher number of rings are toxic, mutagenic, and carcinogenic [59,60]. However, along the time incubation, microbial biomass decreased. This can be explained by the fact that carbon sources which were provided bt the HAPs decreased along the incubation time. Other authors found that depending on its concentration, phenanthrene could decrease the bacterial biomass [61] or activity [62].

The measurement of enzymatic activities was used to evaluate soil fertility [63]. Soil enzymatic activities are considered to be important soil biological activities influenced by contamination occurring in the soil ecosystem. Our studies indicated that enzymes activities were enhanced by the addition of the aloin. The chemical composition of aloe vera contains anthraquinone and PAHs which can be sources of energy to bacterial communities that enable them to produce enzymes in soils. These results are consistent with those of many studies on the effects of HAPs on soil enzymes activities [64,65,66].

The urease activity is based on hydrolysis urea to carbon dioxide and ammonium and it originates mainly from microorganisms, plants and animals [67,68,69,70]. An increase in urease activity levels in soils treated with aloin was observed in our study. Similar results of the stimulating effect of PAHs were reported by [65,71]. The use of hydrocarbons as substrates for microbial growth which use them as a source of carbon and energy is well documented.

Arylsulphatase is produced by bacteria and fungi to limit sulphur, [72] , this enzyme catalyzes the hydrolysis of sulphate esters in the soil [73]. Its activity in soil is correlated with microbial biomass and with the rate of immobilization sulfur [74,75]. This can explain the fact of the increase in arylsulphatase activity found in our results as a consequence of the increase in microbial biomass observed.

β-Glucosidase produces glucose, an important C energy source for microbes in the soil [76], by hydrolyzing the dimers of glucose produced by cellulolytic microorganisms. This enzyme represents a good soil quality indicator and can inform about the capacity of the soil to stabilize organic matter [77,78]. In fact, for soils with aloin only there has been a significant increase which can be a result of the activation of this enzyme with the addition of aloin.

Several researchers reported that acid and alkaline phosphatase activities were therefore considered as a good indicator of soil fertility and play a fundamental role in the soil system [79,80]. Aloinenhaced these two enzymes activity. In fact, the variation of these enzymes depends and are correlated with the phosphate [81] and soil organic matter content [82,83],so contrary to other activities, the results of phosphatase cannot support to discriminate samples, given that this enzyme is both an intra- and extra-cellular and the extracellular part is not very sensitive to variations in environmental conditions to affect microorganisms [84].

The dehydrogenase activity is an indicator of biological activity in soils [85], it only exists in living microbes and represents active viable and intact cells [86].This enzyme acts by oxidizing soil organic matter by acting on the transfer of protons and electrons. Therefore the enzyme participates in the process of respiration of the microorganisms which depends on the conditions and properties of the soil [87,88]. Also, a decrease on the enzyme activity was observed along the incubation time. This was also observed in the work of [89] who noted that typically, addition of PAHs reduced dehydrogenase activity initially, with activity subsequently recovering to

control levels. The higher levels of dehydrogenase activity observed in fluoranthene amended soil may reflect that degradation of this PAH was proceeding rapidly. This would suggest, in the longer term, that PAH amendment, whether of 3-, 4- or 5-ring, did not have a toxic effect overall. This does not preclude specific toxic effects on individual microbial populations which might affect degradation rates.

FDA (fluorescein diacetate) hydrolysis represents a total indicator of soil microbial activity; it has the property to measure the activities of proteases, lipases and esterases[90,91]. Our results showed an increase on this enzyme activity with aloin incubation. However, it decreased along the incubation time.

Using community-level physiological profiles (BiologEcoplates™), we observed that aloe vera exudates had an impact on the community physiology. Indeed, a decrease in metabolic activity was observed with the application of C3 and C4 and increased with the small concentrations. This is in concordance with the work of [56]. Moreover, [92] demonstrated that PAH amendment had a profound effect on functional catabolic bacterial community in sandy pea soil. Moreover, soils tested have more affinity to consume substrates of the categories of amino acids, polymers and various compounds. These observations suggest that the presence of HAPs modified the range of substrates and degradation efficiency. This observation was also noted in the work of [56].

V. CONCLUSION

This work provided clues about a possible positive effect of aloe vera wastes incorporation in agriculture soils despite the known toxic effects of major constituents such as aloin A and B. Indeed, it increased microbiological activities in soils even if this effect does not last in time.

Amendment of Aloe vera residues to soil can be an interesting way to valorize these specific wastes and may promote sustainable agriculture in regions where aloe vera production is the main activity.

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REFERENCES

[1] Boulal, H., Gómez-Macpherson, H., Gómez, J. A. &Mateos, L. Effect of soil management and traffic on soil erosion in irrigated annual crops. *Soil Tillage Res.***115–116,** 62–70 (2011).

[2] Brouder, S. M. & Gomez-Macpherson, H. The impact of conservation agriculture on smallholder agricultural yields: A scoping review of the evidence. *Agric. Ecosyst. Environ.***187,** 11–12 (2014).

[3] Jemai, I., Ben Aissa, N., Ben Guirat, S., Ben-Hammouda, M. &Gallali, T. Impact of three and seven years of no-tillage on the soil water storage, in the plant root zone, under a dry subhumid Tunisian climate. *Soil Tillage Res.***126,** 26–33 (2013).

[4] Zhang, A. *et al.* Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agric. Ecosyst. Environ.***139,** 469–475 (2010).

[5] Ussiri, D. A. N. & Lal, R. Long-term tillage effects on soil carbon storage and carbon dioxide emissions in continuous corn cropping system from an alfisol in Ohio. *Soil Tillage Res.***104,** 39–47 (2009).

[6] Ghazanfar, S. A., Ahmed, W., Ali, M. & Al-, P. O. B. Medicinal Plants of Northern and Central Oman (Arabia) Author (s): Shahina A . Ghazanfar and Ahmed Mohammed Ali Al-Sabahi Reviewed work (s): Published by : Springer on behalf of New York Botanical Garden Press Stable URL : http://www.jstor.org/stabl. **47,** 89–98 (2012).

[7] López, A., De Tangil, M. S., Vega-Orellana, O., Ramírez, A. S. & Rico, M. Phenolic constituents, antioxidant and preliminary antimycoplasmic activities of leaf skin and flowers of Aloe vera (L.) Burm. f. (syn. A. barbadensis Mill.) from the Canary Islands (Spain). *Molecules***18,** 4942–4954 (2013).

[8] Badejo, A. C., Badejo, A. O., Shin, K. H. & Chai, Y. G. A Gene Expression Study of the Activities of Aromatic Ring-Cleavage Dioxygenases in Mycobacterium gilvum PYR-GCK to Changes in Salinity and pH during Pyrene Degradation. *PLoS One***8,** (2013).

[9] Nogales, B., Lanfranconi, M. P., Pi??a-Villalonga, J. M. & Bosch, R. Anthropogenic perturbations in marine microbialcommunities. *FEMS Microbiol. Rev.***35,** 275–298 (2011).

[10] Sun, M. *et al.*Tenax TA extraction to understand the rate-limiting factors in methyl-β-cyclodextrin-enhanced bioremediation of PAH-contaminated soil. *Biodegradation***24,** 365–375 (2013).

[11] Acosta-González, A., Martirani-von Abercron, S. M., Rosselló-Móra, R., Wittich, R. M. &Marqués, S. The effect of oil spills on the bacterial diversity and catabolic function in coastal sediments: a case study on the Prestige oil spill. *Environ. Sci. Pollut. Res.***22,** 15200–15214 (2015).

[12] Sauret, C. *et al.* Influence of PAHs among other coastal environmental variables on total and PAH-

degrading bacterial communities. *Environ. Sci. Pollut. Res.***23,** 4242–4256 (2016).

[13] Stauffert, M., Cravo-Laureau, C. & Duran, R. Structure of hydrocarbonoclastic nitrate-reducing bacterial communities in bioturbated coastal marine sediments. *FEMS Microbiol. Ecol.***89,** 580–593 (2014).

[14] Kimes, N. E., Callaghan, A. V., Suflita, J. M. & Morris, P. J. Microbial transformation of the deepwater horizon oil spill-past, present, and future perspectives. *Front. Microbiol.***5,** 1–12 (2014).

[15] King, G. M., Kostka, J. E., Hazen, T. C. &Sobecky, P. A. Microbial Responses to the *Deepwater Horizon* Oil Spill: From Coastal Wetlands to the Deep Sea. *Ann. Rev. Mar. Sci.***7,** 377–401 (2015).

[16] Rosano-Hernández, M. C., Ramírez-Saad, H. & Fernández-Linares, L. Petroleum-influenced beach sediments of the Campeche Bank, Mexico: Diversity and bacterial community structure assessment. *J. Environ. Manage.***95,** S325–S331 (2012).

[17] Lladó, S. *et al.* Pyrosequencing reveals the effect of mobilizing agents and lignocellulosic substrate amendment on microbial community composition in a real industrial PAH-polluted soil. *J. Hazard. Mater.***283,** 35–43 (2014).

[18] Wang, Y. Bin, Liu, C. W., Kao, Y. H. & Jang, C. S. Characterization and risk assessment of PAH-contaminated river sediment by using advanced multivariate methods. *Sci. Total Environ.***524–525,** 63–73 (2015).

[19] Jenkinson, D. S. Studies on the Decomposition O F Plant Material in. *Evolution (N. Y).***17,** 417–423 (1977).

[20] Huang, X.-F. *et al.*Rhizosphereinteractions: root exudates, microbes, and microbialcommunities 1. *Botany***92,** 267–275 (2014).

[21] Lladó, S. &Baldrian, P. Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. *PLoS One***12,** (2017).

[22] Josic, D. et al. Indigenous pseudomonads from rhizosphere of maize grown on pseudogley soil in serbia.*Bulg. J. Agric. Sci.***18,** 197–206 (2012).

[23] Epelde, L., Becerril, J. M., Hernández-Allica, J., Barrutia, O. &Garbisu, C. Functional diversity as indicator of the recovery of soil health derived from Thlaspicaerulescens growth and metal phytoextraction. *Appl. Soil Ecol.***39,** 299–310 (2008).

[24] Zhang, A. *et al.* Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agric. Ecosyst. Environ.***139,** 469–475 (2010).

[25] Pankhurst, C. E. *et al.*Management practices to improve soil health and reduce the effects of detrimental soil biota associated with yield decline of sugarcane in Queensland, Australia. *Soil Tillage Res.***72,** 125–137 (2003).

[26] Lee, L. S., Carmosini, N., Sassman, S. A., Dion, H. M. &Sepúlveda, M. S. Agricultural Contributions of Antimicrobials and Hormones on Soil and Water Quality. *Adv. Agron.***93,** 1–68 (2007).

[27] Hu, X. F. *et al.* Effects of mining wastewater discharges on heavy metal pollution and soil enzyme activity of the paddy fields. *J. GeochemicalExplor.***147,** 139–150 (2014).

[28] Borowik, A., Wyszkowska, J., Kucharski, J., Baćmaga, M. &Tomkiel, M. Response of microorganisms and enzymes to soil contamination with a mixture of terbuthylazine, mesotrione, and S-metolachlor. *Environ. Sci. Pollut. Res.***24,** 1910–1925 (2017).

[29] Kandeler, E., Tscherko, D. & Spiegel, H. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a chernozem under different tillage management. *Biol. Fertil. Soils***28,** 343–351 (1999).

[30] Wang, X., Xie, H., Guan, H. & Zhou, X. Different responses of MODIS-derived NDVI to root-zone soil moisture in semi-arid and humid regions. *J. Hydrol.***340,** 12–24 (2007).

[31] Zhang, C. B. *et al.*Effects of plant diversity on nutrient retention and enzyme activities in a full-scale constructed wetland. *Bioresour. Technol.***101,** 1686–1692 (2010).

[32] Bouché, Marcel B. "Lombriciens de France: écologie et systématique." (1972): 72-2.

[33] OECD, 2004a. Test no. 222. Earthworm reproduction test (*Eisenia fetida/andrei*). OECD Guidelines for the Testing of Chemicals, Organisation for Economic Co-operation and Development.

[34] Vieira, F. C. B. *et al.*Carbon management index based on physical fractionation of soil organic matter in an Acrisol under long-term no-till cropping systems. *Soil Tillage Res.***96,** 195–204 (2007).

[35] Hossner, L. R. (1996). Dissolution for total elemental analysis. *Methods of Soil Analysis Part 3—Chemical Methods*, (methodsofsoilan3), 49-64.

[36] Gillman, G. P. "A proposed method for the measurement of exchange properties of highly weathered soils." *Soil Research* 17.1 (1979): 129-139.

[37] Anderson, J. P. E. &Domsch, K. H. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.***10,** 215–221 (1978).

[38] Nakamoto, T. &Wakahara, S. Development of Substrate Induced Respiration (SIR) Method Combined with Selective Inhibition for Estimating Fungal and Bacterial Biomass in Humic Andosols. *Plant Prod. Sci.***7,** 70–76 (2004).

[39] Mora, A. P. De, Ortega-Calvo, J. J., Cabrera, F. &Madejón, E. Changes in enzyme activities and microbial biomass after 'in situ' remediation of a heavy metal-contaminated soil. *Appl. Soil Ecol.***28,** 125–137 (2005).

[40] Tabatabai, M. Ali, J. W. B. Stewart, and J. J. Schoenau. "Sulfur in agriculture." *Soil Science* 145.6 (1988): 462-463.

[41] Casida Jr, L. E., D. A. Klein, and Thomas Santoro. "Soil dehydrogenase activity." *Soil science* 98.6 (1964): 371-376.

[42] Kandeler, Ellen and H, G. Short-Term Assay of Soil Urease Activity Using Colorimetric Determination of Ammonium Article in Biology and Fertility of Soils January 1988. *Biol. Fertil. Soils***6,** 68–72 (1988).

[43] Kandeler, E., Tscherko, D. & Spiegel, H. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a chernozem under different tillage management. *Biol. Fertil. Soils***28,** 343–351 (1999).

[44] Brohon, B., Delolme, C. &Gourdon, R. Complementarity of bioassays and microbial activity measurements for the evaluation of hydrocarbon-contaminated soils quality. *Soil Biol. Biochem.***33,** 883–891 (2001).

[45] Green, V. S., D. E. Stott, and M. Diack. "Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples." *Soil Biology and Biochemistry* 38.4 (2006): 693-701.

[46] Garland, J. L. & Mills, A. L. Classification and characterisation of heterotrophic microbial communities on the basis of pattern of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.***57,** 2351–2359 (1991).

[47] Grayston, S. J., Griffith, G. S., Mawdsley, J. L., Campbell, C. D. &Bardgett, R. D. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biol. Biochem.***33,** 533–551 (2001).

[48] Garcia-Villaraco Velasco, A. *et al.*Effect of fire and retardant on soil microbial activity and functional diversity in a Mediterranean pasture. *Geoderma***153,** 186–193 (2009).

[49] Zak, J., Willig, M., Moorhead, D. & Wildman, H. Functional diversity of microbial communities: a quantitative approach. *Soil Biol. Biochem.***26,** 1101–1108 (1994).

[50] Tejeda-Agredano, M. C. *et al.*Influence of the sunflower rhizosphere on the biodegradation of PAHs in soil. *Soil Biol. Biochem.***57,** 830–840 (2013).

[51] Agarwal, T. &Bucheli, T. D. Is black carbon a better predictor of polycyclic aromatic hydrocarbon distribution in soils than total organic carbon? *Environ. Pollut.***159,** 64–70 (2011).

[52] Zhang, P. & Chen, Y. Polycyclic aromatic hydrocarbons contamination in surface soil of China: A review. *Sci. Total Environ.***605–606,** 1011–1020 (2017).

[53] Alrumman, S. A., Standing, D. B. & Paton, G. I. Effects of hydrocarbon contamination on soil microbial community and enzyme activity. *J. King Saud Univ. - Sci.***27,** 31–41 (2015).

[54] El-Tarabily, K. A. Total microbial activity and microbial composition of a mangrove sediment are reduced by oil pollution at a site in the Arabian Gulf. *Can. J. Microbiol.***48,** 176–182 (2002).

[55] Eibes, G., Cajthaml, T., Moreira, M. T., Feijoo, G. &Lema, J. M. Enzymatic degradation of anthracene, dibenzothiophene and pyrene by manganese peroxidase in media containing acetone. *Chemosphere***64,** 408–414 (2006).

[56] Cébron, A. *et al.* Impact of clay mineral, wood sawdust or root organic matter on the bacterial and fungal community structures in two aged PAH-contaminated soils. *Environ. Sci. Pollut. Res.***22,** 13724–13738 (2015).

[57] Bundy, J. G., Paton, G. I. & Campbell, C. D. Microbial communities in different soil types do not converge after diesel contamination. 276–288 (2002).

[58] Johnsen, A. R., Wick, L. Y. & Harms, H. Principles of microbial PAH-degradation in soil. *Environ. Pollut.***133,** 71–84 (2005).

[59] Baran, S., Bielińska, J. E. &Oleszczuk, P. Enzymatic activity in an airfield soil polluted with polycyclic aromatic hydrocarbons. *Geoderma***118,** 221–232 (2004).

[60] Chen, J.-H. the Combined Use of Chemical and Organic Fertilizers and/or Biofertilizer for Crop Growth and Soil Fertility. *Int. Work. Sustain. Manag. Soil-rhizosph. Syst. Effic. Crop Prod. Fertil. Use* 1–11 (2006).

[61] Su, Y. H. & Yang, X. Y. Interactions between selected PAHs and the microbial community in rhizosphere of a paddy soil. *Sci. Total Environ.***407,** 1027–1034 (2009).

[62] Verrhiest, G. J., Clément, B., Volat, B., Montuelle, B. &Perrodin, Y. Interactions between a polycyclic aromatic hydrocarbon mixture and the microbial communities in a natural freshwater sediment. *Chemosphere***46,** 187–196 (2002).

[63] Hofmann, Ed, and A. Seegerer. "* UBER DAS ENZYM SYSTEM UNSERER KULTURBODEN. 1. SACCHARASE." *BiochemischeZeitschrift* 322.3 (1951): 174-179.

[64] Novotný, Č. *et al*. Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi. *Biodegradation***10,** 159–168 (1999).

[65] Margesin, R., Zimmerbauer, A. & Schinner, F. Monitoring of bioremediation by soil biological activities. *Chemosphere***40,** 339–346 (2000).

[66] Andreoni, V. *et al*. Bacterial communities and enzyme activities of PAHs polluted soils. *Chemosphere***57,** 401–412 (2004).

[67] Polacco, Joseph Carmine. "Nitrogen metabolism in soybean tissue culture: II. Urea utilization and urease synthesis require Ni2+." *Plant physiology* 59.5 (1977): 827-830.

[68] Burns, R. G. "Interaction of enzymes with soil mineral and organic colloids." *Interactions of soil minerals with natural organics and microbes*interactionsofs (1986): 429-451.

[69] Mobley, H. L., and R. P. Hausinger. "Microbial ureases: significance, regulation, and molecular characterization." *Microbiological reviews* 53.1 (1989): 85-108.

[70] Nannipieri, P. *et al*.Microbialdiversity and soilfunctions. *Eur. J. SoilSci*.**54,** 655 (2003).

[71] Wyszkowska, J., Kucharski, J. &Lajszner, W. The Effects of Copper on Soil iochemical Properties and Its Interaction with Other Heavy Metals. *Polish J. Environ. Stud*.**15,** 927–934 (2006).

[72] Dick, W. A., and M. A. Tabatabai. "Kinetics and Activities of Phosphatase-Clay COMPLEXES1." *Soil Science* 143.1 (1987): 5-15.

[73] Kertesz, Michael A., and Pascal Mirleau. "The role of soil microbes in plant sulphur nutrition." *Journal of experimental botany* 55.404 (2004): 1939-1945.

[74] Klose, S. &Tabatabai, M. A. Urease activity of microbial biomass in soils. *Soil Biol. Biochem.***31,** 205–211 (1999).

[75] Vong, P. C., Dedourge, O., Lasserre-Joulin, F. &Guckert, A. Immobilized-S, microbial biomass-S and soil arylsulfatase activity in the rhizosphere soil of rape and barley as affected by labile substrate C and N additions. *Soil Biol. Biochem.***35,** 1651–1661 (2003).

[76] Esen, Asim. "beta-Glucosidases: overview." *ACS symposium series (USA)*. 1993.

[77] Bandick, A. K. & Dick, R. P. Field management effects on soil enzyme activities. *Soil Biol. Biochem.***31,** 1471–1479 (1999).

[78] Ndiaye, E. L., Sandeno, J. M., McGrath, D. & Dick, R. P. Integrative biological indicators for detecting change in soil quality. *Am. J. Altern. Agric*.**15,** 26 (2000).

[79] Eivazi, F., and M. A. Tabatabai. "Phosphatases in soils." *Soil Biol. Biochem* 9.3 (1977): 167-172.

[80] Dick, W. A., Cheng, L. & Wang, P. Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biol. Biochem.***32,** 1915–1919 (2000).

[81] Eivazi, F., and M. A. Tabatabai. "Phosphatases in soils." *Soil Biol. Biochem* 9.3 (1977): 167-172.

[82] Kosaric, N. Biosurfactants and their applications for soil bioremediation. *Food Technol. Biotechnol*.**39,** 295–304 (2001).

[83] Nannipieri, P., Sastre, I., Landi, L., Lobo, M. C. &Pietramellara, G. Determination of extracellular neutral phosphomonoesterase activity in soil. *Soil Biol. Biochem.***28,** 107–112 (1996).

[84] Brohon, B., Delolme, C. &Gourdon, R. Complementarity of bioassays and microbial activity measurements for the evaluation of hydrocarbon-contaminated soils quality. *Soil Biol. Biochem.***33,** 883–891 (2001).

[85] Burns, Richard G. "Enzyme activity in soil: some theoretical and practical considerations." *Soil enzymes* (1978).

[86] Tabatabai, M. A. &Bremner, J. M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.***1,** 301–307 (1969).

[87] Gliński, Jan, and WitoldStępniewski. *Soil aeration and its role for plants*. CRC Press, Inc., 1985.

[88] Kandeler, F., Ch Kampichler, and O. Horak. "Influence of heavy metals on the functional diversity of soil microbial communities." *Biology and fertility of soils* 23.3 (1996): 299-306.

[89] Lipińska, A., Kucharski, J. &Wyszkowska, J. Activity of arylsulphatase in soil contaminated with polycyclic aromatic hydrocarbons. *Water. Air. Soil Pollut*.**225,** (2014).

[90] Bandick, Anna K., and Richard P. Dick. "Field management effects on soil enzyme activities." *Soil biology and biochemistry* 31.11 (1999): 1471-1479.

[91] Taylor, J. P., Wilson, B., Mills, M. S. & Burns, R. G. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil Biol. Biochem.***34,** 387–401 (2002).

[92] Yrjälä, K., Keskinen, A. K., Åkerman, M. L., Fortelius, C. &Sipilä, T. P. The rhizosphere and PAH amendment mediate impacts on functional and structural bacterial diversity in sandy peat soil. *Environ. Pollut*.**158,** 1680–1688 (2010).

http://dx.doi.org/10.22161/ijeab/3.4.21 ISSN: 2456-1878

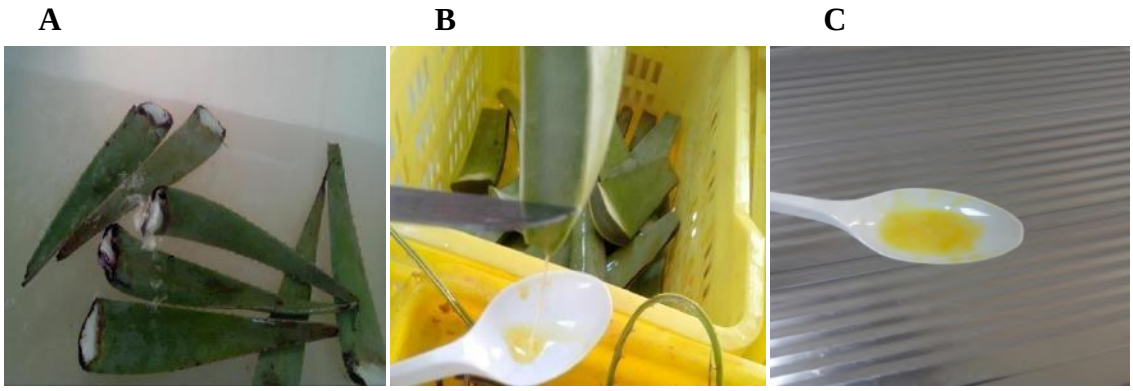

Fig.1: AloinExtraction .A : Leaves of Aloe veracutted, B : Yellow exudate is collected , C : Aloin

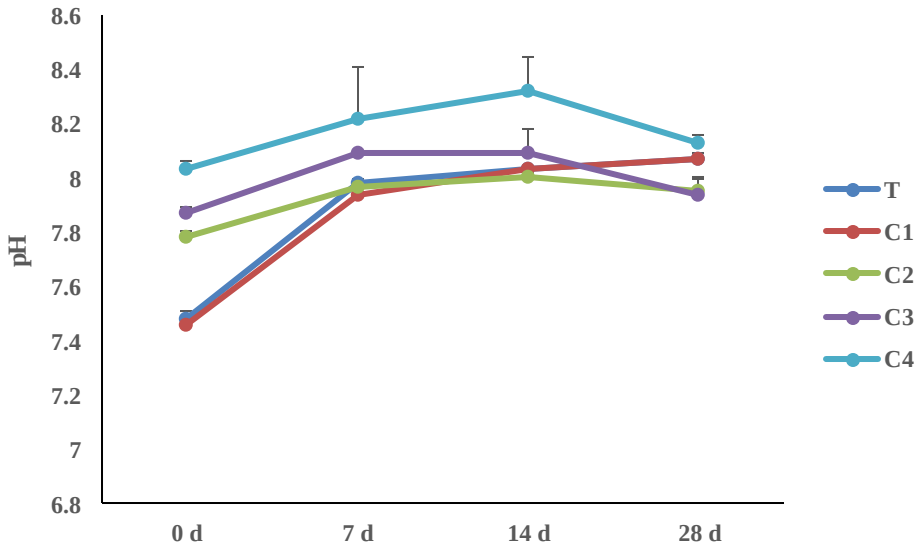

Fig.2: Changes in soil pH after 0, 7, 14 28 and 60 days of incubation with different concentrations of aloin

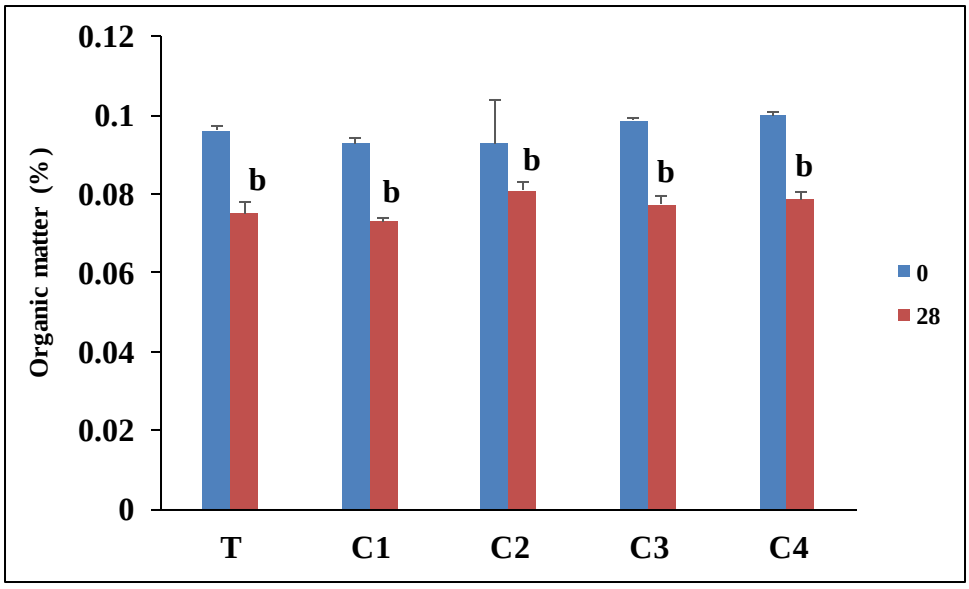

Fig.3: Changes in organic matter (%) after 28 days of incubation with different concentrations of aloin. were analyzed by ANOVA + Tukey'spost test. a: Statistically significant differences (n= 10; P<0.01) in comparison with control condition. b: Statistically significant differences (n= 10; P<0.01) in comparison with soils exposed to the same condition for 0 d.

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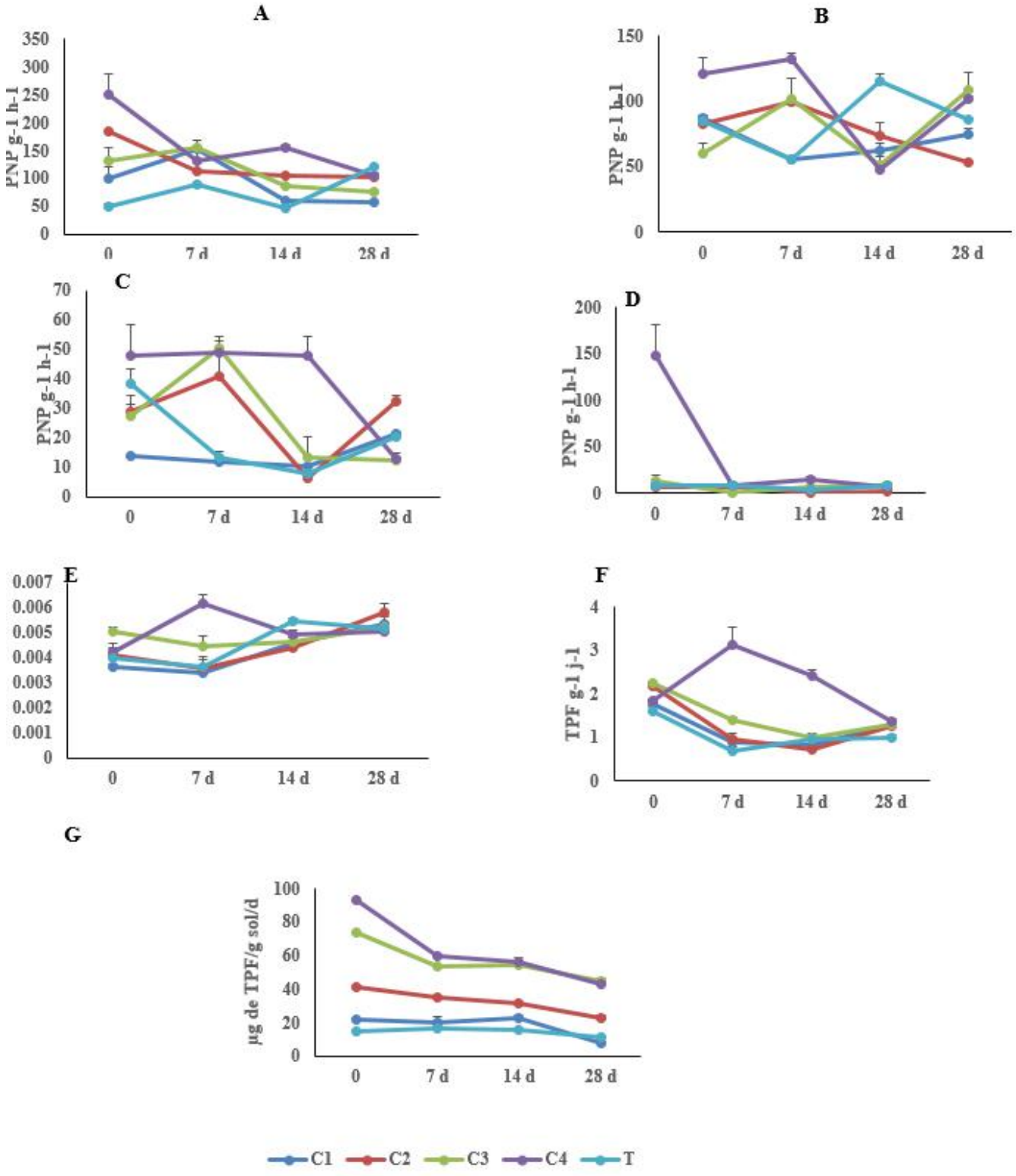

Fig.4: Enzymes activities in soils with different aloinconcentrations : A : β-glucosidase , B : Acid phosphatase, C : Basic phosphatase, D : urease, E : Arylsulphatase, F : Deshydrogenase, G : FDA.

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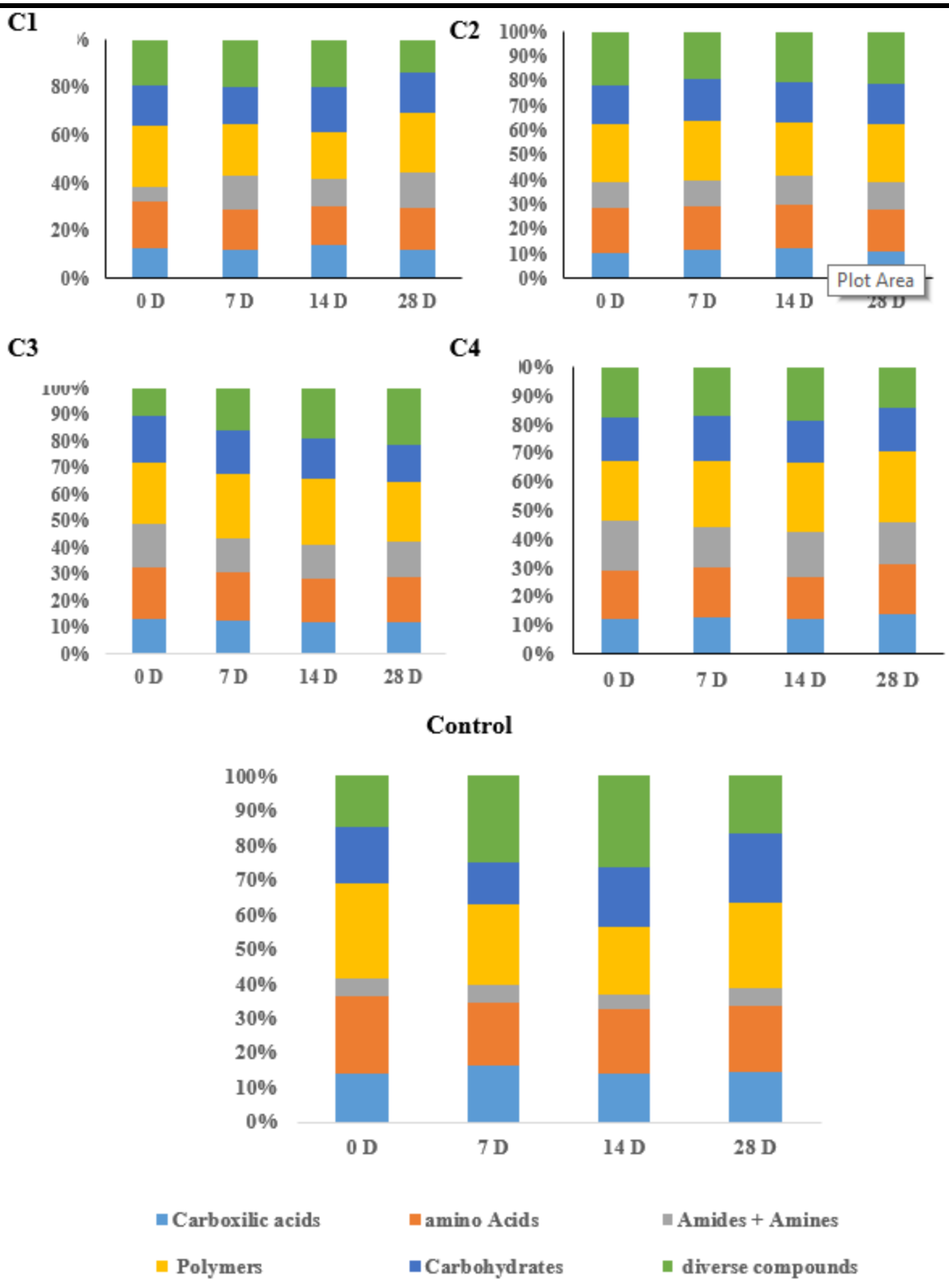

Fig.5: Ecoplates substrates utilization pattern in soils with different aloin concentrations after 0, 7, 14 and 28 days of incubation

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*Table 1. Effect of Aloe vera wastes on Nitrogen content, phosphorus content and CEC. #: Statistically significant differences (n= 10; P<0.01) in comparison with control condition. *: Statistically significant differences (n= 10; P<0.01) in comparison with soils exposed to the same condition for 0 d.*

		Nitrogen (mg/g)	Phosphorus	CEC
Control	0 days	12,19±2,53	7,33±0,28	9,74±2,44
	28 days	7,66*±0,39	5,60*±0,72	13,67*±1,95
C1	0 days	15,41± 3,34	6,40±0,78	13,54±1,09
	28 days	7,27*±1,17	0,09*±0,03	13,79±1,25
C2	0 days	13,51* ±1,91	8,80±0,89	13,43±1,36
	28 days	8,07±0,35	6,71*±0,68	17,91*±3,58
C3	0 days	11,50±2,92	6,73±1,35	13,49±0,28
	28 days	8,54*±2,21	16,47*±0,79	18,97*±0,24
C4	0 days	3,47#±0,73	6,76±1,24	13,60±0,54
	28 days	4,86*±1,09	2,86*±1,89	18,72*±0,44

*Table 2. Substrate induced respiration pattern in soils incubated with aloin in mg CO_2.g-1.h-1. *: Statistically significant differences (n= 10; P<0.01) in comparison with control condition.*

Condition	0	7 d	14 d	28 d
T	43337,00± 791,44	38941,9*± 459,21	34201,3*±2593,49	41361,3±2962,69
C1	47635,69± 9013,11	39725,8*±1091,06	37842,3*±685,06	45256,2±1409,06
C2	47635,69± 9013,11	45213,5±2878,84	41065,4±1075,6	48398,1*±3257,83
C3	28959,83*± 975,77	48340,7±3396,91	46137,1±887,24	38631,9*±1675,2
C4	52655,75* ± 1010,49	52436,75*±1204,45	38327,5*±1525,56	37384,1*±544,479

*Table 3.Evolution of AWCD in soils with aloinafter 0, 7, 14 and 28 days of incubation. . *: Statistically significant differences (n= 10; P<0.01) in comparison with control condition. **

Condition	0	7 d	14 d	28 d
T	0,755 ±0,24	0,670± 0,12	0,707±0,15	0,854± 0,25
C1	0,820± 0,17	1,281 *± 0,23	1,150*± 0,27	1,022*± 0,14
C2	1,20* ± 0,05	1,184* ± 0,14	1,242*±0,17	1,144* ± 0,22
C3	1,520* ±0,10	1,218* ± 0,04	1,242*±0,07	1,230* ± 0,19
C4	1,617 *±0,06	1,264*±0,04	1,276*± 0,06	1,259*± 0,22

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Seaweed Cultivation Techniques *Gracillariaverrucosa* in Pond Ujungpangkah District, Gresik East Java using Broadcast Method

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Abstract**—** *Seaweed is one of the sea cultivation commodities that are easy to be cultivated and have a good market prospect in improving coastal community empowerment. Moreover, the technology used to cultivate seaweed is also simple and inexpensive so it is suitable and easily adapted by coastal communities.Type of seaweed that has been cultivated in brackish water isGracilaria sp.One of the methods used for seaweed cultivation Gracilaria verrucosa is the broadcast method or spread.This method provides an increase in the growth length from 5 cm to 5.8 cm for 42 days and the weight of Gracilaria verrucosa seaweed from 10 grams to 14.1 gr for 42 days. This growth was influenced by ambient temperature including temperature 28-33 º C, salinity 5-17 ppt and degree of acidity (pH) 5-8.*

Keywords**— Broadcast method, environmental factor, Gracilariaverrucosa , growth, weight.**

I. INTRODUCTION

Gracilaria is a species of seaweed that increased production from 1990 to 2010 [1], the world market demand for seaweed continues to increase by 3-5% per year [2]. Intensification of *Gracilaria* cultivation continues to be encouraged to meet the needs of industry, one of them by expanding the production area. *Gracilaria verrucosa* is mostly cultivated in ponds because it can live in waters of 15-30 ppt [3].

Seaweed cultivation business (*Gracilaria* sp.) Often fails due to lack of attention to several factors that can affect the growth rate of seaweed in cultivation, including: location of cultivation, management, seeds, season, location and especially selected cultivation method. Factors mentioned above, greatly affect the level (productivity) production of seaweed cultivated [4].

Gracilaria verrucosa can be cultivated by several methods, one of them using the broadcast method or spread. The method is a method commonly chosen by the farmer's community because the technology is easy, cheap, without the need for extra care [5]. The results of this study are expected to contribute as one source of information in an effort to support the development of seaweed cultivation *Gracilaria verrucossa* with the use of the broadcast method or spread in the pond.

II. MATERIALS AND METHODS

Time and place.The study was conducted for 42 days. In March to May 2018, in the brackish water ponds of Pangkah Wetan Village, Ujungpangkah District, Gresik Regency, East Java Province.

Land preparation. Preparation of land by preparing waring for happa to be made for seaweed cultivation in ponds. Happa is made of black waring with size 3 x 4 x 0.5 with four sides, which on each side are given bamboo as a rebuttal.

Procurement and Selection of Seedlings. Seeds used are good seeds with the following criteria:

- cylindrical rod/tallus appearance, clean, fresh, hard, not slimy, no fishy smell, and not pale
- Seeds with many branches and grow centered from one part of the base and spread
- Seed should be homogeneous not mixed with other types
- Selecting seeds with elongated talus ranges from 15-30 cm

Seedling. Seeds that have been obtained in pieces weighing 10 grams and then in stocking by using the method of spread (broadcast method).

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Seedling maintenance and water quality. Seed maintenance is done once every week, for 42 days of research. Water quality monitoring, such as temperature, pH, and salinity, is also carried out.

Gracilariaverrucosa seaweed growth. Growth and weight of *Gracilaria verrucosa* using formula [6]:

$$\text{Growth} = \frac{In\,(Length\ end) - In\,(Start\ length)}{time} \text{ x } 100\%$$

Weight (g) = final weight - initial weight

III. RESULTS AND DISCUSSION

Growth

Based on existing data from figure 1, the growth of seaweed each week has increased. The initial length was only 5 cm at the beginning of the spread to 5.8 cm. The scattering method used has a weakness that is seaweed is only spread and located in the bottom of the pond less sunlight, thallus many of which are covered in silt deposits resulting in inhibition of long growth in seaweed and also less current. According to [7] the role of the current is to avoid the accumulation of silt and epiphytes attached to the thallus, with the presence of mud attached to the seaweed thallus indicating that the movement of water at the depth is not good because it cannot clean the dirt and the sediment attached to thallus that can block the growth of seaweed itself.

Weight

Based on the graph figure 2 obtained results that indicate that the growth of weight in *Gracilaria verrucosa* seaweed in ponds increased weight (g) every week. The initial weight of spreading using seed seaweed weighing 10 grams increased to 14.1 gr. The lack of growth in this study can be influenced by environmental factors ie temperature. Temperatures in the waters exceed the normal limits of seaweed maintenance that is 20-28ºC [8]. In addition, factors that affect the growth of seaweed is the intensity of sunlight, depth, current, weather and climate.

Environmental factor.

Temperature. Water temperature during the study is between 28-33 ° C. [9] states that seaweed grows and develops well in waters that have a temperature range of 20-28° C. Temperature affects the process of seaweed breeding because it helps the process of photosynthesis in the waters. If in temperatures the water does not match will inhibit the growth process.

Salinity. Based on the results of the average pH measurement at the seaweed cultivation pond-*Gracilaria verrucosa* ranged from 5-17 ppt. The good salinity condition for seaweed growth is between 15-34 ppt

A degree of acidity (pH). The degree of acidity (pH) of the waters during the study ranged from pH 5-8, the pH range still qualified as a process of cultivation in ponds. As in [9] seaweed growth requires optimal sea water pH ranging from 6-9. According to SNI 7579.1 (2010) that the pH of waters required for seaweed cultivation ranges from 7-8.5.

IV. CONCLUSION

The method used in seedling distribution using the spread method. However, by using this method the growth of seaweed is <3%. Long growth increased from 5 cm at baseline to 5.8 cm at the end of the study. The initial weight of the spreading using the seaweed seeds weighing 10 grams increased to 14.1 g until the end of the study for 42 days. Environmental factors include water temperature during the study of 28-33 ° C, salinity 5-17 ppt and pH 5-8.

REFERENCES

[1] FAO.2005–2012. Cultured aquatic species information programme. *Eucheuma* spp. Cultured Aquatic Species Information Programme. Text by G.C. Trono Jr. *In:* FAO Fisheries and Aquaculture Department [online]. Rome.

[2] Bixler, H. and Porse, H. 2011. A Decade of Change in the Seaweed Hydrocolloids Industry. Journal of Applied Phycology, 23, 321-335.

[3] Anggadiredja, J.T., A. Zatnika, H. Purwoto & S. Istini. 2007. Seaweed. The Swadaya spreader. Jakarta. 152p.

[4] Putra, B.D., Riris, A., Isnaini. 2011. *Gracilaria* sp. Seaweed growth rate sp. with

[5] Different Planting Methods in Kalianda Waters, South Lampung. Maspari Journal 03 (2011) 36-41.

[6] Basith, T.A., Sri R., Restina, W.A. 2014. The effect of seeds obtained from selection or non-selection and tissue culture against growth rate, agar content, and Gel strength seaweed *Gracilariaverrucosa* which culture in the brackish water tank with a Broadcast method. Journal of Aquaculture Management and Technology Vol. 3, No.2,pp 18-24.

[7] Subandiyono, Hastuti Sri. 2014. Beronang and the Prospect of Sea Cultivation in Indonesia. UPT UNDIP Press. Semarang.

[8] Mamang, Nurfadly. 2008. Growth Rate of Seaweed Seeds *Eucheuma cottonii* With Thallus Origin Treatment Against Seeds Weight in Lakeba Waters,

http://dx.doi.org/10.22161/ijeab/3.4.22 *ISSN: 2456-1878*

Bau-Bau City, Southeast Sulawesi [Bogor Institute of Technology]. Bogor.

[9] Zatnika, A. 2009. Technical Guidelines for Seaweed Farming. Agency for Assessment and Application of Technology. Jakarta.

[10] Zatnika, A. and Sri istini. 2009. Optimization of Alkali Treatment in Efforts to Improve the Quality of Agar from Seaweed (*Gracilaria* sp.). Agency for Assessment and Application of Technology. Jakarta.

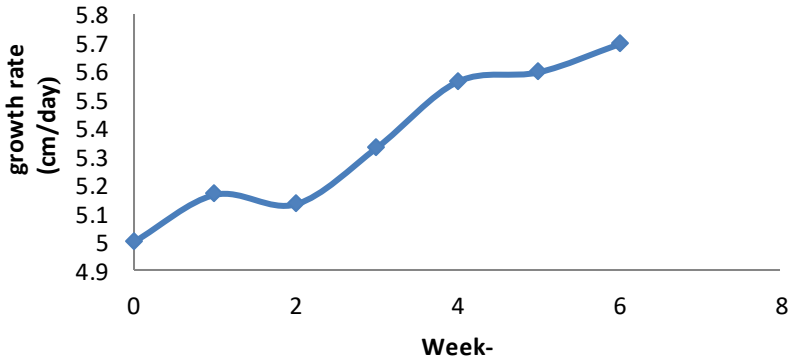

Fig.1: Graph of Seaweed Growth

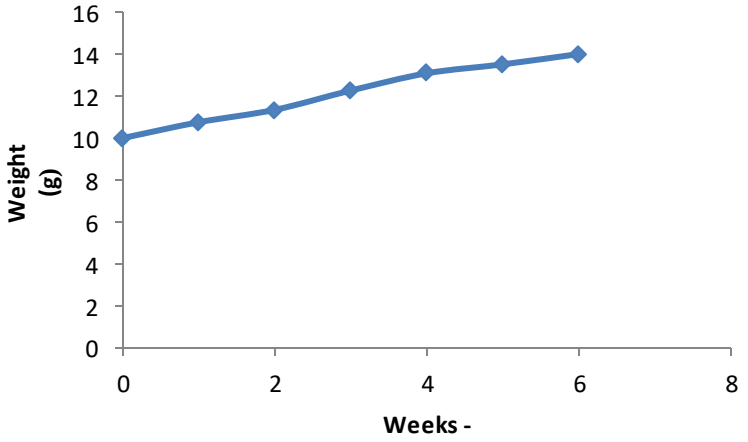

Fig.2: Graph of Growth Rate of Seaweed

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Microbiological Quality of *Kunnu-Zaki* Drinks Sold in Some selected Towns in Osun State, Nigeria

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***Abstract*—** *The microbiological quality of freshly processed and hawked kunnu-zaki drinks, a common Nigerian non-alcoholic beverage was investigated in some selected towns in Osun State, South Western Nigeria. A total of nine (9) towns were assessed. Kunnu-zaki drinks were purchased from these towns on twelve different occasions for a period of six month. The samples were microbiologically analyzed using standard methods. pH values ranged from 3.09 - 4.21 in East and West senatorial district. The TTA of the samples ranged from 0.32 - 0.49 in Central and East senatorial district. All the screened drink samples had varying levels of bacterial contamination ranging from 4.2 x 10^3 to 15.0 x 10^3 CFU/ml, 4.1 x 10^4 to 9.6 x 10^4 CFU/ml and 3.5 x 10^5 to 9.0 x 10^5 CFU/ml for total bacteria, coliform and E. coli count respectively. Total coliform count on MPN ranged from 3 MPN/ml to 93 MPN/ml. 77.78% of the total sampled drinks (n=108) that is (84/108) had bacteria and fungi count. While all the drinks sampled had total coliform count on MPN exceeding the recommended safe level of zero organisms detectable per 100ml. Seven (7) bacteria species were isolated from the kunnu-zaki drink sampled. The bacteria isolated were Escherichia coli, Bacillus species, Staphylococcus species, Pseudomonas species, Streptococcus specie, Enterobacter species, and Klebsiella species. Fungal count ranged from 3.3 x 10^6 to 8.0 x 10^6 CFU/ml respectively. Four (4) fungi were also isolated which include Aspergillus fumigatus, Penicilium italicum, Aspergillus niger and Aspergillus flavus. It can therefore be concluded that the presence of these isolated organisms in kunnu-zaki samples analyzed in Osun State could serve as indicator for the need to promote awareness about the possible health hazards that could arise due to the unhygienic ways of handling and processing of the beverage.*

***Keywords*— Physico-chemical analysis, Bacteria count, Coliform count (MPN), Fungi count and Frequency of Isolated organisms.**

I. INTRODUCTION

Beverage is a liquid drink prepared for human consumption (Robert, 2006). The word "beverage" has been derived from the Latin word "bever" meaning rest from work. After work, one tends to feel thirsty due to fluid loss through perspiration and one is inclined to drink water or other potable beverages to compensate fluid loss. Beverages are potable drinks which have thirst-quenching, refreshing, stimulating and nourishing qualities (Achi, 2005). By refreshing, one means the replenishment of fluid loss from the body due to perspiration. Nourishment is provided by the nutrients in the beverages, especially fruit juices.

Kunnu-zaki (Millet drink) also referred to as "sweet kunnu-zaki" is a popular traditional and important cereal based, non- alcoholic fermented beverage (Adejuyitan *et al*., 2008) made from pearl millet *(Pennisetum glaucum)*, maize (*Zea mays*), or sorghum *(Sorghum bicolor)*, grains but the most commonly used basic ingredient is millet. Maize (*Zea mays*), millet *(Pennisetum* glaucum*)*, rice (*Oryza sativa*) and sorghum (*Sorghum bicolor*) cereals provide mainly carbohydrates and low quality protein (Steinkraus, 1997). It is generally acceptable and widely consumed throughout Nigeria by children and adults as breakfast drink or food supplements and also a weaning food for infants due to its refreshing qualities most especially in the northern parts and it is consumed throughout the year mostly during dry seasons (Elmahmood and Doughari, 2007). Kunnu-zaki is a refreshing drink usually used to entertain visitors, appetizers and is commonly used or served at social gatherings both rural and urban centers, hawked in the motor parks, school premises and market places (Amusa *et al*., 2005). It is a staple beverage that is relatively nutritious and affordable when compared to other carbonated and non-carbonated drinks (Adejuyitan *et al*., 2008). It has low viscosity, a sweet-sour taste depending on the level of fermentation and is milky cream in appearance (Adeyemi and Umar, 1994). It has immense

social, economic, nutritional and medicinal benefits to most consumers (Akoma *et al*., 2006). The preparation of locally fermented foods like kunnu-zaki has become a technology in many homes particularly in rural communities and more recently in the urban areas where more women have developed the skill and commercial production which has helped to alleviate poverty amongst the people (Essien *et al*., 2011).

Kunnu-zaki is been prepared from local cereals which are very common and part of our staple food substances. It is relatively produced in low cost which makes it not expensive because grains and other ingredients used for production are locally sourced and are mostly grown within the savannah region of Nigeria such as Bauchi, Kano, Sokoto and Kastina states (Adebayo *et al*., 2010) and almost throughout the years, most especially the savannah belt of West Africa and the packaging materials are also available, cheap and easily affordable.

Cereals are grains or edible fruits of the grass-monocot family that may be cultivated and used as foods. In India and Africa, cereals products comprise 80% or more of the average diet, 50% in Central and Western Europe and between 20-25% in the U.S. (Onwueeme and Finha, 1991). Millets are cereal crops or grains for foddler and human food (Ray and Sivakumar, 2009). Millets are in the family of cereals grown globally with differential importance across continents and within regions of the world. It is consumed as staple food and drink in most areas. Across Africa several indigenous foods and drinks are made from flour/meal and malt of millet. They are nutritionally equivalent or superior to other cereals (Obilana and Taylor, 2002). In the Northern area of Nigeria Millet is known as Jero, 'Oka baba' in yoruba land and in America, it is known as milo. It can be stored for 6 months without any significant spoilage. Millet are highly tolerant and are very nutritious containing low phytic acid and are rich in dietary fiber, iron, phosphorus, potassium, magnesium, zinc, thiamine, riboflavin calcium and vitamin B (niacin, B_6 and folic acid) which make millet more superior than most cereals, are now being enhanced through biofortification and micronutrient research (Obilana and Taylor, 2002) which are essential for body growth and development. It is easily digested and gluten-free, an excellent choice for anyone with gluten-intolerance (Oladele and Aina, 2007).

Kunnu-zaki processing is mostly done by women using simple household equipment and utensil depending on cereal availability. Kunnu-zaki is produced from malted millet; a portion of the cereal is malted, dried, ground and then mixed with the uncooked portion. The mixture is then added to the cooked portion and stirred vigorously and allowed to ferment (Akoma *et al*., 2012). Spices such as ginger (*Zingiber officinales*), alligator pepper (*Afromonium melegueta*), red pepper (*Capsicum species*), black pepper (*Piper guineense*) and cloves (*Syzygium aromaticum*) are commonly added to improve flavor and taste while honey, sweet potatoes and sugar are also added to act as a sweetener (Ahmed *et al*., 2003). The processed kunnu-zaki is usually packed for sale either in nylon or in a plastic container and can also be stored in refrigerators. Some people in some regions prefer it with pepper and sugar while some with little or no pepper and sugar (Adeyemi and Umar, 1994). All these ingredients perform one function or the other in the course of the preparation. The most abundant constituent of kunnu-zaki is water and it acts as the medium in which all other constituents are dissolved and contain only traces amount of inorganic substances. Spices are usually added in small quantities to improve taste and flavor. Because these are agricultural commodities, they may contain a high level of microbial impurities (Adeyemi *et al*., 1994; Bibek, 2001). The quality and quantity of the products depend largely on the quality of the ingredients and its proper handling in the course of production by the producer. Hence, kunnu-zaki samples for different market in Osun state are purchased to determine the microbial and fungi load.

II. MATERIALS AND METHODS

STUDY LOCATION

Osun State is located in the South Western part of Nigeria. It covers an area of approximately 14,875 square kilometers, lies between longitude 04 00E and latitude 05 558 and is bounded by Ogun in South, Kwara in North, Oyo in West and Ondo States in the East. It total population is approximately 3,416,959.

COLLECTION AREAS

Samples were collected and analyzed from nine (9) major markets using Senatorial districts West, East and Central to ensure adequate representation of samples in Osun State Nigeria, which include three (3) popular towns from each Senatorial district. The towns are: Gbongan, Ikire (Sabo market) and Apomu all in Osun West Senatorial districts, Ipetu-Ijesa, Ikeji-Arakeji and Owena-Ijesa all in Osun East Senatorial district, Ada, Ikirun (Oja tuntun) and Ororuwo all in Osun Central Senatorial district.

COLLECTION OF SAMPLES

Freshly prepared kunnu-zaki samples were purchased in plastic containers (as packaged by the seller) from different Senatorial market locations within Osun State. The samples were labeled and held at 4 ^{0}C by placing them in refrigerated coolers and conveyed in an ice packed cooler and transported to Microbiology and Biochemistry Department Laboratory of Joseph Ayo Babalola University within three hours (3 hours) of collection for microbiological and physico-chemical analysis respectively. A total of 108 kunnu-zaki drinks samples were purchased and sampled. The period of

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study was six months. All samples were subjected to the same analysis.

PHYSICO-CHEMICAL ANALYSIS OF KUNNU-ZAKI

The rate of fermentation by each bacterium on the kunnu-zaki sample was assessed by testing for pH and (TTA) Titratable acidity (AOAC, 2005).

DETERMINATION OF pH OF THE SAMPLES

The pH of the samples was determined using a laboratory pH meter (Jenway 3015, model 10). The pH was measured by putting 10 ml of the kunnu-zaki into a beaker and readings were taken by dipping the electrode into the kunnu-zaki samples (rinsing out the electrode with distilled water), and measurements taken from the display screen when the readings stabilized (Ofori *et al*.,1994).

DETERMINATION OF TOTAL TITRATABLE ACIDITY (TTA)

This is to measure the acidity in a sample. The Total Titratable acidity (TTA) calculated as percentage lactic acid was determined following the method described by (AOAC, 2005).

PROCEDURE

A 10 ml of kunnu-zaki sample was measured into a dried conical flask, 3 drops of phenolphthalein indicator was added and the flask thoroughly shaken. It was then titrated against 0.1 M NaOH (Sodium hydroxide) to a pink colour end-point (compared against a white background) and the titre volume was noted and calculated.

DETERMINATION OF MICROBIAL COUNT

This was carried out using standard microbiological techniques following appropriate dilution on agar plates of Nutrient Agar (NA) for bacteria count, MacConkey Agar (MCA) for coliform count, Eosin Methylene Blue agar (EMB) for *E. coli* and Potato Dextrose Agar (PDA) for fungi count. The agar media were of oxoid grade using pour plate method (Lateef *et al*., 2004).

PROCEDURE

Serial dilution was carried out using 1 ml of kunnu-zaki samples into9 ml of water. Stocks were prepared in test-tubes serially in rows containing 9.0 ml of distilled water representing each sample from each location (APHA, 2001). Then from each suspension 1 ml of dilution was pipette into a set of Petri-dishes and was overlaid with 20 ml of freshly prepared Nutrient agar (NA), MacConkey (MCA) and Eosin methylene blue (EMB) while others were overlaid with 20 ml of freshly prepared potato dextrose agar (PDA) treated with chloramphenicol (1 per 100 ml) prior to sterilization. Colonies which develop after incubation for 18 to 24 h at 37 ^{0}C on the cultured plates were counted using Gallenkamp colony counter and were sub-cultured to obtain pure isolates. The pure isolates were aseptically transferred into Nutrient agar slants and incubated. After 24 h incubation morphological characterization and biochemical tests were carried out on the isolates to aid identification to genus level on bacteria (Cowan and Steel 1985). The purified fungal isolates were identified on the basis of standard cultural, morphological and microscopic characteristics as described by (Samson *et al*., 1988). The fungal isolates were sub-cultured on to a sterile fresh media and incubated at room temperature for 3-5 days. Morphological identification was based on colour, textures spreading rate of each colony on the Potato dextrose agar plate.

DETERMINATION OF MOST PROBABLE NUMBER (MPN)

The multiple tube method was used to estimate coliform numbers. The presumptive, confirmed and completed test was done by adding varying quantities of kunnu-zaki such as 0.1 ml, 1.0 ml and 10 ml to varying quantities and strength of MacConkey broth in test tubes containing Durham tube (APHA, 2001). The MPN of coliforms present in the sample was estimated by making reference to McCrady table or probability table (APHA, 2001).

III. RESULTS AND DISCUSSION

The results show that the pH of the samples ranged from 3.09 - 4.21 in East and West Senatorial district (Table 1).

The result of the analysis revealed that all samples used have an acidic pH range of 3.09 - 4.21. The level of acidity of kunnu has been reported by several researchers including Efiuvwevere and Akoma, (1995) and Akoma *et al*., (2006).

The TTA of the samples ranged from 0.0032 - 0.0049 in central and east Senatorial district (Table 2). East Senatorial district had the highest TTA values of kunnu-zaki drink in the month of July according to the period of samples collection.

All the kunnu-zaki drinks sampled were contaminated with varying level of bacterial load ranging from 4.2 x 10^3 to 15.0 x 10^3 CFU/ml in West and East Senatorial district (Table 3). This is in agreement with the findings of Hatcher *et al*., (1992), Elmamood and Doughari, (2007), Lawal (2012) and Aboh and Oladosu, (2014) who reported a total bacterial count of 5.0 x10^4 to 2.0 x 10^6, 1.0 x 10^2 to 8.9 x 10^4 CFU/ml, 5.0 x 10^4 to 1.79 x 10^5 CFU/ml and 5.1 x 10^2 to 2.0 x 10^8 CFU/ml respectively. The high bacteria count observed in this study might be attributed to factors such as the environment, which include exposure of the foods (kunun-zaki) to air, soil, type of water used in processing, post production operations and personal hygiene of the handlers (Kawo and Abdulmumin, 2009; Aboloma, 2008). Exposure of the foods to air or dust at the point of sale is likely to increase the counts of the bacteria as virtually most of the

bacteria are carried in aerosols by dust and air (Karagozlu *et al*., 2007). Also, during production, most handlers sometime dip their hands into the containers while making kunun-zaki.

The total coliform bacterial count in the kunnu-zaki drink samples using MPN method ranged from 3 MPN/ml to 43 MPN/ml in East, 3 MPN/ml to 93 MPN/ml in West and 3 MPN/ml to 39 MPN/ml in Central. All the samples 108/108 (100%) of the kunnu-zaki samples examined had coliform count (Table 4). The result shows that West district had the highest total coliform count.

Seven (7) bacteria genera were isolated from the kunnu-zaki drink samples. These include *Escherichia coli*, *Pseudomonas species*, *Staphylococcus species*, *Enterobacter species*, *Klebsella species*, *Streptococcus species* and *Bacillus species*. The detection of *S. species* was equally isolated by Olasupo *et al*., (2002) from wara and kunun-zaki, a cereal based, non-alcoholic beverage, also by Aboh and Oladosu, (2014) from kunun-zaki. The presence of organisms like *Bacillus species*, *Streptococcus* has been reported by Adeyemi and Umar, (1994) and Ayo, (2004) who also isolated these organisms from kunun-zaki. The presence of *Klebsiella species* as recorded in this study is usually associated with faecal contamination. Being an enteric bacterium its presence indicates poor practices among handlers. Due to the significance of the faecal-oral route transmission for many bacterial food-borne diseases, basic hygiene measures assume a decisive importance in food safety management (Uzeh *et al*., 2006). The high occurrence of *E. coli* (77.78%) in kunnu-zaki is an indication of faecal contamination. Ironically, most food handlers do not practice good personal hygiene and do not follow good manufacturing practices, which could reduce the occurrence of such bacteria in foods (Bukar *et al*., (2009); Kawo and Abdulmumin, (2009)). Egbere *et al*., (2007) reported the presence of organisms like *Bacillus cereus, Staphylococcus aureus and Escherichia coli* (PHLS, 1996). It is possible that contamination by these pathogens could have occurred during sieving and packaging, as most of the people involved in the production, packaging and hawking do not take necessary precautions, and as such contamination could be very prominent (Elmamood and Doughari, 2007).Gadaga *et al*., (2004) reported that pathogens have been isolated from some fermented foods and some laboratory tests have shown the possibility of pathogens to survive and grow in some fermented foods. He said post processing contamination is often cited as the major cause of food poisoning, this agrees with the observation made as regards the source of kunnu collected for this study which is not really hygienic. Gadaga *et al*., (2004) equally said that *Escherichia coli*, is the most commonly encountered pathogens in African fermented food. This tally with the types of organism isolated in this study. The occurrence of bacterial pathogens in fermented foods suggests a need for caution in the consumption of this beverage. The source of contamination may also have come from the spices used as additives (Essien *et al*., 2011 and Lawal, 2012).

Fungal count ranged from 3.3 x 10^6 to 8.0 x 10^6 CFU/ml in West and Central district (Table 5). A total of Four (4) different moulds were isolated from the kunnu-zaki drink samples in the course of study. These were *Aspergillus fumigatus, Penicilium italicum, Aspergillus niger and Aspergillus flavus.*

The frequency of occurrence of the organisms isolated from kunnu-zaki drink samples are shown in Table 6. *Escherichia coli* and *Streptococcus species* were found in 77.78% (84/108) of samples examined, *Pseudomonas species* (33%), *Staphylococcus species* (66.67%), *Enterobacter species* (55.56%), *Klebsella species* (44.44%), *Bacillus species* (33.33%), *Aspergillus flavus* was found in 77.78% (84/108) of the samples examined, *Aspergillus fumigatus* (44.44%), *Penicilium italicum* (66.67%) and *Aspergillus niger* (55.55%). *Aspergillus species* is ubiquitous, aerobic fungi that can survive in air, dust. The presence of fungi may be attributed to the acidic nature of the sample since it has been observed that molds are capable of utilizing organic acids. Also the presence of fungi in the food may lead to food poisoning and contaminated fungi result in the production of undesirable odour, colour changes and loss of taste of the sample. The presence of these organisms in the sample may be due to the nutritional composition of the millet; these nutrients are present in different proportions. *Aspergillus flavus* is a common mold in the environment, and can cause storage problems in stored grains. It can also be a human pathogen, associated with *Aspergillosis* of the lungs and sometimes causing corneal, otomycotic of aflatoxin and nasoorbital infections. *Aspergillus flavus* spores are allergenic (Klich, 2007). Ijabadeniyi (2007) and Omemu (2011) isolated *Penicillium, Aspergillus, Mucor* and *Rhizopus* during the early stage of maize fermentation for ogi production.

The bacterial and fungal counts showed that all the kunnu- zaki sampled had a high prevalence of these contaminants and spoilage organisms which of course is of great concern. They are therefore considered microbiologically unsafe for human consumption when not hygienically prepared and utensils cleaned regularly. Clusters of infection caused by the organisms have been linked to poorly maintained utensils, poor personal hygiene, improper storage facilities, and poor water supply (Oyelana and Coker, 2012). Also it can be linked to unhygienic human handlers, dispensing of the extract into bottles, addition of flavors and sweeteners and process of cooling of the extract (Omemu *et al*., 2007a).

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Many native African beverages are little known outside the parent continent. Effort should be made to improve in the quality, shelf-life and production process of these indigenous beverages so that large- scale production for export outside the continent can be carried out. Many people now prefer imported and exotic beverages because of their attractive form, long shelf life, ease of transportation and other forms of utility which consumers associate with them (Achi, 2005). As of now, there are no industries involved in production of Kunnu-zaki. Kunnu-zaki is widely believed to be of immense social, economic and medicinal importance to its numerous consumers (Akoma *et al*., 2006).

Table.1: pH Values of Kunnu-zaki Drink Sampled from Some Selected Towns in Osun State.

pH VALUES

	MONTHS											
	JUL		AUG		SEP		OCT		NOV		DEC	
TOWNS												
Gbongan	3.93	3.90	3.86	3.92	3.89	3.92	3.88	3.87	3.93	3.88	3.91	3.92
Ikire	4.03	3.98	4.00	3.99	4.01	4.03	4.02	4.01	4.00	4.01	3.97	4.00
Apomu	4.20	4.18	4.16	4.19	4.20	4.14	4.13	4.17	4.19	4.16	4.21	4.15
Ipetu-Ijesha	3.83	3.84	3.86	3.33	3.50	3.85	3.55	3.40	3.78	3.80	3.88	3.60
Owena	3.89	3.51	3.65	3.99	3.55	3.19	3.09	3.67	3.68	3.88	3.85	3.89
Ikeji-Arakeji	3.60	3.50	3.58	3.63	3.45	3.52	3.57	3.48	3.56	3.59	3.60	3.58
Ada	3.94	3.90	3.91	3.93	3.80	3.79	3.88	3.91	3.93	3.69	3.89	3.91
Ikirun	3.75	3.69	3.72	3.74	3.68	3.75	3.73	3.66	3.69	3.72	3.71	3.77
Ororuwo	3.73	3.72	3.71	3.70	3.67	3.73	3.69	3.71	3.70	3.66	3.72	3.69

Table.2: Total Titratable Acidity Values for Kunnu-zaki Drink Sampled from Some Selected Towns in Osun State.

TITRABLE ACIDITY VALUES

	MONTHS											
	JUL		AUG		SEP		OCT		NOV		DEC	
TOWNS												
Gbongan	0.0038	0.0041	0.0036	0.0038	0.0039	0.0037	0.0035	0.0040	0.0039	0.0037	0.0039	0.0041
Ikire	0.0036	0.0040	0.0042	0.0037	0.0041	0.0041	0.0039	0.0036	0.0038	0.0040	0.0033	0.0037
Apomu	0.0035	0.0038	0.0039	0.0036	0.0038	0.0036	0.0040	0.0038	0.0041	0.0041	0.0038	0.0036
Ipetu-Ijesha	0.0037	0.0041	0.0043	0.0039	0.0040	0.0038	0.0039	0.0041	0.0038	0.0037	0.0035	0.0039
Owena	0.0049	0.0047	0.0037	0.0040	0.0038	0.0036	0.0037	0.0038	0.0037	0.0039	0.0040	0.0038
Ikeji-Arakeji	0.0040	0.0039	0.0038	0.0041	0.0036	0.0038	0.0037	0.0034	0.0041	0.0040	0.0039	0.0038
Ada	0.0037	0.0045	0.0038	0.0038	0.0039	0.0039	0.0037	0.0038	0.0039	0.0041	0.0040	0.0041
Ikirun	0.0036	0.0038	0.0040	0.0040	0.0041	0.0039	0.0032	0.0037	0.0044	0.0040	0.0039	0.0041
Ororuwo	0.0039	0.0041	0.0037	0.0039	0.0038	0.0040	0.0036	0.0041	0.0040	0.0039	0.0039	0.0038

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Table.3: Bacterial Count (CFU/ml) of Kunnu-zaki Drink Sampled from Some Selected Towns in Osun State.

BACTERIAL COUNT (CFU/ml)

	MONTHS											
	JUL		AUG		SEP		OCT		NOV		DEC	
TOWNS												
Gbongan	9.7 ×10³	9.0 ×10³	8.2 ×10³	9.5 ×10³	4.2 ×10³	8.0 ×10³	7.9 ×10³	7.5 ×10³	8.4 ×10³	8.6 ×10³	6.5 ×10³	7.0 ×10³
Ikire	6.5 ×10³	7.0 ×10³	7.5 ×10³	8.0 ×10³	8.0 ×10³	9.0 ×10³	7.6 ×10³	9.2 ×10³	9.3 ×10³	8.8 ×10³	10.0 ×10³	9.5 ×10³
Apomu	8.0 ×10³	8.8 ×10³	8.0 ×10³	9.1 ×10³	9.6 ×10³	8.9 ×10³	7.3 ×10³	7.4 ×10³	6.0 ×10³	9.2 ×10³	8.5 ×10³	6.4 ×10³
Ipetu-Ijesha	6.6 ×10³	7.8 ×10³	6.8 ×10³	6.1 ×10³	9.5 ×10³	7.4 ×10³	8.6 ×10³	6.6 ×10³	8.8 ×10³	9.3 ×10³	7.7 ×10³	8.1 ×10³
Owena	15.0 ×10³	6.1 ×10³	5.3 ×10³	4.9 ×10³	7.6 ×10³	9.7 ×10³	6.0 ×10³	7.1 ×10³	6.6 ×10³	9.1 ×10³	5.5 ×10³	7.1 ×10³
Ikeji-Arakeji	9.0 ×10³	7.5 ×10³	7.1 ×10³	5.2 ×10³	5.5 ×10³	7.3 ×10³	8.0 ×10³	8.5 ×10³	9.2 ×10³	7.3 ×10³	7.0 ×10³	8.1 ×10³
Ada	6.6 ×10³	4.7 ×10³	8.2 ×10³	6.7 ×10³	7.3 ×10³	8.4 ×10³	6.6 ×10³	9.1 ×10³	7.5 ×10³	6.2 ×10³	7.5 ×10³	7.5 ×10³
Ikirun	10.0 ×10³	8.2 ×10³	8.3 ×10³	9.9 ×10³	5.0 ×10³	8.9 ×10³	9.5 ×10³	9.0 ×10³	8.1 ×10³	9.7 ×10³	6.0 ×10³	9.1 ×10³
Ororuwo	7.5 ×10³	9.1 ×10³	8.6 ×10³	9.5 ×10³	8.0 ×10³	7.2 ×10³	9.5 ×10³	7.1 ×10³	7.2 ×10³	6.7 ×10³	9.0 ×10³	6.2 ×10³

Table.4: Coliform Count (MPN McCredy or Probability Table) of Kunnu-zaki Drink Sampled from Some Selected Towns in Osun State.

COLIFORM COUNT MPN/ml

	MONTHS											
	JUL		AUG		SEP		OCT		NOV		DEC	
TOWNS												
Gbongan	9	3	7	3	3	43	11	28	75	93	3	4
Ikire	20	43	28	21	20	39	3	23	4	23	13	11
Apomu	7	3	4	7	7	4	3	11	9	11	23	28
Ipetu-Ijesha	11	23	21	7	3	4	4	20	28	43	3	3
Owena	4	7	4	11	13	3	21	21	28	3	4	7
Ikeji	11	21	13	7	4	3	7	20	11	43	28	39
Ada	3	4	11	9	20	21	4	3	7	7	3	3
Ikirun	7	9	13	4	3	11	21	11	13	3	4	4
Ororuwo	3	4	11	39	13	9	3	11	39	4	11	3

Legend:

MPN= (Most Probable Number)

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Table.5: Fungi Count of Kunnu-zaki Drink Sampled from Some Selected Towns in Osun State.

	FUNGI COUNTS (SFU/ml)											
MONTHS	**JUL**		**AUG**		**SEP**		**OCT**		**NOV**		**DEC**	
TOWNS												
Gbongan	4.5 ×10^6	5.0 ×10^6	3.3 ×10^6	6.5 ×10^6	4.0 ×10^6	5.0 ×10^6	4.1 ×10^6	4.3 ×10^6	6.0 ×10^6	4.7 ×10^6	5.1 ×10^6	4.6 ×10^6
Ikire	4.2 ×10^6	5.8 ×10^6	3.3 ×10^6	5.9 ×10^6	5.0 ×10^6	6.0 ×10^6	5.7 ×10^6	6.7 ×10^6	6.9 ×10^6	6.4 ×10^6	5.0 ×10^6	6.0 ×10^6
Apomu	5.0 ×10^6	4.7 ×10^6	6.0 ×10^6	6.8 ×10^6	6.9 ×10^6	5.6 ×10^6	5.0 ×10^6	5.2 ×10^6	6.3 ×10^6	7.8 ×10^6	6.5 ×10^6	5.8 ×10^6
Ipetu-Ijesha	3.9 ×10^6	5.8 ×10^6	4.6 ×10^6	4.0 ×10^6	7.6 ×10^6	4.8 ×10^6	7.0 ×10^6	5.0 ×10^6	5.8 ×10^6	5.0 ×10^6	4.9 ×10^6	5.5 ×10^6
Owena	4.8 ×10^6	4.3 ×10^6	4.2 ×10^6	3.8 ×10^6	6.0 ×10^6	6.8 ×10^6	4.1 ×10^6	5.3 ×10^6	5.0 ×10^6	6.4 ×10^6	4.2 ×10^6	5.9 ×10^6
Ikeji-Arakeji	4.9 ×10^6	6.4 ×10^6	5.8 ×10^6	4.0 ×10^6	3.5 ×10^6	5.1 ×10^6	6.3 ×10^6	6.0 X10^6	4.9 X10^6	5.8 ×10^6	4.9 ×10^6	6.0 ×10^6
Ada	5.3 ×10^6	3.8 ×10^6	6.0 ×10^6	5.8 ×10^6	6.0 ×10^6	5.8 ×10^6	4.8 ×10^6	6.1 ×10^6	5.7 ×10^6	4.9 ×10^6	6.0 ×10^6	5.3 ×10^6
Ikirun	5.0 ×10^6	5.9 ×10^6	5.1 ×10^6	8.0 ×10^6	4.3 ×10^6	6.9 ×10^6	5.7 ×10^6	4.6 ×10^6	6.0 ×10^6	6.5 ×10^6	4.5 ×10^6	7.0 ×10^6
Ororuwo	6.0 ×10^6	5.7 ×10^6	5.5 ×10^6	5.9 ×10^6	5.5 ×10^6	5.8 ×10^6	7.9 ×10^6	5.5 ×10^6	5.0 ×10^6	4.8 ×10^6	6.5 ×10^6	5.0 ×10^6

Legend:
Sfu/ml = Spore forming unit per ml

Table.6: Frequency of Occurrence of Organisms Isolated From Kunnu-Zaki Drink Sample From Some Selected Towns in Osun State.

Isolates	**Gbongan**	**Ikire**	**Apomu**	**Ipetu**	**Owena**	**Ikeji**	**Ada**	**Ikirun**	**Ororuwo**	**% Occurrence**
Pseudomonas species	-	+	-	-	+	+	-	-	-	**33.33**
Bacillus species	-	-	+	-	+	-	+	-	-	**33.33**
Klebsiella species	-	+	+	-	+	+	-	-	-	**44.44**
Aspergillus fumigates	-	-	-	+	+	+	-	+	-	**44.44**
Aspergillus Niger	+	+	+	-	+	-	-	+	-	**55.55**
Enterobacter species	+	+	-	+	-	-	-	+	+	**55.56**
Staphylococcus species	+	-	+	-	+	+	-	+	+	**66.67**
Penicilium italicum	+	-	+	-	+	+	+	+	-	**66.67**
Escherichia coli	+	+	+	+	+	-	+	+	-	**77.78**

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Streptococcus species	-	+	+	+	-	+	+	+	+	**77.78**
Aspergillus flavus	+	+	+	+	+	-	+	-	+	**77.78**

IV. CONCLUSION

It is well known that kunnu-zaki is one of the locally made beverages that people purchase regularly for its cheap cost, sweet taste and its nutritional values. The presence of these isolated organisms in kunnu-zaki samples analyzed in Osun State could serve as indicator for the need to promote awareness about the possible health hazards that could arise due to handling and processing of the beverage. The range of microorganism isolated pose serious threat to food safety and hence the need to ensure microbial safety during the production and distribution of this drink that is widely consumed in some towns in Osun State, Nigeria.

REFERENCES

[1] Aboh, M. I., Oladosu, P. (2014). Microbiological assessment of kunun-zaki marketed in Abuja municipal area council (AMAC) in Federal Capital Territory (FCT), Nigeria. *African Journal Microbiology Resource*, 8 (15): 1633 – 1637.

[2] Aboloma, R. I. (2008). Microbiological analysis of bread samples from bakery to sale – point in Ado – Ekiti, Ekiti State, Nigeria. *Biology Environmental Science Journal Tropical*, 5 (3): 77 - 81.

[3] Achi, O. K. (2005). The potential for upgrading traditional fermented foods through biotechnology. African Journal of Biotechnology, 4 (5): 375 - 380.

[4] Adejuyitan, J. A., Adelakun, O. E., Olaniyan, S. A. and Popoola, F. I. (2008). Evaluating the quality characteristics of kunu produced by dry-milled sorghum. African Journal of Biotechnology, 7 (13): 2244 - 2247.

[5] Adeyemi, I. A. and Umar, S. (1994). Effect of manufacture on quality characteristics of kunu-zaki, a millet based beverage. Nigeria Food Journal, 12: 34 - 40.

[6] Akoma, O., Agarry, O. O. and Nkama, I. (2013). A study on the production and consumption pattern of Kunun-zaki: A cereal based ethnic fermented beverage of Northern Nigeria. British Journal Applied Science Technology, 3: 1220 - 1227.

[7] Amusa, N. A. and Ashaye, O. A. (2009). Effect of processing on nutritional, microbiological and sensory properties of kunun-zaki (a sorghum based non-alcoholic beverage) widely consumed in Nigeria. Pakistan Journal Nutrition, 8 (3): 288 - 292.

[8] AOAC. (2005). Association of official analytical chemists. Official methods of analysis (22nd edition). Washington D. C, USA, 1 (5): 115 – 119.

[9] APHA. (2001). Compendium of methods for the microbiological examination of foods, 4th edition, F. L. Downes and K, Ito (editions), American public health association, Washington, D. C., 34 - 170.

[10] Ayo, J. A. (2004). Microbiological evaluation of "kunun-zaki" and "zoborodo" drink (beverages) locally produced and sold in a polytechnic community in Nigeria. Nigeria Food Journal 22: 119 – 126.

[11] Egbere, O. J., Adik, C., Inyang, C. U., Henry, U. I. and Ogbonna, C. I. C. (2007). Microorganisms associated with fermentation and spoilage of kunun zaki. Nigeria Journal of Biotechnology, 18 (1-2): 49 – 55.

[12] Elmahmood, A. M. and Doughari J. H. (2007). Microbial quality assessment of kunnu-zaki beverages sold in Girei town of Adamawa State, Nigeria. African Journal Science, 1: 011 – 015.

[13] Essien, E., Monago, C., and Edor, E. A. (2011). Evaluation of the nutritional and microbiological quality of kunun (a cereal based non-alcoholic beverage) in Rivers State, Nigeria. The Internet Journal of Nutrition and Wellness, 10 (2).

[14] Gadaga, T. H., Nyanga, L. K. and Mutukumira, A. N. (2004). The occurrence, growth and control of pathogens in African fermented food. African Journal of Food Agriculture Nutrition and Development, 4: 5358 – 5374.

[15] Ijabadeniyi, A. O. (2007). Microbiological safety of garri, lafun and ogiri in Akure metropolis, Nigeria. African Journal of Biotechnology, 6 (22): 2633 - 2635.

[16] Klich, M. A. (2007). Aspergillus flavus: the major producer of Aflatoxin. Molecular Plant Pathology, 8 (6): 713 - 722.

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Evaluate the Efficiency of Gamma Irradiation and Chitosan on Shelf-Life of Strawberries Fruits

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Abstract*—Chitosan play an important role as an antifungal against Botrytis cinerea and the effect was a concentration dependent. The obtained results of in vitro experiment demonstrated that chitosan (4%) decreased radial growth of B. cinereato 2 %. Invivo the severity of infection reduced from 59.8 and 100.0 to 9.7, 33.8 and 40.1 in first, second and third week's storage periods at 13℃, respectively. Also, chitosan coating (4%) significantly caused an increase in fruit firmness whereas TSS was decreased with an increase by increasing in storage time. However,Vitamin C gave fluctuated results by increasing storage time. Gamma irradiation at 2.5 KGy reduced severity (%) of infected fruits from 55.5, 100 and 100 to 31.7, 45.9 and 49.9 and in healthy fruits severity (%) reduced from 48.9, 100 and 100 to 23.3, 25.1 and 29.1 in different storage periods 1, 2 and 3 weeks, respectively. Similarly, chitosan as well as gamma irradiation combination induced a significant increase of peroxidase enzyme (POD) activity. Induced changes in surface morphology and damage of cell structure caused by using chitosan shown by scanning electron microscopy. Also, gamma irradiation causes changes in hyphea structure and in surface morphology but combination of gamma irradiation with chitosan was more effective in altering fungus morphology and cell structure damage and no spore forming. This providing the efficiency of combination on reducing disease severity (%) of strawberry.*

Keywords*— *gamma irradiation, chitosan coating, strawberry fruits.

I. INTRODUCTION

Strawberries (*Fragaria x ananassa* Duch.) was a highly perishable fruit in a postharvest stage due to fungal infections. The shelf-life of fresh fruits at low temperature (0-4ºC) was around 5 days.

Braun and Sutton (1987) showed the postharvest decay represent major losses in horticultural industry. Losses during storage and shipment of fruits by *Botrytis cinerea* and *Rhizopus stolonifer* caused gray mould and soft rot, diseases, respectively.

Application of fungicides is most effective method to control postharvest disease. However, chemical control program face imminent problem first there are reports of on increasing number of fungicide-resistant strains of postharvest fungi and second due to health risk concerns. Thus, there is a growing need to one tactic that is being actively pursued involves: the use of bio-active substances**(Tarek 2004)**.

Chitosan, a high molecular weight cationic polysaccharide has been shown to be fungicidal against several fungi **(El-Ghaouth *et al*., 1990)**.

Vargas *et al*., (2006) found that, chitosan treatment of strawberry fruits delayed the occurrence of fungal infections compared with the uncoated fruits which started to decay from the beginning of storage.

Gianfranco Romanazzi, *et al.* (2013) found that the commercial chitosan formulation was effective in the control of gray mold and Rhizopus rot of strawberries when immersed in this solution and preserved for 4 days at 20±1C°.**Shiekh, *et al.* (2013)** confirmed that the chitosan is edible active coatings, maintain the quality and expand shelf-life of fresh fruits and prevent microbial damage.

Milena Petriccione *et al.* (2015); Reported that chitosan coating significantly reduced water loss and delayed the qualitative changes in color,titratable acidity and ascorbic acid content of strawberry also chitosan coating enhanced the activity of some antioxidant enzymes, preventing flesh browning and reducing membrane damage.

Chu *et al.* (2015); gamma irradiation was evaluated for its in vitro and in vivo antifungal activity against *Botrytis cinerea* on cut rose varieties. The irradiating dose required to reduce the population by 90% was 0.99 kGy. Gamma irradiation showed complete inhibition of spore germination and mycelia growth of *B. cinerea* especially 4.0 kGy in vitro.

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Combinatory treatments have also widely been investigated to give synergistic effects. Gamma irradiation in combination with other treatments (e.g., heat, washing, modified atmosphere storage and edible coating process) give an effective result in extending shelf-life of the fruits. **(Hussain *et al*., 2013)**.

II. MATERIALS AND METHODS

Strawberry fruits collected from different fields of El-Sharkia governorate were classified into two groups healthy and decayed fruits. Decayed fruits were examined after 3 day of storage at 13°C. The developing fungal colonies were picked up and examined.

Isolation, purification and identification of causal organisms:

Rotted fruits of strawberry were rinsed several time with sterilized water, surface disinfected by 70% ethanol, dried and cut into small pieces. These parts were cultivated in sterilized Petri dishes contained potato dextrose agar (PDA) and incubated at 20°C for 3 days. The growing fungi were isolated and purified on PDA and identified. The purified cultures were maintained on PDA and identified according to **Raper and Thom (1968)** in Mycological Lab.2 (ML2), Faculty of Science, Zagazig University. The media used for identification was Czapek's – Dox agar medium.

Isolation purification in vitro antifungal activity of chitosan:

The antifungal activity of chitosan against *Botrytis cinerea* were determined using PDA plates amended with (1، 2 and 4%) chitosan. The PDA plates were prepared then inoculated with disks (3mm diameter) of fungal growth taken from 7 days old culture of *Botrytis cinerea*. The linear growth of the fungus was measured when control plates reached full growth.

Preparation of inoculums

Botrytis cinerea was isolated from infected Strawberries and maintained on Potato dextrose agar (PDA). Condia of *B. cinerea* were recovered by filtering the mycelial suspension of 2 weeks old culture through 3 layers of sterile cheese cloth. The concentration of the conidial suspension was adjusted to 2 x 10^5 conidia per mL.

***In vivo* antifungal activity of chitosan**

Strawberries were immersed in a conidial suspension of *B. cinerea* containing 0.1% tween 80 and allowed to air dry at room temperature for 2 hrs. in order to fixed fungal infection. Different concentrations (0, 1, 2 and 4%) of chitosan were added individually to Erlenmeyer flasks (250ml capacity). Each contain 100 ml sterilized potato dextrose agar (PDA) media. The prepared media were poured in sterilized Petri dishes. After solidification, the dishes were inoculated singly at the center with equal discs (3 mm diameter) of fungal growth taken from 10 days old culture grown on PDA medium incubated at 20 °C. The linear growth of tested fungi was measured when the control plates reached full growth and the percentage of growth inhibition (%) calculated .Three replicates were used for each treatment.

After treated healthy and infected strawberries with chitosan or with gamma irradiation Strawberry fruits were examined for diseases assessment (Severity %) through different storage periods (weeks) under 13°C.

Radiation: Strawberry fruits were exposed to different gamma irradiation doses 1.0, 1.5 and 2.5 KGy in Indian Co^{60} gamma cell at the dose rate was 2.45kGy/hr at the time of experiment. Each treatment was replicated three times, each replicate contain 15 fruits. All treatments fruits and control were packed in perforated plastic containers and stored the Strawberry fruits were examined for disease assessment at different storage periods.

Chitosan treatment: chitosan solutions were prepared by dissolving 1, 2 and 4 gm of chitosan in 100 mL of distilled water with 2 mL acetic acid. Then heating with constantly agitation for 24 h. The obtained solution was adjusted to pH 5.5 by sodium hydroxide 0.1N; than 0.1 mL of tween 80 was added **(El-Ghaouth *et al*., 1991)**. Sprays of the different coating chitosan concentrations were applied and then stored the treated fruits.

Quality parameters:

1- **Total soluble solids (TSS):** TSS content expressed in ^{0}(Brix) was determined using a ago (Japan) NI refractometer according to **Kader (1991)**.

2- **Firmness:** Firmness (Firm) was measured as the maximum penetration force reached during tissue breaking of each fruit with hand penetrometer equipped with 1-9 mm diameter plunger (g/Cm^2) according to **Kader (1991)**.

3- **Ascorbic acid (Vitamin C):** Ascorbic acid content was determined by titration in the presence of 2.6 dichlorophenol- indophenol dye as an indicator against 2% oxalic acid solution as substrate. Ascorbic acid was calculated as milligram L - ascorbic acid per 100 mL of juice as described by **Lucoss (1994).**

Determination of peroxidase activity:

Samples of infected strawberry fruits treated with each antioxidant at 8 g/L, caraway oil at 700 µl/L and 2.5 kGy radiation dose, were collected after 10 days storage at 13°C for peroxidase activity assay. Also, infected fruits without treatment were used as control. Enzyme extract was obtained by grinding fruits tissues (2 ml/g fruits tissue) in 0.1 M sodium phosphate buffer at pH (7.1) in a porcelain mortar and extracted. The extracted tissues were strained through four layers of

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cheesecloth. Filtrates were centrifuged at 3000 rpm for 20 min. at 6°C. The clear supernatants were collected and considered as crude enzyme extract. Peroxidase activity was expressed as changes in absorbance/min at 425 nm according to the method of **Allam and Hollis (1972).**Determination of peroxidase enzyme was conducted in Central Lab. of Biotechnology, Plant Pathology Research Institute, Agricultural Research Centre, Egypt.

Scanning electron microscopy: Mycelia of *B. cinerea* grown in PD broth medium treated with chitosan 4 % and that from non-treated (control) were fixed in 2.5% glutaraldhyde at 4°C for 24 hr and post–fixed in 1.0% osmium tetraoxide for one hr at room temperature (**Harley and Fergusen, 1990**). The specimens were then dehydrated with ascending concentrations of acetone, critical point dried, and finally sputter coated with gold. The examination and photographing was done through Joel Scanning Electron Microscope (JSM – 1200 EX).

Conclusion

This study demonstrated that chitosan play an important role as an antifungal against *Botrytis cinerea* . Also, chitosan coating (4%) significantly caused an increase in fruit firmness whereas TSS was decreased with an increase by increasing in storage time. However ,Vitamin C gave fluctuated results by increasing storage time. Gamma irradiation at 2.5 KGy reduced severity (%) . but combination of gamma irradiation with chitosan was more effective in altering fungus morphology and cell structure damage and no spore forming. This providing the efficiency of combination on reducing disease severity (%) of strawberry.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Experimental design and statistical analysis:

All treatments in this study were arranged in complete randomized design. The obtained data were subjected to analysis of variance using the general linear module procedure of **SAS (1985),** where appropriate treatment means were separated using Duncan's multiple range test **(Duncan 1955)** and all percentages were transferred to angles before statistical analysis.

III. RESULTS

Antifungal activity of different chitosan concentrations on *Botrytis cinerea*

The obtained data from Table (1) and Fig. (1) show that the correlation between increased chitosan concentrations with decreased the linear growth of *Botrytis cinerea*.

Table.1: Effect of different chitosan concentrations on radial growth of Botrytis cinerea

chitosan concentrations %	Linear growth (cm)	inhibition %
0	9.0	0.0
1	7.0	30
2	5.0	50
4	2.0	80

* Means having the same letters in each column are statistically insignificant at 5% level

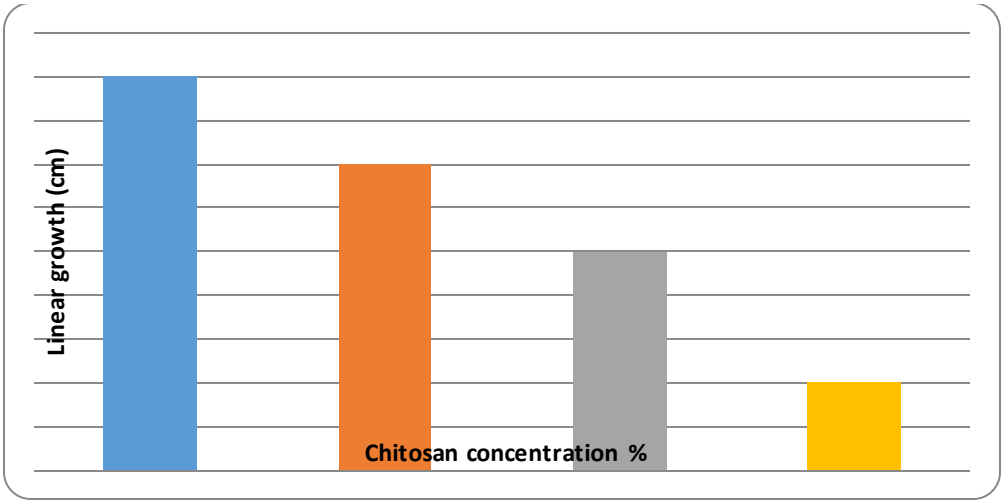

Fig.1: Effect of different gamma irradiation and chitosan treatment on (severity%) of strawberries fruits gray mold at different storage periods (weeks) at 13 ℃.

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Table.2: Effect of different gamma irradiation and Chitosan treatment on (severity %) of strawberries fruits gray mold at different storage periods (weeks) at 13 ℃.

Storage periods (weeks)	Gamma doses kGy	Severity %		Chitosan %	Severity %	
		Infected	Healthy		Infected	Healthy
1	0	55.5A	48.9A	0	59.8A	42.4A
	1	45.3B	40.1B	1	31.1B	21.6B
	1.5	38.4C	29.8C	2	20.1C	7.2C
	2.5	31.7D	23.3D	4	9.7D	2.4D
2	0	100.0A	100.0A	0	89.4A	77.66A
	1	73.8B	45.5B	1	57.3B	30.1B
	1.5	65.6C	41.7C	2	39.9C	20.4C
	2.5	45.9D	25.1D	4	33.8D	16.9D
3	0	100.0A	100.0A	0	100.0A	100.0A
	1	80.8B	54.4B	1	62.5B	40.4B
	1.5	69.7C	46.3C	2	53.4C	28.1C
	2.5	49.9D	29.1D	4	40.1D	19.2D

* Means having the same letters in each column are statistically insignificant at 5% level.

Data in Table (2) show that effect of different gamma irradiation doses (1, 1.5 and 2.5 KGy) and different chitosan concentrations (0, 1, 2 and 4%) coating on severity (%) of strawberry fruits at 13°C for different periods (1, 2, 3 weeks).

The obtained data show that as chitosan % increased the severity % decreased. The lowest severity % obtained at 4% chitosan. Also as the storage period increase the severity % increased. Moreover, as storage period increase the severity (%) increased, and different doses of gamma ray decreased the severity (%) and at 2.5 KGy is the effective dose decrease severity (%) in different storage periods.

Effect of chitosan treatments concentrations, storage time (weeks) and *Botrytis cinerea* infection on some strawberries quality parameters.

Data in Table (3) show that interaction between storage time and chitosan treatments on quality parameters of strawberry fruits, Data indicate that, treating strawberries with chitosan significantly decreased the values of TSS by increasing storage time (1, 2, 3 weeks) while an opposite effect was obtained in firmness which increased by using chitosan coating at different concentrations (0, 1, 2 and 4%), since at 4% chitosan give the highest values of firmness at different storage periods. Vitamin gave fluctuated values by increasing storage time.

Table.3: Effect of chitosan treatment concentrations, storage time (weeks) and Botrytis cinerea infection on some strawberries quality parameters at 13 ℃.

Storage periods (weeks)	Chitosan %	TSS (Brix)		Firmness (g/Cm^2)		Vitamin C	
		Healthy	Infected	Healthy	Infected	Healthy	Infected
1	00.0	7.21A	8.21A	423.3A	404.1A	0.020A	0.030A
	1	7.01B	6.73B	422.5B	400.0B	0.027B	0.025B
	2	6.88C	6.87C	448.7C	453.7C	0.019A	0.018C
	4	5.9B	7.1D	450.1D	457.6C	0.018A	0.015C

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2	00.0	5.9A	6.33A	342.7A	299.1A	0.019A	0.023A
	1	6.13B	7.1B	345.8B	301.8B	0.020B	0.025B
	2	5.7C	6.12A	352.1C	330.9B	0.023B	0.022A
	4	5.4C	5.91C	359.3D	345.5C	0.019A	0.021A
3	00.0	6.01A	6.01A	225.8A	198.01A	0.029A	0.027A
	1	5.79B	5.93B	235.3B	200.0B	0.028B	0.025B
	2	5.68C	5.01C	240.2C	214.8C	0.029A	0.024B
	4	5.35D	4.13D	245.7C	220.6D	0.031C	0.030C

* Means having the same letters in each column are statistically insignificant at 5% level

Combination of gamma irradiation and chitosan on strawberry fruits gray mold at different storage periods (weeks) at 13°C.

Data in Table (4) show that combination effect of gamma ray (2.5 KGy) and chitosan (4%) on severity (%) of gray mold on strawberry fruits. The combination between gamma ray (2.5 KGy) and chitosan (4%) was more effective to reduce severity (%) as compared when we used chitosan (4%) alone or when used gamma rays at (2.5 KGy) alone, since combination reduced severity (%) from 55.5, 48.9 to 8.5, 2.1 for infected and healthy fruits respectively at first week, from 100.0, 100.0 to 19.9, 8.7 for infected and healthy fruits respectively at second week and at third week severity (%) of infected and healthy fruits decreased from 100.0, 100.0 to 24.7, 18.9 respectively.

Table.4: Combination of gamma irradiation and chitosan on strawberry fruits gray mold (severity %) at different storage periods (weeks) at 13 ℃.

Storage periods (weeks)	Treatments	Severity %	
		Infected	Healthy
1	Control	55.5A	48.9A
	Chitosan (4%)	10.8B	4.5B
	2.5 KGy	31.7C	26.3C
	2.5 KGy + Chitosan (4%)	8.5D	2.1D
2	Control	100.0A	100.0A
	Chitosan (4%)	33.8B	16.9B
	2.5 KGy	48.9C	25.1C
	2.5 KGy + Chitosan (4%)	19.9D	8.7D
3	Control	100.0A	100.0A
	Chitosan (4%)	40.1B	19.2B
	2.5 KGy	49.9B	29.1C
	2.5 KGy + Chitosan (4%)	24.7C	18.9B

* Means having the same letters in each column are statistically insignificant at 5% level.

Effect of gamma irradiation (2.5 kGy), chitosan (4%) and combination between gamma irradiation and chitosan on peroxidase enzyme activity in strawberry fruits infected with *B. cinerea* and stored for one week

Results in Fig(2) Show that strawberry fruits inoculated with *B.cinerea* treated with combination of chitosan (4%) and gamma irradiation 2.5kGy induce higher activity of peroxidase (POD) enzyme. followed by chitosan(4%) and gamma irradiation 2.5 kGy irrespectively as compared with control fruits after one week storage periods.

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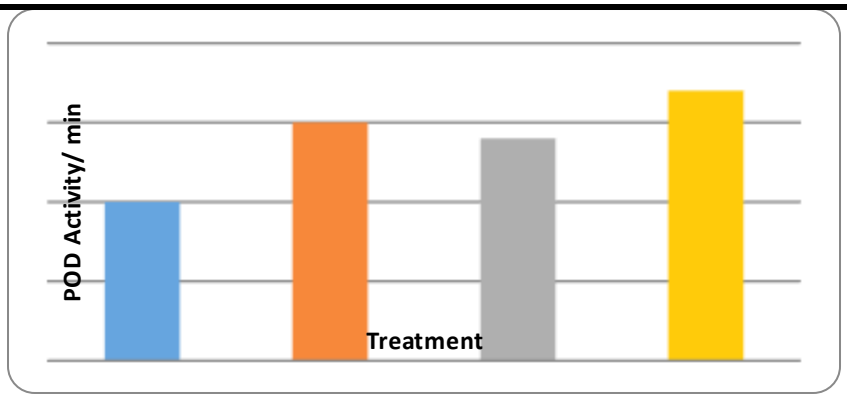

Fig.2: Effect of gamma irradiation (2.5 kGy),chitosan (4%) and combination between gamma irradiation and chitosan on peroxidase enzyme activity in strawberry fruits infected with B. cinerea and stored for one week

Scanning electron microscopy

Fig. (3) showed the morphological changes occurred in hyphae and conidiophores of *B. cinerea* treated with chitosan(4%) , gamma irradiation 2.5 kGy and combination between chitosan (4%) and gamma irradiation2.5kGy irrespectively

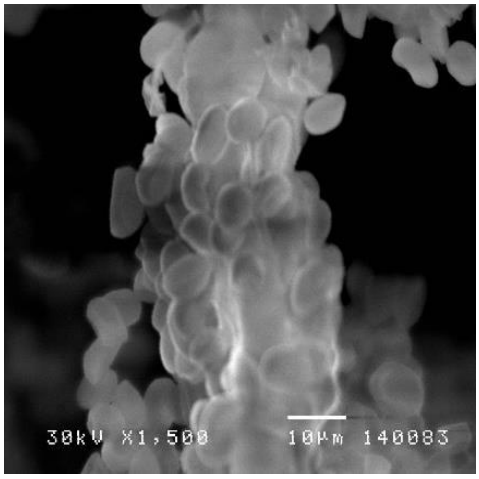

Fig.3 A) Control

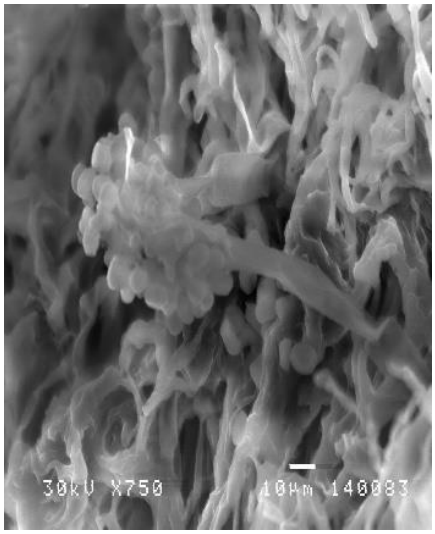

Fig.3 B) Chitosan treatment

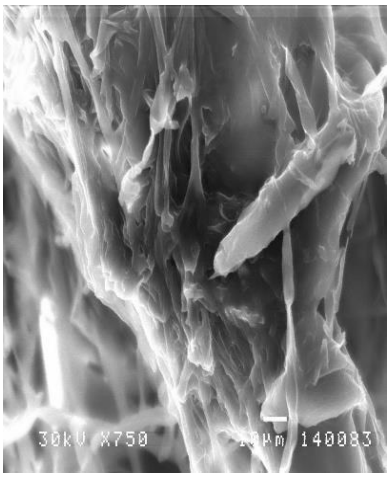

Fig. (3C) Gamma irradiation treatment

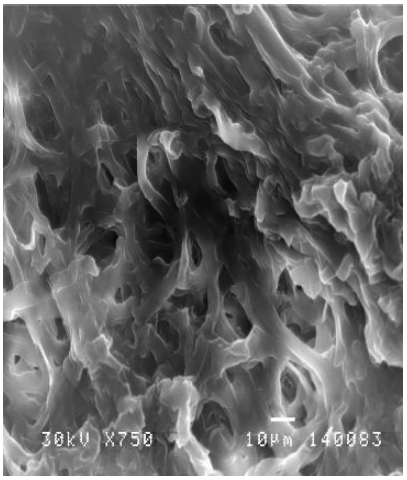

Fig. (3D) combination treatment

Fig.3: Scanning electron microscopy examinations of B. cinerea as affected by chitosan and gamma irradiation

It was found that control fungus *B. cinerea* have normal hyphea, sporangium, sporangiophore and normal cell wall and spore(Fig. 3A).

Chitosan treatment(4%) induced changes in surface morphology and cause damage to cell structure of *B.cinerea* and sporangiophore without spore(Fig. 3B).

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Gamma irradiation induced changes in surface morphology and cause damage to hypha also an affected sporangiophore(Fig. 3C).

The combination effect of chitosan(4%) and gamma irradiation2.5kGy on *B. cinerea* show more destructive effect in surface morphology and more effective damage to cell structure , corrugate surface and no spore found (Fig. 3D).

IV. DISCUSSION

Several studies have been performed to extend strawberry fruits shelf-life, using alternative methods rather than chemicals to avoid residues such as fungicide residues for the fruit itself **(Peng and sutton, 1991)** and to avoid pathogen populations from developing resistance to pesticides **(Bakkali *et al*., 2008)**.

Chitosan, a high molecular weight cationic polysaccharide, has been shown to be fungicidal against several fungi **(El-Ghouth *et al*., 1990)**.

The obtained results show that chitosan (4%) reduced the severity % of gray mold on different storage period and these results are in agreement with **Li and Yu (2000).** Confirmed the potential effect of chitosan to protect postharvest brown rot of peach caused by *M. fructicola* by decreasing the incidence, prolonging the incubation period and reducing of brown rot is correlated with chitosan induction of defence response, in addition to its antifungal property. **Romanazzi *et al*.(2000)** reported that strawberries dipped in 1% and 0.5% chitosan decreased the gray mold infection from natural inoculum after 10-days storage at 0C°. Followed by 4 days shelf-life. **Casariego(2004)** confirmed that chitosan films were also reported to inhibit the growth of fungi and yeasts in the area of contact, forming a halo of inhibition on the inoculated plates.

Atia*et al*., (2005)suggested that the mechanism by which chitosan coating reduced that decay of strawberries appear to be related to its fungistatic property rather than to its ability to induce defense enzymes such as chitinase, chitosanase and β-1,3-glucanase.and its capacity to stimulate plant defence mechanisms **(Aziz, *et al*.,2006)**

Ribeiro *et al.* (2007)explained that strawberry in non-climacteric fruits, but has a high postharvest respiration rate, which leads to a rapid deterioration at room temperature, coating with 1% chitosan reduced the growth rate of microorganisms in strawberries.

Romanazzi (2010)confirmed that pre-harvest and postharvest chitosan treatments of table grapes, strawberries and sweet cherries reduce their decay under field and during storage.

Besides its antifungal activity, chitosan also has the potential for inducing defenserelated enzymes **(Bautista-Bonas *et al*.,2006)** and phenolics in plants **(Benhamou,1996).**

Ben-Shalom *et al*., (2003) demonstrated that POD activity was elicited by chitosan in cucumber, resulting in an increase in resistance against*B. cinerea*. **Liu *et al*.(2007)**confirmed that chitosan inhibit the growth of *B. cinerea* and *P. expasumin vitro* and potently induce defense reactions in tomato fruits.

Li *et al.* (2000)used chitosan as a semi-permeable coating and found that it can maintain the qualities of the treated fruit and prolong its storage life, chitosan slows down the aging process of peaches by decreasing respiration rate and ethylene production, reducing malondialldehyde(MDA) production, stimulate superoxide dismutase(SOD) activity and maintaining membrane integrity.

Chitosan has a double mechanism of action: it reduces the growth of decay causing fungi, and it induces resistance responses in host tissues. With this double effectiveness chitosan can be considered as the first compound of a new class of plant protection products **(Atia *et al*., 2005)**.

Hernandez-Lauzardo*etal*. (2011) demonstrate the mode of action of chitosan on different fungal pathogen. They reported that molecules of chitosan can penetrate the intracellular level and interact with intracellular structure and cause damage.

Greater effects of chitosan to inhabit the growth of *B. cinarea* and cause serious damage to cell structure as well as the ability to form an impervious layer around the cell, therefore, chitosan could be considered as a potential alternative for synthetic fungicides (**Silva Junior *et al*.,2014)**.

SEM show that chitosan causing changes on morphology of *B. cinerea* and cause damage to cell structure also gamma irradiation cause changes in surface morphology and cause damage to hypha also effect on sporangiophore but combination between chitosan(4%) and gamma irradiation 2.5 kGy show more destructive effect in surface morphology and more damage to cell structure. these result arein agreement with **Swelim (2004) who** confirmed that scanning electron microscope showed that the decrease in sporulation and morphology abnormalities of *Fusarium solani* were occurred after irradiation with 6 , 8 and 10 kGy. Meanwhile low dose levels of 1, 2 and 3 kGy cause malformation and compactness of mycelia as well as absence of sporulation in *F. verticillioides*.

Our results indicated that treating strawberries with chitosan significantly decreased the value of TSS by increasing storage time(weeks) while an opposite effect was obtained in firmness which increased by chitosan coating but vitamin C would not be detected in clear level of amounts. These results are in agreement with **El-**

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Gaouth (1991) and Luna *et al*., (2001) who reported that greater firmness of fruits such as strawberries, tomatoes and peaches were obtained when fruits coated with a chitosan. Also, **Dam and Nguyen 2011** suggested that, all chitosan treatments enhanced the firmness of strawberries fruits compared to untreated fruits.

Gamma irradiation doses reduced the severity (%) of strawberry fruits in our obtained results and 2.5 kGy doses was the most effective doses decreased the severity % these obtained results are in agreement with **Shadia and Ehab (2011)** who confirmed that gamma radiation decreased the percentage of infection of strawberry fruits artificially inoculated with *B. cinerea* and naturally infected at 2.5 KGy compare with control.

The combination of chitosan and gamma radiation indicated that this treatment was the more effective in reducing severity (%) as compared when use every one alone.

REFERENCES

[1] **Allam, A. I. and Hollis, S. P. (1972)**: Sulfide inhibition of oxidase in rice root phytopathology, 62: 634-639.

[2] **Ana Niurka Hernandez-Lauzardo, Miguel Gerardo Velazquez-del Valle and Maria Guad Guerra-Sanchez, (2011):** Current status of action mode and effect of chitosan against phytopathogenis fungi. African J. Microbiol. Res., 2: 4243-4247.

[3] **Atia, M. M. M.; Buchenauer, H., Aly, A. Z. and Abou-Zaid, M. I. (2005):** Antifungal activity of chitosan against *Phytophthora infestans* and activation of defence mechanisms in tomato late blight. PolymBuul., 20: 83-88.

[4] **Aziz, A., Trotel-Aziz, P., Dhuicq, L., Jeander, P., Coudercher, M., Vernet, G., (2006):** Chitosan Oligomers and copper sulfate induce grapevine mildew. Phytopathology, 96: 1188-1194.

[5] **Bakkali, F.; Averbeck, S.; Averbeck, D. and Idaomar, M. (2008):** Biological effects of essential oils. A review: Food and Chem. Toxicol., 46: 446-475.

[6] **Bautista-Banos, S., Hernandez-Lauzardo, A. N., Velazquez-del Valle, M.G., Hernandez- Lopez, M., AitBarka, E. Bosquez-Molina, E., Wilson, C.L., (2006):** Chitosan as a potential natural compound to control pre and postharvest disease of horticultural commodities. Crop. Prot., 25, 108-118.

[7] **Benhamou, N., (1996):** Elicitor-induced plant defense pathways. Trends Plant Sci., 1: 233-240.

[8] **Ben-Shalom, N., Ardi, R., Pinto, R., Aki, C., Fallik, E., (2003)**: Controlling gray mould caused by *Botrytis cinerea* in Cucumber plants by means of chitosan. Crop. Prot., 22: 285-290.

[9] **Braun, P. G. and Sutton, J. C., (1987):** Inoculum sources of *Botrytis cinerea* in Fruit rot strawberries in Ontario. Can. J. Plant Pathol., 9: 1-5.

[10] **Casariego, A. (2004):** Obtencion de pellicles antimicrobian as a partir de quitosana. CienciayTecnologia de los Alimentos, 14: 11-14.

[11] **Dam, S. M. and Nguyen, H. X. P. (2011):** The effect of the neem seed extract (*Azadirachtinindica*), Chitosan and $CaCl_2$ on strawberry (*Fragariaananassa*) storage. The 12th Asian Food Conference, Thailand.

[12] **Duncan, D. B. (1955):** Multiple range and multiple F. tests. Biometrics, 11: 1-42.

[13] **El-Ghaouth, A.; Arul, J. and Ponnampalam, R., (1990):** The effect of chitosan on growth and morphology of *Rhizopusstolonifer*. (Abst.). Phytopathology, 80: 1020.

[14] **Eun- Hee Chu, Eun-Jung Shin and Hae-Jun Park (2015):** Effect of gamma irradiation and its convergent treatment for control of postharvest *Botrytis cinerea* of cut roses. Radiation physics and chemistry, 115: 22-29.

[15] **Harley, M.M. and Fergusen, I.K. 1990:** The role of SEM in pollen morphology and plant systematic. Association special, 41: 45-68, Clarendon press, oxford.

[16] **Hongye Li and Ting Yu (2000):** Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. Journal of the Science of Food and Agriculture, 81(2): 269-274.

[17] Hongye Li and Ting Yu, (2001): Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest Peach fruit. Journal of the Science of food and Agriculture, 18 (2): 269-274.

[18] **Hussain, P. R., Dar, R. S. and Wani, A. M. (2013):** Impact of radiation processing on quality during storage and post-refrigeration decay of plum (*Prunusdomestica* L.) cv. Santaroza. Rad. Phys. and Chem.. 4(1): 1016-1020.

[19] **Jia Liu, Shiping Tian, Xianghong Meng and Yong X.U., (2007):** Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruits. Postharvest biology and Technology 44: 300-306.

[20] **Kader, A. A. (1991):** Quality and its maintenance in relation to the posthavest physiology of strawberry. The strawberry into the 21st. century: Proc, third North Amer. Strawberry Conf. Timber press, Portland. OR, PP. 145-152.

[21] **Lucoss, E. H. (1994):** Determination ascorbic acid in large numbers of plant samples. Ind. Eng. Chem. Anal., 15: 649-652.

[22] **Luna, D.; Bustamanta, L. M.; Gonzalez, G.; Dominguez, S. J.; Bautista, B. S.; Shirai, K. and Bosquez, M. E. (2011):** Treatments on the quality of papaya fruit during storage. Proceeding of the Eighth international congress on Engineering Food. Technomic publishing Co. Inc., Lancaster, Abstr., PP.: 1042-1046.

[23] **Milena petriccione, Francesco Mastrobuoni, Maria Silvia pasquariello, Luigi Zampella, Elvira nobis, Giuseppe Capriolo and Marco Scortichini(2015)**; Effect of chitosan coating on postharvest quality and antioxidant enzyme system response of strawberry fruit during cold storage. Foods ISSN 2304-8158, 4: 501-523.

[24] **Peng, G. and Sutton, J. C. (1991):** Evaluation of microorganism for biocontrol of *Botrytis cinerea* in strawberry. Can. J. plant pathol., 13: 247-257.

[25] **Raper, K. B. and Thom, C. (1968):** A manual of the penicillia. Hafner publishing company, New York and London.

[26] **Rayees Ahmed Shiekh, Maqsood Ahmed Malik, Shaeel Ahmed Al-ThaBatti and Muneer Ahmed Shiekh (2013)**; Chitosan as a Novel Edible coating for fresh fruits. Review. Food Sci. Technol. Res., 19(2): 139-155.

[27] **Ribeiro, C.; Vicenta, A. A.; Teixeira, J. A. and Miranda, C. (2007):** Optimization of edible coating composition to treat strawberry fruit senesce. Postharvest Biol. and Tech., 44: 63-70.

[28] **Romanazzi, F.; Nigro, F. and Ippolito, A. (2000):** effect to di trattamenti pre postraccolta con chitosan sui marciumidellafragola in conservazionre. Rivista di Frutticoltura, 62(5): 71-75.

[29] **Romanazzi, G. (2010):** Chitosan treatment for the control of postharvest decay of table grapes, strawberries and sweet cherries. Fresh Produce 4 (Special Issue), 111-115.

[30] **Romanazzi**, **G., Feliziani, E., Santini, M. and Landi, L. (2013)**: Effectiveness of postharvest treatment with chitosan and other resistance inducers in the control of storage decay of strawberry. Postharvest biology and technology, volume 75. January, pages 24-27.

[31] **SAS "Statistical Analysis System" (1985):** SAS/STAT user's guide: statistics, vesion 6. 0.3 Edition. SAS Institute Ic. Cary, N. C. USA.

[32] Shadi, A. A. and Ehab, A. S. (2011): Effect of some Antioxidants, caraway oil (*Carium carvum* L.) and Gamma Radiation on Gray Mold of strawberry fruits during storage. J. Rad. Res. Appl. Sci., vol., 4(B): 1415-1428.

[33] **Silva Junior S., Stamford N., P., Lima M.A.B., Arnaud, T.M.S., Pintado, M.M. and Sarmento, B. F., (2004):** Characterization and inhibitory activity of chitosan on hypha growth and morphology of *Botrytis cinerea* plant pathogen. International Journal of Applied Research in Natural Products, 7(4): 31-38.

[34] **Swelim, M.A., (2004)**: Effect of gamma irradiation on growth and cellular structure of two *Fusarium* species. Isotope and Rad. Res., 36, 135-150.

[35] **Tarek, S.S.S. (2004):** Integrated control for minimizing postharvest diseases of strawberry. M.Sc. thesis, Fac. Agric. Ain shams Univ., 165pp.

[36] **Vargas, M.; Albors, A.; Chiralt, C. and Gonzalez – Martinez, S. (2006):** Quality of cold-stored strawberries as effected by chitosan-oleic acid edible Coatings. Postharvest Biol. and Tech., 41: 164-171.

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Evaluation of four plants compost teas against fungi responsible for Corn damping-off in Côte d'Ivoire

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Abstract*— *Corn (Zea mays L.) is one of the most important cereals in Côte d'Ivoire. However, Corn seeds and seedlings are susceptible to infection by a number of soilborne fungi which caused seeds decay before or after germination. The objective of this study is to evaluate the suppressive effect of Four plants compost teas (Chromolaena odorata, Ricinus communis, Nicotiana tabacum, Azadiracta indica) on Corn damping off. In vitro assays showed a most suppressive effect of C. odorata and R. communis compost teas on mycelial growth of pathogenic fungi. Results of in vivo trials showed significant reduction of Corn seedlings diseases incidence and high seed germination percent after treatment with C. odorata, R. communis and A. indica compost teas. No efficiency effect was noted with N. tabacum compost tea. This study demonstrated the usefulness of compost tea as an efficient biological tool for the control of fungi responsible of corn damping-off.

***Keywords*— Corn, damping-off, fungi, compost tea.**

I. INTRODUCTION

Corn (*Zea mays* L.) is an annual tropical herbaceous plant of the family Poaceae and it is one of the most important cereal grains grown worldwide. Corn is the world's top most cereal crop in terms of total production and productivity after wheat and rice (FAO, 2006). Corn is the second most important food crop in Côte d'Ivoire after rice with an annual production of about 600 000 tones (FAO, 2014). This commodity is widely grown for its starch-rich grains. It is also used as a forage plant in some localities. Corn is also the principal staple cereal diet of most of the Ivorian people who mainly lives in the North. In the last twenty years, Corn cultivation has experienced a considerable development of its market with urbanization and especially the establishment of food industries (Boone *et al*., 2008).

However, several diseases are observed on Corn and cause significant yield reduction (Sétamou *et al*., 1998). This decrease in yield is linked to the action of certain fungi that can have adverse effects on the aerial organs (ears, leaves and stems) or on the root system thus reducing the productivity of the plant. According to Harvey *et al* (2006), fungi such as *Aspergillus* sp., *Pythium* sp., *Fusarium* sp. and *Rhizoctonia* sp. would be responsible for root rot and seeding of corn.

Synthetic fungicides remain the most widely used control measure against fungal plant diseases. Although relatively effective, synthetic fungicides have two major drawbacks: their generally widespread lack of long-term efficacy caused by the development of resistance in plant pathogens (Avis, 2007). The fungicides, however, can have a negative effect on human health and the environment (Perez-Garcıa *et al*. 2011)

A possible alternative to synthetic chemical fungicides is to exploit the antimicrobial activities of compost teas. The potential of compost tea in the suppression of plant pathogens has been demonstrated (Litterick *et al*., 2004). Recently, compost teas from sheep manure compost showed antimicrobial activities against phyllosphere (Koné *et al*., 2010) and rhizosphere (Dionne *et al*., 2012) pathogens of tomato (*Solanum lycopersicum* L.) plants. In addition, Compost teas was considered safer for health and the environment (Siddiqui *et al*., 2009).

The objective of this study is to identify the fungi responsible of seed-borne fungi in Corn seeds collected from different areas and evaluate compost tea of four plants on some fungal pathogens.

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1. MATERIALS AND METHODS

1.1. SAMPLING AND ISOLATION OF FUNGI

Samples of ungerminated corn kernel showing rot symptom were removed 15 days after planting from Corn fields in three cultivation areas of Northern Côte d'Ivoire (Katiola, Korhogo, Odienne). Samples were transported to the laboratory for fungi isolation. To obtain fungal population from rotten Corn kernel, samples were disinfested in 2% sodium hypochlorite for 3 min, rinsed twice in distilled water for 5 min and air-dried at 26ºC for 1 to 2 h in a sterile Lamina flow hood. Sterilized kernels were placed into Petri dishes contained PDA medium, each

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medium containing 3 kernels was incubated at 28±2°C. Mycelial growths from the corn kernel were transferred (picked with flamed needle from the periphery of growth) to fresh PDA plates. Sub-culturing was carried out to obtain pure isolates which were maintained on PDA. To confirm the identification of isolated fungi, microscopic observation of the morphological characteristics of the isolates, grown for two weeks in Petri dishes containing Potato Dextrose Agar (PDA), was performed using identification keys (Botton et al. 1990, Barry and Barnett, 1972). The frequency of each isolated fungi was calculated by using the following formula (1)

$$F\,(\%) = \frac{Nc}{Nt} \times 100 \quad (1)$$

Where, **F**: Frequency (%), **Nc**: Number of genus or species isolated, **Nt**: total number of isolates

1.2. PATHOGENICITY TEST

1.2.1. INOCULATION OF SEEDS

Isolated fungi were analysed for their capacity to inhibit seed germination and the development of roots. Briefly, kernels corn was surface-disinfected for 3 min in 2% sodium hypochlorite, rinsed twice with distilled water. Corn kernels were immersed for 1mimute in a $10^6 mL^{-1}$ micro conidial suspension and placed into Petri dishes on sterile water saturated filter paper. three dishes containing 15 seeds each were used for each isolated fungi and un-inoculated seeds were used as controls. After 8 days, disease symptoms were estimated by determining the percentage of germinated seeds.

1.3. EVALUATION OF ANTIFUNGAL ACTIVITY OF COMPOST TEA

1.3.1. PREPARATION OF COMPOST TEA

Four extracts prepared from different compost (C_1, C_2, C_3, and C_4) were used (Table1). Original composts were produced according to an aerobic process (Znaidi, 2002). Extract production consists on suspending composts in tap water (1 :5, v/v) in 20 litter plastic container and stirring the mixture daily for about 10 min during an extraction period of 5 days (Weltzein, 1992). After the incubation period, the mixture was filtered through cheesecloth (250 µm) and the obtained extract were stored at 4 °C.

Table.1: compost ingredients

Composts	Compositions
C_1	20 % Cm+ 80 % *Chromolaena odorata* leaves
C_2	20 % Cm+80 % *Ricinus communis* leaves
C_3	20 % Cm+80 % *Azadirachta indica* leaves
C_4	20 % Cm+80 % *Nicotiana tabacum* leaves

Cm : Cattle manure

1.3.2. EFFECT OF COMPOSTS TEAS ON THE MYCELIAL GROWTH OF CORN DAMPING-OFF FUNGI

Antifungal activity of compost tea was evaluated using three fungi cultured on agar plates at concentration 60 $g\,L^{-1}$ and unamended media were used as controls. The prepared composts teas were added to conical flasks containing previously sterilized and cooled agar medium. After thorough mixing, 15mL of media were poured into sterilized Petri dishes 9 cm in diameter. Fungal plugs (0.5mm in diameter) were removed with a cork borer from the growing margin of each fungus colony and placed at the center of the test plate. Five replications were made for each treatment and the cultures were incubated at room temperature. Colony diameter was measured in two directions daily until the fungus covered the whole of the agar in the control plate. Data were expressed as growth rate (mm/day) relative to control. Percentage inhibition (mycelial growth) was determinate.

1.3.3. *IN VIVO* EFFECT OF COMPOST TEAS AGAINST KERNEL CORN PATHOGENS

Corn seeds were disinfected for 3 min in 2% sodium hypochlorite and rinsed with sterile distilled water. After drying, seeds were placed under sterile conditions in Petri plates on filter papers soaked with sterile distilled water. Seed germination was assessed after 4 days in a growth chamber at 27 °C (Hibar *et al*., 2005). For each fungal pathogen, germinated seedlings were drenched with 100 ml of conidia suspension (10^6 conidia mL^{-1}). For non-inoculated control distilled water was used. Then inoculated seedlings were transferred to plastic pots filled with the following substrates: T_0: CMS (Corn Meal Agar) +20 mL of distilled water (control), T_1: CMS+20 mL of C_1, T_2: CMS+20 mL of C_2, T_3: CMS +20 mL of C_3, T_4: CMS +20 mL of C_4. The experiment was carried out in a growth chamber at 27°C with a 12- hour-photoperiod. For each treatment 15 pots were used and 3 seeds were planted per pots. Seedlings were watered daily. As a measure of disease severity, seedling stands were counted 25 days after inoculation. Low percent seedling stands indicated high disease severity, whereas high percent-seedling stands indicated high disease suppressiveness. Percent-seedling stands were calculated and the mean value of five replications was considered.

1.4. STATISTICAL ANALYSIS

Means and standard error of the mean were calculated for the mycelial growth inhibition and germinated seeds after composts teas treatment measured for the three sets of experiments in each case. These means were statistically compared using the LSD Fischer test was used to determine if they were significantly different at $P < 0.05$.

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II. RESULTS

2.1. Identification and frequency of isolated fungi

In total, seven fungal genera associated with corn kernel rot was isolated on PDA medium and were identified based on the morphologic and cultural characteristics as Aspergillus sp, *Colletotrichum* sp, *Fusarium* sp, *Pythium* sp, *Rhizoctonia* sp, *Rhizomucor* sp, *Trichoderma* sp. *Trichoderma* sp. was the most commonly isolated fungal species among all of the isolates obtained (48.06%) followed by *Fusarium* sp. (34.73%) and *Rhizoctonia* sp (13.53%). *Aspergillus* sp (3.42%) and *Colletotrichum* (6.23%) had the lowest isolation rates (Figure 1).

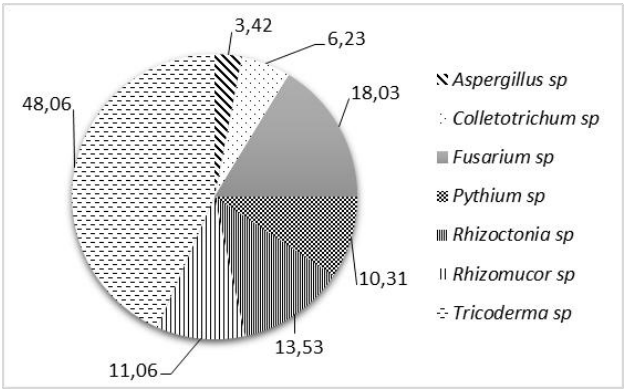

Fig.1: Proportion of fungal species in the mycoflora of Corn grain

2.2. PATHOGENICITY TESTS

The pathogenicity test with fungi associated with corn kernel rot was carried out. Results based on the *in vitro* seeds inoculation test showed significant pathogenic effects of *Pythium*, *Fusarium* and *Rhizoctonia* on seeds germination and seedlings health due to high infection. *Pythium* sp and *Rhizoctonia* reduced significantly seeds germination with percentage germination of 5 and 2% respectively. Seeds infected by *Fusarium* sp showed percentage germination of 55%. The seed samples inoculated with *Aspergillus* sp, *Colletotrichum* sp, *Rhizomucor* sp and *Trichoderma* sp showed similar percentage germination with the control seeds (Figure 2).

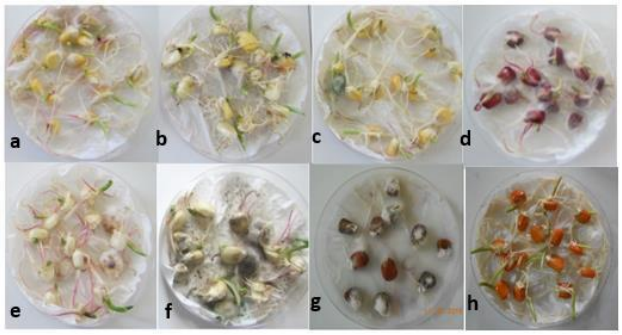

Fig.2: Fungi virulence assay on corn seed

a: *Rhizomucor* sp, **b:** *Colletotrichum* sp, **c:** *Aspergillus* sp, **d:** *Fusarium* sp, **e:***Trichoderma* sp, **f:** *Pythium* sp, **g:** *Rhizoctonia* sp, **h:** control

2.3. SUPPRESSION OF MYCELIAL GROWTH BY PLANTS COMPOST TEA

Results showed that the tested compost teas induced a significant interaction with the tested phytopathogenic fungi. The radial growth of *Pythium*, *Rhyzoctonia* and *Fusarium* noted after 7 days of incubation, was significantly ($p < 0.05$) reduced in compararison to the controls. In fact, *Chromolaena odorata* (C_1) and *Ricinus communis* (C_2) compost tea reduced significantly mycelial growth of all tested fungi with inhibition rates ranged from 85 to 100%. The compost tea C_3 was also effective against *Rhizoctonia* sp and *Fusarium* sp with inhibition ratios of 94.09 and 95.78 % respectively. Compost tea C_4 showed the less inhibitory effect reducing mycelial growth of *Pythium* and *Fusarium* by approximately 45 and 50% respectively (Table 2).

Table.2: In vitro effect of compost teas on mycelial growth of Pythium, Rhizoctonia, Fusarium

Compost teas	Mycelial inhibition rate (%)		
	Pythium	Rhyzoctonia	Fusarium
C_0	$34{,}59\pm5{,}30^{e}$	$22{,}36\pm2{,}63^{c}$	$43{,}33\pm2{,}88^{d}$
C_1	$92{,}82\pm1{,}93^{a}$	$94{,}09\pm1{,}93^{b}$	$97{,}46\pm0{,}00^{b}$
C_2	$85{,}23\pm1{,}93^{b}$	$96{,}62\pm1{,}46^{b}$	$100{,}0\pm0{,}00^{a}$
C_3	$65{,}40\pm7{,}30^{c}$	$94{,}09\pm1{,}46^{b}$	$95{,}78\pm1{,}46^{b}$
C_4	$45{,}56\pm3{,}34^{d}$	$100{,}00\pm0{,}00^{a}$	$50{,}21\pm6{,}97^{c}$
F	10.42	8.40	10.35
P	0.015	0.038	0.015

Within a column, means with the same letter are not significantly different according to Fisher's LSD test ($P = 0.05$).

2.4. *IN VIVO* EVALUATION OF THE EFFECT OF COMPOSTS ON CORN KERNELS GERMINATION

The percentage germination of corn kernels varied significantly according to compost tea treatments. In fact, the lowest percentage germination ranged from 16.29 to 53% was observed on the inoculated soil without compost tea treatment (T_0) and soil treated with *nicotiana tabacum* compost (T_4) (Table 3). In these subtract seedling showed disease symptoms include rotted seed that is soft and brown, rotted roots with a wet and slimy appearance. Above ground symptoms include damping-off after emergence and seedlings that turn yellow, wilt and die. Results recorded on inoculated soil treated with *C. odorata* and *R. communis* compost showed highest percentage germination (100%). *C. odorata* and *R. communis* exhibited remarkable potency in suppressing seeds rot and seedling blight (Figure 3).

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Table.3: In vivo effect of compost teas on corn kernels germination.

Compost teas treatment	***Germination rate (%)***		
	Pythium	*Rhyzoctonia*	*Fusarium*
T_0	$44{,}44 \pm 8{,}11^d$	$16{,}29 \pm 3{,}39^d$	$43{,}40 \pm 10{,}02^c$
T_1	100^a	100^a	100^a
T_2	100^a	100^a	100^a
T_3	$91{,}11 \pm 8{,}01^b$	$94{,}07 \pm 5{,}59^b$	$95{,}55 \pm 5{,}87^b$
T_4	$53{,}12 \pm 5{,}87^c$	$43{,}70 \pm 5{,}59^c$	$47{,}41 \pm 6{,}78^c$

Within a column, means with the same letter are not significantly different according to Fisher's LSD test ($P = 0.05$).

T_0 control ; T_1 : *C. odorata* compost tea treatment ; T_2 : *R. communis* compost tea treatment ;T_3 : *A. indica* compost tea treatment ; T_4 : *N. tabacum* compost tea treatment

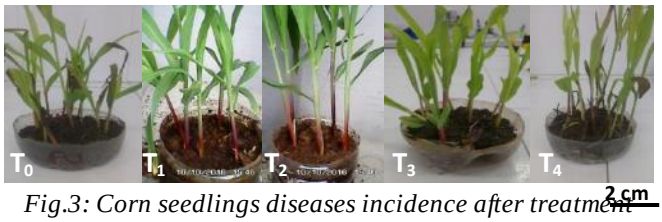

Fig.3: Corn seedlings diseases incidence after treatment with compost teas

T_0: Inoculated soil without compost tea treatment, **T_1** : *C. odorata* compost tea treatment ; **T_2** : *R. communis* compost tea treatment ;**T_3** : *A. indica* compost tea treatment ; **T_4** : *N. tabacum* compost tea treatment

III. DISCUSSION

Based on morphological characteristics, seven fungal genera were identified in total after isolation from corn kernels samples showing rot symptoms. These genera include *Aspergillus* sp, *Colletotrichum* sp, *Fusarium* sp, *Pythium* sp, *Rhizoctonia* sp, *Rhizomucor* sp, *Trichoderma* sp. In this present study most of the obtained fungus genera (*Colletotrichum* sp, *Rhizomucor* sp, *Trichoderma* sp, *Aspergillus* sp) were saprophytic. Survey conducted by Niaz *et al*. (2009) showed that these fungi were frequently found in corn kernel. The abundant growth of saprophytic fungi on Corn seeds implies that storage problems should also be studied. It is now imperative that efforts should be made to continuously evaluate the seed health of Corn seeds produced in Côte d'ivoire. This current survey showed that *Fusarium* sp, *Pythium* sp and *Rhizoctonia* sp. induced corn kernel rot and seedling blight. Recent study conducted by Tesfaye and Dawit (1998) in Ethiopia showed that several phytopathogenic species included *Fusarium* spp were found to be associated with damaged corn kernel. (Girma, 2009) also identified *Fusarium* species associated with Corn grain in Ethiopia. Kommedahl (1981) observations revealed that symptoms caused by these pathogens are: failure to emerge, wilting, chlorosis or yellowing, root rot and poor rot development. Concerning *Phythium* sp , Zhang *et al*. (2000) confirms that a variety of *Pythium* spp. have the capacity to reduce germination and cause lesions on roots of corn and soybean seed and seedlings. Investigations conducted by Dorrance *et al*. (2004) showed that *Pythium* spp. frequently are associated with seed and seedling diseases, and commonly have been isolated from corn.

The aqueous extracts of composts leaf of *Chromolaena odorata*, *Azadiracta indica* and of *Ricinus communis* showed a more significant antifungal activity against *Pythium* sp., *Rhyzoctonia* sp. and *Fusarium* sp. Compost treatments significantly reduced the incidence of the disease on seed germination. No disease was observed in soils treated with *Chromolaena odorata* and *Ricinus communis* composts. Our results are in agreement with those obtained by Scheuerell and Mahaffee in 2004. These authors have shown that the irrigation by compost extract of a culture substrate inoculated with *Pythium ultimum* at reduces the effect of cucumber blight caused by this pathogen. The study conducted by El-Masry *et al*. (2002) showed that the compost extract can control several pathogenic fungi like *Pythium debaryanum, Sclerotium bataticola* and *Fusarium oxysporum* f. sp. *lycopersici*. In addition, Khaled *et al*. (2005) showed that the treatment of the growing medium, used for growing tomato plants, by the various compost extracts interfered with *F. oxysporum* infection. *F.* sp. *radicis-lycopersici* and its expression, which greatly reduced the incidence of the disease. Compost tea effectiveness could be explained by antifungal substances contained in leaves of each plant. Monisha *et al*. (2013) and Koumaglo *et al*. (2009) suggested that the aqueous extract of *Chromolaena odorata* and *Ricinus communis* leaves had antifungal properties due to the presence of compounds such as phenols, tannins and flavonoids. Le Page and Bousquet (2007) showed that several organic chemicals present in compost or released by compost inhabiting microorganisms provided disease suppressive effects, including phenolic compounds, volatile fatty acids and salicylic acid.

To summarize the present study showed that compost tea prepared from various plants leaves had negative effect on corn kernel pathogenic fungi (*Fusarium* sp, *Pythium* sp, *Rhizoctonia* sp). They could constitute a promising alternative for a biological control of seed and seedling diseases and reduces the abusive use of synthetic fungicides. However, a more field comprehensive survey is needed to confirm our findings in the field under natural conditions.

REFERENCES

[1] Avis, T.J., (2007). Antifungal compounds that target fungal membranes: applications in plant disease control. Can. J. Plant Pathol. 29, 323–329.

[2] Barnett H.L. et Hunter B.B. (1972). Illustrated genera of imperfecti fungi.Third edition. Burgess Publishing Company.Minapolis, USA. 241 p.Biotechnol. 18:551-558.

[3] Boone P., Stathacos C.J.D. et Wanzie R.L. (2008). Evaluation sous régionale de la chaîne de valeurs du maïs, rapport technique ATP N°1. Bethesda, MD : projet, Abt Associates Inc.

[4] Botton B., Breton A., Fevre M., Gauthier S., Guy P.H., Larpent J-P., Reymond P., Sanglier J-J., Vayssier Y., Veau P. (1990). Moisissures utiles et nuisibles, importance industrielle. Collection Biotechnologies. 2è édition, Masson, Paris, 498 p.

[5] Dionne, A., Tweddell, R.J., Antoun, H., Avis, T.J., (2012). Effect of non-aerated compost teas on damping-off pathogens of tomato. Can. J. Plant Pathol. 34, 51–57.

[6] Dorrance A.E, Jia H, Abeny T.S. (2004). Evaluation of soybean differentials for their interaction with *Phytophthora sojae*. Plant Health Prog. doi: 10.1094/PHP-2004-0309-01-RS.

[7] El-Masry M.H, Khalil A.I, Hassouna M.S, Ibrahim H.A.H. (2002). In situ and in vitro suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. World J. Microbiol.

[8] FAO, (2006). FAOSTAT. Food and Agriculture Organization of the United Nations

[9] FAO, (2014). FAOSTAT. Food and Agriculture Organization of the United Nations

[10] Girma Tegegne, Fekede Abebe, Temam Hussien, Tewabech Tilahun, Eshetu Belete, Melkamu Ayalew, Girma Demese and Kiros Meles.(2009) (Eds). Review of Maize, Sorghum and Millet Pathology Research. In: Abraham Tadesse (Ed.), *Increasing Crop Production through Improved Plant Protection-Volume II*.

[11] Harvey P.R. Warren R.A. et Wakelin S. (2006). Emerging soil-borne constraints to irrigated maize: a *Pythium-fusarium* root disease complex, 6th triennial conference, *Maize Association of Australia*, 8p

[12] Hibar, K., M. Daami-Remadi, H. Khaireddine and M. El Mahjoub. (2005). Effet inhibiteur *In vitro* et *In vivo* du *Trichoderma harzianum* sur *Fusarium oxysporum* f. sp. *Radicis-lycopersici*. *Biotechnol. Agron. Soc. Environ*., 9(3): 163-171.

[13] Kommedahl, T.; Windels, C.E. Root-, stalk-, and ear infection *Fusarium* species on corn in the USA. *Fusarium* (1981): Diseases, Biology, and Taxonomy; Pennsylvania State University: University Park, PA, USA,; pp 94–103.

[14] Koné, S.B., Dionne, A., Tweddell, R.J., Antoun, H., Avis, T.J., (2010). Suppressive effect of non-aerated compost teas on foliar fungal pathogens of tomato. Biol. Control 52, 167–173.

[15] Koumaglo K. H., Dotse K., Bettini F. et Bayle J.C. (2009). Chemical composition of *Chromolaena odorata* (L) King and Robinson (Asteraceae) essential oil from Togo: Drying and collecting site effects. 13 p

[16] Le Page C, Bousquet F (2007). Sustainable development and biodiversity – 2, serie of Composing for Sustainable Agriculture (volume 3). M.L. Sukhadia University Botany Department, Udaipur, Rajasthan, India. Dinesh K. Maheshwari Editor.

[17] Litterick, A.M., Harrier, L., Wallace, P., Watson, C.A., Wood, M., (2004). The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production – a review. Crit. Rev. Plant Sci. 23, 453–479.

[18] Monisha, K. (2013). Effect of Phytochemical Constituents of *Ricinus Communis*, *Pterocarpus Santalinus*, *Terminalia Belerica* on Antibacterial, Antifungal and Cytotoxic Activity *International Journal of Toxicological and Pharmacological Research*; 5(2): 47-54.

[19] Niaz, I.; Dawar, S. (2009) Detection of seed borne mycoflora in Corn (*Zea mays* L.). *Pak. J. Bot.*, *41*, 443–451.

[20] Perez-Garcıa, A., Romero D., and A. de Vicente. (2011). Plant Protection and Growth Stimulation by Microorganisms: Biotechnological Applications of *Bacillis* in Agricultura." *Current Opinion of Biotechnology* 22: 187–193.

[21] Scheuerell, s.j., mahaffee, w.f. (2004). Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. *Phytopathology*, *94*, 1156–1163.

[22] Sétamou M, Cardwell K.F, Schulthess F et Hell K. (1998). Effect of insect damage to maize ears, with special reference to the ear borer *Mussidiani grivenella*, on *Aspergillus flavus*in fection and aflatoxin production in preharvest maize in the Republic of Benin. *Journal of Economic Entomology*, **91**: 433-433.

[23] Siddiqui, Y., Meon, S., Ismail, R., Rahmani, M., (2009). Bio-potential of compost tea from agro-waste to suppress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. Biol. Control 49, 38–44.

[24] Tesfaye Woubet and Dawit Abate. (1998). Toxigenic *Fusarium* species in maize grain in Ethiopia. Pp.132-134. In: Maize Production Technology for the future: Challenges and Opportunities. Proceedings of the 6th Eastern and Southern Africa Regional Maize Conference. CIMMYT/EARO.

http://dx.doi.org/10.22161/ijeab/3.4.25 *ISSN: 2456-1878*

[25] Weltzien, H.C. (1992). Biocontrol of foliar fungal diseases with compost extracts. In: *Micobial ecology of leaves*. (Eds.): J.H. Andrews, S.S. Hirano. pp 430-450. Springer Verlag, New York.

[26] Zhang, W., D.Y. Han, W.A. Dick, K.R. Davis H.A.J. Hoitink. (1998). Compost and compost water extract induced systemic acquired resistance in cucumber and Arabidopsis. *Phytopathol*., 88 : 450-455.

[27] Znaïdi, I.A. (2002). Etude et évaluation du compostage de différents types de matières organiques et des effets des jus de composts biologiques sur les maladies des plantes. Master of Science Degree N° 286. *Mediterranean Organic Agriculture, CIHEAM Mediterranean Agronomic Institute,* 94 p.

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Sustainable Agricultural Bioindustry Development: Integration of Cassava Cultivation with Beef Cattle Husbandry in North Sulawesi Province

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Abstract— *This paper reviews the potential sustainable agricultural bioindustry development based on animal feed and organic fertilizer through an integration between crops cultivation with livestock production. This bio-industrial development could be carried out successfully in Indonesia, including in the region of North Sulawesi Province. Cattle feed bioindustry could be developed from biomass of cassava plantation, such as the cassava leaves, tubers and cassava peelers. Whereas, the solid and liquid organic fertilizers bioindustry could be developed from cattle feces and urine. Agricultural bioindustry can be carried out in all areas of North Sulawesi Province, because almost in every district has beef cattle and cassava plants. The largest cassava production in North Sulawesi Province are in the regencies of Bolaang Mongondow, Sangihe Island and Talaud Island. Whereas the highest population of beef cattle are in the regencies of Bolaang Mongondow, North Bolaang Mongondow, Minahasa, North Minahasa and South Minahasa. Therefore, this type of bioindustry will be well implemented in the three regencies of Bolaang Mongondow, Minahasa and North Minahasa, as there are large cassava plants and with a high livestock population in these three areas. Although numbers of beef cattle population are also higher in some other regencies, but the production of cassava in those areas are still very small.*

Keywords—Animal feed, Beef cattle, Bioindustry, Cassava, Organic fertilizer.

I. INTRODUCTION

Cassava is one of the staple foods in addition to rice and maize in Indonesia and is also a staple food for most of the population residing in tropical climates. Cassava is also a source of food that plays an important role for food security in developing countries, especially in addressing the impacts of climate change (Koswara, 2013; Krisdiana, 2015). According to ISB (2016) in 2015 Indonesia produced more than 24 million tons of cassava per year and later in 2016, the national production reached 27 million tons. Indonesia is among the world's three largest cassava producers after Nigeria and Thailand. Indonesia also could become the world's largest cassava producer country if the cassava cultivation diversification is done intensively and continuously and the opportunity to export cassava is still to be done to other countries including China, Korea and Europe.

Cassava is widely consumed wherever the plant is cultivated either regionally, nationally, or ethnically, such as for staple foods, snacks, dessert and so on. Around 200 million people or nearly a third of the sub-Saharan African population also make cassava an important regional food. In Nigeria for example, cassava tubers and leaves not only serve as an important source of calories but also as a major source of income for rural households. Cassava is also food and income sources for over 30 million farmers and many craftsmen and traders in Nigeria (Abdoulaye *et al*., 2014). Also, in Ghana about 46% of the country's GDP is contributed by cassava trade. Almost every farming family in the country grows cassava that contributes daily caloric intake of at least 30% of the population in the country.

At present time, cassava has been commercially processed into various types of food in the form of fried or other types of chips made from cassava flour with some additional flavorings (Koswara, 2013; Ahza *et al*., 2015). The tender taste can replace the potatoes. Cassava is used in cholent in some households, can be processed into cassava starch flour used in bread, noodles, cakes and cookies. However, cassava can not last long in a fresh state, so it must be processed first into other more durable

forms, such as cassava chips, cassava flour, fermented cassava, and others.

In 2015 the Government of Indonesia has launched a concept for developing bioindustry farming system in the Agricultural Development Master Plan of the year 2015-2040 (MOA, 2014). To accelerate the improvement of quality, added value and competitiveness of agricultural products (including plantation and livestock), sustainable agricultural bioindustry development based on animal feed and organic fertilizer through an integration between crops cultivation with livestock is expected to become one of the bioindustrial development that will be successful in Indonesia, including in the region of North Sulawesi Province. Integration system of cassava cultivation and beef cattle production has a great potential for the bioindustry development, which is in the form of bioindustry of animal feeds and organic fertilizer. Cattle feed bioindustry could be developed from biomass of cassava plantation including the cassava leaves and cassava peelers. Whereas, organic fertilizer bioindustry could be developed from cattle feces and urine. In the concept of agricultural bioindustry it is not only to produce processed food products but also production of non-food products and energy. The implementation of this concept aimed at optimizing the potential of land resources that are formed in the agricultural area scale.

II. POTENTIAL OF CASSAVA CULTIVATION IN NORTH SULAWESI PROVINCE

In North Sulawesi Province, corn and tuber crops are alternative food after rice. Cassava is one of the staple food of the people in the archipelago region of North Sulawesi including Talaud and Sangihe Islands. Cassava tubers that are often planted and consumed are the white and yellow/ivory tubers. Cassava planted in this area is more prevalent in dry land areas with sufficient water availability and rainfed water. As reported that cassava plants are tolerant of soils that have low fertility and drought as well resistant to pests and diseases (Aboki *et al*., 2013). Cassava plants in North Sulawesi are also planted among the coconut plants with a wide spacing of the coconut.

Based on data from ISB of North Sulawesi Province (2016), the average cassava productivity in this area is still quite low, which is only 12.280 tons/ha. In addition, there is also a decline in the harvested area and the cassava production in the period of 2010-2015. Cassava production in 2010 was 84,083 tons but it was decreased in 2015 to only 44,134 tons. This is mainly due to the decreasing in harvested area which was 6,424 ha in 2010 and it was decreasing steadily within 5 years period to 3,594 ha in 2015 (Table 1). This decrease in harvested area is due in part to changes in the use of agricultural land (land conversion) that turns into land for housing, factories/industries and so on.

The largest cassava production in North Sulawesi Province in 2015 are in the regencies of Bolaang Mongondow (7,998 tons), Sangihe Island (6,851 tons) and Talaud Island (6,212 tons). In addition, in the Minahasa regency and the surrounding areas also have a quite large cassava production which are between 2,417 and 4,506 tons. The production of cassava in these areas exceeds the need for consumption of local communities. So that some of them are exported to other areas in need. While in some other areas cassava is only grown by farmers for their own consumption purposes.

III. INNOVATIONS OF CASSAVA CULTIVATION TECHNOLOGIES

The principal objective of rural agricultural development is to improve the welfare of peasant communities as measured by the level of real income or average consumer spending per capita. Technological improvements, such as improved varieties, are the most important factors in improving agricultural productivity and poverty reduction in the long term. The use of cassava superior varieties accompanied by integrated crop management can produce as much as 30-35 ton/ha, and even when using higher input, it can reach up to 80-100 ton/ha (ICFCRD, 2012). Now, there are also some types of superior and fast harvested cassava available, such as Malang-1 variety, with a production between 45-59 tons/ha or an average of 37 tons/ha, and Malang-2 variety, with an average production between 34-35 tons/ha. Up to 2014, the Indonesian Agency for Agricultural Research and Development (IAARD) has released as many as 11 varieties of cassava, including: Adira-1, Adira-2, Adira-4, Malang-1, Malang-2, Darulhidayah, UJ-3, UJ-5, Malang-4, Malang -6, and UK-2 (ILTCRI, 2016).

To improve cassava productivity, technology should be adopted in the production process and the adoption rate of new technology is highly correlated with the profitability, level of risk associated with it, capital requirements, agricultural policy and socio-economic characteristics of farmers. The adoption of innovation is the final step in the decision-making process to make full use of innovations will have a positive impact on the farmers or user's welfare level. Increased adoption of agricultural technology provides an opportunity to increase production, substantial income and to reduce food insecurity (Nata *et al*., 2014). The adoption of agricultural technology depends on the personal, social, cultural and economic factors as well as on the

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Table.1: Harvested area, Yield and Production of Cassava in North Sulawesi Province, in 2015.

No.	City/Regency	Harvested Area (Ha)	Yield (Ton/Ha)	Production (Ton)
1.	Bitung City	179	12.100	2,165.90
2.	Bolaang Mongondow	654	12.230	7,998.42
3.	Bolaang Mongondow East	134	12.260	1,642.84
4.	Bolaang Mongondow North	60	12.250	735.00
5.	Bolaang Mongondow South	103	12.270	1,263.81
6.	Kotamobagu City	28	12.070	337.96
7.	Manado City	82	12.060	988.92
8.	Minahasa	356	12.240	4,357.44
9.	Minahasa North	367	12.280	4,506.76
10.	Minahasa South	275	12.280	3,377.00
11.	Minahasa South-East	197	12.270	2,417.19
12.	Sangihe Island	553	12.390	6,851.67
13.	Sitaro Island	-	-	-
14.	Talaud Island	501	12.400	6,212.40
15.	Tomohon City	105	12.050	1,265.25
	North Sulawesi Province	**3,594**	**12.280**	**44,134.32**

Source: ISB of North Sulawesi Province (2016).

characteristics of the innovation itself. Increased adoption of agricultural technology also has a positive impact on poverty reduction and improving human welfare (Challa and Tilahun, 2014).

Ojo and Ogunyemi (2014) have analyzed factors influencing the adoption of improved cassava production technology at the Ekiti State, Nigeria. The results of socio-economic analysis revealed that approximately 73.3 percent of respondents adopted improved cassava production technology. The cost and return analyses showed that cassava production benefits the adopters of improved cassava production technology and has a higher and significant net return rate compared to the non-users. Furthermore Adetule *et al*. (2017) has also undertook research at the Ekiti State, Nigeria, on factors influencing the adoption of improved crop varieties by cassava farmers. The study showed that the majority (55.8%) of farmers finally adopted, 19.3% were in the trial phase, 7.2% were still evaluating, 13.8% had shown interest to know more about improved varieties while only 3.9% said that they newly realize better cassava cultivars are available in the city.

A study aimed to assess the impact of adoption of improved cassava varieties on the welfare of cassava-producing households was carried out by Afolami *et al*. (2015) in two states in Southwestern Nigeria. TME 419 is the most widely adopted varieties among cassava varieties introduced in the state with 60.2% of farmers adopting this varieties. The results also showed that adoption of improved cassava varieties in the study area increases the annual income and annual consumption expenditure by cassava farm households thus improving their welfare. The adoption of improved cassava varieties is therefore pro-poor community with the adopters having lower poverty rates than the non-adopters. Significant relationships were found between marital status of farmers, agriculture as main occupations, farming experience, access to cassava cutting in villages, radio use and adoption of improved cassava varieties.

Pingmuanglek *et al*. (2017) has compared the conventional system of cassava starch production by incorporating an increase of one change in the practice of crop production, transportation and starch production. In an improved scenario of crop production, some nutritional management practices are applied together with optimization of fertilization. Flour mills have been modified to improve water recycling and reduce losses to reduce input of cassava. Reduction of starch loss was achieved in fiber and pulp separation processes in which the largest starch loss occurs. The results showed that technological and management improvement scenarios can reduce consumption of all resources and emissions including cassava (4%), fertilizer (50%), water (30%), wastewater (40%) and energy (8%). All dregs of cassava can be used to produce ethanol as well as for animal feed. All waste water can be reused for irrigation in the cassava plantation areas.

According to Qurrahman *et al*. (2014), the use dry land for intensive cassava production without applying sustainable cultivation techniques has the potential to cause soil damage, so it is necessary to use the organic fertilizer. Otherwise, agricultural activities will be high investment with low yields due to the uncontrolled use of synthetic chemicals that become dangerous for the ecosystem. To protect the ecosystem, organic farming needs to be practiced properly without the use of

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hazardous chemicals and replace them with organic fertilizers, bio-pesticides, and others. The results of Mathias and Kabambe (2015) study stated that the use of organic fertilizer can improve soil structure to facilitate root penetration and cassava tubers formation. Similarly, nutrients contained in organic fertilizers are released more slowly and stored longer in the soil allowing longer residual effects. Although manure and dolomite can be replaced by adding urea fertilizer to 500 kg/ha, but manure is superior in terms of soil treatment (Radjit *et al*., 2014). One effort to increase the availability of organic fertilizer is to conduct a system of cassava crop farming with beef cattle production.

Perfect soil treatment is considered the most important to improve cassava productivity. This is because the dry lands cultivated for cassava plants by farmers generally have relatively hard soil layers so that the good soil processing that could make the land become looser is needed, which makes the cassava plants can grow optimally (Ariningsih, 2016). Deliyana *et al*. (2016) showed that minimum tillage and herbicides application caused the highest increased in growth of cassava compared to the complete soil preparation + herbicide, minimum soil preparation and complete soil preparation only. The complete soil preparation system + herbicide produced highest cassava production and the amount of nutrient transported crops (i.e. N, P and K and C-plant) compared to the minimum soil preparation + herbicide, minimum soil preparation and complete soil preparation. The complete soil preparation and herbicides are more advantageous than complete soil preparation, minimum soil preparation and minimum soil preparation + herbicide in the second growing season.

In the irrigation method, the most important technological component is the volume of water supply as needed in the cassava plantation on dry land and frequent drought that causes the decreasing of crop yield and causing the dry cassava crop and non-optimal production, cassava tubers become small, or even cause the crop failure (Ariningsih, 2016). To anticipate the occurrence of drought, the drought tolerant superior varieties such as Malang-6, Malang-4 and Adira-4 varieties should be used. All these three varieties are suitable as raw materials for starch industry because they have high yield potential and have high starch levels as well, although the flavor of the tubers is rather bitter (Wahyuni, 2015).

IV. BEEF CATTLE FARMING CONDITION IN NORTH SULAWESI PROVINCE

North Sulawesi province has big potential or opportunity for cattle raising. This is due to the support of natural resources (lands, feeds), human resources and has good market prospects and potential. In addition, cattle business is also a source of regional revenue through the inter-provincial and inter-island trades.

At present, about 60% of the meat requirement is fulfilled by pork, however the Government of North Sulawesi Province will develop an artificial insemination program to increase beef production to meet the national needs. North Sulawesi Province has a large area with a total land area of 1,527,219 hectares that can be used for crops, horticulture and plantation areas. In North Sulawesi Province, cattle are maintained in an integrated manner with plants known as plant integration systems with livestock. Integration of cattle business with crops can gain a positive influence on the cultivation, social and economic community.

Cattle business in North Sulawesi Province is generally extensive or livestock still traditionally maintained. Cattle rearing is done with a binder and moved system with feeding in the form of green grass and corn straw. Cattle is brought to the grazing area in the morning with the distance from the house about 1-3 km, then in the afternoon the cow was taken back to its shade accompanied by additional feed grass and corn straw. The allocation of daily cattle management can take about 2-4 hours/day depending on the distance of the grazing areas and the number of cows owned by farmers.

The Agriculture and Livestock Service Office of North Sulawesi Province has undertaken various measures to develop livestock farms in the province. One of these policies was to provide cattle to several farmer groups. To increase the livestock productivity, it is necessary to increase the calf birth, shortening the calving interval, extend the production period. Successful cattle raising is strongly influenced by various interrelated factors, such as education, input use, marketing, planning and others. The success of cattle business depends on three elements: breeds, feed and management. The North Sulawesi province has the potential to be a cattle center in the eastern part of Indonesia.

Based on the Agricultural Census data of 2013 (ISB, 2014) it was reported that the highest population of livestock breed in the North Sulawesi Province is Pigs (123,943 heads), while the number of beef cattle is only 96,628 heads and goats are 10,776 heads (Table 2). In addition, there are also few numbers of horses and dairy cattle kept farmers in this province. In general, beef cattle are found throughout most of the North Sulawesi province, with the highest number are in the following regencies: Bolaang Mongondow (19,993), Minahasa (16.089), as well as in regencies of North Bolaang Mongondow, North Minahasa and South Minahasa with an average amount of 12,197 heads.

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V. CASSAVA AS FEED INGREDIENTS FOR ANIMALS

One way in anticipating the problem of limited sources of feed for livestock and as an effort for feed cost efficiency, is to find the new feed materials in the form of agricultural wastes which have not been generally used by farmers or known as unconventional feed materials. In some areas there are several types of agricultural crops and plantations wastes that have not been used as feed materials by farmers other than rice straw and corn leaves. These include: corn beard, soybean shell, cassava peelers, cocoa husk, cocoa shell and coffee shell; but in some other areas there are already using the material as animal feed.

Table.2: Population (heads) of Beef cattle, Goats and Pigs raised by farmers in North Sulawesi Province, in 2015.

No.	City/Regency	Beef Cattle	Goats	Pigs
1.	Bitung City	2,145	681	4,519
2.	Bolaang Mongondow	19,993	1,642	14,839
3.	Bolaang Mongondow East	2,372	1,384	571
4.	Bolaang Mongondow North	12,287	1,769	361
5.	Bolaang Mongondow South	3,255	691	39
6.	Kotamobagu City	1,678	239	1,005
7.	Manado City	2,290	315	3,755
8.	Minahasa	16,089	542	32,987
9.	Minahasa North	12,651	1,185	13,434
10.	Minahasa South	11,654	440	14,589
11.	Minahasa South-East	3,126	851	6,862
12.	Sangihe Island	1,595	644	7,584
13.	Sitaro Island	71	112	9,536
14.	Talaud Island	748	128	7,625
15.	Tomohon City	2,674	153	6,237
	North Sulawesi Province	**92,628**	**10,776**	**123,943**

Source: Agricultural Census data of 2013 (ISB, 2014).

All parts of the cassava plant in general, can be used as animal feed. Leaf parts can be used as a source of protein that can be given in dry or silage form. Stems can be mixed with leaves as ingredients in concentrate feeds. Tubers can be converted into pellets. There are also cassava peelers which are the main cassava plant wastes in developing countries. Cassava peelers can be given to cattle directly or after fermented.

Feed ingredients derived from postharvest cassava wastes include cassava tubers, cassava stems, cassava leaves, dried cassava and cassava pomade, are classified as feeds with an easily digestible carbohydrate source. Cassava pomade can be dried prior to use or can be used as a substrate to produce single cell proteins. Some research results in the use of cassava as ruminants feed has been done for both small and large ruminants. The utilization of cassava in the form of dried cassava or cassava pomades is the most commonly used as a best concentrate for beef or dairy cattle.

The use of cassava pomade as an energy source in a concentrate mixture of up to 45% to replace corns did not affect the milk production of dairy cow (Suksombat and Lounglawan, 2004). This will greatly benefit small farmers because the price of the cassava pomade is cheaper than the price of corn. The effects of feed containing cassava (tubers and leaves) were investigated for feed intake, weight gain, feed conversion ratio, egg production performance and egg quality for 5 weeks of experimental feeding in laying hens in Myanmar. The results showed that, up to 40% of corn can be replaced with cassava to increase egg production and egg quality. Moreover, the substitution of cassava leaves with cassava tubers was more efficient in reducing cholesterol content in egg yolks (Kyawt *et al*., 2015).

VI. ALTERNATIVE ENERGY SOURCE FROM ANIMAL WASTES

Wastes in the form of feces and cow urine are commonly used as organic fertilizer by most of farmers. However, most of these types of wastes are immediately taken to the filed without being previously composting. Though the feces are still hot and can disrupt the growth of plants. Therefore, to overcome this problem, biogas installation can be provided, and farmers will get gas as fuel, solid organic fertilizer, and liquid organic fertilizer from the remaining fermentation of organic materials in the biogas digester. It also can reduce the pollutions caused by stacks of feces.

Hasiholan *et al*. (2016) examined the production, productivity, hydraulic retention time, and quality of

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biogas made from cassava tubers and leaves with cow dung as the starter. It was concluded that the substrates compositions significantly affected the total biogas production, but it did not have significantly effect on biogas productivity. The highest biogas production (6,995 ml) was obtained from the composition of 25% of cassava tubers and 75% of cattle manure. The farmers' perception and their willingness to adopt the biogas technology in Indonesia is very good. Cattle manure will not only produce biogas that can be used by the local people for cooking, but it is also needed to overcome air pollution and disease hazard arising from untreated cow dung (Asmara *et al*., 2013).

The process of biogas making begins by inputting the livestock wastes in the form of dirt, feed residues, urine and wastewater into the biogas reactor. The stages of the biogas manufacturing process are as follows: a) The biogas input materials (in the form of fresh livestock organic wastes) mixed with water, with a ratio of 1 part of wastes and 1 part of water. b) The mixture is stirred, then flowed into the biogas reactor to the maximum extent of the discharge hole. c) Fermented for 2-3 weeks period, with the position of the gas control valve and the gas valve expenditure to the stove are in a closed state. d) The result of the fermentation process can be seen at the end of the second week, the mild biogas will be accumulated at the top of the reactor dome. e) The first gas formed is removed until a typical biogas smell comes out. f) If the use of biogas is used every day, then the filling of biogas input materials is also carried out every day. g) Biogas production will take place continuously, depending on the filling and maintenance of the installation. h) The entry of pesticides, disinfectants, detergent/soap/shampoo solutions into the biogas reactors, should be avoided (Widodo and Asari, 2011). The raw biogas cannot be used directly as fuel because it still contains CO2 and H2S which will decrease the heating value and could cause corrosion in the biogas storage vessels. Enrichment of biogas could be done by removing the CO2 and H2S contents which will significantly improve the quality of the biogas (Akila *et al*., 2017).

VII. BIOSLURRY FROM BIOGAS DIGESTER AS ORGANIC FERTILIZER

The sludge comes out of a biogas installation is called the bio-slurry and this can be used as an organic fertilizer in the solid or liquid form (Amir, 2016). The solid in the wet or dry form can be used directly as crops fertilizer as it has been decomposed during the fermentation process in the biogas digester/reactor. This solid contains very small quantities of pathogenic microorganisms so that it is very good to be used a media for planting mushrooms or plant nurseries. Oktavia and Firmansyah (2016) introduced a biogas technology to reduce the economic costs of households in Tanjung Bulan Village in Prabumulih City, South Sumatra. It was concluded from the study that the biogas program was very beneficial for the community in the Village. There were three main benefits perceived by the community, including a) Biogas could be used as a substitute for the liquid petroleum gas (LPG) which has been used previously by the community. b) The biogas byproducts have been widely used as organic fertilizers. (3) From the social point of view, the program has also educated the community to utilize the livestock manure that has been considered by the community as waste can provide an economic and environmental benefits.

The by-products of the biogas technology which are processed into organic fertilizers could increase soil nutrients, save costs for fertilizer purchases and increase public incomes (Rajendran *et al*., 2012). The process of making organic liquid fertilizer is as follows: The sludge output from the biogas reactor is filtered by a fine sieve and the water is collected in a plastic drum. To improve the quality of the fertilizer, it is necessary to add such other ingredients as bone meal, egg shell flour and blood meal. All these ingredients are thoroughly mixed and fermented for a 7 days period. The fluid is then filtered again by using a cloth then the cloth is squeezed. The liquid is then collected in plastic drums and allowed to stand for 3-4 days and frequently stirred or aerated to remove the residual gases. Furthermore, the liquid is left without stirring for 2 days to settle the particles and the liquid becomes clearer. The liquid is then packed in plastic bottles and ready to be used or sale.

VIII. INTEGRATION OF CASSAVA CULTIVATION WITH BEEF CATTLE HUSBANDRY

The model of integration between crops and livestock or often called integrated agriculture, is to combine the livestock production and farming activities. This model is often called as zero-waste farming or bio-industrial farming, because the livestock wastes are used as fertilizer, and the agricultural wastes as animal feeds.

The agricultural bioindustry is an agricultural system which in principle manages and/or utilizes optimally all biological resources including agricultural biomass and/or organic wastes, for the welfare of society in an ecosystem in harmony. The key word of this bioindustry farming system lies in all biological resources, biomass, and agricultural wastes (including livestock), science and technology, bioprocess, utilization and genetic engineering. Therefore, this farming system will produce high value food products and bioproducts, zero waste, biorefinery and sustainable.

The interaction between crops and livestock should be complementary, supportive and mutually beneficial, to encourage increased production efficiency and increased

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profitability of its farm operations. Some references state that the integration model is directed to the concept of food products, feed sources, renewable energy and soil fertility (Anugrah *et al.*, 2014; Amir, 2016).

The concept of cassava crops and cow production integration is presented in Fig. 1 (Amir, 2016). This zero-waste model is directed to a net production as an effort to extend the production cycle by optimizing the by-products. As it has been explained previously that the main products of beef cattle production are meat/carcass and manure as its by-products. Livestock manure is then processed into the biogas and fertilizer for cassava plant. Cassava has the main product of tubers with tubers peelers and leaves as the by-products which could be processed into cow feed in the form of silages and concentrates. By applying this integration model, the concept of bio-industrial agriculture is expected to be formed by itself.

The activities of agricultural bioindustry in the form of integration of beef cattle production with cassava plants can be carried out in almost all areas of North Sulawesi Province. This is because almost in every district has beef cattle and cassava plants, except in the Sitaro Island that does not have cassava plantation. The implementation of this agricultural bioindustry in North Sulawesi Province will be able to be implemented well in three regencies, namely Bolaang Mongondow, Minahasa and North Minahasa. In relation to these three areas, there are large cassava plants (Table 1) and with a high livestock population (Table 2). Cassava plants are also present in a quite large amount in the regency islands of Sangihe (553 ha) and Talaud (501 ha), but the beef cattle exists in these regions are only in a few number. Therefore, beef cattle population in both areas should be increased through various government programs.

The number of beef cattle population is also high in the regencies of North Bolaang Mongondow (12,287 heads) and South Minahasa (11,654 heads). However, the production of cassava in these regions are still very small. Therefore, it is necessary to increase the amount of existing cassava productions by increasing the planted areas or using superior cassava varieties, so that it can support the integration activities between beef cattle production with the cassava plants.

IX. CONCLUSION

Agricultural bioindustry can be carried out in all areas of North Sulawesi Province, because almost in every district has beef cattle and cassava plants. This bioindustry will be well implemented in three regencies including Bolaang Mongondow, Minahasa and North Minahasa, as there are large cassava plants and with a high livestock population in these three areas. Although numbers of beef cattle population are also higher in the regencies of North Bolaang Mongondow and South Minahasa, but the production of cassava in these regions are still very small. So that, to support the agricultural bioindustry, it is necessary to increase the amount of existing cassava productions in these areas by increasing the planted areas or using the superior cassava varieties.

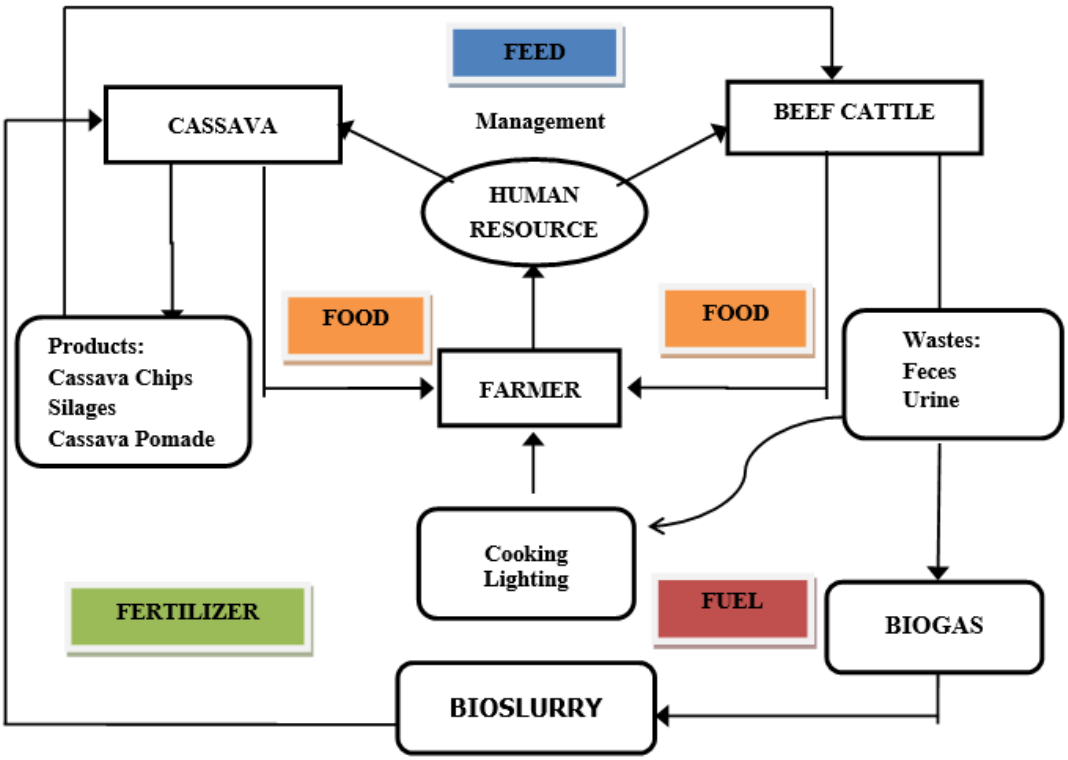

Fig. 1: The Diagram Concept of Integration between Cassava Cultivation and Beef Cattle Husbandry (Adopted from Amir, 2016).

REFERENCES

[1] Abdoulaye, T., Abass, A., Maziya-Dixon, B., Tarawali, G., Okechukwu, R., Rusike, J., Alene, A., Manyong, V. and Ayedun, B. (2014). Awareness and adoption of improved cassava varieties and processing technologies in Nigeria. *Journal of Development and Agricultural Economics*. 6(2): 67-75.

[2] Aboki, E., Jongur, A.A.U., Onuand, J.I. and Umaru, I.I. (2013). Analysis of Technical, Economic and Allocative Efficiencies of Cassava Production in Taraba State, Nigeria. *IOSR Journal of Agriculture and Veterinary Science* (IOSR-JAVS). 5(3): 19-26.

[3] Adetule, F.S., Owoeye, R.S. and Sekumade, A.B. (2017). Factors Affecting the Adoption of Improved Planting Materials by Cassava Farmers in Ekiti State, Nigeria. *International Journal of Sustainable Development Research*. 3(3): 27-31.

[4] Afolami, C.A., Obayelu A.E. and Vaughan, I.I. (2015). Welfare impact of adoption of improved cassava varieties by rural households in South Western Nigeria. *Agricultural and Food Economics*. Springer; Italian Society of Agricultural Economics (SIDEA). 3(1): 1-17.

[5] Ahza, A.B., Fidiena, T.I. and Suryatman, S. (2015). Physical, Sensorial and Chemical Characteristics of Simulated Chips of Cassava (*Manihot esculenta* Crantz): Rice (*Oryza sativa* L.) mix. *Procedia Food Science*. 3(2015): 82-95.

[6] Akila, E., Pugalendhi, S. and Boopathi, G. (2017). Biogas Purification using Coconut Shell Based Granular Activated Carbon by Pressure Swing Adsorption. *International Journal of Current Microbiology and Applied Sciences*. 6(4): 1178-1183.

[7] Amir, A. (2016). The Potency of Zero Waste Model through an Integration of Dairy Cattle and Cassava Plants. *Journal of Livestock Technology Science*. 5(1): 17-26. (in Indonesian).

[8] Anugrah, I.S., Sarwoprasodjo, S., Suradisastra, K., Purnaningsih, N. (2014). Integrated Agricultural System: Its Concept, Implementation, and Role in Agricultural Development in Bali Province. *Agro-economic Research*, 32(2): 157 – 176. (in Indonesian).

[9] Ariningsih, E. (2016). Increasing cassava production under region base program in West Java and South Sulawesi Provinces. *Agriculture Policy Analysis*. 14(2): 125-148. (in Indonesian).

[10] Asmara, A., Hutagaol, M.P. dan Salundik. (2013). Potential analyses of production and public perception in biogas development at the centre of dairy cattle farming in Bogor Regency. West Java Province, Indonesia. *Journal of Indonesian Agribusiness*. 1(1): 71-80. (in Indonesian).

[11] Challa, M. and Tilahun, U. (2014). Determinants and Impacts of Modern Agricultural Technology Adoption in West Wollega: The Case of Gulliso District. *Journal of Biology, Agriculture and Healthcare*. 4(20): 63–77.

[12] Deliyana., Lumbanraja, J., Sunyoto and Utomo, M. (2016). Effect of land preparation methods on the growth, production and fertilizer absorption by cassava *(Manihot esculenta* Crantz*)* cultivation at 2nd planting period in Gedung Meneng village of Bandar Lampung. Lampung Province, Indonesia. *Journal of Agrotech Tropics*. 4(3): 233 – 239. (in Indonesian).

[13] Hasiholan, U., Haryanto, A. and Prabawa, S. (2016). Produksi Biogas Dari Umbi Singkong Dengan Kotoran Sapi Sebagai Starter. *Lampung Journal of Agriculture Technics*. 5(2): 109-116. (in Indonesian).

[14] ICFCRD. (2012). General Guidance for Integrated Crops Management of Cassava Cultivation. *Indonesian Centre for Food Crops Research and Development*. Indonesian Agency for Agricultural Research and Development. Bogor. Indonesia. (in Indonesian).

[15] ILTCRI. (2016). Description of Outstanding Varieties of Legumes and Tubers. *Indonesian Legumes and Tuber Crops Research Institute*. Indonesian Centre for Food Crops Research and Development. Indonesian Agency for Agricultural Research and Development. Malang. East Java. Indonesia. (in Indonesian).

[16] ISB. (2014). Agricultural Census of 2013. *Indonesian Statistical Board*. Jakarta, Indonesia. (in Indonesian).

[17] ISB. (2016). Statistics of Indonesian Agriculture. *Indonesian Statistical Board*. Jakarta, Indonesia. (in Indonesian).

[18] ISB of North Sulawesi Province. (2016). Agricultural Statistics of North Sulawesi Province. *Indonesian Statistical Board of North Sulawesi Province*. Manado, Indonesia. (in Indonesian).

[19] Koswara, S. (2013). Modul for Roots and Tubers Processing. Part VI: Cassava Processing. *Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center*. Research and Community Service Institution, Bogor Agricultural University. Bogor, Indonesia. (in Indonesian).

[20] Krisdiana, R. (2015). Disseminations, Preferences and Economic Contributions of Outstanding Variety of Cassava in Central Java, Indonesia. *Proceedings*

of Seminar on Research Results of Indonesian Legumes and Tuber Crops Research Institute. Held on 5 June 2014. Indonesian Centre for Food Crops Research and Development. Indonesian Agency for Agricultural Research and Development. Malang. East Java. Indonesia. Pp.: 561-568. (in Indonesian).

[21] Kyawt, Y.Y., Toyama, H., Htwe, W.M., Thaikua, S., Imura, Y. and Kawamoto, Y. (2015). Effect of Whole Cassava Meal as Substitutes for Maize in the Diets on the Performance Characteristics and Egg Quality of Laying Hens. *Journal of Warm Regional Society of Animal Science, Japan*. 58(1): 19-28.

[22] Mathias, L. and Kabambe, V.H. (2015). Potential to increase cassava yields through cattle manure and fertilizer application: Results from Bunda College, Central Malawi. *African Journal of Plant Science*. 9(5): 228-234.

[23] MOA. (2014). The Main Strategic of Agriculture Development in 2015-2045: Sustainable Agriculture Bioindustry, Solution for Future Indonesian Development. *Ministry of Agriculture*. Jakarta, Indonesia. (in Indonesian).

[24] Nata, J.T., Mjelde, J.W. and Boadu, F.O. (2014). Household adoption of soil-improving practices and food insecurity in Ghana. *Agriculture & Food Security*. 3(1): 17.

[25] Ojo, S.O., and Ogunyemi A.I. (2014). Analysis of Factors Influencing the Adoption of Improved Cassava Production Technology in Ekiti State, Nigeria. *International Journal of Agricultural Sciences and Natural Resources*. 1(3): 40-44.

[26] Oktavia, I. and Firmansyah, A. (2016). The use of biogas technology as alternative source of for energy in the surrounding operational area of PT. Pertamina EP Asset 2 Prabumulih Field. *CARE Journal of Conflicts Resolution, CSR and Empowerment*. 1(1): 32-36. (in Indonesian).

[27] Pingmuanglek, P., Jakrawatana, N. and Gheewala, S.H. (2017). Supply chain analysis for cassava starch production: Cleaner production opportunities and benefits. *Journal of Cleaner Production*. 162(2017): 1075-1084.

[28] Qurrahman, B.F.T., Suriadikusuma, A. and Haryanto, R. (2014). Analysis of potential damage of soil for cassava (*Manihot utilisima*) production in dry land of Tanjungsiang District, Subang Regency. West Java Province, Indonesia. *Journal of Agronomy*. 1(1): 22-32. (in Indonesian).

[29] Radjit, B.S., Widodo, Y., Saleh, N. and Peasetiaswati, N. (2014). The technology for increasing productivities and profits of cassava farming in Utisol dry land. *Crops Science and Technology*. 9(1): 51-62. (in Indonesian).

[30] Rajendran, K., Aslanzadeh, S. and Taherzadeh, M.J. (2012). Household biogas digesters-A review. *Energies*. 5: 2911-2942.

[31] Suksombat, W. and Lounglawan, P. (2004). Silage from agricultural by-products in Thailand: Processing and storage. *Asian-Aust. J. Anim. Sci*. 17(4): 473-478.

[32] Wahyuni, T.S. (2015). The growth and tolerance of cassava plant accession on dry condition. In (Kasno *et al*., Editors) Technology Innovation on Legumes and Tubers Cultivation to Support Sustainable Agriculture Bioindustry. *Proceedings of Seminar on Research Results of Indonesian Legumes and Tuber Crops Research Institute*. Held on 5 June 2014. Indonesian Centre for Food Crops Research and Development. Indonesian Agency for Agricultural Research and Development. Malang. East Java. Indonesia. Pp.: 747-758. (in Indonesian).

[33] Widodo, T.W. and Asari, A. (2011). Innovation on mechanization technology to support the energy provision for farmer's households. Agroinnovation. Sinar Tani. 1-7 June 2011 Edition. No. 3408. Year XLI. (in Indonesian).

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Seasonal Phenology of Reptiles in a Mediterranean Environment ("Castel di Guido" Natural Park, Northern Latium, Italy)

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***Abstract*—** *The present work reports the seasonal phenology of the reptiles of the "Castel di Guido" Natural Park near Rome (Northern Latium, Italy). During field observations, between September 2014 and July 2016, transects were carried out along the ecotones of the park, in order to describe the period of seasonal activity of the reptiles present. The area is characterized by Mediterranean mesothermal climate. In the Mediterranean area, reptiles have a broader annual activity range than other European regions, greatly reducing winter latency. Reptile activities start very early, in some case it is possible to observe the snake Hierophis viridiflavus and lizards, such as Podarcis muralis and Podarcis siculus, in thermoregulation activity in the middle of winter. The mild climate that is recorded on average in autumn favors the activity in the period between September and December; Zamenis longissimus is observed regularly in October. Testudo hermanni mates regularly in autumn and it is active until the first decade of December. The research shows that during the winter period reptiles can be observed in activity; for some species, Chalcides chalcides, Podarcis sp., Hierophis viridiflavus, this seems to be a remarkable datum that broad considerably the annual phenology reported in the literature.*

***Keywords* — M*editerranean environment, phenology, reptiles, winter activity*.**

I. INTRODUCTION

The present study illustrates the phenology of the reptiles of the "Castel di Guido" Natural Park (Northern Latium, Italy) (Figure 1); the data relative to the reptiles species observed in the course of herpetological research are reported. In many species, biological activity and reproduction times are determined by a combination of endogenous cycles and exogenous signals; in particular for reptiles, ectoderm species that have body temperatures generally close to those of their environment, temperature and humidity influence many aspects of their biology (Whittier & Crewe, 1987).

In the literature, the phenology of reptiles varies a lot according to the latitude and the different habitats, as well as to the seasonal climatic trends that can vary from year to year (Sindaco *et al*., 2006; Corti *et al*., 2010). In Europe, certainly a distinction in phenological activities is observed in the continental climate range compared to the Mediterranean climate zone where the activities of reptiles begin very early compared to other environments in Europe (Bologna *et al*., 2000; Bologna *et al*., 2007).

In some cases it is possible to see in thermoregulation some lizards, such as *Podarcis muralis* (Laurenti, 1768) and *Podarcis siculus* (Rafinesque Schmaltz, 1810), in December and January; also *Hierophis viridiflavus* has been observed in mid-winter activity (Pizzuti Piccoli, 2016; Cattaneo, 2017).

The work doesn't concern *Emys orbicularis* (Linnaeus, 1758), *Anguis veronensis* Pollini, 1818, *Hemidactylus turcicus* (Linnaeus, 1758) and *Tarentola mauritanica* (Linnaeus, 1758), for which observations were based exclusively on the absence presence data. (Pizzuti Piccoli *et al*., 2017a; Pizzuti Piccoli *et al*., 2017b).

II. STUDY AREA

The present study was carried out in the Castel di Guido Natural Park, within the homonymous public farm, located in the Municipality of Rome, in the stretch between the 16th and the 20th km of the main road Aurelia. The public farm is under the direct management of the Municipality of Rome since 1978 and it produces cereals, fodder and cattle, bred in the stable (Italian Friesian cow) and in a wild state (Maremmana cow). The farm extends for 1966 ha and is characterized by hilly areas that degrade towards the coastal plain; the maximum altitude reached is 80 m above sea level, while the minimum altitude reaches about 10 m asl. The activity

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of man has further modified the territory, leading to the formation of mosaics of small natural areas, interesting because characterized by relict vegetation (Chirici *et al*., 2001).

Climatically the area is part of the Mediterranean Transitional Region, with mild climate, due to the proximity of the sea (Blasi, 1994). The minimum temperatures are recorded in January (average value 3.0 ° C), the highest in July and August (average value 30.0 ° C); rarely there are values below 0 ° C and above 40 ° C. In autumn there is maximum rainfall (over 275 mm), but also in spring there are frequent rains (175 mm) (Table 2) (Mangianti & Perini, 2001).

The study area is characterized by an evident vegetation complexity and by a great floristic richness, which can be seen in the various habitats present. Of the 1966 hectares of the Farm, 17% (366 hectares) is occupied by crops such as durum wheat, maize, barley, olive groves, and alfalfa meadows, 22% (430 hectares) by natural coppice woods with prevalence of *Quercus ilex* Linnaeus, 1758 and *Quercus pubescens* Willd., 1805, 22% (433 hectares) is used for permanent pasture, 28% (552 hectares) is covered by pine woods and areas of reforestation, while the remaining part of the territory is occupied by roads, farmhouses, stables and irrigation canals. (Filesi, 2001; Bartolucci & De Lorenzis, 2004).

The birds of the Park are represented by numerous resident and migratory species (Cecere, 2006); among the most representative mammals we find *Hystrix cristata* Linnaeus, 1758, *Vulpes vulpes* Linnaeus, 1758, *Meles meles* Linnaeus, 1758, *Martes foina* Erxleben, 1777, *Erinaceus europaeus* Linnaeus, 1758 and *Muscardinus avellanarius* Linnaeus, 1758 (Imperio *et al*., 2007). Remarkable the presence, recently proven, of the wolf, *Canis lupus* Linnaeus, 1758. The amphibians of the area are represented by five species: common toad *Bufo bufo* (Linnaeus, 1758), emerald toad *Bufotes balearicus* (Boettger, 1880), Italian tree frog *Hyla intermedia* Boulenger, 1882, green frog *Pelophylax bergeri* (Gunther, 1986) / *Pelophylax* kl. *hispanicus* (Bonaparte, 1839) and smooth newt *Lissotriton meridionalis* (Boulenger, 1882) (Pizzuti Piccoli & De Lorenzis, 2015). Reptiles are well represented with the confirmed presence of 14 species present (Table 1). (Pizzuti Piccoli *et al*., 2017).

III. MATERIAL AND METHODS

Data collection took place between September 2014 and July 2016; surveys were carried out weekly; the detection method adopted was that of the linear transect with "sight counts", V.E.S. = Visual Encounter Surveys (Heyer, 1988; Crosswhite *et al*., 1999; Greenwood & Robinson, 2006). As a transept was chosen a linear path of 2,200 meters, crossing all the representative environments and the ecotonal zones present; the presence of animals in the five meters on the right and on the left of the transept were considered (Hofer *et al*., 2002).

Field work was conducted following the regulations and with all the necessary authorizations for this kind of study.

IV. RESULTS

The data collected for the species are shown below.

Testudo hermanni Gmelin, 1789

During the field surveys, 38 specimens of *Testudo hermanni* were observed and captured. The species in the Mediterranean area is normally found from March to November, with bimodal activity in June and September (Calzolai & Chelazzi, 1991, Mazzotti *et* al., 2002). In the site the first observations date back to the first days of March and the species mates regularly in autumn; noteworthy the finding of a moving specimen on 10 December 2015(Figure 2).

Chalcides chalcides (Linnaeus, 1758)

Along the transept 14 specimens of *Chalcides chalcides* were observed; all the specimens presented the coloring with longitudinal dark stripes. In the literature, the species is active from spring to early summer, from the end of July a phase of sporadic activity begins until September and it has a winter latency from October to March (Sindaco *et al*., 2006).

Field observations confirm the start of activities in March, with observations also in August; in the study area individuals in activity were found until the end of October.

Podarcis muralis (Laurenti, 1768), *Podarcis siculus* (Rafinesque Schmaltz, 1810) and *Lacerta bilineata* Daudin, 1802

Podarcis siculus, appears to be more numerous than *Podarcis muralis*, the latter presents the typical color of the subspecies *nigriventris* (Sindaco *et al*., 2006). The two species appear to be well separated in habitat habitation. *P. siculus* appears to be confined to prairie areas, while *P. muralis* has been observed mainly in areas with tall trees, often above the trunks of trees.

Lacerta bilineata is present above all in the areas close to the watercourses and in the borders of *Rubus sp*. The phenology observed (Figure 3) indicates an intense activity for the three species between May and June; for *Lacerta bilineata* we also assist to significant activities in April; a further peak we find in the month of September (end of August - September for *Podarcis siculus*). In general, specimens were already active during the first sunny days of January, with a progressive increasing of

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biological activity with the peak in May and June. In the late autumn period we find specimens in activity until November. Between November and January the observations refer to specimens in basking activities, we have no data on trophic activity in this period; trophic activity and reproductive activity are observed from March. The observations are consistent with the data reported in the literature (Capula *et al*., 1993; Caldonazzi *et al*., 2002; Di Cerbo e Ditizio, 2008; Tenan, 2007).

Elaphe quatuorlineata (Bonnaterre, 1790)

For this species, the data in literature report individuals in activity between March and October (Pozio, 1976; Capizzi *et al*., 1996; Cattaneo, 2005; Cattaneo, 2017). The individuals observed during the research were found in the time range of April and July in both study years, confirming the phenological information in literature (Figure 4).

Hierophis viridiflavus (Lacépède, 1789)

Among the snakes, *Hierophis viridiflavus* is the most frequent; it has been observed 32 times along the transects in the ecotonal bands and, to a lesser extent, has been found in areas covered by tree vegetation and close to household goods. Found mainly on the ground, still or moving. For the species, the bibliographic data show activities from February to October (Capula *et al*., 1997; Capula & Luiselli, 1995; Filippi & Luiselli, 2000). In the study area, the species is observed regularly, albeit with few specimens, even in the months of November December and January.

Zamenis longissimus (Laurenti, 1768)

The species is present in the park, where it was found with five individuals in the ecotonal zones. The observations fall within the season range (between March - April and October - November) reported in the literature (Sindaco & Silvano, 1991; Gomille, 2002); interesting the observation of two individuals in October.

Natrix helvetica (Lacepède, 1789)

Natrix helvetica is the least common snake; it should be noted that the particular ecology of the animal, that needs presence of water bodies, makes it less observable in the habitat investigated with the transects. However its presence is confirmed with the capture of some individuals in the phenological range, described in literature for the species, between March and September (Gentili & Zuffi, 1995; Kindler *et al*., 2017).

Vipera aspis (Linnaeus, 1758)

In the area *Vipera aspis* was observed only in the period between April and July, despite being known, for the coastal Tyrrhenian locations, observations throughout all the year (Zuffi et al., 1999b; Grano et al., 2017).

V. CONCLUSION

The periods of activity of the observed species often appear in line with those in other Mediterranean coastal areas (Cattaneo, 2005, Mayor et al., 2006; Corti et al., 2010; Pizzuti Piccoli, 2016). The research shows that, during the winter period, reptiles can be observed in activity; for some species, Chalcides chalcides, Podarcis sp., Hierophis viridiflavus, this seems to be a remarkable fact that would greatly broaden the annual phenology (Figure 5). It is evident that these activities in the late autumn and winter can be correlated with seasonal climatic trends, which often offer mild, or even warm, winters (Table 2), favoring the activity of reptiles (Bologna et al., 2000; Bologna et al. ., 2007). In conclusion, the work carried out offers a contribution to the knowledge of the phenology of reptiles in the Mediterranean environment of the Tyrrhenian coast, with particular reference to the area of the Roman coast. It will certainly be important to investigate the phenology of the observed reptiles for the site, in order to obtain a more appropriated knowledge of the reptiles present.

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REFERENCES

[1] Bartolucci F. & De Lorenzis A. (2004). La flora vascolare. Collana i quaderni dell'Oasi "Castel di Guido". Vol 1. *Comune di Roma*.

[2] Blasi C. (1994). Fitoclimatologia del Lazio. *Regione Lazio, Roma*.

[3] Bologna M. A., Capula M. & Carpaneto G. (eds) (2000). Anfibi e rettili del Lazio. *Fratelli Palombi Ed., Roma*.

[4] Bologna M.A., Salvi D. & Pitzalis M. (2007). Atlante degli anfibi e dei rettili della Provincia di Roma. *Gangemi editore, Roma*.

[5] Caldonazzi, M., Pedrini, P., Zanghellini, S. (2002). Atlante degli Anfibi e dei Rettili della Provincia di Trento. 1987-1996 con aggiornamenti al 2001. *St. trent. Sci. Nat., Acta Biol. 77: 1-173*.

[6] Calzolai R. & Chelazzi G. (1991). Habitat use in a central Italy population of *Testudo hermanni* Gmelin (Reptilia Testudinidae). *Ethology & Ecology & Evolution 3: 1-14*.

[7] Capizzi D., Capula M., Evangelisti F., Filippi E., Luiselli L. & Trujllo V. (1996). Breeding frequency, clutch size, reproductive status and correlated

behaviours in sympatric *Elaphe quatuorlineata* and *Elaphe longissima* (Reptilia: Colubridae). *Revue Ecologie (terre et vie) 51: 297 – 311*.

[8] Capula M., Filippi E., Luiselli L. & Trujillo V. (1997). The ecology of the Western Whip Snake (*Coluber viridiflavus* Lacépède, 1789) in Mediterranean Central Italy (Squamata: Serpentes: Colubridae). *Herpetozoa 10 (1/2): 65 - 79*

[9] Capula, M. & Luiselli, L. (1995). *Hierophis viridiflavus* (western whip snake). Communal nesting. *Herpetological Review 26, 38–39*.

[10] Capula M., Luiselli L. & Rugiero L. (1993). Comparative ecology in sympatric *Podarcis muralis* and *P. sicula* (Reptilia: Lacertidae) from the historical centre of Rome: What about competition and niche segregation in an urban habitat?. *Boll. Zool., 60: 287-291*.

[11] Cattaneo A, (2005). L'erpetofauna della Tenuta Presidenziale di Castelporziano (Roma) *Atti Mus. Stor. nat. Maremma*, 21: 49-77

[12] Cattaneo A. (2017). I serpenti della tenuta di Castelporziano, tra passato e presente. *Segretariato Generale della Presidenza della Repubblica*.

[13] Cecere J. G (2006). L'Avifauna - ricerche e check-list. Collana i quaderni dell'Oasi "Castel di Guido". Vol 3. *Comune di Roma*.

[14] Chirici G., Corona P., Filesi L., Vannuccini M. (2001) .Lineamenti ambientali della Tenuta di Castel di Guido. In: Corona P., 2001. I rimboschimenti della Tenuta di Castel di Guido: Materiali di studio. *Supplemento al n° 4/2000 di Innovazione e Agricoltura, periodico di ARSIAL, Roma: 23-30*.

[15] Corti C., Capula M., Luiselli L., Razzetti E. & Sindaco R. (2010). Fauna d' Italia. Reptilia. Calderini Editore. Bologna.

[16] Crosswhite D.L., Fox S.F. & Thill R.E. (1999). Comparison of Methods for Monitoring Reptiles and Amphibians in Upland Forests of the Ouachita Mountains. *Proc. Oklaoma Acad. Sci., 79: 45-50*.

[17] Di Cerbo A. & Di Tizio (2008). Ramarro occidentale. In: Di Tizio L., Pellegrini M., Di Francesco M. & Carafa N. Atlante dei rettili d'abruzzo. *Ianeri – Talea Edizioni. 208 pp*.

[18] Filesi L. (2001). Vegetazione attuale e potenziale della Tenuta di Castel di Guido. In: Corona P. *I rimboschimenti della Tenuta di Castel di Guido: Materiali di studio*, 2001. *Supplemento al n° 4/2000 di "Innovazione e Agricoltura", periodico di ARSIAL, Roma: 31-42*.

[19] Gentilli A. & Zuffi A. L. (1995). Thermal ecology of a grass snake (*Natrix natrix*) population of Northwestern Italy (Reptilia, Serpentes, Colubridae). *Amphibia-Reptilia 16: 401-404*

[20] Gomille, A. (2002). Die Äskulapnatter – *Elaphe longissima*. *Frankfurt am Main: Edition Chimaira*.

[21] Grano M., Meier G. & Cattaneo C., (2017). Vipere italiane. Gli ultimi studi sulla sistematica, l'ecologia e la storia naturale. *Castel Negrino, Aicurzio, 197 pp*.

[22] Greenwood J.J.D. & Robinson R.A. (2006). General census methods. Pp. 87–183 in: Sutherland W.J. (ed.), Ecological Census Techniques, 2nd Edition. *Cambridge University Press*.

[23] Heyer R.W. (1988). Measuring and monitoring biological diversity: standard methods for amphibians. *Smithsonian Institution Press, 297 pp*.

[24] Hofer U., Misslin S. & Camponovo I. (2002). Monitoraggio delle popolazioni di Saettone (*Elaphe Longissima*), di Biacco (*Hierophis viridiflavus*), e di Natrice dal collare (*Natrix natrix*) in località Boschi, Stabio TI. *Boll. Soc. ticinese Sc. nat.*, 90: 59-67.

[25] Imperio S., Panchetti F., Cecere J. G.& Maurizi E (2007). I Mammiferi: Insettivori, Lagomorfi e Roditori. Collana i quaderni dell'Oasi "Castel di Guido". Vol 4. *Comune di Roma*.

[26] Kindler, C., Chèvre M., Ursenbacher S., Böhme W., Hille A., Jablonski D., Vamberger M. & Fritz U. (2017).Hybridization patterns in two contact zones of grass snakes reveal a new Central European snake species. *Scientific Reports 7: 7378, DOI:10.1038/s41598-017-07847-9; 12 pp*.

[27] Mangianti F. e Perini L. (2001). *Osservazioni Meteorologiche dell'anno 1998 - 1999 - 2000 - 2001. Ufficio Centrale di Ecologia Agraria e difesa delle piante coltivate dalle avversità meteoriche. Osservatorio Meteorologico Torre Calandrelli. Roma, Collegio Romano*.

[28] Mazzotti S, Pisapia A., Fasola M. (2002).Activity and home range of *Testudo hermanni* in Northern Italy. *Amphibia-Reptilia 23:305-312*

[29] Pizzuti Piccoli A. & De Lorenzis A. (2015). Gli anfibi. Collana i quaderni dell'Oasi "Castel di Guido". Vol 5. *Comune di Roma*.

[30] Pizzuti Piccoli A. (2016). Note sui rettili presenti nell'Oasi Naturale del Bosco di Palo (Lazio settentrionale, Italia). *Naturalista sicil., S. IV, XL (2),pp. 53-65*.

[31] Pizzuti Piccoli A., Scalas I. & Di Stano L. (2017a). First Record of *Emys orbicularis* (Boulenger, 1882), (Reptilia, Testudinati) in the "Castel di Guido" Natural Park (Northern Latium, Italy): a Case of Interest for Species Conservation. *International Journal of Environment, Agriculture and Biotechnology (IJEAB). Vol-2, Issue-1, Jan-Feb-2017*.

[32] Pizzuti Piccoli A., De Lorenzis A. & Fortuna F. (2017b). Osservazioni preliminari sui Rettili

dell'Oasi LIPU Castel di Guido (Lazio settentrionale, Italia). *Naturalista sicil*., 41: 147 – 159.

[33] Pozio, E. (1976). La biologia e il ciclo di vita di *Elaphe quatuorlineata* (Lacepède). *Bull. Soc. Zool. Fr. 101: 741–746*.

[34] Sindaco R., Doria G., Razzetti E. & Bernini F. (eds) (2006). Atlante degli Anfibi e dei Rettili d'Italia. *Societas Herpetologica Italica, Ed. Polistampa, Firenze, 792 pp*.

[35] Sindaco R. & Silvano F. (1991). La composizione dell'ofidiofauna padana e appenninica in un'area dell'Italia nord-occidentale (Reptilia, Serpentes). *Rivista Piemontese di Storia Naturale · 12: 81-87*.

[36] Tenan S. (2007). Ramarro occidentale. In: Bonato l., Fracasso G., Pollo R. & Semenzato M. Atlante degli anfibi e dei rettili del Veneto. *Associazione Faunisti Veneti – Nuovadimensione. 240 pp*.

[37] Whittier J.M., Crewe D. (1987). Seasonal reproduction: patterns and control. In: Hormones and Reproduction in Fishes, Amphibians and Reptiles (eds. Norris D.O., Jones R.E.), pp. 338–449. *Plenum Press, New York and London*.

[38] Zuffi A.L., Macchia M., Ioalè P. & Giudici F. (1999). Winter activity in a coastal popilation of Vipera aspis (Reptilia, Viperidae). *Revue d'Ecologie. 54:365 - 374*

Table 1. The reptile species observed in the "Castel di Guido" Natural Park.

Species observed	
European pond terrapin	*Emys orbicularis*
Hermann's tortoise	*Testudo hermanni*
Mediterranean house gecko	*Hemydactilus turcicus*
European common gecko	*Tarentola mauritanica*
Italian slowworm	*Anguis veronensis*
Italian three-toed skink	*Chalcides chalcides*
Common wall lizard	*Podarcis muralis*
Italian wall lizard	*Podarcis siculus*
Western green lizard	*Lacerta bilineata*
Four-lined snake	*Elaphe quatuorlineata*
Western whip snake	*Hierophis viridiflavus*
Aesculapian snake	*Zamenis longissimus*
Grass snake	*Natrix helvetica*
Asp viper	*Vipera aspis*

Table 2. Monthly averages of meteorological data in the period 1987 – 2017 (Rome Ciampino Station) - Source www.ilmeteo.it.

Month	**T min**	**T max**	**Rain**	**Humidity**
January	3 °C	12 °C	103 mm	77 %
February	4 °C	13 °C	99 mm	75 %
March	5 °C	15 °C	68 mm	72 %
April	8 °C	18 °C	65 mm	73 %
May	11 °C	23 °C	48 mm	71 %
June	15 °C	27 °C	34 mm	68 %
July	17 °C	30 °C	23 mm	67 %
August	18 °C	30 °C	33 mm	66 %
September	15 °C	27 °C	68 mm	69 %
October	11 °C	22 °C	94 mm	74 %
November	7 °C	16 °C	130 mm	78 %
December	4 °C	13 °C	111 mm	78 %

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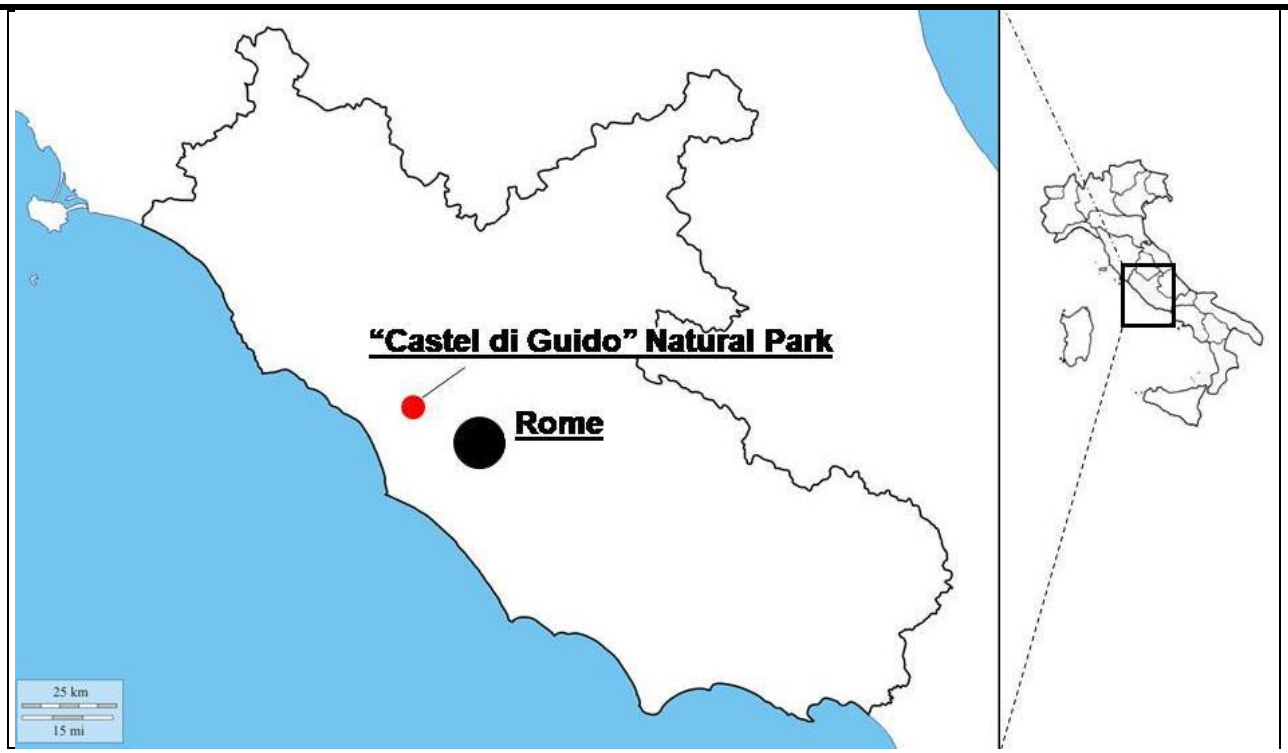

Fig.1: The "Castel di Guido" Natural Park.

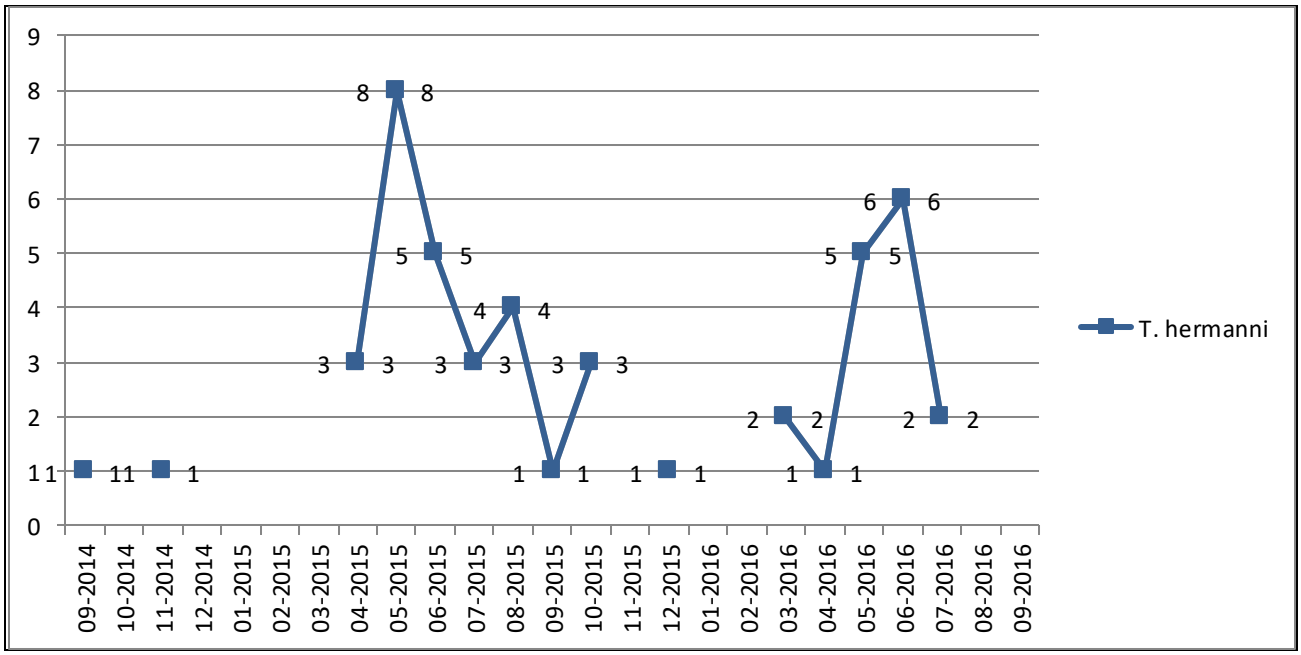

Fig.2: Testudo hermanni specimens observed during the study period.

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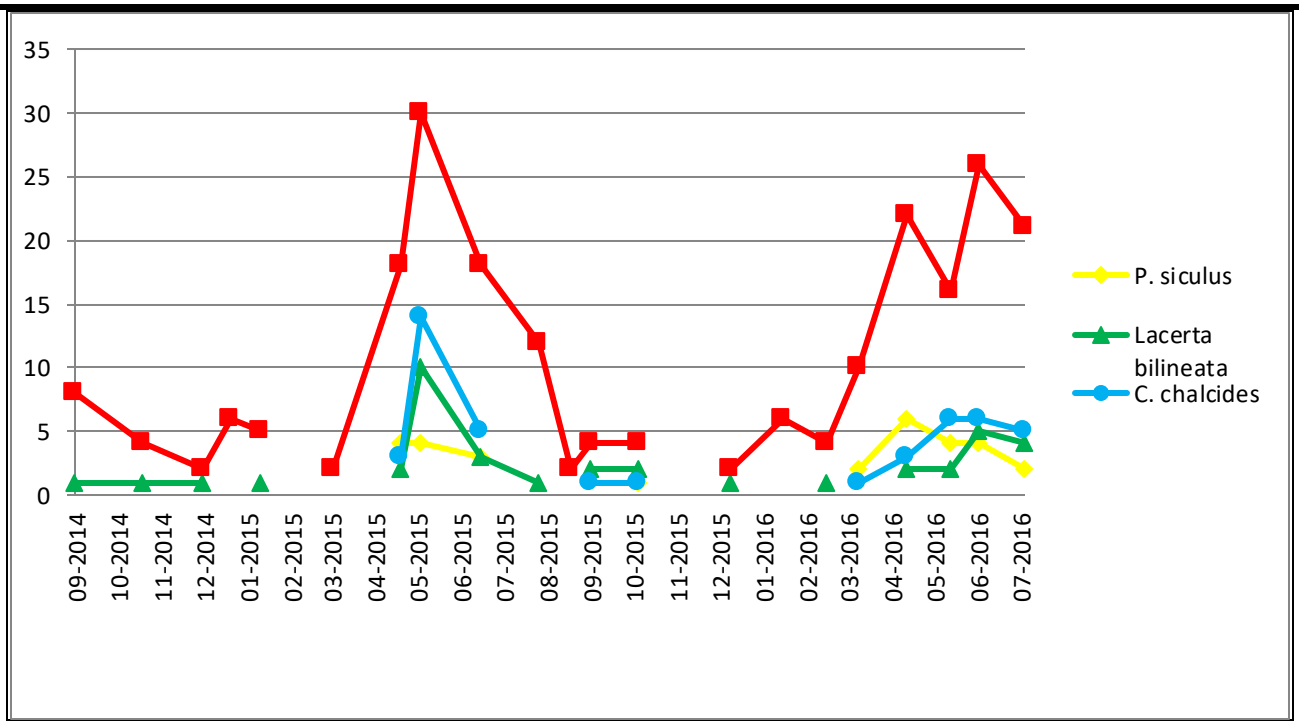

Fig.3: Observations during the study period of the lizards.

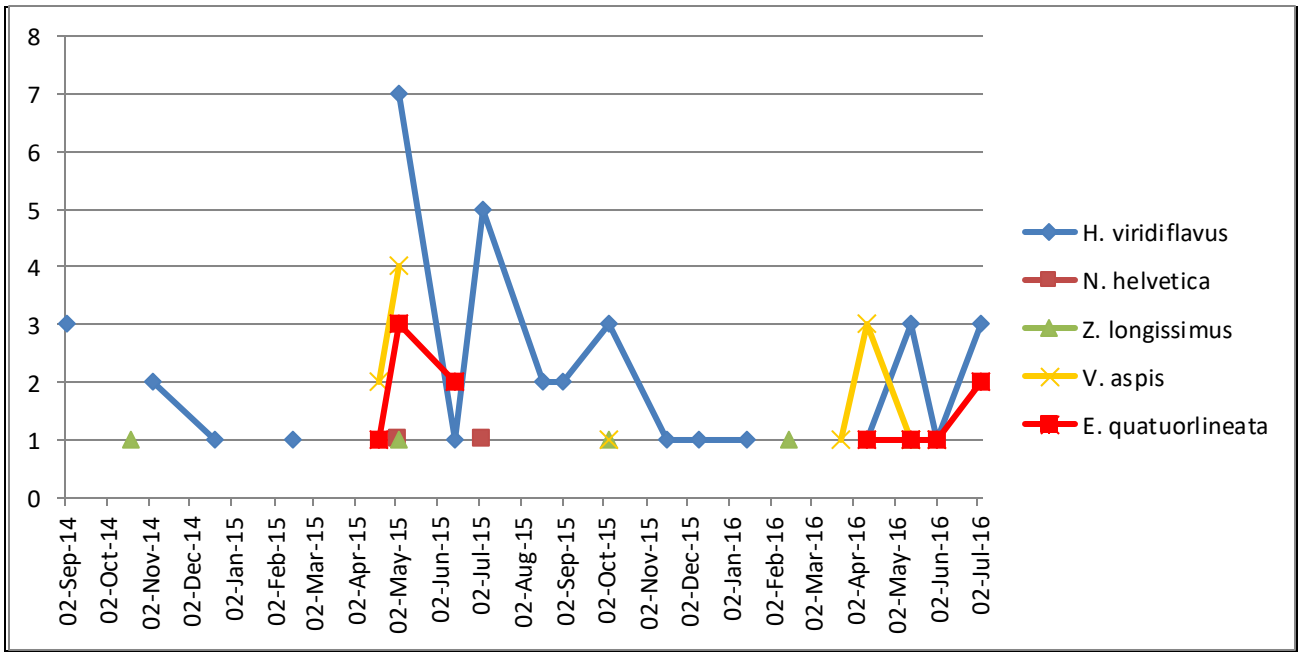

Fig.4: Observations of snake species during the study period.

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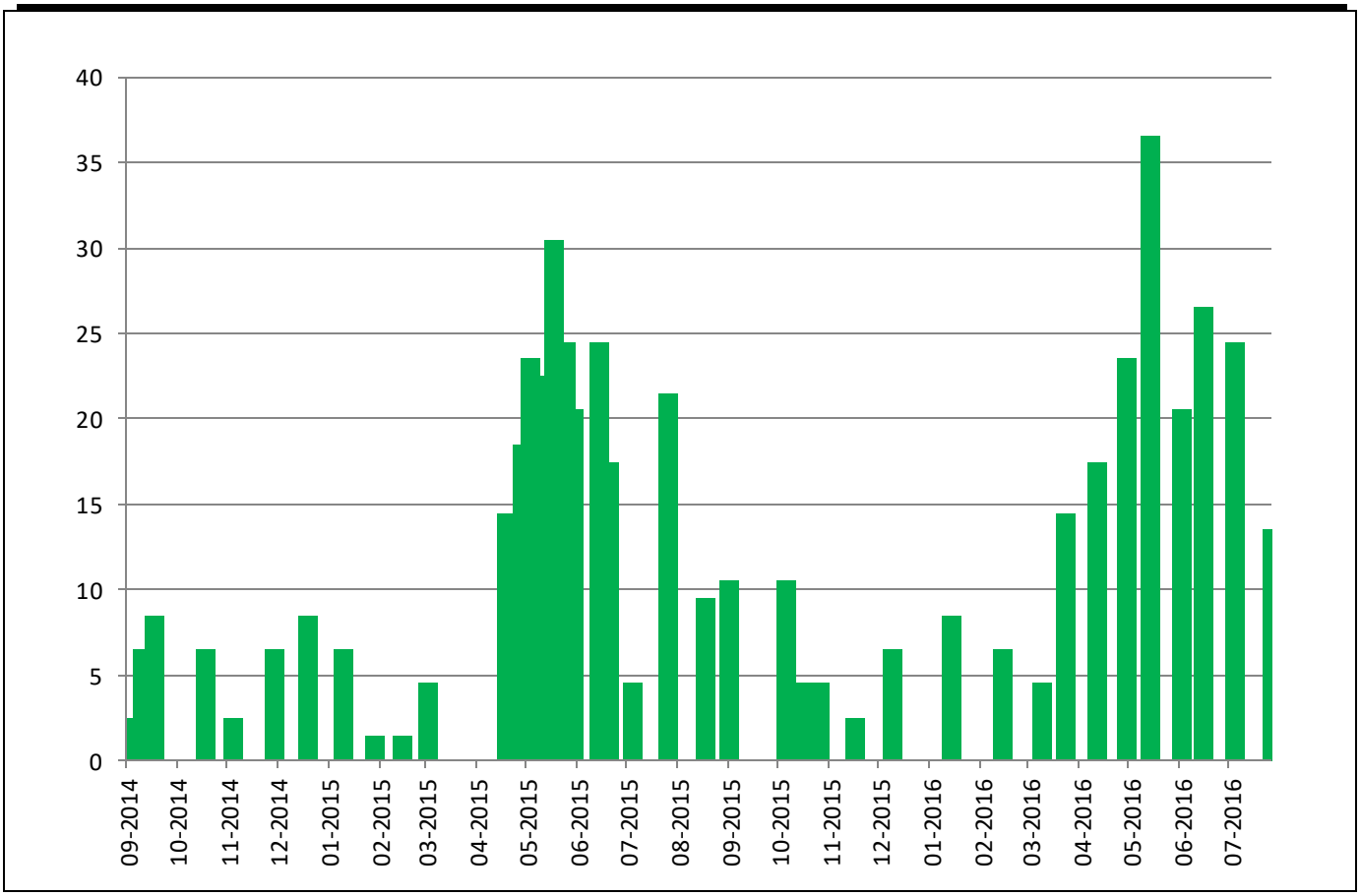

Fig.5: Seasonal observations of all reptile species during the study period.

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Agricultural Restructuring in Vietnamese Mekong Delta: Economic Analysis of Rotational Sesame Production on Rice Field among Small-scale Farmers.

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***Abstract*—** *The study examined the economic analysis of sesame production compliant withagricultural restructuring plan in rural areas of Vietnamese Mekong Delta. Conditional non-probability sampling technique was employed to select 90 respondents who have produced sesame rotationally on rice field in summer-autumn crop season. Primary data were analyzed using both descriptive and inferential statistics including percentage, frequency and farm budget model. Gross Margin analysis was used to estimate cost, returns sesame production in the study area. The study revealed that the average cost, revenue, gross margins of production per hectare was 17.60, 37.38 and 20.56 million VND, respectively.Moreover,the average rate of returnsalsoindicated that with every 1,000 VND invested to sesame production, a farmer made a profit of 1,390 VND. As a result, it can be concluded that sesame farming is profitable in the context of agricultural restructuring strategy from rice to other crops in Mekong Delta region. It is recommended that smallholders should take initiative in participation in sesame cooperatives and 'big field' model to be more beneficial to inputs price, harvested machine and formal credit in the beginning of each season.*

***Keywords*— economic analysis, sesame, smallholders, profitable, gross margins, 'big field' model.**

I. BACKGROUND

Sesame is one of the plants that has oil and has high levels of oil in the grain of which products are both used in food and other purposes such as industry, handicraft industry, pharmaceuticals, and biofuel production (RichardBell, 2008).Rotational and intercropping sesame cultivation have the positive effect of limiting pests and diseases, increasing productivity and improving the quality of land. In Thailand, sesame crop is mainly rotated with rice crop. Recommendations in Thailand suggest that rotation cultivation destruction of post-harvest residues are always necessary in order to prevent some important diseases to rice and sesame. (Pornparn and Sorasak, 2001).

The study by Ibrahim et al. (2014) also reported that sesame is one of the major industrial crops produced in northern Kordofan, Sudan and mainly for sesame oil production. Random sampling technique was used to survey 205 farmers. Results showed that the technical efficiency of crop production ranged from 11% to 100% with an average error of 84% for sesame. Average technical performance indicates that there are opportunities to increase by 16% the total output of sesame by introducing certain inputs by adopting existing technologies by farm households have a good technique.

When evaluating the profitability of sesame cultivars in Nigeria, Abu et al. (2012) used Cobb-Douglas analysis to determine the technical efficiency, distribution and economic efficiency of sesame-seed farmers in the state of Nasarawa. They used a targeted sampling technique to collect data from 194 sesame households. Technical analysis shows that production scale and pesticides are not significantly correlated to technical efficiency, while seed, labor and fertilizer are statistically significant for efficiency.

In recent years, sesame cultivation area in Vietnamese Mekong Delta has been increasing rapidly because of the agricultural restructuring plan in some localities. In An Giang, Can Tho, Dong Thap and Long An provinces, there are about 7,000 ha of sesame, occupying 17% of national area, of which Dong Thap and An Giang are the two provinces with the highest average productivity of 1.2- 1.4 tons/ha (MARD, 2017).

In Vietnam, many researchers consider that the competitiveness of the national vegetable oil industry is still

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weak because the raw material for production is mainly sesame oil, peanut oil and rice bran, the remaining 90% of raw materials have to be imported from abroad. Vegetable oil extraction outside the jungle also includes other crops, including soybean and groundnut, both of which are suffering from a lack of raw materials. Due to this shortage, Vietnam has to import 1.0 - 1.3 million tons of soybean annually (7 times the domestic production of soybean) to process vegetable oil and animal feed. (Vietrade, 2012).

Sesame cultivation on rice field in Mekong Delta is still spontaneous with smallfarms, farmers mainly use their own experience in using fertilizers and pesticides and the low application of mechanizationintopeoducing stagesis still a big matter of concern.For the above reasons, economic analysis of sesame planting model in Mekong Delta is necessary to promote new directions for this crop variety and potential economic development of the whole region in the future.

II. METHODS

Research area

Can Tho, Dong Thap and Long An provinces were chosen as the study area in Vietnamese Mekong Deltasince data from MARD, DARD and related research results have indicated that by 2016 these are the three largest areas of sesame production and have relatively potential growth when comparing to farming models in other provinces.

Sampling techniques

Conditional non-probability sampling methodwas used to collect primary data since sesame households located scatteredly in rural regions and their production usuallyfluctuate each year due to the risk related to market price.

Table.1: Proportion of Sesame Farmers Selected from Communes

Province	District	Commune	No. of households
Cần Thơ	Ô Môn	Thới Long	30
Đồng Tháp	Cao Lãnh	Tịnh Thới	30
Long An	Vĩnh Hưng	Khánh Hưng	30
Total			**90**

Source: Field Survey, 2016

Due to the limitation of time and research budget, sample size was 90 units (sesame growers). The structure of sample observations is illustrated that 30 farmers located in O Mon District (Can Tho), 30 farmers from Cao Lanh District (Soc Trang) and the last 30 ones from Vinh Hung District (Long An).

Primary data were collected by interviewing personally 90 farmers in the three districts using structured questionnaire to record information on household resources and sesame production in 2016 crop year.

Method of data analysis

Descriptive statistics like means, percentages, standard deviation and frequencies were used in analyzing socioeconomic characteristics of respondents.

Gross Margin (GM) analysis (Olukosi and Erhabor, 1988) was used to determine the mean gross margin per hectare, the mean total revenue per hectare, the mean total variable cost per hectare, the highest cost incurred by the respondents as well as the mean output obtained by the respondents.

The GM analysis of sesame production in Mekong Delta was expressed as:

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

Where

GM = Gross margins per hectare

TR = Total revenue per hectare

TVC=Total variable cost per hectare

The estimation of GM served as a profit index of sesame producers in the study area. The higher the GM the more likely a sesame farm was considered to be profitable and the smaller the GM, the lesser the profit possibility.

III. EMPIRICAL RESULTS

Socio-economic characteristics of sesame growers

Table 2 showed that majority (93.3%) of the respondents were male and be within the age group Older than 50 years old (52.2%). The productive group of sesame in Vietnamese Mekong Delta, young farmers who are always active and ready to adopt new technique, occupied just a proportion of 12%. This can be considered as a disadvantage of sesame production since older farmers are more likely to apply traditional seasonal crops calendar and not willing to change to another new ways of using fertilizers and pesticides.

Table.2: Socioeconomics characteristics of sesame farming households

Item	No. of respondents	Percentage (%)
Gender		
Male	84	93.3
Female	6	6.7
Age		

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From 21 to 30	7	7.8
From 31 to 40	11	12.2
From 41 to 50	25	27.8
Older than 50	47	52.2
Education		
Uneducated	2	2.2
Primary school	36	40.0
Secondary school	38	42.2
High school	11	12.2
Undergraduate	3	3.3
Family size		
From 1 to 3	4	4.4
From 3 to 5	38	42.2
More than 5	48	53.3
Total farm size (ha)		
From 0.1 to 1	44	48.9
From 1 to 1.5	8	8.9
More than 1.5	38	42.2
Input contract		
Yes	6	6.7
No	84	93.3
Output contract		
Yes	0	0
No	90	100

Source: Field Survey, 2016

Figures on educational level indicated that 42.2% of sesame growers have secondary education. This was followed by the primary and high school groups with 40% and 12.2% respectively. There was only 3.3% of undergraduate respondents.

Most of farmers (53.3%) belonged to households with more than 5 members, followed by those with the family size of 3 to 5 persons constituting 42.2%. It can also be seen that 48.9% of sesame growers own from 0.1 to hhhhhhhhhn6y51 hectare land size for production. This result indicated that the largest proportions of total farm holdings in the study area are small scale holdings.

Input contracts obtained by 6.7% of respondents here were small contracts for purchasing fertilizers, pesticides between farmers and local agencies. Most of local farmers have to buy inputs on credit and give partial payment. About contract of consumption, 100% of sesame households have no guarantee for selling their products. After harvesting, farmers retains a small portion for food and seeds, and the rest for sale. For that reason, unexpectedly output price alwaysdependson middle-men or local traders and becomes a great concern of farmers.

Table.3: Distribution of respondents by sesame output per hectare

Yield (kg/ha)	No. of respondents	Percentage
From 100 – 300	4	4.4
From 301 – 600	8	8.9
From 601 – 900	22	24.4
From 901 – 1200	17	18.9
From 1201 – 1500	19	21.1
More than 1500	20	22.2
Total	90	100
Mean	1100.92	
SD	514.594	

Source: Field Survey, 2016

Table 3 showed that majority of the farmers (24.4%) obtained sesame yield of 601-900kg per hectare and followed by those respondents (22.2%) who obtained sesame output of more than 1500 kg per hectare. Those respondents who obtained sesame yield of 1200 – 1500 kg per hectare accounted for 21.1% of the total sample. Only 3.3% of the respondents have sesame yield of less than 300 kg per hectare. The mean sesame stands at 1100.92 kg per hectare.

Table.4: Mechanization application in each step of sesame production

	Level 1 0%	Level 2 < 25%	Level 3 < 50%	Level 4 < 75%	Level 5 > = 75%	Total
Step 1. Ploughing	21	5	15	18	21	*80*
Step 2. Planting	64	1	6	5	2	*78*
Step 3. Fertilizing	80	0	1	0	1	*82*
Step 4. Spraying pesticide	14	7	23	20	24	*88*

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Step 5. Pumping	2	12	20	18	35	*87*
Step 6. Harvesting	78	0	1	2	6	*87*
Step 7. Transporting	24	16	20	6	2	*68*
Step 8. Shell seperation	27	0	14	18	25	*84*
Step 9. Storage	69	4	0	0	0	*73*

Source: Field Survey, 2016

Mechanization was mainly used in soil preparation, spraying pesticides, irrigation and shellseparation steps. Level 5 (more than 75% of application) was highly used in the two stagespumping (35 respondents) and shell separation (25 respondents), followed by spraying pesticides (24 respondents) and ploughing (21 respondents) steps. In these production stages, mechanical machinery is used primarily by re-using machinery from rice production because of their same function withmany types of crops. At the same time, planting, fertilizing, harvesting, storage (drying and stocking) stages were done with an average of 0% mechanization.Especially,farmers have to harvest sesame by hands because there are no specialized harvested machine to use for this important stage in Mekong region. This is the reason why farmers will have to use more hired labor costs for the production of sesame in hand-work steps.

Table.4: Cost and return analysis of sesame production in Vietnamese Mekong Delta

	Mean (thousand VND/ha)	Std. Dev.
Total Revenue (TR)	37386.60	18600.478
Seed cost	514.21	284.408
Fertilizer cost	4989.79	2473.417
Pesticides cost	4630.72	4035.756
Family labour cost	7278.51	6760.751
Hired labour cost	5382.78	3642.505
Mechines cost	649.46	909.290
Hired machines cost	1438.99	1545.778
Total Variable Costs (TVC)	17606.04	7532.430
Gross Margins (GM)	20558.08	14681.324
Average Rate of Returns	1.39	

Source: Field Survey, 2016

In the crop season of 2016, seasame production achieved a total revenue of 37.38 million VND. However, the result would be better if there was no price pressure from the middle-man and natural plant disease affected productivity.

The average profit of sesame farmers was 20.56 million VND/ha, lower than that one of 34.26 million VND/ha in Tan's research (2016) on sesame in Dong Thap province. With an average profit of more than 20 million VND per hectare in a 2.5-month crop, this was a relatively high result compared to the same period of summer-autumn rice crop. A typical example of Dao (2015) study in 2012, Cao Lanh district, Dong Thap province, summer-autumn rice crop yield was 5.8 tons/ha, with11 million VND/ha of profit and another study by Cuong (2013) also indicated that the profit of summer-autumn rice crop in O Mon District, Can Tho City is 11.63 million VND/ha. Therefore, the profit of sesame plants is 2 times higher than that of rice in the same summer-autumn crop.

In general, based on the results of the analysis of the financial performance indicators, we can conclude that with every 1,000 VND invested into production, it is about 1,390VND of profit to be made. It can be seen that sesame cultivation on rice field in Mekong Delta provinces has a positive performance, although it is not too high.In next years, ifsmall-scale area will possibly be concentrated into bigger farms,sesame production will totallybring such a potential economicdevelopment for the whole region in the context of restructuring the mainly-wet rice agriculture into other positively more profitable crops.

IV. CONCLUSIONS AND RECOMMENDATIONS

This study analyzed the economic indicators of smallholder sesame growers in the three provinces Can Tho, Long An and Dong Thap in Vietnamese Mekong region. Rotational sesame production on the rice field has become one of the most popular models chosen by farmers in recent years. Through the Gross Margins item, the model has proven that sesame plants are more economically efficient than rice production in the same crop season.The results of this study would help providing significant reference sourcefor farmers who possibly want to change their main product and local policy makers who give decisions about

which crops to produce in regions in the context of severve climate change.

In the year 2016, the production of sesame in the Mekong Delta achieved high yield and returns but the financial efficiency was not much positive for famers to totally change from rice to sesame in a long-term period. Cost items in the production process were occupied such a big proportion, especially fertilizer, pesticide and labor costs. For that reason, farmers must use fertilizer properly under the guidance of agricultural officers, avoiding the use of old practices that could affect negatively to their products. The Department of Agriculture and Rural Development should organize more frequent training courses to help people improve knowledge, farming skills and technology related to sesame production.

The formation of cooperatives for sesame is very necessary to help making production process stable, reducing input price, stabilizingoutput price of sesame by using contracts to help farmers avoid the market risks. In addition, the government should mobilize farmers to convert the sesame in accordance with the master plan into "big fields", thereby reducing the area of summer-autumn rice season. At the same time, to invest in irrigation system to ensure sufficient supply of water for sesame in the dry season, promote mechanization in production, planning raw material areas and associate with enterprises to buy products for farmers.

REFERENCES

[1] Abu, G.A., P.I. Ater and D. Abah.(2012). Profit Efficiency among Sesame Farmers in Nasarawa State, Nigeria. *Current Research Journal of Social Sciences*, 4(4):261-268.

[2] Cuong, N.V. (2013). Factors affecting the financial efficiency of rice production in O Mon district, Can Tho city. Post graduate thesis. Can Tho University.

[3] Dao, L.X. (2015). Sesame on the rice field achieved high economic efficiency. http://snnptnt.dongthap.gov.vn/wps/portal/snnptnt/!ut/p/c0/04_SB8K8xLLM9MSSzPy8xBz9CP0os_jQEDc3n1AXEwN3SwsDA8_AABM3b3MvI4NAI_2CbEdFAKkL7ck!/?WCM_GLOBAL_CONTEXT=/wps/wcm/connect/SNNPTNT/sitsnnptnt/sitamohinhsanxuathieuqua/mo+hinh+canh+tac+me+tren+dat+lua+hieu+qua+kinh+te+cao, retrieved on 1/8/2017.

[4] FAOSTAT(2017). Food and Agriculture Organization of the United Nations. FAOSTAT Database. Available at http://www.fao.org/faostat/en/#data/QC, retrieved on 4/8/2017.

[5] Ibrahim, E., Mohammed, A. and Adam, E. A. (2014). Identification of factors that influence technical efficiency of cash crops production (sesame and groundnut) in North Kordofan state, Sudan. *International Journal of Scientific & Technology Research*, 3 (10): 89-94.

[6] MARD, 2017. *Synthesis of agricultural production results as of June 15, 2017*. Day retrived: 2/7/2017.

[7] Olukosi, J.O. and Erhabor, P.O. (1988). Introduction to Farm Management Economics: Principles and Applications. Agitab Publishers Ltd., Nigeria, p.16.

[8] Pornparn,S. and Sorasak, M.(2001). Sesamum indicum L. In a guide book for field crops production in Thailand. Thailand Field Crop Research Institute. 87-95.

[9] F., E., & Okpoko, P. (2018). Tourism and Agro-Processing Industries: A Qualitative Study of Abakaliki Rice Mill. *International Journal Of Horticulture, Agriculture And Food Science*, *2*(2), 7-14. doi: 10.22161/ijhaf.2.2.2

[10] Richard,B.(2008). Bioethanol and bidiesel overview. WREC, Bangkok, Thailand.

[11] Tan, P.M. (2016). Analysis of production efficiency of sesame farmers in Dong Thap province. Post graduate thesis. Can Tho University.

[12] Vietrade (2012). Forecast of production and consumption of vegetable oil in Vietnam until 2025.http://www.vietrade.gov.vn/nong-sn-khac/2775-du-bao-san-xuat-va-tieu-thu-dau-thuc-vat-tai-viet-nam-den-nam-2025.html, retrieved on 20/10/2016.

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Eroding Fabrics of Communal Land Ownership in Papua New Guinea

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***Abstract*—** *This paper analyses the perceptions of 120 landowner-households of Nanadai Clan of Gaire Village in Central Province and Sek Clan of Madang Province concerning breaking apart of communal ownership of customary land in PNG. Previous researches have argued that there is lack of a clear distinction between individual and communal property rights in some parts of Papua New Guinea. The existing weak land administration system and mechanisms have contributed immensely towards tearing apart the bond and connections between clan members and the dismantling of communal land ownership in Papua New Guinea thus, compromising national land administration values and standards. Current practices reveal that customary land is held at the sub-clan, family and individual levels, while the major clans just bear ownership name-tag. The existing land legislation in Papua New Guinea recognises that ownership of customary land is vested in the clans, however, the realities on the ground from the findings of this research indicate otherwise. Therefore, this paper calls for the strengthening of the weak land administration functions and mechanisms together with the review of all existing laws to improve the standards of land administration system in the country. This paper argues that communal land ownership in Papua New Guinea is slowly breaking apart causing disharmony between*

Keywords*— *Communal ownership, Clans, Land Administration System and Land Administration Standards.

I. NATURE OF CUSTOMARY LAND PROBLEM IN PNG

Man and land in Papua New Guinea are inseparable and the association between the two is at the heart of the economic, cultural and spiritual foundations of society, which invariably underpins the individual's and group's sense of social identity and belonging (Koczberski, Numbasa, Germis and Curry, 2017; Sillitoe, 1999). This link to social and cultural identity also underpins the common view among landowners that land is inalienable. Even customary land that has been acquired by the state or converted to freehold title is rarely seen as being alienated permanently from customary ownership (Chand and Yala, 2006; Filer and Lowe, 2011; Curry et al., 2012). Customary land tenure arrangements vary across the country, but generally, under customary tenure, rights to land are based on a mixture of descent, residence and participation in communal activities (Cooter, 1991; Larmour, 1991; Curry, 1997; Koczberski et al., 2017 & 2009). Exclusive individual landownership and inheritance are generally limited in PNG.

ILG incorporation is already being seeing as the major problem because what the major clan holds is just the skeleton or structural frame of ownership but the control and use of the customary land is fully vested in individuals and family units in some communities in PNG (Karigawa 2016). Traditionally, land ownership through communal arrangements keeps the clans/tribes in Papua New Guinea intact but in the modern economy; it becomes an obstacle to economic and other forms of development on customary land (Karigawa, Babarinde and Holis, 2016; Curry et al., 2012).

Lakau (1991) and Armitage (2002) have argued that legislations in PNG dealing with land directly or indirectly are too many and most of these laws are not compatible to one another creating more problems for the already weak land administration system in PNG. This argument is supported by Martin (2005); Grant, Ting and Williamson (1999) whilst Green Peace Australia Pacific (2012) stated that land grabbing issues in PNG is a result of the weak land administration system.

The purpose of this study is to investigate the causes of communal land ownership break-down and suggest way forward to mitigate these challenges and thereby prevent further disintegration of customary land tenure in PNG. The paper consists of six sections. After the introduction and problem statement in the first section, Section 2 presents an overview of communal land ownership in PNG, followed

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by an outline of the hypothesis and research questions in Section 3. The research method and findings are presented in Sections 4 and 5 respectively, while the concluding section (Section 6) summarizes the paper and offers some advice in terms of policy implications of the findings.

II. AN OVERVIEW OF COMMUNAL LAND OWNERSHIP IN PNG

The complexity of the manner in which customary land is owned in PNG cannot be denied. Communal land ownership is recognised by the existing land legislation, which denies individual ownership - a bond that has created a strong relationship between man and his land over the years. Champagne (2017) has observed that distribution of land resources has worked for many indigenous nations for thousands of years. The tribal entities managed the land collectively. However, there are rules that uphold the rights of tribal sub-groups for access to land sufficient for their livelihood. The land is held not only for gathering food and resources, but tribal members have an obligation to maintain the land in good use for future generations.

Since the families, clans or villagers that use land expect to live in the same area for many future generations, the tribal members have a vested interest in maintaining the ecological and cultural soundness of their allocations. However, during the course of maintaining the land for future generations by individuals, their fathers transfer ownership from the main clan to individuals and families. This is the birth of a mixed communal ownership in PNG. Cousins (2009) in reviewing the work of Bruce (1986) regarding communal ownership in African nations stated that "communal tenure systems are in fact mixed tenure regimes, comprising variable bundles of individual, family, sub-group and larger group rights and duties in relation to a variety of natural resources". Therefore, communal ownership of land began to shift towards ownership, control, use and disposition by specific groups within the main community, together with land obligations that are vested in those specific groups (sub-clans) and individuals.

Curry et al. (2012) strongly argued that the "adaptations and modifications to customary land tenure by landowners in response to these key drivers offer lessons to inform land reform policies". They further stated that "whilst customary land tenure is recognised in PNG's Constitution, it has largely been considered problematic in discussions of land reform." Land reform in PNG and elsewhere in the Pacific has been dominated by the assertion that customary tenure is incapable of providing secure property rights necessary for facilitating investment and the commercial use of land. Thus, attempts at land reform in PNG have been based on the notion that secure individual property rights through land titling and tenure conversion are a prerequisite for building a favourable investment climate and fostering economic development.

The analysis from the African countries and other indigenous countries around the globe reveals that there is significant shift from communal ownership to individual ownership. Elahi (2013) argues that PNG should shift from communal to privatised ownership to make land accessible for agricultural development and this has been supported by some other studies (e.g. Karigawa, Babarinde and Holis, 2016; Curry et al., 2012), which claim that communal ownership is an obstacle to economic development in PNG. Thus, there are already clear indications that PNG is slowly moving towards private ownership of customary land although it is not legally recognised yet.

Although there are already laws in place protecting customary land from being sold and leased, there are continuous sales of customary land across PNG. In most cases, land sales tend to be through informal verbal agreements between the transacting parties, with an individual's access and use rights to the land loosely defined (Curry et al., 2012). Members of the broader landowner groups are sometimes not aware that land has been 'sold' to an 'outsider' but this can sooner or later become a major source of discontent within the major landowner group. Disputes over 'purchased' customary land (and even over land initially gifted to migrants) have been increasing over the past 10 years. These disputes arise not so much because migrants and landowners have different understandings of land use rights – e.g. the right to plant oil palm - but rather because they have different perceptions of land 'ownership', which means that their respective interpretations of the obligations and expectations associated with land transactions can be very different (Curry et al., 2012).

Therefore, a significant challenge for policy makers in PNG will be how to deal with the proliferation of informal (and sometimes illegal) land transfers taking place, as landowners develop their own arrangements for land mobilisation outside government structures, and as they seek to capitalise on the demand for urban and rural land by land-poor migrants". How policy makers can develop an effective reform program and land administration system to accommodate the range of informal and semi-formal arrangements already well established will be one of the principal challenges for land reform in PNG. Customary land in PNG has gone through a lot of land

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reforms in the past to present. Past land reforms were geared towards security of tenure while current land reforms are more about transforming customary land into a saleable commodity that can be transacted in the open market (Curry et al., 2012).

III. HYPOTHESIS AND RESEARCH QUESTIONS

Having regard to the above situation analysis, this paper attempts to test one hypothesis and answer four research questions. The hypothesis states that: *Communal land ownership is slowly breaking apart in PNG communities.* The four research questions are as follows:

i) What are the main causes of communal land ownership break-down in PNG?
ii) What are the flaws in the land administration system in PNG?
iii) What are the views of customary landowners regarding the protection by existing land laws and the customary land title?; and
iv) What is the way forward for customary land ownership in PNG?

IV. METHOD

The paper uses a stratified random sample to gather the perceptions of respondents selected from two sub-clans in PNG. A stratified random sample is a sampling method that requires the population to be divided into smaller groups called strata from which random samples are taken.

This research is based on two sub-clans of Laurina Clan of Gaire Village in Central Province and Sek Clan of Sek Island in Madang Province. A representative sample size of 120 landowners, representing 67% of the total population of 360 landowners was interviewed. Table 1 illustrates the sampling frame and sample size.

Table.1: Sample Population

Stratified Random Sample Selection							
Major Clan Name	Sub-clan	Total population (main clans)	Total Population (sub-clan)	Sample (%)	Target	Total Number Returned	Total Returned (%)
Laurina	Nanadai	500	160	50	80	64	80
Sek Clan	Panuwadan	700	200	50	100	56	56
Total		1,200	360	100	180	120	67

Source: Author, 2018

Primary data was obtained through questionnaires, interviews and site observations, while secondary data was sourced from relevant literature and public records. Data was analysed using SPSS, excel and statistics, particularly Chi-square Test (χ^2). At this juncture, it is worthy of note to add that the research leading to this paper faced two main limitations. First, funding constraints made it impossible for the author to investigate more clans in other parts of PNG. Second, the researcher was unable to investigate a matrilineal system, thus both sample populations are from the patrilineal system in PNG.

V. FINDINGS AND DISCUSSIONS

In this section, an attempt is made to test the hypothesis and answer the four research questions posited in Section 3 of the paper.

i) Test of Hypothesis

H_o: Communal land ownership is not slowly breaking apart in PNG communities.

H_1: Communal land ownership is slowly breaking apart in PNG communities.

This above hypothesis (H_1) can be tested using current indications of customary land ownership in PNG. The protection over customary land by existing laws gives full recognition to the clans and tribes to own and control customary land while individuals, families and smaller groups have user rights over the land. Any land dealings on customary land are done through their ILGs. Findings of the research indicate that major clans and the sub-clans have very little control over the land. Currently ownership of the land vests in families and individuals. Figure 1 shows the responses of landowner households regarding ownership of land at the current time in PNG.

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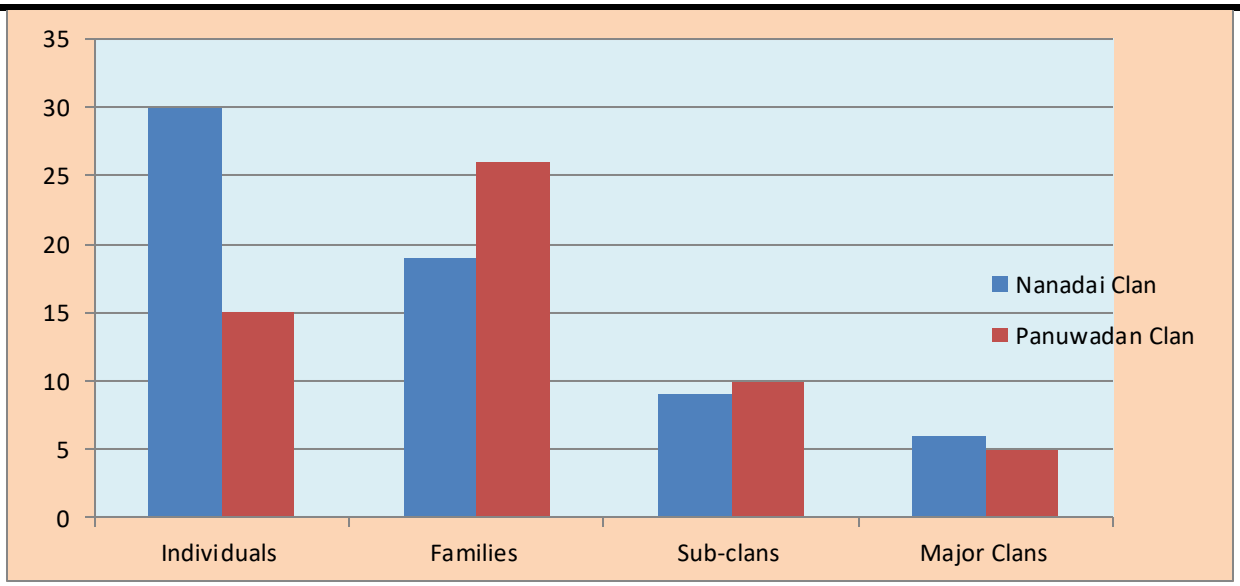

Fig.1: Various levels of customary land ownership

Source: Author, 2018

On the hand, the results from the landowners regarding the flaws in the land administration system are presented in Figure 2.

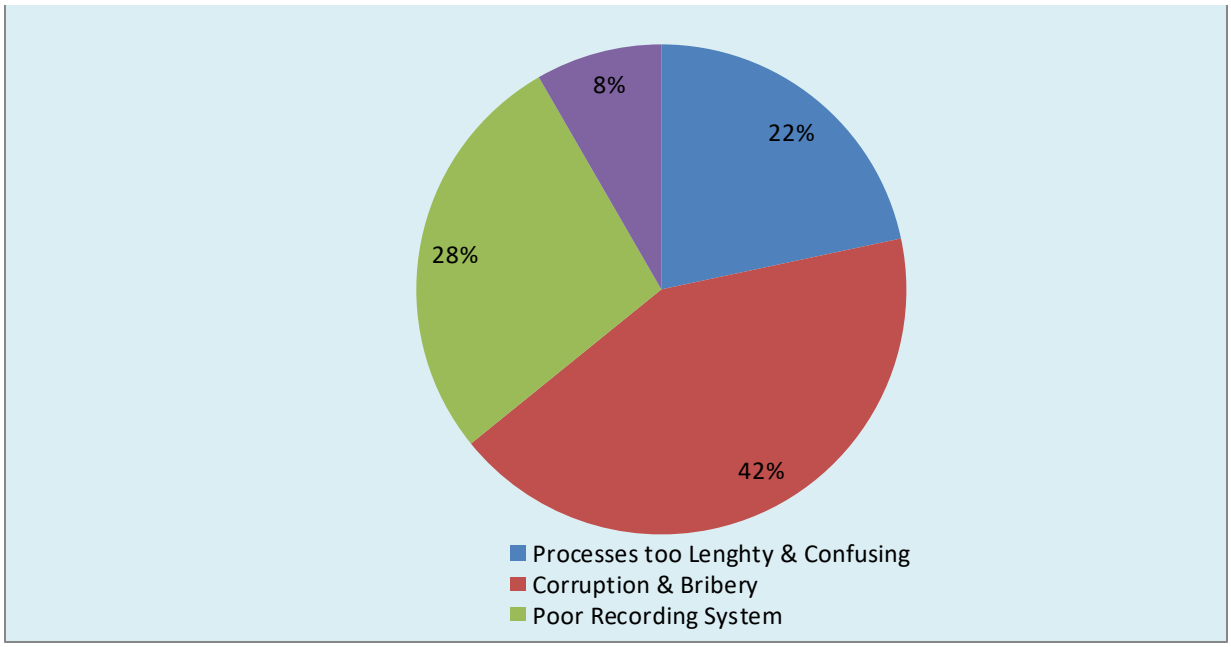

Fig.2: Land Administration flaws in PNG

Source: Author, 2018

The results from the perception of landowners regarding the land administration system in PNG already reveal that there is high level of corruption and bribery in the system thus destroying the land administration system in the country. This is followed by poor recording system, lengthy processes and confusing to the land owners and old systems still in use. This paper argues these factors have contributed immensely towards tearing apart of the customary land tenure system in the country.

Moreover, current indications reveal that many members of the major clans do not have the right to use land that is owned by the other member of the clan. Its use must come with consent from the one who claims to be the owner. Land disputes are becoming common between members of

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the same clan. The respondents argued that land is more secured and easily accessed when it is individualised than when it owned by the community under the ILGs. The proponents of strong individual rights have suggested that a registered individual title, backed by effective land administrative systems, provides the greatest certainty and security. For example, Carson (2009) pointed out the same argument regarding communal ownership in Africa claiming that "there are ambiguities in the legal systemand institutional configurations that were inherited during the colonial era and reproduced after independence." The results have indicated that communal land ownership in PNG is no longer intact and it is gradually breaking apart. About 54% and 70% of the respondents from Panuwadan and Nanadai clans respectively claimed that PNG customary land interests and rights have been inherited by families and individuals. Therefore, based on these findings, our hypothesis is supported by available data.

ii) **Research Question 1:** *What are the main causes of communal land ownership break-down in PNG?*

The research has identified five main causes (Figure 2) of communal land ownership break-down in PNG. The two very significant results are: (a) Benefits are not equally distributed, which accounts for 46% of reasons given by the respondents and (b) Increase in population (27% of responses obtained). These two are followed by land disputes within the clan (13%), shortage of land or land not easily accessed (4%). It is contended that the weak land administration system and incompatibility of land laws (Lakau 1991), together with other land-related issues have caused this break-down in the tenure system. Thus, there will be problems of incorporating ILGs under big clans, particularly when it comes to property listings of the ILG.

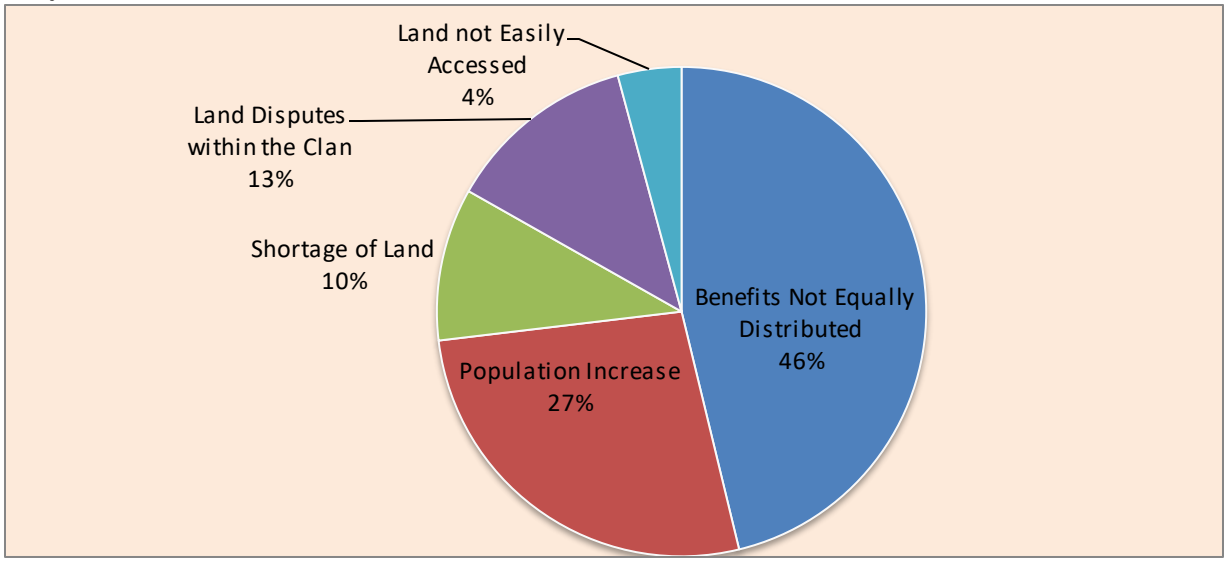

Fig.3: Causes of Communal Ownership Break-down in PNG

Source: Author, 2018

The most likely scenario is that ILGs will have to negotiate with the individuals and families for release of the land to be listed under its property listing. However, our past experience reveals that individuals claiming ownership over customary land that was held and controlled by major clans in the past have been claiming bigger cuts from the proceeds of the land than any other ordinary land owners.

iii) **Research Question 2:** *What are the flaws in the land administration system in PNG?*

Land administration systems (LAS) are about addressing land problems by providing basic infrastructures for implementing land-related policies and land management strategies to ensure social equity, economic growth and environmental protection. Moreover, land administration is the manner in which the rules of land tenure are applied and made operational. The land administration system in PNG has faced a lot of challenges in the past to present date and appears to be weak as claimed by Armitage (2002), Goldman (2005) and Lakau (1991). This paper supports the findings of previous studies in this regard as indicated in Figure 3.

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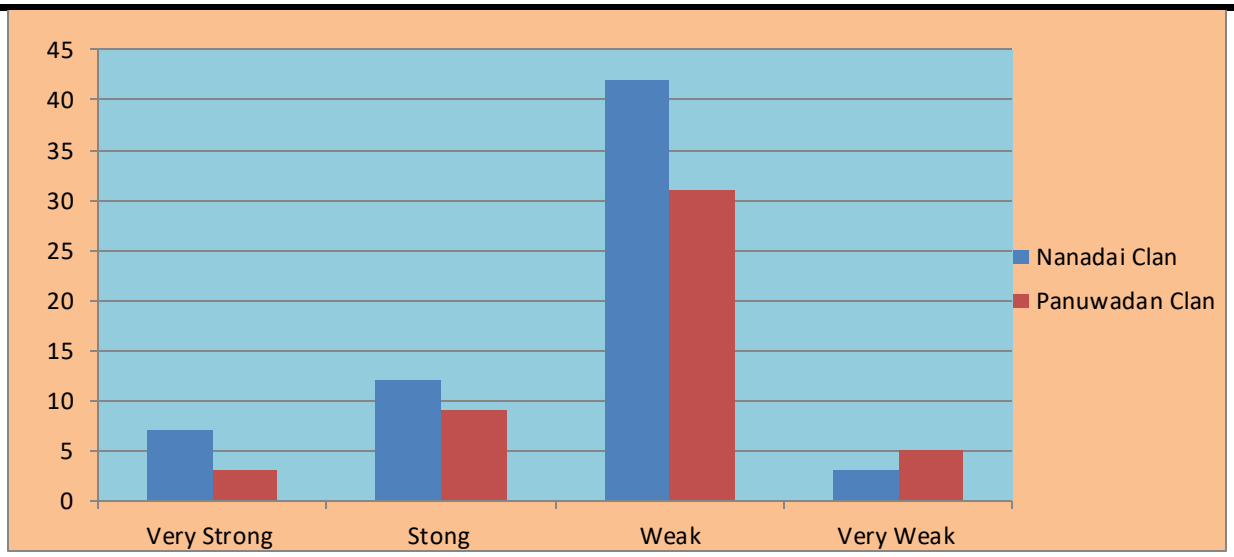

Fig.4: Land Administration System (LAS) in PNG

Source: Author, 2018

This research supports the findings of Grant, Ting and Williamson (1999), when they stated that "the humankind-land relationship is dynamic and change is occurring at a pace faster than at any other time in history". Global economic, social and technological factors, the need for sustainable development of land, and macro–economic as well as micro–economic reforms are having a substantial impact on land administration systems. Most land administration systems today are not adequate enough to cope with the increasingly complex range of rights, restrictions and responsibilities in relation to land, which are influenced by such factors as water, indigenous land use, noise and pollution together with other land-related activities. In short, land information and land administration systems need to be re-engineered and allowed to evolve to face the increasing complexity of the humankind-land relationship. For PNG to meet world standards in terms of valuation and land administration system, it has to address the flaws in the land administration system. This paper argues that the break-down of the tenure system is the result of the weak land administration system that PNG has experienced over the past many years. The variables used in the Chi-square Test are as follows: Land administration system, land laws, land disputes and security of tenure thus yielding a Chi-square Value of (χ^2) of 2.01 and P Value of 0.61 or 61%. Thus, the weak but positive correlation shown in Table 2 and the Chi-Square Test Value of 2.01 and P Value of 0.61 together with the results in Figure 3 calls for re-engineering of the land administration system to meet the increasing and complex nature of customary land tenure in PNG to avoid the total break-down of the customary land tenure system.

Table.2: Correlation Analysis of Tenure Breakdown and Land Administration System in PNG

Variables	Pearson Correlation (r)			
	Nanadai Clan		**Panuwadan Clan**	
Variable 1 Break-Down of Communal Land Ownership	1	0.379**	1	0.377**
		0.000		0.000
	64	64	56	56
Variable 2 Land Administration System in PNG	0.379**	1	0.377**	1
	0.000		0.000	
	64	64	56	56

**. Correlation is significant at the 0.01 level (2-tailed) for both sample groups

Source: Author, 2018

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The Pearson Correlation (r) of 0.38 indicates that there is a positive but weak correlation between communal ownership and the land administration system, has 61% chances of breaking down in PNG societies if the land administration system is not overhauled and existing land laws are not reviewed to meet the current ownership status quo and development aspirations of landowners in Papua New Guinea.

By re-engineering the land administration paradigm, it should address issues such as multiple titles, ILG fissioning, land dispute resolutions, land grabbing, fraudulent land registration, and other land administration issues.

i) **Research Question 3:** What are the views of customary landowners regarding the protection by existing land laws and the customary land title?

The existing land laws together with the Constitution of the Independent State of PNG give full protection over customary land. Therefore, it can be concluded that there is full tenure security. However, even though the laws are very clear on the sale, lease and other dealings on customary land, landowners are still defying the protection given by law and continuously sell customary land to foreigners. The findings of this research in Figure 3 reveal that landowners are dissatisfied that land laws are not protecting the rights of the landowners and there is already a sense of insecurity among the landowners over their customary land. However, it is hoped that the recent amendments of the ILG Act, the Land Registration Act and the Land Act currently under review will bring new hopes to the landowners.

Moreover, the results reveal that about 23% of the landowners (Figure 4) claim that the customary land title that is currently issued to the ILGs is not clearly defined by law. The title is claimed to be a freehold interest but the characteristics of the customary land title does not fit into any of the freehold interest categories. Therefore, it is very confusing to say that it is a freehold interest. Moreover, the ILG Act states that upon registration, all customs cease to operate for the duration of the title but on the other hand, the nature of the land remains customary land, thus this paper argues that there are still some elements of custom embedded in it.

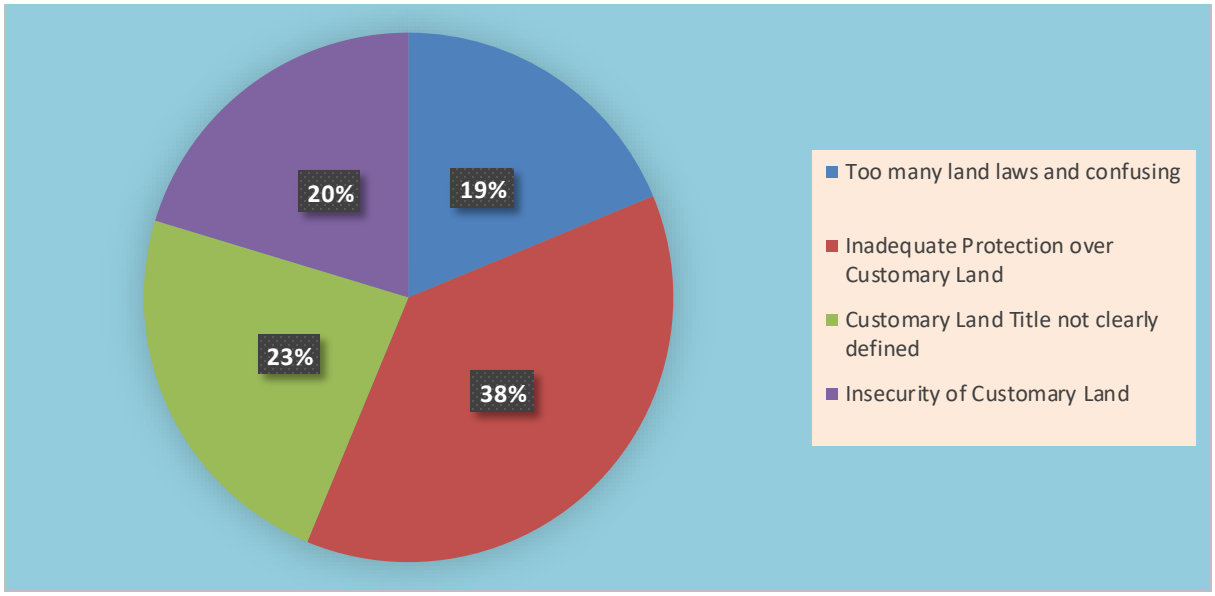

Fig.4: Views of Landowners regarding PNG's Existing Land Laws

Source: Author, 2018

Therefore, it can be concluded that by default, the law calls the title a freehold interest but in reality, it is not a freehold interest. This paper argues that the best category that this title can fall under is the *sui generis* group of properties because the customary land title is very unique and special. *Sui generis* groups or classes of properties are unique and special classes of properties that are set aside from the rest of the properties. Thus, the customary land title in PNG best fits categorization into the *sui generis* class of properties.

VI. CONCLUSION AND POLICY IMPLICATIONS

The purpose of this paper is to analyse the perceptions of landowners in regard to communal land ownership break-

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down in PNG and suggest ways to resolve the issues. Two customary landowner groups were investigated through stratified random sampling of two sub-clans namely Nanadai Clan of Gaire Village of Central Province and Panuwadan Clan of Sek Island in Madang Province. Both sub-clans are from the patrilineal societies and appear to be from the coastal regions of Papua New Guinea. Due to varying customs across all communities in PNG, the views of the landowners vary according to the way they interact with their land. This paper argues that communal landownership in PNG is slowly breaking apart.

There is a positive but weak correlation (38%) between communal land ownership break-down and land administration system indicating that the land administration system in PNG is weak for purposes of managing the affairs of customary land tenure in these challenging times. Furthermore, there is a negative perception by the landowners regarding the land laws in PNG suggesting that current land laws are not protecting the landowners' rights fully thus huge tracks of customary land were taken away from the landowners Green Peace Australia (2012). The findings supported the argument by Champagne (2017) stating that Indigenous nations are confronted with small and often shrinking land bases that do not provide the necessities of food and resources for growing populations. Privatisation of land takes land and resources out of collective tribal management. It is difficult to reclaim privatised land allotments once tribal members are granted them, usually by government policies. Thus, the concept of land ownership in PNG is particularly problematic, as is the idea that before "ownership" all things were held in common with everybody having equal rights to the same thing, or belonged to nobody as claimed by Du Plessis & Frantz (2013). Du Plessis & Frantz (2013) in reviewing the work of Bennett (2004) highlighted that "it is more likely that, before the concept of individual ownership emerged, only rights of use were protected". With the introduction of commerce, an exchange value had to be attached to a commodity, and in this context ownership provided the answer in securing the property. With ownership came the idea of "absoluteness" that implied that one person could hold all the entitlements in a certain property, and dispose of it at free will. This differs remarkably from the pre-colonial era where different interests in the same property could vest in different holders, and where these interests are furthermore flexible and ever changing. Therefore, this paper argues that the findings from the literature together with the findings of this research assert that communal ownership in PNG is slowly breaking apart.

The findings of this paper are important for policy formulation and implementation and review of the existing land laws in Papua New Guinea for good and secured tenureship particularly on customary land. The complexity of the customs cannot be denied and customary landownership in PNG evolved around these complex customs.

Thus, to answer the last research question, this paper recommends key strategies that could be adopted to mitigate the challenges facing customary land tenure as follows:

- ***Re-engineering the Land Administration System***

Land administration is the foundation of tenureship in any country, thus it forms the basis for valuation, land administration mechanisms and property management. The land administration system in PNG is an adopted system from the colonial era. Thus it is believed that land problems had been inherited all along. Many of the concepts used are foreign concepts that PNG needs to revise to suit the needs of our tenure system and land development aspirations of the landowners in the country. Re-engineering the Land Administration System should mitigate issues such as:

a) Double titling;
b) Land grabbing;
c) ILG fissioning;
d) Security of Tenure;
e) Flexibility for collateral purposes and
f) Many other land administration related issues

The above issues are believed to be some of the contributing factors towards the break-down of communal ownership in PNG

- ***Review of all land laws***

The results from this research have indicated that laws are not protecting the rights of the land owners and there is already a sense of insecurity regarding the laws. Moreover, the Land Act 1996 states clearly that customary land should not be sold to any other persons except to the State. However, there is evidence of increasing customary land sales. Thus there is a great deal of need to toughen the existing laws to protect landowners from losing their land. Moreover, these laws must be compatible to each other to avoid confusion among the landowners. The titles given to landowners must be given the full strength like any other titles.

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- ***Institutional Involvement – A wake up call to PNGIVLA***

The Papua New Guinea Institute of Land Administrators and Valuers (PNGIVLA) must take a leading role and be active in the formulation of the Land Policies and review of Land legislation and any other land-related dealings in PNG. The experts in Valuation, Land Administration and Property Management in PNG are the members of the Institute. The Institute must be vocal in all bad land dealings and must put forward proper mechanisms to mitigate the issues of land administration, valuation and property management before calling for adopting world standards. The Institute must be neutral to fight for justice for the landowners. It must be at the forefront to stand side-by-side with the Department of Lands and Physical Planning fighting against the giants of land grabbing to reclaim land for the land owners that were lost in the past and continue to fight to protect the land rights and resources of the indigenous people of PNG. Moreover, the paper argues that PNGIVLA should be active in recommending its members for Valuer Registration because the strength of the institute lies with its registered valuers and financial members.

It is the view of this paper that PNGIVLA will compromise the world standard in valuation, land administration and property management disciplines if its backyard contains a backlog of unresolved issues or is not actively involved in decision-making regarding efficient land dealings in PNG.

- **Codification of PNG Norms and Customs with respect to Communal Ownership**

This concept is adopted from Karigawa, Babarinde and Holis (2016) and Du Plessis & Frantz (2013). This paper understands that with the advent of constitutionalism in Papua New Guinea, customary laws (made of traditional norms and customs) will of necessity be elevated alongside Statutory provisions (such as Acts of Parliament) and Common Law being recognised and accepted as one of the sources of law in the country. However, these norms and customs appear to be very complex in nature. Dealing with these norms and customs is not an easy task for land administrators and valuers when it comes to customary land dealings. Therefore, this paper argues that the on-going codification of customs in PNG, like that of South Africa and other some other African countries be pursued to a logical conclusion to cover all the 22 provinces in PNG. The codification of traditional norms, values and customs will create flexibility needed in dealing with customary land in PNG.

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REFERENCES

[1] Armitage, L. (2002), Customary Land Tenure in Papua New Guinea: Customs and Commerce at the Interface, A Case Study of Ahi Peoples' Land, Lae, Morobe Province, Queensland University of Technology, Brisbane; Available at:
[2] http://www.prres.net/Papers/Armitage indigenous property rights custom & commerce PNG.pdf, accessed on 15 June 2014.
[3] Armitage, L. (2000), Indigenous property rights: Custom & Commerce at the Interface, Brisbane: Queensland University of Technology; Available at: http://scholar.google.com/scholar?biw=320&h=303&um=1ie=UTF8lr&cites93615458325954, accessed on 20 August, 2015.
[4] Attributes of Good Governance, Available at: http://www.wri.org/blog/2015/02/qa-mark-robinson-role-good-governance-sustainable-development, accessed on 13 December, 2016.
[5] Australian Aid, 2008; Available at: http://www.ausaid.gov.au/publications/pdf/MLW VolumeOne Bookmarked.pdf, accessed on 13 December, 2017).
[6] Bennett TW *Customary law in South Africa* (Juta, Cape Town 2004).
[7] Bendor, J. and Swistak, P. (2001), The Evolution of Norms, *American Journal of Sociology*, Volume 106, Number 6,pp. 1493–1545.
[8] Brundtland Commission (1987), Report of the World Commission on Environment and Development, *United Nations*.
[9] Burton, J. (1991), 'Social Mapping,' in P. Larmour (Ed.), Customary Land Tenure: Registration and Decentralisation in Papua New Guinea. Port Moresby: Australian National University, New Guinea Research Unit (Bulletin 40).
[10] Champagne D (2017) Is Keeping Communal Land Possible? *For indigenous land is essential*
[11] Chand, S., Ondopa, J. and Nao, L. (2014), Use of land lease as collateral for accessing formal sector finance

in Papua New Guinea (National Research Institute Issues Paper No. 7), Port Moresby: National Research Institute.

[12] Commission of Inquiry into the Special Agriculture and Business Leases, Available at: http://www.coi.gov.pg/sabl.html, accessed on 24 January, 2017.

[13] Claassens, Aninka, 2005, 'The Communal Land Rights Act and women: Does the Act remedy or entrench discrimination and the distortion of the customary?'. Occasional Paper 28, Cape Town: Programme for Land and Agrarian Studies, University of the Western Cape.

[14] Cooter, R. D. (1989), Issues in Customary Land Law, Port Moresby: Institute of National Affairs (Discussion Paper 39).

[15] Cousins, Ben and Aninka Claassens, 2004. 'Communal land rights, democracy and traditional leaders in post-apartheid South Africa'. In *Securing Land and Resource Rights in Africa: Pan-African Perspectives*, ed. M. Saruchera, 139-54. Cape Town: Programme for Land and Agrarian Studies, University of the Western Cape.

[16] Cousins, Ben, 2008. "Characterising 'communal' tenure: nested systems and flexible boundaries", in: Aninka Claassens and BenCousins (eds). *Land, Power and Custom: Controversies generated by South Africa's CommunalLand Rights Act.* Cape Town: UCT Press and Athens: Ohio University Press (109-137).

[17] Cousins, Ben, 2008. "Contextualizing controversies: communal tenure reform in post-apartheid South Africa", in: Aninka Claassens and Ben Cousins (eds). *Land, Power and Custom: Controversies generated by South Africa's CommunalLand Rights Act.* Cape Town: UCT Press, and Athens: OhioUniversity Press (3-31).

[18] Definition and Indicators of Sustainable Development, Available at: http://www.sustainablemeasures.com/Training/Indicators/Def-Br1.html, accessed on 25 January, 2017.

[19] Dixon, K. (2006), Working with mixed commons/anticommons property: mobilising customary land in Papua New Guinea, Harvard Environmental Law Review (Vol. 31); Available at: www.law.havard.edu>vol31_1>dixonpdf, accessed on 15 October, 2015.

[20] Du Plessis & Frantz (2013)African Customary Land Rights in a Private Ownership Paradigm

[21] Elkington, J. (1997), Cannibals with Forks – Triple bottom line of 21st century business, Stoney Creek, CT: New Society Publishers.

[22] FAO (2012), Voluntary guidelines on the responsible governance of tenure (VGGT), UN, Rome; Available at: http://www.fao.org/docrep/016/i2801e/i2801e.pdf, accessed on 12 February, 2017.

[23] Filer, C. (2012), The New Land Grab in Papua New Guinea, Resource Management in Asia-Pacific Program, Crawford School of Economics and Government, Australian National University; Available at: http://actnowpng.org/sites/default/files/The%20new%20land%20grab%20in%20Papua%20New%20Guinea%20Colin%20Filer.pdf., accessed on 6 April, 2017.

[24] Filer, C. (2007), Local Custom and the Art of Land Group Boundary Maintenance in Papua New Guinea, Australia National University; Available at: http://press.anu.edu.au/apem/customary/mobile_devices/ch07s02.html,accessed on 4 October, 2015.

[25] Fingleton, J. S. (1998), Legal Recognition of Indigenous Groups, Rome; Available at:

[26] http://press.anu.edu.au/apem/customary/mobile_devices/ch07s02.html, accessed on 15 May, 2014.

[27] Goldman, L. (2005), PNG Gas Project Social Impact Assessment,' Brisbane: Exxon Mobil; Available at: http://press.anu.edu.au/apem/customary/pdf/ch06.pdf; accessed on 14 December, 2015.

[28] Grant, D., Ting, L. and Williamson, I. (1999), The Evolving Role of Land Administration in Support of Sustainable Development: A review of the United Nations: International Federation of Surveyors, Bathurst Declaration on Land Administration for Sustainable Development: Sustainable development is just rhetoric without appropriate land administration systems, *Position Paper*, Department of Geomatics, University of Melbourne, Australia.

[29] Green Peace Australia Pacific (2014), Available at: http://www.greenpeace.org/australia/en/what-we-do/, accessed on 12 December, 2015.

[30] Karigawa, L. (2015), Issues Affecting Incorporated Land Groups (ILGs) and their Sustainability in Papua New Guinea: A Case Study of Morobe Province, An MPhil Thesis in the Department of Surveying and Land Studies, Papua New Guinea University of Technology, Lae, Papua New Guinea.

[31] Kalinoe, L. K. (2001), Incorporated Land Groups in Papua New Guinea, MLJ 4; [2003-04] 29 *Melanesian Law Journal* 73; Available at:

http://www.google.com.pg/search?q=ilg+ing&biw=320&bih=508&prmd=1.5#q=ilg+in+png, accessed on 12 November, 2015).

[32] Kocmanova, A. and Simberova, I. (2014), Determination of Environmental, Social and Corporate Governance Indicators: Framework in the Measurement of Sustainable Performance, *Journal of Business Economics and Management*, Volume 15, Issue 5, pp. 1017-1033.

[33] Kwapena, F. (2014), Empowering Customary Landowners to give Value to their Land: NRI Commentry, Port Moresby; Available at: www.pngpeles.com>index.php>272-empowering-customary-landowners-to-give-value-to-their-land; accessed on 15 May, 2015.

[34] Lakau, A. (1991), State Acquisition of Customary Land for Public Purposes in Papua New Guinea, Department of Surveying and Land Studies, Papua New Guinea University of Technology, Lae.

[35] Larcom, S. (2016), Natural Resource Contests and Precolonial Institutions in Papua New Guinea, Available at: http://onlinelibrary.wiley.com/doi/10.1111/joac.12169/abstract, accessed on 7 April, 2017.

[36] Leavoy, P. (2016), Sustainability Reporting: The What and Why? Available at:

[37] http://www.environmentalleader.com/2014/09/15/sustainability-reporting-the-what-and-why/, accessed on 22 January, 2017.

[38] Making Land Work - Volume One: Reconciling customary land and development in the Pacific; Available at:

[39] http://dfat.gov.au/about-us/publications/Documents/MLW_VolumeOne_Bookmarked.pdf, accessed on 21 January, 2017.

[40] Minister of Land's Indictment of the Department of Lands (DLPP); Available at: http://lands.gov.pg/Services/Governance/Complaints_Desk.html, accessed on 23 January, 2017.

[41] North Dakota Oil Pipeline Battle, USA; Available at: https://www.nytimes.com/2016/11/02/us/north-dakota-oil-pipeline-battle-whos-fighting-and-why.html?_r=0, accessed on 12 February, 2017.

[42] Ogle. L. and Tararia, A. (2001), Incorporated Land Groups and the Registration of Customary Land: Recent Developments in PNG; Available at: http://milda.aidwatch.org.au/, accessed on 14 December, 2015.

[43] Oliver, N. and Fingleton, J. (2008), Settling customary land disputes in Papua New Guinea. In Making Land Work, Volume Two: Case Studies on Customary Land and Development in the Pacific; AusAID: Canberra, Australia, pp. 223–240.

[44] O'Riordan, T. (1989), The challenge for environmentalism, in *New Models in Geography*, Peet, R. and Thrift, N. (Eds), Unwin Hyman: London; pp. 77–102.

[45] Petrosyan, A. F. (2010), A Model for Incorporated Measurement of Sustainable Development Comprising Remote Sensing Data and Using the Concept of Biodiversity, *Journal of Sustainable Development*, Vol. 3, No. 2, pp. 9-26.

[46] Power, A. P. (2001), Land Mobilisation in Papua New Guinea. Port Moresby, PNG (ed.) 2000, Land group incorporation: village guide and legal guide, Anutech/AusAID, Canberra; Available at: www.pngbuai.com>land-development, accessed on 19 December, 2014.

[47] Robinson, G., Available at: http://www.wri.org/blog/2015/02/qa-mark-robinson-role-good-governance-sustainable-development, accessed on 10 February, 2017.

[48] Stratified Random Sample; Available at: http://study.com/academy/lesson/stratified-ramdom-sample-definition-quiez.html, accessed on 12 December, 2015.

[49] Sustainability; Available at: http://www.kennucorked.com>sustainability-definition.html, accessed on 13 December, 2015.

[50] Ogle. L & Tararia, A. (2007). Incorporated Land Groups and the Registration ofCustomary Land-Recent Developments in PNG. http://scholar.google.com/scholar?um=1&ie-UTF-8&lr&cites=10774025897395858495, 14th August, 2015

[51] Tumare, J., Babarinde, J. A. and Tagicakibau, M. (2015), A Conceptual Framework for Multipurpose Land Information System (MPLIS) Application for Land Management in Papua New Guinea, *Journal of Geomatics and Property Studies*, Vol. 1, pp. 69-80.

[52] UNICEF (2012), At a glance: Papua New Guinea; Available at: https://www.unicef.org/infobycountry/papuang_statistics.html, accessed on 10 April, 2017.

[53] Weber, M. (2005), What role for politics in the governance of complex innovation systems? New concepts, requirements and processes of an interactive

technology policy for sustainability, in: U. Petschew, J. N. Rosenau & E. U. von Weizsacker (Eds), Governance and Sustainability, New Challenges for States, Companies and Civil Society, pp. 100-118, Sheffield: Greenleaf.

[54] Weiner, J. F. (2000), Audit of Foi Incorporated Land Groups: Results and Analyses, Chevron Report to Minister of Petroleum and Energy, Australia, Available at:

[55] http://press.anu.edu.au/apem/customary/mobile_devices/ch07s02.html, accessed on 12 May, 2015.

[56] Weiner, J. F. and Glaskin, K. (Eds.) (2007), Customary Land Tenure and Registration in Australia and Papua New Guinea: Anthropological Perspectives, Asia-Pacific Environment Monograph 3, pp. 1-297.

[57] World Resources Institute (2015), Q&A with Mark Robinson: The Role of Good Governance in Sustainable Development by Madaleine Weber - February 18; Available at: http://www.wri.org/blog/2015/02/qa-mark-robinson-role-good-governance-sustainable-development, accessed on 25 January, 2017.

[58] Yala, C. (2010), The genesis of the Papua New Guinea land reform programmes, Port Moresby: The National Research Institute.

[59] Young, M. (1990), Fieldwork Report: Logging or Conservation on Woodlark (Muyuw) Island. Department of Anthropology, Research School of Pacific Studies, Australian National University; Available at: www.ulg.ac.be>PARROTS>YOUNG, accessed on 14 December, 2015.

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Observation for spoilage in fish and beef in a daily simulated local market style of Southwestern, Nigeria.

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***Abstract*—** *The degree of spoilage of fish and beef in a daily simulated market style of southwest, Nigeria was determined by Trimethylamine (TMA) levels in each sample using the standard pictrate technique. 100g of each of the three different parts of a bull (liver, meaty and fatty tissues) and fresh African cat fish (Clarias gariepinus) (liver, meaty portion and head) were purchased twice a week for five weeks from the abattoir and Oja-Oba market in Akure respectively. The samples were subjected to ambient temperature and their degree of spoilage was assessed after 3 hours, 6 hours and 9 hours of purchase. The results showed that, there was no significant difference (P≥ 0.05) in the effect of time (hours) on TMA concentration in different parts of the fish and bull samples, though, the mean concentration of TMA in the fish samples increased with time. However, the degree of spoilage is slower in the bull compared to the fish samples but higher in the late evening (9 hours). Hence, buying and selling of beef in the late evening should be discouraged to avoid consumption of unwholesome meat with high TMA concentrations, while fish should be stored-frozen and sold in deep freezers.*

Keywords: Beef spoilage, Fish spoilage, Trimethylamine (TMA).

I. INTRODUCTION

Fish and meats are important components of human diet. Their nutritive value and palatability are widely appreciated. Despite the relevance of fish and meat in human diet as well as in the economy of Nigeria, a large quantity of fish and beef are prone to spoilage which could be as a result of activity of pathogenic bacteria and fungi thus, making fish or meat unfit for human consumption. Spoilage of fish and beef is a problem for livestock producers, retailers and consumers; it could result to food wastage and consequently economic losses leading to reduction in the profit made by farmers. The practice of freezing and selling of spoilt fish and beef is a great public health hazard. As a result, there is a considerable universal interest in spoilage rate of tropical fish and meat from animals in response to ambient temperature in order to curtail the rising incidence of diseases resulting from food borne sources. Fish bacteria are very sensitive to temperature and the rate at which bacteria multiply depends upon environmental temperature and this leads to spoilage (Doyle, 2007, Nuin *et al*., 2008). Trimethylamine (TMA), (CH3)3N is a tertiary amine which is gaseous at normal temperature and has a characteristic smell of rotting fish. Trimethylamine content is an indicator of meat/fish quality, and picric acid reaction to form an intensely yellow coloured pi-complex and is a standard method for its determination (Francisco et al., 2010). The TMA content determines the flavor of meat and it is also an important factor which determines the natural quality of meat (Mohammod *et al*., 2013). Trimethylamine (TMA) levels are used universally to determine microbial deterioration leading to spoilage of both fish and meat. The amount of TMA produced is a measure of the activity of spoilage bacteria in the flesh, thus it is an indicator of spoilage (Dalgaard *et al*., 2006).

Unfortunately, there is notable dearth of information in terms of previous studies describing or comparing the degree of spoilage of fish and beef sold at various markets in the south west Nigeria, at different hours of the day in response to ambient temperature. Hence, this study was conducted to; determine and compare the degree of spoilage of fish and beef in response to time and ambient temperature by chemical analysis of Trimethylamine (TMA) levels in fish and beef samples measured using the

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standard picrate technique described by Muray and Gibson, (1972a and b) and to ascertains the wholesomeness of fish and beef offered for sale at different hours of the day at Oja-Oba market in Akure, Southwestern, Nigeria.

II. MATERIALS AND METHODS

Study Area

This experiment was carried out in the nutrition laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure, Ondo state, Nigeria

Collection of samples

Three samples each of different part of a bull (liver, meaty and fatty portion) and fresh African cat fish (*Clarias gariepinus*) (liver, meaty portion and head) were purchased twice a week for five weeks from abattoir and Oja-Oba market in Akure respectively and transported to the laboratory. The baseline temperature of the samples were recorded immediately after purchased with a resistance thermometer probe and tagged as the control. The samples were then subjected to ambient temperature and their degree of spoilage was evaluated after 3 hours, 6 hours and 9 hours of purchase. Spoilage was assessed by chemical analysis of the level of Trimethylamine (TMA) in each of the purchased samples were measured using the standard pictrate technique described by Muray and Gibson, (1972a and b).

Statistical analysis

Data obtained was analyzed (SAS version 9.1) using a factorial analysis to test the significant difference in the mean values of TMA concentration and ambient temperature of fresh fish and beef samples at different stages of spoilage while separation of means was done using the Duncan's Multiple Range Test,

the difference in mean was considered significant at $P \leq 0.05$.

III. RESULTS

Table 1 shows the mean values of TMA concentration (µg TMA /100g) of liver, meaty portion and head of catfish at different hours of the day. There was significant difference ($P \leq 0.05$) in the TMA concentration of the liver, meaty portion and head of catfish as the time (hours) increases from 0 – 9 hours. However, there was no significant difference ($P \geq 0.05$) in the TMA concentration among the liver, meaty portion and head of catfish at 0 hours, 3 hours, 6 hours and 9 hours.

Table.1: Effect of time (hours) on TMA concentration (µg TMA /100g) of different parts of fish (Mean ± SD)

Time (hours)	Liver	Meaty portion	Head
0	2.090 ±0.006^{d}	2.148±0.009^{d}	2.036± 0.007^{d}
3	3.665±0.040^{c}	3.591±0.051^{c}	3.770± 0.067^{c}
6	5.552±0.087^{b}	5.424 ±0.110^{b}	5.789 ±0.055^{b}
9	10.303±0.131^{a}	10.067±0.120^{a}	10.966 ±0.218^{a}

(a-d) = mean values on the same row with different superscript are significantly different ($p<0.05$). SD = Standard Deviation

Table 2 shows the mean values of TMA concentration (µg TMA /100g) of liver, meaty and fatty tissues of bull at different hours of the day. There was significant difference ($P \leq 0.05$) in the TMA concentration of the liver, meaty and fatty tissues of the bull as the time (hours) increases from 0 – 9 hours with fatty tissues giving the higher mean values of TMA concentration (µg TMA /100g) at 3 hour (3.389±0.014^{a}), 6 hour (4.447±0.052^{a}) and 9 hour (8.237± 0.046^{a}) respectively.

Table.2: Effect of time (hours) on TMA concentration (µg TMA /100g) of different parts of bull (Mean ± SD)

Time (hours)	Liver	Meaty portion	Fatty tissue
0	1.948 ±0.004^{d}	1.894±0.007^{d}	1.795±0.013^{d}
3	3.243±0.008^{b}	3.161±0.012^{c}	3.389±0.014^{a}
6	4.335±0.007^{b}	4.290±0.008^{c}	4.447±0.052^{a}
9	7.767±0.048^{a}	7.173±0.118^{b}	8.237± 0.046^{a}

(a-d) = mean values on the same row with different superscript are significantly different ($p<0.05$). SD = Standard Deviation

Table 3 shows the mean values of ambient temperature (^{0}C) of liver, meaty portion and head of catfish at different hours of the day. At 0 hour, the analysis of variance shows that the ambient temperature of liver, meaty portion and head of catfish different significantly ($P \leq 0.05$) from the ambient temperature of the liver, meaty portion and head of catfish at 3 hours, 6 hours, 9 hours. However, there was no significant different ($P \geq 0.05$) in the ambient temperature among the liver, meaty portion and head of catfish at 3 hours, 6 hours and 9 hours.

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Table.3: Effect of time (hours) on ambient temperature (^0C) of different parts of fish (Mean ± SD)

Time (hours)	Liver	Meaty portion	Head
0	29.390±0.382[a]	27.290 ±0.215[c]	27.610± 0.230[b]
3	30.800 ±1.274[a]	29.620±1.007[a]	30.280±1.073[a]
6	31.650 ±1.147[a]	30.520 ±1.080[a]	30.900±0.995[a]
9	30.170 ±0.381[a]	29.390 ±0.300[a]	30.900±0.995[a]

(a-b) = mean values on the same row with different superscript are significantly different ($p<0.05$). SD = Standard Deviation

Table 4 shows the mean values of ambient temperature (^0C) of liver, meaty and fatty portion of the bull at different hours of the day. The analysis of variance shows that there was significant difference ($P\leq0.05$) in ambient temperature of the liver, meaty and fatty tissues of the bull as the time (hours) increases from 0 – 9 hours with fatty tissues giving the higher mean values of ambient temperature at 3 hour (34.320±1.361[a]), 6 hour (36.280±1.222[a]) and 9 hour (32.691±0.489[a])respectively.

Table.4: Effect of time (hours) on ambient temperature (^0C) of different parts of bull (Mean ± SD) Deviation

Time (hours)	Liver	Meaty portion	Fatty tissue
0	27.510 ± 0.250[b]	28.830±0.239[b]	28.360±0.290[b]
3	32.230±1.072[b]	31.250±0.883[c]	34.320±1.361[a]
6	33.790±1.111[b]	32.400±0.808[c]	36.280±1.222[a]
9	29.440±0.338[bc]	29.590±0.368[bc]	32.691±0.489[a]

(a-c) = mean values on the same row with different superscript are significantly different ($p<0.05$). SD = Standard Deviation

IV DISCUSSION

From the study, the TMA concentration of different parts of the fish and bull at different hours of the day were not significantly different ($P\geq 0.05$) from each other though, the mean value of TMA concentration of different parts of fish (liver, meaty portion and head) as well as different parts of bull (liver, meaty and fatty portion) increased as the time (hours) increased. The mean values of TMA concentration in the head of the sacrificed fish was slightly lower at the start of the experiment than that of liver and meaty portion of the same fish, but the opposite was the case at the end of the experiment (9 hours after) where the TMA concentration was higher in the fish head than that of liver and meaty portion. This implies that rate of spoilage is faster in fish head compare to other parts such as liver and meaty portion. This could be as a result of myriad of spoilage microorganisms inhabiting the gills of the fish. The longer the period of exposure of fish or beef to ambient temperature, the higher the level of TMA in the sample, hence, the faster the rate of spoilage, this increase in TMA level in response to time and ambient temperature was also observed by Adeyemo *et al.*, (2008).This study also revealed that the mean values of TMA concentration in the fatty tissues of the bull was slightly lower at the start of the experiment compared to liver and meaty portion of the same animal but at 3 hours, 6 hours, and 9 hours of the experiment, the mean values of TMA concentration of the fatty tissues were slightly higher than other parts of the bull (liver and meaty portion), similar to observations by Zeev *et al.*, (2002).This could be due to increase in ambient temperature at this hour of the day which enhanced the proliferation of microorganism and hence, the degree of spoilage. These findings indicated that the rate of spoilage is faster and higher in fatty tissues than other parts of the bull.

Comparatively, this study showed that the rate of spoilage is faster in fish liver both at the start and end of the experiment than that of bull. More so, the meaty portion of the fish spoils faster than that of the bull due to higher level of TMA in the meaty portion of fish both at the start and end of the experiment. The activities of microorganisms are largely responsible for spoilage of fish and meat leading to increase in the TMA level in the affected fish and meat (Adeyemo *et al.*, 2008).

High ambient temperature enhances the multiplication of putrefactive organisms leading to spoilage. Spoilage bacteria respond to temperature and the extent at which bacteria proliferate depends largely on environmental temperatures which result into spoilage of fish and meat. The higher the ambient temperatures, the faster the spoilage rate of different parts of the fish and meat in the tropical environments. The assessment of rate of spoilage of different parts of fish by exposure to ambient temperature revealed no statistically significant difference at ($P\geq 0.05$) at 3hours, 6hours, and 9hours of the experiment. At zero hour,

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the analysis of variance shown that the ambient temperature (^{0}C) of fish liver, meaty portion as well as fish head different significantly at (P≤0.05) from exposure of aforementioned samples to ambient temperature (^{0}C) at 3 hours,6 hours, 9 hours, this difference in ambient temperature could be attributed to vagaries nature of climate and weather. The assessment of rate of spoilage of different part of bull by exposure to different ambient temperatures at 3 hour, 6hour, and 9 hour showed that mean values of ambient temperatures of fatty tissues were significantly different at (P ≤ 0.05) than other parts such as and meaty portion. This indicates that degree of spoilage is higher in fatty tissues than other parts of bull, as a result, fatty tissues spoil faster when compared with others due to increase in ambient temperature which corroborates the growth of putrefactive organisms.

IV. CONCLUSION

This experiment is relevant to this study environment because marketing of fish and beef is done under unmanipulated tropical temperature which enhances the growth of natural mesophilic microorganisms on tropical fish species and meat from animals. This observation of fish and beef in response to daily ambient temperature enables the understanding of the gradual spoilage of the fresh meat and fresh fish which in turn provide useful information for marketing and distribution of fish and meat in the tropical environment. Based on the results of this experiment, it can be concluded that the degree of spoilage is slower in beef compared to fish under the same condition such as period of exposure to different ambient temperature.

V. RECOMMENDATIONS

It is therefore recommended that buying and selling of meat in the late evening should be discouraged to reduce the risk of consumption of unwholesome meat by the public due to decay/putrefaction which increased TMA recorded in this study indicates. Consumers should endeavor to purchase meat within a short time after slaughtering process to prevent the consumption of unhygienic meat especially where storage facility is in doubt. Fish should be well frozen, stored and sold in deep freezers. Fishmongers and butchers should be enlightened via extension services on hygienic handling of fish and meat to avert multiplication of bacteria.

REFERENCES

[1] Adeyemo, O.K.., Aluko, O.O., Agbede, S.A. (2008). Effect of temperature on spoilage of fresh and frozen fish sold in markets in Ibadan, Nigeria. *African Journal of Livestock Extension*, 6: 65-68.

[2] Dalgaard, P., Madsen, H.L., Samieian, N. and Emborg, J. (2006). Biogenic amine formation and microbial spoilage in chilled garfish (*Belone belone*) - effect of modified atmosphere packaging and previous frozen storage. *Journal of Applied Microbiology*, 101: 80-95.

[3] Murray, C.K. and Gibson D.M. (1972a). An investigation of the method of determining trimetylamine in fish muscle extracts by formation of its picrate salt. Part I. *Journal of food Technology*, 7: 35-46

[4] Murray, C.K. and Gibson D.M. (1972b.) An investigation of the method of determining trimetylamine in fish muscle extracts by formation of its picrate salt. Part II. *Journal of food Technology*, 7: 47-51

[5] Nuin M, A. B., Cruz, Z., Argarate, N., George, S., Le Marc, Y., Olley, J., and Pin, C.(2008). Modelling spoilage of fresh turbot and evaluation of a time-temperature integrator (TTI) label under fluctuating temperature. *International Journal of Food Microbiology* 127(3): 193-199

[6] Doyle, E.M. (2007).Microbial food spoilage Losses and control strategies. Food Research Institute, University of Wisconsin Madison,WI 53706.

[7] Zeev, K., Boris, T., Rachel, G., and Avraham, L. (2002). Determination of volatile biogenic amines in muscle food products by ion mobility spectrometry. *Analytica Chimica Acta* 463:155-163.

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Vegetative Propagation of *Argania spinosa* (L.) Skeels Cuttings: Effects of Nutrient Solution

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Abstract**—** *The effects of the mineral composition of nutrient solution (Hoagland and Arnon (HA), Quoirin and Lepoivre (QL), Murashige and Skoog (MS), and Woody Plant Medium (WPM)), cutting type (softwood, semi-hardwood and hardwood) and cutting position (basal, medial, and apical) on sprouting and rooting performance of Argania spinosa cuttings were investigated. According to the results, the nutrient solution, cutting type and cutting position had an effect on the sprouting and adventitious rooting ability of A. spinosa cuttings. The leafy semi-hardwood cuttings taking from the basal positions and irrigated with Hoagland solution performed best and produced the highest number of roots (44.63), root length (28.86 cm), and had the highest rooting and survival percentage (63.81% and 96.09%, respectively). The nutrient solution applications caused a notable increase in sprouting and rooting potential of the argan tree. The highest values were recorded for HA and QL, while the MS and WPM gave the poorest result and the greatest mortality rate of cuttings.*

The cuttings type had also a pronounced effect on vegetative propagation of A. spinosa. The leafy semi-hardwood cuttings performed better than the leafy softwood cuttings, whereas leafless hardwood cuttings were completely unable to sprout and root even when treated with nutrient solutions. Thus, vegetative propagation of A. spinosa can best be achieved using basal leafy semi-hardwood cuttings irrigated with Hoagland nutrient solution.

Keywords**— *Argania spinosa, cutting, nutrient solution, rooting, vegetative propagation.***

I. INTRODUCTION

Argania spinosa (*L.*), commonly known as the argan tree, is a thorny evergreen tree. Its main natural distribution extent is limited to the south-west of Morocco, from the Atlantic coast and Souss plains to bordering slopes of the Anti-Atlas and High Atlas mountains, between Oued Tensift to the north and Oued Draa to the south (Emberger 1925; Msanda, 1993). The argan forest is recognised by UNESCO since 1998 as a biosphere reserve with a rich natural biodiversity. It's an indispensable component of the ecological balance of semi-arid south-western ecosystems of Morocco (Msanda *et al*., 2005). In fact, *A. spinosa* is recommended as a climate change mitigation species because it acts as a barrier against desertification, protects the soil against erosion, retains water moisture, restores soil fertility and provides a very favorable area for intercropping or fallow management by its deep root system (Morton and Voss, 1987; M'hirit et *al*., 1998). The argan tree is still reproduced through seeds but does not guarantee the production of a high-performance material. Although the seeds can germinate readily, the gradual destruction of the argan forest coupled with unsustainable seeds harvesting methods, overgrazing and the reconversion of the argan woodland into intensive agriculture systems are posing an increasing threat to natural regeneration, reducing the population size, and could result in fewer individuals of this species (Nouaim *et al*., 2002). Therefore, an alternative method such as clonal propagation is required for mass multiplication of elite genotypes obtained in breeding programs or selected from natural populations (Hartmann *et al*., 1997). Compared to other vegetative propagation techniques, the use of cuttings is considered as the most efficient and low-cost method (Leakey, 2004). It's an efficient method for producing large numbers of uniform plants, but it does not always succeed due to some difficulties of rooting and subsequent growth that can be observed (De Vries and Dubois, 1988; Dubois and De Vries, 1991). The rooting potential of stem cuttings varies considerably among plant species, some can easily root while others remain recalcitrant even with the application of growth regulators (De Klerk *et al*., 1999). Rooting in stem cuttings of woody species is a complex

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process that is affected by a combination of physiological processes in the leaf and stem portions of the cuttings. Each of these processes is influenced by numerous morphological and anatomical factors which result from interactions with the genetic background, age, ontogenetic phase, culture conditions of the stock plant and post-severance treatments such as plant growth regulators and composition of the nutriment solution (Németh, 1986; Hartmann *et al*., 2002). Based on the species being propagated, the cutting maturity and a specific type of cutting are usually required. Some species root more easily with softwood, others require semi-hardwood cuttings, and still, others may root easier when hardwood cuttings are used (Greenwood and Hutchison, 1993; Hartmann *et al*., 2002). Moreover, adventitious root ability may vary between cuttings from different nodal positions, especially in woody species (Hansen and Kristensen, 1990; Bredmose *et al*., 2004). The position of the cutting on the stock plant affects the bud break and the shoot growth (Bredmose and Hansen, 1996; Husen and Pal, 2007; Otiende *et al*., 2017). The nutritional approach suggested that nutritional factors rather than growth substances are involved in regulating plant growth and development including rooting process (Fageria and Moreira, 2011). The metabolism of adventitious rooting is influenced by mineral nutrients of the stock plant or of its cuttings during propagation (Haissig, 1986). Indeed, the mineral nutrients are able to influence adventitious rooting, either by inhibiting or increasing the number of adventitious roots or by modulating the root length. Therefore, rooting may be increased if stock plants or cuttings are properly supplied with a combination of low to moderate amounts of other macro- or micro-nutrients ordinarily required for satisfactory plant vigour (Eliasson, 1978). This study was undertaken to investigate the influence of the mineral composition of the nutritive solution, cutting position and cutting type on sprouting and rooting ability of *Argania spinosa* cuttings.

II. MATERIALS AND METHODS

1. Sources and preparation of cuttings

Cuttings were collected from adult *Argania spinosa* trees from the Arboretum of Oued Cherat, province of Bouznika in Morocco (33°81'96" N ; 7°11'03" W; 45 m altitude), which is located within 2000 m of the Moroccan Atlantic coast and with an average annual rainfall of 460 $mm.yr^{-1}$. The selected trees were growing in wild conditions and showing superior phenotypes with very good crown diameter, fruits shape and caliber. Shoots were collected by using sterile pruning scissors from the tree crown in the early morning and were kept in perforated plastic bags inserted in a cool box to prevent drying during collection before being taken to the laboratory. Prior to insertion in the rooting medium, cuttings were stored in a dark cold room (4°C) during 48 hours. The terminal node of the shoots was excised, then cuttings were excised and kept separately according to the position on the stock plant (basal, medial and apical), then they were classified with regard to cutting types. Actually, hardwood (leafless) cuttings were taken in winter. However, softwood (green and tender wood; leafy) cuttings were taken in spring and semi-hardwood (semi-lignified; leafy) cuttings were taken in summer. Healthy cuttings were screened to (10 ± 0.5) cm length with at least six nodes. Leaves were excised from the down part so that only four leaves remained on the cuttings to minimize water stress caused by transpiration. To minimise fungal attacks, a chemical surface disinfection was applied on these cuttings for 10 min with a 0.2% (w/v) fungicide solution (Dithane-M45) and subsequently washed with sterile distilled water. The apical cut ends of the treated cuttings were sealed with tree wound dressings to reduce the water loss, prevent diseases and decay. The base of each cutting was wounded (basal cut just below a node), then it was soaked for 5 min in 3000 $mg.L^{-1}$ of indole-3-butyric acid (IBA), this concentration proved to be the most successful in a study conducted by our team (currently being published). Prepared cuttings were immediately placed in 1000 cc polyethylene (PE) pots containing sterilized sieved sand placed in greenhouse under the natural conditions of luminescence and humidity and a mean temperature of 32 ± 2 °C, at the biotechnology unit of the Regional Center of Agricultural Research of Rabat, INRA-Morocco. To harden the plants, rooted cuttings with roots of more than 1 cm long and without symptoms of fungal diseases were transferred to 4500 cc polyethylene (PE) pots containing a mix of sterilized sieved sand, sterilized forest soil and peat moss (1:1:1 v/v). These pots were then placed in the green house, at a spacing of 20 cm × 20 cm. The growing cuttings were monitored for a two year period. Cuttings were regularly watered every 2 days with tap water and received weekly applications of nutrient solution Hoagland and Arnon (HA; Hoagland and Arnon, 1950), Quoirin and Lepoivre (QL; Quoirin and Lepoivre, 1977), Murashige and Skoog (MS; Murashige and Skoog, 1962), and Woody Plant Medium (WPM; Lloyd and Mc Cown, 1980). The macronutrients were added separately from stock solutions. A combined stock solution is made up containing all micronutrients except iron. Iron is added as ferrous sulphate heptahydrate ($FeSO_4.7H_2O$). The pH of the nutrient solution was adjusted to a value of 5.6 ± 0.2.

2. Experimental design and treatments

The experiment was organised according to a randomized complete block design (RCBD). Thirty two replications were used in four blocks for each treatment. The experiment tested three cutting types (leafy softwood (SW), leafy semi-hardwood (SHW) and leafless

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hardwood (HW) stem cuttings), three cutting positions (apical, medial and basal position), four nutrient solution treatments (Hoagland and Arnon (HA), Quoirin and Lepoivre (QL), Murashige and Skoog (MS), and Woody Plant Medium (WPM)) and their interactions.

3. Data collection

After 12 weeks, the cuttings were scored for the number of leaves (NL), leaf size in cm^2 (LS), number of sprouts (NS), length of the longest sprout cm (SL), sprouting rate (SP %), number of roots (NR), length of the longest root in cm (RL), and rooting rate (RP %). Finally, the survival rate (SR %) was recorded 48 weeks after rooting induction, a period that has been considered sufficient to measure the survival of rooted cuttings.

4. Statistical analysis

The data collected were submitted to tests of analysis of variance (ANOVA) for treatment effects of the general linear model (GLM) procedure in SAS program version 9.1 (SAS Institute, Cary, NC, USA) for all the evaluated parameters. Comparisons between treatments were performed by using Duncan's Multiple Range Test (DMRT) with at least 95% level of statistical reliance, and as a result, homogenous groups were acquired and interpreted. All data were reported as means ± standard deviation (SD). Before statistical analysis, data were converted by angular transformation (X = arcsine √Y) for sprouting, rooting and survival ratios.

III. RESULTS

1. Effect of the nutrient solution, cutting type and position on the number of leaves and leaf size of *Argania spinosa* cuttings

1.1. Number of leaves

The highest number of leaves was obtained from hardwood cuttings, followed by the semi-hardwood cuttings, although softwood cuttings tended to produce the lowest result (Table 1). The lignification trend in the number of leaves was also similar for cutting position. Over the entire experimental period, basal position had a highest leaf number, followed by the medial position (Table 1). In our study, the highest number of leaves for basal hardwood cuttings was observed with MS solution (36.25 ± 0.73), while softwood cuttings treated with WPM solution presented the lowest number of leaves and exhibited several nutrient-deficiency symptoms and chlorosis on their leaves.

1.2. Leaf size

Leaf size had a mean per cutting significantly greater in hardwood cuttings, followed by semi-hardwood then softwood cuttings (Table 1). The highest mean for leaf size was observed in cuttings taken from the basal position followed by cuttings of medial position then apical position (Table 1). The results presented herein indicate that leaf size of basal softwood cuttings treated with WPM or HA was generally lower (9.90 ± 0.46 cm^2 and 11.19 ± 0.49 cm^2, respectively) than that for basal hardwood cuttings irrigated with either QL or MS (24.96 ± 0.53 cm^2 and 28.93 ± 0.71 cm^2, respectively).

The mean number of leaves and size were significantly ($P < 0.01$) affected by the nutrient solution, cutting type and cutting position, while interactive effect of nutrient solution, cutting type and cutting position had a significant ($P < 0.01$) effect only for leaves size (Table 2).

Table.1: Effects of nutrient solution, cutting type and cutting position on mean values of the number of leaves (NL) and leaf size cm^2 (LS) of Argania spinosa cuttings.

Nutrient solution	Cutting position	SW		SHW		HW	
		NL	LS	NL	LS	NL	LS
HA	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{g}	00.00 ± 0.00^{d}	00.00 ± 0.00^{e}
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	21.38 ± 0.89^{c}	15.64 ± 0.74^{de}	28.38 ± 0.84^{c}	19.35 ± 0.65^{d}
	Basal	17.50 ± 0.75^{b}	11.19 ± 0.49^{b}	22.00 ± 0.59^{bc}	17.20 ± 0.55^{cd}	29.31 ± 0.73^{c}	20.66 ± 0.62^{d}
QL	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{g}	00.00 ± 0.00^{d}	00.00 ± 0.00^{e}
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	24.00 ± 0.75^{ab}	18.05 ± 0.51^{bc}	32.00 ± 0.88^{b}	23.36 ± 0.56^{c}
	Basal	**19.25 ± 0.79^{a}**	**13.61 ± 0.53^{a}**	**24.56 ± 0.72^{a}**	19.78 ± 0.78^{ab}	32.75 ± 0.64^{b}	24.96 ± 0.53^{bc}
WPM	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{g}	00.00 ± 0.00^{d}	00.00 ± 0.00^{e}
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	20.63 ± 0.91^{c}	13.66 ± 0.60^{f}	27.19 ± 1.32^{c}	17.10 ± 0.92^{d}
	Basal	16.88 ± 0.72^{b}	09.90 ± 0.46^{b}	21.62 ± 0.86^{c}	15.07 ± 0.65^{ef}	28.94 ± 0.86^{c}	18.90 ± 0.6d^{c}
MS	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{g}	00.00 ± 0.00^{d}	00.00 ± 0.00^{e}
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	**24.50 ± 0.70^{a}**	**20.18 ± 0.71^{a}**	33.19 ± 0.75^{b}	25.47 ± 0.62^{b}
	Basal	**20.19 ± 0.83^{a}**	**14.71 ± 0.69^{a}**	**25.31 ± 0.56^{a}**	**21.49 ± 0.51^{a}**	**36.25 ± 0.73^{a}**	**28.93 ± 0.71^{a}**

Within each treatment, value marked by the same letter are not significantly different (Duncan's Multiple Range *Test (DMRT), P < 0.05, mean ± SD, n = 32).*

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Table.2: Analysis of variance (ANOVA) for effect of nutrient solution, cutting type, cutting position and their interactions on the number of leaves and leaves size (cm^2) of Argania spinosa cuttings.

Source of variance	Dependent variable	df	F-value	P-value
Cutting type				
	Nb. of leaves	2	1119.0	0.000
	Leaves size (cm^2)	2	1028.4	0.000
Cutting position				
	Nb. of leaves	2	3323.5	0.000
	Leaves size (cm^2)	2	2875.7	0.000
Nutrient solution				
	Nb. of leaves	3	25.222	0.000
	Leaves size (cm^2)	3	80.297	0.000
Cutting type * Cutting position * Nutrient solution				
	Nb. of leaves	12	01.206	0.275
	Leaves size (cm^2)	12	03.235	0.000

Nb. = number.

2. Effect of the nutrient solution, cutting type and position on the number of sprouts and sprouts length of *Argania spinosa* cuttings

2.1. Number of sprouts

Semi-hardwood cuttings gave the best sprouting yield and produced the largest number of sprouts, followed by softwood, whereas hardwood cuttings failed to produce shoots (Table 3). Cuttings taken from the medial internodes were the ones which produced the greatest number of sprouts in comparison with the basal position, though the apical position didn't sprout at all (Table 3). In our study, analysis of variance revealed that the cuttings taken from the medial position of semi-hardwood branch treated by the QL solution produced the maximum number of sprouts (1.81 ± 0.13), followed by WPM (1.75 ± 0.09) then basal softwood cuttings irrigated by HA and MS solutions (1.31 ± 0.11 and 1.13 ± 0.05, respectively). Besides, cuttings irrigated with WPM solution were characterized by short and thin shoots, with small, narrow leaves and cuttings irrigated with MS were observed to develop more shoot tip necrosis.

2.2 Sprouts length

The influence of the cuttings type on sprouts length revealed that semi-hardwood cuttings performed best and produced longer shoots than softwood cuttings (Table 3). Moreover, cuttings at the middle position showed the highest shoot length values per cutting (Table 3). The results revealed that the longest sprouts were recorded on the medial position of the semi-hardwood cuttings with the use of QL (16.88 ± 0.6 cm) (Figure 1), followed by the WPM solution (16.13 ± 0.66 cm).

Significant differences ($P < 0.05$) existed between the mean number of sprouts and sprouts length produced by cuttings at the various stages of development and at different position on the branch of the stock plant, as well as the differences between the cuttings sprouts length produced by cuttings treated by different nutrient solutions (Table 4). Although the mean number of sprouts were not significantly ($p = 0.075$) affected by nutrient solution and also there were no significant differences in term of interaction for sprouts number and length ($p = 0.188$ and $p = 0.179$, respectively).

Table.3: Effects of nutrient solution, cutting type and cutting position on mean values: number of sprouts (NS) and sprout length in cm (SL) of Argania spinosa cuttings.

Nutrient solution	Cutting position	SW		SHW		HW	
		NS	SL	NS	SL	NS	SL
HA	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{e}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	01.75 ± 0.09^{ab}	15.31 ± 0.62^{ab}	00.00 ± 0.00	00.00 ± 0.00
	Basal	01.31 ± 0.11^{ab}	11.25 ± 0.63^{ab}	01.63 ± 0.12^{ab}	14.56 ± 0.61^{bc}	00.00 ± 0.00	00.00 ± 0.00
QL	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{e}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	01.81 ± 0.13^{a}	16.88 ± 0.60^{a}	00.00 ± 0.00	00.00 ± 0.00
	Basal	01.44 ± 0.12^{a}	12.13 ± 0.75^{a}	01.44 ± 0.12^{b}	15.69 ± 0.88^{ab}	00.00 ± 0.00	00.00 ± 0.00
WPM	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{e}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	01.75 ± 0.09^{ab}	16.13 ± 0.66^{ab}	00.00 ± 0.00	00.00 ± 0.00
	Basal	01.38 ± 0.12^{a}	11.94 ± 0.58^{a}	01.44 ± 0.12^{b}	15.19 ± 0.73^{ab}	00.00 ± 0.00	00.00 ± 0.00
MS	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{e}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	01.44 ± 0.12^{b}	12.88 ± 0.66^{cd}	00.00 ± 0.00	00.00 ± 0.00
	Basal	01.13 ± 0.05^{b}	10.06 ± 0.55^{b}	01.44 ± 0.12^{b}	12.18 ± 0.76^{d}	00.00 ± 0.00	00.00 ± 0.00

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Within each treatments, value marked by the same letter are not significantly different (Duncan's Multiple Range Test (DMRT), $P < 0.05$, mean ± SD, n = 32).

Table.4: Analysis of variance (ANOVA) for effect of nutrient solution, cutting type, cutting position and their interactions on the number of sprouts and sprout length (cm) of Argania spinosa cuttings.

Source of variance	Dependent variable	df	F-value	P-value
Cutting type				
	Nb. of sprouts	2	0652.9	0.000
	Sprout length (cm)	2	1235.1	0.000
Cutting position				
	Nb. of sprouts	2	0509.9	0.000
	Sprout length (cm)	2	0922.0	0.000
Nutrient solution				
	Nb. of sprouts	3	02.308	0.075
	Sprout length (cm)	3	08.121	0.000
Cutting type * Cutting position * Nutrient solution				
	Nb. of sprouts	12	01.346	0.188
	Sprout length (cm)	12	01.364	0.179

Nb. = number.

3. Effect of the nutrient solution, cutting type and position on number of root and longest root length of the *Argania spinosa* cutting

3.1. Number of roots

The highest number of roots was produced from semi-hardwood cuttings, followed by softwood cuttings, while, the hardwood cuttings (which included developing leaves) failed to root completely. In addition, the cuttings from the basal part of the branch exhibited a markedly superior number of roots than medial position. However, cuttings from the apical position didn't root at all (Table 5). The greatest number of roots was obtained on cuttings sourced from the basal position of semi-hardwood which were irrigated with HA (44.63 ± 0.69) (Figure 1), followed by QL solution (40.63 ± 0.88), whereas the poorest roots number was recorded for the basal softwood cuttings irrigated with MS and WPM solutions (31.06 ± 0.89 and 32.88 ± 0.82) respectively.

3.2 Root length

Semi-hardwood cuttings showed the longest roots, followed by softwood. Furthermore, the basal position has given the highest values, followed by the medial position (Table 5). Basal semi-hardwood cuttings treated with HA solution showed the longest roots (28.86 ± 0.75 cm) (Figure 1), followed with QL (26.19 ± 0.68 cm). However, the shortest roots were recorded for the basal softwood cuttings irrigated with MS and WPM solution (22.00 ± 0.84 cm and 22.38 ± 0.56 respectively). The results indicate that nutrient solution, cutting type, cutting position has very significantly ($P < 0.01$) affected both the mean number of roots and their length, while interactive effects of nutrient solution, cutting type and cutting position was significant ($P < 0.01$) only for the number of roots produced (Table 6).

Fig. 1: (A) Argania spinosa semi-hardwood cuttings inserted in a sterilized sieved sand rooting medium, distributed according to a Randomized Complete Block Design (RCBD) under non-mist greenhouse conditions (1 week). (B) Sprouted basal leafy softwood cuttings irrigated with Quoirin and Lepoivre (QL) nutrient solution (12 weeks). (C) Rooted basal leafy semi-hardwood cuttings freshly removed from the sand rooting medium (48 weeks). (D) Well-developed roots of basal leafy semi-hardwood cuttings irrigated with Hoagland & Arnon (HA) nutrient solution (72 weeks).

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Table.5: Effects of nutrient solution, cutting type and cutting position on mean values of number of roots (NR) and root length in cm (RL) of Argania spinosa cuttings.

Nutrient solution	Cutting position	SW		SHW		HW	
		NR	RL	NR	RL	NR	RL
HA	Apical	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{f}	00.00 ± 0.00^{d}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	40.19 ± 0.83^{bc}	26.25 ± 0.84^{b}	00.00 ± 0.00	00.00 ± 0.00
	Basal	38.56 ± 0.89^{a}	25.06 ± 0.83^{a}	44.63 ± 0.69^{a}	28.86 ± 0.75^{a}	00.00 ± 0.00	00.00 ± 0.00
QL	Apical	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{f}	00.00 ± 0.00^{d}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	38.63 ± 0.70^{bcd}	25.06 ± 0.54^{bc}	00.00 ± 0.00	00.00 ± 0.00
	Basal	35.38 ± 0.88^{b}	23.63 ± 0.68^{ab}	40.63 ± 0.88^{b}	26.19 ± 0.68^{b}	00.00 ± 0.00	00.00 ± 0.00
WPM	Apical	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{f}	00.00 ± 0.00^{d}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	38.13 ± 0.72^{cd}	24.00 ± 0.66^{bc}	00.00 ± 0.00	00.00 ± 0.00
	Basal	32.88 ± 0.82^{c}	22.38 ± 0.56^{b}	39.31 ± 0.86^{bcd}	24.69 ± 0.86^{bc}	00.00 ± 0.00	00.00 ± 0.00
MS	Apical	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{f}	00.00 ± 0.00^{d}	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	35.31 ± 0.66^{e}	23.19 ± 0.81^{c}	00.00 ± 0.00	00.00 ± 0.00
	Basal	31.06 ± 0.89^{c}	22.00 ± 0.84^{b}	37.19 ± 0.80^{de}	23.88 ± 0.94^{bc}	00.00 ± 0.00	00.00 ± 0.00

Within each treatments, value marked by the same letter are not significantly different (Duncan's Multiple Range *Test (DMRT), P < 0.05, mean ± SD, n = 32).*

Table.6: Analysis of variance (ANOVA) for effect of nutrient solution, cutting type, cutting position and their interactions on number of roots and root length (cm) of Argania spinosa cuttings.

Source of variance	Dependent variable	df	F-value	P-value
Cutting type				
	Nb. of roots	2	6186.0	0.000
	Root length (cm)	2	2732.4	0.000
Cutting position				
	Nb. of roots	2	5605.3	0.000
	Root length (cm)	2	2582.0	0.000
Nutrient solution				
	Nb. of roots	3	08.504	0.000
	Root length (cm)	3	08.503	0.000
Cutting type * Cutting position * Nutrient solution				
	Nb. of roots	12	03.852	0.000
	Root length (cm)	12	01.246	0.247

Nb. = number.

4 Effect of the nutrient solution, cutting type and position on sprouting percentage, rooting percentage and survival rate of *Argania spinosa* cuttings

4 .1 Sprouting percentage

Sprouting response was dependent on explants type, with a maximum sprouting being obtained through semi-hardwood cuttings, followed by softwood, though hardwood cuttings did not sprout at all for any of the four nutrient solutions. Moreover, medial cuttings had the highest sprouting yield, followed by cuttings of the basal position (Table 7). The highest sprouting percentage of cuttings was recorded when the medial semi-hardwood cuttings were irrigated with the QL solution (85.69 ± 01.07 %), whereas the lowest sprouting ratio was recorded on basal softwood cuttings treated by MS solution (64.59 ± 1.70 %).

4.2 Rooting percentage

The semi-hardwood cuttings rooted significantly better than other cuttings type, with a higher rooting percentage (Table 7). Rooting yield varied among nodal positions, with a sequential decline in performance from basal to apical position. Basal semi-hardwood cuttings irrigated with HA produced relatively more roots (63.81 ± 1.89) %, followed by QL (58.59 ± 1.37) %, and the basal position of softwood cuttings irrigated with MS solution exhibited the lowest rooting rate (46.09 ± 0.68) %.

4.3 Survival rate

Semi-hardwood cuttings rooted more frequently and had lower mortality rates than softwood and hardwood

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cuttings (Table 7). The overall survival of rooted cuttings increased significantly between apical and basal positions. Indeed, basal position had the highest survival results, followed by cuttings of the medial position, whereas the cuttings from the apical position were completely decayed (Table 7). The cuttings taken from the basal position of semi-hardwood cuttings irrigated with HA solution had the highest survival rate per cutting (96.09 ± 0.29) %, followed by the QL solution (93.75 ± 0.39) %. However, basal softwood cuttings treated with MS solution had the poorest survival rate (48.44 ± 1.17) %. Some softwood cuttings irrigated with MS solution showed symptoms of apical necrosis and were unable to grow.

The analysis of variance indicates a significant difference for the mean of sprouting, rooting rate and survival percentage for each factor studied separately (nutrient solution, type and position of cuttings) ($p < 0.05$), though the nutrient solution × cutting type × cutting position combinations were significant only for the survival rate (Table 8).

Table 7: Effects of of nutrient solution, cutting type and cutting position on mean values of sprouting percentage (SP), rooting percentage (RP), and survival rate (SR) of *Argania spinosa* cuttings.

Nutrient solution	Cutting position	SW			SHW			HW		
		SP	RP	SR	SP	RP	SR	SP	RP	SR
HA	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{f}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	82.66 ± 0.87^{ab}	58.59 ± 1.37^{abc}	93.75 ± 0.39^{a}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Basal	70.84 ± 0.91^{ab}	57.81 ± 1.56^{a}	74.22 ± 0.98^{a}	80.22 ± 1.04^{ab}	63.81 ± 1.89^{a}	96.09 ± 0.29^{a}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
QL	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{f}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	85.69 ± 01.07^{a}	57.81 ± 1.95^{abc}	83.01 ± 1.39^{bc}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Basal	76.41 ± 1.74^{a}	54.69 ± 1.37^{a}	71.63 ± 1.14^{ab}	82.66 ± 0.87^{ab}	58.59 ± 1.37^{ab}	93.75 ± 0.39^{ab}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
WPM	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{f}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	83.54 ± 0.80^{ab}	56.25 ± 1.17^{abc}	75.10 ± 1.21^{c}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Basal	74.50 ± 0.58^{a}	53.91 ± 1.37^{a}	67.97 ± 0.98^{b}	79.54 ± 1.17^{ab}	57.81 ± 0.87^{abc}	77.98 ± 1.26^{c}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
MS	Apical	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	00.00 ± 0.00^{f}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Medial	00.00 ± 0.00^{c}	00.00 ± 0.00^{c}	00.00 ± 0.00^{d}	78.66 ± 1.43^{ab}	47.66 ± 1.46^{c}	53.91 ± 1.37^{e}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
	Basal	64.59 ± 1.70^{b}	46.09 ± 0.68^{b}	48.44 ± 1.17^{c}	76.31 ± 1.50^{b}	50.78 ± 1.76b^{c}	64.06 ± 1.17^{d}	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00

Within each treatments, value marked by the same letter are not significantly different (Duncan's Multiple Range *Test (DMRT), $P < 0.05$, mean ± SD, $n = 32$).*

Table.8: Analysis of variance (ANOVA) for effect of nutrient solution, cutting type, cutting position and their interactions on sprouting percentage, rooting percentage, and survival rate of Argania spinosa cuttings.

Source of variance	Dependent variable	df	F-value	P-value
Cutting type				
	Sprouting percent	2	2254.1	0.000
	Rooting percent	2	0733.9	0.000
	Survival percent	2	2264.3	0.000
Cutting position				
	Sprouting percent	2	1954.2	0.000
	Rooting percent	2	0708.0	0.000
	Survival percent	2	2058.4	0.000
Nutrient solution				
	Sprouting percent	3	03.325	0.022
	Rooting percent	3	04.601	0.005
	Survival percent	3	55.811	0.000
Cutting type * Cutting position * Nutrient solution				
	Sprouting percent	12	00.849	0.601
	Rooting percent	12	00.746	0.703
	Survival percent	12	10.690	0.000

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IV. DISCUSSION

The present results indicate a significant effect of cutting type on the success of sprouting, rooting and survival rates for *A. spinosa* cuttings. Vegetative propagation of argan trees can best be achieved using semi-hardwood cuttings followed by softwood cuttings. The findings from this study for high sprouting and rooting ability of semi-hardwood cuttings are in accordance with results obtained for other tree species, such as *Moringa oleifera*, where semi-hardwood and hardwood cuttings performed best and produced the highest number of shoots and the longest shoots, while the shortest shoots were produced on softwood cuttings (Antwi-Boasiako and Enninful, 2011); *Stevia rebaudiana*, where semi-hardwood cuttings were more successful than softwood cuttings (Abdullateef and Osman, 2012); *Duranta repens*, for which semi-hardwood and hardwood cuttings rooted significantly better than softwood cuttings with a higher percentage of rooted cuttings, a greater number of roots and longer roots length per rooted cutting (Okunlola, 2013); *Bougainvillea glabra*, in which root number was higher for semi-hardwood compared with softwood cuttings (Seyedi *et al*. 2014). Semi-hardwood cuttings are generally considered easy to propagate due to its low production of secondary metabolites in comparison with hardwood cuttings (Hartmann *et al*. 1997).

In addition, the hardwood cuttings prepared had no leaves, therefore their dependence on photosynthetic activity for rooting and bud formation was denied, which adversely influenced their sprouting, rooting and survival efficiency. This lack of sprouting and rooting performance of hardwood cuttings could also be pronounced by the early appearance of leaves which could lead to resource depletion before rooting (Antwi-Boasiako and Enninful, 2011). Moreover, this increase in rooting potential of semi-hardwood cuttings may be due to their lignification, which increases their ability to withstand dry or other adverse conditions. Therefore, they survived in moist soil until the roots formed, whereas softwood cuttings are delicate, dried-out rapidly and much more subject to attacks by various fungal diseases (Longman, 1993). In fact, the maintenance or presence of leaves on semi-hardwood and softwood cuttings influenced sprouting and rooting due to their ability to produce auxins and carbohydrates (Hartmann et al., 1990).

The analysis of variance indicated that significant differences existed in terms of sprouting and rooting ability of *A. spinosa* cuttings taken from different cutting positions of stock plants shoots. The highest root number, root length, rooting percentage and survival rate was observed in cuttings taken from a basal position, followed by those taken from a medial cutting position. However, the cuttings made from an apical position hadn't rooted at all. The observations showed that rooting response of *A. spinosa* is a function of nodal position and the cuttings rooted better if taken from a basal position of the stock plant. These results are in agreement with those obtained previously for other tree species such as *Azadirachta indica* (Palanisamy and Kumar, 1997), *Rosa hybrida* (Bredmose *et al*., 2004; Otiende *et al*., 2017), *Dalbergia sissoo* (Husen, 2004), *Ulmus villosa* (Bhardwaj and Mishra, 2005), *Tectona grandis* (Husen and Pal, 2007), *Dalbergia melanoxylon* (Amri *et al*., 2010) and *Pterocarpus santalinoides* (Ky-Dembele *et al*., 2016). Better rooting of cuttings made from basal position followed by cuttings from middle position then from an apical position. This may be associated with certain environmental factors such as luminous intensity, photoperiod, temperature (Hansen, 1986) or by changes in the extent of lignification and the degree of secondary thickening along the stem (Girouard, 1969; Hartmann *et al*., 1997). There are also physiological and anatomical factors which may influence the performance of cuttings from different nodal positions such as leaf water potential, leaf age, stomatal distribution, stem diameter, wood structure and xylem elements (Hartmann and Kester, 1990). These factors could influence the distribution of available mineral nutrients, endogenous growth regulators and carbohydrates in both the mother plant and the cuttings sampled (Leakey and Coutts, 1989). Leakey (1983) stated that the poor rooting of apical cuttings may be due to less favorable water relations and increased susceptibility to water stress in young cuttings. Otherwise, this may be due either to an inhibitory effect of auxins produced during root initiation (Smith and Wareing, 1972) or to low carbohydrate concentrations in the apical portion (Palanisamy and Kumar, 1997).

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Table.9: Ion concentrations of nutrient solutions (Hoagland and Arnon (HA), Quoirin and Lepoivre (QL), Murashige and Skoog (MS), and Woody Plant Medium (WPM)).

	Nutrient solution			
Macronutrients (mM)	HA	QL	WPM	MS
NH_4^+	02.0	05.0	05.0	20.6
NO_3^-	14.0	33.0	09.7	39.4
PO_4^-	02.0	02.0	01.3	01.3
K^+	06.0	19.8	12.6	20.0
Ca^+	04.0	05.1	03.0	03.0
Mg^+	01.0	01.5	01.5	01.5
SO_4^-	01.0	01.6	07.2	01.7
Micronutrients (µM)				
Fe^{++}	64.0	100.0	100.0	100.0
Zn^{++}	02.0	29.9	29.9	29.9
Cu^{++}	00.5	01.0	01.0	01.0
B^{+++}	25.0	100.0	100.0	100.0
Mn^{++}	02.0	44.8	132.0	100.0
MoO_4^-	00.5	01.0	01.0	01.0
I^-	–	00.5	–	05.0
Co^{++}	–	00.1	–	00.1
Cl^-	50.0	–	01.3	06.0
Na^+	64.0	200.0	200.0	200.0
Summary values (mM)				
Total N	16.0	38.0	14.7	60.0
NO_3^-/NH_4^+	07.0	06.6	02.0	01.9
Total Molarity	30.2	68.5	42.2	94.0

Finally, there was a significant difference between the four nutrient solutions studied. The results presented herein indicate that MS and WPM media were superior to HA and QL for the number of leaves and leaf size. The sprouting percentage, the number of shoots and shoot length on QL and WPM media might be preferred to the one on HA and MS, while HA and QL were superior to WPM and MS for survival rate, rooting percentage, number of adventitious roots and their elongation. A number of studies have pointed out that nutrient uptake is closely related to the sprouting and rooting proprieties: Blomstedt *et al*. (1991) found that root proliferation of *Eucalyptus regnans* was greater by using HA or WPM in comparison with MS. Wang (1991) also observed a higher degree of multiple shoot formation of *Prunus communis* explants on WPM and QL than on MS. However, Abousalim and Mantell (1994) indicated that the shoot-tip necrosis is a frequent and persistent problem in shoot cultures of *Pistacia vera*, by adding a standard MS solution. Pérez-Tornero and Burgos (2000) reported also that WPM produced chlorosis in the leaves of *Prunus armeniaca*. The differences observed among these nutrient solutions could be explained by their total ionic strength. Indeed, HA solution has a total ion concentration equivalent to 32% of MS, WPM 45% and QL is 73% MS (Table 9). In fact, plants growing on a concentrated nutrient solution developed a short, compact, and densely branched root system, while on dilute solutions or water the roots were long and more sparsely branched (Forde and Lorenzo, 2001). This increase in the level of mineral nutrition benefits the shoot rather more than the root, whereas nutrient deficiency often induces an increased root/shoot ratio (Chapin, 1980). Otherwise, after separation from the stock plant, cuttings have a fixed mineral nutrient pool. The mineral nutrient pool in the cutting may even decline as a result of leaching during propagation (Blazich *et al*., 1983). Therefore, the low rooting and survival percentages of MS solution may be

explained by its excessive nitrogen content for the *A. spinosa* cuttings, while the leaves chlorosis of WPM solution could be due to its low nitrogen content. The full-strength MS is high in ammonium (20.6 mM) and nitrate ions (39.4 mM) followed by QL and WPM (5 mM), whereas HA is a solution with the lowest ammonium content (2 mM). Macronutrients of HA contain a $NO3^{-}/NH4^{+}$ ratio (7) much higher than the other nutrient solutions. However, MS has the lowest $NO3^{-}/NH4^{+}$ ratio (1.9). High endogenous nitrogen levels in cuttings seem to enhance shoot growth if the level of nitrogen exceeds the optimum for root primordium initiation and development (Brouwer, 1962). Furthermore, the decrease in rooting potential of *A. spinosa* cuttings due to an increased nitrogen supply in MS solution may be due to a decrease in the processes of carbon assimilation and partitioning (Druege *et al*., 2000). Basu and Ghosh (1974) reported that the rooting cofactor activity was inversely related to nitrogen supply. High ratios of total available carbohydrates/total nitrogen (C/N) and total phosphorus/total nitrogen (P/N) increased anthocyanin pigmentation in the shoot induced an increased rooting cofactor activity in the cuttings tissues. Our observations indicated that MS and WPM produced shoot-tip necrosis, shoot hyperhydricity and the lowest rooting percentage and survival rate. This observed responses may be due to the slightly low calcium contents of WPM and MS (3 mg) in comparison with HA (4 mg) and QL (5 mg) (Table 9). Indeed, the low availability of calcium may influence rooting because the endogenous pool of these minerals is poorly transported in the phloem (Eliasson, 1978). Moreover, It has been shown to act as a secondary messenger at the crosstalk of auxin and nitric oxide signaling pathways of adventitious roots formation (Lanteri *et al*., 2006), to be involved in cell division and root primordia elongation process (Burstrom, 1968; Imaseki, 1985).

V. CONCLUSION

The results of this study revealed that sprouting and rooting success of *Argania spinosa* cuttings were significantly influenced by nutrient solution, cutting type and nodal position. The hardwood cuttings taken from the basal position with MS solution gave the best number of leaves (36.25) and leaves size (28.93cm²). The highest number of sprouts (1.81), sprouts length (16.88 cm) and sprouting percentage (85.69 %) were recorded in leafy semi-hardwood cuttings from medial position and treated by QL solution. The leafy semi-hardwood cuttings taken from basal position and irrigated with Hoagland solution produced in average more roots (44.63), longer roots (28.86 cm), and had higher rooting and survival percentages: 63.81 % and 96.09 %, respectively. The results of the present study clearly indicated that the cuttings from adult A. *spinosa* trees can be propagated using basal semi-hardwood cuttings receiving Hoagland nutrient solution. Further work could focus on the needs of each nutrient for *A. spinosa* cuttings to produce high quality materials for planting argan orchards and promoting sustainable arganiculture programs.

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REFERENCES

[1] Emberger, L. (1925). Le domaine naturel de l'arganier. Bulletin de la Société Botanique de France, 72(4), 770-774. doi:10.1080/00378941.1925.10832788

[2] Msanda, F. (1993). Ecologie et cartographie des groupements végétaux d'Anzi (Anti-Atlas occidental, Maroc) et contribution à l'étude de la diversité génétique de l'arganier (Argania spinosa (L.) Skeels) (Doctoral dissertation, Grenoble 1).

[3] Msanda, F., El Aboudi, A., and Peltier, J. P. (2005). Biodiversité et biogéographie de l'arganeraie marocaine. Cahiers Agricultures, 14(4), 357-364.

[4] Morton, J. F., and Voss, G. L. (1987). The argan tree (Argania sideroxylon, Sapotaceae), a desert source of edible oil. Economic Botany, 41(2), 221-233. doi: 10.1007/BF02858970.

[5] M'hirit, O., Benzyane, M., Benchekroune, F, El Yousfi, S.M. and Bendaanoun, M. (1998). L'Arganier: Une espèce fruitière-forestière a usages multiples. Ed. Pierre Mardaga, Sprimont, Belgique. 150 p.

[6] Nouaim, R., Mangin, G., Breuil, M. C., and Chaussod, R. (2002). The argan tree (Argania spinosa) in Morocco: Propagation by seeds, cuttings and in-vitro techniques. Agroforestry Systems, 54(1), 71-81.doi: 10.1023/A:1014236025396.

[7] Hartmann, H. T., Kester, D. E., Davies, F. T., and Geneve, R. L. (1997). Plant propagation: principles and practices (6th edition, pp 770). Prentice-Hall Incorporated. New Jersey.

[8] Leakey, R. R. B., (2004). Physiology of vegetative reproduction. In: Burley, Jeffrey, Evans, Julian, and Youngquist, J. A. (eds) Encyclopaedia of Forest Sciences (1st edition, pp. 1655-1668). Academic Press, London.

[9] De Vries, D. P., and Dubois, L. A. (1988). The effect of BAP and IBA on sprouting and adventitious root formation of Amanda rose single-node softwood

cuttings. Scientia Horticulturae, 34(1-2), 115-121. doi : 10.1016/0304-4238(88)90081-7.

[10] Dubois, L. A., and De Vries, D. P. (1991). Variation in adventitious root formation of softwood cuttings of Rosa chinensis minima (Sims) Voss cultivars. Scientia Horticulturae, 47(3-4), 345-349. doi: 10.1016/0304-4238(91)90018-T.

[11] De Klerk, G. J., Van Der Krieken, W., and de Jong, J. C. (1999). Review the formation of adventitious roots: new concepts, new possibilities. In Vitro Cellular and Developmental Biology Plant, 35(3), 189-199. doi: 10.1007/s11627-999-0076-z

[12] Németh, G. (1986). Induction of rooting. In Trees (Volume 1, pp. 49-64). Springer, Berlin, Heidelberg.

[13] Hartmann, H. T., Kester, D. E., Davies, F. T., and Geneve, R. L. (2002). Plant propagation: principles and practices (7th edition, pp. 880). Prentice-Hall Incorporated, Upper Saddle River, New Jersey.

[14] Greenwood, M. S., and Hutchison, K. W. (1993). Maturation as a developmental process. In Clonal Forestry (Volume 1, pp. 14-33). Springer, Berlin, Heidelberg.

[15] Hansen, J., and Kristensen, K. (1990). Axillary bud growth in relation to adventitious root formation in cuttings. Physiologia Plantarum, 79(1), 39-44. doi:10.1111/j.1399-3054.1990.tb05863.x

[16] Bredmose, N., Kristiansen, K., and Nielsen, B. (2004). Propagation temperature, PPFD, auxin treatment, cutting size and cutting position affect root formation, axillary bud growth and shoot development in miniature rose (Rosa hybrida L.) plants and alter homogeneity. The Journal of Horticultural Science and Biotechnology, 79(3), 458-465. doi:10.1080/14620316.2004.11511790.

[17] Bredmose, N., and Hansen, J. (1996). Topophysis affects the potential of axillary bud growth, fresh biomass accumulation and specific fresh weight in single-stem roses (Rosa hybrida L.). Annals of Botany, 78(2), 215-222. doi:10.1006/anbo.1996.0115

[18] Husen, A., and Pal, M. (2007). Effect of branch position and auxin treatment on clonal propagation of Tectona grandis Linn. f. New Forests, 34(3), 223-233. doi: 10.1007/s11056-007-9050-y.

[19] Otiende, M. A., Nyabundi, J. O., Ngamau, K., and Opala, P. (2017). Effects of cutting position of rose rootstock cultivars on rooting and its relationship with mineral nutrient content and endogenous carbohydrates. Scientia Horticulturae, 225(1), 204-212. doi: 10.1016/j.scienta.2017.07.009

[20] Fageria, N. K., and Moreira, A. (2011). The role of mineral nutrition on root growth of crop plants. In Advances in agronomy (Volume 110, pp. 251-331). Elsevier Inc. Academic Press.

[21] Haissig B.E. (1986). Metabolic processes in adventitious rooting of cuttings. In: Jackson M.B. (eds) New Root Formation in Plants and Cuttings. Developments in Plant and Soil Sciences (Volume 20, pp 141-189). Springer, Dordrecht.

[22] Eliasson, L. (1978). Effects of nutrients and light on growth and root formation in Pisum sativum cuttings. Physiologia Plantarum, 43(1), 13-18. doi: 10.1111/j.1399-3054.1978.tb01560.x.

[23] Hoagland, D. R., and Arnon, D. I. (1950). The water-culture method for growing plants without soil (2nd edition, pp. 347). Circular. California Agricultural Experiment Station.

[24] Quoirin, M., and Lepoivre, P. H. (1977). Improved media for in vitro culture of Prunus sp. In Symposium on Tissue Culture for Horticultural Purposes, 78, 437-442. doi10.17660/ActaHortic.1977.78.54.

[25] Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15(3), 473-497. doi: 10.1111/j.1399-3054.1962.tb08052.x.

[26] Lloyd, G., and McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel Kalmia latifolia, by use shoot-tip culture. Combined Proceedings of the International Plant Propagation Society, 30, 421-427.

[27] Antwi-Boasiako, C., and Enninful, R. (2011). Effects of growth medium, a hormone, and stem-cutting maturity and length on sprouting in Moringa oleifera Lam. The Journal of Horticultural Science and Biotechnology, 86(6), 619-625. doi:10.1080/14620316.2011.11512813

[28] Abdullateef, R. A., and Osman, M. (2012). Effects of stem cutting types, position and hormonal factors on rooting in Stevia rebaudiana Bertoni. Journal of Agricultural Science, 4(1), 49. doi: 10.5539/jas.v4n1p49.

[29] Okunlola, A. I. (2013). The effects of cutting types and length on rooting of Duranta Repens in the nursery. Global Journal of Human-Social Science Research, 13(3), 1-4. https://socialscienceresearch.org/index.php/GJHSS/article/view/774.

[30] Seyedi, A., Esmaeili, A., Zadeh, K., and Posiabidi, M. (2014). Comparative evaluation of the rooting in cuttings in (Bougainvillea glabra L.). International Journal of Farming and Allied Sciences, 3(8), 872-875.

[31] Longman, K. A. (1993). Tropical trees: propagation and planting manuals. In Rooting cuttings of tropical trees (Volume 1, pp. 137). Common wealth Science Council.

[32] Hartmann, H. T., Kester, D. E. and Davis, T. F. (1990). Plant Propagation. Principles and Practices (5th edition, pp. 647). Prentice-Hall Incorporated. Englewood Cliffs, New Jersey.

[33] Palanisamy, K., and Kumar, P. (1997). Effect of position, size of cuttings and environmental factors on adventitious rooting in neem (Azadirachta indica A. Juss). Forest Ecology and Management, 98(3), 277-280. doi:10.1016/S0378-1127(97)00116-3

[34] Husen, A. (2004). Clonal propagation of Dalbergia sissoo Roxb. by softwood nodal cuttings: effects of genotypes, application of IBA and position of cuttings on shoots. Silvae Genetica, 53(1-6), 50-55. doi: 10.1515/sg-2004-0009.

[35] Bhardwaj, D. R., and Mishra, V. K. (2005). Vegetative propagation of Ulmus villosa: effects of plant growth regulators, collection time, type of donor and position of shoot on adventitious root formation in stem cuttings. New Forests, 29(2), 105-116. doi:10.1007/s11056-005-0240-1.

[36] Amri, E., Lyaruu, H. V. M., Nyomora, A. S., and Kanyeka, Z. L. (2010). Vegetative propagation of African Blackwood (Dalbergia melanoxylon Guill. and Perr.): effects of age of donor plant, IBA treatment and cutting position on rooting ability of stem cuttings. New Forests, 39(2), 183-194. doi: 10.1007/s11056-009-9163-6

[37] Ky-Dembele, C., Bayala, J., Kalinganire, A., Traoré, F. T., Koné, B., and Olivier, A. (2016). Clonal propagation of Pterocarpus santalinoides L'Hér. ex DC.: the effect of substrate, cutting type, genotype and auxin. Southern Forests: Journal of Forest Science, 78(3), 193-199. doi: 10.2989/20702620.2016.1150696.

[38] Hansen, J. (1986). Influence of cutting position and stem length on rooting of leaf-bud cuttings of Schefflera arboricola. Scientia Horticulturae, 28(1-2), 177-186. doi: 10.1016/0304-4238(86)90137-8

[39] Girouard, R. M. (1969). Physiological and biochemical studies of adventitious root formation. Extractible rooting cofactors from Hedera helix. Canadian Journal of Botany, 47(5), 687-699. doi.org/10.1139/b69-098.

[40] Leakey, R. R. B., and Coutts, M. P. (1989). The dynamics of rooting in Triplochiton scleroxylon cuttings: their relation to leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. Tree Physiology, 5(1), 135-146. doi: 10.1093/treephys/5.1.135.

[41] Leakey, R. R. B. (1983). Stockplant factors affecting root initiation in cuttings of Triplochiton scleroxylon K. Schum., an indigenous hardwood of West Africa. Journal of Horticultural science, 58(2), 277-290. doi :10.1080/00221589.1983.11515121.

[42] Smith, N. G., and Wareing, P. F. (1972). The distribution of latent root primordia in stems of Populus robusta, and factors affecting the emergence of preformed roots from cuttings. Forestry: An International Journal of Forest Research, 45(2), 197-209. doi:10.1093/forestry/45.2.197.

[43] Blomstedt, C., Cameron, J., Whiteman, P., and Chandler, S. F. (1991). Micropropagation of juvenile Eucalyptus regnans (mountain ash). Australian Journal of Botany, 39(2), 179-186. doi: 10.1071/BT9910179.

[44] Wang, Q. (1991). Shoot multiplication of pear in double-phase medium culture. In International Symposium on Plant Biotechnology and its Contribution to Plant Development, Multiplication and Improvement, 289, 349-350. doi:10.17660/ActaHortic.1991.289.90

[45] Abousalim, A., and Mantell, S. H. (1994). A practical method for alleviating shoot-tip necrosis symptoms in in vitro shoot cultures of Pistacia vera cv. Mateur. Journal of Horticultural Science, 69(2), 357-365. doi:10.1080/14620316.1994.11516465.

[46] Pérez-Tornero, O., and Burgos, L. (2000). Different media requirements for micropropagation of apricot cultivars. Plant Cell, Tissue and Organ Culture, 63(2), 133. doi.10.1023/A:1006430718024

[47] Forde, B., and Lorenzo, H. (2001). The nutritional control of root development. Plant and Soil, 232(1-2), 51-68. doi:10.1023/A:1010329902165.

[48] Chapin, F. S. (1980). The mineral nutrition of wild plants. Annual Review of Ecology and Systematics, 11(1), 233-260. doi: 10.1146/annurev.es.11.110180.001313.

[49] Blazich, F. A., Wright, R. D., and Schaffer, H. E. (1983). Mineral nutrient status of Convexa holly cuttings during intermittent mist propagation as influenced by exogenous auxin application. Journal-American Society for Horticultural Science, 108, 425-429.

[50] Brouwer, R. (1962). Nutritive influences on the distribution of dry matter in the plant. Nutritive influences on the distribution of dry matter in the plant. Instituut Voor Biologisch en Scheikundig Onderzoek Van Land Bouwgewassen, (205), 20p.

[51] Druege, U., Zerche, S., Kadner, R., and Ernst, M. (2000). Relation between nitrogen status, carbohydrate distribution and subsequent rooting of chrysanthemum cuttings as affected by pre-harvest nitrogen supply and cold-storage. Annals of Botany, 85(5), 687-701. doi: 10.1006/anbo.2000.1132.

[52] Basu, R. N., and Ghosh, S. K. (1974). Effect of nitrogen nutrition of stock plants of Justicia gendarussa L. on the rooting of cuttings. Journal of

Horticultural Science, 49(3), 245-252. doi:10.1080/00221589.1974.11514576.

[53] Lanteri, M. L., Pagnussat, G. C., and Lamattina, L. (2006). Calcium and calcium-dependent protein kinases are involved in nitric oxide-and auxin-induced adventitious root formation in cucumber. Journal of Experimental Botany, 57(6), 1341-1351. doi:10.1093/jxb/erj109.

[54]Burstrom, H. G. (1968). Calcium and plant growth. Biological Reviews, 43(3), 287-316. doi.10.1111/j.1469185X.1968.tb00962.x.

[55] Imaseki, H. (1985). Hormonal control of wound-induced responses. In Hormonal regulation of development (3rd edition, pp. 485-512). Springer, Berlin, Heidelberg.

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Impact and Awareness of Soil Health Card on Soybean Production Technology in Ujjain block of Ujjain District, M.P, India

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***Abstract*—** *The SHC is a simple document, which contains useful data on soil based on chemical analysis of the soil to describe soil health in terms of its nutrient availability and its physical and chemical properties. The SHC is a simple document, which contains useful data on soil based on chemical analysis of the soil to describe soil health in terms of its nutrient availability and its physical and chemical properties. An amount of* Rs 568 crore *(US$89 million) was allocated by the government for the scheme. In 2016 Union budget of India,* 100 crore *(US$16 million) had been allocated to states for making soil health cards and set up labs. The target for 2015–16 was to collect 100 lakh soil samples and test these for issue of soil health cards. The government plans to distribute 14 crore soil health cards by 2017. it is therefore, important to find out the impact and awareness associated with farmer towards the usefulness and application of Soil Health Card on their agricultural production, diversification and cropping pattern. Considering the above points, an effort will be made to conduct study with the following objective with 120 beneficiaries of KVK Ujjain, M.P. The main findings is majority of beneficiaries were found to have high level of awareness about various components of soybean production technology followed by medium and low level of awareness about various components of Soybean production technology.*

Introduction

Soil is one of the elements required for farming as it provides nutrients to the plants. Healthy soil containing all the elements for growth and development of crop and on the other hand soil deprived of one or more elements either reduces production or degrades quality of crops. Proportion and quantity of macro and micro nutrients refers to the soil health. As far as agriculture production is concerned, soil health plays a vital role in ensuring sustainable production with optimizing the utilization of fertilizers and reducing its wastage. Soil Health Card (SHC) is a scheme launched by the Government of India in February 2015 in Gujarat. The SHC is a simple document, which contains useful data on soil based on chemical analysis of the soil to describe soil health in terms of its nutrient availability and its physical and chemical properties. status of farmers.

Madhya Pradesh is known as the Soybean bowl of India, because major chunk of Soybean production is contributed by Madhya Pradesh State alone. Nutrients are essential for plants' growth and development. When soil nutrients are missing or in short supply, plants suffer from nutrient deficiency and stop growing. Then, application of fertilizers to soils as per requirement is very important to provide balanced nutrients to the plants grown on it. Considering the growing importance of soil testing.

Keywords*— *Soil Health Card, Soybean Production, Soybean bowl of India.

OBJECTIVE

To know the awareness of SHC holders regarding its utility.

REVIEW LITERATURE

Patel and Chauhan (2012) in their study revealed that more than one third (35.00%) of farmers had neutral attitude towards soil health card programme, while 20.00 per cent of farmers had strongly favourable attitude. Equal number (17.00%) of farmers had unfavourable and strongly unfavourable attitude towards soil health card programme. Rest of them (11.00%) had favourable attitude towards soil health card programme.

Hossen *et al* (2013) reported that most of the respondents (77.00%) had positively observed the climate change occurred and only 23.00 per cent did not realize

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about climate change. They further 26 found that most of the farmers (57.2%) had no idea about soil carbon but they followed various soil management practices (crop rotation 90.3%, irrigation 98.96% and fertilization 96.8%) for better crop production which helps to increase soil organic carbon in the farmer's field.

Abebe and Abera (2014) indicated that there was a significant difference between farmers and Agricultural and Rural Development workers regarding their perception towards gender and gender mainstreaming, moderately female farmers have higher degree of perception than male farmers in rural areas. Relatively female workers have higher degree of perception than male workers in Agricultural and Rural Development workers.

METHODOLOGY

For fulfilment of these objectives, the multistage sampling technique has been adopted for selection of sample for present study. Ujjain district comprises of six development blocks. All the six development blocks of the district comes under the SHC for Soybean production out of which one block i.e. Ujjain was selected due to higher number of SHC holders in the block .Ujjain block constitutes of twenty five villages out of which four villages, were selected by the SHC Center for improved cultivation practices of Soybean production, namely Undasa, Madhaopura, Narvar and Chandesara villages. List of 300 SHC holders (2015-16) of the selected four villages was obtained from KVK, Ujjain and 120 farmers have been selected randomly for present study.

RESULT & DISCUSSION

Level of Awareness of Soil Health Card beneficiaries regarding Soybean production technology:

Awareness is defined as knowledge about something exists, or understanding of a situation or subject at the present time based on information or experience. It is a quality or state of being aware. For the present investigation, the awareness level of selected beneficiaries of SHC regarding Soybean production technology was assessed and was presented in the table below:

Table: Distribution of beneficiaries according to their extent of awareness in respect of SHC regarding Soybean production technology. (n=120)

S.No	*Components*	*Extent of Awareness*		
		Least	*Partial*	*Full*
1.	Ploughing and land preparation	22 (18.33)	40 (33.34)	58 (48.33)
2.	Improved varieties of Soybean (JS-9305 and JS-335)	18 (15.00)	45 (37.50)	57 (47.50)
3.	Seed treatment (*Carbendazim+captan@3 gm/kg seed*)	20 (16.67)	48 (40.00)	52 (43.33)
4.	Soil type	22 (18.33)	41 (34.16)	57 (47.50)
5.	Method of sowing*(By Seed-Drill & acc. to the availability of machinery)*	15 (12.50)	46 (38.33)	59 (49.16)
6.	Cropping pattern	30 (25.00)	32 (26.66)	58 (48.33)
7.	Cropping diversification	28 (23.33)	52 (43.33)	40 (33.34)
8.	Type of fertilizer	30 (25.01)	45 (37.49)	45 (37.50)
9.	Fertilizer dose application	35 (29.16)	43 (35.83)	42 (35.00)
10.	Integrated weed management	28 (23.33)	36 (30.00)	56 (46.67)
11.	Integrated pest management	32 (26.66)	42 (35.00)	46 (38.33)
12.	Level of production	30 (25.00)	40 (33.34)	50 (41.66)

(Figure in parentheses shows percentage)

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The above table describes the distribution of SHC beneficiaries as per their obtained mean score of knowledge in the sub component of the programme.

i. Awareness regarding ploughing and land preparation:

Regarding awareness of field preparation showed, out of the total beneficiaries, majority of the beneficiaries (48.33%) pertained high level of awareness followed by partial awareness (33.34%) and least awareness (18.33%).

ii. Awareness regarding improved varieties of Soybean:

Regarding awareness of improved varieties of Soybean JS-9305 and JS-355 showed, out of the total beneficiaries, majority of the beneficiaries (47.50 %) pertained high level of awareness followed by partial awareness (37.50%) and least awareness (18.33%).

iii. Awareness regarding seed treatment:

Regarding awareness of *seed treatment (*Carbendazim+captan@3 gm/kg seed*)* showed, out of the total beneficiaries, majority of the beneficiaries i.e. (43.33%) pertained high level of awareness followed by partial awareness (40.00%) and least awareness (16.67%).

iv. Awareness regarding soil type:

Regarding awareness of *soil type* showed, out of the total beneficiaries, majority of the beneficiaries (47.50%) pertained high level of awareness followed by partial awareness (34.16 %) and least awareness (18.33%).

v. Awareness regarding *method of sowing:*

Regarding awareness of *method of sowing* (By Seed-Drill & acc. to the availability of machinery) showed, out of the total beneficiaries, majority of the beneficiaries (49.16 %) pertained high level of awareness followed by partial awareness (38.33%) and least awareness (12.50%).

vi. Awareness regarding *cropping pattern:*

Regarding awareness of *cropping pattern* showed, out of the total beneficiaries, majority of the beneficiaries (48.33%) pertained high level of awareness followed by partial awareness (26.66%) and least awareness (25.00%).

vii. Awareness regarding *cropping diversification:*

Regarding awareness of *cropping diversification* showed, out of the total beneficiaries, majority of the beneficiaries (43.33%) pertained partial level of awareness followed by high awareness (33.34%) and least awareness (23.33%).

viii. Awareness regarding *type of fertilizer:*

Regarding awareness of type of fertilizer (*FYM, Organic and Inorganic)* showed, out of the total beneficiaries, majority of the beneficiaries (37.50%) pertained high level of awareness followed by partial awareness (37.49%) and least awareness (25.01%).

ix. Awareness regarding *Fertilizer dose application:*

Regarding awareness of *Fertilizer dose application (N:P:K; 20:40:20)* showed, out of the total beneficiaries, majority of the beneficiaries (35.83%) pertained partial level of awareness followed by high awareness (35.00%) and least awareness (29.16%).

x. Awareness regarding integrated weed management:

Regarding awareness of integrated weed management showed, out of the total beneficiaries, majority of the beneficiaries (46.67%) pertained high level of awareness followed by partial awareness (30.00%) and least awareness (23.33%).

xi. Awareness regarding integrated pest management:

Regarding awareness of integrated pest management showed, out of the total beneficiaries, majority of the beneficiaries (38.33%) pertained high level of awareness followed by partial awareness (35.00%) and least awareness (26.66%).

xii. Awareness regarding level of production:

Regarding awareness of level of production showed, out of the total beneficiaries, majority of the beneficiaries (41.66 %) pertained high level of awareness followed by partial awareness (33.34%) and least awareness (25.00%) respectively.

Regarding awareness of field preparation showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively. This result revealed that soybean growers have more awareness of field preparation because they are fully determined towards gaining the high production.

Regarding awareness of improved varieties of Soybean JS-9305 and JS-355 showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively. The result revealed that the improved varieties of soybean give yield than the local variety of soybean.

Regarding awareness of *seed treatment (*Carbendazim+Captan@ 3gm/kg seed*)* showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively. This increases the diseases resistance of seeds which leads into higher production.

With respect to awareness of *soil type* showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively. The main reason behind the result is applicability of soil health card and high numbers of literate beneficiaries.

Regarding awareness of *method of sowing* (By Seed-Drill & acc. to the availability of machinery) showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively.

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Regarding awareness of *cropping pattern* showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively.

Regarding awareness of *cropping diversification* showed, out of the total beneficiaries, majority of the beneficiaries pertained partial level of awareness followed by high awareness and least awareness respectively.

Regarding awareness of type of fertilizer (*FYM, Organic and Inorganic)* showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively.

Regarding awareness of *Fertilizer dose application (N:P:K; 20:40:20)* showed, out of the total beneficiaries, majority of the beneficiaries pertained partial level of awareness followed by high awareness and least awareness respectively.

Regarding awareness of integrated weed management showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively.

Regarding awareness of integrated pest management showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively.

Regarding awareness of level of production showed, out of the total beneficiaries, majority of the beneficiaries pertained high level of awareness followed by partial awareness and least awareness respectively.

REFERENCES

[1] Arya,R.L., Arya,Sonam, Kurel,R.S. (2012) Indira Agriculture Competition Explorer, P.N.365.

[2] Awasthi, H. K.; Singh, P. R. and Sharma, R. N. (2000). Knowledge and attitude of dairy farmers towards improved dairy practices, Maharashtra *J.Extn. Edn*.,**19** :290-293.

[3] Darandale, A.D. (2010). A study on attitude of tribal farmers towards organic farming practices in maize crop. Unpublished M. Sc. (Agri.) Thesis, AAU, Anand.

[4] Gulkari (2011) Attitude of beneficiaries towards National Horticulture Mission Unpublished M. Sc. (Agri.) Thesis, AAU, Anand.

[5] Patel (2011) An Impact Analysis of Peasantry Modernization in Agriculture under Integrated Tribal Development Project of Vadodara District. Ph.D. (Agri.) Thesis, AAU, Anand.

[6] Satyaprakash, Kumari, N., Kumar, A. and De, D. (2010). To study the attitude of beneficiaries towards the technology dissemination programme of ATMA. J. C. S., 18: 206-209.

http://dx.doi.org/10.22161/ijeab/3.4.33 ISSN: 2456-1878

Clusia rosea (Gal Goraka), an Alien Invasive Species Used as Fuelwood for Tea Drying in the Maskeliya Region, Sri Lanka

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***Abstract*—** *Fuelwood is the major source of energy for tea drying in Sri Lanka. High moisture content and presence of latex in the wood are the two main problems in using Cluisia as a fuelwood in tea drying. This study was carried out at Moussakellie tea factory and Hapugastenne estate in Maskeliya to evaluate Clusia rosea as a fuelwood species in tea drying. Two fuelwood combinations were evaluated by estimating Specific Fuelwood Consumption (SFC). Moisture content of Clusia wood were measured in relation to stem girth and methods of processing. Wood production of Clusia coppice managed under a six year rotation was measured.*

The study clearly shows that Clusia rosea is a suitable fuelwood species for tea drying. Clusia showed a higher energy value as inclusion of it in the fuelwood mixtures caused a significant reduction in the Specific Fuelwood Consumption (SFC). Clusia wood can be mixed up to 50% with wild wood for tea drying without causing deposition of latex on heat tubes by maintaining flue gas temperature above 100^0C. Results of the study also showed that splitting and peeling are effective methods in removing moisture from Clusia wood. The study also identified the relationship between moisture content and moisture loss with stem girth. The biomass production of Clusia is found to be comparable with common fuelwood species used in tea drying. The study also revealed some characteristics of Clusia that contributes to its invasive behavior.

Keywords*— *Alien invasive species Clusia rosea, coppice management, latex in fuelwood and tea drying.

I. INTRODUCTION

Tea industry is the largest industrial fuelwood consumer in Sri Lanka utilizing approximately 33% of the total industrial consumption (Haskoning, 1989; Koneswaramoorthy *et al*, 2004). Fuelwood is used for generation of heat for withering green leaf and tea drying in Sri Lanka (De Silva, 1994; Gunasena and Mohamed, 1998). About 22.4 MJ of thermal energy or 1-2kg of fuelwood is required to produce 1kg of made tea (Mohamed, 1998). The quantity of fuelwood is dependent upon the type of wood, efficiency of the furnace and the drier, type of manufacture and waste heat recovery system. A fuelwood to made tea ratio of 1:1 can be considered as very satisfactory. However, the efficiency of most furnaces used in the tea industry are very low, only have 55-65%. *Hevea brasiliennsis, Eucalyptus grandis, Paraserianthus falcataria, Calliandra calothyrsus* and *Gliricidia sepium* are the main species of fuelwood used in Sri Lankan tea industry. These species possesses characteristics that are typical of fuelwood species such as high growth rate, high calorific value, low leaf: stem ratio, high coppicing ability and also good burning properties (NAS, 1980; Gunasena and Pushpakumara, 1998). However, increasing fuelwood demand for the tea industry is difficult to meet through the established species as remaining underutilized lands in the tea growing regions are not suitable for growing them due to low soil depth and high rockiness.

Clusia rosea grows as an evergreen shrub to large tree. It is a hardy plant and exhibits Crassulacean Acid Metabolism (CAM) (Lee *et al*, 1989). This is a photosynthetic mechanism which aids in conserving moisture. *Clusia rosea* is a tree that can grow on infertile rocky lands. It can even establish on wet mosses growing on rock surfaces. It is also known for high growth rate and high biomass production. It is commonly known as pitch-apple, cupey or autograph tree (Florida Native Plant Society, 2013). *Clusia rosea* is commonly identified in Sri Lanka as "*Gal goraka, Ambul gas, Gal idda*" in Sinhala and "*Pulichcha*" in Tamil. It is listed as

an invasive species in Sri Lanka (MMD&E, 2015). *Clusia rosea*, a member of the family *Clusiaseae*, is a terrestrial or epiphytic tree or shrub native to Mexico, Florida and Central America, the Caribbean and northern South America (Wright, 1868; Francis, 1994; Dehgan, 1998; Starr *et al*, 2003; Gilman and Watson, 2014). The growth habit of *Clusia* is that it produces a cluster of stems (stools) from its base.

Clusia has naturalized in certain up country areas (high elevations) in Sri Lanka (Pebotuwage *et al*, 2012). It is widely used as a fuelwood for tea drying and for domestic cooking by the local communities in the Maskeliya region (Hitinayake *et al*, 2012). They peel and split the wood to accelerate drying prior to use them in cocking stoves. The villagers use the *Clusia* roots to tie the bundles of fuelwood. *Clusia* poles are used for construction of temporary houses and also as fence posts. The leaves of *Clusia rosea* are used to feed the goats.

There are two problems of using *Clusia* fuelwood in tea drying. *Clusia* wood has a yellow-white, resinous latex (Gilman and Watson, 1993). Before the furnace get heated to higher temperature, the latex present in *Clusia* wood evaporates and get deposited on the metal tubes in the furnace that convey heat, also on other parts of the furnace and the drier, reducing their efficiency (Raveendran, 2015). High moisture content of *Clusia* wood is the other issue for using it as a fuelwood (Raveendran, 2015).

The objective of the present study was to evaluate *Gal goraka* (*Clusia rosea)* as a fuelwood species in tea drying. Hence, estimation of specific fuelwood consumption (SFC) of fuelwood mixtures involving *Clusia rosea*, method to minimize deposition of latex on furnace especially on tubes that convey heat and also on drier when using *Clusia rosea* as a fuelwood, to study the moisture loss of *Clusia rosea* in relation to the girth classes, drying period and method of processing and to evaluate the biomass production of *Clusia* coppice were identified as the specific objectives of the study.

II. MATERIALS AND METHODS

This study was conducted during 2015 at Mousakelleie tea factory and Hapugastenne tea estate located in the Maskeliya region, Nuwara Eliya district. Both Mousakelleie tea factory and Hapugastenne estate are located in the up country wet zone at elevations 1372m and 1205m amsl, respectively. The mean annual rainfall in this area ranges between 2763-3517mm and average annual temperature is 20.4^{0}C (Anon, 2018).

Experiment 01: Specific fuelwood consumption (SFC) of fuelwood mixtures containing *Clusia rosea*

As said before when *Clusia* is used as a fuelwood in tea drying, the latex present in its wood will get deposited on heat conveying tubes and other parts of furnace reducing its efficiency. Hence *Clusia* is always used in combination with wild wood by the tea factories in the Maskeliya region as a means of minimizing this problem. Two fuelwood mixtures containing *Clusia* commonly used in the tea factories in the Maskeliya region were selected for evaluation under this experiment: 75% wild wood + 25% *Clusia* and 50% wild wood + 50% *Clusia*. Wild wood contains approximately 80% *Albizzia* and 20% *Calliandra*. These fuelwood mixtures were evaluated by estimating their Specific Fuelwood Consumption (SFC).

Drier output (kg of made tea per hour), fuelwood feeding rate (kg of fuelwood fed per hour), flue gas temperature and inlet temperature of the drier were measured. Specific Fuelwood Consumption (SFC) was estimated by dividing fuelwood feeding rate (kg/hr) by the drier output (kg/hr).

During the experimental period efforts were made to maintain the flue gas temperature and drier inlet temperature at a constant range and also to maintain the fuelwood feeding rate normally practiced at the factory. It took one day to complete a replicate of a fuelwood mixture. Four replicates from each mixture was used in the experiment. After 4 days, heat tubes were examined to check whether the latex of *Clusia rosea* is deposited on them.

Data were analyzed by applying sample t-test using SAS package.

Experiment 02: Moisture loss from *Clusia rosea* in relation to the girth classes, method of processing and drying period

The experiment consisted of 24 treatment combinations due to three factors (stem girth class, method of processing and drying period) and their levels (4x3x2). The stems of felled trees were separated into four girth classes (4-8cm, 8-12cm, 12-16cm and >16cm). Two methods of processing that is peeling and splitting of wood were evaluated by comparing with the control (unprocessed wood). Moisture content of wood was measured at the time of harvesting and after four weeks in the storage shed. Samples were oven dried at 103°C till they reach a constant weight (oven dried weight). Moisture content was measured using following formula:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight (g)} - \text{Oven dried weight (g)}}{\text{Initial weight (g)}} \times 100\%$$

The experiment design was a three factor factorial completely randomized design. Mean separation was done using Least Square Mean separation method (LSM).

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Experiment 03: Biomass production of *Clusia rosea*

Fuelwood production of naturally regenerated *Clusia rosea* stand managed under simple coppicing system on a six year rotation was measured. A plot of 4x5m was harvested to measure the biomass production. The bottom girth, top girth and the length of each stem were taken. The girth of stems ranged from 4-16 cm. Wood volume was measured using following formula:

$$\text{Wood volume} = \frac{(A1 + A2)\ L}{2}$$

Key: A1- Cross sectional area at the top of stem, A2- Cross sectional area at the bottom of stem, L-Length of the stem

After harvesting the stems in the selected plot, number of fruits and number of seeds were counted to understand the regeneration potential of *Clusia rosea*.

The experimental design was a single factor completely randomized design. Mean separation was done using Least Significant Difference method (LSD).

III. RESULTS AND DISCUSSION

Experiment 01: Specific Fuelwood Consumption (SFC) of fuelwood mixtures consisting *Clusia rosea*

Fuelwood mixture 1 showed significantly high SFC when compared to the mixture 2. The SFC of mixture 1 and 2 were 1.02 and 0.84, respectively. Average fuelwood feeding rate for mixture 1 and 2 were 257 kg/hr and 203 kg/hr, respectively. High fuelwood feeding rate (high SFC) is an indication for low efficiency of the fuelwood mixture (i.e. it consumes relatively high quantity of fuelwood to produce a kilogram of black tea). The results also indicates that *Clusia* has got a higher energy value when compared to wild wood as the mixture 2 that contained higher proportion of *Clusia* wood showed a low SFC.

Average drier inlet temperature was 268^0F for mixture 1 and 266^0F for mixture 2. The inlet temperature was slightly low with mixture 2 as fuelwood mixture contained relatively high proportion of *Clusia*. Maintaining a high inlet temperature will be a challenge when proportion of *Clusia* is high in the fuelwood mixture. Average flue gas temperatures maintained at the ID fan (temperature of hot air near the blower fan) with mixture 1 and 2 were 108^0C and 110^0C, respectively. The study also showed that these temperatures were high enough to minimize the deposition of latex on heat conveying tubes when above two mixtures of fuelwood is used.

Experiment 02: Moisture loss from *Clusia rosea* in relation to the girth classes, method of processing and drying period

1. Moisture content at the time of harvesting

Heating value of wood is largely determined by its moisture content. Drying of wood increases the calorific value of most type of fuelwood from about 4000 kcal/kg to 6500 kcal/kg (Jayatunge, 2014). In order to get maximum fuel use efficiency and performance, fuelwood requires to be cut to a uniform length of about 30cm and a diameter of 15cm and to have a maximum moisture content of 15% (Jayatunge, 2014).

The results of the experiment shows that moisture content was significantly high (P=0.05) in girth class 4-8 cm when compared to other girth classes at the time of harvesting (Table 1). But the difference between girth classes 8-12cm (57.57%) and 12-16cm (56.49%) was non-significant. The highest moisture content was showed with the girth class 4-8cm (59.92%) and the lowest was with girth class >16 cm (53.58%). The highest moisture content was showed by the unprocessed wood in girth class 4-8cm and the lowest by the peeled wood in girth class 4-8 cm. This shows that moisture content is high when the wood is thin and also when the bark is intact. This relationship is largely determined by the presence or absence of the bark and the proportion of the bark in the wood sample. This is because the bark contains more moisture than the inner parts of the stem and also the proportion of the bark in a wood sample is higher when the stem girth is smaller.

When consider the methods of wood processing, differences in mean moisture content between peeled (52.64%) and split wood (58.49%) was significant (P=0.05), but the differences between split wood and unprocessed wood (59.54%) was non-significant (P=0.05). The differences in moisture content between unprocessed and peeled wood across all girth classes except girth class >16 cm is significant (P=0.05). As said before, the above differences are largely determined by the presence or absence of the peel, as peel contains more moisture than the inner part of the stem. Differences in moisture content among girth classes of peeled wood was non-significant (P=0.05).

Table.1: Moisture content (%) at the time of harvesting.

Girth class (cm)	Processing method		
	Unprocessed wood - Control	Split wood	Peeled wood
4-8cm	64.53 ± 1.24^a	64.17 ± 1.20^a	51.08 ± 0.73^d
8-12cm	61.24 ± 1.52^b	59.41 ± 0.58^c	52.06 ± 1.05^d
12-16cm	58.04 ± 0.23^c	57 ± 1.41^c	54.44 ± 0.27^d
>16cm	54.37 ± 1.28^d	53.4 ± 1.42^d	52.98 ± 0.17^d
Mean	59.54±4.12	58.49±4.28	52.64±1.41

2. Moisture content after four weeks from harvesting

Moisture content of *Clusia* wood varied significantly (P=0.05) after four weeks in storage in relation to girth class and method of processing (Table 2). The highest

moisture content was recorded in wood with girth class >16cm (34.83%) and the lowest was with class 4-8cm (21.08%). But the moisture content didn't differ significantly between girth classes 8-12cm (29.27%) and 12-16cm (32.53%). This shows that the rate of moisture loss from wood decreases with increasing girth.

Moisture content after four weeks in the storage differed significantly (P=0.05) when wood was processed differently. The highest moisture content was recorded with unprocessed wood (35.65%) and the lowest with wood stored after removing the peel (24.75%). Also, it was found that moisture content was significantly different (P=0.05) between unprocessed wood and split wood in relation to girth class. The highest moisture content was recorded by the unprocessed wood in girth class >16 cm and lowest moisture content by the split wood in girth class 4-8 cm. The differences between mean moisture content between split wood and peeled wood in girth class 4-8 cm and >16 cm were non-significant (P=0.05).

Table.2: Moisture content (%) after four weeks from harvesting.

Girth class (cm)	Processing method		
	Unprocessed wood-Control	Split wood	Peeled wood
4-8cm	28.44±1.40^{b}	16.8±1.30^{c}	18.01±0.86^{c}
8-12cm	33.51±0.35^{b}	32.24±0.93^{b}	22.08±2.36^{c}
12-16cm	39.65±1.07^{a}	30.32±1.34^{b}	27.64±0.61^{bc}
>16cm	41±1.13^{a}	32.2±5.65^{b}	31.3±2.70^{b}
Mean	35.65±5.43	27.89±7.26	24.75±5.62

3. Moisture loss after harvesting

Differences in moisture loss among girth classes during the first four weeks after harvesting was significant (P=0.05) (Table 3). The highest mean moisture loss (38.84%) was recorded with girth class 4-8 cm and the lowest (18.75%) with girth class >16 cm. This shows that when girth increases the rate of moisture loss was low.

Also moisture loss varied significantly (P=0.05) among wood that were processed differently. The highest moisture loss (30.6%) was recorded with split wood and the lowest (23.9%) with unprocessed wood. The moisture loss differed significantly between unprocessed wood and split wood in girth class 4-8 cm. The highest moisture loss was showed in split wood in girth class 4-8 cm. Moisture loss didn't differ significantly among different wood processing methods in girth class 8-12 cm. The lowest moisture loss was recorded with unprocessed wood in girth class >16 cm.

Table.3: Moisture loss (%) during the first four weeks after harvesting.

Girth class (cm)	Processing method		
	Unprocessed wood-Control	Split wood	Peeled wood
4-8cm	36.1±2.65^{b}	47.37±0.09^{a}	33.07±1.6^{b}
8-12cm	27.72±1.86^{b}	27.17±1.51^{b}	29.99±3.42^{b}
12-16cm	18.39±1.3^{c}	26.68±2.75^{b}	26.8±0.9^{b}
>16cm	13.36±0.15^{d}	21.19±4.2^{c}	21.69±2.53^{c}
Mean	23.89±9.42	30.6±10.83	27.88±4.82

Experiment 03: Biomass production of *Clusia rosea* in a coppice stand

A *Clusia* stand managed under simple coppice system on a six year rotation was clear felled (harvested) by cutting the stems at the ground level. The resulted stems (copse or rods) were sorted based on their average girth (Table 4). Majority of the stems resulted after coppicing the stools (stumps) were in the girth class 8-12 cm (9.8 stems per stool) followed by girth classes 4-8cm and 12-16cm. Also the stems of the girth class 8-12cm recorded the highest wood volume. Wood volume per stool with 4-8cm, 8-12cm and 12-16cm girth classes were 5830cm^3, 15842cm^3 and 6036cm^3, respectively (Table 4). In the *Clusia* stand there were about 4845 stools per ha. Hence, the total wood volume harvested from a hectare of *Clusia* coppice under six year rotation was 129 cubic meters and it is highly comparable with commonly used fuelwood species in tea drying. For example, fuelwood production of *Eucalyptus grandis and Pariserianthus falcataria* are 40-200 cu. m per ha and 30-50 cu. m per ha, respectively (NAS, 1980).

Table.4: Size of stems and wood volume resulted from harvesting a 4x5m plot in the Clusia coppice.

Girth class (cm)	Average Number of stems	Wood volume per stool (cm^3)
4-8cm	7.3	5830
8-12cm	9.8	15842
12-16cm	2.3	6036
Total	**19.4**	**26708**

In addition to aggressive growth rate which will enable the species to over compete the other species easily, the study also revealed some other important reasons underlying its invasive behavior. The seed production of *Clusia* was estimated. The average number of seeds per fruit and the average number of fruits per stool was 41.5 and 55, respectively and therefore, the average number of seeds per stool was about 2283. As mentioned above, there were 4845 stools per ha in the *Clusia* stand. Hence, about 11.06 million seeds are produced by a one hectare stand. This is in addition to prolific vegetative propagation ability of species where even a small piece of stem can get easily establish as a plant.

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IV. CONCLUSIONS

The results of this study shows that *Clusia rosea* can be considered as a suitable fuelwood species for tea drying. The inclusion of *Clusia* in the fuelwood mixtures has decreased the Specific Fuelwood Consumption (SFC), significantly. The study also shows that *Clusia* wood can be mixed up to 50% with wild wood for tea drying without causing considerable problem due to the deposition of latex on heat tubes. This was achieved by maintaining flue gas temperature near to the blower fan above 100°C. It was found that splitting and peeling are effective methods to dry *Clusia* wood. The study also identified the relationship between moisture content and moisture loss with the stem girth. The biomass production of Clusia is found to be comparable with common fuelwood species used in tea drying. Further, earlier studies have showed that calorific value of *Clusia* is 4155 kcal/kg (Hitinayake *et* al, 2012) and which is well above the minimum accepted (3500 kcal/kg) for fuelwood species (Gunasena and Pushpakumara, 1998). The study also revealed some important characteristics of *Clusia* that contributes to its invasive behavior.

REFERENCES

[1] Anon. (2018). Climate-Maskeliya. Climate-Data.org. Available at: https://en.climate-data.org/location/1061541/ [Accessed: 15 June 2018]

[2] De Silva, W.C.A., (1994). Status of energy utilization by the tea industry in Sri Lanka, *Sri Lanka Journal of Tea Science*, Vol. 63: 36-57.

[3] Cheek, M.D. and Lalla, R. (2017). A description of the naturalized *Clusia rosea* Jacq. (Clusiaceae) populations in South Africa. *Bothalia* 47(1), a2229. Available at: https://doi.org/ 10.4102/abc.v47i1.2229 [Accessed 15 June 2018]

[4] Dehgan, B. (1998). Landscape Plants for Subtropical Climates. University Press of Florida, USA. 656pp.

[5] Florida Native Plant Society (FNPS), 2013, *Clusia rosea*. Available at: http://www.fnps.org/plants/plant/clusia-rosea [Accessed 15 June 2018].

[6] Francis, J.K. (1994). *Clusia* rosea Jacq., Cupey: *Clusia*ceae, *Clusia* Family. International Institute of Tropical Forestry, U.S. Department of Agriculture, Forest Services. 4pp.

[7] Gilman, E.F. and Watson, D.G. (2014). *Clusia rosea*: Pitch Apple. Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, USA.

[8] Gunasena, H.P.M., and Mohamed, M.T.Z. (1998). Use of biomass energy in processing tea. The Tea Research Institute, Talawakele, Sri Lanka.

[9] Gunasena, H.P.M. and Pushpakumara, D.K.N.G., (1998). Selection criteria for fuelwood tree species. Proceedings of the ninth national workshop on multipurpose trees. MPTS Network of Sri Lanka, Kandy, Sri Lanka. pp. 111-121.

[10] Haskoning, (1989). Energy Conservation in the Tea Industry of Sri Lanka. Haskoning Royal Dutch Consulting Engineers and Architects, 206pp.

[11] Hitinayake, H.M.G.S.B., Priyadarshana, G.V.U. and Mahendra, P. (2012). Potential of *Clusia rosea* (Gal Goraka) as a Fuelwood Species. *Sri Lanka Journal of Agricultural Sciences*. Vol. 49: 98-105.

[12] Jayatunge, N.G.P. (2014). Engineering aspects of tea processing (Revised edition). National Institute of Plantation Management, Sri Lanka. 130pp.

[13] Koneswaramoorthy, S., Mohamed, M.T.Z. and Galahitiyawa, G. (2004). Developing and evaluating solar energy techniques for tea drying. *Journal of National Science Foundation*. Vol. 32 (1&2): 49-60.

[14] Lee, H.S.J., Schmitt, A.K. and Luttge, U. (1989). The Response of the C_3—CAM Tree, Clusia rosea, to Light and Water Stress: II. Internal CO_2 Concentration and water use efficiency. *Journal of Experimental Botany*. Oxford University Press Vol. 40, No. 211. pp. 171-179.

[15] MMD&E. (2015), Invasive Alien Species in Sri Lanka: Training Manual for Managers and Policy Makers. Biodiversity Secretariat, Ministry of Mahaweli Development and Environment

[16] Mohamed, M.T.Z. (1998). Use of biomass energy in processing tea. Proceedings of the ninth national workshop on multipurpose trees, MPTS Network of Sri Lanka, Kandy, Sri Lanka. pp. 152-163.

[17] NAS (1980). Firewood Crops: Shrubs and tree species for energy production. National Academy of Sciences. Washington D.C. 237pp.

[18] Peabotuwange, I., Bandara, I.N., Samarsinghe, D., Perera, N., Madawala, M., Amarasinghe, C. (2012). Range extension for *Duttaphrynus kotagamai* (Amphibia: Bufonidae) and preliminary checklist of herpatofauna from the Uda Mäliboda trail in Samanala nature reserve, Sri Lanka. Amphibian and Reptile Conservation 5: 52–64.

[19] Raveendran, K. (2015). *Personal communication*. Tea Research Institute, Talawakelle, Sri Lanka.

[20] Starr, F., Starr, K. and Loope, L. (2003). '*Clusia rosea*: Autograph tree'. United States Geological Survey-Biological Resources Div., Haleakala Field Station, Maui, Hawaii.

[21] Wright, C., (1868). A tropical air-plant. American Naturalist 2, 368–370. Available at: https://doi.org/10.1086/270263 [Accessed 15 June 2018].

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Morphophyziological and Anatomical Characteristics of Leaves in Accessions of Wild Einkorn (*Triticum boeoticum* Boiss.)

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***Abstract*—** *The aim of this study was to assess the degree of variation between 32 accessions of wild einkorn (Triticum boeoticum* Boiss.*) on the basic morphophysiological and anatomical characteristics of the flag and subflag leaves. The experiment was carried out during 2016 – 2017 growing seasons in the randomized block design in four replications and 10 m^2 plot size. Significant differences among the accessions for all studied characters were recorded. The epidermis of the studied 32 accessions was constructed by strongly elongated prosenhyme cells with flexous walls. The stomatas were with oval to elliptic shape, about 1.5 times longer than wide. The most variable character was the total chlorophyll content. Accessions with numbers B6E0416, B6E0413, B6E0398 and B6E0392 had the largest amount of chlorophyll pigments exceeding the average standard almost twice. The water-to-biomass ratio in the flag leaf was the greatest for B6E0378, B6E0389 and B6E0401, while for the subflag leaves B6E0379, B6E0401 and B6E0385 were with the highest amount of water per unit of dry mass. The correlation between intensity of transpiration and the fresh and dry mass of leaves were slightly negative for flag leaf and slightly positive for subflag leaf. The water content of the subflag leaf had a stronger influence on the morphophysiological parameters compared to the water content of the flag leaf. PC-analysis grouped accessions according to similarity on the basis of investigated morphophysiological and physiological characters in two components in the factor plane.*

***Keywords*—wild einkorn, anatomy of leaf, morphophysiological characters of leaves, correlation, PC-analysis.**

I. INTRODUCTION

Drought is known to limit plant productivity in many regions of the world (Chartzoulakis *et al*., 2002). Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hu & Schmidhalter, 1998). Photosynthesis is the key process of primary metabolism, and its capacity can influence plant performance and productivity (Lawlor & Tezara, 2009; Pinheiro & Chaves, 2011). The extent to which photosynthetic capability is maintained during periods of water stress and the ability of rapid recovery of photosynthesis after rewatering may play an important role in plant adaptation to drought environments. In order to preserve photosynthesis under drought conditions, plants have evolved physiological processes to maintain to some extent tissue turgor and stomatal opening (Chartzoulakis *et al*., 2002). Stomata regulate CO2 diffusion into, and water diffusion out of, plant leaves (Chaves *et al*., 2002). Under water-deficit conditions, plants close stomata to prevent major water loss; this, consequently, reduces photosynthesis via decreased influx of CO2 (Pinheiro & Chaves, 2011). In the long-term response to water deficit, stomatal conductance can be influenced by leaf anatomical traits such as stomatal density and size, which can vary to acclimate to the environment (Xu and Zhou, 2008; Franks & Beerling, 2009; Ouyang *et al*., 2017). Leaf anatomical characteristics are considered the true indicators of stress influence (Aberentthy *et al*., 1998). Number of epidermal cells decreases progressively with the increase in water stress, but number of stomata decreases slightly (McCree & Davis, 1974). Drought resistant wheat genotypes had greater stomatal frequency than susceptible genotypes in drought conditions, and drought susceptible genotypes had higher frequency than drought resistant in irrigated conditions (Nayeem, 1989). Thickness of leaf, cuticle, epidermis, hypodermis, and number of stomata generally increased under water stress while the number of hair and stomatal length decreased (Hameed *et al*., 2002).

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Wild wheat species have great potential as a source of genetic traits to improve the drought resistance of wheat cultivars because wild wheat species are highly tolerant to drought stress (Budak *et al*., 2013). The wild wheat species, *Triticum boeoticum* Boiss., is more tolerant to drought than other wheat relatives, such as *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf., *Triticum araraticum* Jakubz. and common wheat cultivars (Sultan *et al*., 2012; Hui Liu *et al*., 2015).

There is little information regarding to the variation of morphophysiological and anatomical characteristics leaves of *Triticum boeoticum* Boiss. The importance of the internal exposed surface of the leaves for plant activity is well recognized, especially in certain phenological stages of development of the crop, i.e. the critical period (from 20 days before flowering to10 days after flowering) and the grain filling period. These phases are of great importance for the generation of number of grains and its final weight, respectively. Water, oxygen and carbon dioxide are exchanged through this surface and the rates of most cellular activities depend on this exchange (Filgueira & Golik, 2003).

The aim of this study was to assess the degree of variation between 32 accessions of wild einkorn (*Triticum boeoticum* Boiss.) on the basic morphophysiological and anatomical characteristics of the flag and subflag leaves as indicators of dry resistance.

II. MATERIAL AND METHODS

Field experiment

The study was conducted in the experimental field of IPGR – Sadovo, in the period 2016 - 2017 with 32 accessions from the ex situ collection, belonging to the species *Triticum boeoticum* Boiss. The experiment was carried out in the randomized block design in four replications and 10 m^2 plot size, after the predecessor peas. Normal agronomic and cultural practices were applied to the experiment throughout the growing seasons. Type of bush (at tillering), ligule-presence, auricles -length, leaf-flag attitude (at the beginning of heading), and leaf pubescence were determined according to international descriptor for genera *Triticum* (Anonymous, 1984). In phase of end of heading were made biometric measurements of the following parameters: length and width of flag and subflag leaves. From each accession, 30 leaves were collected for biometrical measurements. Leaf area was calculated by the formula of Kerin *at al*. (1997), Chanda *et al*. (2002) and Berova *et al*. (2004):

A=k*l*b, where:

k- coefficient, different for each genera (0.65);

l - length of the leaf along the central vain;

b - maximum leaf width.

Laboratory experiment

Fresh (FW, g) and dry weight (DW, g) of flag and subflag leaves are determined using a precision electronic analytical balance OHAUS AS60-USA. Dry weight of leaves is determined by drying the leaves at 104ºC for 1 hour or until reaching a constant mass in three consecutive measurements (Beadle, 1993).

Water content (WC) in flag and subflag leaves is determined by calculating the water to dry weight ratio- gH2O/gDW.

Intensity of the transpiration (T) in flag and subflag leaves is determined by method of Ivanov *et al*. (1950) with modifications by Georgiev & Valchev (1991).

Chlorophyll content meter (CCM-200, Opti-science, Inc., NH, USA) is used to measure the total chlorophyll count in the leaves.

The microscopic observations of the epidermal cells of the 32 accessions were made with light microscope Olympus CX22LED, with total magnification 400. The following characters of flag leaves are analyzed: length and width of stomata and length and width of epidermal cells.

Statistical analyzes

The mean data from all characters were used to analyze the variance according to Lydansky (1988). LSD test was carried out to explore the significance of differences between mean standard and respective accession in the data set.

Phenotypic correlations were calculated by using of phenotypic variances and covariance. The phenotypic correlations thus calculated were tested for significance (Lydansky, 1988).

PC-analysis was applied to group accessions according to similarity on the basis of morphophysiological and physiological characters in two components in the factor plane.

Statistical analyses were performed using the statistical program SPSS 19.0.

III. RESULTS AND DISCUSSION

Leaf anatomy

For genus *Triticum* is typical the isolateral leaf structure, stomata are situated at both sides of leaf (amphystomatic leaf) and absence of accompanying cells – stomata of anomocyte type. It is considered that the bigger number of accompanying cells is typical for the evolutionary primitive plant families of monocotyledons (Ninova, 1995), so the absence of these cells in genus Triticum is a sign of evolutionary higher stage (Uzundzhalieva *et al*., 2017).

The epidermis of the studied 32 accessions from *Triticum boeoticum* Boiss. species was constructed by strongly elongated prosenhyme cells with flexous walls (Fig.1). The cell length varied from 444.71 µm for B6E0414 to 1468.14 µm for (B6E0412A). The length of epidermal

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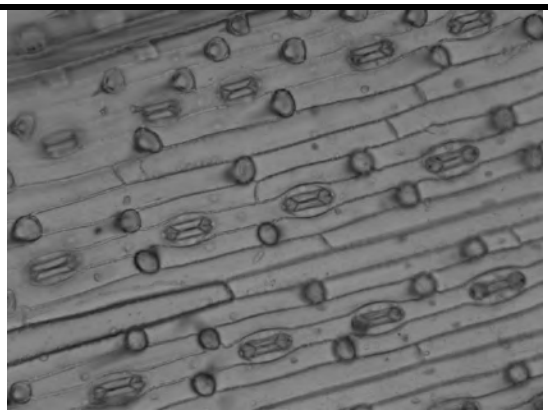
cells in seventeen of accessions was above 1000 µm. The width of the epidermal cells in five of the studied samples had proven differences in compare with the mean standard of the trial. The smallest cells had B6E0414 – 444.71 µm long and 34 µm wide (Table 1). The stomatas were with oval to elliptic shape (Fig.1), about 1.5 times longer than wide. The average length and width of stomata was respectively 381.14 µm and 235 µm. The longest stomata had B6E0397 (449 µm), B6E0410 (466 µm) and B6E0413 (470.14 µm), while the widest stomata had B6E0401 (279.56), B6E0398 (279.57 µm), B6E0380 (280.86 µm), B6E0410 (293.71 µm), B6E0390 (297.71 µm), B6E0400 (347.57 µm). The values of coefficient of variation (CV, %) were above 20% for length of epidermal cells and width of stomata (21.06% and 21.30%, respectively) (Table 1).



Фиг.1 Epidermal cells in *Triticum boeoticum* Boiss.

Table.1: Anatomical characters of flag leaf in 32 accessions of Triticum boeoticum Boiss.

Accessions	Length of epidermal cells, µm	Width of epidermal cells, µm	Length of stomata, µm	Width of stomata, µm
St	1009.57	172.86	381.14	235.00
B6E0378	796.28	180.71	402.29	264.57*
B6E0379	965.00	180.57	335.00*	246.86
B6E0380	717.14	191.43	371.14	280.86***
B6E0381	1068.00	184.43	392.71	234.57
B6E0382	1040.86	183.86	389.14	245.86
B6E0383	909.28	152.57	405.43	215.14
B6E0385	836.43	182.23	417.14	246.29
B6E0386	1278.43	192.29	444.29**	228.43
B6E0387	1163.00	160.29	385.71	242.29
B6E0388	732.00	180.00	359.29	194.14**
B6E0389	1113.57	187.86	439.43**	274.86**
B6E0390	761.28	209.29**	403.29	297.71***
B6E0392	984.00	167.57	386.00	263.71*
B6E0397	1164.57	232.71***	449***	159.43***
B6E0398	755.14	205.57**	346.00	279.57***
B6E0399	1049.00	171.00	347.29	222.57
B6E0400	1120.71	209.00**	446.86**	347.57***
B6E0401	1338.14	180.86	396.43	279.56***
B6E0401A	1190.00	178.71	379.00	261.57*
B6E0402B	862.86	169.29	314.14**	215.86
B6E0405	1078.71	186.29	392.86	224.86
B6E0410	1145.14	186.71	466.00***	293.71***
B6E0412A	1468.14**	185.14	346.71	238.14
B6E0412B	1082.86	151.14	390.86	224.29
B6E0413	1062.86	150.57	470.14***	188.43***
B6E0414	444.71*	34.00***	122.43***	70.00***
B6E0415	836.86	141.57*	318.29**	203.29*
B6E0416	1176.71	150.14	404.00	186.86***
B6E0418B	1289.57	162.57	363.29	183.71***
B6E0420	1002.86	121.43***	381.57	242.71
B6E0421	889.43	164.29	343.71	190.14***
B6E0423	974.43	184.71	387.86	266.29*
LSD0.5	335.04	23.87	39.64	24.33
LSD0.01	442.18	31.50	52.31	32.11
LSD0.001	568.03	40.47	67.20	41.25
CV, %	21.06	19.40	16.25	21.30

*p<0.05, **p<0.01, *** p<0.001

Morphological characters

Accessions from analyzed wild einkorn (*Triticum boeoticum* Boiss.) are characterized with prostrate type of bush (>70º), present of legule and medium size of auricles. The leaves are hairy, which is a typical characteristic for accessions from species *Triticum boeoticum* Boiss. (Empilli *et al*., 2000). Leaf-flag attitude was dropping (91-135º) in 24 of accessions, while in

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B6E0397, B6E0401, B6E0412A and B6E0423 it was semi-upright (15-45º). Accessions with number B6E0379, B6E0390, B6E0392, B6E0416 had horizontal leaf-flag attitude.

1. Flag leaf

1.1. Morphophysiological characters

In Table 2 are presented the results from the biometric analysis of the morphophysiological characters of the flag leaves - length (L, cm) and width (W, cm) of leaves, leaf area (LA, cm^2), fresh weight of flag leaf (FW, g), dray weight of flag leaf (DW, g). Length of the flag leaf varied between 6.83 cm and 15.33 cm. Accessions with numbers B6E0416 and B6E0413 are characterized with proven the longest leaves, respectively 15.33 cm and 15.17 cm, while B6E0380 and B6E0423 were proven the shortest (7.03 cm and 6.83 cm). The average width of the flag leaf was 0.52 cm. In B6E0389 (0.73 cm) and B6E0416 (0.80 cm) are recorded the largest values for this characters against the average experience standard at level of statistical significance $p \leq 0.01$ and $p \leq 0.001$. With the largest leaf area were B6E0416 (8.15 cm^2), B6E0389 (5.66 cm^2) and B6E0413 (5.57 cm^2), while with the smallest B6E0423 (1.50 cm^2) and B6E0380 (1.68 cm^2). The fresh weight of flag leaf of samples B6E0416, B6E0413 and B6E0400 was the largest and exceeds the average standard value of about 38.0%. Accession B6E0423 had the lowest fresh mass value that was below the average standard. In the morphophysiological indicator dry biomass, samples B6E0416, B6E0413 and B6E0400 accumulate the most biomass, the value of which was about 30.0% above the average standard. On the other hand, sample B6E0423 had the lowest biomass accumulation, respectively 0.0149 cm^2 (Table 2).

1.2. Physiological characters

The water-to-biomass ratio shows the water content in the flag leaf. This ratio was the greatest for samples B6E0378 (1.84), B6E0389 (1.78) and B6E0401 (1.73). In accession with number B6E0389, good hydration is combined with a large leaf area, whereas samples B6E0378 and B6E0401 had leaf area below the average standard. The lowest water content had B6E0401A, for which one of the smallest leaf area was also measured (Table 2).

Transpiration refers to evaporation from plant tissue. The process is quite passive, driven by the water vapor difference between the stomatal cavity (or intercellular space) and the surrounding air. When stomata are open, almost all transpiration occurs through the stomata, but plants also transpire through the cuticular layer, which is referred to as cuticular transpiration (Kubota, 2016). The morphological characteristics of the leaves and the plant as a whole, as well as the factors of the environment, influence the intensity of the transpiration (Tzvetkov & Anev, 2017).

The highest intensity of transpiration was reported for B6E0380 (0.540 $mg/cm^2/1$ min), B6E0388 (0.465 $mg/cm^2/1$ min) and B6E0423 (0.440 $mg/cm^2/1$ min), with B6E0380 having leaf area, water content and dry mass of leaf below the value of the average standard. For the remaining accessions B6E0388 and B6E0423, similar values were observed for leaf areas and dray weight of flag leaf, indicating low transpiration efficiency in these accessions. For samples with the highest dry mass of leaf, water content and leaf area values, the intensity of transpiration was about the average standard (Table 2).

The chlorophyll content is an important experimental parameter in the agronomy and in the plant biology research (Lamb *et al*., 2012). It shows alteration depending on many edaphic and climatic factors such as salt stress, light, water stress, air pollution, fertilizing and also it shows alteration depending on time in vegetation period (Sevik *et al*., 2012). In our experiment the amount of chlorophyll expressed as a total chlorophyll content index (CCI) ranged from 2.51 to 20.89. One of the reasons for the strong variation in the value of CCI is the difference in time of occurrence of the seed filling phase of the accessions as well as its duration. Accessions with numbers B6E0416, B6E0413, B6E0398 and B6E0392 had the largest amount of chlorophyll pigments exceeding the average standard almost twice. The first two samples are characterized with maximum values of the leaf area, fresh and dry mass of leaves (Table 2).

2. Subflag leaf

2.1. Morphophysiological characters

The length of the subflag leaf ranged between 14.03 cm and 27.47 cm, and samples with numbers B6E0383 (27.47 cm), B6E0399 (27.37 cm), B6E0413 (26.83 cm) and B6E0416 (25.93 cm) exceed significantly the average values of the experiment. They had also the largest leaf area. The smallest leaf area had B6E0423, the difference from the standard was almost three times. The width of the subflag leaf ranged from 0.6 cm to 1.1 cm, with the magnitude of range greater than this of the flag leaf. Accessions B6E0399 and B6E0416 had the widest leaves, and samples with numbers B6E0414 and B6E0415 had the narrowest leaves. With the highest fresh mass of the subflag leaves were B6E0399, B6E0386 and B6E0383, their average values being higher than the average standard by 35.0%. B6E0399 and B6E0383 were also indicative of the previous characters. The lowest fresh mass of subflag leaf is reported for B6E0401A. The largest dry mass had B6E0399, B6E0392 and B6E0416. Their values exceed the average standard by more than 30.0%. The lowest dry mass had B6E0401A. It was the only one of all accessions with an average dry mass below 0.05 g (Table 3).

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Table.2: Morphophysiological characters flag leaf in the end of the heading phase and total leaf chlorophyll content index (CCI)

Accessions	FW, g	DW, g	WC, g H_20/g DW	L, cm	W, cm	LA, cm^2	T mg/cm^2/1 min	Total CCI
St	**0.0777**	**0.0322**	**1.42**	**10.35**	**0.52**	**3.67**	**0.306**	**8.28**
B6E0378	0.0580	0.0174	1.84	7.83	0.33*	2.26	0.300	6.54
B6E0379	0.0732	0.0280	1.68	9.56	0.53	3.33	0.330	11.81
B6E0380	0.0497	0.0212	1.37	7.03*	0.37	1.68	0.540*	13.07
B6E0381	0.0771	0.0322	1.48	8.4	0.53	2.94	0.403	8.77
B6E0382	0.0746	0.0341	1.27	10.3	0.53	3.63	0.233	7.70
B6E0383	0.1152	0.0456	1.52	13.1	0.6	4.99	0.289	6.69
B6E0385	0.0797	0.0300	1.65	9.83	0.53	3.47	0.335	12.88
B6E0386	0.1046	0.0432	1.39	11.67	0.63	5.27	0.294	6.89
B6E0387	0.0781	0.0307	1.52	10.63	0.63	4.69	0.295	8.80
B6E0388	0.0645	0.0254	1.59	8.26	0.43	2.32	0.465	3.57
B6E0389	0.1014	0.0362	1.78	11.5	0.73**	5.66*	0.276	3.31
B6E0390	0.101	0.0387	1.66	11.33	0.53	4.02	0.368	5.84
B6E0392	0.093	0.0366	1.53	10.2	0.5	3.32	0.388	16.33*
B6E0397	0.056	0.0242	1.32	8.93	0.47	2.78	0.372	11.90
B6E0398	0.067	0.0268	1.50	9.29	0.5	3.02	0.299	18.33*
B6E0399	0.099	0.0418	1.38	11.33	0.67	5.31	0.376	12.79
B6E0400	0.1209	0.0480	1.52	12.93	0.53	4.47	0.333	3.63
B6E0401	0.0797	0.0293	1.73	10.4	0.53	3.63	0.395	2.93
B6E0401A	0.0409	0.0208	1.00	8.03	0.33*	1.76	0.285	2.51
B6E0402B	0.0566	0.0234	1.40	8.83	0.3**	1.74	0.307	6.13
B6E0405	0.0779	0.0332	1.35	9.53	0.47	2.91	0.129	3.43
B6E0410	0.0809	0.0361	1.23	10.5	0.53	3.68	0.106	3.39
B6E0412A	0.0732	0.0308	1.36	10	0.53	3.53	0.275	3.52
B6E0412B	0.0701	0.0296	1.35	11.67	0.53	4.08	0.181	4.07
B6E0413	0.1212	0.0537*	1.26	15.17***	0.57	5.57	0.355	15.14
B6E0414	0.0437	0.0191	1.29	9.86	0.43	2.77	0.135	14.46
B6E0415	0.0631	0.0311	1.02	11.38	0.47	3.71	0.215	7.27
B6E0416	0.1256	0.0578*	1.16	15.33***	0.8***	8.15***	0.271	20.89**
B6E0418B	0.0755	0.0329	1.31	10.97	0.57	4.26	0.273	4.71
B6E0420	0.0616	0.0252	1.43	10.03	0.43	2.94	0.359	2.51
B6E0421	0.0730	0.0309	1.36	10.7	0.57	3.94	0.179	6.86
B6E0423	0.0310	0.0149	1.15	6.83*	0.33*	1.50	0.440	11.24
LSD0.5	0.050	0.019	0.579	2.76	0.16	1.98	0.226	7.915
LSD0.01	0.067	0.025	0.771	3.66	0.21	2.63	0.301	10.446
LSD0.001	0.086	0.032	0.998	4.72	0.27	3.39	0.389	13.419
CV, %	30.23	30.66	14.12	18.82	21.62	37.61	31.28	59.53

*p<0.05, **p<0.01, *** p<0.001

Length of leaf (L, cm), and width of leaf (W, cm), leaf area (LA, cm^2), fresh weight of leaf (FW, g), dray weight of leaf (DW, g), water content (WC, g H_20/g DW), transpiration (T, mg/cm^2/1 min), total leaf chlorophyll content index (CCI), coefficient of variation (CV, %)

2.2. Physiological characters

Accessions with numbers B6E0379, B6E0401 and B6E0385 were with the highest amount of water per unit of dry mass. B6E0423 had the smallest water content. With the lowest water content per unit of dry mass was B6E0423, the difference with the leading accessions was about 1 g. For this sample, the lowest values for leaf width and leaf area were also reported. The highest intensity of transpiration was found in samples B6E0381, B6E0388 and B6E0398 and respectively the lowest intensity in accession B6E0401A. In B6E0381 and B6E0398, strong transpiration was combined with relatively high values of fresh and dry mass. Compared to them, in sample with number B6E0388, transpiration was ineffective. In B6E0401A there was, also an ineffective transpiration (Table 3).

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Table. 3: Morphophysiological characters of subflag leaf in the end of the heading phase

Accessions	FW, g	DW, g	WC, g H_20/g DW	L, cm	W, cm	LA, cm	T, mg/cm^2/1 min
St	0.227	0.083	1.728	20.23	0.84	11.37	0.231
B6E0378	0.2365	0.0938	1.41	19.73	0.83	11.88	0.195
B6E0379	0.2478	0.0808	2.52*	19.57	0.97	12.4	0.209
B6E0380	0.1968	0.0677	1.90	15.67	0.73	7.72	0.275
B6E0381	0.2468	0.0804	2.07	18.33	0.73	8.93	0.332
B6E0382	0.2537	0.0899	1.76	23.77	0.8	12.36	0.259
B6E0383	0.3096	0.1095	1.81	27.47**	0.9	16.07*	0.285
B6E0385	0.2745	0.0862	2.14	20.77	0.97	13.08	0.238
B6E0386	0.3209	0.1112	1.88	22.73	0.97	14.37	0.316
B6E0387	0.2599	0.0899	1.87	21.8	0.97	13.76	0.257
B6E0388	0.1891	0.0655	1.93	15.9	0.83	8.79	0.329
B6E0389	0.2489	0.0888	1.78	20.9	1.03**	13.96	0.217
B6E0390	0.2401	0.0791	2.07	19.77	0.97	12.49	0.222
B6E0392	0.3397	0.1251	1.72	23.33	1*	15.17	0.254
B6E0397	0.2021	0.0686	1.94	19.33	0.97	12.2	0.260
B6E0398	0.2069	0.0730	1.93	14.72*	0.8	7.85	0.317
B6E0399	0.4006*	0.1482**	1.72	27.37**	1.1***	19.48***	0.175
B6E0400	0.3017	0.1049	1.84	23.63	0.83	13.18	0.285
B6E0401	0.2183	0.0674	2.21	19.23	0.87	11.1	0.253
B6E0401A	0.1061	0.0443	1.36	16.23	0.6***	6.33*	0.082*
B6E0402B	0.1315	0.0506	1.57	14.03*	0.67*	6.11*	0.171
B6E0405	0.2924	0.1080	1.78	21.2	0.87	12.39	0.179
B6E0410	0.1973	0.0832	1.50	19.5	0.77	10.02	0.166
B6E0412A	0.2502	0.0935	1.71	21.53	0.93	13.39	0.217
B6E0412B	0.1775	0.0720	1.47	22.37	0.63**	9.13	0.156
B6E0413	0.2508	0.1091	1.23	26.83**	0.8	13.98	0.235
B6E0414	0.1466	0.0573	1.55	19.7	0.63**	8.14	0.207
B6E0415	0.1192	0.0533	1.22	18.67	0.63**	7.71	0.116
B6E0416	0.2780	0.1134	1.48	25.93*	1.07**	18.03**	0.202
B6E0418B	0.1668	0.0675	1.48	17.37	0.97	10.93	0.166
B6E0420	0.1472	0.0540	1.72	17.5	0.7*	7.96	0.264
B6E0421	0.1472	0.0540	1.72	18.33	0.83	9.92	0.246
B6E0423	0.1222	0.0610	1.11	14.10*	0.53***	4.99**	0.299
LSD0.5	0.130	0.046	0.758	4.86	0.14	4.01	0.134
LSD0.01	0.173	0.061	1.009	6.45	0.19	5.33	0.178
LSD0.001	0.224	0.079	1.307	8.31	0.24	6.87	0.231
CV, %	35.07	33.05	18.57	21.75	20.72	34.31	29.50

*p<0.05, **p<0.01, *** p<0.001

Length of leaf (L, cm), and width of leaf (W, cm), leaf area (LA, cm^2), fresh weight of leaf (FW, g), dray weight of leaf (DW, g), water content (WC, g H_20/g DW), transpiration (T, mg/cm^2/1 min), coefficient of variation (CV, %)

Correlation between investigated characters

Correlations between the morphophysiological and physiological characters reported for the flag and the subflag leaves were with moderate and strong positive values with a proof of up to 1% (table 4 and table 5).

There were some differences in the calculated correlation between the morphophysiological and physiological indicators of both types of leaves. The relationship between intensity of transpiration with the fresh and dry mass of leaves, were slightly negative for flag leaf and slightly positive for subflag leaf, respectively. The water content of the subflag leaf had a stronger influence on the morphophysiological parameters compared to the water content of the flag leaf, with significant at p≤0.05. For both types of leaves, the CCI value affected positively on the most of the characters, with stronger impact on the flag leaf (table 4 and table 5).

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Table. 4: Correlation between morphophysiological and physiological characters of flag leaf

Characters	FW	DW	WC	L	W	LA	T	CCI
FW	1.000	0.971**	0.171	0.886**	0.654**	0.862**	-0.083	0.477**
DW		1.000	-0.061	0.915**	0.670**	0.889**	-0.164	0.501**
WC			1.000	-0.081	0.036	-0.040	0.353**	-0.059
L				1.000	0.583**	0.861**	-0.261	0.408*
W					1.000	0.894**	-0.262	0.423**
LA						1.000	-0.268	0.538**
T							1.000	0.068
CCI								1.000

*p<0.05, **p<0.01, *** p<0.001

FW - fresh weight of leaf, DW - dray weight of leaf, WC - water content in the leaf, L- length of leaf, W - width of leaf, LA - leaf area, T - Intensity of the transpiration in the leaf, CCI - Chlorophyll content index.

Table. 5: Correlation between morphophysiological and physiological characters of subflag leaf

Characters	FW	DW	WC	L	W	LA	T	CCI
FW	1.000	0.948**	0.407*	0.774**	0.758**	0.886**	0.271	0.308
DW		1.000	0.119	0.835**	0.699**	0.896**	0.123	0.330*
WC			1.000	0.032	0.418*	0.231	0.481**	0.036
L				1.000	0.544**	0.872**	-0.031	0.193
W					1.000	0.876**	0.088	0.315
LA						1.000	0.025	0.303
T							1.000	0.125
CCI								1.000

*p<0.05, **p<0.01, *** p<0.001

FW - fresh weight of leaf, DW - dray weight of leaf, WC - water content in the leaf, L- length of leaf, W - width of leaf, LA - leaf area, T - Intensity of the transpiration in the leaf, CCI - Chlorophyll content index.

Principal component analysis (PC-analysis)

PC-analysis was applied to group accessions according to similarity on the basis of investigated morphophysiological and physiological characters in two components in the factor plane. The values of the two components to each of the study parameters for flag and subflag leaves were calculated empirically (Table 6). The analysis shows that the first component explains 63.16 % of the total variation in the trial with flag leaves and 62.16% of the total variation in the experiment with subflag leaves, the second - 20.57 % and 21.11%, respectively for the experiments with flag and subflag leaves. Two factors explain total 83.73 % of the variation in the experience with flag leaves and 83.27% in the experience with subflag leaves. First factor had an important role to justify alteration of FW, DW, L, W and LA, while second factor was in positive correlation with WC and T (Table 6).

Distribution of evaluated accessions in the coordinate system of PC1 and PC2, presents the grouping of accessions according to similarity of traits: FW, DW, L, W, LA, WC and T both for experiment with flag leaf (in left) and experiment with subflag leaf (in right) (Fig. 2). The accessions grouped in the upper left quadrants had positive values for PC1 and negative values for PC2 (high FW, DW, L, W, LA and low WC and T). The samples classified in the upper right quadrants had positives values for both factors (PC1 and PC2). Accessions in the below left quadrants had respectively negative values for both factors. The samples in the below right quadrants are characterized with negative values for PC1 and positive values for PC2. Some of the accessions are separated as “detached” from other. For the both experiments these accessions were B6E401A, B6E415 and B6E0388. B6E401A and B6E415 had low values of all characters included in the factor analyses. B6E0388 is characterized with high value of T and moderate value of WC in the both types of analyzed leaves. B6E0416 is characterized with the highest values of L, W, LA, FW, DW as well as with low values of WC and T of the flag leaf, while B6E399 for the subflag leaf.

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Table. 6: Factor analysis of traits using principal components analysis in the trials with flag and subflag leaves in 32 genotypes from Triticum boeoticum Boiss.

Characters	Rotated Component Matrix in the trial with flag leaf		Rotated Component Matrix in the trial with subflag leaf	
	Components		Components	
	1	2	1	2
FW - fresh weight of leaf,	0,95	0,17	0,92	0,30
DW - dray weight of leaf	0,95	-0,06	0,95	0,05
WC - water content in the leaf	0,01	0,84	0,15	0,87
L- length of leaf	0,92	-0,23	0,91	-0,16
W - width of leaf	0,89	0,00	0,79	0,35
LA - leaf area	0,96	-0,11	0,98	0,08
T - Intensity of the transpiration in the leaf	-0,08	0,81	0,01	0,80
Eigen values	4,42	1,44	4,35	1,48
Proportional variance, %	63,16	20,57	62,16	21,11
Cumulative variance, %	63,16	83,73	62,16	83,27

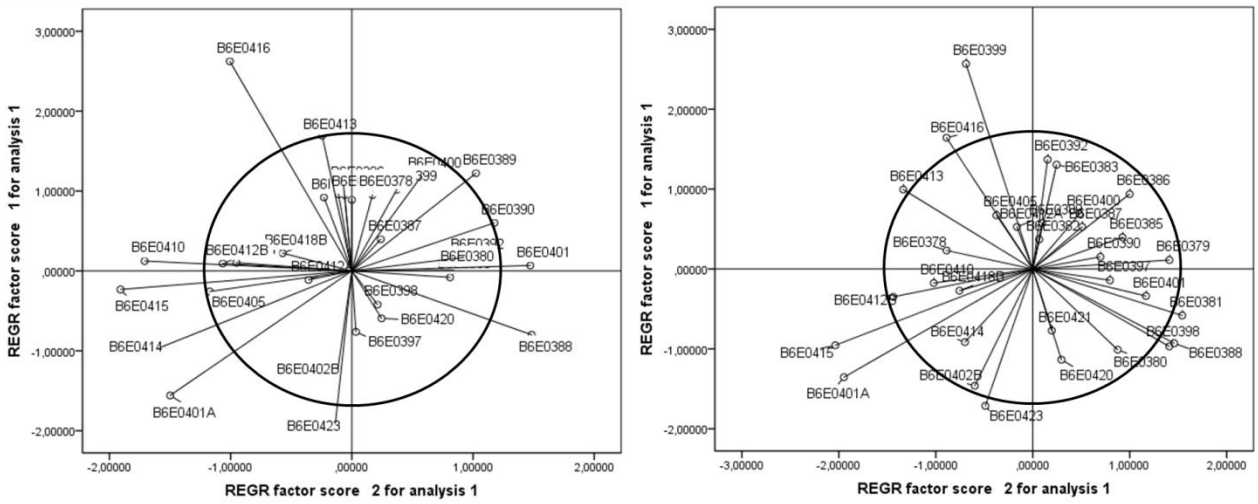

Fig.2: Distribution of evaluated accessions within the factor plane according to similarity of traits: FW - fresh weight of leaf, DW - dray weight of leaf, WC - water content in the leaf, L- length of leaf, W - width of leaf, LA - leaf area, T - Intensity of the transpiration in the leaf, both for experiment with flag leaf (in left) and experiment with subflag leaf (in right)

IV. CONCLUSION

Analysis of variance showed highly significant differences among the accessions for all anatomical, morphological and physiological characters included in the study for the flag and subflag leaves. The most variable character was the total chlorophyll content. B6E0416 and B6E0413 are characterized with the largest leaf area, fresh and dry mass of the flag leaves and high total chlorophyll content. Low transpiration efficiency of flag leaf was detected for B6E0380, B6E0388 and B6E0423. An ineffective transpiration of subflag leaf had number B6E0388 and B6E0401A. The correlation between intensity of transpiration and the fresh and dry mass of leaves were slightly negative for flag leaf and slightly positive for subflag leaf. The water content of the subflag leaf had a stronger influence on the morphophysiological parameters compared to the water content of the flag leaf. The total chlorophyll content in the leaves expressed through CCI value affected positively on the most of the morphophysiological and physiological characters, with stronger impact on the flag leaf. PC-analysis grouped accessions according to similarity on the basis of investigated morphophysiological and physiological characters in two components in the factor plane. First factor had an important role to justify alteration of fresh weight of leaf,

dray weight of leaf, length of leaf, width of leaf, and leaf area, while second factor was in positive correlation with water content in the leaf and intensity of the transpiration.

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REFERENCES

[1] Aberentthy, G. A., Fountain, D. W. and McManus, M. T. (1998). Observation on the leaf anatomy of Festuca novae Zlandiae and biochemical response to water deficit. New Zealand Journal of Botany, 36: 113–23.

[2] Anonymous (1984). The International comecon list of descriptors for the genus Triticum L. The N.I. Vavilov All-Union Institute of Plant Industry, Leningrad. USSR.

[3] Beadle, C. L. (1993). Growth analysis. In: Photosynthesis and production in a changing environment: A field and laboratory manual. Hall DO, Scurlock JM, Bolhar-Nordenkampf HR, Leegood R, Long S (eds.). Chapman and Hall, London, pp. 36-46.

[4] Berova, M., Kerin, V., Stoeva, N., Vasilev, A. and Zlatev, Z. (2004). Manual exercise of Plant Physiology. Academic Publishing Agricultural University. Plovdiv.

[5] Budak, H., Kantar, M., Yucebilgili, K. (2013). Drought tolerance in modern and wild wheat. The Scientific World Journal, 548246: 1–16.

[6] Chanda, S.V. and Singh, Y.D. (2002). Estimation of leaf area in wheat using linear measurements. Plant Breeding and Seed Science (Radzikov, Poland), 46(2), 75-79.

[7] Chartzoulakis, K., Patakas, A., Kofidis, G., Bosabalidis, A. and Nastou, A. (2002). Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. Scientia Horticulturae, 95, 39–50.

[8] Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P., Osório, M. L., Carvalho, I., Faria, T. and Pinheiro, C. (2002). How plants cope with water stress in the field. Photosynthesis and growth. Annals of Botany, 89, 907–916.

[9] Empilli, S., Castagna, R. and Brandolini, A. (2000). Morpho-agronomic variability of the diploid wheat Triticum monococcum L. Plant Genetic Resources, 2000, 124, 36-40.

[10] Filgueira, R. R. and Golik I. S. (2003). Anatomical and physiological characteristics of two argentine wheat cultivars. Ciência Rural, Santa Maria, 33(4), 641-645.

[11] Franks, P. J. and Beerling, D. J. (2009). Maximum leaf conductance driven by CO2 effects on stomatal size and density over geologic time. Proceedings of the National Academy of Sciences, USA 106, 10343–10347.

[12] Georgiev, G. and Valchev, D. (1991). Influence of SBB 1450 on the water regime and formation of the leaf surface of some varieties of barley. Plant physiology, BAS, XVII, Book 3, 24-32.

[13] Hameed, M., Ubeda, M., Muhammad, A. and Altaf-Ur-Rehman, R. (2002). Variation in leaf anatomy in wheat germplasm from varying drought-hit habitats. International Journal of Agriculture & Biology, 4(1), 12-16.

[14] Hu, Y. and Schmidhalter, U. (1998). Spatial distributions of inorganic ions and carbohydrates contributing to osmotic adjustment in the elongating wheat leaf under saline conditions. Australian Journal of Plant Physiology, 25, 591–597.

[15] Hui Liu, Muhammad Abdul Rab Faisal Sultan, Xiang li Liu, Jin Zhang, Fei Yu, and Hui xian Zhao (2015). Physiological and comparative proteomic analysis reveals different drought responses in roots and leaves of drought-tolerant wild wheat (Triticum boeoticum). PLoS One, 10(4): e0121852.

[16] Kerin, V., Tsonev, T., Moetska-Berova, M., Vassilev, A.,Zlatev, Z. (1997). Methods of indicating stress in plants. Modern methods of analysis in plant physiology, AU, Plovdiv, pp. 100-109.

[17] Kubota Chieri. 2016. Chapter 10.Growth, Development, Transpiration and Translocation as Affected by Abiotic Environmental Factors.In Plant Factory,An Indoor Vertical Farming System for Efficient Quality Food Production, 151–164. Edited by: Toyoki Kozai, Genhua Niu and Michiko Takagaki.

[18] Lamb, J. J., Eaton-Rye, J. J. and Hohmann-Marriott, M. F. (2012). An LED-based Fluorometer for Chlorophyll quantification in the laboratory and in the field. Photosynth Res, 114, pp 59-68.

[19] Lawlor, D. W. and Tezara, W. (2009). Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany, 103, 561–579.

[20] Lydansky, T. 1988. Statistical methods in biology and agriculture. Zemizdat. Sofia.

[21] McCree, K. J. and Davis, S. D. (1974). Effect of water stress and temperature on leaf size and on size and number of epidermal cells in grain sorghum. Crop Science, 14, 751–5.

http://dx.doi.org/10.22161/ijeab/3.4.34 *ISSN: 2456-1878*

[22] Nayeem, K. A. (1989). Genetical and environmental variation in stomatal frequency and distribution in wheat Triticum spp. Cereal Research.Communication, 17: 51–7.
[23] Ninova, D. (1995). Anatomy and morphology of plants. Zemizdat, Plovdiv, 320 pp.
[24] Ouyang, W., Struik, P. C., Yin, X., Yang, J. (2017). Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. Journal of Experimental Botany, 68 (18), 5191–5205.
[25] Pinheiro, C. and Chaves, M. M. (2011). Photosynthesis and drought: can we make metabolic connections from available data?Journal of Experimental Botany, 62, 869–882.
[26] Sevik, H., Guney, D., Karakas, H., Aktar, G. (2012) Change to amount of chlorophyll on leaves depend on insolation in some landscape plants. International Journal of Environmental Sciences, 3 (3), 1057-1064.
[27] Sultan, M., Hui, L., Yang, L. J., Xian, Z. H. (2012). Assessment of Drought Tolerance of Some Triticum L. Species through Physiological Indices. Czech Journal of Genetics and Plant Breeding,48, 178–184.
[28] Tzvetkov, N. and Anev, S. (2017). Guide to Plant Physiology Exercises. University ff Forestry, Sofia, 1-104.
[29] Uzundzhalieva, K. and Desheva, G. (2017). Plant Genetic Resources from Medicinal Plant Species – ex situ and in vivo conservation in the Institute of Plant Genetic Resources – Sadovo. Jubilee scientific conference with international participation 135 years of agricultural science in Sadovo and 40 years Institute of Plant Genetic Resources - Sadovo, May 29-31, 2017, 86-100.
[30] Xu, Z. and Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany, 59, 3317–3325.

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Molecular Characterization of Three Cultivars of Tomato (*Lycopersicon Esculentum L.*) in South-West Nigeria Using SSR Markers

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Abstract**—** *Molecular characterisation of local tomato cultivars – Ibadan Local (IbL), Ife and JM94/46 (JM) were assessed using simple sequence repeat (SSR) markers. Out of ten SSR primer pairs used, three primer pairs were able to differentiate amplified genomic DNA of the cultivars. Unweighted Pair Group Method Using Arithmetic Average (UPGMA) cluster analysis of the data showed a close relationship between IbL and Ife with a genetic distance (GD) of 0.067; Ife and JM had GD of 0.2 and JM and Ife had GD of 0.25.*

Keywords**— Genetic Distance, Local cultivars, Nigeria, SSR Markers, Tomato.**

I. INTRODUCTION

The genetic analysis of relatedness between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources (Weising et al. 1995). The use of molecular markers in the characterization of much diversified materials offers a unique opportunity to define significant marker- trait associations of biological and agronomic interest; it also has proven to be a valuable tool in the evaluation of genetic variation both within and between species (Powell et al. 1996).

The genome of tomato plant is one of the most investigated plant genomes (Foolad 2007) and recent studies show that several researchers have characterized tomato varieties of interest using molecular markers. Random amplified polymorphic DNA (RAPD) marker studies in tomato has been conducted by El-Hady et al. (2010), Comlekcioglu et al. (2010), Naz et al. (2013), Mazzucato et al. (2008), Sharifova et al. (2013), Pal and Singh (2013), (Tabassum et al. 2013), Thamir et al. (2014) and Shah et al. (2015) while Amplified fragment length polymorphism (AFLP) marker studies in tomato was recently done by Berloo et al. (2008).

Simple Sequence Repeats (SSR) marker studies have recently been carried out by Benor et al. (2008), El-Awady et al. (2012), Korir et al. (2014) and Singh et al. (2014) .The high degree of polymorphism and the large number of bands obtained per assay shows that SSR is the most informative marker system for tomato genotyping for purposes of rights protection and for the tomato industry in general (Korir et al. 2014). SSR markers have the advantages of being co-dominant, reproducible, multiallelic, highly polymorphic, and assayable by PCR (Miskoska– Milevska et al, 2011).

Tomato fruits are a significant source of nutrition for substantial portions of the world's human population because this vegetable crop is widely cultivated and consumed extensively as both a fresh vegetable and concentrated processed products (Hamner and Maynard, 1942; Beecher, 1998). In tropical Africa, the area used for tomato cultivation is about 300,000 ha with an estimated annual production of 2.3 million tonnes; Nigeria is the largest producer accounting for 541,800 ha and an annual production of 2,143,500 tonnes (FAOSTAT 2014). Nigeria ranks 14th in the world in production, and 3rd in hectares of land cultivated (FAOSTAT, 2014) There is however paucity of documented work on diversity studies of Nigerian cultivars of tomato; such work would provide the background work for the application of modern biotechnology techniques in solving agricultural problems by providing new advances for the development and production of indigenous stress tolerant cultivars. The aim of this research was to analyze and characterize the genetic variability of some Nigerian Tomato cultivars.

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The choice of the tomato cultivars from South West Nigeria was based on agronomic studies carried out at the National Institute for Horticultural Research and Training (NIHORT) suggesting that Ibadan local (IbL) and Ife cultivars are farmer preferred varieties in the south-western part of Nigeria and are reported to be resistant to certain diseases and relatively high yielding (Badra *et al*., 1984; Anno-Nyako and Ladunni, 1984).

II. MATERIALS AND METHODS

2.1 Sample collection and preparation

This research work was carried out in the Central Biotechnology Laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State. The tomato cultivars, Ibadan local (IbL), JM94/46 and Ife cultivars were collected as seedlings from the National Institute of Horticultural Research and Training (NIHORT), Ibadan, Oyo State and cultivated in a nursery bed for four weeks to produce fresh leaves.

2.2 Extraction of DNA

DNA extraction was carried out using modified Dellaporta (1983) procedure. Fresh leaves (0.15 – 0.2 g) from young tomato plants (3-4 weeks old) were harvested and ground freshly in liquid nitrogen with a plastic pestle in 1.5 ml eppendorf tube. 800 µl of extraction buffer (100mM Tris-HCl, pH 8.0, 50mM EDTA, pH 8.0, 500mM NaCl, 1% PVP) and 20 µl of 0.7% β-mercarptoethanol was quickly added and mixed until the tissues became dispersed in the buffer. Afterwards, 100 µl of 20% Sodium deodecyl sulphate (SDS) was added and mixed thoroughly for 1 min and incubated at 65°C in a water bath (GFL), mixing intermittently 5-6 times for 15 minutes. Samples were removed from 65°C and allowed to cool to room temperature (30 ± 2°C) before 300 µl of ice-cold potassium acetate was added and mixed by gently inverting 5-6 times and incubated on ice for 20 min. Samples were centrifuged (Eppendorf 5417C) at 14,837.76 g for 10 minutes and the supernatant was carefully transferred to new eppendorf tubes. 700 µl of ice-cold isopropanol was added and inverted gently 8-10 times, incubated at -80°C for 1 hr and then centrifuged 14,837.76 g for 10 minutes. The supernatant was tipped off ensuring the removal of the last drops of isopropanol. Pellets were re-suspended in 250 µl of high salt Tris-EDTA (TE) and 4 µl of 10 mg/ml RNase (Sigma) and incubated at 37°C for 30 min with constant gentle shaking. A 500 µl portion of ice-cold isopropanol was added, mixed by inverting 8-10 times, incubated at -80°C for 1 hr and centrifuged at 14,837.76 g for 10 minutes. Supernatant was tipped off removing last drops of isopropanol and then washed twice in 70% ethanol, centrifuging at 14,837.76 g for 10 min each time. Pellet was allowed to dry and 100 µl of sterile distilled water was added. Samples were stored at 4°C overnight to dissolve pellets. The concentration in ng/µl was measured at 260-280 nm with Nanodrop spectrophotometer (ND1000).

2.3 Polymerase Chain Reaction (PCR)

Ten SSR primer pairs (Suliman-Pollatschek *et al*., 2002) were used for the Polymerase Chain Reaction. PCR was carried out with Peltier thermal cycler-PTC200 using PCR conditions as described by Rajput *et al*. (2006). A 25 µl of PCR mix contained 2.5µl 10X reaction buffer (100mM Tris-HCl pH 9, 15mM $MgCl_2$, 500M KCl and 0.1% Gelatin), 3µl dNTPs (200 mM), 2µl of each forward and reverse primers 5 pica moles/ml primer, 1µl of 50 ng/ml genomic DNA and 0.8 U/ml *Taq* polymerase (Sigma) in addition to deionised water to complete the reaction mix. Only one DNA sample and both forward and reverse primer were added to any single reaction. The PCR programme used: one cycle (an initial denaturing step) at 94°C for 3 min; 35 cycles at 94°C for 1 min (denaturing); 55°C for 1 min; 72°C for 1 min 30 sec and one cycle (final extension) at 72°C for 7 min, kept at 4°C. The PCR amplification products were temporarily stored at -20°C. Electrophoresis of the amplified DNA products was carried on 3% agarose gel and 6% polyacrylamide gel for the determination of bands. The size of the alleles was determined by comparison with Hyper ladder V marker (Bioline) loaded on adjacent gel tracks.

Table.1: List of primers and sequence

S/N	SSR Repeat	Forward Primer 5' 3'	Reverse Primer 5' 3'
1	Tom 8-9 ATT7	GCA TTG ATT GAA CTT CAT TCT CGT CC	ATT TTT GTC CAC CAA CTA ACC G
2	Tom 11-28 CTT5/CT5	ATT GTA ATG GTG ATG CTC TTC C	CAG TTA CTA CCA AAA ATA GTC AAA CAC
3	Tom 31A-32A	AAT GTC CTT CGT ATC	CTC GGT TTT AAT TTT TGT

	TA11	CTT TCG T	GTC T
4	Tom 39A-40A AATT4	TAA CAC ATT CAT CAA AGT ACC	TTG CGT GAT CCA GTA AT
5	Tom 41-42TCC6	GAA ATC TGT TGA AGC CCT CTC	GAC TGT GAT AGT AAG AAT GAG
6	Tom 43-44TCC6	GCA GGA GAT AAT AAC AGA ATA AT	GGT AGA AGC CCG AAT ATC ATT
7	Tom 47-48 AT10	CAA GTT GAT TGC ATT ACC TAT TG	TAC AAC AAC ATT TCT TCT TCC TT
8	Tom 49-50 AT10	AGA AAA CTT TTT GAA TGT TGC	ATT ACA ATT TAG AGA GTC AAG G
9	Tom 55-56ATTT5	ATT TCT GTA ACT CCT TGT TTC	TGA CTT CAA CCC GAC CCC TCT T
10	Tom 57-58CT8	TCT AAG TGG ATG ACC ATT AT	GCA GTG ATA GCA AAT GAA AAC

2.4 Gel electrophoreses

2.4.1 Agarose gel electrophoresis of tomato genomic DNA

Using procedures as described by Rajput *et al*., (2006), 3% Agarose gel was prepared by weighing 4.5g of agarose powder and melting in 150 ml 1% TBE buffer (10.8g Tris-base; 5.5g boric acid; 20mM EDTA in 1 L) in a microwave oven (100 ^{o}C) until completely dissolved. The gel was allowed to cool slightly (about 40 ^{o}C) by continuous stirring on the magnetic stirrer (Thermolyne Cimarec 2) and then poured into the gel tank to set with the appropriate combs. 5 µl of gel loading dye was added to 5 µl of PCR product and spun down in the centrifuge to mix thoroughly. The samples were loaded on the gel; with Hyper ladder V marker (Bioline) loaded on adjacent gel tracks to determine the size of the alleles by comparison and allowed to run for 2-3 hr at 100 volts (Voltmeter EC 105). The gels were stained with 10 mg/ml ethidium bromide, visualized on a 302 nm UV transilluminator and photographed with a UVP bioimaging system (GDS-800).

2.4.2 Polyacrylamide gel electrophoresis (PAGE) of tomato genomic DNA

The long and short plates were washed until squeaky clean and wiped with ethanol. Long and short plates were treated with gel slick and 3µl bind silane in 95% ethanol respectively. When the plates dried (10 min) they were arranged on the gel caster. 600 µl of ammonium persulphate (NH_4SO_4) and 60 µl of temed were added to 60 ml of polyacrylamide solution and gradually poured between the plates before solidification. The comb was inserted and the plates clamped together and allowed to dry for about 1 hr. The clamp and comb were removed, the plates mounted on the gel ridge and the anode and cathode filled with 1X TBE (10.8g Tris-base; 5.5g boric acid; 20mM EDTA in 1 L) buffer to the lane levels and pre-ran for about 45 – 60 min at 1000 amps. A mixture of PCR product to bromophenol blue dye was prepared in the ratio 2:1 and denatured in the PCR machine for 5 min and immediately placed on ice. The power was disconnected to insert the comb and to quickly load the samples and allowed to run for 2 hr. The plates were separated and the short plates were placed in 200 ml acetic acid:1800 ml distilled water fixing solution with continuous shaking for 20 min. The plate was rinsed 2-3times with distilled water and transferred to staining solution consisting of 2 g of $AgNO_3$ in 2000 ml distilled water and 3 ml of 37% formaldehyde agitating well for 30 min. It was rinsed briefly in ultrapure water (5-10 sec) and transferred to 1 L of chilled developing solution consisting of 60g of sodium carbonate in 2000 ml distilled water with 3 ml of 37% formaldehyde and 40µl of sodium thiosulphate. The plate was agitated very well in the developing solution and when the first bands were visible; the fresh solution was replaced with the remaining 1 L and agitated till all the bands were visible. The plate was dipped into the fixing solution shaking for 2-3 min to stop the reaction and then rinsed in ultrapure water twice while shaking. The plate was allowed to dry by leaving at room temperature.

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2.5 Molecular Characterisation of the three local tomato cultivars

Characterization/amplification of the three tomato cultivars with simple sequence repeat (SSR) markers and the genetic and phylogenic data analysed using NTSYS (Applied Biostatistics Inc. version 2.0) software by the clustering method of the Unweighted Pair Group Method using Arithmetic Average (UPGMA).

2.6 Genetic similarity estimation and cluster analysis

All distinct DNA fragments were scored as present {1} or absent {0} for each of the markers. The genetic similarity (GS) estimates between two cultivars *i* and *j* was estimated following the methods of Nei and Li (1979), which is defined as:

(1) $Sij = 2Nij / (Ni + Nj)$

Where *Nij* is the number of bands present in the cultivars *i* and *j*, and *Ni* and *Nj* representing the number of bands present in cultivar *i* and *j*, respectively.

For phylogenetic analysis, only data from the polymorphic SSR loci were subjected to NTSYS statistical software. The 3 cultivars were clustered based on the estimated genetic distance, and the phylogenetic analysis was carried out with the clustering method of the Unweighted Pair Group Method Using Arithmetic Average (UPGMA).

III. RESULTS

3.1 Estimation of genetic similarity

Genetic similarity among the cultivars was deduced from the banding patterns on the agarose and polyacrylamide gel electrophoreses in Fig. 1 and Fig. 2 which showed polymorphism among IbL, JM94/46 and Ife cultivars with primers T3, T8 and T10. Monomorphic bands were disregarded. The primers were able to differentiate the three cultivars by the presence or absence of amplified bands. Polymorphism for T10 was between 150 – 200 bp for T10; and 200 – 250 bp for T3 and T8. A total of 35 bands were obtained with 10 SSR primer pairs (TABLE 4) out of which 10 were polymorphic. Genetic similarity estimates between IbL (1) and Ife (3) was highest at 0.90. JM showed the least similarity to the other two cultivars at 0.65. The presence or absence of bands at any loci differentiates one cultivar from the other and were statistically analysed by UPGMA cluster analysis (Nei and Li, 1979) to obtain the dendogram and genetic similarity coefficients shown in Fig. 3 and TABLE 2 respectively.

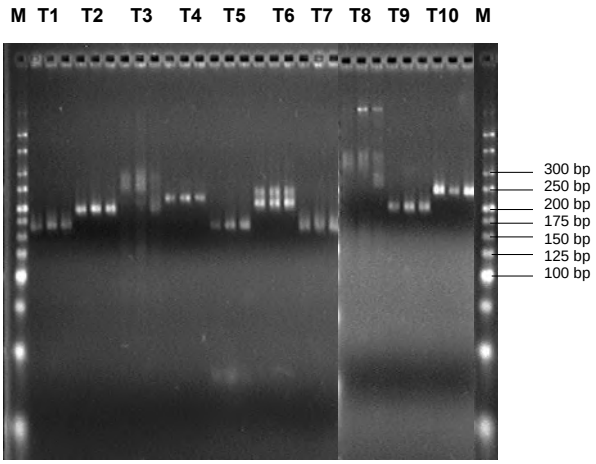

Fig.1: Agarose gel showing the alleles using 10 SSR primer pairs (T1-T10), M-Hyper ladder V to determine the allele sizes. Cultivars are arranged: Ibadan local (IbL), JM94/46 (JM), Ife

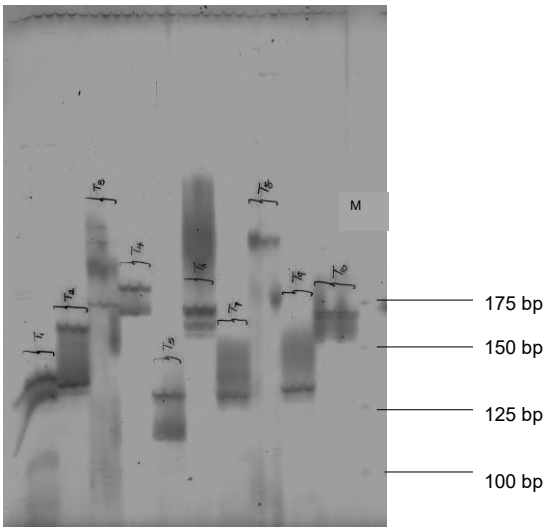

Fig. 2: PAGE gel showing the alleles using 10 SSR primer pairs (T1-T10), M-Hyper ladder V marker to determine the allele sizes. Cultivars are arranged: Ibadan local (IbL), JM94/46 (JM), Ife

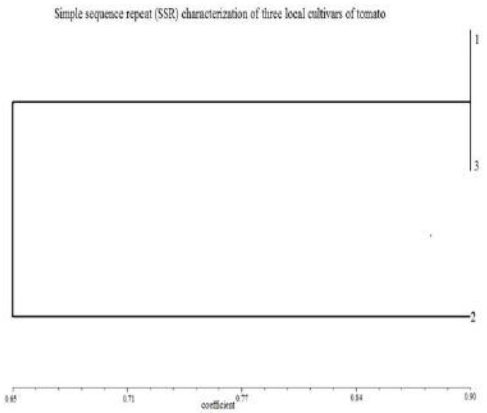

Fig.3: Dendogram of simple sequence repeat (SSR) primers for characterization of three tomato cultivars. 1-IbL; 2-JM; 3-Ife

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Genetic distances obtained using three SSR markers constructed by UPGMA clustering of Nei and Li (1979).

Table.2: Genetic similarity coefficients among three tomato cultivars

	Line1	Line2	Line3
Line 1	0.0000		
Line 2	0.2000	0.0000	
Line 3	0.0667	0.2500	0.0000

Line1-IbL; Line2-JM; Line3-Ife

GD 01-02 = 0.2; 01-03 = 0.0667; 02-03 = 0.25

GD = genetic distance

3.2 Primer evaluation/Characterization of primers

Out of the ten primer pairs (TABLE 1) used in the characterization of the three tomato cultivars, three of them were polymorphic i.e. primers T3, T8 and T10 (TABLES 3 and 4). The polymorphic information content (PIC) of the polymorphic markers was evaluated using the formula:

(2) PIC = 1 – ∑ pi2

Pi = frequency of ith allele (Weir, 1990)

pi2 = (relative frequency)2 = total sum of frequency/each frequency

From the data, Tom 57-58 (T10) had three alleles with bands either present or absent at each locus between the range of 150-175bp. It had the highest polymorphic inforation content (PIC) value of 0.816 (81.6 %). Tom 31A-32A (T3) had five alleles with bands either present or absent between 140-300bp and (PIC) value of 0.778 (77.8 %). The least PIC value of 0.375 (37.5 %) was recorded for Tom49-50 (T8) with two alleles and bands present or absent between 160-350bp. Average PIC value was calculated to be 0.656 (65.6 %).

Table.3: Polymorphic information content (PIC) of polymorphic markers

Primer/ Cultivar	**Ibadan local**	**JM94/46**	**Ife**	**Sum of freq**	**freq(i)**	**{freq(i)}^{2}**	**PIC**	
T3A	1	1	1	3	0.2500	0.063		
T3B	0	1	0	1	0.0830	0.007		
T3C	1	0	1	2	0.1660	0.028		
T3D	1	1	1	3	0.2500	0.063		
T3E	1	1	1	3	0.2500	0.063		
				12		0.222	0.778	
T8A	0	0	1	1	0.2500	0.063		
T8B	1	1	1	3	0.7500	0.563		
				4		0.626	0.375	
T10A	0	1	0	1	0.143	0.021		
T10B	1	1	1	3	0.429	0.184		
T10C	1	1	1	3	0.429	0.184	0.816	
				7				
Average PIC value								0.656
Highest								0.816
Lowest								0.375

Table.4: Characteristics of Polymorphic SSR markers used in the study

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S/N	LD.	SSR Name/ Repeat	Forward Primer 5' 3'	Reverse Primer 5' 3'	No of Alleles	Allele size (bp)	PIC
1	T3	Tom 31A-32A TA11	AAT GTC CTT CGT ATC CTT TCG T	CTC GGT TTT AAT TTT TGT GTC T	5	140-300	0.778
2	T8	Tom 49-50 AT10	AGA AAA CTT TTT GAA TGT TGC	ATT ACA ATT TAG AGA GTC AAG G	2	160-350	0.375
3	T10	Tom 57-58 CT8	TCT AAG TGG ATG ACC ATT AT	GCA GTG ATA GCA AAT GAA AAC	3	150-175	0.816

IV. DISCUSSION

Molecular markers are an effective tool for efficient selection of desired agronomic traits because they are based on the plant genotypes and also are independent of environmental variations (Sunilkumar et al, 2016).

4.1 Molecular Characterisation with simple sequence repeat (SSR) markers

Ten (10) SSR primer pairs were used for molecular characterisation of three Nigerian cultivars of tomato. This was carried out to determine their genetic similarity and variability. The primer pairs were used to amplify specific segments of the tomato genome in order to generate the relevant data. Three (3) of the ten primer pairs amplified polymorphic segments of the three tomato cultivars and the data obtained was used to estimate the genetic similarity (TABLE 2) and to determine the genomic cluster of the cultivars on the phylogenic tree (Fig. 3). The data was also used to determine the polymorphic information content (PIC) of the primers (markers) (TABLE 3).

4.2 Agarose gel electrophoresis

Fig. 1 shows the resolution of the amplified alleles of the tomato DNA on agarose gel electrophoresis. The allele sizes ranged between 140-350 bp.

4.3 Polyacrylamide gel electrophoresis (PAGE)

Although the popularity of PAGE gels is declining, mainly due to the drudgery of the method and to comparable efficiency and simplicity of agarose gel; they usually give a higher resolution than agarose gels because the amplified DNA is denatured before running them on PAGE gel. Fig. 2 is the PAGE gel of the three tomato cultivars. The allele sizes ranged between 100-350 bp. The alleles are more distinct and data easier to record with PAGE gel. Due to the close genetic relationship among modern tomato cultivars and their narrow genetic base (Alvarez et al., 2001; Zhang et al., 2003), PAGE gels could be more efficient in distinguishing between tomato cultivars.

4.4 PIC of primers

The highest PIC was recorded for primer pair T10 with PIC value of 0.816, and lowest was 0.375 for primer pair T8. The PIC value for T3 was also high at 0.778. Average PIC value of the three polymorphic primers was 0.656. With the value of 1.0 being the highest/max, the two primers, T3 and T10 are highly polymorphic. The highest number of alleles was recorded with primer T3. García-Martínez et al. (2006) reported PIC values between 0.035 and 0.775 for tomato germplasm evaluated with amplified fragment length polymorphism (AFLP) while Bredemeijer et al. (2002) obtained PIC values of 0.40 evaluating 500 varieties of tomato with SSR markers. These results may suggest that highly polymorphic markers are ideal to conduct assessments aimed at understanding the genetic diversity of plant crops.

4.5 Genetic similarity/diversity of cultivars

The genetic distance (GD) among the three cultivars as estimated showed the highest GD between JM and Ife (0.25); least GD was between IbL and Ife (0.0667) and between IbL and JM (0.2). These values show that the cultivars are all closely related. Close genetic relationship has been reported in tomato cultivars due to lack of variability that was ascribed to the self-pollinating nature of modern tomato cultivars combined with their narrow genetic base (Alvarez et al., 2001; Zhang et al., 2003). Also, the genetic similarity estimated according to SSR data suggests the potential of SSR markers in discriminating among plants of close or distant genetic backgrounds (El-Awady et al, 2012). This study shows that the genetic similarity between the three tomato

cultivars suggests the need for more analysis using tomato varieties across the geo political zones of Nigeria for the purpose of maintaining the tomato germplasm, understanding its genetic diversity and as a prerequisite for effective breeding programme.

V. CONCLUSION

The SSR marker system is useful for studying genetic diversity among tomato inbred lines collected from diverse geographical locations. The combination of polymorphism and the large number of bands obtained per assay shows that SSR is the most informative marker system of tomato genotyping. The work of Smulders *et al*. (1997), Bredemeijer *et al*. (2002), He *et al*. (2003), Frary et al. (2005), Garcia- Martinez *et al*. (2006) and Song *et al*. (2006) confirmed the utility of SSRs for studying genetic diversity and variability in the genus *Solanum* and for selecting tomato cultivars.

This study showing the genetic similarity between the three tomato cultivars suggests the need for more analysis using tomato varieties across the geo political zones of Nigeria for the purpose of maintaining the tomato germplasm, understanding its genetic diversity and as a prerequisite for effective breeding programme. More efforts should be directed at preserving our indigenous germplasm for research and economic purposes. It is also very essential to carry out the characterization of cultivated and economically useful; as well as neglected and underutilized indigenous genetic resources in the Nigerian eco-system.

REFERENCES

[1] Alvarez A.E, Van De Wiel C.C.M., Smulders M.J.M. And Vosman B. (2001). Use of Microsatellites to Evaluate Genetic Diversity and Species Relationships in the Genus *Lycopersicon*. *Theoretical Applied Genetics*, **103:** 1283−1292.

[2] Anno-Nyako F. And Ladunni A. (1984). Evaluation of Tomato Germplasm Under Field Conditions For Reaction To Tomato Virus Disease. *National Institute for Horticultural Research and Training (NIHORT) Annual Report*, Pp 23-24

[3] Badra T., Denton O. And Anyim O. (1984). Tomato Germplasm Evaluation. *National Institute for Horticultural Research and Training (NIHORT) Annual Report*, Pp 22-23

[4] Beecher G.R. (1998). Nutrient Content of Tomatoes and Tomato Products. *Proceedings of the Society for Experimental Biology and Medicine*, **218** (2): 98-100

[5] Benor, S., Zhang, M., Wang, Z., & Zhang, H. (2008). Assessment of Genetic Variation in Tomato (*Solanum Lycopersicum L.*) Inbred Lines Using SSR Molecular Markers, *35*, 373–379.

[6] Berloo, R. Van, Zhu, A., Ursem, R., Verbakel, H., Gort, G., & Eeuwijk, F. A. Van. (2008). Diversity and Linkage Disequilibrium Analysis Within a Selected Set Of Cultivated Tomatoes, 89–101.

[7] Bredemeijer G.M.M., Cooke P.R.J., Ganal M.W., Peeters R., Isaac P., Noordijk Y., Rendell S., Jackson J., Wendehake M.S.R.K., Dijcks M., Amelaine M., Wick-Aert V., Bertrand L. And Vosman B. (2002). Construction and Testing of a Microsatellite Database Containing More Than 500 Tomato Varieties. *Theoretical and Applied Genetics*, **105**: 1019-1026

[8] Comlekcioglu, N., Simsek, O., & Boncuk, M. (2010). Genetic Characterization of Heat Tolerant Tomato (*Solanum Lycopersicon*) Genotypes by SRAP and RAPD Markers, *9*(4), 2263–2274.

[9] Dellaporta S. J., Wood J. and Hicks J. B. (1983). A Plant DNA Mini Preparation: Version II. *Plant Molecular Biology Report*, **1:** 19–21

[10] El-Awady, M. A. M., El-Tarras, A. A. E., & Hassan, M. M. (2012). Genetic Diversity and DNA Fingerprint Study in Tomato (*Solanum Lycopersicum* L.) Cultivars Grown In Egypt Using Simple Sequence Repeats (SSR) Markers, *11*(96), 16233–16240.

[11] El-Hady, E.A.A.A., Haiba, A.A.A., El-Hamid, N.R.A. and Rizkalla, A.A. 2010. Phylogenetic Diversity and Relationship of Some Tomato Varieties by Electrophoretic Protein and RAPD Analysis. Journal of American Science 6(1): 434-441.

[12] FAOSTAT (2014). Global tomato production in 2012. Rome, FAO.

[13] Foolad, M. R. (2007). Genome Mapping and Molecular Breeding of Tomato, *2007*.

[14] Frary A., Xu Y., Liu J., Mitschell S., Tedeschi E. And Tanksley S. (2005). Development of a Set of PCR-Based Anchor Markers Encompassing the Tomato Genome and Evaluation of their Usefulness for Genetics and Breeding Experiments. *Theoretical And Applied Genetics*, **111**: 291−312

[15] Garcia-Martinez S., Andreani L., Gracia-Gusano M., and Geuna F. (2006). Evaluation of Amplified Fragment Length Polymorphism and Simple Sequence Repeat for Tomato Germplasm Fingerprinting: Utility For Grouping Closely Related Traditional Cultivars. *Genome*, **49**: 648−656

[16] Hamner K.C. And Maynard L.A. (1942). Factors Influencing the Nutritional Value of the Tomato. A Review of Literature, U.S. Dept. of Agric. Misc. Publication No. 52

[17] He, C., Poysa, V., and Yu, K. (2003). Development and Characterization of Simple Sequence Repeat (SSR) Markers and Their Use in Determining Relationships among *Lycopersicon Esculentum* Cultivars. *Theoretical And Applied Genetics*, **106**: 363–373

[18] Korir, N. K., Diao, W., Tao, R., Li, X., Kayesh, E., Li, A., & Zhen, W. (2014). Genetic Diversity and Relationships among Different Tomato Varieties Revealed By EST-SSR Markers, *13*(1), 43–53.

[19] Mazzucato, A., Papa, R., Bitocchi, E., Mosconi, P., Nanni, L., Negri, V., Veronesi, F. (2008). Genetic Diversity, Structure and Marker-Trait Associations in a Collection of Italian Tomato (*Solanum Lycopersicum* L.) Landraces, 657–669.

[20] Milevska, E. M., Popovski, Z. T., Dimitrievska, B. R., & Bandzo, K. (2011). Determination Of Genetic Diversity Among Different Tomato Varieties Using SSR Markers I, *XVI* (March), 9–17.

[21] Naz, S., Zafrullah, K., Shahzadhi, K. and Munir, N., 2013, Assessment of genetic diversity within germplasm accessions in tomato using morphological and molecular markers. Journal of Animal and Plant Sciences 23(4): 1099-1106.

[22] Nei M. And Li W.H. (1979). Mathematical Model for Studying Genetic Variation In Terms Of Restriction Endonucleases. *Proceedings of National Academy Of Science*, USA. **76**: 5269–5273

[23] Pal, D. and Singh, M., 2013, molecular profiling & RAPD analysis of commercial hybrid parental lines in tomato & chili. Int. J. Innovative Res. In Sci. Engineering and Techno, 2(9): 4288-4292.

[24] Powell, W., Morgante, M., Andre, C. Hanafey, M., Vogel, J., Tingey, S., Rafalski, A., 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2: 225–238.

[25] Rajput S.G., Wable K.J., Sharma K.M., Kubde P.D and Mulay S.A. (2006). Reproducibility Testing Of RAPD and SSR Markers in Tomato. *African Journal of Biotechnology*, **5** (2): 108-112

[26] Shah, M.A., Khan, A.I., Awan, F.S., Sadaqat, H.A., Bahadur, S., Rasheed, A. and Baloch, F.S. (2015). Genetic diversity of some tomato cultivars and breeding lines commonly used in Pakistani breeding program. Turkish Journal of Agriculture and Forestry 3(3): 126-132.

[27] Sharifova, S., & Resources, G. (2016). Assessment of Genetic Diversity in Cultivated Tomato *(Solanum Lycopersicum L.*) Genotypes Using Rapd P, (September).

[28] Singh, M., Singh, N.P., Arya, S., Singh, B. and Vaishali 2014. Diversity analysis of tomato germplasm (*Lycopersicom esculentum)* markers using SSR. International Journal of Agricultural Science and Research 4(4): 41-48.

[29] Smulders M.J.M., Bredemeijer G., Rus-Kortekaas W., Arens P. and Vosman B. (1997). Use of Short Microsatellites from Database Sequences to Generate Polymorphisms among *Lycopersicon Esculentum* Cultivars and Accessions of Other *Lycopersicon* Species. *Theoretical and Applied Genetics*, **97**: 264–272

[30] Song J., Chen J., Chen H.Y., Liu Y. and Zhuang T.M. (2006). Research of Genetic Diversity of Tomato Using SSR Markers. *Journal Of Shanghai Jiaotong University* **24**: 524–528

[31] Suliman-Pollatschek S., Kashkush K., Shats H., Hillel J. And Lavi U. (2002). Generation and Mapping of AFLP, SSRs and SNPS in *Lycopersicon Esculentum. Cellular & Molecular Biology Letters*, **7**: 583 – 597

[32] Sunilkumar, M. K., Bommesh, J. C., Vijeth, S., Gokavi, N., Shankargouda, S., & Ravi, Y. (N.D.). Molecular Characterisation in Tomato (*Solanum Lycopersicum* L.) - A Review.

[33] Tabassum, N., Sony, S. K., Bhajan, S. K., & Islam, M. N. (2013). Analysis of Genetic Diversity in Eleven Tomato Cultivars. PTC & B *23*(1), 49–57.

[34] Thamir, A.J., Al-Saadi, A.H. and Abbass, M.C. (2014). Genetic diversity of some tomato *Lycopersicon esculentum* Mill varieties in Iraq using random amplified polymorphism DNA (RAPD) markers. Journal of Babylon University Pure and Applied Sciences 9(22): 2342-2351.

[35] Weir B.S. (1990). Genetic Data Analysis-Methods for Discrete Population Genetics data. (Sunderland: Sinauer Associates, Inc)

[36] Weising, K., Atkinson, R.G. and Gardner, R.C. (1995). Genomic fingerprinting by microsatellite-primed PCR: A critical evaluation. PCR Methods and Applications 4: 249-255.

[37] Zhang H.Y., Yu D.N., Wang R.P., Li M.F. and Li Q.Z. (2003). An Analysis of Genetic Diversity of the Germplasm Resources of *L. Esculentum* and the Application with RAPD. *Journal of Plant Genetic Research*, **4**: 151–156

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Growth Analysis of Baby Corn (*Zea mays* L.) Under the Effect of Integrated Nutrient Management

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***Abstract*—** *Maize (Zea mays L.) is the most versatile crop having wider adaptability in varied ecologies. Presently baby corn is gaining popularity among Indian farming communities mainly due to its short duration, high market rate, nutritive value and also its multiuse. Baby corn requires higher population and plant nutrition than normal grain corn. Therefore the nutrient management is of immense importance for higher corn production. The present study was thus carried out during Kharif season 2015 at the Instructional Dairy Farm (IDF), Nagla, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand to analyse the growth of baby corn under the effect of integrated nutrient management. The experimental design was Randomized Block Design with 11 treatments consisting of sole application of NPK fertilizer, sole application of Azotobacter and Azospirillum, and application of Azotobacter and Azospirillum along with NPK fertilizer. The study revealed that Leaf area index was significantly higher at 50 DAS and harvest under 100% NPK+Azot+Azos. Application of 75% NPK+Azot+Azos had significantly higher* $\overline{CGR}$ *at 25-50 DAS while 100% NPK+Azot+Azos gave significantly higher* $\overline{CGR}$ *at 50 DAS – harvest. The* $\overline{RGR}$ *values remained non significant at both the stages, however the highest* $\overline{RGR}$ *was recorded at application of 100% NPK+Azot+Azos. The* $\overline{NAR}$ *remained non significant at 25 – 50 DAS but at 50 DAS – harvest, the* $\overline{NAR}$ *values recorded significantly higher at 100% NPK+Azot+Azos that remained non significant with all the treatments except control and seed treatment with Azotobacter. The* $\overline{LAR}$ *too was recorded non significant by different integrated nutrient management practices at 25-50 DAS but at 50 DAS-harvest, the significantly highest* $\overline{LAR}$ *was recorded under control, whereas the lowest* $\overline{LAR}$ *was found at application of 100% NPK that remained statistically at par with all other treatments except control. Higher dose of nitrogen coupled with biofertilizers improved the plant growth.*

***Keywords*— azotobacter, azospirillum, leaf area index, crop growth rate, relative growth rate, net assimilation rate, leaf area ratio.**

I. INTRODUCTION

Maize is the third most important staple food crop in the world after wheat and rice but in term of productivity, it ranks first followed by rice, wheat and other millets. Presently baby corn is gaining popularity among Indian farming communities mainly due to its short duration, high market rate, nutritive value and also its multiuse. Baby corn is dehusked immature maize ear, harvested within 2-3 days of silking but prior to fertilization **(Pandey *et al.*, 1998)**. Baby corn is used as *vegetable, salad, soup, pickle, kheer, murabba, chutney, manchurian, halwa* etc. Baby corn is highly nutritive as 100 g of baby corn contains 89.1% moisture, 0.2 g fat, 1.9 g protein, 8.2 mg carbohydrate, 0.06 g ash, 28.0 mg calcium, 86.0 mg phosphorus, 11.0 mg ascorbic acid **(Das *et al.*, 2009).** Baby corn has a great potential to fetch foreign currency through international trade. There is a great demand of baby corn in international market mainly because of its freshness, taste, nutrition, free from pesticides and its multiuse.

In general, morphology, physiology and agronomy of baby corn differed from grain corn as the varieties grown for baby corn must have high vigour and prolificacy. It responds to higher doses of fertilizer that may normally cause lodging in other cereal crops. Thus nutrient management is a very important aspect for proper growth of baby corn. The leaf area, LAI and dry accumulation per plant was noticed significantly higher at application of 240 kg N ha^{-1} over 0, 60, 120 and 180 kg ha^{-1} N **(Kaledhonkar, 2003). Verma *et al.* (2006)** also found that

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150 per cent recommended NPK gave the maximum plant height, leaf area index of maize. **Kumar *et al.* (2007)** noticed that leaf area index and total dry matter production of sweet corn were influenced favourably with increasing levels of N, P_2O_5 and K_2O up to 150:60:40 kg ha^{-1}, respectively. **Suryavanshi *et al*. (2008)** reported that 150 kg N gave higher leaf area index (LAI) and dry matter production of maize compared to either 50 or 100 kg N ha^{-1}. Chemical fertilizers may solve the problem but organics are required to minimize the harmful effects of chemicals. Higher leaf area index was observed in different crops when inoculation was done with *Pseudomonas*, *Azospirillum* and *Azotobacter* strains **(Siddiqui and Shaukat, 2002). Prasad *et al*. (2003)** reported that application of 5 t ha^{-1} vermicompost along with 14 and 10 t ha^{-1} poultry manure and FYM gave higher leaf area index. **Jayaprakash *et al.* (2004)** studied the effect of organics on maize and found that application of vermicompost @ 2 t ha^{-1} and FYM @ 10 t ha^{-1} resulted in significantly higher LAI compared to no organics. Best way out is integration of chemicals and organics. The combination of FYM and mineral fertilizer significantly increased the leaf number and leaf area index of maize **(Haq, 2006). Gable *et al.* (2008)** reported significantly higher all growth parameters of maize were at application of 100 % recommended dose of fertilizer (225:50:50 kg NPK ha^{-1}) followed by 75 % RDF + 25 % N through *Leucaena* lopping + biofertilizer. **Panwar (2008)** observed that integrated nutrient management had significant effect on growth parameters of maize crop. **Megawer and Mahfouz (2010)** reported that inoculation of canola seeds by either *Azotobacter*, *Azospirillum* or the mixed inoculum and adding half recommended dose of nitrogen showed high leaf area and save half of the mineral nitrogen recommended dose. Thus the present study was carried out to find out the best integrated nutrient management practice for baby corn under which the crop would show best results in terms of growth.

II. MATERIAL AND METHODS

The experiment was conducted at the Instructional Dairy Farm (IDF), Nagla, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India. The Instructional Dairy Farm is located in the *Tarai* belt of Shivalik range of Himalayas with humid sub-tropical type of climate at latitude of 29°N and longitude of 79.3°E and situated at an altitude of 243.84 m above the mean sea level. The climate of the *Tarai* region is broadly humid sub-tropical with harsh winter and hot dry summers. The soil of the experimental field was slightly silty clay loam (Nagla series, Mollisol) in texture, from dark greyish brown to dark grey in humus with weak, fine to medium granular structure.

Eleven treatments were tested in a Randomized Block Design 3 replications the treatments were Control (no application), 50% NPK, 100% NPK(180:60:40), Seed treatment with Azotobacter @200g/10Kg seeds, Seed treatment with Azospirillum @200g/10Kg seeds, Seed treatment with Azospirillum + Azotobacter, 50% NPK + Seed treatment with Azotobacter, 50% NPK + Seed treatment with Azospirillum, 50% NPK+ Seed treatment with Azospirillum + Azotobacter, 50% NPK+ Seed treatment with Azospirillum + Azotobacter and 100%NPK+seed treatment with Azospirillum + Azotobacter. The variety sown was V.L. Baby corn-1 – released from Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora,Uttarakhand.

III. RESULTS AND DISCUSSION

Leaf Area Index (LAI)

The LAI remained non significant under different integrated nutrient management practices at 25 DAS. At 50 DAS and harvest, significantly higher LAI was recorded with application of 100% NPK+*Azot*+*Azos* that remained at par with 100% NPK, 50% NPK+*Azot*+*Azos* and 75% NPK+*Azot*+*Azos* at 50 DAS and with 100% NPK and 75% NPK+*Azot*+*Azos* at harvest stage. Among the biofertilizer treatments, the LAI at 50 DAS remained non significant, however combined seed treatment produced the higher LAI followed by seed treatment with *Azotobacter*. At harvest, seed treatment with *Azot*+*Azos* produced significantly highest LAI than seed treated with either of biofertilizers. Nitrogen is the main constituent of chlorophyll that keeps leaf greener for longer period and also improved photosynthesis that finally resulted into better leaf growth and development. The data pertaining to LAI is given in table 1.

Mean Crop Growth Rate ($\overline{CGR}$)

At 25-50 DAS, the highest **($\overline{CGR}$)** was recorded with application of 75% NPK+*Azot*+*Azos* that remained significantly at par with application of 100% NPK, 50% NPK+*Azot*+*Azos* and 100% NPK+*Azot*+*Azos*, whereas at 50 DAS – harvest, the higher **($\overline{CGR}$)** values were recorded with application of 100% NPK + *Azot* + *Azos* that was significantly similar to 100% NPK, 50% NPK + *Azot*, 50% NPK+*Azot*+*Azos* and 75% NPK+*Azot*+*Azos*. Among the biofertilizer treatments, the **($\overline{CGR}$)** at 25-50 DAS and 50 DAS-harvest remained non significant with each other, however combined seed treatment recorded higher **($\overline{CGR}$)** at both the stages followed by seed treatment with

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Azotobacter. The lowest ($\overline{CGR}$) was recorded under control at both the stages. The higher ($\overline{CGR}$) value might be caused due to better plant growth at combined application of nitrogen and bioferilizers.

Relative Growth Rate ($\overline{RGR}$)

The data pertaining to ($\overline{RGR}$) indicated that it declined with advancement of crop age. The highest and the lowest ($\overline{RGR}$) values were recorded with application of 100% NPK+*Azot+Azos* and under control at both 25- 50 DAS and at 50 DAS – harvest crop stages, respectively. Among the biofertilizer treatments, the highest ($\overline{RGR}$) values were recorded at seeds treated with *Azotobacter+Azospirillum* followed by seed treatment with *Azotobacter*. Similarly, at 25-50 DAS, 50% NPK+*Azot+Azos* recorded the highest ($\overline{RGR}$) value followed by 50% NPK+*Azot*. At 50 DAS – harvest stage the highest ($\overline{RGR}$) value was recorded at 50% NPK+*Azot* followed by 50% NPK+*Azot+Azos*. The data pertaining to CGR and RGR is given in table 2.

Net Assimilation Rate ($\overline{NAR}$)

The net assimilation rate was recorded non significant by different integrated nutrient management practices at 25-50 DAS. At 50 DAS-harvest, significantly higher ($\overline{NAR}$) value was recorded with application of 100% NPK+*Azot+Azos* that remained statistically at par with all the treatments except control and seed treatment with *Azospirillum*. The ($\overline{NAR}$) was recorded non significant among the treatments where either alone or combined biofertilizers were used for seed treatment, but combined seed treatment with biofertilizer and seed treatment with *Azotobacter* produced the highest ($\overline{NAR}$) followed by seed treatment with *Azospirillum*. The ($\overline{NAR}$) was recorded significantly lower with alone application of 50% NPK than all treatments having 50% NPK+ seed treatments with biofertilizers.

Leaf Area Ratio ($\overline{LAR}$)

The leaf area ratio was recorded non significant by different integrated nutrient management practices at 25-50 DAS. At 50 DAS-harvest, the significantly highest ($\overline{LAR}$) was recorded under control, whereas the lowest ($\overline{LAR}$) was found with application of 100% NPK that remained statistically at par with all other treatments except control. Among the biofertilizers treatments, ($\overline{LAR}$) remained non significant, however seed treatment with *Azospirillum* recorded the highest ($\overline{LAR}$) followed by seed treatment with *Azot+Azos*. The LAR remained non significant among the treatments having combined application of 50% NPK + biofertilizers, but the highest ($\overline{LAR}$) was noticed with application of 50% NPK+*Azos* followed by 50% NPK+*Azot*. The data pertaining to NAR and LAR is given in table 3.

Conclusion

Combined application of nitrogen and bioferilizers improved photosynthesis that finally resulted into better leaf growth and crop growth. The present study concluded the benefits of integrated nutrient management including use of biofertilizers in combination of chemical fertilizers and its positive effect on growth of baby corn.

Table.1: Effect of integrated nutrient management on leaf area index at different growth stages of baby corn

Treatment	Leaf Area Index		
	25 DAS	50 DAS	Harvest
Control	0.14	2.50	3.07
Azotobacter	0.17	2.80	3.37
Azospirillum	0.16	2.70	3.23
Azot +Azos	0.17	3.00	3.77
50% NPK	0.18	3.20	3.80
100% NPK	0.21	4.00	4.47
50% NPK + *Azotobacter*	0.19	3.60	4.20
50% NPK +*Azospirillum*	0.18	3.50	3.93
50% NPK + *Azot+Azos*	0.2	3.70	4.27
75% NPK + *Azot+Azos*	0.22	4.20	4.60
100% NPK + *Azot+Azos*	0.21	4.30	4.67
SEm±	0.08	0.23	0.09
LSD (p=0.05)	ns	0.68	0.28

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Table.2: Effect of integrated nutrient management on crop growth rate and relative growth rate at different growth stages of baby corn

Treatment	($\overline{CGR}$) (g/m²/day)		($\overline{RGR}$) (mg/g/day)	
	25-50 DAS	50 DAS-harvest	25-50 DAS	50 DAS-harvest
Control	2.81	2.44	60.10	23.90
Azotobacter	4.02	4.30	62.16	29.35
Azospirillum	3.82	3.93	61.67	28.10
Azot +Azos	4.12	4.52	62.63	29.88
50% NPK	4.43	5.00	62.92	30.70
100% NPK	5.41	6.96	64.35	34.63
50% NPK + *Azotobacter*	4.87	6.15	63.95	33.76
50% NPK +*Azospirillum*	4.52	5.48	63.19	32.59
50% NPK + *Azot+ Azos*	5.07	6.37	64.13	33.67
75% NPK + *Azot+ Azos*	5.51	7.30	64.56	35.35
100% NPK + *Azot + Azos*	5.47	7.67	64.75	37.14
SEm±	0.20	0.55	1.62	3.33
LSD (p=0.05)	0.61	1.63	Ns	Ns

Table.3: Effect of integrated nutrient management on net assimilation rate and leaf area rate at different growth stages of baby corn

Treatment	($\overline{NAR}$) (mg/cm²/day)		($\overline{LAR}$) (cm²/g)	
	25-50 DAS	50 DAS-harvest	25-50 DAS	50 DAS-harvest
Control	0.35	0.06	175.6	372.8
Azotobacter	0.42	0.11	146.4	276.3
Azospirillum	0.42	0.10	146.2	285.2
Azot +Azos	0.42	0.11	150.1	278.7
50% NPK	0.42	0.11	152.0	279.8
100% NPK	0.43	0.14	152.6	251.2
50% NPK + *Azotobacter*	0.42	0.13	153.6	266.0
50% NPK +*Azospirillum*	0.40	0.12	156.2	281.4
50% NPK + *Azot+ Azos*	0.42	0.13	153.4	260.2
75% NPK + *Azot+ Azos*	0.40	0.14	161.0	258.6
100% NPK + *Azot + Azos*	0.41	0.15	158.6	255.6
SEm±	0.22	0.02	7.1	12.0
LSD (p=0.05)	Ns	0.04	Ns	35.7

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REFERENCES

[1] Das, S., Ghosh, G., Kaleem, M.D. and Bahadur, V. (2009). Effect of different levels of nitrogen and crop geometry on the growth, yield and quality of babycorn (*Zea mays* L.) cv. 'golden baby'. *Acta Horti*. 809: 161-166

[2] Gable, D.B., Kubde, K.J., Katore, J.R., Fiske, A.V. and Deshmukh, M.R. (2008). Effect of integrated nutrient management on growth and yield of maize-chickpea cropping system. *J. of Soils and Crops*. 18(2): 392-397.

[3] Haq, S.A. (2006). Integrated Nutrient Management in maize (*Zea mays* L.) under irrigated agro-ecosystem of Kashmir Valley. *M.Sc. Research Thesis*. p.120.

http://dx.doi.org/10.22161/ijeab/3.4.36 *ISSN: 2456-1878*

[4] Jayaprakash, D.C., Sawant, P.S. and Singh, R.S. (2004). Effect of vermicompost on growth and yield of maize as well as nutrient uptake. *Indian J. Agron*. 23(1): 121-123.

[5] Kaledhonkar, P.R. (2003). Evaluation of promising quality protein maize (*Zea mays)* cultivars in Konkan region. Thesis, M. Sc. Agriculture Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri. 72p.

[6] Kumar, A.M.A., Gali, S.K. and Hebsur, N.S. (2007). Effect of different levels of NPK on growth and yield parameters of sweet corn. *Karnataka J. Agric. Sci*. 20(1): 41- 43.

[7] Megawer, E. A. and S. A. Mahfouz. (2010). Response of canola (*Brassica napus* L.) to bio-fertilizers under Egyptian conditions in newly reclaimed soil. *International J. Agric. Sci*. 2(1): 12-17.

[8] Pandey, A.K., Prasad, Kamta, Mani, V.P., Chauhan, V.S. and Singh, Prem. (1998). Improved maize production technology for mid-hills of N-W Himalayas. Technical Bulletin 11: 1-46.

[9] Panwar, A.S. (2008). Effect of Integrated Nutrient Management in maize (*Zea mays*)- mustard (*Brassica compestris var toria*) cropping system in mid hills altitude. *Indian J. Agric. Sci*. 78(1): 27-31.

[10] Prasad, B.K., Singh, D.N. and Singh, S. N. (2003). Effect of long-term use on fertilizer, lime and manures on growth and yield of sweet corn. *J. Indian Soc. Soil Sci*. 34: 271-274.

[11] Siddiqui, I.A. and S.S. Shaukat. (2002). Mixtures of plant disease suppressive bacteria enhance biological uptake control of multiple tomato pathogens. Biology and Fertility of Soil. 36(4): 284-297.

[12] Suryavanshi, V.P., Chavan, B.N., Jadhav, V.T. and Baig, M.I.A. (2008). Response of maize to nitrogen and phosphorous application in *Vertisols*. *International J. Tropical Agric*. 26: 293-296.

[13] Verma, A., Nepalia, V. and Kanthaliya, P.C. (2006). Effect of integrated nutrient supply on growth, yield and nutrient uptake by maize (*Zea mays* L.)-wheat (*Triticum aestivum*) cropping system. *Indian J. Agron*. 51(1): 3-6.

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Effect of treated wastewater irrigation on physiological and agronomic properties of beans *Vicia faba*

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Abstract*—The current study investigated the effect of two doses (50%, and 100 %) of treated wastewater (TWW)on biometric and physiologic parameters of Vicia faba beansafter 40 days of exposure.*

Our data showed a decrease in shoots and roots length and weight in plants amended with TWW. Moreover, a significant decrease in Chlorophyll 'a', 'b' and carotene content was observedin plants irrigated with 100% of TWW.

These findings provided new insights on TWW reuse which can cause different types of stress as it may affect the development of cultivated crops.

Keywords—Treated wastewater, Vicia faba, growth, chlorophyll, carotene.

I. INTRODUCTION

Tunisia is a country where the agricultural sector is a priority representing its most important natural resource. However, the annual rainfall is very irregular both in space and time and almost this country belongs to arid and semi-arid climate [1]. In arid and semi-arid regions, variations in rainfall accompanied by successive periods of drought generate undesirable impacts on water availability [2].

In Tunisia, the reuse of treated wastewater (TWW) is part of freshwater resources mobilization strategy and sustainable development of water resources [3]. Nevertheless, once properly treated, wastewater could replace freshwater and decrease this pressure on natural resources to be conserved for other purposes. However, from a quantitative point of view, wastewater is a source of water always available. Indeed, TWW can ensure the balance of the natural water cycle and preserve resources by reducing harmful discharges into the natural environment [4]. On the other hand, and as has been reported by numerous study the TWW reuse could cause harmful effects on soils and even on living beings such as plant, invertebrates and microorganisms [5,6,7,8,9].

In the end of the past century the use of microbial, animal and human cell culture for toxicity evaluation [10,11,12] have been replaced using animal and vegetable bioindicators. Numerous international studies supported by United Nations Environment Program (UNEP), World Health Organization (WHO) and US Environmental Protection Agency (US-EPA) have validated plant-based bioassays for toxicity monitoring [13,14,15].

Vicia faba is commonly used as a model for cytological, physiological and toxicological studies [16,17,18,19] for many reasons such as its availability around the year, easy to cultivate and does not require sterile conditions. This plant could be used for toxicity assessment of various contaminants in soils i.e. heavy metals, aromatic compounds, pesticides etc.

Vicia faba is one of the oldest domesticated food legumes. Its importance in terms of food and agriculture is reflected by the occupied area worldwide (3.6 million hectares) in more than 50 countries and gives a total production of 4 million tons per year.

Many studies have investigated the effect of TWW on agronomic and physiological aspect of numerous vegetables and improve that those effluent constitute a

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reliable source of nutrients i.e. (nitrogen, phosphorus, potassium) and organic matter that enhance soil fertility and productivity [20,21,22,23,24]. However, the chemical composition of TWW could influence vegetable growth, uneven fruit maturity and quality and quantity of yields due to the potential presence of heavy metals, surfactants and pharmaceuticals [25]. The metallic and salt stress have gained an increasing attention, and this was reported by several studies [26,27,28,29,30] which founded that growth factors and physiological properties of many plants are affected when exposed to wide varieties of contaminants that can potentially exist in TWW.

In this context, the current study was, therefore, carried out to evaluate the effect of municipal TWW irrigation on agronomic (growth dynamic properties) and physiological properties (chlorophyll and carotenoid contents) of *Vicia faba*plants.

II. MATERIALS AND METHODS

2.1. Soil sampling:

The soils used for this research were collected from an organic farming plot in the region of Chott-Mariem. The soils were sampled from the depth of 0-15 cm. Before use, samples were air-dried and crushed to pass a (<2 mm) screen.

2.2. Water sampling:

Secondary TWW have been collected in glass bottles from wastewater treatment plant of Northern Sousse, Tunisia, managed by the National Office of Sanitation (ONAS).

2.3. Experimental design:

Dry Certified seeds of beans (*V. faba Aguadulce*) obtained fromlocal productionwere germinated on moistened filter paper at 22°C, when the primary roots were about 2–3-cm long, the seedlings were transplanted in the containers containing 1 kg of soils. Before transplantation soils were moistened with deionized water (Control), diluted TWW (50%) and TWW (100%) brought to 70 % of its holding capacity and this was maintained during the experimentation, five replicates per condition were used.

2.4. Growth measurements:

Five replicates were taken for each treatment were used to calculate the mean of each measurement. Plants were collected after 40 days of exposure to three conditions including control one. The measurements taken were the following:

- Length of the root and shoot system.
- Fresh weights of the root and shoot system
- Number of nodes.

2.5. Chemical content: Photosynthetic pigments

For this purpose, 1g of fresh leaves, was extracted by grinding in a mortar using 20 ml 80% acetone, with small amount of pure (Silica Quartz), and 0.5 g calcium carbonate to equalize the cellular sap acidity. The extract was filtered and collected in Eppendorf's tubes.

The optical density (DO) of the extract was measured at wave lengths 663, 645, and 440.5 nm [31] to estimate chlorophyll 'a' and 'b', and carotenes respectively, using a Spectrophotometer (VWR-UV-3100-PC) and a vitreous cell (thickness of photo route 1 cm). Three replicates were used for each treatment, and the amount of pigment present in each sample was calculated according to the following equations:

- mg Chlorophyll "a" / g-tissue

$= 12.7.DO_{663} - 2.69.DO_{663} * v/(w.1000)$

- mg Chlorophyll "b" / g-tissue

$= 29.9.DO_{663} – 4.68.DO_{663} * v/(w.1000)$

- mg Carotenoids / g-tissue

$= 46.95.DO_{440.5}. (DO_{440.5} – 0.268 *$ Chlorophyll "a" + "b")

Whereas, W, the fresh weight by grams for extracted tissue; V, the final size of the extract in 80% acetone; DO, optical density at specific wave length.

2.6. Statistical analysis:

The non-parametric Mann–Whitney U-test was used to compare the data from plants exposed to 50% and 100% of TWW with data from the control soil (irrigated with deionized water).

III. RESULTS

The difference of shoots and roots weight of *V. faba* beans after 40 days of exposure to 50% and 100% of TWW is given in **Figure 1 a**and **b**. Results showed a significant variation between treatments. Indeed, shoots weight (**Fig.1a**) decreased significantly comparing to control plants irrigated with fresh water with a value of 17,93 ± 0,75 g.

However the root weight (**Fig.1b**) increased significantly in plants exposed to soils irrigated with diluted TWW (8,16 ± 0,23 g). In contrast, a slight decrease was recorded in root's weight of plants exposed to 100% which reaches 7,6 ± 0,2 g.

On the other hand, **Figure 2 a** and **b** illustrate the length variation of faba bean's plant shoot and root after 40 days of exposure to 50% and 100% of TWW.

Accurately to weight variation, the length of shoots (**Fig.2a**) decrease progressively with TWW dose to reach 51,33 ± 4,6 mm in plants exposed to 100 % of TWW.

Thus, roots length (**Fig.2b**) decreased significantly in plants exposed to 50 % of TWW and reach 15,66 ± 1,15 mm which represents approximately 50% of control mean (28 ± 3,46 mm).

Finally, the number of nodes in *V.faba* plants after exposure to TWW are shown in **Figure 3**, whatever, the number of nodes didn't show any significant changes between treatments, it's almost the same in all the experiments.

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Figure 4 a and **b**reported the effect of 40 days of irrigation with two doses (50% and 100%) of TWW on chlorophyll "a" and "b". It was noticed that chlorophyll "a" content decrease significantly in the plants exposed to 50% of TWW to reach 0,6 ± 0,21 mg/g-fresh tissue compared to control plant where the content was 1,37 ± 0,04 mg/g-fresh tissue.

However, the concentration of chlorophyll "b" **(Fig.4b)**decreased by the increasing of TWW dose reaching its lowest 0.61 ± 0,09 and 0,77 ± 0,03 mg/g-fresh tissue when exposed respectively to 50% and 100% of TWW.

By following carotenoid content after exposure of bean plants to TWW it appears from results illustrated in **Fig.5** that TWW irrigation inhibits the carotenes formation and this was clear in plants exposed to 100% of TWW where means reach its minimum at 0.253 ± 0.001 mg/g-fresh tissue.

IV. DISCUSSION

In our study, TWW application on *Vicia faba* beans for 40 days was assessed through the measurement of biometric parameters which were modified after exposure to 50% and 100% of TWW.

Under exposure to numerous pollutants that can reach soils through TWW reuse in irrigation [32,33,34,35], plants can be subject to different types of stress mainly metallic and salt stress.

Globally, a decrease of the length and weight of the shoots and roots was recorded by increasing TWW dose. This decrease could be related to the high amount of salts present in TWW as reported by [36,37,38,39,40,41] who assessed the effect of salts on different plants and they found that it could cause several changes through negative effects on photosynthesis process, changes in enzymatic activity, decrease on the carbohydrates level and growth hormones.

Otherwise, metal content in TWW can exert an inhibitory effect on growth parameters, then they are strongly poisonous to the metabolic activities. However, an exceeded dose of heavy metals such as (Cd, Zn, Pb, Cr…) could cause phytotoxicity and this was proved by many authors [42,43,44,45].

Interestingly, chlorophyll is a clue element for plant's life which contributes to ATP production from the sun's light energy, it is indeed a good biomarker to asses plant's state under stress or exposure to toxics.

Our results regarding a decrease in chlorophyll 'a', 'b' are in concordance with several studies [46,41] which reported that salinity lead to the decrease of chlorophyll rates in barely and beans plant. The second factor that may influence photosynthetic process in *V.faba* beans is the trace elements uptake and this was by inhibiting chlorophyll biosynthesis and reducing the activity of enzymes involved in CO_2 fixation [47,48,45].

Indeed, in stressed plants the carbon metabolism seems to be inhibited, then, in general the amount of amino acids was lower than in normal plants as proved by Gadallah, 1999 and this was due to the inhibition of amino acids incorporation into proteins under salt/metal stress.

As a part of national strategy to face water scarcity and to save freshwater resources, TWW constitute a sustainable way to manage water resources in the Mediterranean arid and semi-arid regions. But, like every strategy with all its positive effects (i.e. natural fertilizer, source of nutrients, availability), it presents many undesirable effects mainly (heavy metals, high amount of NA^+ and Cl^- cations, pathogens…).

However, this can obviously affect normal development of plants and functional properties of soils receiving this non-conventional water. Moreover, many studies have been assessed the effect of TWW reuse on soils, plants and soil organisms [50,51,52,53,54,55] and in most cases they found that those effluent modify the physicochemical properties of soils, and physiological properties of plants and other organisms.

V. CONCLUSION

Our study showed that TWW reuse affect strongly growth and physiological parameters of *Vicia faba* beans, and this by decreasing shoots and roots length and weight than chlorophyll 'a' and 'b' content. Results also highlighted the effect of TWW on carotene content which decreased after 40 days of exposure.

Overall, TWW reuse as an alternative to save freshwater resources, it could be a good way ensuring the transfer of nutrients, organic matter and minerals in soils.

But, if these effluents are not subject to a periodic control, they can be a source of pathogens and potentially hazardous chemical substances (salts, heavy metals and surfactants), accumulated in soils, then as a result unfavorable effects on crop quality and productivity.

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REFERENCES

[1] CGRE, (1991).84-90, Bulletins mensuels et annuels, Centre de Gestion des Ressources en eau de l'lNAT, INAT, Tunisie.

[2] Khouri, N. Kalbermatten, J.M. and Bartone, C.R., (1994). The reuse of wastewater in agriculture: A guide for planners. In Khouri, N. Kalbermatten, J.M. and Bartone, C.R. Water and sanitation.

[3] Al Atiri R., Rezgui, F., Bel Hassen, M.A., (2002). Réutilisation des eaux usées. Cas de la Tunisie. In : Forum de la gestion de la demande en eau. Rabat, Maroc, mars 2002.

[4] Bouchet, C., (2008). Recyclage et réutilisation des eaux usées : où en sommes-nous ? L'eau, l'industrie, les nuisances, n 308, p. 33-42.

[5] Yadav RK, Goyal B, Sharma RK, Dubey SK et Minhas PS., (2002). Post-irrigation impact of domestic sewage effluent on composition of soils, crops and groundwater—A case study. Environmental International 28 : 481–486.

[6] Rattan R.K., Datta S.P., Chhonkar P.K., Suribabu K., Singh A.K., (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. Agriculture, Ecosystems and Environment 109, 310–322.

[7] Solis C., Andrade E., Mireles A., Reyes-Solis I.E., Garcia-Calderon N., Lagunas-Solar M.C.,Pina C.U.et Flocchini R.G., (2005). Distribution of heavy metals in plants cultivated withwastewater irrigated soils during different periods of time. Nuclear Instruments and Methods in Physics Research B 241, 351–355.

[8] Abbas, H., Nasr, R., & Seif, H. (2006). Study of waste stabilization pond geometry for the wastewater treatment efficiency. Ecological Engineering, 28(1), 25-34.

[9] Herpin U., Gloaguen T. V., da Fonseca A. F., Montes C. R., Mendonça F. C., Piveli R. P., Breulmann G., Forti M. C. Et Melfi A. J., (2007). Chemical effects on the soil-plant system in a secondary treated wastewater irrigated coffee plantation- A pilot field study in Brazil. Agricultural Water Management 89, 105 – 115.

[10] Battersby, N. S. (2000). The biodegradability and microbial toxicity testing of lubricants–some recommendations. Chemosphere, 41(7), 1011-1027.

[11] Parng, C., (2005). In vivo zebra fish assays for toxicity testing. Curr. Opin. Drug Disc. Dev. 8, 100–106.

[12] Wilsnack, R., Meyer, F., Smith, J., (1973). Human cell culture toxicity testing of medical devices and correlation to animal tests. Artif. Cell. Blood Substit. Biotechnol.1,543–562.

[13] Grant, W. F. (1999). Higher plant assays for the detection of chromosomal aberrations and gene mutations—a brief historical background on their use for screening and monitoring environmental chemicals. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 426(2), 107-112.

[14] Rizzo, L. (2011). Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. Water research, 45(15), 4311-4340.

[15] Iqbal, M. (2016). Vicia faba bioassay for environmental toxicity monitoring: a review. Chemosphere, 144, 785-802.

[16] Feng, S., Wang, X., Wei, G., Peng, P., Yang, Y., & Cao, Z. (2007). Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity. Chemosphere, 67(6), 1133-1137.

[17] Yi, M., Yi, H., Li, H., & Wu, L. (2010). Aluminum induces chromosome aberrations, micronuclei, and cell cycle dysfunction in root cells of Vicia faba. Environmental Toxicology: An International Journal, 25(2), 124-129.

[18] Giorgetti, L., Talouizte, H., Merzouki, M., Caltavuturo, L., Geri, C., & Frassinetti, S. (2011). Genotoxicity evaluation of effluents from textile industries of the region Fez-Boulmane, Morocco: a case study. Ecotoxicology and environmental safety, 74(8), 2275-2283.

[19] Shahid, M., Pinelli, E., Pourrut, B., Silvestre, J., & Dumat, C. (2011). Lead-induced genotoxicity to Vicia faba L. roots in relation with metal cell uptake and initial speciation. Ecotoxicology and environmental safety, 74(1), 78-84.

[20] Meli, S., M. Porto, A. Belligno, S. A. Bufo, A. Mazzatura, and A. Scopa. (2002). Influence of irrigation with lagooned urban wastewater on chemical and microbiological soil parameters in a citrus orchard under Mediterranean condition. Science of the Total Environment 285: 69–77.

[21] Rusan, M. J. M., Hinnawi, S., & Rousan, L. (2007). Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination, 215(1-3), 143-152.

[22] Kiziloglu, F. M., Turan, M., Sahin, U., Kuslu, Y., & Dursun, A. (2008). Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (Brassica olerecea L. var. botrytis) and red cabbage (Brassica olerecea L. var. rubra) grown on calcareous soil in Turkey. Agricultural water management, 95(6), 716-724.

[23] Bedbabis, S., Ferrara, G., Rouina, B. B., & Boukhris, M. (2010). Effects of irrigation with treated wastewater on olive tree growth, yield and leaf mineral elements at short term. Scientia Horticulturae, 126(3), 345-350.

[24] Libutti, A., Gatta, G., Gagliardi, A., Vergine, P., Pollice, A., Beneduce, L., ... & Tarantino, E. (2018). Agro-industrial wastewater reuse for irrigation of a

vegetable crop succession under Mediterranean conditions. Agricultural Water Management, 196, 1-14.

[25] Pedrero, F., Kalavrouziotis, I., Alarcón, J. J., Koukoulakis, P., & Asano, T. (2010). Use of treated municipal wastewater in irrigated agriculture—Review of some practices in Spain and Greece. Agricultural Water Management, 97(9), 1233-1241.

[26] Rusan, M. J. M., Hinnawi, S., & Rousan, L. (2007). Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination, 215(1-3), 143-152.

[27] Kaymakanova, M. (2009). Effect of salinity on germination and seed physiology in bean (Phaseolus vulgaris L.). Biotechnology & Biotechnological Equipment, 23(sup1), 326-329.

[28] Khalid, S., Shahid, M., Dumat, C., Niazi, N. K., Bibi, I., Gul Bakhat, H. F. S., ... & Javeed, H. M. R. (2017). Influence of groundwater and wastewater irrigation on lead accumulation in soil and vegetables: Implications for health risk assessment and phytoremediation. International journal of phytoremediation, 19(11), 1037-1046.

[29] Tabassum, R. A., Shahid, M., Dumat, C., Niazi, N. K., Khalid, S., Shah, N. S., ... & Khalid, S. (2018). Health risk assessment of drinking arsenic-containing groundwater in Hasilpur, Pakistan: Effect of sampling area, depth, and source. Environmental Science and Pollution Research, 1-12.

[30] Shahid, M., Shamshad, S., Farooq, A. B. U., Rafiq, M., Khalid, S., Dumat, C., ... & Niazi, N. K. (2018). Comparative effect of organic amendments on physio-biochemical traits of young and old bean leaves grown under cadmium stress: a multivariate analysis. Environmental Science and Pollution Research, 1-12.

[31] Smith, J. H., & Benitez, A. (1955). Chlorophylls: analysis in plant materials. In Modern Methods of Plant Analysis/Moderne Methoden der Pflanzenanalyse (pp. 142-196). Springer, Berlin, Heidelberg.

[32] Salgot, M., Vergés, C., & Angelakis, A. N. (2003). Risk assessment in wastewater recycling and reuse. Water Science and Technology: Water Supply, 3(4), 301-309.

[33] Conkle, J.L., White, J.R., Metcalfe, C.D., 2008. Reduction of pharmaceutically active compounds by a lagoon wetland wastewater treatment system in Southeast Louisiana. Chemosphere 73, 1741–1748.

[34] Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ. Pollut. 152, 686–692.

[35] Ibekwe, A.M., Gonzalez-Rubio, A., Suarez, D.L., (2018). Impact of treated wastewater for irrigation on soil microbial communities. Sci. Total Environ. 622–623, 1603–1610.

[36] Hamada, A. M. (1995). Alleviation of the adverse effects of NaCl on germination, seedling, growth and metabolic activities of maize plants by calcium salts. Bull. Fac. Sci. Assiut Univ, 24, 211-220.

[37] Misra, A. N., Sahu, S. M., Misra, M., Singh, P., Meera, I., Das, N., ... & Sahu, P. (1997). Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biologia plantarum, 39(2), 257-262.

[38] Dantas, B. F., Ribeiro, L. D. S., & Aragão, C. A. (2005). Physiological response of cowpea seeds to salinity stress. Revista Brasileira de Sementes, 27(1), 144-148.

[39] Mazher, A. A., El-Quesni, E. F., & Farahat, M. M. (2007). Responses of ornamental and woody trees to salinity. World J Agric Sci, 3, 386-395.

[40] Memon, S. A., Hou, X., & Wang, L. J. (2010). MORPHLOGICAL ANALYSIS OF SALT STRESS RESPONSE OF PAK CHOI. Electronic Journal of Environmental, Agricultural & Food Chemistry, 9(1).

[41] Qados, A. M. A. (2011). Effect of salt stress on plant growth and metabolism of bean plant Vicia faba (L.). Journal of the Saudi Society of Agricultural Sciences, 10(1), 7-15.

[42] Di Toppi, L. S., & Gabbrielli, R. (1999). Response to cadmium in higher plants. Environmental and Experimental Botany, 41(2), 105-130.

[43] Wojcik, M., & Tukiendorf, A. (2004). Phytochelatin synthesis and cadmium localization in wild type of Arabidopsis thaliana. Plant Growth Regulation, 44(1), 71-80.

[44] Mohanpuria, P., Rana, N. K., & Yadav, S. K. (2007). Cadmium induced oxidative stress influence on glutathione metabolic genes of Camellia sinensis (L.) O. Kuntze. Environmental Toxicology: An International Journal, 22(4), 368-374.

[45] Guo, J., Dai, X., Xu, W., & Ma, M. (2008). Overexpressing GSH1 and AsPCS1 simultaneously increases the tolerance and accumulation of cadmium and arsenic in Arabidopsis thaliana. Chemosphere, 72(7), 1020-1026.

[46] Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. Environmental chemistry letters, 8(3), 199-216.

http://dx.doi.org/10.22161/ijeab/3.4.37 *ISSN: 2456-1878*

[47] Tort, N., & Turkyilmaz, B. (2004). A physiological investigation on the mechanisms of salinity tolerance in some barley culture forms. JFS, 27, 1-16.

[48] De Filippis, L. F., & Ziegler, H. (1993). Effect of sublethal concentrations of zinc, cadmium and mercury on the photosynthetic carbon reduction cycle of Euglena. Journal of Plant Physiology, 142(2), 167-172.

[49] Gadallah, M. A. A. (1999). Effects of proline and glycinebetaine on Vicia faba responses to salt stress. Biologia plantarum, 42(2), 249-257.

[50] Klay, S., Charef, A., Ayed, L., Houman, B., Rezgui, F., (2010). Effect of irrigation with treated wastewater on geochemical properties (saltiness, C, N and heavy metals) of isohumic soils (Zaouit Sousse perimeter, Oriental Tunisia). Desalination 253, 180–187.

[51] Hidri, Y., Fourti, O., Eturki, S., Jedidi, N., Charef, A., Hassen, A., (2014). Effects of 15-year application of municipal wastewater on microbial biomass, fecal pollution indicators, and heavy metals in a Tunisian calcareous soil. J. Soils Sediments 14, 155–163.

[52] Carlos, F.S., Schaffer, N., Andreazza, R., Morris, L.A., Tedesco, M.J., Boechat, C.L., Camargo, F.A. de O., (2018). Treated Industrial Wastewater Effects on Chemical Constitution Maize Biomass, Physicochemical Soil Properties, and Economic Balance. Commun. Soil Sci. Plant Anal. 49, 319–333.

[53] Elfanssi, S., Ouazzani, N., Mandi, L., (2018). Soil properties and agro-physiological responses of alfalfa (Medicago sativa L.) irrigated by treated domestic wastewater. Agric. Water Manag. 1–10.

[54] Lüneberg, K., Schneider, D., Siebe, C., Daniel, R., (2018). Drylands soil bacterial community is affected by land use change and different irrigation practices in the Mezquital Valley, Mexico. Sci. Rep. 8, 1–15.

[55] Pan, M., Chu, L.M., (2018). Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China. Sci. Total Environ. 624, 145–152.

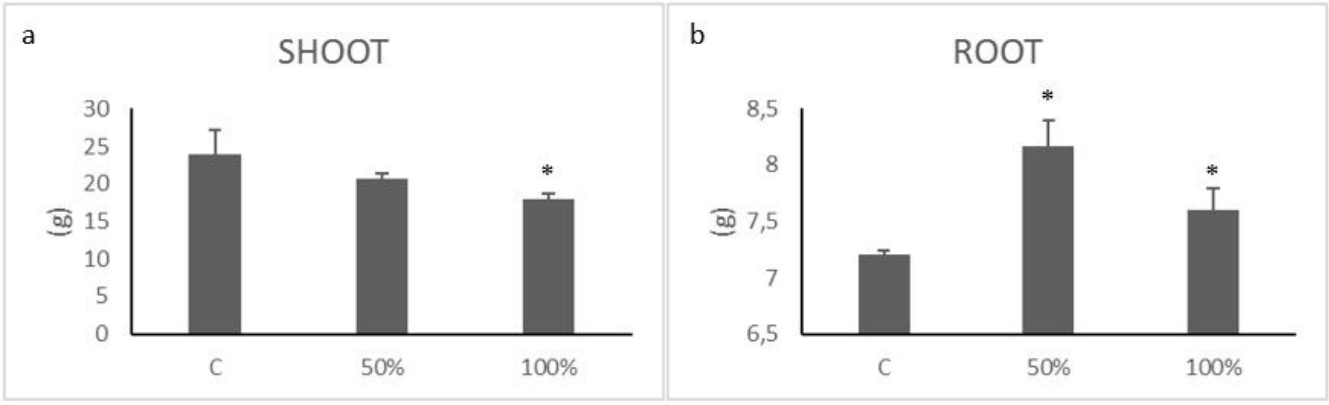

Fig.1: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on Vicia faba(a) shoots and (b) roots weight after 40 days of exposure. Results represent the Mean ± SD of at least 5 replicates.(*) Statistically significant differences (p < 0.05) comparing to control.

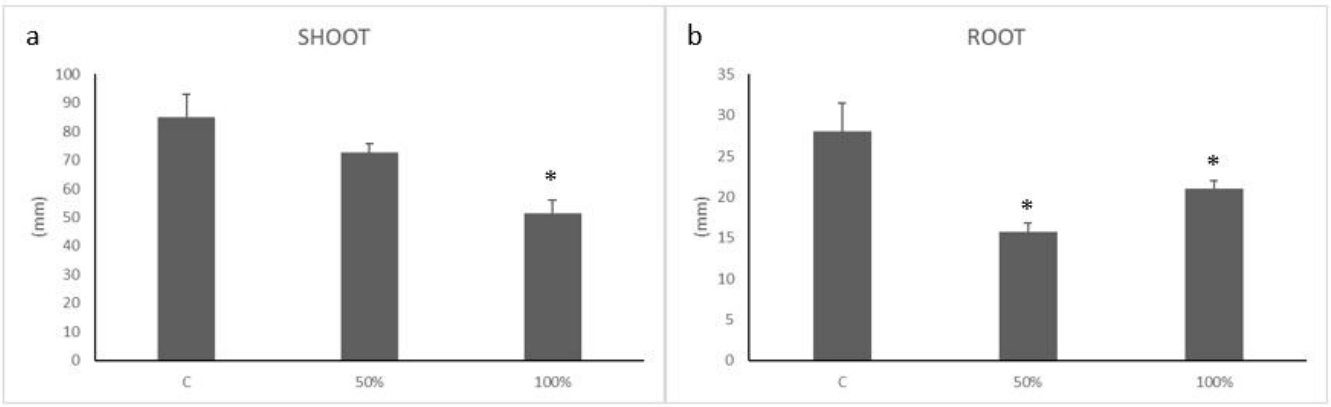

Fig.2: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on Vicia faba (a) shoots and (b) roots legnth after 40 days of exposure. Results represent the Mean ± SD of at least 5 replicates.(*) Statistically significant differences (p < 0.05) comparing to control.

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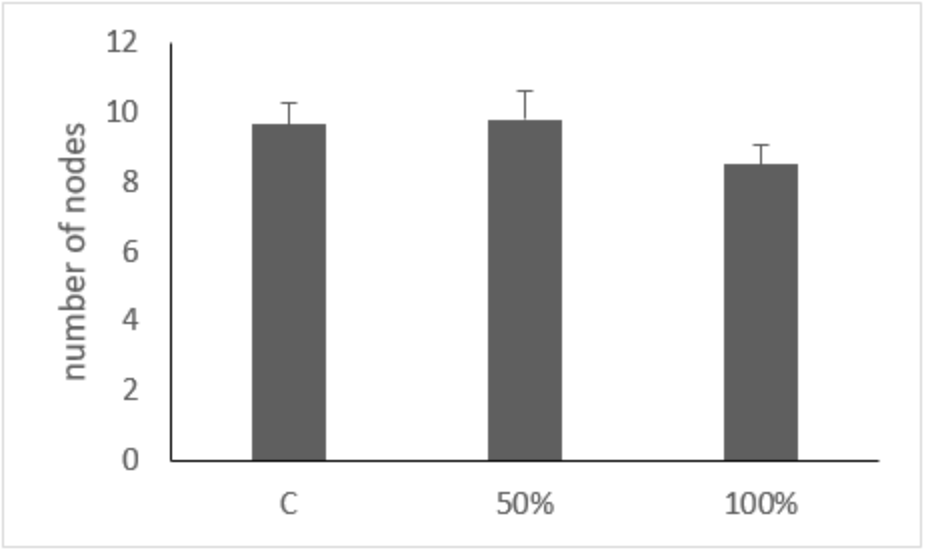

Fig.3: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on the number of nodes of Vicia faba after 40 days of exposure. Results represent the Mean ± SD of at least 5 replicates.(*) Statistically significant differences ($p < 0.05$) comparing to control.

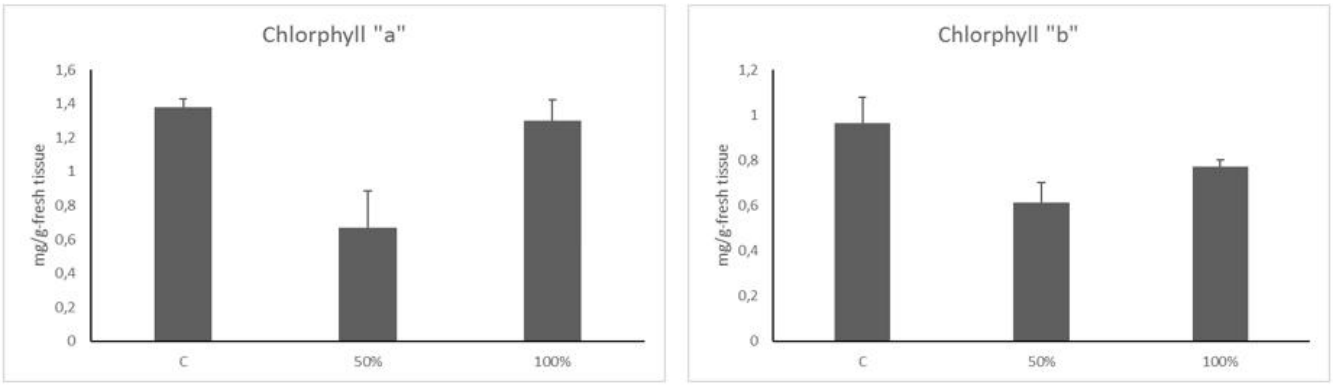

Fig.4: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on (a) chlorophyll 'a' and (b) chlorophyll 'b' content of Vicia faba after 40 days of exposure. Results represent the Mean ± SD of at least 5 replicates.(*) Statistically significant differences ($p < 0.05$) comparing to control.

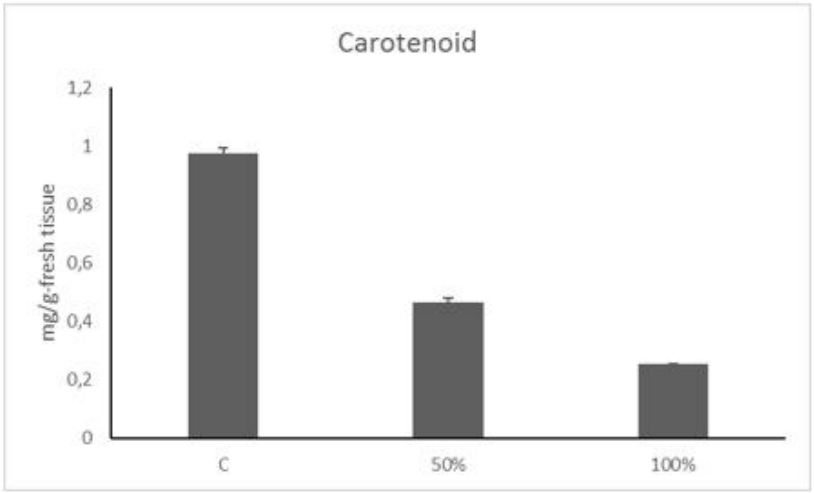

Fig.5: Effect of TWW irrigation (C: control, Diluted TWW: 50% and TWW: 100%) on carotene content of Vicia faba after 40 days of exposure. Results represent the Mean ± SD of at least 5 replicates.(*) Statistically significant differences ($p < 0.05$) comparing to control.

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Germination, vegetative and flowering behavior of Balsam (*Impatiens balsamina* L.) in response to natural photoperiods

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Abstract— *A lack of application of photoperiod and light intensity to manipulate the growth of current spring annuals has, in part, been due to the lack of information identifying the photoperiodic and light intensities requirements of various species. Present pot experiment was carried out at Horticulture Garden, Department of Horticulture, Sindh Agriculture University Tandojam, during spring 2017, which was laid out in a three replicated Complete Randomized Design (CRD). Two varieties of balsam (V1= Tom Thumb, V2 = Double Camcellia) were studied under NP_1= Control (Normal day length), NP_2=3 hrs (8:00 am- 11:00 am), NP_3= 6 hrs (8:00 am – 2:00 pm), NP_4= 9 hrs (8:00 am-5:00 pm), NP_5= Natural shade. Results from the research data revealed significant ($P<0.05$) photoperiodic effect on the growth and flower quality of balsam. The study revealed that maximum seed germination (44.81 %), germination index (0.62 gi) plant height (13.96 cm), leaves plant^{-1} (37.60), days to 1st flower (52.62), flowers plant^{-1} (3.40), days to flower persistence (8.74), weight of single flower (0.59 g), chlorophyll content (34.1 SPAD) was recorded from variety Tom Thumb, whereas the variety Double camellia had minimum seed germination (38.51 %), germination index (0.55 gi) plant height (11.42 cm), leaves plant^{-1} (26.10), days to 1st flower (51.72), flowers plant^{-1}(2.80), days to flower persistence (6.98), weight of single flower (0.52 g), chlorophyll content (31.29 SPAD) The results for natural photoperiods on vegetative and flowering behavior of Balsam are significant effect on different growth parameters. The results indicated that maximum seed germination (74.99 %), germination index (1.18 gi) plant height (17.28 cm), leaves plant^{-1} (58.33), days to 1st flower (64.75), flowers plant^{-1} (5.41), days to flower persistence (13.16), weight of single flower (1.26 g), chlorophyll content (46.79 SPAD) was recorded from NP_4= 9 hrs (8:00 am-5:00 pm) as compared to seed germination (63.88%), germination index (0.66 gi) plant height (14.92 cm), leaves plant^{-1} (48.16), days to 1st flower (45.96), flowers plant^{-1} (5.00), days to flower persistence (11.16), weight of single flower (0.62 g), chlorophyll content (36.46 SPAD) was recorded from NP_1= Control (Normal day length).*

Keywords— flowering behaviour, natural photoperiods, Complete Randomized Design.

I. INTRODUCTION

Balsam, (*Impatiens balsamina* L.) is an ornamental plant in the Balsaminaceae family (Gardeners, 2017). It is a quick growing summer annual flower, with gardenia-like blooms (Tooke and Battey, 2000). Continuous blooms grow on top of a bushy plant with leaves. The balsam is originated in Asia, North America and South Africa and there are numerous annual and perennial varieties (Christopher, 2013). The blooms appear in about 60-70 days and colours include shades of white, pink, rose, violet, and red (Park *et al.*, 2003; Wang *et al.*, 2009). Different parts of the plant are used as traditional remedies for disease and skin afflictions. Juice from the leaves is used to treat warts and snakebite, and the flower is applied to burns (Wang *et al.*, 2009). This species has been used as indigenous traditional medicine in Asia for rheumatism, fractures, and other ailments (Park *et al.*, 2003). Changes in temperature and day-length trigger physiological and seasonal developmental processes of ornamental plants that enable ornamental plants withstand severe climatic conditions (Adams *et al.* 2005). Climate change is expected to increase the air temperature in the summer, while the natural decreasing photoperiod remains unaffected (Kim *et*

al., 2009). As shown previously, an increase in air temperature inhibits CO_2 assimilation, with a concomitant increased capacity for zeaxanthin-independent dissipation of energy exceeding the photochemical capacity of plants (Busch *et al*.,2008). Flowering behaviour in plant cycle shows the adaptability of plants to seasonal changes (Kim *et al*., 2009) and increase in duration of photoperiod reduced time to first visible bud. Temperature and day length are related in the sense that as the natural day length becomes longer or shorter, the temperature warms or cools, respectively (Ha, 2014). A lack of application of photoperiod and light intensity to manipulate the growth of current spring annuals has, in part, been due to the lack of information identifying the photoperiodic and light intensities requirements of various species. The proposed study is mainly aimed at examining the effect photoperiod on germination of vegetative and flowering traits Balsam (*Impatiens balsamina* L) under light intensity.

II. MATERIALS AND METHODS

Present study was conducted during summer, 2017 at Horticulture Garden, Department of Horticulture, Sindh Agriculture University, Tandojam. Seed of two balsam verities (V_1= Tom Thumb, V_2 = Double Camellia) were sown in 20-cm diameter earthen pots in late February 2017 using a silt FYM-based medium in a 1:1 ratio based on volume. Potted seed of both balsam varieties were germinated with five natural photoperiod treatments resulted from the installation of black shade cloth. For control treatment pots were simply kept under normal day length in sun light, while pots of both varieties were kept under shade for normal day length, while for other photoperiod pots were covered with black cloth and uncovered as per photoperiod hours. Such a design has been used to exclude any direct illumination and to obviate any microclimate alterations due to the presence of the shade cloth. A two factor (**Factor -A**: Varieties (V) = 02 (V_1 = Tom Thumb, V_2 = Double Camellia); **Factor-B**: Natural Photoperiods (NP) = 04 (NP_1= Control (Normal day length= 12 hrs= 6 am to 6 pm), NP_2=3 hrs (8:00 am-11:00 am), NP_3= 6 hrs (8:00 am – 2:00 pm), NP_4= 9 hrs (8:00 am-5:00 pm), NP_5= Natural shade) Completely Randomized Design (CRD); was set out with three replications where each replication was had four pots. Frame structure was made by placing wooden pegs of about 4 ft in height from ground length on both side of the plant row than a black thick cloth was covered leaving 3 inches from the ground surface for aeration, where plants were observed for seed Germination (%), germination index (GI), plant height (cm), leaves plant^{-1}, days to 1st flower, flowers plant^{-1}, days to flower persistence, weight of single flower (g), chlorophyll content (SPAD).

Procedure of recording observations

Seed Germination (%): Germination percentage was calculated as per following formula. Total number of seeds was sown and at seventh day germinated seedlings were calculated from each treatment than were divided with total number of seeds sown which were multiplied by 100 as per follows:

GP = Seeds germinated / Total No. of seed x 100

Germination index (GI): Germination /emergence index (GI/E) was calculated by following formula used by Association of Official Seed Analsis (AOSA, 1990)

$$\text{GI or EI} = \frac{\text{No of germinated or emerged seeds}}{\text{Days of first count}} + \ldots\ldots + \frac{\text{No of germinated or emerged seeds}}{\text{Days of final count}}$$

Plant height (cm): Four plants of each variety's were selected at random from pots and their hight was meausred from ground surface to the top with foot scale and the average tallness was worked out in cm at the time of the flowering.

Leaves plant^{-1}: Average number of leaves per plant was calculated on visually from six randomly plant of each variety under each treatment

Days to 1st flower: Days to flower emergence from each variety of the randomly selected plants were noted as they 1st appeared after germination and average was worked out.

Flowers plant^{-1}: Flowers from each variety of the randomly selected plants were counted visually at maturity as they appeared and then average was worked out.

Days to flower persistence: This observation was recorded from the day of flower emergence till the flower withered or dropped on plant than days were counted and average was worked out.

Weight of single flower (g): Flowers of each variety were collected and tagged at random and weighed as an individual on weighing balance machine to record the

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weight in g.

Chlorophyll content (SPAD): Chlorophyll content each plant from each treatment was noted on chlorophyll meter. Leaves from top, mid and bottom where tagged and then placed while attached on the plant digital meter (SPAD METER) then reading were noted and there after average was done.

Average temperature and RH recorded during entire experiment:

February= 18-22 °C, March = 25-27°C, April= 25-32 °C

RH= 75-85%

Statistical analysis:

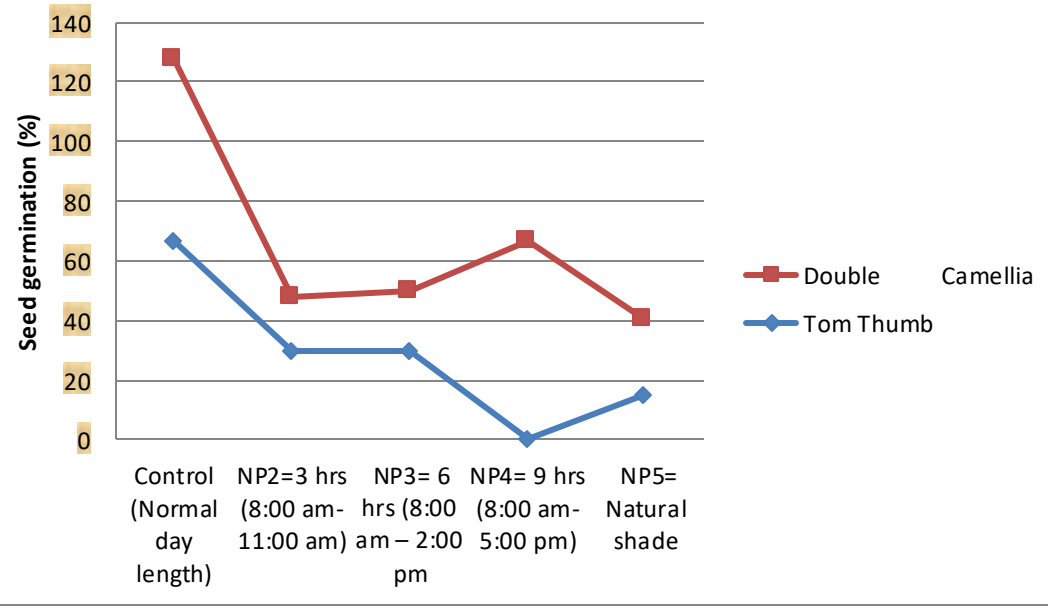
The data was statistically analyzed using Statistix-8.1 computer software (Statistix, 2006). The Deccan's Multiple Range Test was applied to compare treatments superiority in case results are significant at P≤ 0.05 probability level.

III. RESULTS AND DISCUSSION

Seed Germination (%)

Results pertaining to germination (%) as affected by different natural photoperiods on vegetative and flowering behavior of Balsam are presented in the Fig-1. Both the varieties responded well when exposed to natural photoperiod from 8:00 am to 5:00 pm. Tom Thumb had maximum seed germination (83.33%) followed by Double camellia (66.66). However when both varieties were exposed to low photoperiod from 8:00 am to 11:00 am produced less seed germination (29.62 and 18.51 % respectively). Results also revealed that the maximum seed germination (74.99) was observed from NP_4= 9 hrs (8:00 am-5:00 pm) followed by control (12 hrs= 6 am to 6 pm) where plant were grown in full day sunlight (63.88). Further data showed that NP_2=3 hrs (8:00 am- 11:00 am), NP_3= 6 hrs (8:00 am – 2:00 pm and NP_5= Natural shade had maximum seed germination of balsam (24.06, 24.99 and 20.36 %, respectively).

Fig. 1: Seed germination (%) of balsam in response to natural photoperiods



Germination index

The results for varieties seed germination index (Fig. 2) revealed that the variety Tom Thumb produced maximum (0.62 GI) germination index as compared to Double Camcellia (0.55 GI). Both the varieties responded well when exposed to natural photoperiod from 8:00 am to 5:00 pm. Tom Thumb had maximum germination index (1.30 GI) followed by Double camellia (1.07 GI). However when both varieties were exposed to low photoperiod from 8:00 am to 11:00 am produced less germination index (0.28 GI and 0.22 GI respectively). Results also revealed that the maximum germination index (1.18) was observed from NP_4= 9 hrs (8:00 am-5:00 pm) followed by control (12 hrs= 6 am to 6 pm) where plant were grown in full day sunlight (0.66 GI). Further data showed that NP_2=3 hrs (8:00 am-11:00 am), NP_3= 6 hrs (8:00 am – 2:00 pm and NP_5= Natural shade had minimum germination index of balsam (0.25, 0.46 and 0.39 GI).

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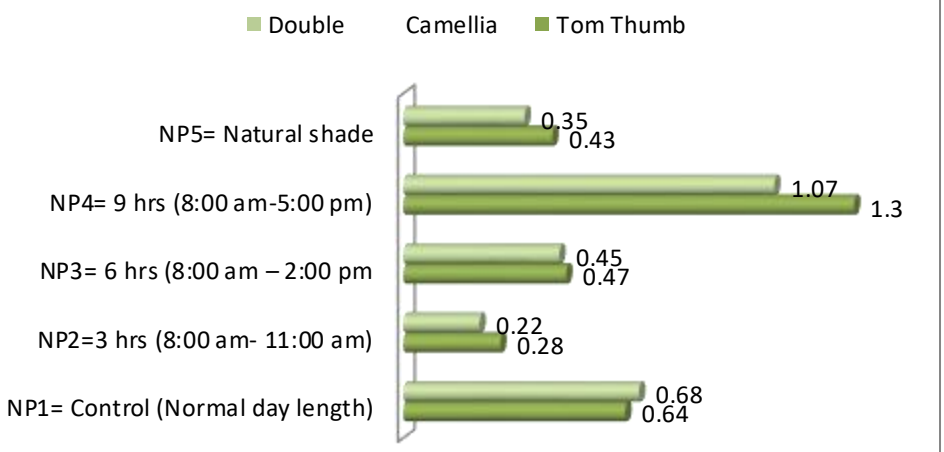
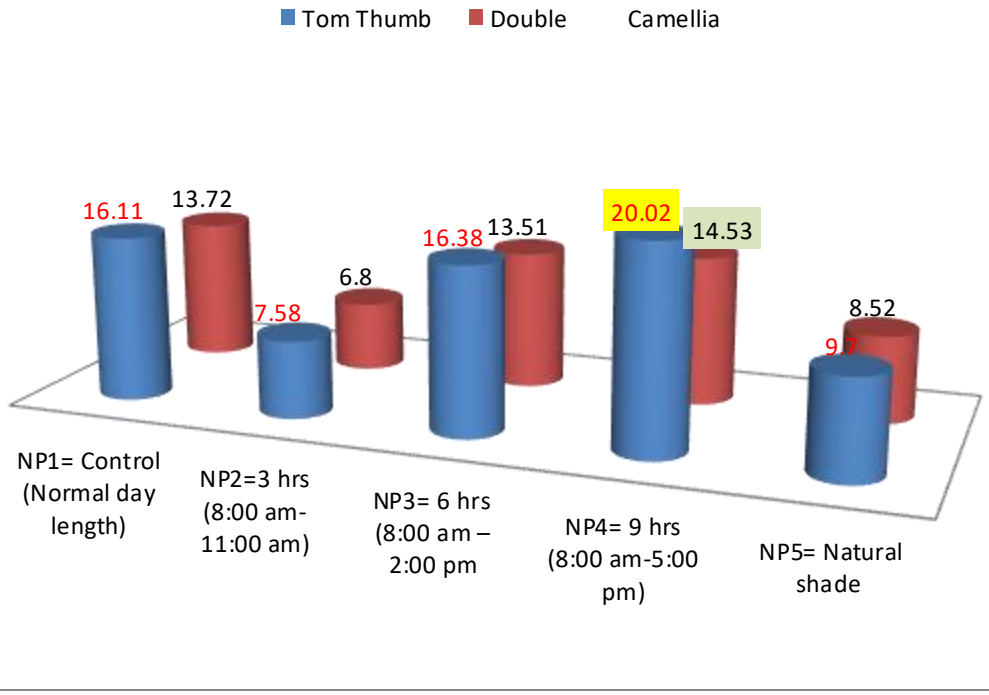
Fig. 2: Germination index (GI) of balsam in response to natural photoperiods



Plant height (cm)

The data regarding for plant height as affected by different natural photoperiods on vegetative and flowering behavior of Balsam are presented in the Fig-3 It can be seen from the results that plant height varied significantly ($P<0.05$) for the treatments and varieties. The results for varieties revealed that the variety "Tom Thumb" produced maximum plant height as compared to "Double Camcellia". Both the varieties responded well when exposed to natural photoperiod from 8:00 am to 5:00 pm. "Tom Thumb" had maximum plant height (20.02 cm) followed by "Double camellia" (14.53 cm) followed by normal day length of 12 hours. However when both varieties were exposed to low photoperiod from 8:00 am to 11:00 am produced less plant height (7.58 and 6.80 cm, respectively) as well as under natural shades their response was also negative.

Fig. 3: Plant height (cm) of balsam in response to natural photoperiods



Leaves plant^{-1}

The data regarding leaves plant^{-1} as affected by different natural photoperiods on vegetative and flowering behavior of Balsam are presented in the Fig-4 It

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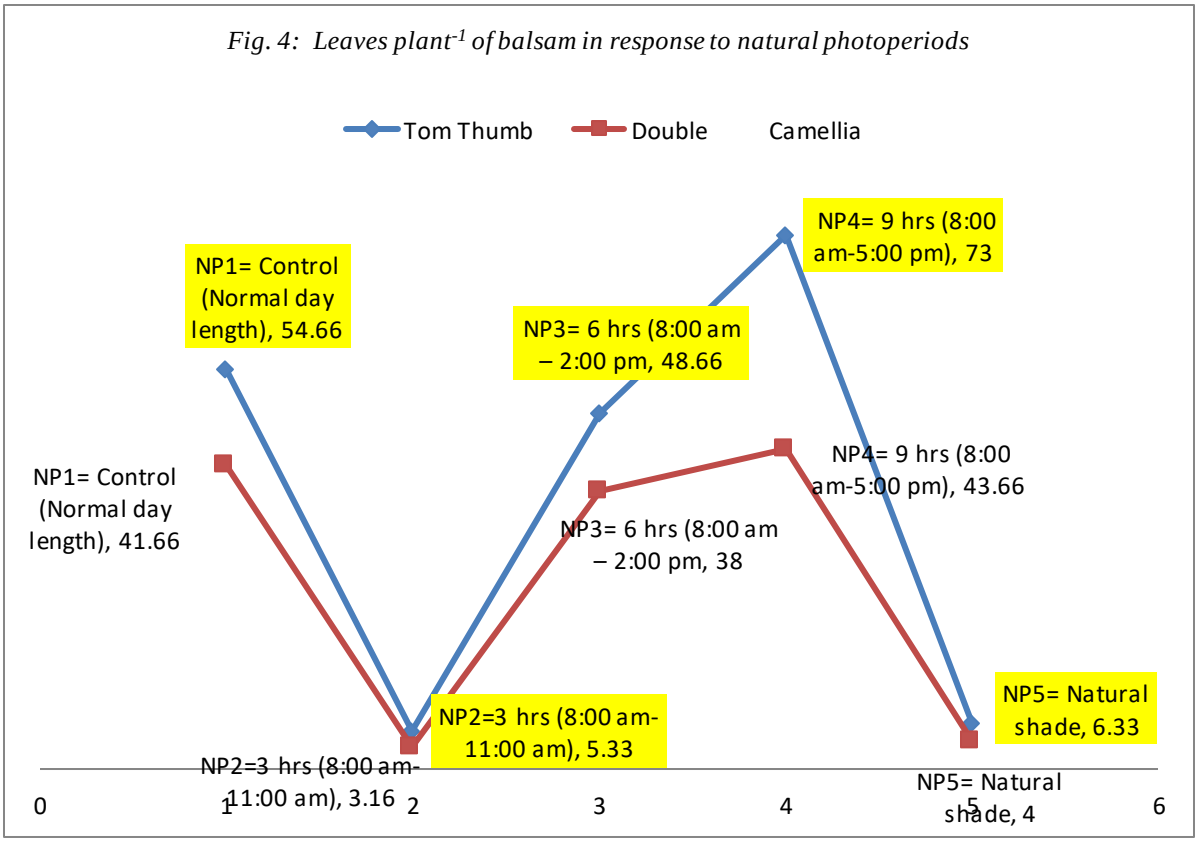
can be seen from the results that leaves plant^{-1} varied significantly (P<0.05) between the treatments and varieties. Both the varieties responded well when exposed to natural photoperiod from 8:00 am to 5:00 pm. "Tom Thumb" had maximum leaves plant^{-1} (73.00) followed by "Double camellia" (43.66) followed by normal day length (12 hours) where "Tom Thumb" produced 54.66 leaves where as "Double camellia" produced 41.66 leaves on single plant . However when both varieties were exposed to low photoperiod from 8:00 am to 11:00 am produced less leaves plant^{-1} (5.33 and 3.16, respectively) followed by plants grown under natural shades 6.33 and 4.0 ("Tom Thumb" and "Double camellia", respectively) .

Fig. 4: Leaves plant^{-1} of balsam in response to natural photoperiods



Flowers plant^{-1}

The data regarding for flowers plant^{-1} as affected by different natural photoperiods on vegetative and flowering behavior of Balsam are presented in the Fig-5 It can be seen from the results that flowers plant^{-1}varied significantly (P<0.05) between the treatments and varietal interaction.

The results revealed that both the varieties responded well when exposed to natural photoperiod from 8:00 am to 5:00 pm. Balsam variety "Tom Thumb" had maximum flowers plant^{-1} (6.16) followed by "Double camellia" (4.66) followed by natural photoperiod of 12 hours where "Tom Thumb" developed 5.66 flowers on single plant and "Double camellia" produced 4.33 flowers on single plant. However when both varieties were exposed to low photoperiod from 8:00 am to 11:00 am produced less flower plant^{-1} (4.33 and 1.16, respectively) followed by plants grown under shade (0.66 and 0.66, respectively).

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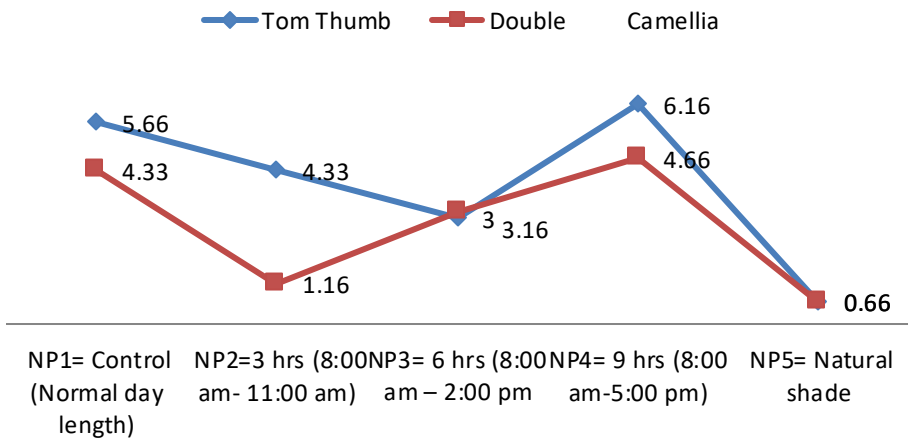
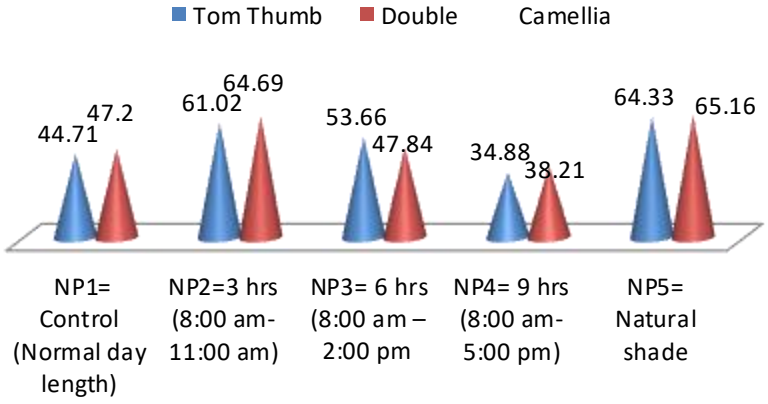
Fig. 5: Flowers plant^{-1} of balsam in response to natural photoperiods



Days to 1st flower plant^{-1}

The data (Fig. 6) regarding for days to first flower plant^{-1} as affected by different natural photoperiods on vegetative and flowering behavior of Balsam presented varied significant ($P<0.05$) difference between the treatments and varieties. Results revealed that both the varieties responded well in terms of days to 1st flower appearance when exposed to 09 hours of natural photoperiod from (8:00 am- 5:00 pm). Here the variety "Tom thumb" had produced first flower plant^{-1} within minimum number of days (34.88) followed by "Double Camelia" (38.21) followed by natural photoperiod of 12 hours where variety "Tom thumb" had developed first flower after 44.71 days followed by "Double Camelia" that had first flower after 47.2 days of sowing. However when both varieties were grown under Natural shade took maximum days to first flower appearance plant^{-1} for both the varieties (64.33 and 65.16, respectively) followed by 6 hours of photoperiod exposure (8:00 am- 11:00 am).

Fig. 6: Days to 1st flower of balsam in response to natural photoperiods



Days to flower persistence

The data regarding for days to flower persistence as affected by different natural photoperiods are presented in the Fig.7. It can be seen from the results that days to flower persistence varied significantly ($P<0.05$) for varieties under treatments. The results for varieties revealed that the variety Tom Thumb produced maximum (13.33) days to flower persistence as compared to Double Camcellia (13.00) well when exposed to natural photoperiod of 09 hours from 8:00 am to 5:00 pm, followed by control where plants were

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grown in full day sunlight of 12 hours where "Tom Thumb" had maximum days to flower persistence (12.00) followed by Double camellia (9.33). However when both varieties were exposed to low photoperiod from 8:00 am to 11:00 am aloted less days to flower persistence (3.34 and 2.01, respectively) followed by natural shade (4.66 and 3.04, respectively) for both the varieties of Balsam.

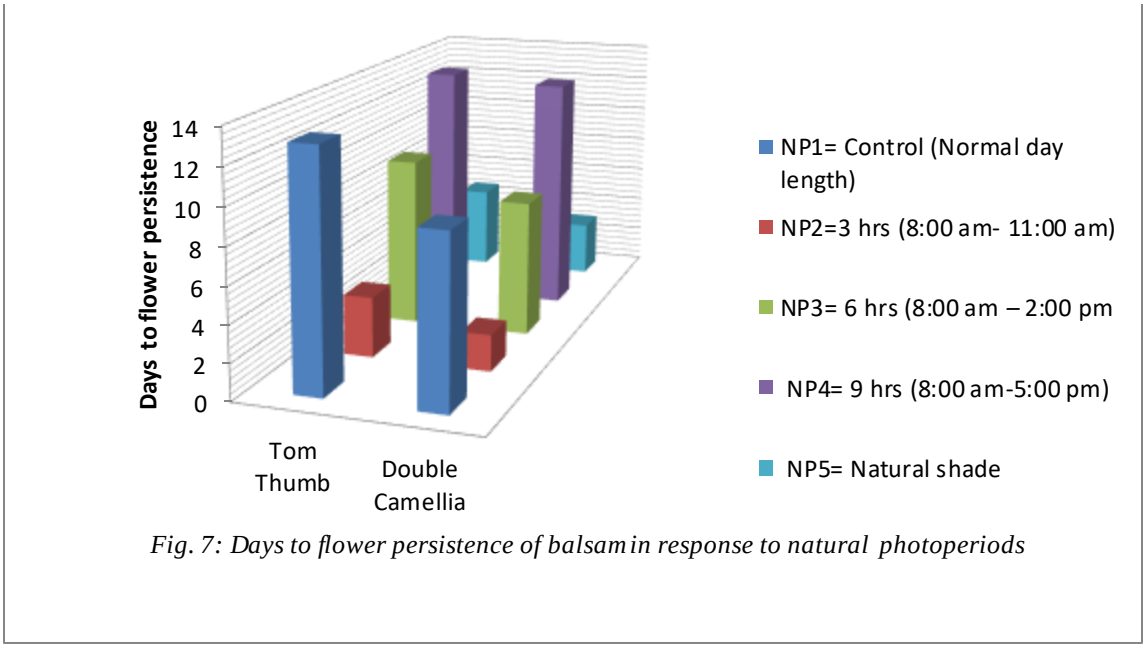
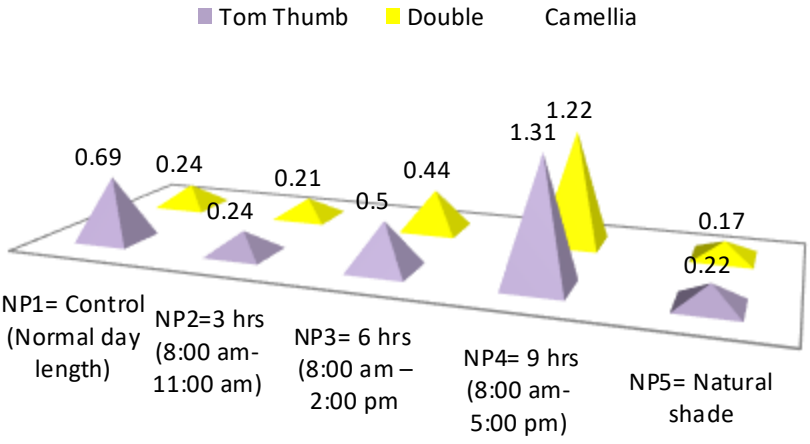


Fig. 7: Days to flower persistence of balsam in response to natural photoperiods

Weight of single flower (g)

The data regarding weight of single flower (g) plant^{-1} as affected by different natural photoperiods have been presented in the Fig. 8. It can be inferred from the data that weight of single flower plant^{-1} varied significantly ($P<0.05$) for varieties under treatments. Both the varieties responded well when plants were grown under natural photoperiod of 09 hours from 8:00 am to 5:00 pm here plants of variety "Tom Thumb" had displayed maximum weight of single flower plant $^{-1}$ (1.31g) followed by "Double camellia" (1.22g). Balsam varieties grown in full day sunlight of 12 hours developed flowers having maximum weight in variety "Tom Thumb" (0.69 g) where as variety "Double camellia" had flower weight of 0.44 g when grown under 6 hrs of photoperiod (8:00 am – 2:00 pm). However, when both varieties were grown under natural shade produced less weight of single flower plant $^{-1}$ (0.22 and 0.17 g, respectively).

Fig. 8: Weight single flower plant^{-1} of balsam in response to natural photoperiods



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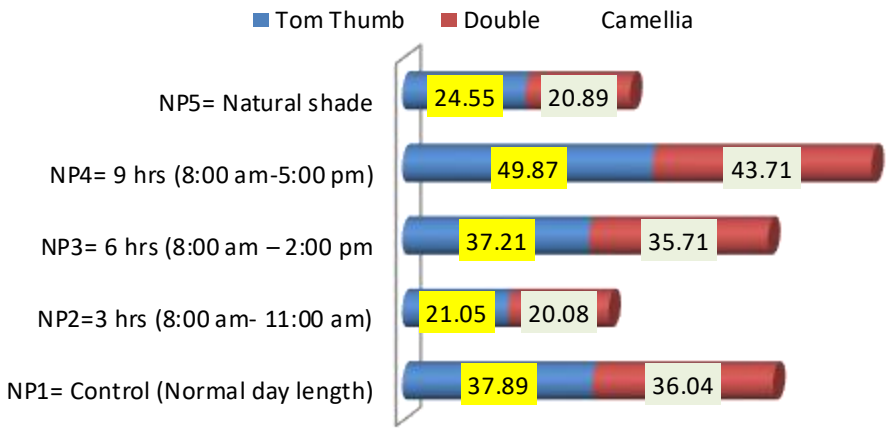
Chlorophyll content (SPAD)

The results (Fig.9) for both varieties of Balsam revealed that Both the varieties responded well when exposed to natural photoperiod from 8:00 am to 5:00 pm where, "Tom Thumb" had developed maximum chlorophyll content (49.87) followed by "Double camellia" (43.71). These varieties when grown under normal day length (12 hrs) developed chlorophyll content of maximum (37.89) in "Tom Thumb" followed by "Double camellia" (36.04). However when both varieties were exposed to low photoperiod from 8:00 am to 11:00 am produced less chlorophyll content (21.05 and 20.08, respectively) followed by plants grown under shade (24.55 and 20.89, respectively).

Fig. 9: Chlorophyll content (SPAD) of balsam in response to natural photoperiods



Correlations (Pearson)

	GP%	G.index	chlorph	flowpet	flowrpp	fstfemer	weight
Gindex	0.7571						
chlorph	0.7349	0.7747					
flowpet	0.7350	0.6996	0.8226				
flowrpp	0.6964	0.6530	0.7365	0.7370			
1stflower	-0.6730	-0.7221	-0.7928	-0.7304	-0.7448		
weight	0.8246	0.8933	0.8851	0.8150	0.7462	-0.7890	
height	0.4658	0.4553	0.7592	0.7247	0.5790	-0.5775	0.5393

Germination percentage (GP%) has been positively correlated with plant height (0.4658), chlorophyll content (0.7349) and days to flower persistence (0.7350), however this parameter had maximum correlation with flower weight (0.8246), however, germination % had negatively been correlated with days to 1st flower appearance (-0.6730). Chlorophyll content had been positively correlated at maximum with weight of flower (0.8851) and days to flower persistence (0.8226) followed by plant height (0.7592). Days to first flower emergence has been negatively correlated with flower weight (-0.7890) and plant height (-0.5775). Weight of single flower has shown positive correlation with germination index (0.8933), chlorophyll content (0.8851). Days to 1st flower emergence has shown negative correlation with all the parameters studied.

IV. DISCUSSION

It is obvious the present results had significant effects on varieties and natural photoperiod and varieties. For obtaining good germination keeping the soil moist is favorable and seeds are sown in early season covering with 1/8" of soil layer and water is given continuously to keep the soil moist till the germination is completed. The germination initiation may range between 10-15 days (Li *et al.*, 2011; Hua *et al.*, 2012; Christopher, 2013). The present study had significant effects on varieties and natural photoperiod and varieties. Interactive effect showed that maximum seed germination was recorded from Tom Thumb variety under NP4= 9 hrs (8:00am -5:00am) natural photoperiod, whereas the lowest seed germination was observed under plants grown in shade. Both varieties of Balsam produced maximum parameters less than 9 hours of photoperiod. Akbarian *et al.* (2016) observed best seed germination and quality flower seedlings under combinations of 25% blue and 75% red and fluorescent lamps for 10 hours of photoperiod. Kim *et al.* (2009) reported that flowering behavior in plant cycle shows the adaptability of plants to seasonal changes; and increase in

duration of photoperiod reduced time to first visible bud. Temperature and day length are related in the sense that as the natural day length becomes longer or shorter, the temperature warms or cools, respectively. Tooke and Battey (2000) found that the completion of flower development in *Impatiens balsamina* requires continuous inductive (short-day) conditions. Plants were grown in long-day (LD) conditions of 8 hr of light provided by tungsten and fluorescent bulbs at 271 to 309 $\mu mol.m^{-2}$ sec^{-1}, followed by 16 hr of tungsten light at 4.2 to 5.2 $\mu mol.m^{-2}$ sec^{-1}. Short-day (SD) conditions were 8 hr of tungsten and fluorescent light (as above) followed by 16 hr of darkness. Kim *et al*., (2009) indicated that the chemical, biological and physiological process is influenced by temperature. The cut flowers produced during summer when temperature exceeds 38°C, the biological processes are adversely affected. The leaf number below the 1st flower was affected by the addition of supplemental lighting with one some species of ornamental plants (Erwin and Warner 2002; Blanchard and Runkle, 2011). Under the conditions of extremely high temperatures, the plant proteins are denatured, affecting these processes and subsequently the flower quality has been adversely affected due to florigen present in flower (Wahocho *et al*., 2016). Erwin and Warner (2002) cultivated ornamental plants at 16 ^{0}C or 20 ^{0}C in combination with short day (SD, 8 hours) or long day (LD, 16 hours). Time to flower (first horizontal petals) at 16^{0}C increased from 56 to 64 days as so increased from 1 week to continuous conditions in SD, while LD decreased time to flower from 64 to56 days. Time to flower at 20 ^{0}C varied from 73 to 87 days with additional SD exposure resulting in flower and LD in faster flowering.

V. CONCLUSION

From present study It is concluded that Balsam variety **"**Tom Thumb" variety grown under NP4= 9 hrs (8:00am - 5:00am) natural photoperiod had significant effect on all the vegetative and flower traits studied. The results also revealed that the decrease in daylight adversely affected the traits investigated and photoperiod treatment comprised of 9 hours (8 am – 5:00) severely increased the flowers $plant^{-1}$ as well as the flower quality. So, Balsam can be successfully grown for commercial purpose under 9 hrs (8:00am -5:00am) of natural photoperiod.

REFERENCES

[1] Adams, S. R., and F.A. Langton. 2005. Photoperiod and plant growth: a review. J. Hort. Sci. Biot., 80 (1): 2–10

[2] Akbarian, B., M. Matloobi and N. Mahna. 2016. Effects of LED Light on Seed Emergence and Seedling Quality of Four Bedding Flowers Keywords: Bedding flowers, Light-emitting diode, Light quality, Seedling emergence, Seedling's quality. J. Ornam. Plants., 6 (2): 115-123.

[3] Blanchard, M.G. and E.S. Runkle. 2011. The influence of day and night temperature fluctuations on growth and flowering of annual bedding plants and greenhouse heating cost predictions. Hort. Sci., 46 (4): 599–603.

[4] Busch, F., P.A. Norman and I. Ensminger. 2008. Increased Air Temperature during Simulated Autumn Conditions Impairs Photosynthetic Electron Transport between Photosystem II and Photosystem II. Plant Phys., 147 (1): 402-414.

[5] Christopher, C. 2013. Impatiens. Encyclopedia of Cultivated Plants: From Acacia to Zinnia. Christopher Cumo, ed. ABC-CLIO, Pp. 523.

[6] Erwin, J.E. and R.M. Warner. 2002. Determination of photoperiodic response group and effect of supplemental irradiance on flowering of several bedding plant species. Acta Hort., 580 : 95-100.

[7] Gardeners, 2017. Plant Botanical Names. http://gardenersnet.com/gardening/ plantbotanicalnames.htm

[8] Ha, T.M. 2014. A Review of plants flowering physiology: The control of floral induction by juvenility, temperature and photoperiod in annual and ornamental crops. Asian J. Agri. and Food. Sci., 2(3):186-195.

[9] Hua, L., Z. Peng, L.S. Chia, N.K. Goh and S.N. Tan. 2012. Separation of kaempferols in *Impatiens balsamina* flowers by capillary electrophoresis with electrochemical detection. J Chromat., 909 (2): 297–303.

[10] Kim, D.H., M.R. Doyle, S. Sung and R.M. Amasino. 2009. Vernalization: winter and the timing of flowering in plants. Ann.Rev. Cell Develo., 25: 277-299.

[11] Li, H.J., J.J. Yu and P. Li. 2011. Simultaneous qualification and quantification of baccharane glycosides in Impatientis Semen by HPLC–ESI-MSD and HPLC–ELSD. J. Pharm. Biomed. Anal., 54 (4): 674–80.

[12] Park, J.H., J.M. Kim and W.I. Do. 2003. Pharmacognostical studies on the folk medicine bong seon wha dae. Korean J. Pharm., 34 (3): 193–96.

[13] Statistix. 2006. Statistics 8 user guide, version 1.0. Analytical software, P.O. Box 12185, Tallahassee fl 32317 USA. Copyright @ 2006 by Analytical software.
[14] Tooke, F. and N.H. Battey. 2000. A leaf-derived signal is a quantitative determinant of floral form in *Impatiens*. Plant Cell., 12(10): 1837–1848.
[15] Wahocho, S.A., T.F. Miano, N. Memon and N.A. Wahocho. 2016. Photoperiodic effect on vegetative growth and flower quality of zinnia (*Zinnia elegans* Jacq.). Sarhad J. Agri., 32 (4): 316-324.
[16] Wang, Y.C., D.C. Wu DC, J.J. Liao, C.H. Wu, W.Y. Li and B.C. Weng. 2009. In vitro activity of *Impatiens balsamina* L. against multiple antibiotic-resistant Helicobacter pylori. Am. J. Chin. Med., 37 (4): 713–22.

Picture gallery of experiment

Experimental set up of Balsam varieties under different Natural photoperiod

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Measuring Chlorophyll content of Balsam plant

Researcher taking observations from Balsam plant

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Effect of glycerol, peanut oil and soybean lecithin contents on the properties of biodegradable film of improved cassava starches from Côte d'Ivoire

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***Abstract*—** *Edible films have been successfully used in the food packaging industry for several decades. Today natural polysaccharides, including cassava starch, are increasingly being used in the production of such biodegradable edible films and food packaging. In Côte d'Ivoire, there are improved cassava varieties whose starches have not yet been tested in the production of biodegradable films. In this study, the optical and mechanical properties and the water solubility of starch-based composite films of four improved cassava varieties with added glycerol, peanut oil and soy lecithin were determined. Starch was obtained by cold water extraction from native cassava from the varieties Bocou 1, Bocou 2, Yavo and TMS. Films preparation was made bycasting method with cassava, glycerol (25-30 %), peanut oil (5-10 %) and soybean lecithin (0-5 %). Increasing the glycerol content, increased L*color value and elongation at break and decreased a*, b*, colourdifference (ΔE^*_{ab}) and tensiles trength of the composite films. Also, increasing the oil content from 5 to 10%, increased the opacity, b*, ΔE^*_{ab}, water solubility, elongation at break but decreased L*, a* and tensiles trength. Similarly, increasing the soy lecithin content from 0 to 5%, increased the opacity, L*, b* and ΔE^*_{ab}, but decreased a*, of the starch-based composite films. The results suggest an ideal formulation of 4% starch/25% glycerol/5% oil/5% soy lecithin for a film with optimum mechanical properties with low solubility.*

***Keywords*— Biodegradable film, Cassava starch, Emulsified film, Mechanical properties, Starch-based film.**

I. INTRODUCTION

Scientific literature now contains several studies aimed at improving the properties of materials already used or using new materials to produce packages having properties that may tend towards synthetics. Due to increasing pressure from society and legislation to reduce non-degradable synthetic packaging, research on the production of biodegradable alternatives prepared from natural biopolymers has been encouraged (Adjouman *et al*., 2017). Biodegradable films are flexible and thin matrices used for coating and packaging in a diversity of product groups and industries. Main applications include food packaging, fruit coatings, pharmaceuticals and cosmetics. Films are predominantly made of natural biopolymers, such as polysaccharides, proteins and other abundant natural elements (Bergo *et al*., 2010).

Starch is a polysaccharide composed of amylose, a linear polymer consisting of glucose with α (1–4) bonds, and amylopectin, a polymer with α (1–6) bonds. The amylose vs amylopectin ratio and, consequently, the form

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and functionality vary between cultivars within and between species (Copeland *et al.*, 2009). Cassava (*Manihot esculenta* Crantz) roots are among the most important sources of starch worldwide. According to the Food and Agricultural Organisation (FAO), the global cassava production in 2015 was estimated at 281.1 million tonnes with 54 % produced in Africa (FAO, 2016). In Côte d'Ivoire cassava is the second major food crop after yam, with an estimated 5.1 million tonnes produced in 2015 (FAO, 2016).Unlike other starches, cassava starch allows obtaining transparent and flexible films (Vicentini and Cereda, 1999). However, for the production of edible films and optimised application in food technology plasticizers, hydrophobic agents and emulsifiers have to be added to the film composition (Bergo*et al.*, 2010). Adding vegetable oils to hydrophilic films, for instance, reduces the water vapor barrier properties of films based on proteins (Fabra*et al.*, 2009) and those based on polysaccharides (Koelschand Labuza,1992). Those emulsion-based films with enhanced physico-chemical and mechanical properties generally arise from oil droplets with small diameter, a high degree of homogeneity and highly stable film-forming emulsions (Nilsuwan *et al.*, 2016; Debeaufort *et al.*, 1995). For good dispersion/homogenization of the oil in the film-forming matrix, emulsifiers are therefore commonly added. They are nonpolar substances which bind to both water and oil, thus improving emulsification and increasing the stability of the emulsion. Furthermore, soybean lecithin incorporated into protein-based emulsified films has previously shown effective in stabilizing the emulsion film (Prodpran *et al.*, 2007).Lipid compounds are used to modulate the water barrier properties of films; however, in hydrophilic suspensions they adversely affect the mechanical and optical properties of the resulting films (Yang and Paulson, 2000). The most effective plasticizers are generally those whose structure resembles the structure of the polymer they plasticize. For starch-based films polyols such as sorbitol and glycerol are the commonly used plasticizers (Mali *et al.*, 2005). With increasing glycerol concentration, the breaking strain has shown to improve and the tensile strength to decrease (Alves *et al.*, 2007).

In Côte d'Ivoire, there are improved cassava varieties whose starches have not yet been tested in the production of biodegradable films. The aim of this study is to determine the effect of plasticizer, lipid and emulsifier contents on the mechanical properties, solubility, opacity and color parameters of films based on starch from improved cassava varieties grown in Côte d'Ivoire.

II. MATERIALS AND METHODS

Cassava natives starches from four improved varieties Bocou 1, Bocou 2, Yavo and TMS, belonging to Centre National de Recherche Agronomique in Côte d'Ivoire were used in this study. Cassava plants were harvested at maturity, 12 months after plantation. Glycerol (bidistilled, 99.5% purity) and soybean lecithin were purchased from VWR Prolabo Chemicals (Leuven, Belgium). The CORA brand peanut oil used in this study was purchased at a supermarket in Belgium.

2.1. Amylose content

The amylose content of the starch samples was determined by colorimetric reaction and subsequent measuring of the absorbance of the amylose-iodine blue complex formed (Morrison and Laignelet, 1983).

2.2. Starch paste clarity

The paste clarity of the starches was determined as previously described by Craig et al. (1989). Approximately 0.11 g of starch was weighed into quartz screw tubes. The mass was supplemented to 10 g with distilled water. The closed tube with the well homogenized content was left in aboiling water bath at 100°C for 30 min with uniform stirring. The solution obtained was cooled and the paste clarity or percent transmittance (% T) was determined using a Shimadzu UV-2401-PC (Kyoto-Japan) spectrophotometer at 650 nm against a blank sample containing distilled water.

2.3. Film preparations and thickness measurements

Film preparation and thickness measurements were determined in accordance with previously described protocol (Adjouman *et al.*, 2017). The emulsified films were prepared in two steps. First, 4 g of cassava starch (w/w, starch) was mixed with glycerol (1–1.2 g) and with two thirds of distilled water, the final mixture being heated from 30°C to 75°C for 20 min. Peanut oil (0.2–0.4 g), soybean lecithin (30–60 mg) and distilled water (a third of the total mixture) (Table 1) was also heated together for 20 min from 30°C to 75°C. Both solutions were heated with constant stirring at 750 rpm/min. Thereafter, the solution of peanut oil, soybean lecithin and distilled water was homogenised at 24,000 rpm for 2 min using an Ultra-Turrax T25 basic (IKA Werke, Staufen /Germany). The homogenised solution was subsequently mixed with the starch and glycerol and then heated from 75°C to 95°C at 750 rpm stirring for 25 min. A 20 g aliquot of the final solution was transferred and spread with even thickness on the surface of a Petri dish (9 cm diameter) and oven-dried in a ventilated oven model (Memmert UF-110, Schwabach, Germany) at 35°C for 24 h. The dried films were removed and stored in a desiccator at 25°C for 48 h, before testing. The thickness of all films was determined using a hand micrometer (NSK, Japan) at 10 random positions of the films.

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Table.1: Cassava starch-based film formulations

Formulation	Starch (g)	Oil 5 and 10% (g/g starch)	Glycerol 25 and 30% (g/g starch)	Lecithin 0 and 5% (mg/g oil)	Potassium sorbate (g)
F1: G25 H5 L0	4	0.2	1	0	0.2
F2: G25 H5 L5		0.2	1	30	
F3: G30 H5 L0		0.2	1.2	0	
F4: G30 H5 L5		0.2	1.2	30	
F5: G25 H10 L0		0.4	1	0	
F6: G25 H10 L5		0.4	1	60	
F7: FG30 H10 L0		0.4	1.2	0	
F8: G30 H10 L5		0.4	1.2	60	

G25 H5 L0 : 25 % glycerol/ 5 % oil/ 0% lecithin, G25 H5 L5 : 25 % glycerol/ 5 % oil/ 5% lecithin, G30 H5 L0 : 30 % glycerol/ 5 % oil/ 0% lecithin, G30 H5 L5 : 30 % glycerol/ 5 % oil/ 5% lecithin, G25 H10 L0 : 25 % glycerol/ 10% oil/ 0% lecithin, G25 H10 L5 : 25 % glycerol/ 10 % oil/ 5% lecithin, G30 H10 L0 : 30 % glycerol/ 10 % oil/ 0% lecithin, G30 H10 L5 : 30 % glycerol/ 10 % oil/ 5% lecithin.

2.4. Film water solubility

Film water solubility was determined as previously describe (López *et al.*, 2008). From each film pieces of 2 x 3 cm were cut and stored in a desiccator, containing silica gel beads, for 7 days. Pieces were weighed to the nearest 0.0001 g and placed into test beakers with 80 ml deionized water. The samples were left under constant stirring at 200 rpm for 1 h at room temperature. After 1 h, any remaining pieces of film were subsequently collected by filtration, dried again in an oven at 60°C to constant weight and the proportion of total soluble matter was calculated.

2.5. Film opacity

Film opacity was determined in accordance with previously described protocol (López *et al.*, 2008). Film samples were cut into rectangles of1 x 3 cm and placed inside a spectrophotometer cell. The absorbance spectrum (400–700 nm) was recorded for each sample using a Shimadzu (UV-2401-PC Kyoto/Japon) spectrophotometer. Film opacity was determined by an integration procedure and expressed as absorbance units per nanometers (AU. nm).

2.6. Film color

The film L*, a* and b* color values were determined using a Miniscan XE HunterLab colorimeter (Virginia, USA) against the standard plate (L* = 93.5; a* = -0.61 and b* = 0.12). Color was measured on 8 x 8 cm segment of film. The total color difference (ΔE^*_{ab}) was calculated using the following equation 1:

$$\Delta E * ab = \sqrt{(L*)2 + (a*)2 + (b*)2} \quad (1)$$

Film specimens were pre-conditioned at 62% RH and 25°C for 72 h prior to taking color measurements.

2.7. Mechanical properties

Mechanical properties were studied using a TA.TX2 Stable Micro Systems Texture Analyzer. ASTM D882-02(2002), standard test method for tensile properties of thin plastic sheeting with some modifications was used. The films were cut into 25 mm wide and 80 mm long strips, using a scalpel and mounted between the grips (A/TG) of the texture analyser. The ends of the strips were mounted between cardboard grips. Strength resistance and deformation at break were recorded in tensile mode, during extension at 10 mm min^{-1}, with an initial 50-mm distance between the grips, until the specimens broke.

2.8. Statistical analysis

UsingStatistica 7.1 software (Statistica), multivariate analyses of variance (MANOVA) was performed, to compare the means of the various properties of the film formulations (cassava variety and glycerol, peanut oil and soy lecithin added). Duncan's multiple range test at the 5% threshold, allowed locating the differences.

III. RESULTS AND DISCUSSION

3.1. Amylose content and paste clarity

Paste clarity and amylose content of cassava varieties used in this study are presented in Table 2. The highest value of amylose was obtained with the TMS variety, followed by the varieties Yavo, Bocou 2 and Bocou 1. In contrast, the clearest starch gel was obtained with the Bocou 1 variety, followed by Bocou 2, Yavo and TMS. Starches with low amylose content have high paste clarity. Amylose contents are known to influence clarity of starch pastes. High amylose content may result in more opaque starch pastes (Schmitz *et al.*, 2006).

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Table.2: Amylose content and paste clarity of starches in cassava varieties

Cassava starch	Bocou 1	Bocou 2	Yavo	TMS
Amylose content* (%)	16.28 ± 2.2[a]	17.33 ± 1.2[b]	18.37 ± 1.5[c]	20.36 ± 2.1[d]
Starch paste clarity* (% T)	71.02 ± 3.2[a]	69.77 ± 2.5[b]	61.33 ± 2.1[c]	48.86 ± 1.3[d]

*% T : percent transmittance, *the means followed by a common letter are not significantly different by Duncan's multiple range test at p <0.05.*

3.2. Film water solubility

Glycerol, oil and lecithin added showed a significant difference in the water solubility of the starch films ($p < 0.05$). Increasing the concentration of glycerol from 25% to 30%, oil from 5% to 10% and lecithin from 0% to 5% resulted in increased water solubility of the composite films from all varieties (Table 3).

Table.3: Water solubility of cassava starch-based composite films

Formulation	Solubility (%)			
	Bocou 1*	Bocou 2*	Yavo*	TMS*
G25 H5 L0	47.14 ± 1.48[a]	42.88 ± 1.87[a]	40.49 ± 2.75[a]	37.15 ± 1.76[a]
G25 H5 L5	49.13 ± 1.71[ab]	43.90 ± 1.00[ab]	41.61 ± 2.56[ab]	39.61 ± 0.58[ab]
G25 H10 L0	62.75 ± 3.36[d]	61.67 ± 3.36[d]	58.32 ± 2.43[d]	52.99 ± 2.43[d]
G25 H10 L5	66.34 ± 1.33[e]	64.68 ± 0.81[e]	60.50 ± 1.42[e]	56.16 ± 1.24[e]
G30 H5 L0	51.85 ± 1.24[b]	45.95 ± 1.82[b]	45.01 ± 2.18[b]	44.35 ± 2.14[b]
G30 H5 L5	53.82 ± 1.30[c]	47.89 ± 1.07[c]	48.65 ± 1.93[c]	47.65 ± 2.02[c]
G30 H10 L0	73.19 ± 2.84[f]	69.53 ± 0.74[f]	62.32 ± 0.56[f]	59.65 ± 0.82[f]
G30 H10 L5	74.63 ± 2.76[g]	72.96 ± 1.23[g]	64.50 ± 1.42[g]	63.07 ± 0.78[g]

**The means followed by a common letter are not significantly different by Duncan's multiple range test at p <0.05.*

Increasing the glycerol content from 25% to 30% allowed an increase in the water solubility of the films of the starches of the four varieties of cassava. Such observations were made in previous studies on films based on bitter and soft cassava starch on the film-forming capacity of corn starch amended (Lopez *et al.*, 2008; Belibi, 2014). The demonstrated positive correlation between the solubility of the films and the plasticizer concentration may be due to the low molecular weight of the glycerol which enables it to be easily inserted between the polymer chains (Cuq *et al.*, 1997) and to the hydrophilic nature of glycerol (Lopez *et al.*, 2008). Furthermore, the increase in the glycerol concentration in biodegradable films was found to induce a marked decrease in the crystallinity of the starch films (Belibi, 2014). The less crystallites are formed in the films the more readily they will swell in the water and disintegrate. Consequently, this will express in higher water solubility (Maizura *et al.*, 2007).

Increasing the oil content from 5% to 10% increased the water solubility of the starch films of all cassava varieties which is likely to be due to hight degree of solubility of the fatty acids contained in the peanut oil. An increase in fat content increases solubility in water as long as the concentration of fatty acids is lower than the saturation level for an expected volume of water (Fakhouri *et al.*, 2009). Previous work on gelatin films with lauric, palmitic and stearic acids revealed indeed a positive correlation between water solubility and fatty acid content (Fakhouri *et al.*, 2003). Another study revealed that solubility of soy protein films showed a significant increase with the addition of lauric acid (Rhim *et al.*, 2002) supported by similar findings in wheat gluten films with added fatty acids (Gontard *et al.*, 1994).

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The increase in soy lecithin content from 0 to 5% led to an increase in the water solubility of the starch films of all cassava varieties. It was hoped that the inclusion of compounds having hydrophobic characteristics (i.e. soy lecithin) could reduce the water solubility of cassava starch films; however, this behavior was not observed in this study. A study of edible composite films based on wheat gluten and lipids suggested that the increase in solubility due to addition of compounds with hydrophobic characteristics is related to the breakdown of the intermolecular bonds in the protein network and to the formation of weak interactions with hydrophobic substances (Gontard *et al.,* 1994). The subsequent increase in the solubility of cassava-based films when the concentration of soy lecithin is being raised is likely to be related to the breakdown of the intermolecular bonds in the starch network.

3.3. Film opacity

The opacity values of the starch films of the different cassava varieties as a function of the different formulations are presented in Table 4. At a given concentration of glycerol addition of oil allowed an increase in the opacity of the films of cassava starches. Similarly, at a given concentration of glycerol and oil, addition of soy lecithin caused an increase in the opacity of cassava starch films. Highest values were found with films from TMS starch, followed by Yavo, Bocou 2 and Bocou 1. Added oil and lecithin and oil-lecithin combination showed significant differences in the opacity of the starch films of all cassava varieties ($p < 0.05$).

Table.4: Opacity of cassava starch-based composite films

Formulation	Opacity (AU nm^{-1})			
	Bocou 1*	Bocou 2*	Yavo*	TMS*
G25 H5 L0	110.64 ± 6.98^{a}	166.70 ± 15.05^{a}	169.21 ± 8.36^{a}	191.42 ± 4.61^{a}
G25 H5 L5	172.82 ± 6.40^{c}	257.27 ± 10.35^{c}	223.92 ± 3.41^{c}	251.42 ± 8.50^{c}
G25 H10 L0	155.65 ± 6.86^{b}	169.36 ± 3.47^{b}	208.15 ± 11.85^{b}	221.39 ± 3.30^{b}
G25 H10 L5	272.55 ± 11.19^{d}	322.62 ± 8.86^{d}	332.50 ± 12.09^{d}	348.66 ± 2.49^{d}
G30 H5 L0	132.82 ± 6.39^{a}	162.67 ± 7.02^{a}	181.56 ± 3.14^{a}	196.58 ± 1.77^{a}
G30 H5 L5	200.56 ± 5.13^{c}	264.62 ± 11.39^{c}	237.33 ± 8.49^{c}	259.06 ± 3.11^{c}
G30 H10 L0	171.47 ± 14.05^{b}	156.27 ± 7.15^{b}	209.09 ± 16.28^{b}	217.65 ± 6.71^{b}
G30 H10 L5	291.98 ± 5.07^{d}	299.29 ± 1.00^{d}	337.12 ± 17.07^{d}	348.57 ± 7.63^{d}

**The means followed by a common letter are not significantly different by Duncan's multiple range test at $p < 0.05$.*

The increase in oil content from 5 to 10% allowed an increase in the opacity of the films of the starches of all cassava varieties, which is in line with previous study (Perez-Mateos *et al.,* 2009). Similar results were found in a study on the physical and barrier properties of composite films of apple pectin and manioc starch incorporated with *Laurus Nobilis* oil and oleic acid (Taqi *et al.,* 2014). The authors attributed this behaviour to the physical properties of oils at room temperature, which generally depends on the oil concentration in the film. The opacity of the films would therefore be linked to the oil droplets dispersed throughout the starch network and to the original color of the oil used. Similar observations were made previously on the whiteness of gelatin film color in relation to the sunflower oil dispersed in the emulsion (Perez-Mateos *et al.,* 2009) and opacity increase as a function of the fatty acid level in a film within a study on the effects of fatty acid addition to the properties of corn starch and gelatin films (Fakhouri *et al.,* 2009).

Increase in lecithin content from 0 to 5% allowed an increase in opacity of films from starches of all cassava varieties. We attribute this increase to original state of yellow soybean lecithin and a hydrophobic compound. Incorporation of the hydrophobic substances into the hydrocolloid polymer HPMC matrix caused a decrease in the luminance of the films which resulted in an increase in their opacity in previous work (Quezada-Gallo *et al.,* 2000). An oil-soy lecithin combination also allowed an increase in opacity of starch films of four cassava varieties which seems to be accumulation of their individual effects. Opacity was more pronounced in TMS starch films than in starch films derived from the three other varieties used in this study and is related to the intrinsic characteristics of each starch variety specifically the starches paste clarity (Table 2).

3.4. Film color

The color parameters (Fig. 1) of the films were significantly influenced by the addition of glycerol, oil and lecithin ($p < 0.05$). Increasing the glycerol content, led to increased L* (Fig. 1a) and decreased a* (Fig. 1b), b* (Fig. 1c) and ΔE^*_{ab} (Fig. 1d), of starch films from four cassava varieties. Conversely, increasing the oil content, resulted in a decrease in L* and a*, and an increase in b* and ΔE^*_{ab}, of the starch films formulated using cassava varieties. Lecithin addition, resulted in an increased L*, b* and ΔE^*_{ab}, but decreased a*, of starch films of cassava varieties. The increase in b*, resulted in an increase in ΔE^*_{ab} of the resulting films.

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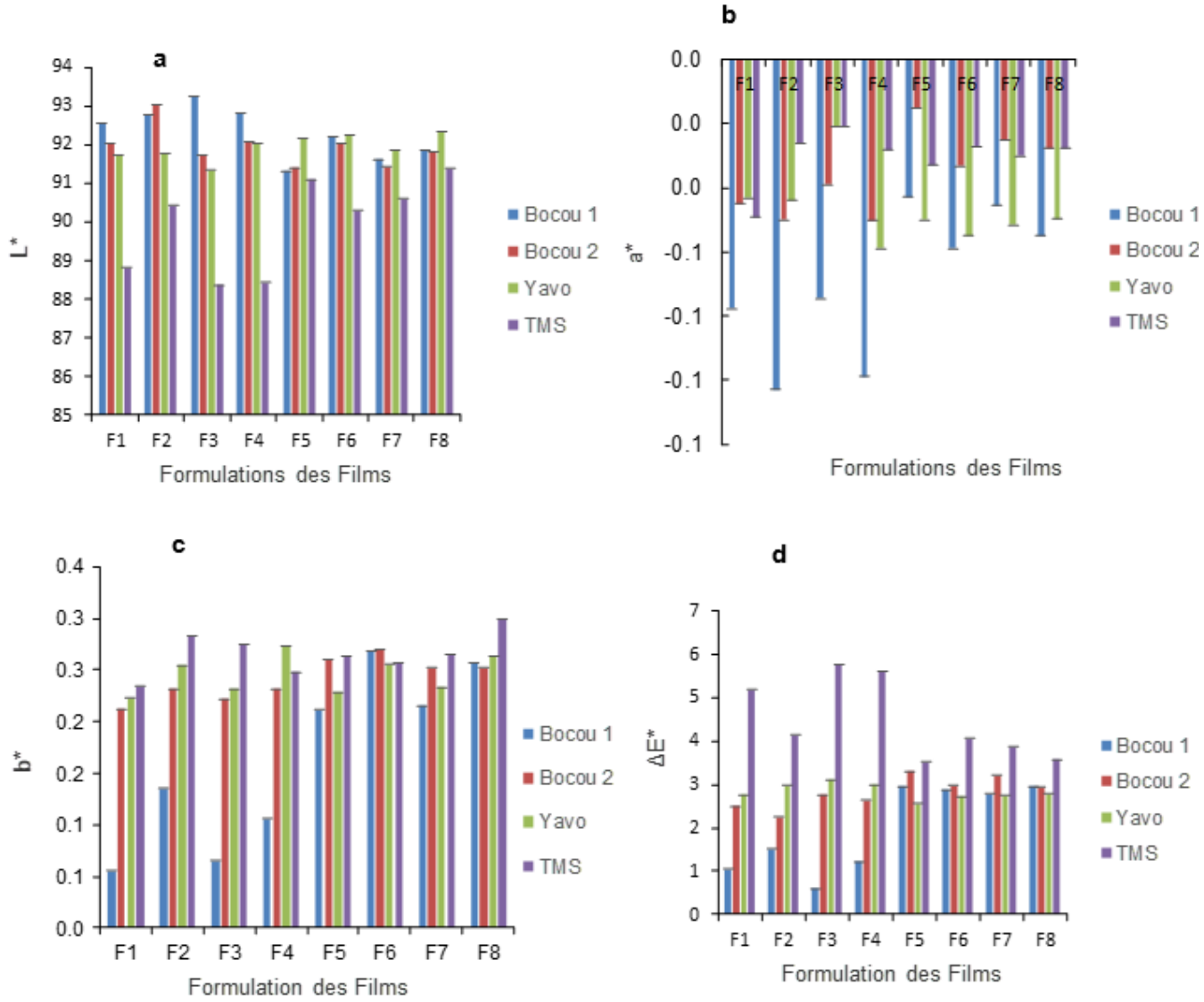

Fig.1: Effect of glycerol, oil and soy lecithin on the colour parameters of films based on cassava starch, a) color parameter L^*, b) color parameter a^*, c) color parameter b^*, d) ΔE^* indexes

The study on HPMC films plasticized with 15 and 30% glycerol was showed a slight increase in L* luminance but not a decrease in a*, b* and ΔE^*_{ab} indexes (Al Mahdi, 2006). However, our results concerning effect of oil on color parameters are consistent with studies on emulsified films based on gelatine of fish shell and palm oil (Tongnuanchan *et al*., 2015) and on the stability of the emulsion and the properties of gelatin films affected by palm oil and emulsifiers (Nilsuwan et al. 2016). Previous studies in films with soy lecithin as emulsifier reported highest b* and ΔE^*_{ab} values compared with other emulsifiers (Nilsuwan *et al*., 2016). Therefore, the colors of peanut oil and soy lecithin directly determined the color of the cassava starch films in this study.

3.5. Mechanical properties

Table 5 shows values of tensile strength and elongation at break of composite films based on cassava starch.

Table.5: Tensile strength and elongation at break values of cassava-based composite films

Formulations	Tensile Strength at break (MPa)				Elongation at break (%)			
	Bocou 1*	Bocou 2*	Yavo*	TMS*	Bocou 1*	Bocou 2*	Yavo*	TMS*
G25 H5 L0	2.82 ± 0.3^a	5.11 ± 1.2^a	5.26 ± 1.1^a	5.99 ± 1.6^a	15.08 ± 2.3^a	8.64 ± 1.8^a	8.20 ± 5.1^a	7.39 ± 1.7^a
G25 H5 L5	3.94 ± 1.6^b	6.22 ± 1.1^b	6.40 ± 1.2^b	7.92 ± 0.9^b	27.54 ± 3.2^b	18.17 ± 2.4^b	16.34 ± 2.1^b	17.82 ± 3.3^b

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G25 H10 L0	0.96 ± 0.2^{e}	1.39 ± 0.3^{e}	1.26 ± 0.1^{e}	2.11 ± 0.5^{e}	73.66 ± 5.7^{e}	59.82 ± 3.4^{e}	60.12 ± 2.8^{e}	44.36 ± 4.7^{e}
G25 H10 L5	1.28 ± 0.1^{e}	1.49 ± 0.1^{e}	1.65 ± 0.2^{e}	2.23 ± 0.4^{e}	88.42 ± 1.6^{e}	73.28 ± 4.3^{e}	75.44 ± 4.8^{e}	54.23 ± 3.7^{e}
G30 H5 L0	2.08 ± 0.3^{c}	3.54 ± 0.4^{c}	3.90 ± 0.4^{c}	3.94 ± 0.4^{c}	35.77 ± 5.3^{c}	27.97 ± 2.6^{c}	21.47 ± 2.2^{c}	29.23 ± 6.0^{c}
G30 H5 L5	1.78 ± 0.6^{d}	2.51 ± 0.6^{d}	2.80 ± 0.6^{d}	2.98 ± 0.7^{d}	47.69 ± 4.3^{d}	35.16 ± 3.4^{d}	30.27 ± 4.2^{d}	30.91 ± 4.5^{d}
G30 H10 L0	0.71 ± 0.1^{f}	1.07 ± 0.3^{f}	1.09 ± 0.2^{f}	1.13 ± 0.1^{f}	95.44 ± 1.1^{f}	91.18 ± 2.8^{f}	87.41 ± 3.7^{f}	65.09 ± 2.1^{f}
G30 H10 L5	0.45 ± 0.1^{g}	0.69 ± 0.1^{g}	0.72 ± 0.1^{g}	0.89 ± 0.1^{g}	99.21 ± 1.4^{g}	99.50 ± 0.7^{g}	94.49 ± 3.8^{g}	85.56 ± 1.9^{g}

**The means followed by a common letter are not significantly different by Duncan's multiple range test at $p < 0.05$.*

Increasing glycerol content from 25% to 30% resulted in a decrease in tensile strength at break and an increase in the elongation at break of films from cassava starches varieties. It has already been pointed out by several authors that increase in level of plasticizer such as glycerol in starch films leads to a decrease in tensile strength and an increase in elongation at break (Alves *et al*., 2007; Bertuzzi *et al*., 2012; Sanyang *et al*., 2015). It has been shown that glycerol reduces the rigidity of the starch network, leading to a less orderly film structure and increasing the capacity of the polymer chain movement (Sothornvit and Krochta, 2005). These properties render glycerol a suitable plasticizer able to reduce intramolecular forces between starch chains and to promote hydrogen bond formation between plasticizer and starch molecules. An increase in elongation of films may be related to the fact that plasticizers decrease intermolecular bonds between amylose, amylopectin and amylopectin amylose phases of the starch and substituting them by hydrogen bonds formed between starch molecules and plasticizer (Sanyang *et al*., 2015). Such perturbation and reconstruction of starch molecular chains reduces stiffness and promotes film flexibility by allowing greater mobility of the chain. Zavareze *et al*. (2012) have shown that elongation of the polymers depends on the mobility of their molecular chains.

Increasing oil content from 5% to 10% resulted in a decrease in tensile strength and an increase in elongation at break of starch films from all four cassava varieties. Similar results have been obtained on films based on cinnamon oil incorporated cassava starch (Souza et al. 2013) and a variety of other films with other polymers (Lopez *et al*., 2008; Al Mahdi, 2006). The addition of lipids or oil to protein-based or polysaccharide-based films such as starch can reduce intermolecular interactions between polymer chains. The result is a decrease in stiffness with a concomitant increase in the extensibility/elasticity of the film obtained (Lopez *et al*., 2008; Souza *et al*., 2013). The combination of glycerol and lecithin influenced tensile strength and elongation at break of films based on cassava starch ($p < 0.05$).

At 25% glycerol, addition of soybean lecithin resulted in an increase in tensile strength and an increase in elongation at break for all varieties; however, at 30% the addition resulted in decrease in tensile strength and increases an elongation at break of the starch-based films. This behavior observed at 30% glycerol when the soy lecithin is added could be due an apparent synergistic effect between glycerol and emulsifiers (Rodriguez *et al*., 2006). This interaction means that the films with glycerol in the presence of an emulsifier mechanically behave in the form of films with a larger amount of plasticizer. At 25% glycerol, an increase in tensile strength was observed that is likely to be due to the good dispersion and distribution of the added oil in the film caused by the addition of 5% lecithin. It is well known that mechanical properties of emulsified films are improved when the hydrophobic compound is small and distributed more homogeneously (Debeaufort and Voilley, 1995). Resistance and elongation at break of starch films from four cassava vary between varieties. This difference in mechanical properties of films may be explained by the difference in amylose content of starches constituting these films. Indeed, as the amylose content of the starch increases at constant glycerol concentration, the tensile strength at break of the corresponding films increases while the elongation at break decreases (Alves *et al*., 2007).

IV. CONCLUSION

Starches of improved cassava varieties in Côte d'Ivoire can be used in the production of biodegradable films with plasticizer, lipid and emulsifier. Effect of plasticizer, lipid and emulsifier concentration on films from four improved cassava varieties was evaluated. The increased levels of glycerol, oil and soybean lecithin influenced the properties of cassava-based starch films. The resultant films from improved cassava starch with glycerol, peanut

oil and soybean lecithin had satisfying mechanical properties and low water solubility. However, to further optimize other biodegradable compounds must be added to formulation.

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REFERENCES

[1] Adjouman, Y. D., Nindjin, C., Tetchi, F. A., Dalcq, A. C., Amani, N. G. and Sindic, M. (2017). Water vapor permeability of edible films based on improved cassava (*Manihotesculenta* Crantz) native starches. *Journal of Food Processing and Technology*,8, 665. doi: 10.4172/2157-7110.1000665

[2] Al Mahdi, R. (2006). Mise au point et caractérisation de films comestibles à base de polysaccharides et de matières grasses (doctoral thesis). Institut National Polytechnique de Lorraine, Lorraine, France.

[3] Alves, V. D., Mali, S., Beléia, A. and Grossmann, M. V. E. (2007).Effect of glycerol and amylose enrichment on cassava starch film properties. *Journal of Food Engineering*,78, 941-946.

[4] ASTM D882-02. (2002). *Standard test method for tensile properties of thin plastic sheeting*. Philadelphia : American Society for Testing and Materials.

[5] Belibi, P. C. (2014). Development and characterization of biofilms based on cassava starch reinforced by bi and tri-dimensional mineral fillers. Thesis. Université de Haute Alsace, Mulhouse, France. 156p

[6] Bergo, P., Sobral, P. J. A. and Prison, J. M. (2010).Effect of glycerol on physical properties of cassava starch films. *Journal of Food Processing and Preservation*, 34,401-410.

[7] Bertuzzi, M. A., Gottifredi, J. C. and Armada, M. (2012).Mechanical properties of a high amylose content corn starch based film, gelatinized at low temperature. *Brazilian Journal of Food Technology* (Campinas),15, 219-227.

[8] Copeland, L., Blazeka, J., Salmana, H. and Tanga, M. C. (2008).Form and functionality of starch. *Food Hydrocholloids*,23(6), 1527-1534.

[9] Craig, S. A. S., Maningat, C. C., Seib, P. A. and Hoseney, R. C. (1989). Starch paste clarity. *Cereal Chemistry*, 66, 173-182.

[10] Cuq, B., Gontard, N., Cuq, J. L. and Guilbert, S. (1997).Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *Journal of Agricultural and Food Chemistry*,45, 622-626.

[11] Debeaufort, F. and Voilley, A. (1995).Effect of surfactants and drying rate on barrier properties of emulsified edible films. *International Journal of Food Science and Technology*, 30, 183-190.

[12] Fabra, M. J., Jiménez, A., Atarés L., Talens, P. and Chiralt, A. (2009).Effect of fatty acids and beeswax addition on properties of sodium caseinate dispersions and films. *Biomacromolecules*, 10,1500-1507.

[13] Fakhouri, F. M., Batista, J. A., Grosso, C. R. F. (2003).Efeito de coberturas comestíveis aplicada semgoiabas*in natura* (*Psidiumguajava*L.) I. Desenvolvimento e caracterização de filmes comestíveis de gelatina, triacetina e ácidos graxos. *Brazilian Journal of Food Technology*,6,301-308.

[14] Fakhouri, F. M., Fontes, L. C. B., Innocentini-Mei, L. H. and Collares-Queiroz, F. P. (2009).Effect of fatty acid addition on the properties of biopolymer films based on lipophilic maize starch and gelatin. *Starch/Stärke*, 61,528-536.

[15] FAO. (2016). Food and Agriculture Organisation 359 of the United Nations. Food outlook : biannual report on global food markets. http://www.fao.org/3/a-i6198e.pdf Accessed 04.03.2017.

[16] Gontard, N., Duchez, C., Cuq, J. L. and Guilbert, S. (1994).Edible composite films of wheat gluten and lipids water vapour permeability and other physical properties. *International Journal of Food Science and Technology*, 29, 39-50.

[17] Koelsch, C. M. and Labuza, T. P. (1992).Functional, physical and morphological properties of methyl cellulose and fatty acid-based edible barriers. *LWT - Food Science and Technology*, 25, 404-411.

[18] López, O. V., García, M. A. and Zaritzky, N. E. (2008). Film forming capacity of chemically modified corn starches. *Carbohydrate Polymers*, 73, 573-581.

[19] Maizura, M., Fazilah, A., Norziah, M. H. and Karim, A. A. (2007). Antibacterial activity and mechanical properties of partiallyhydrolyzedsagostarch-alginate

edible film containinglemongrassoil. *Journal of Food Science*, 72, C324-C330.

[20] Mali, S., Sakanaka, L. S., Yamashita, F. and Grossmann, M. V. E. (2005). Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect. *Carbohydrate Polymers*, 60, 283-289.

[21] Morrison, W. R. and Laignelet, B. (1983). An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *Journal of Cereal Science*, 1, 9-20.

[22] Nilsuwan, K., Benjakul, S. and Prodpran, T. (2016). Influence of palm oil and glycerol on properties of fish skin gelatin-based films. *Journal of Food Science and Technology*, 53, 2715-2724.

[23] Pérez-Mateos, M., Montero, P. and Gómez-Guillén, M. C. (2009). Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends. *Food Hydrocolloids*, 23, 53-61.

[24] Prodpran, T., Benjakul, S. andArtharn, A. (2007).Properties and microstructure of protein based film from round scad (*Decapterusmaruadsi*) muscle as affected by palm oil and chitosan incorporation. *International Journal of Biological Macromolecules*, 41, 605-614.

[25] Quezada-Gallo, J. A., Debeaufort, F., Callegarin, F. and Voilley, A. (2000).Lipid hydrophobicity, physical state and distribution effects on the properties of emulsion based edible films. *Journal of Membrane Science*, 180, 37-46.

[26] Rhim, J. W., Gennadios, A., Weller, C. L. and Hanna, M. A. (2002). Sodium dodecyl sulfate treatment improves properties of cast films from soy protein isolate. *Industrial Crops and Products*, 15, 199-205.

[27] Rodríguez, M., Osês, J., Ziani, K. and Maté, J. I. (2006).Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. *Food Research International*, 39, 840-846.

[28] Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R. and Sahari, J. (2015). Effect of plasticizer type and concentration on tensile, thermal and barrier properties of biodegradable films based on sugar palm (*Arengapinnata*) starch. *Polymers*, 7, 1106-1124.

[29] Schmitz, C. S., de Simas, K. N., Joao, J. J., de Mello Castanho Amboni, R. D. and Amante, E. R. (2006). Cassava starch functional properties by etherification-hydroxypropylation. *International Journal of Food Science and Technology*, 41, 681-687.

[30] Sothornvit, R. and Krochta, J. M. (2005).Plasticizers in edible films and coatings. In J. H. Han (Ed.), *Innovations in food packagings* New York : Elsevier.pp. 403-433.

[31] Souza, A. C., Goto G. E. O., Mainardi, J. A., Coelho, A. C. V. andTadini, C. C. (2013).Cassava starch composite films incorporated with cinnamon essential oil : antimicrobial activity, microstructure, mechanical and barrier properties. *LWT-Food Scienceand Technology*, 54, 346-352.

[32] Taqi, A., Mutihac, L. and Stamatin, I. (2014).Physical and barrier properties of apple pectin/cassava starch composite films incorporating *Laurus nobilis* L. oil and oleic acid .*Journal of Food Processing and Preservation*, 38, 1982-1993.

[33] Tongnuanchan, P., Benjakul, S., Prodpran, T. and Nilsuwan, K. (2015). Emulsion film based on fish skin gelatin and palm oil physical, structural and thermal properties. *Food Hydrocolloids*, 48, 248-259.

[34] Vicentini, N. M. and Cereda, M. P. (1999).Uso de filmes de fécula de mandioca empóscolheita de pepino (*Cucumissativus*L.).*Brazilian Journal of Food Technology*, 12, 87-90.

[35] Yang, L. andPaulson, A. T. (2000).Effects of lipids on mechanical and moisture barrier properties of edible gellan film. *Food Research International*, 33, 571-578.

[36] Zavareze, E. R., Pinto, V. Z., Klein, B., El Halal, S. L. M., Elias, M. C., Prentice-Hernández, C. and Dias, A. R. G. (2012).Development of oxidised and heat–moisture treated potatos tarch film. *Food Chemistry*, 132, 344-350.

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Multinomial Logit Estimation of Income Sources by Watermelon Farmers in Northeastern Nigeria

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Abstract**—** *The main objective of the research was to use multinomial logit model to estimate income sources of watermelon farmers in northeastern Nigeria. A total of 434 farmers were sampled through multi-stage sampling procedure covering three Local Government Areas of Yobe state, Nigeria. The sources were personal savings, friends and relatives, Bank loans and cooperative/thrift societies. The results revealed that farm size, age and level of education were significant at 5% probability level and positively influenced the utilization of income from friends and relatives. Farmers' level of education, total cost of production and farm size significantly influenced farmers to obtain loans from banks. The marginal effects were 0.0504, 2.75 and 0.0038 showing the degrees of probabilities the variables can influence bank loans. Watermelon farmers can only obtain loans from cooperative and thrift society based on their farm size, total revenue, age, total cost and their level of output. These variables were significant at 1% and 5% probability levels with appropriate signs. The study concluded that 60% of the farmers fund their farm through personal savings and was difficult to get bank loans. It was recommended that micro-savings be encouraged among farmers and cooperative/thrift societies should be encouraged and adequately developed through the Non-Governmental Organizations.*

Keywords— income, Sources, Utilization, Farmers, Nigeria

I. INTRODUCTION

The survival strategies among rural households of developing countries are intertwined with agricultural activities. But financing the sector at the rural base level is a major predicament to both individuals, cooperate bodies and government (Tiku and Enoibor, 2012).

It is recognized that increase in finance and investment are needed at all the food chain, with special interest in increasing the access to finance by the agricultural households and communities that are most vulnerable to food insecurity and poverty. Source of agricultural financing is imperative to the development of agrarian economy, through financial services ranging from short-medium and long-term loans to leasing, to crop and livestock insurance, covering the entire agricultural value-chain in inputs supply, production and distribution, wholesaling, processing and marketing (Miller and Jone, 2010) .

The agricultural sub-sector is saddled with peculiar risks, risks that can hardly be diversified, calculated or quantified making it almost near impossible for commercialization. This factor has left rural farmers at the mercy of their little income and most times informal sector financing (Emmanuel and Enimus, 2015).

The need to finance agricultural activities is primarily to alleviate poverty among the rural poor in developing economy where 70% and above are employed. Fassil and Mekonnen (2016) observed that farm households diversify their income sources for at least two reasons: pull factors and push factors. The pull factor is diversification undertaken for asset accumulation objectives, whereas push factor is diversification undertaken to reduce vulnerability and build resilience to shocks. Increase financial support to agriculture could lead to capital accumulation. According to Jhingan (1999) in Emmanuel and Enimus (2015), the vicious Cycles of poverty in under-developed countries can be broken through capital accumulation. It is capital formation that leads to utilization of available resources. Thus, capital formation leads to increase in the size of the national output, income and employment, thereby solving

the problems of inflation and balance of payment and making the economy free from the burden of foreign debts. Muhammed and Haruna (2015) stressed that agriculture is and will continue to be a major building block in the achievement of the Millennium Development Goals (MDGs). Agricultural based small scale business (ASSBS) include businesses that engage in the supply of Agricultural inputs, services to farming/Agribusiness, trading produce, storing and transportation, processing and retailing of farm produce. Recent statistics shows that agricultural production needs to increase by 70 percent by 2050 in order to feed the world, while demographic growth, climate change and urbanization put pressure on available cultivable land (Muhammed and Haruna, 2005).

To support the laudable importance of agriculture in Nigeria, Adegoke, *et al* (2015) revealed that the Central Bank of Nigeria has established a USD 350millon risk sharing facility to reduce the risk of farmers and agribusinesses. It will also reduce interest rates paid by farmers from 18% to 8%. The Federal Government is also recapitalizing the Bank of Agriculture (BoA) to lend at single digit interest rates to farmers. Financial services include weather index-based insurance Schemes as proposed by Government, because many farmers will not be able to afford the cost of insurance premiums. In addition, subsidies were proposed to support and reduce the high fixed cost of insurance products. Area-based food insurance scheme is expected to be established in areas prone to floods. All this laudable programmes has remained on paper and implementation is near zero.

In 2017, cost of importation of food items into Nigeria remain very high, most homes go to bed hungry and agricultural productivity in the country is unsustainable. It is on the bases of this that Emmanuel and Enimus (2015), reechoed the Neo-classical growth theory of convergence thesis in conjunction with Cobb-Douglas production function, where output is a function of labour, capital and the level of technology and there are constant to each factor separately. Solow in 1956 opened a new chapter in development economics by pioneering an economic growth model based on the assumption that increasing capital accumulation and technical efficiency are the sources of economic growth. According to Thirwall (1999), capital accumulation is as much the endogenous consequences of growth as the exogenous cause growth.

In the Harrod-Domar model, the prime mover of the economy is investment and it has a dual role: create demand and capacity (Jhingan, 2007).

It is based on these roles we make attempt to investigate the level of involvement of commercial banks and other informal financial institutions in promoting the production of watermelon in the northeast of Nigeria.

The significant of the study is to underscore reasons that most watermelon farmers in the country are faced with the problem of sourcing for income to finance their agricultural activities. They rely on informal sources which are very precarious, unstable and un-assured. The findings will help the researchers to unravel the major determinants of farmers' choices of income source, which is very critical in agricultural development of the country.

The description of watermelon and the nutritional importance show that watermelon (*Citrullus lanatus*) is a member of the Cucurbit family (Cucurbitaceae). The crop is grown commercially in areas with long frost free warm periods. Seed requirement is 3kg/ha. Nutritionally, an average fruit is made up of 93% water by weight and about 7% consists of small amount of protein, fat, mineral and vitamin (Adekunle *et al*, 2003). The major components of the fruits are carbohydrates and vitamins.

Table.1: Nutritive Value Per 100kg edible portion

Nutrients	**Calories**
Energy	16.0 kcal
Protein	0.2 g
Fat	0.2 g
Carbohydrates	3.3 g
Calcium	11.0 mg
Phosphorus	12.0 mg
Iron	7.9 mg
Thiamine	20.0 µg
Riboflavin	40.0 µg
Vitamin C	1.0 mg

Source: Adekunle *et al*, 2003

Generally, the study will uncover the necessity of agricultural financing; it is an attempt to recognize the financial needs of the entirety of agricultural value chain in watermelon production. The study will advance knowledge of identifying the income gaps among farmers which is a major force that drives agricultural processes.

The paper is an exposition of how watermelon farmers do respond to their specific requirements for obtaining credit supply. It is a tailored approach designed to monitor the dynamics of farmers' choices in sources of income to finance production in the face of limited assistance from government and the unwilling nature of commercial banks to make agricultural financial supply to the farmers a priority.

The specific objectives of the study are to:

i. identify the major sources of income to watermelon farmers;

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ii. estimate the determinants of the choice of the source of income; and

iii. make policy recommendations for the enhancement of watermelon production in the area.

II. MATERIALS AND METHODS

Study area: the research was conducted in the 2015 and 2016 farming season in Yobe State northeast of Nigeria. The State was carved out from Borno State on 27th August, 1991. The State is predominantly a rural State with only five medium size fairly populated towns viz: Damaturu, Potiskum, Gashua, Nguru and BunuYadi. The State has 17 Local Government Areas (L.G.As.). The study was conducted in three LGAs. Purposive sampling method was used to select Bade, Nguru and Potiskum LGAs because the form the major watermelon producing fringe of Lake Chad agro-ecological zone.

Data Collection: primary data was collected from watermelon farmers and traders in Bade, Nguru and Potiskum using a structured questionnaire. The questionnaire was administered by qualified enumerators drawn from Federal University, Gashua, Yobe State and the State ministry of Agriculture. The multistage procedure was employed to select respondents randomly among the watermelon farmers. Proportionality factor was applied to select the respondent in relation (ratio) to the sample frame obtain from the water melon farmers association. With this, Hundred and thirty (130) farmers from the 10 political wards of Bade, One hundred and forty (140) farmers from the 10 political wards of Nguru and One hundred and sixty four (164) farmers from 10 political wards of Potiskum, making a total of four hundred and thirty four (434) respondents used for the study.

Table.2: Variables used in the multinomial logit model

Variable Name	Nature of Variable	Unit	Variable description
Dependent variable sources of income	Discrete	1	Personal savings
		2	Friends and relatives
		3	Bank loans
		4	Cooperative/thrift society
Independent variables			
Output	Continuous	100Kg = 40 fruits of watermelon	Total output is meant to be an asset/incentive to attract bigger loans from banks
Years of farming experience	Continuous	Years= No. of years spent in cultivating watermelon	It was hypothesized to positively/negatively influence a household to use a better source of income to improve on his production.
Household size	Continuous	No. of persons living together	It was hypothesizes to positively influence better sources of income. As more persons in a household will mean more family labour and higher productivity.
Total cost	Continuous	Total cost of production	In Naira: it is expected that the higher the cost of production the higher the demand for money.
Revenue	Continuous	Total revenue minus total variable cost	It was hypothesized that higher revenue will lead to better standing in the bank to obtain better financial assistance.
Age	Continuous	No. of years of the household head	Age of household can be a proxy to experience and was hypothesized to positively influence a household to select a given source of income.
Level of education	Continuous	Schooling No. of years	Education of household head in years was hypothesized to influence the farmer, more years in school meant higher probability to select a higher source of income.

Source: Survey data, 2017

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Analytical Framework: The descriptive statistics: that is the use of Tables, Charts and graphs was employed to describe socio-economic characteristics of the respondents. In order to determine the factors that influence the choices of sources of income, the multinomial logit was employed. Choices involving more than two alternatives can be best explained by probit or logit model and predict the probability that an individual with certain set of characteristics chooses one of the alternatives. The models could be multinomial logit, conditional logit and multinomial probit. In this case the multinomial logit was used. The four sources of income available to watermelon farmers identified were: Personal Savings, Bank Loans, Friends and relatives and co-operative/thrift societies.

Since we are dealing with categorized dependent variable, numerical values were assigned to the qualitative variables (dummies)

1 = Personal Savings

2 = Friends and Relatives

3 = Bank Loans 4 = Cooperatives/Thrift Societies.

The farmer has four alternatives having no particular ordering. The probability that the i^{th} farmer uses alternative j is $P_{ij} = \rho$ [individual I chooses alternative j].

Setting up the model structure

Assuming a single explanatory factor X_i in the multinomial logit specification (Hoffman and Duncan, 1988)[10] the probabilities of individual I choosing alternative j = 1,2,3, and 4 are:

$$P_{11} = \frac{1}{1 + exp(\beta_{12} + \beta_{22}x_i) + exp(\beta_{13} + \beta_{23}x_i), j = 1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

$$P_{12} = \frac{exp(\beta_{12} + \beta_{22}x_i)}{1 + exp(\beta_{12} + \beta_{22}x_i) + exp(\beta_{13} + \beta_{23}x_i), j = 2} \quad \ldots\ldots\ldots\ldots\ldots 2$$

$$P_{13} = \frac{exp(\beta_{13} + \beta_{23}x_i)}{1 + exp(\beta_{12} + \beta_{22}x_i) + exp(\beta_{12} + \beta_{23}x_i), j = 3} \quad \ldots\ldots\ldots\ldots\ldots 3$$

$$P_{14} = \frac{exp(\beta_{14} + \beta_{24}x_i)}{1 + exp(\beta_{12} + \beta_{22}x_i) + exp(\beta_{14} + \beta_{24}x_i), j = 4} \quad \ldots\ldots\ldots\ldots\ldots 4$$

The parameters specific to the 1st, 2nd, 3rd and 4th alternative sources of income are β_{11} and β_{21}, β_{12} and β_{22} and β_{14} and β_{24} respectively. To solve an identification problem and to make the probabilities sum to one, the parameters of the last (j^{th}) or the most frequently use source of income set to zero. In this case personal savings was set to zero.

In this report 434 farmers were investigated, our objective is to understand the determinants that lead a farmer to use a particular source of income against other alternatives. The factors included in the explanatory variables are output of the farmer, years of farming experience, household size, total cost of production, revenue generated, age of the farmer and level of education.

$P_{ij} = \rho$[individual use of income alternative j] we consider that

Y_{i1}, Y_{i2}, Y_{i3} and Y_{i4} are personal savings, friends and relatives, bank loans and thrift societies as indicators of source of income by individual i.If personal savings is used;

$Y_{i1} = 1$, $Y_{i2} = 0$, $Y_{i3} = 0$ and $Y_{i4} = 0$ ……5

If friends and relatives is used

$Y_{i1} = 0$, $Y_{i2} = 1$, $Y_{i3} = 0$ and $Y_{i4} = 0$ ……6

If Bank loans

$Y_{i1} = 0$, $Y_{i2} = 0$, $Y_{i3} = 1$ and $Y_{i4} = 0$ ……7

If cooperative /thrift societies

$Y_{i1} = 0$, $Y_{i2} = 0$, $Y_{i3} = 0$ and $Y_{i4} = 1$ ……8

Generally, the Multinomial logit defines probabilities as a function of X_i of unknown parameter μ

$P_1 = (P_5X_i, \Theta)$ ……9

In the standard MNM, the probability function defined as by Maddala (1983)[11], Wanyaina *et al* (2010)[12], the reference source of income is Personal Savings. Hence, for each source of income there are 4 – 1 =3.

A farmer is likely to use at least more than one income source depending on his socio-economic characteristics. The decision to use a particular source of income is a behavioural response arising from a set of alternative and constraints facing the farmer. In this study, the alternative is as earlier defined.

III. RESULTS AND DISCUSSION

The socio-economic factors among farmers that influences the use of particular sources of income for water melon production in Yobe State is presented in Table 2. The variables also used in the model in Table 3 reveals the percentage of sources of income utilized by farmers. Personal savings rank highest 59.45% among alternative sources of financing watermelon in Yobe State as revealed in Table 3. The impact of Bank loan is very small as it is hardly accessed by farmers in the study area. Reasons might not be far from factors of illiteracy, ignorance, interest rate, administrative bottlenecks, cultural barriers etc.

Table.3: Distribution of watermelon farmers by source of income

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S/No.	Source of income	No. of respondents	Percentage
1	Personal Savings	258	59.45
2	Friends/Relatives	77	17.74
3	Bank loans	35	8.06
4	Cooperatives/Thrift Society	64	14.75
	Total	**434**	**100**

Source: Survey data, 2016

The religion factor is also very opposed to loan collection in the areas. The people of the area are predominantly Muslims and they seldom take credit facilities that have to do with interest payment. That is why the result shows that 91.94% of source of financing watermelon production in Yobe State surround personal savings, friends/relatives and thrift societies.

The descriptive statistics of the respondents and its implications is given in Table 4. It revealed that the average output of watermelon in the study area is 882644.2kg per hectare and the mean farmer's years of experience is 14 years. This means that watermelon farmers in Yobe State have sufficient farming dependence to guide them take sacrosanct decision in terms of where, when and how to obtain credit facilities in funding watermelon production. The Table 4 equally shows that the average household size is 16 persons. This figure agreed with the practice of polygamy in the area where a man is permitted to marry up to four (4) wives despite their socio-economic statues.

Table.4: Descriptive statistics on sample characteristics of watermelon farmers

Variables	Variable description	Mean	Std. Deviation
Sincome	Sources of income	1.797235	±1.100238
Outpkg	Output	882644.2	1174024
Yrsfexp	Years of farming experience	14.19816	10.32911
Hhs	Household size	16.23963	15.31298
Fsize	Farm size	3.814516	4.48807
Tcost	Total cost	142190.9	92971.23
Revenue	Revenue	1277539	2498079
Age	Age of farmer	39.72333	12.23625
Ledu	Level of education	8.605991	5.396009

Source: Survey Data, 2016.

The average farm size is four (4) hectares. This is possible because Yobe State has large expand of Sahel Savannah land, which most times left uncultivated. So, farmers take advantage of the availability of land in the area to cultivate large farm size without necessarily having a corresponding harvest per unit area. This study negated some literatures that conclude that most arable crops are cultivated within 1 to 2 hectares of land in Nigeria (Amalu, 2005).

The Table 4 further revealed that the average age of household head is 40 years. The implication is that most farmers are in their active age and they have the capacity to access financial facilities to boast their production if given opportunities. In this age bracket they have acquired sufficient experience in life to take risks in farm management decisions, including acquisition of farm income financing and risk taking. The Study agreed with Reddy *et al.,* (1990) that agricultural production is confronted with risk and uncertainty condition, as agricultural production being biological and seasonal in nature.

The study revealed that average revenue is ₦1277539 and average cost of production is N142190.9 making about 88.9% profit. This is possible because the cost of maintaining watermelon farm in the study area is quite low and the existence of high patronage for it an attractive means of income. The average schooling period is 8 years. The meaning is that most watermelon farmers stop schooling after primary school and 2 years of possible Arabic education or the entire six years in Arabic education and no higher school. The implications are that majority of the respondent can only read and write in Hausa and Arabic but little western education literacy. This has affected farmers greatly, because illiteracy inhibit farmers from accessing bank loans and instill in them fear of expansion in their scope of business and acquisition of modern technologies and innovations. The importance of education in capacity building cannot be overemphasized in this regards as nobody or a nation can grow above his/her level of education.

Multinomial logit Results

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Friends/relatives: The result of multinomial logit revealed in Table 5that factors that influence watermelon farmers to get their sources of income from friends and relatives include age and level of education and farm size; there are significant at 5%,5% and 1% probability level respectively. The result of the marginal effect in Table 6 also revealed that level of education was positive and significant at 5% implying that a 1percent increase in the level of education of the respondent increases the probability of the farmer getting credit facility from friends and relatives by 0.45percent. As farmer get older the chances of getting loans from friends and relatives decreases by 0.17%, this is understandable because as farmers get older they tend to have accumulated resources that can sustain their farming cost, equally they become more risk conscious and avoidance. Meanwhile, increase in farm size will open up more opportunities for farmers to get loans from friends and relatives by probability level of 4%.

Bank loans

The level of education, total revenue and farm size where significant at 5% probability level as revealed in Table 5 and 6. The marginal effect of the level of education was negatively related to bank loans. The inverse relationship exhibited show that an increase in the level of education by 1%, the probability of obtaining loan by watermelon farmers from banks reduces by 0.38%. The result is in disagreement with a priori expectation. It is expected that increase level of education should get the farmers more opportunities to gravitate towards obtaining loans from banks. The negative sign may not be unconnected to the earlier reasons tied to their religion and cultural belief and other discouraging element from the bank especially the area of insufficient initial bank deposit and collateral facilities. More so, total cost was significant at 10% showing that the marginal effect was positive, an increase in total cost by 1% will increase the probability of farmers obtaining bank loan by 9%. Farm size is also very critical in farmers getting loans from banks. Farm size was positively significant at 5%, revealing that a unit increase in farm size will bring about 0.038% probability of getting loan from the bank.

Table.5: Multinomial logistic regression results

Friends and Relatives

Variables	Coeff.	STD Error	Z	P>/Z/
outpkg	2.48e-07	1.78e-07	1.39	0.165
hhs	.0123315	.0110369	1.12	0.264
ledu	-.0619127	.0343521	-1.80	0.071
yrsfexp	-.0068663	.0285903	-0.24	0.810
age	.0294865	.0153734	1.92	0.055
tvc	5.39e-06	3.50e-06	1.54	0.123
tr	-3.81e-08	1.30e-07	-0.29	0.770
fsize	-.353887	.0958012	-3.69	0.000
cons	-1.803768	.8246075	-2.19	0.029

Bank loans

Variables	Coeff.	STD Error	Z	P>/Z/
outpkg	-2.76e-07	2.21e-07	-1.25	0.211
hhs	-.0161975	.014204	-1.14	0.254
ledu	.0856162	.0422189	2.03	0.043
yrsfexp	.0413835	.0311402	1.33	0.184
age	.0109238	.0191073	0.57	0.568
tvc	.0000103	3.42e-06	3.00	0.003
tr	1.20e-08	9.91e-08	0.12	0.904
fsize |	-.1795159	.0994485	-1.81	0.071
cons |	-3.9317	1.067693	-3.68	0.000

Co-operative/Thrift Society

Variables	Coeff.	STD Error	Z	P>/Z/

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outpkg	-6.84e-07	2.75e-07	-2.48	0.013
hhs	-.0081796	.0114432	-0.71	0.475
ledu	.0364356	.033604	1.08	0.278
yrsfexp	.0352179	.0245082	1.44	0.151
age	.0353457	.0151214	2.34	0.019
tvc	.0000136	3.56e-06	3.81	0.000
tr	1.49e-07	6.80e-08	2.19	0.028
fsize	-.4499722	.1258513	-3.58	0.000
cons	-3.337394	.8662805	-3.85	0.000

(sincome==1 is the base outcome) LR chi2(24) = 85.36 Prob> chi2 = 0.0000
Log likelihood = -307.45366 Pseudo R2 = 0.1219

Table.6: Marginal Effect (probabilities) After Multinomial Logit

Variable	Personal income	Friends/ Relative	Bank loan	Cooperative/Thrift Society
Output	-6.840e-7*	-9.13e-07*	-3.95e-07*	-8.80e-09*
Yrsexp	-.0003005**	-.0044289**	.0021825**	.0044956*
Hhs	-.0013792**	-.004744*	.0012356**	-.0018822*
Fsize	.0399668***	-.0112483***	.0003832**	-.0172e07**
Tcost	8.09e-07*	-9.66e-07*	2.75e-09***	5.03e07*
Revenue	-4.80e07*	-2.66e-07*	8.56e08***	1.16e-07*
Age	-.0017693**	.0044965**	-0006271*	.0012642*
Ledu	.0045307**	-.0032717**	-.0050494*	.00982*

N/B *, **, and *** = Significant at 1%, 5% and 10% respectively
Source: Survey data, 2016

Cooperative/thrift society

The result of the multinomial logit shows that output, age, total cost, total revenue, and farm size was significant at 1% probability level respectively. The marginal effect indicated that a 1% increase in output will lead to a less than 8% decrease in the use of cooperative/thrift society as a source of income to support the farming activity. Age had a positive coefficient and a 1% increase in age will lead to 1.3% probability of obtaining loan from cooperative/ thrift society. Meanwhile, total cost was positively significant and the marginal effect revealed that a 1% increase in total cost will result to a 5% probability of getting credit facilities from cooperative/ thrift society. Total revenue also had a positive sign shows that a 1% increase in total revenue will provide a 12% probability of farmers to obtain loans from cooperative/thrift society. Finally, Farm size equally had a significant influence on farmers collecting loans from cooperative/ thrift societies. A unit increase in farm size will lead to a less than 0.19% probability of farmers obtaining loans. The implication is that as farmers get larger farm land, the expansion will necessitate farmers to source for bigger and more stable sources of income to finance their farms.

IV. DISCUSSION

The current findings revealed that personal savings ranked highest as of source income in financing watermelon production activity in the study area. The result is in consonant with Fassil and Mekonnen (2016). Their study on the determinants of off-farm income diversification show that personal income accounted for 51%. They opined that reduction in poverty can only be achieved through removal of entry to barrier to off-farm activities (access to finance, market, education and infrastructure) needs to overcome and expanded by government.

The study equally discovers that accessibility to loan facility is very difficult. Kirsten and Moldenhauer (2006) acknowledges this, when they carried a survey in South Africa, with a conclusion that households have multiple livelihood strategies with agriculture generally playing a small role in the house income generation thereby needing external interventions. But Nandudu (2017) echoed that banks still don't trust farmers with agricultural loans. Smallholders' farmers have to look for alternatives of financing if they are to increase production and upgrade to commercial farming. "Providing credit to small holders farmers involves large transaction cost for a financial institution. This makes it hard to asses a farmer who is relying on his collateral to get loan because he or she may

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not be sure of what he will get to pay the loan, making it more risky" (Nandudu 2017).

The current research unravel that education is very imperative in decision making on choices of loan for agricultural activities and poverty reduction. The finding is in agreement with Janjua and Kamal (2011) on the study of the role of education and income in poverty alleviation. They concluded that income growth plays a moderately positive role in alleviating poverty, but that income distribution does not play a key role in poverty alleviation in the sample overall. Secondly, it concludes that education is the most significant contributor to poverty alleviation.

Education is important mechanism for enhancing both the financial and physical health of the farmers Feinstein *et al*, (2006), concluded that there are substantial and important causal effects of education on health productivity. Shirazi (1994) investigates the incidence of poverty and socioeconomic profiles of the poor in Pakistan, and concluded that as the educational level of the head of the household increases the probability of that household being poor decreases.

The study also discovers that cooperative society is vital among water melon farmers in Yobe State. The relevant of cooperative in a system is a function of its viability. Most farmers with sizable land holders, years of farming experience and adequate output had access to credit facilities from cooperative/ thrift society. Bello (2005) discussing the role of cooperative societies in Economic development posited that for over 160 years now cooperative societies have been an effective way for people to exert control over their economic livelihoods as they play increasingly important role in facilitating job creation, economic growth and social development. Underscoring the importance of cooperatives Najamuddeen *et al* (2012) appealed to government to intensify its effort in financing capacity building and provision of technical facilities to cooperative societies.

V. CONCLUSION

From the foregoing, the probability of getting money from friends and relatives depends on age, farm size and level of education. Obtaining loan from bank has to do with farmers' level of education, total cost and farm size indicating their repayment ability. The cooperative/thrift societies can give loans to watermelon farmers on the basis of their output, age, farm size, total cost and total revenue; equally all these are indicators for possible repayment of the credit facility. It is concluded here that 60% of the watermelon farmers gets their money from personal savings to fund their farm activities. Majority of watermelon farmers (92%) fund their farms from informal sources and only 8% can have access to banks loans.

RECOMMENDATIONS

From the finding, it is recommended that micro-finance banks, Bank of Agriculture and Commercial banks should make loans available to watermelon farmers by using their farm lands as colla teral, and encouraging opening of micro-saving accounts to improve on their bank relationship and familiarization of banking formalities. NGOs should educate the farmers on the benefit of cooperative societies. The farmers could pool their sources together to take the advantage of economies of scale in terms of buying inputs and marketing their produce.

Government should establish factories that can utilize the watermelon and if possible convert it into juice and other derivatives. With this the farmers can be sure of ready market and banks could be willing to give loans to farmers because default rate of repayment will be reduced.

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REFERENCES

[1] Adegoke, J., Ibe, C. and Araba, A. (2015). A Report by Advisory Committee on National Agricultural Resilience Framework in Nigeria. Federal Ministry of Agriculture and rural Development Pp: 65 Garki, Abuja: Green Ink, www.fmard.gov.ng

[2] Adekunle, A.A., Fatunbi,A.O.,Adisaando, S. and Adeyemi, A. (2003). Growing watermelon Commercially in Nigeria. An illustrated guide IITA/FAO Publication www.fao.org/sd/erp//w/kit/bwks-illust.guidebook

[3] Amalu, U.C (2005). Poverty Alleviation and Agriculture in Sub-Saharan African: The Nigerian Experience. *J.food Agri. and Envi. 3(2): 230-236 Helsinki Finland: WFL Publisher.*

[4] Bello, D.A. (2010). The Role of Cooperative societies in Economic Development Online at http://mpra.ub.uni-muechende/23161. MPRA paper no.23161 Date download 15/07/2017

[5] Emmanuel, OE and S.Enimus (2015). Financing Agriculture to support Economic Development in Nigeria: the need to bank to Re-strategies. Proceedings of 29th Farm Management Association of Nigerian

annual National conference (Ed) Haruna, U., Izge,A.U., Abdulhamid, A. IIiyasu, A., AbdurrahmanS.L andKatanga, Y.N Agriculture: A Renewed focus for Economic Development in Nigeria. 23rd -26th November, 2015. PP 582-587

[6] Fassil, E. and E.Mekonne (2016)..Determinants of off farm income diversification and its effect on rural household poverty in GamoGofa Zone,Southern Ethiopia. *J. Dev. Agric. Econ. 8(10):217-227. DOI: 10.4897/JDAE 2018-0736, 471CD8760570, ISSN: 20069774*

[7] Jhingan, ML.2007. Macro-economic theory, 11th revised Edition,Delhi: Vrinda publication (p) Ltd.8187125454 pp:60 ISBN: 8187125454

[8] Feinstein L., Sabates,R., Anderson T.M. Sorhaindo, A. and Hammond, C. (2006). Measuring the effects of education on health and civil engagement: proceedings of the Copenhagen symposium, @OECD 2006, date downloaded 15/07/2017

[9] Hoffman, SD and GJ Duncan (1988). Multinomial and Conditional Logit Discrete – Choice Model in Demography. Demography 25(3): 415-427

[10] Janjua, P.Z. and Karmal,U.A. (2011). The role of Education and Income in Poverty Alleviation; A Cross Country Analysis. The Lahore Journal Of Economics 16(1):143-172, date download 16/07/2017

[11] Kirsten, J. and Moldenhauer, W (2006). Measurement and Analysis of Rural Household Income in a Dualistic Economy: The Case of South Africa. UPSpace – Natural and Agricultural Science. URL Repository http://hdl.handle.net/2263/2286

[12] Maddala, G.S (1977). Econometrics (Ed). MacGraw-New York, USA: Hill BookCompany,pp.516,

[13] Miller, C and L.Jone (2010). Agricultural Value Chain Financing: Approach, Tools, Lessons and innovations. Practical Action: U.K Publishers. (Available at) download date 09/29/2016

[14] Muhammed, Y. and TH.Haruna (2015). Sustainability impact of Microfinance Banks on the performance of Agric-Based Small Scale Businesses in Kaduna Metropolis. Proceedings of 29th Farm Management Association of Nigerian annual National conference Duste (Ed) Haruna, U., Izge,A.U., Abdulhamid, A. IIiyasu, A., AbdurrahmanS.L andKatanga, Y.N Agriculture: A Renewed focus for Economic Development in Nigeria. 23rd -26th November, 2015. PP 539-550

[15] Najamuddeen, G., Abubakar, B.Z., Kebber,M.G., Magaji, A.S. and Ukashutu., U. (2012). The role of cooperative societies in comminuty development in Sokoto State, Nigeria. *Scientific Journal of Review* 1(3):1 -15

[16] Nandudu, P. (2017). Banks still don't trust farmers with agricultural loans. Saturday Vision, July 15, 2017. Date download: 1507/07/2017

[17] Reddy, S.S., Ram, P.R., Sasty, N.T.V and Devi, I.B.(2004). Agricultural Economics, Oxford &New Delhi: IBA publishing Co. Pvt.Ltd, pp: 361 ISBN 8120415965

[18] Shirazi, N.S. (1994). An Analysis of Pakistan's Poverty and its Alleviation through hfaq (unpublished doctoral dissertation). International Islamic University, Islamabad.

[19] Tiku, NE and Enoibor, J H, (2012). Analysis of Resource use Efficiency in Yam Production in Yakurr Local Government of Cross River State, Nigeria. Agric.J 7(5):297-304 ISSN: 1816-9155

[20] Thirlwall, A.P (1999). Growth and Development: With special reference to Developing Economics (6th Ed) Chaitenhan: Edward Elgar, pp: 32-56

[21] Wanyama, M.1., Mose, L, Odendo, M., Okuro, J.O., Owuor, G. and .Mohammed, L. (2010). Determinants of income diversification strategies amongst rural households in maize based farming systems of Kenya.*Afric.J. Food Sci. 4(12): 754-763*

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Detection of Kids milk Quality using Methylene Blue Reduction test

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Abstract— Back ground and Objectives: *Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. Rapid, simple and inexpensive microbiological quality determination methods including Methylene Blue Reduction (MBRT) test could be commonly used as a quick method to assess the microbiological quality of raw and pasteurized milk. The aim of study is to determine quality of kids milk using Methylene Blue Dye Reduction Test*

Methods: *A total of 37 samples comprising of kids milk collected at different levels of collection and processed. Accordingly 12 different milk samples from hypermarket, 8 different milk samples from unlicensed hawker (retail market), 11 different samples with additives from hyper market samples and 6 different samples with high price. Samples were collected. One ml of the Methylene Blue Thiocyanate solution added into a test tube then 10 ml of milk poured into test tube. Tubes incubated at 37 ^oC*

Results: *Results showed that all types of milk that purchased from super market, local market and high price milk types shown no change of methylene Blue color appear on the base of time, that indicate very good quality of the milk. On the base of milk types with additive materials only one milk showed change in colour but after confirm test the colour remained blue and not changed.*

Conclusion: *Methylene blue reduction test is rapid economic method that can be used for detection of milk quality. Approximately all the kids of milks that is purchased in our market and local markets showed sterility and the source contamination if take place may be by storage condition and transvers vehicle.*

Keywords— kids milk, Methylene blue reduction test, decoulorization.

I. INTRODUCTION

Milk is the nutrient rich liquid secreted by the mammary gland of mammals. It provide the primary sources of nutrition for newborns before they are able to digest other type of food the early lactation of milk is known as colostrum's and carries the mother antibodies to the baby, it can reduce the risk of many diseases of the offspring. [1,4,5]

Good quality milk should have a pleasantly sweet and clean flavor with no distinct after taste. Because of the perishability of milk and the nature of milk production and handling procedures, the development of off-flavors/odors is not uncommon. To prevent flavor/odor defects in milk, proper milk handling procedures from the farm to the consumer are essential. These defects of milk smell may be classified according to; absorbed/transmitted, bacterial/microbial and chemical/enzymatic processes.6,[10,11,12,21]

Contaminated raw milk may act as a source of many harmful bacteria leading to various diseases, such as undulant fever, Salmonellosis, Dysentery and Tuberculosis. Raw milk with bacteria count below a specified limit is known as "certified" milk and is considered healthy. [2]

The shelf life of pasteurized milk can be affected by large number of somatic cells in raw milk. Increased somatic cell numbers are positively correlated with concentration of plasmin, a heat stable protease and of lipoprotein lipase in freshly produced milk. Activities of these enzymes can supplement those of bacterial hydrolases, hence shortening the time to spoilage. The major determinants of quantities of these enzymes in milk supply are the initial cell numbers of psychotropic bacteria, their generation times, abilities to produced specific enzymes, time and temperature at which the milk is stored before processing. [15]

Methylene blue reduction test is based on the fact that the color imparted to milk by the addition of a dye such as methylene blue will disappear more or less quickly. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism causes the color to disappear. The methylene blue reduction test has lost much of its popularity because of its low correlation with other bacterial procedures. This is true particularly in

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those samples which show extensive multiplication of the psychotropic species. [23]

The methodology employed the enzymatic reduction of methylene blue by a metabolically active organism turning the Methylene Blue colorless. [24]

Aim of the study: Our aim of this study was to determine quality of kid's milk that purchased from different Markets in Erbil City by using Methylene Blue Dye Reduction Test.

II. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 37 samples comprising of kids milk collected at different levels of collection and processed. Accordingly 12 different milk samples from hypermarket, 8 different milk samples from unlicensed hawker (retail market), 11 different samples with additives from hyper market samples and 6 different samples with high price. Samples were collected. All the samples were kept in an icebox and transported to the laboratory under chilled conditions within 2 hours and analyzed using Methylene blue dye reduction test method as described. [27]

Fig.2.1: (A& B) Different milk samples used in experiment

2.2. Methylene Blue Reduction Test:

All glassware and rubber stoppers Sterilized either in an autoclave or in boiling water. One ml of the Methylene Blue Thiocyanate solution added into a test tube then 10 ml of milk poured into test tube and shacked to mix dye with milk in test tube.

Tubes placed in the water bath immediately for incubation. The temperature maintained at 37 ^{o} C . 25, [28,29]

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Fig.2.2: Inoculation of milk samples into methylene blue

The initial time noted down and the test tube examined after half an hour then subsequent readings are taken at hourly interval and results are interpreted as follows:

Classification.–The suggested classification is listed.

Class 1. Excellent, not decolorized in 8 hours.

Class 2. Good, decolorized in less than 8 hours but not less than 6 hours.

Class 3. Fair, decolorized in less than 6 hours but not less than 2 hours.

Class 4. Poor, decolorized in less than 2 hours.

III. RESULTS

3.1. Sample from supermarket:

Different type of milk that purchased from super market and their quality are shown in Table 3.1 and figure 3.1, All milk types shown no change of methylene Blue colour appear on the base of time, that indicate excellent quality of the milk bellow.

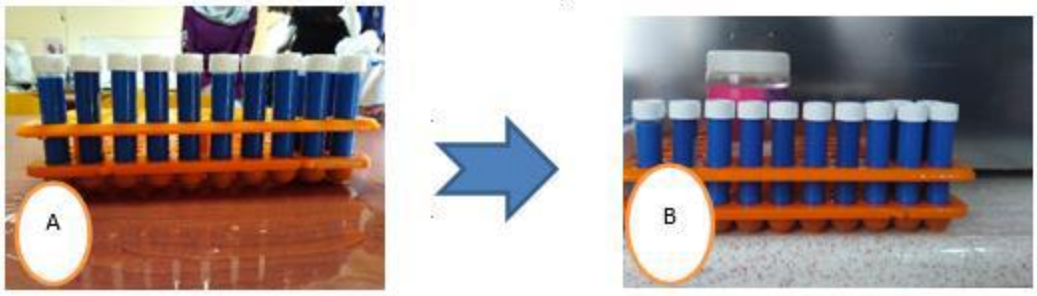

Fig.3.1: Different milk samples purchased from super market (Before and after incubation)

3.2. Samples from Retail Market: After purchased samples from retail market present or absence of bacteria studied and no change of Methylene Blue color appeared by the time table 3.2.and figure 3.2.

Table 3.2. Different milk samples purchased from retail market

No.	Type of Milk sample	Cod	Milk Quantity(ml)	Initial Time (hour)	Final time (hour)
✓	Almarai Milk	Am M	10	9.04 a.m.	-
✓	Yorsan Milk	Yo M	10	9.04 a.m.	-
✓	KDD Milk	K M	10	9.04 a.m.	-
✓	Hamaouda Milk	H M	10	9.04 a.m.	-
✓	Selin Milk	Se M	10	9.04 a.m.	-
✓	Alrabie Milk	Ar M	10	9.04 a.m.	-

✓	Sutas Milk	Su M	10	9.04 a.m.	-
✓	Sa Milk	Sa M	10	9.04 a.m.	-

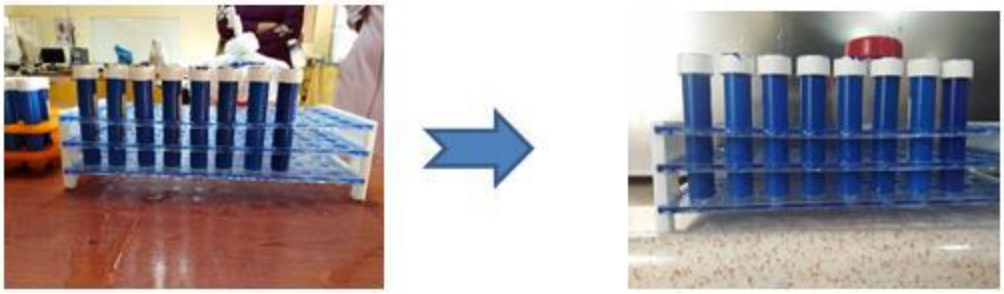

Fig.3.2: Different milk samples purchased from retail market

3.3. Samples from high per market with (High price 500-1000ID)

After purchased different type of milk with high price no change of colour obtained by time as shown in Table 3.3 and figure 3.3.

Table.3.3: Different milk samples from high per market (High price 500 ID)

No	Milk	Cod	Milk Quantity(ml)	Initial Time (hour)	Final time (hour)
1.	Shizer milk	Sh M	10	9.04 a.m.	-
2.	Kalleh milk	K M	10	9.04 a.m.	-
3.	Pinar Kido milk	Pk M	10	9.04 a.m.	-
4.	Safio milk	SaM	10	9.04 a.m.	-
5.	Nada milk	N M	10	9.04 a.m.	-
6.	Bouny milk	B M	10	9.04 a.m.	-

Fig.3.3: Different milk samples from high per market (High price 500 ID)

3.4. Sample of milk with different additives:

As shown in table and figure 3.4. After incubation of samples with methylene blue the color of Almarai (Chocolate) changed after 1.5 hours. So tests triplet by purchased samples from different market to confirm the result. That showed no change of color in confirm test.

Table.3.4: Different milk samples with different additives

No	Milk	Cod	Milk Quantity(ml)	Initial time(hour)	finalTime (hour)
1.	Pinar Kido (Strawberry)	Pk S	10	8.40 a.m.	-
2.	Pinar Kido (Chocolate)	Pk Ch	10	8.40 a.m.	-
3.	Kalleh (Banana)	K B	10	8.40 a.m.	-
4.	Kalleh (Straw berry)	KS	10	8.40 a.m.	-

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5.	Kalleh (Chocolate)	K Ch	10	8.40 a.m.	-
6.	Almarai nijoom (Strawberry)	Am S	10	8.40 a.m.	-
7.	Almarai (Banana)	Am B	10	8.40 a.m.	-
8.	Almarai (Chocolate)	Am Ch	10	8.40 a.m.	10.20a.m.
9.	Alarabie (Banana)	Ar B	10	8.40 a.m.	-
10.	Yorsan (Strawberry)	Yo. S	10	8.40 a.m.	-
11.	Yorsan (Chocolate)	Yo. Ch	10	8.40 a.m.	-

Fig.3.4: Different milk samples with different additives before and after addition of MB.

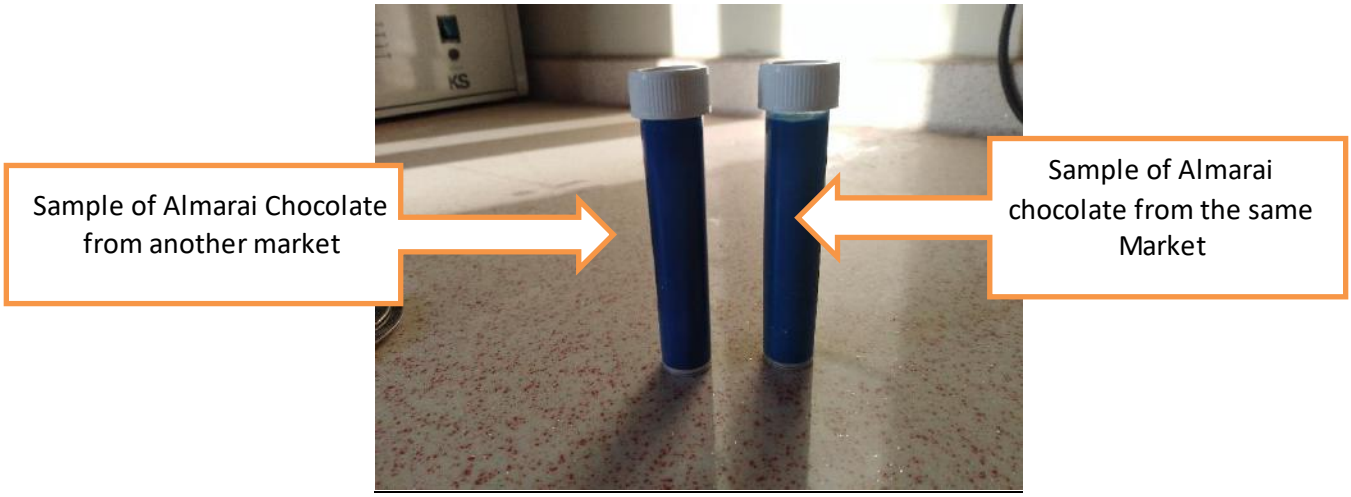

Fig.3.5: Repeat the test above to confirm the result by taking sample of milk from different source.

IV. DISCUSSION

Milk is valuable and consumed on daily basis. As milk contains fat, protein, carbohydrates, minerals, vitamins and other various ingredients dispersed in water, it is considered as a complete diet. at the same time, it is highly vulnerable to bacterial contamination and hence is easily perishable. [30]

Some of these bacteria that grow in milk, during the production of metabolites, may cause an unacceptable sensory alteration, such as off flavor, odor, change in texture or appearance, termed as spoilage. [31,32]

These microorganisms should be termed as specific spoilage organisms for milk, as other microorganisms may also grow in milk but without causing any sensory changes.

The microbial quality of raw milk is important for the production of dairy products and it also influences their shelf life. [33]

Milk of high quality contains a few hundreds of bacteria per milliliter whereas bad milk contains millions of bacteria per milliliter. [34]

The presence of coliform bacteria in milk points out fecal contamination. [35]

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Methylene Blue Dye Reduction Test, commonly known as MBRT test is used as a quick method to assess the microbiological quality of raw and pasteurized milk. This test is based on the fact that the blue color of the dye solution added to the milk get decolorized when the oxygen present in the milk get exhausted due to microbial activity. In our study when collect sample of milk that from super market and used Methylene Blue Reduction tests to determine their quality as shown in Table (3.1 -3.2) and figure (3.1-3.2). All milk types shown no change of Methylene Blue color appear on the base of time, that indicate excellent quality of the milk bellow repeated the test to confirm the results and get the same result the sample were collected from supermarket low price (250 ID).

Collected high price kids milk (500-1000ID). After addition of Methylene Blue into the milk samples and detect the quality using time as showed in table 3.3 and figure 3.3. That the color of samples never changed by the time this indicate that the quality of the samples that collected from super market was in excellent quality. In both tests can see that the milk samples was posturized and stored in a good conditions such as temperature and external damage of packet or bottle that the milk present in it.

When studied the quality of milk by using the same method Methylene blue reduction test for the kid milk with additive materials such as addition of fruit or flavors into the milk we found that one of the samples as showed in table 3.4. and figure 3.4 the color of one of the milk with chocolate flavor changed after 2 hours that indicate present of bacteria and reduced the coloure by breakdown the methylene blue colure and the chocolate color appeared to confirm this test we repeated the test by getting the same sample from same market and another market . found that color never changed because of change the color in the first test is one of the factors that cause spoilage of milk such ad damage of milk bottle or present a pore in the milk sample that help inter of bacteria and grow in the milk sample.

When done experiments of the types of milk on the market were changing the color to white means that were contamination. Emphasis of contamination repeats the test for same type of the milk (chocolate) in the same market it does not change the color. It meaning that were not contamination may the first time contamination. When transfer from the factory to the market or services drop of the milk cause vent packed of the milk, may not well storage.

From the study that done by on raw, pasteurized UHT milk samples collected from different locations in Bangladesh assay it can be concluded that the microbial were not satisfactory as indicated by their high bacterial loads and presence of coliform bacteria. However after pasteurization and UHT treatment they were found to be safe for the consumers. Even after this, proper refrigeration temperature should be maintained particularly in case of detection of the quality of the milk using MBR test. [36]

Collected 240 raw milk samples and 72 pasteurized milk samples from different places of Madurai District for a period of six months and were analyzed for microbial quality. Among the raw milk samples only 19.1% of samples were good quality and 28.3% are poor quality. In the pasteurized milk samples 81.9% of samples were excellent for human consumption.[11]

V. CONCLUSION

- From this research work, it is clearly seen that all type of kids milk were sterile and no bacteria detect in milk.
- Its save to consume all type of kids milk that examined in the research with care to storage condition that include storage temperature.
- Present or absence of pore on the surface of milk can or containers.

VI. RECOMMENDATION

1- Using plate count and molecular studies to confirm the test.
2- Study on Detection of adulteration in milk Most of the chemicals used as adulterants are poisonous and cause health hazards.
3- Our recommendations for consumers obtain milk that keep in refrigerator because combination of preservative coupled with subjection to pasteurization and refrigeration could help in extending the shelf-life.
4- Checking expiration date.
5- Checking storage temperature.
6- Checking tetra pack products have vent or concavity.
7- Sure the milk product protective to sun light.
8- Milk products put into sterile tetra pack shelf-safe carton when opened sure it storage in clean place.

REFERENCES

[1] Meher R.K, Sathy S, Nayak N. Qualitative analysis and microbial test of pasteurized milk . International Journal of Pharmacy and Biological Sciences 2015; 5 (2) :215-219.
[2] Abbas N M, Khattak B, Sajid A, Islam U. T, Jamal Q, Munir S. Biochemical and bacteriological analysis of cow's milk samples collected from district Peshawar ,

International Journal of Pharmaceutical Sciences Review and Research 2013; (21) 2: 221-226.

[3] Sourav B., Kamal K., Aftab U . Microbiological quality analysis of raw, pasteurized, UHT milk sample collected from different locations in Bangladesh. Stamford Journal of Microbiology 2014; (4)1: 5-8.

[4] Popescu A. and Angel E. Analysis of milk quality and it is importance for milk processors. University of Agricultural Sciences and Veterinary Medicine Bucharest, Romania; 2009; (42) 1 : 501- 506.

[5] Kajal M. F. I., Wadud, A. Islam M N and Sarma1 P. K. Evaluation of some chemical parameters of powder milk available in my men Singh town . J. Bangladesh Argil University 2012; (10) 1: 95-100.

[6] Kahuta G. Milk Quality and on-farm factors leading to milk spollagein bugaaki SUB count – kyenjojo district. Masters of science degree in livestock development, planning and management (MLD) of makorere university. 2013;15. http://ddl. Handle.net/10570/2097.

[7] Banik K S, Das K . K., Uddin A. ,Microbiological quality analysis of raw, pasteurized UHT milk samples collected from different location in Bangladesh . Journal of microbiology. 2014; (4) 1:5-8.

[8] Anwer S.S, Ali A.G, Hamadamin Z.C, Jaafar Y.H. Isolation and identification of fungi from fast food restaurants in Langa bazar ,International journal of environment . agriculture and biology .2017;(12) 4 : 1517-1522.

[9] Ledenbach HL, Marshall TR. Type of spoilage of microorganism. Microbiological spoilage of dairy products. 2009; (13) 7: 41-67. https://pdfs.semanticscholar.org/9acd/74648432f4db848d8d19d9f1f8ef474111b0.pdf.

[10] Banic.SK, Das. K.K, Uddin.MA . Micribiological quality analysis of raw pasteurized, UHT milk samples collected from different locations in Bangladesh). Stamford journal of Microbiology 2014; (4) 1:5-8.

[11] Nandy SK, Venkatesh KV. Effect of Carbon and Nitrogen on the cannibalistic behavior of B. Subtilis. Appl. Biochem. Biotechnol 2008; 151: 424-432. (DOI 10.1007/s12010-008-8210-7).

[12] Nandy SK, Bapat P, Venkatesh KV. Sporulating Bacteria Prefers Predation to Cannibalism in Mixed Cultures. FEBS Lett 2007; 581: 151-156.

[13] Jackson CJ. Factors in the Reduction of Methylene Blue in Milk. Journal of Dairy research 1936; (7) 1: 31-40.

[14] Grag S.R. and Usha Mandokt V. Reliability of routine quality control test for grading raw milk sample, 1997;3: 1-16.

[15] Singhal RS, Kulkarine P, Rega DV. Indices of Microbial quality in milk and milk products. Handbook of indices of food quality and authenticity. Chapter 4. 1997; Cambridge. England .

[16] Banik S, Das K, Uddin M. Microbiological quality analysis of raw, pasteurized, UHT milk samples collected from different locations in Bangladesh. Stamford Journal of Microbiology. 2014; (4) 1: 5-8.

[17] Haug A, Hostmark A T and Harstad OM. Bovine milk in human nutrition . Lipids Health. Dis. 2007; 6: 25-34.

[18] Winterhoff C, Beyerbach M., Homuth M., Strutzberg K. and Gerlach, G.-F. Evaluation of an ELISA for the detection of Mycobacterium avium subspecies paratuberculosis-specific antibodies in milk. Deutsche Tierarztliche Wochenschrift. 1998; 109: 230-4.

[19] Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B. and Givskov, M. Food Spoilage - interactions between food spoilage bacteria. International Journal of Food Microbiology. 2002; 78: 79-97.

[20] Harding F. Milk Quality, 1st Edition, Chapman and Hall India, 1995; 16-20, 4547.

[21] FAO. FAO Manuals for Standard Examination of Food.1996; pp 1-30.

[22] Dairy Development Authority (DDA), (2001) Annual Report, Kampal.

[23] Sudhasaravanan R, and Binukumari S. Microbial Quality of Raw and Pasteurized Milk Samples Collected From Different Regions of Madurai District, (T.N) India.IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 2015; 9: 7

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Study on heavy metals levels and its risk assessment in edible fish (*Himantura imbricate*) from Persian Gulf

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***Abstract*—** *Heavy metals are contaminants of great environmental concern due to their multiple origins (natural and anthropogenic), the ability to accumulate in organs and tissues, and the deleterious effects they can cause in organisms. Studies on the accumulation of metals in seafood, such as fish, have increased in importance due to the risk for human health when consuming fish contaminated by metals. The present work was aimed at verifying the concentrations of cadmium (Cd), Nickel (Ni) and lead (Pb) in the muscular tissue of Himantura imbricate (from the Persian Gulf in Hormozgan province, Iran. Samples were analyzed by Atomic Absorption Spectroscopy. There were significant variations among heavy metal accumulation levels of the species and their regions. The heavy metal concentrations found in regions varied for Cd: 0.14, Ni: 0.33, Pb: 0.02 in Qeshm and Cd: 0.25, Ni: 0.48, Pb: 0.03, µg/g in Suoroo. The heavy metal concentrations of fish in Qeshm were lower than those of fish from Suoroo regions. This research showed that heavy metal concentrations in muscle of investigated specie were also lower than the maximum levels set by law.*

Keywords*— *Himantura imbricate, risk assessment, Atomic Absorption Spectroscopy and Persian Gulf.

I. INTRODUCTION

In the recent years, world consumption of fish has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. In addition to its important source of protein, fish typically have rich contents of essential minerals, vitamins and unsaturated fatty acids [1]. Among the pollutants, non-degradable pollutants (persistent pollutants) such as heavy metals in sediments and mud and sludge concentrated as potential marine pollution and at the same time accumulate in aquatic and body tissue and concentrated And fish consumption may be toxic to humans and severe adverse effects such as disorders of the nervous system, renal, genetic mutations, and so on to be created, It is of utmost importance. Among the heavy metals Pb, Ni, and Cd indices, oil pollution and pollution from industrial activities in the marine ecosystem, the capacity of ecosystems to accept the changes in the environment and although, by its nature has the ability to cope with change But today it is clear that destruction has been the speed of natural regeneration. And the process for irreversible environmental degradation is growing, so measures to protect the environment should ponder [2]. In addition, today one of the major concerns in the discharge of heavy metals into the marine environment is all over the world. And is well established that heavy metals cause toxicity and accumulation of ecological significance are many, these elements have devastating effects on the marine ecosystem and species diversity [3]. Lead one of four metals that have the most damaging effects on human health. Bio-synthesis of hemoglobin disorders and anemia, high blood pressure, kidney damage, miscarriage and preterm birth, nervous system disorders, brain damage, infertility in men, decreased learning ability and behavioral disorders and hyperactivity in children from the negative effects of increasing the concentration of lead in body[4]. Nickel toxicity varies widely and is affected by salinity and the presence of other ions is placed. Industrial and commercial use of nickel-containing stainless steel, plating, painting and ceramics are. Nickel also from anthropogenic sources enters the water system. Small amounts of nickel in people who are allergic to this heavy metal can cause severe inflammation of the skin [4]. Cadmium is one of the natural elements in building the body's cells and many enzymes and hormones involved. The metal body with many vital macromolecules are irreversibly linked and

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threatened to disrupt the biological activity of cells. Cadmium also causes gastrointestinal disturbances such as nausea, vomiting, dry mouth, fever, headache and neurological disorders and respiratory diseases are also on the human body in high concentrations in the prostate, bone, muscle and liver accumulates [5]. The most important control methods, choice of different fish species widely to the physiological effects of heavy metals can be used [6]. Thus, the concentration of heavy metals in the tissues of aquatic can be a prelude to detect the level of aquatic pollution [7]. Such as indicator species to measure the amount of pollution can be traced to the Fish table Himantura imbricate.

II. MATERIALS AND METHODS

Heavy metals are hazardous pollutants that waste and sewage into the sea. Aquaculture can act as a measure of pollution in aquatic ecosystems. Therefore, measurement and evaluation of a number of toxic elements and heavy metals (lead, nickel, iron, zinc and copper) in the muscle tissue of blue Fish table Himantura imbricate stations (Qeshm and Souroo) in the Persian Gulf was the basis for this study . After determining the three stations, in every season of every station, 30 type of Himantura imbricates were sampled, so that were collected randomly in every season of collect 90 type and total two season 180 sample. After the biometrics, autopsy was performed and was isolated muscle tissue.so that for dry crap muscle tissues put in the freeze dryer (VaCo5 model) at 40 - ° C for 8 to 10 hours.so after running out the time and ensure complete dry of muscle tissue, the samples are removed balloons and in the petri dishes were placed numbered [8]. In order to digest the samples were ground with a porcelain mortar laboratory. the First, was measured (0.5gr) amount of dried samples tissue by" Sartorius scales"-made in Germany- with accuracy equivalent 0.001grams.The sample is poured shed into a microwave vial (ETHOS1, model) and after the addition of 7 ml of concentrated nitric acid 65% (after the using porcelain mortar any time, washed with nitric acid 5% and was completely rinse with distilled water) so 1 ml hydrogen peroxide (30%), closed the door's vials and those are placed in a special chamber, next transferred to the microwave and according to the order digest the sample. After digestion and cooling time, the samples were removed from the device and were pure through Whitman filter paper number 42. The content of the filter was washed with distilled water. So samples liquid ready discharged into the beakers and were dried in the laboratory's temperature. After dried, samples mixed through distilled water and samples deliver to the volume 50 with pure distilled water.So sample kept in polyethylene containers and stored at 4 ° C (to avoid any reduction in the volume). Obviously storage time should not be long and after digestion of the samples, they injected the atomic absorption and their actual chemical concentrations were calculated. The chemical digestion is based on accepted MOOPAM [8]. Data analysis was performed using SPSS 21 software and analysis of means to help T- test were compared with the presence or absence of a significant difference at 95% (P- value <0.05) was determined. As well as charts and tables Excel2007 software was used.

III. RESULTS

Analysis of variance showed that the concentrations of lead, nickel and cadmium in muscle tissue of Fish table Himantura imbricate between regions Qeshm and Souroo there is a significant difference (P-value< 0.05). As the studied concentration in the muscle of fish table Himantura imbricate Souroo higher than from Qeshm and the difference was statistically significant (P-value<0.05). (Table1 and Figures 1).

Table.1: compares the results of the average of the elements nickel, lead and cadmium in muscle tissue of Fish table Himantura imbricate in Qeshm and Souroo (mean ± SD), (n = 30)..

Index / Area	Qeshm	Souroo
Nickel (micrograms per gram)	0.02±0.33	0/03±0.48
Lead (micrograms per gram)	0/002±0.024	0/003±0.035
Cadmium (micrograms per gram)	0.01±0.25	0.02±0.34

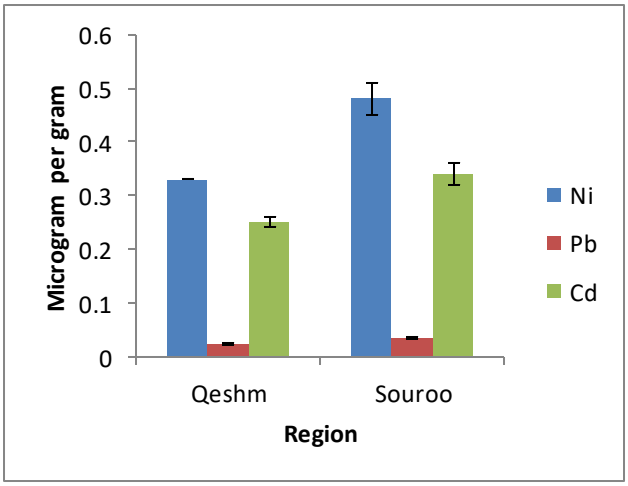

Fig.1: Comparison of nickel, lead and cadmium in muscle tissue of Fish table Himantura imbricate muscle in Qeshm and

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Souroo

IV. DISCUSSION

Exploration, extraction and transportation of oil in the Persian Gulf, in addition to direct contamination, due to large amounts of heavy metals, chemical pollution of the Gulf marine and aquatic life is [9]. Fabris and colleagues (2006) showed that the concentration of heavy metals such as arsenic, cadmium, iron, zinc and mercury in fish and lobster J. Edwardsis P. bassenis ground now and abalone H. rubra to the location where the fish live in it. Depends on the concentration of the species in different parts of the coastal waters of Victoria in Australia there is a significant difference, but a pattern and there was no consistent trend across regions at a concentration of heavy metals. There are significant differences between the concentrations of heavy metals in different areas can be discussed and not because of different management application, environmental conditions, evacuation of wastewater, the presence of industrial plants and aquaculture activities in the areas [10]. Chen (2002), showed significant differences in the concentrations of lead, cadmium, mercury, silver, copper and iron Chi-Ku Lagoon was found in samples from different regions. He also said that in areas where the origin of pollutants from sewage or fresh water input. Cadmium, mercury and copper were present in the environment, while the entrance to the remote areas of the mouth and go wetland reduced concentrations of these elements [11]. Dural and colleagues (2007), with several experiments showed that the concentration of heavy metals in aquatic organisms in different regions (the Persian Gulf, Gulf Egypt, the Gulf Askndryvn, in the South Atlantic salt marshes and wetlands Spain California) due to different environmental conditions such as: temperature, salinity, pH and light industrial activities .... and there is a significant difference[12]. Turkmen and colleagues (2005) reported that concentrations of heavy metals in fish muscle, according to the area where the fish is caught. And according to the species of fish can be very diverse and vary, also showed. Although not different between the concentrations of heavy metals in different parts of sampling fish there are significant differences [13]. Meador et al. (2005), the concentration of three cadmium, mercury and lead in sediments and fish in several areas in Alaska and California have measured the results showed concentrations of lead and cadmium in sediments rural areas of California due to human activity is the because gasoline is increasing [14]. A significant impact on aquatic habitats so that the concentration of heavy metals, heavy metals in organisms that live in the Gulf are less than the amount of heavy metals in the body of organisms in coastal waters and estuaries, bays and inlets are present [15]. Unfortunately, in the discharge of sewage and solid waste and industrial development and dredging operations off the coast and ports, unloading of agricultural pesticides and fertilizers, as well as Persian Gulf oil extraction operations are heavily polluted with heavy metals and hydrocarbons is [16]. More pollutants into aquatic systems are eventually are deposited in the sediment. Sediments, aquatic environments are a critical component for performance and nutrition provide habitat for many organisms and in many cases the accumulation of metals in sediments than in the water [15]. And semi-benthic benthic species vulnerable to contaminants in sediments and contaminants are water-soluble, this species also play a constructive role in this environment and therefore their demographic shifts affect all societies and threaten the balance of ecosystems [17].

Generally, the most important reasons for the high concentration of lead, nickel and cadmium in muscle tissue in Himantura imbricate in Souroo compared to Qeshm in various industries along the coast, discharge of industrial effluents and urban coastal waters is that their wastewater in a variety of heavy metals, and this increases the concentration of these metals. On the other hand there dhow building yards along the waterfront of the island and the use of color and anti-corrosion material (which contains zinc chromate and lead oxides area and finally moved to the coastal waters and adjacent areas and water pollution in this area are), too Boat traffic (tourism and fishing activities) and the presence of lead and nickel in gasoline and publish it in the air, then lead and nickel from combustion and quickly deposited on the soil, The nickel-containing sediments by rivers to the Persian Gulf could also be other reasons for this increase. The most important part is edible muscle tissue of fish that can directly impact on human health. Based on concentrations measured in terms of weight compared with existing standards (Table 2), was lower than the limit concentration, and muscle consumption of lobster in three regions of the Hormozgan province (Qeshm and Souroo) will not be a threat to the these metals.

Table2. World standards for permitted levels of nickel, lead and cadmium (micrograms per gram) in fish muscle tissue

Reference	Metal					International standard
	Cu	Fe	Zn	Ni	Pb	
FAO/WHO, 2010	10	-	30	-	2.14	FAO/WHO
Pourang et	10	-	35	-	1.5	NHMRC

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al., 2004						
Pourang et al., 2004	-	100	35	1	5	FDA

In the first study to estimate the amount of entry and the risk of heavy metals nickel, lead, zinc, iron and copper were the crab. Results showed that all estimates of daily log metals is no danger in taking this species of fish found not consumers. It should be noted that in Crab various other metals such as mercury and organic pollutants such as polyaromatic hydrocarbons, and accumulate. It is therefore essential that health authorities such as the Ministry of Health and other organizations in comprehensive background check estimate the amount of risk in different groups of consumers such as children and pregnant women do, and the accumulation of heavy metals in fish consumed annually carcinogenic and non-commercial and examine..

REFERENCES

[1] Medeiros RJ, dos Santos LM, Freire AS, Santelli RE, Braga AMCB, Krauss TM, et al. Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. Food Control 2012; 23:535e41.

[2] GanJavi,M., Ezzat panahi, H., Givianrad, M. H., Shams, A., 2010. Effect of conned tuna fish processing steps on lead and cadmium contents of Iranian tuna fish Food Chemistvy 118, 525-528

[3] Agah, H., Leer Makers, M., Marc Elskens, S., 2008. Accumulation of trace metals in the muscle and liver tissues of five speciies from the Persian Gulf.Environ Monit Assess 157, 499-514.

[4] EPA, 1997. Drinking water standards Environment of Criteria and Assessment.

[5] Merian, E., 1992. Metals & their compounds in environment. Journal of Environmental Healt Criteria . 4, 63 -53.

[6] Merian, E., 1992. Metals & their compounds in environment. Journal of Environmental Healt Criteria . 4, 63 -53.

[7] Dugo, G., Lapera, L., Bruzzes, A., Pellicano, T. M., Lotorco, V., 2006. Concentration of Cd, Cu, Pb, Se and Zn in cultured sea bass (Dicentrarchus labrax) tissue from Tyrrhenian sea and Silcilian sea by derivative stripping potentiometer. Food Control 17, 146- 152.

[8] Moopam ., 1999. Manual of oceanographic observations and pollutant analysis methods . 3rd ed, Kuwait, 321p.

[9] Matta, J.,Milad,M.,Manger,R.,&Tosteson, T. 1999. Heavy metals,lipid peroxidation, and cigateratoxicity in the liver of the Caribben barracuda(spHyraena barracuda) . Biological Trace Element Research,70 ,pp. 69-79.

[10] Fabris, G., Turoczy, N. j., Stagnitti, F., 2006. Tract metals concentration in edible tissue of snapper, flathead and ablone from coastal waters of Victoria, Australia Ecotoxicology and Environmental Safety 63, 286-292.

[11] Chen, M. H., 2002. Baseline metal concentration in sediments and fish and determination of bio indicators in the subtropical, Baseline Marine Pollution Bulletin 44, 703-714.

[12] Dural, M., Goksu, M. Z. L., Ozak, A. A., 2007. Investigation of heavy metal levels in economically important fish species from the Tuzla lagoon. Food Chemistry 102, 415- 421.

[13] Turkmen, A., Turkmen, M., TQE, Y., Akyu, I., 2005. Heavy metals in three commercially valuable fish species from Iskenderun Bay, North East Mediterranean Sea, Turkey. Food Chemistry 91, 167-172.

[14] Meador, J., Erest, D., kagley, A., 2005. A comparison of the non- essential elements cadmium , mercury and lead foud in fish and sediment from alska and California. Science of the Total Enviroment 339, 189-205.

[15] Al- Yousef, M. H., Ei- shahawi, M. S., Al- Ghais, S. M., 2000. Trace metals in liver, skin and muscle of Lethrinus lentjan fish species in relation to body length and sex. Science Total Environment 256, 87-94.

[16] Ashraf V., 2005. Accumulation of heavy metals in kidney and heart tissues of EpinepHelus microdon fish from the Persian Gulf. Journal of Environmental Monitoring and Assessment, 101: 311- 316.

[17] Bellas, J., Beiras, R., Carlos, J. and Fernandez, N. 2005. Toxicity of organic compounds to marine invertebrate embryos and larve: A comparison between the Sea Urchin embryogenesis bioassay and alternative test species. Journal of Ecotoxicology, 14: 337- 353.

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Effects of Livelihood Sustenance Activities on Off-Farm Income of Poultry Farmers in IMO State, Nigeria

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Abstract**—** *The study analysed the off farm income and its effect on livelihood sustenance of poultry farmers in Imo state.Multistage sampling technique was used to select 120 respondents. Data for the study were obtained with the aid of structured questionnaire and analysed using descriptive statistics and ordinary least square bivariate regression model. Results showed that: the mean off-farm income of poultry farmers was N410223 per annum. Livelihood sustenance activities of poultry farmers positively and significantly affected their off-farm income. It is recommend that government should come up policies that will center on establishment of more livelihood sustenance activities for poultry farmers that will generate increased off-farm income and promote agricultural development simultaneously.*

Keywords**— Income, Livelihood sustenance, Off-farm Poultry Farmers.**

I. INTRODUCTION

In many developing countries, and particularly in Africa, agricultural income represents an essential component of rural households' subsistence. However, this type of income exhibits a high seasonality and leads to uncertain outcomes, mainly due to market prices volatility and environmental hazards. Consequently, household members partly allocate their working time to activities which provide a more stable income so as to cope with adverse shocks (Ellis, 2000).

Rural areas usually provide two categories of income sources to their dwellers; Farm and the non-farm economy. In the rural areas of Nigeria, the majority of households are involved in farm activities and many of them get their income from non-farm activities (World Bank, 2008). Thus, in the rural area, it is hard to find peasants who do only farming.

According to (FAO, 2012), out of 3 billion people living in rural areas in the world, 2.5 billion people derive their livelihood from non-agricultural enterprises. For instance, Haggblade *et al* (2010) observed that non-farm income accounts for between 65% and 80% of total income of rural households in developing countries. Oxford policy management (Opm, 2004), noted that majority of households across all income strata in Nigeria are involved in several off-farm activities, whose importance has increased over the last 25 years.

In Nigeria, majority of the farm household populace either depend entirely on farming for survival and generation of income, or depend on farming to supplement their main sources of income (World Bank, 2010). Sample studies of rural income portfolios showed that on average, roughly 50 percent of rural households income in sub-Saharan African are generated from engagement in non-farm activities and transfer from urban areas or abroad, with remittance and pension payments being the chief categories of such transfer (Ellis 2000; Ellis & Freeman, 2004). Evidence from a sample of rural villages in Tanzania (Chapmen & Tripp, 2004; Ellis & Madox, 2003) shows that on average, half of the household income came from crops and livestock and the other half from non-farm wage employment, self-employment and remittance. The proportion of non-farm income was higher for the upper income groups than for the lowest income groups. Therefore, the poorest households were more reliant on agriculture, and the reliance on agriculture decreased with increased diversification into non-farm activities.

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Off-farm activities have become an important component of livelihood strategies among rural households in most developing countries. Several studies have reported a substantial and increasing share of off-farm income in total household income (Ruben and van den Berg, 2001; de Janvry and Sadoulet, 2001; Haggblade *et al*., 2007). Reasons for this observed income diversification include declining farm incomes and the desire to insure against agricultural production and market risks (Kijima *et al*., 2006; Matsumoto *et al*., 2006; Reardon, 1997). However, when farming becomes less profitable and more risky as a result of population growth and crop and market failures, households are pushed into off-farm activities leading to "distress-push" diversification. In other cases, however, households are rather pulled into the off-farm sector, especially when returns to off-farm employment are higher or less risky than in agriculture, resulting in "demand-pull" diversification. The study by Oseni & Winters (2009) found that 31% of farm households in Nigeria participate in various non-farm activities and that non-farm income makes up 27% of total annual household income, on average. The authors indicated that southern households earn more from non-farm activities than northern households where about 50% of household income is from non-farm sources. According to Ibekwe *et al* (2010), more than 40% of the income from households in South-East Nigeria came from off farm activities. Non-farm self-employment is the most common forms of off-farm activities in Nigeria followed by non-farm wage employment (Oseni & Winter, 2009). In a more recent study by Enyia,(2016), non farm income activities accounted for 36.4% of Fadama household income and 48.1% of non Fadama household income in Imo State, Nigeria.

A livelihood comprises capabilities, material and social resources and activities required for a means of living which also takes into account the role played by structures, policies and processes in influencing the choice of livelihood strategies by the rural poor. It is considered sustainable when it can cope with and recover from stresses and shocks, maintain or enhance its capabilities and assets, while not undermining its natural resource base (Scoones, 2000, Carney, 1998, Kanji, Macgregor & Tacoli, 2005). A Review of different livelihood definitions, reveal that the term livelihoods is a multi-faceted concept referring to what people do to make a living with the assets at their disposal and what they accomplish by doing it in a particular context (Niehof, 2004). The concept of livelihood is therefore about individuals, households or communities making a living, attempting to meet their various consumption and economic necessities, coping with uncertainties and responding to new opportunities (de Haan and Zoomers, 2005).

The contribution of farm activities to household income in the developing world in general and Nigeria in particular is substantial. While agricultural related activities still constitute the largest share of total income among rural households, a number of empirical studies show the growing importance of Rural Non-Farm (RNF) activities in developing and transition countries. While recognizing the urgent need to maintain a robust agricultural sector, it is increasingly becoming clear that the agricultural sector alone cannot be relied upon as the core activity for rural households as a means of improving livelihood and reducing poverty. This study therefore seeks to provide an in-depth understanding of the effect of off farm income on livelihood sustenance of poultry farmers in Imo state.

The specific objectives of the study were to examine the socio economic characteristics of the poultry farmers, determine the off-farm income of poultry farmers, and determine the effects of livelihood sustenance activities on off-farm income of poultry farmers.

II. METHODOLOGY

This study was conducted in Imo state, Nigeria. Imo State lies between Latitude $5^0 10'$ and $6^0 35'$ North of the equator and between Longitude $6^0 35'$ and $7^0 31'$ East of the Greenwich meridian. The State has a population of about 4.13 million people (NPC, 2013). It is bounded on the East by Abia state, on the North by Anambra and Abia State, and on the West by Rivers State. The State is divided into 27 administrative units called Local Government Areas which are grouped into 3 agricultural zones viz Owerri, Okigwe and Orlu. Agriculture is the predominant occupation of the people, for almost all the farm families either as primary or secondary occupation. The ecological zone favours the growing of tree crops, roots and tubers, cereals, vegetables and nuts (Onyenwaku *et al*, 2010).The major crops cultivated in the state are maize, melon, rice, groundnut, vegetables, yams, cassava, oil palm, and rubber. Major animals reared include chicken, turkey, goats, sheep and pigs.

Multistage random sampling technique was used for the study. In each agricultural zone, two Local Government Areas (LGAs) were purposively selected. In each of the selected LGA, five communities were randomly selected, and from each community, one village was randomly selected to give a total of five villages. Four farmers were randomly selected from each of the villages to give a sample size of 120 poultry farmers for the study. These farmers were selected from the list of households who are into poultry production in the selected villages and this list was obtained from the Agricultural Development Programme (ADP) extension agents and Imo State Fadama III Coordination office (SFCO). Primary data were collected through the use of a set of structured questionnaire administered to the respondents. The

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primary data that were collected for the study included the socio-economic characteristics of the farmers, flock size, annual income from the farm, off-farm income, access to credit, etc. Data collected were analyzed with descriptive statistics, such as percentages, and mean, as well as ordinary least squares bivariate regression model.

The bivariate regression model as used by Rahman, 2005, Rahman & Alamu, 2003) is implicitly specified as

Y = f (x, e)

Where,

Y= Mean off-farm income (₦)

X = Livelihood sustenance activities (Dummy variable, if the poultry farmer earns off-farm income from 5-9 livelihood sustenance activities = 1, and if the poultry farmer earns off-farm income from 1 – 4 livelihood sustenance activities = 0).

e = error term.

It is expected *a priori* that the coefficient of x > 0.

Four functional forms of the model; linear, semi-log, double-log, and exponential were fitted to the data to select the lead equation on the basis of having the highest value of coefficient of determination (r^2), highest variable significance, and conformity to *a priori* expectation.

III. RESULTS AND DISCUSSION

The socio-economic characteristics of poultry farmers are presented in Table 1.

Table.1: Socio-economic characteristics of Poultry Farmers

Age (years)	Frequency	Percentage (%)	Mean
≤30	11	9.2	
31 -40	37	30.8	
41-50	63	52.5	
≥51	9	7.5	
Total	120	100	41years
Sex			
Female	49	40.8	
Male	71	50.2	
Total	120	100	
Education Level (Years)			
0(No Formal Education)	3	2.5	
1 – 6	16	13.3	
7 – 12	65	54.2	
13 - 18	36	30.0	
Total	120	100	10 years
Marital Status			
Married	94	78.3	
Single	26	21.7	
Total	120	100	
Farming Experience (Years)			
≤ 20	72	60.0	
21-30	38	31.7	
31 – 40	10	8.3	
Total	**120**	**100**	**20.3 years**
Household Size (Number of Persons)			
1-5	47	39.2	
6-10	69	57.5	
≥11	4	3.3	
Total	**120**	**100**	**6 persons**
Extension Contact (Number of Visit/Year)			
0 (No visits)	85	70.8	
1-5	30	25.0	
6 - 10	4	3.4	
≥11	1	0.8	

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Total	**120**	**100**	**1.0 visits**
Membership of Cooperative			
Member	89	74.2	
Non Member	31	25.8	
Total	**120**	**100**	

Source: Survey Data, 2016

Table 1 shows that majority (52.5%) of the poultry farmers in the study area fall within the age bracket of 41 – 50 years of age with a mean age of 41 years. This implies that majority of the poultry farmers are young. The table also shows that the mean education level is 10 years. This indicates that the poultry farmers in the study area are literate enough to read and write in English language. The result indicates that mean farming experience of poultry farmers is 20.3years. The mean household size was found to be 6 persons, while mean extension contact was 1.0 visit per year. This indicates that poultry farmers are poorly visited by extension agents.

Off-farm income from livelihood sustenance activities

The mean off-farm income from the poultry farmers' livelihood sustenance activities is presented in Table 2.

Table.2: Mean off-farm income from poultry farmers' livelihood sustenance activities

Livelihood sustenance activities	Mean off-farm income (₦)	Percentage
Interest received in cash from off-farm loan	63482	15.5
Off-farm service earnings (salaries, wages, pensions, etc)	106123	25.9
Sale of purchased crop	51446	12.5
Sale of purchased animals and animals products	73489	17.9
Sale of equipment	23112	5.6
Sale of fertilizers	39546	9.6
Sale of non-agricultural items	33189	8.2
Sale of agro-chemicals	15294	3.7
Lease of rented land	4542	1.1
Total	410223	100

*Source: Survey Data, 2016

Data in the table show that the mean annual off-farm income of the poultry farmer was ₦410223 per annum indicating that the poultry farmers earned moderate annual off-farm income. About 26% of the off-farm income was contributed by off-farm service earnings (salaries, wages, pensions, etc), while 17.9%, 15.5% and 12.5% of the off-farm income were contributed by sale of purchased animals and animals products, interest received in cash from off-farm loan, and sale of purchased crop respectively. Also, 9.6%, 8.2%, 5.6%, 3.7% and 1.1% of off-farm income were from sale of fertilizers, sale of non-agricultural items, sale of equipment, sale of agro-chemicals, and lease of rented land respectively. This finding implies that off-farm income of the poultry farmers came from various livelihood sustenance activities in the study area.

Effect of Livelihood Sustenance activities of Poultry farmers on off-farm income

To determine the effect of livelihood sustenance of poultry farmers on off-farm income, four functional forms of the bivariate regression analyses were fitted to the data so as to select the lead equation. Results of the bivariate regression analyses were presented in Table 3.

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Table.3: Results of Bivariate Regression Analyses on Effect of livelihood sustenance activities of poultry farmers on off-farm income

Explanatory variable	Linear	Semi-log	Double-log	Exponential
Constant	316.112 287.015	164.009 121.318		
Livelihood sustenance activities (x)	14.247 (2.323)*	3.069 (1.872)	0.082 (4.677)**	0.007 (2.549)*
r^2	0.5531	0.4821	0.8934	0.6924
F-value	145.553**	109.58**	992.667**	266.308**
Sample size (n)	120	120	120	120

Figures in parentheses are t-ratios
* Significant at 5%
**Significant at 1%
Source: Survey Data, 2016

The table shows that the double-log function produced the highest value of coefficient of determination (r^2), highest variable significance, and conformed to *a priori* expectation and was therefore selected as the lead equation and used for discussion.

The value of r^2 was 0.8934, which implies that about 89% of the variation in off-farm income was accounted for by the action of poultry farmers livelihood sustenance activities.
The r^2 value of 0.8934 gave F-value of 992.667 which was significant at 1% level of probability, implying that the double-log function gave a good fit to the data.
The coefficient of livelihood sustenance activities (x) was positive and significant at 1% level, implying that increase in livelihood sustenance activities employed by the poultry farmers lead to increase in off-farm income.
Therefore, there was a positive effect of poultry farmers' livelihood sustenance activities on their off-farm income in Imo State, Nigeria.

IV. CONCLUSION AND RECOMMENDATIONS

The mean off-farm income earned by poultry farmers was ₦410223. Livelihood sustenance activities of poultry farmers positively and significantly affected their off-farm income. The study recommends that government should come up with policies that will center on establishment of more livelihood sustenance activities for poultry farmers that will generate increased off-farm income and promote agricultural development simultaneously.

REFERENCES

[1] Carney, D. (1998). Implementing the sustainable livelihood approach. "In D. Carney eds., Sustainable Rural Livelihoods: What Contribution Can We Make. London: DFID. 143

[2] De Hann, L.J., & Annelies Z. (2005). Exploring the Frontier of livelihood research. *Development and Change, 36 (1): 27-47.*

[3] De Janvry, A., Sadoulet, E., & Murgai, R. (2002). Rural development and rural policy. In: B.L. Gardner and G.C. Rausser (ed.). Handbook of Agricultural Economics. 1593-1658. Amsterdam: Elsevier. Retrieved October 11, 2013 from mpra.ub.uni-muenchen.de/13365

[4] Ellis, F. (2000). Rural Livelihood Diversity in Developing Countries: Analysis, Methods, Policy, Oxford: Oxford University Press.

[5] Ellis, F., & Freeman, H. A. (2004). Rural Livelihoods and Poverty Reduction Strategies in Four African Countries, *Journal of Development Studies*, 40(4):18-23.

[6] Ellis, F. & Mdoe, N. (2003). Livelihoods and Rural Poverty Reduction in Tanzania, *World Development*, 31(8):1367-1384.

[7] Enyia C.O (2016), Livelihood diversification Strategies among Fadama and non Fadama users in Imo State, Nigeria. An unpublished Ph.D thesis, Federal University of Technology Owerri.

[8] Food and Agriculture Organisation,(2012). Livelihood Diversification and Natural Resource Access: Livelihood Support Programme; Working Paper No. 9; Food and Agriculture Organisation. Retrieved December 4, 2015 from ttp:www.fao.org /DOCREP/006/ AD689E/AD689E00.

[9] Haggblade, S., Hazell, P., & Reardon, T., (2007). "Introduction", in Haggblade, S, P. Hazell, and T. Reardon, (Eds), Transforming the Rural Nonfarm Economy, Baltimore, Johns Hopkins University Press.

[10] Ibekwe, U. C, Eze C.C, Ohajianya, D.O, Orebiyi J.S, Onyemauwa, C.S., & Korie, O. C. (2010). Determinants of non farm income among farm

households in South East Nigeria. *Academia Area, 2010; 2(8): 29-33*.

[11] Kanji, N., Macgregor, J. & Tacoli, C. (2005).'Understanding market-based livelihoods in a globalizing world: combining approaches and methods'. London: *International Institute for Environment and Development* (IIED).

[12] Niehof, A. (2004). The significance of diversification for rural livelihood systems. J. Food Policy. 29(4): pp.321-338. Retrieved October 23, 2013 from http://www.sciencedirect.com /science/article/B6VCB-4DBSWD2

[13] National Population Commission (2013): National Population Commission updated population figure for Imo State of Nigeria 2013 census report. http://www.qtsnigeria.com/.

[14] Onyenweaku, C E, & Nwachukwu, I.N. (2010). Productivity growth in food crop production in Imo State, Nigeria. *African Crop Science Journal*, 18(3):89-95

[15] OPM (2004). *Oxford Policy Management, DFID Rural and Urban Development Case Study – Nigeria*. June 2004.

[16] Oseni, G. & Winters, P. (2009). Rural Nonfarm Activities and Agricultural Crop Production in Nigeria. *Agricultural Economics*, 40(2), 189-201

[17] Reardon, T. (2007). Agribusiness transitions in the Developing World. Chapter 5 in Haggblade, Hazell and Reardon (eds), The Rural Nonfarm Economy: Opportunities and Threats in the Developing World. Baltimore: Johns Hopkins University Press.

[18] Ruben, R., Van Der Berg, M. (2001): Nonfarm employment and rural farm households in Honduras. *World Development* 29(3):549-560.

[19] Scoones, I., (2000). Sustainable Rural Livelihoods: A framework for analysis. IDS Working Paper 72. Institute of Development Studies.

[20] World Bank (2008). World Development Report 2008 Overview: Agriculture for development. Washington DC: International Bank for Reconstruction and Development/World Bank

[21] World Bank (2010): World Bank updates poverty estimates for developing World. Retrieved December 3, 2015 from http://econ.worldbank.org/.

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Variability in Selected Soil Properties of Soils of Dissimilar Parent Materials in the Humid Tropics

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Abstract**—** *The study investigated variability of selected soil properties of soils derived from different parent materials (that is false bedded sandstone and Imo clay shale) in the humid tropic. Soil samples were collected from horizons based on profile differentiation. Data collected were subjected to statistical analysis using coefficient of variation (CV) and correlation. There were variations in soil properties of the two studied areas. Results showed that soil properties differed in their degree of variation among the parent materials. Sand content had little variation in both soils (CV = 6.56 % and CV = 17.23 %) for false bedded sandstone and Imo clay shale respectively. Clay had moderate variation in both soils (CV =40.17% and 36.50 %) for false bedded sand stone and Imo clay shale respectively. Bulk density and pH had little variation in both soils CV =7.30 % and CV =4.29) in false bedded sandstone while (CV =6.16 % and 4.85 %) in Imo clay shale. Organic matter varied highly in false bedded sandstone (CV =51.32 %) while in Imo clay shale it varied moderately (CV = 42.30 %). Organic matter had positive significant relationship with clay content in both soils (r =0.98, 0.99, P < 0.05) for false bedded sandstone and Imo clay shale respectively. pH had a negative relationship with Available phosphorus in false bedded sandstone (r =- 0.45, P < 0.05) while in Imo clay shale pH had positive significant relationship with Available phosphorus (r =0.96, P< 0.05). pH had non- significant positive relationship with organic matter in both soils (r = 0.63, 0.06, p < 0.05) for false bedded sandstone and Imo clay shale respectively. A more intensive soil sampling from the study area with the inclusion of more parameters will provide a better and reliable representation of the variability of soil properties even at a regional scale.*

Keywords**— variability, Pedon, Parent material, Tropical soils.***

I. INTRODUCTION

Most soil users in South-eastern Nigeria have regarded the soils to be the same in every respect simply because they are all in the same geographical location. Information on variation in soil properties enable potential soil users to appreciate the behavior of the various types of soils in the region, so that they can be utilized appropriately to derive optimum productivity [3]. Soils exhibit tremendous variability in their biological, chemical and physical properties [20]. According to [18] variation in soil properties has long been known and has been the subject of much research.

Pedologists have identified fundamental soil forming processes that influence soil properties: parent material, topography, climate, time and organisms [28]. Parent materials affect the morphological and physico-chemical characteristics of the soils under the same agro-ecological condition [14][13]. However, living organisms such as vegetation also have an important role in a number of processes involved in soil formation including organic matter accumulation, profile mixing and biogeochemical nutrient cycling [20]

[25] working on Alfisols of Southwestern Nigeria observed that soil pH was the least variable (low variability) property, irrespective of depth. The variability of soil properties like organic matter, available phosphorus, total nitrogen and Cation Exchange Capacity, increases with depth. Properties such as pH and porosity are among the least variables, while those pertaining to water or solute transport are among the most variable.

Soils of South-eastern Nigeria are formed from diverse parent materials [26]. Information on how these parent materials and their corresponding pedogenic processes influence soil properties in South-eastern Nigeria is limited. [20] reported on the variability in soil properties in relation to topography. Based on the above, we investigated the variation in properties of soils derived from two different parent materials in South-eastern Nigeria.

II. Materials and Methods

2.1. Study area

The study was conducted in two different locations namely Amuro (Okigwe LGA), which has Imo clay shale as its parent material located between latitude 5° 48'N and longitude 7° 20'E and Mbiakpa Ibakesi (Ini LGA) which has false bedded sandstone as its parent material located between latitude 5° 25' N and longitude 7° 44'E. The two study locations are in Imo State which lies between latitude 4° 40' N and longitude 6° 40'E and Akwa Ibom State which lies between latitude 5° 03' N and longitude 7° 93' E, both in Southern Nigeria Imo state lies within the humid tropics. Temperatures are high and change slightly during the year (mean daily temperature about 27℃) [21]. The average annual rainfall is about 2400mm and there is a distinct dry season of about 3-month dryness. Imo State has rainforest vegetation characterized by multiple tree species [26]. Agriculture is a major socio-economic activity in the study area. Agricultural crops mostly cultivated in the study area include yam (*Dioscorea Spp*), cassava (*Manihot Spp*), oil palm (*Elaies guineensis*) and maize (*Zea mays*). Akwa Ibom State also lies within the humid tropics with temperature of 27℃ to 31℃. Average annual rainfall is about 2500mm to 3000mm. Relative humidity of Akwa Ibom state is about 75% - 80% [11].

2.2 Field study

Prior to field study, a reconnaissance visit was made at each of the study locations in the early 2016; and this was followed by field sampling. Sampling sites were selected using free survey sampling techniques. One profile pit was dug at each of the various sampling site, summing to a total of two profile pits dug for the study. The study sites and profile pits were geo-referenced with the aid of a hand held Global Positioning System (GPS) receiver. The profile pits were described using [10] guidelines. Delineation of horizon boundaries was accomplished before actual sample collection for laboratory analyses and samples were collected according to horizons. A total of 30 soil samples were collected for the study. The soil samples were air-dried, crushed, sieved through 2mm sized sieve mesh. Ten grams (10 g) of each sample was finely grounded and preserved for determination of organic carbon and total nitrogen. Undisturbed soil samples for determination of bulk density were collected using core sampler.

2.3 Laboratory analysis

The particle size analysis was determined using the hydrometer method [12]. 5% calgon (sodium hexametaphosphate) solution was used vas dispersing agent. Gravimetric Moisture Content was determined by gravimetric method. This method involves weighing representative soil samples into moisture cans. Samples were oven dried and weight also obtained. Moisture calculated using formula

θ = Mw / M

:. Mc = Mw / Ms X 100 / 1

Where θ = gravimetric moisture content, Mt = total soil mass, Mw = mass of water, Ms = mass of solid particles.

Bulk density were taken in-situ using core samplers. The samples collected were oven dried after which bulk density calculated using the formula

Bulk density {pB} = $\frac{\text{mass of dry soil}}{\text{Volume of soil core sampler}} = \frac{Ms}{Vt}$

Bulk volume of soil = volume of cylinder = (πr^2h) Where r = radius, h = height of cylinder.

Soil pH was determined using a soil pH meter and this was done in both distilled water and 0.1 N KCl in a soil water ratio of 1:2:5. The pH of the resulting suspension was then read from a pH meter. Organic Carbon was determined by acid dichromate digestion and wet oxidation method [29]. Organic matter was obtained from organic carbon by multiplying by 1.724. Total Nitrogen was determined by the regular Kjeldhal method [6].Available Phosphorus was determined using Bray 2 method [5].

Exchangeable bases (Ca, Mg, Na and K). Exchangeable Na and K was extracted using 1N NH_4OAC using flame photometer [16].Exchangeable acidity was determined by extracting the soil with 0.1N KCl solution and titrating the aliquot of the extract with 1N NaOH [19]. Effective cation Exchange Capacity (CEC) was determined by the summation of exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) and exchangeable acidity (H^{+} and Al^{3+}). The summation of the exchangeable bases and acidity gave the effective cation exchange capacity (ECEC) value.

2.4 Statistical analysis

The data was analyzed using the Coefficient of Variation (CV) procedure, ranking of variability according to [4]. The percentage CV values were graded as little variation (0-20%) moderate variation (20-50%) and high variation (50-100%). Correlation was used to determine the degree of relationship among soil properties of the different parent materials using SPSS. The experiment was replicated two times. Only the mean values are reported.

III. RESULTS AND DISCUSSIONS

3.1. Physical properties of soil.

The profile distributions of the physical properties of the soils are presented in Table 1. The distribution of clay down the profile showed moderate variation (cv 20-50 %) Table 1. This observation is in line with [25] who stated that % clay range from moderate to high variability. The distribution of total porosity down the profile showed little variation (cv < 20 %) (Table 1). This observation is in line with [25] who stated that properties such as soil pH and porosity are among the least variables. The mean bulk

density values (0.73 – 1.55 gcm^{-3}) recorded across the soils investigated were below the value quoted as the minimum bulk density at which root restricting conditions will occur (1.75 – 1.80 Mgm^{-3}) [27] showing that the soils were not compacted [2]. Generally in normal soil bulk density ranges from 1 – 1.60 gm/cc [7]. Generally, bulk density increased down the profile pit in all the soils. The results of coefficient of variation analysis showed little variation in the vertical distribution of the bulk density values (Table 1). This result is consistent with findings by [3] who observed little to moderate variation in the vertical distribution of bulk density in soil of contrasting lithosequence in Southeastern Nigeria. Moisture contents of the soils showed little variations (cv < 20 %) among the soils. This result is contrary to findings by [3] who observed high variation in the vertical distribution of moisture contents in soil of contrasting lithosequence in Southeastern Nigeria. With exception of the pedon developed on shale, the distribution of moisture contents in the profile pit increased with depth, indicating that the epipedon contains lesser quantity of moisture compared to the sub- surface horizons and could be a reflection of the clay contents of these horizons. Clay has high rate of adsorption and can retain water easily. Moisture content increased with increasing clay content as the clay would cause impaired drainage especially at the sub-surface horizons [23].

Table.1: physical properties of studied sites

Site	Origin of soils	Depth (cm)	Sand (g/kg)	Silt (g/kg)	Clay (g/kg)	Bulk density (g/cm3)	Total porosity (%)	MC(%)
Mbiakpa Ibakesi	Falsebedded sandstone	0-20	842.4	80	77.6	1.45	45.30	12.39
		20-35	782.4	100	117.6	1.43	46.10	12.84
		35-69	842.4	60	97.6	1.57	40.80	15.27
		69-108	732.4	50	217.6	1.58	40.40	15.19
		108-192	722.4	60	217.6	1.71	35.50	12.95
		mean	784.4	70	145.6	1.55	41.62	13.73
		STDV	5.154	2	6.723	0.1132	4.28	1.40
		CV	6.57	28.57	40.17	7.30	10.28	10.10
Amuro	Imo clay shale	0-15	702.4	160	137.6	0.67	74.1	19.00
		15-25	622.4	180	177.6	0.70	73.6	15.00
		25-50	522.4	155.2	322.4	0.73	72.5	17.80
		50-69	482.4	156.0	361.6	0.77	70.9	19.30
		69-150	482.4	170	347.6	0.77	70.7	17.80
		mean	562.4	164.2	273.4	0.73	72.4	17.80
		STDV	9.70	1.06	9.98	0.04	1.487	1.70
		CV	17.23	6.45	36.50	6.05	2.10	20.5

3.2. Chemical properties

The chemical properties of soil of the studied area are presented in Table 2. The pH values of the soils were moderately acidic with mean values ranging from 5.07 to 5.89. The acidic nature of the soils shows the inherent characteristics of soils of the study area irrespective of their parent materials. [1] [15] [3] reported similar findings in some soils of Southeastern Nigeria. The organic matter content of soils was low with a mean value ranging from 0.64 to 2.69 %. Organic matter decreased with depth in all the pedons (Table 2). The higher proportions of organic matter at the epipedon could be due to the fact that most of the organic residues are incorporated or deposited on the soil surface. Top soil organic matter contents are directly related to organic carbon inputs and there have been a number of studies demonstrating improvements in soil quality and fertility after organic carbon additions [8]. The higher concentration of organic matter at epipedal horizons was further revealed by the moderate to high variation (cv > 35 %) observed in the organic matter contents of the varying horizons in all the pedons.

The nitrogen content of soils was low with the mean total nitrogen content in both soils being 0.01 %. However, the nitrogen contents of the surface horizons were higher than the sub-surface horizons and may be attributed to the organic matter contents of these horizons. The higher

nitrogen at epipedal horizons was further revealed by the moderate to high variation (cv > 35 %) observed in the nitrogen contents of the varying horizons in all the pedons. The Effective Cation Exchange Capacity (ECEC) of soils was generally low with the mean value ranging from 2.57 to 2.99 $cmolkg^{-1}$. Soils of South-eastern Nigeria had earlier been reported to be made of low ECEC and basic cations [24] [22].

Table.2: Chemical properties of studied sites

Origin of soils	Horizon	pH(H20)	Om gkg^{-1}	TN g/kg^{-1}	Av. P mg/kg	TEB ($cmolkg^{-1}$)	Al ($cmolkg^{-1}$)	EA ($cmolkg^{-1}$)	ECEC ($cmolkg^{-1}$)	BS(%)	A l sat (%)
False bedded sandstone											
	Ap	6.01	26.40	1.24	13.38	1.62	-	0.44	2.06	78.64	-
	AB	5.60	24.70	1.18	3.15	1.05	-	0.46	1.15	69.53	-
	Bt1	5.61	23.60	0.80	10.85	2.03	1.04	0.41	3.48	58.33	29.88
	Bt2	5.43	9.80	0.88	18.83	1.87	1.01	0.35	3.23	57.89	31.32
	Bt3	5.41	6.40	0.69	11.76	1.43	1.03	0.46	2.91	48.79	35.34
	mean	5.61	18.20	0.96	11.69	1.60	0.69	0.42	2.57	62.64	32.18
	STDV	0.241	0.93	0.02	5.69	0.38	0.59	0.05	0.96	11.58	2.87
	CV(%)	4.39	51.33	25.00	48.50	24.00	85.80	0.85	37.20	18.49	8.92
Imo clay shale											
	Ap	6.50	26.90	1,20	7.42	2.14	-	1.40	3.54	60.45	-
	A	6.60	23.00	1.08	2.52	1.14	-	1.00	2.14	58.51	-
	Bt1	6.11	14.80	0.86	0.35	0.92	-	3.40	4.32	21.29	-
	Bt2	6.05	11.00	0.50	2.66	1.33	-	1.20	2.53	52.57	-
	Bt3	5.90	10.70	0.44	2.54	1.14	-	1.00	2.14	52.27	-
	mean	6.23	17.30	0.96	3.11	1.39	-	1.60	2.99	49.04	-
	STDV	0.30	0.73	0.06	2.58	0.46	-	1.02	0.91	15.93	-
	CV (%)	4.85	42.30	58.33	82.96	33.17	-	63.75	30.57	32.48	-

3.3 Relationship among selected physiochemical properties. The relationships among selected soil properties are shown in Table 3. The result shows that organic matter had a positive significant relationship with clay in all the soils suggesting that organic matter increase as clay content increases (r = 0.97,0.99 P< 0.05).Soil pH had significant positive relationship with available P in Imo clay shale (r =0.96 P< 0.05) while in false bedded sandstone, it had no significant. Similar result had been reported by [9] [17][20] on the relationship between pH and available P. The significant relationship between P and soil pH is an asset in soil management as status of soil pH can be used to predict P-availability and unavailability for crops. This is critical since P- anions react quickly with some cations such as Al, Fe and Ca to become less soluble even with slight pH change. Organic matter had a non significant negative relationship with available P in false bedded sandstone (r =-0.45 P <0.05) while in Imo clay, shale it had non significant positive relationship (r = 0.73). [20] reported a non significant relationship between organic matter and available P in an upperslope and midslope of a river slope in southeastern Nigeria.

Table.3: Relationship among selected properties of soils in the study area (n = 5)

Site	Soil property	r
Mbiakpa Ibakesi (False bedded sandstone)	Soil pH Vs OM	0.64^{NS}

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	Soil pH Vs Av P	-0.45 ^{NS}
	O.M Vs Av P	-0.45 ^{NS}
	OM Vs clay	0.98 **
Amuro (Imo clay shale)	Soil pH Vs OM	0.06 ^{NS}
	Soil pH Vs Av P	0.96 **
	O.M Vs Av P	-0.73 ^{NS}
	OM Vs clay	0.99 **

IV. CONCLUSION

From the results, five diagnostic horizons in soil taxonomy were recognized and defined on the basis of clay movement and illuviation, horizon thickness, organic matter content and presence of anthropogenic activities. Soil physico-chemical properties varied in many respect. However, large scale studies may be necessary in future investigation for increased accuracy of prediction. In addition, more attributes of soil resources should be investigated to create greater confidence.

REFERENCES

[1] Abua, M.A., R.A. Offiong, A.I. Iwara and U.W. Ibor 2010. Impact of newly constructed roads on adjoining soil properties in Tinapa resort, southeastern Nigeria. Annals of Humanities and Development Studies. 1(1): 176 – 184.

[2] Ahukaemere, C.M., B.N. Ndukwu, and L.C. Agim 2012. Soil Quality Soil Degradation as influenced by Agricultural Land Use Types in the Humid Environment. International Journal of Soil, Forest and Erosion 2(4): 175 – 179.

[3] Ahukaemere, C.M., E.U. Onweremadu., B.N Ndukwu and U.N. Nkwopara. 2016. Properties of Soils of contrasting lithosequence in southeastern Nigeria. FUTO Journal Series 2(1): 48 - 56.

[4] Aweto, A.O. 1982. Variability of upper slope soils developed in sandstones in Southwestern Nigeria. The Niger in geographic Journal 25: 27 – 37.

[5] Bray, R.H, and L.T. Kurtz 1945. Determination of total organic and available forms of phosphorus in soils. Soil Sci. 59: 39 – 45.

[6] Bremner, J.M, 1965. Total nitrogen. In C.A Black (Ed). Methods of Soil Analysis. Agron Monogr 8. American Assoc. Agronomy Madison, WI, Part 2, Pp 1149 -1178.

[7] Das, K.D., 2015. Introductory Soil Science. 4th ed. Kalyani Publishers, New Delhi, India. 879 pp.

[8] Dick, W.A and E.G Greorich, 2004. Development and maintaining soil organic matter levels, In: managing soil quality challenges in modern Agriculture (eds Pschjonning, S. Elmholt and B.T Christen Gen), PP 103 – 120 (ABI publishing, Wallingford.

[9] Dodor, D.E. and K. Oya, 2000. Phosphorus sorption characteristics of major soils in Okawana. Japan. Commun. Soil Sci Plant anal, 31: 277 – 288.

[10] FAO (Food and Agricultural Organization) 2006. World reference base for resources 84 world soil resources report, ISSS- AISSIBG, FAO Rome, Italy.

[11] Federal Department of Agricultural Land Resources. FDALR 1985. The reconnaissance soil survey of Imo state Nigeria (1: 250, 000) Soil Report 133 pp.

[12] Gee, G.W and J.W. Bauder .1986. Particle size analysis. In: Klute. A. (Ed.). Methods of soil analysis Part 1. Agronomy Monogr. No 9, ASA Madison, WI, Pp 91 -100.

[13] Irwark, S., A.K Surueu, . and I.H. Aydogdu, 2007. Effect of different parent materials on the mineral characteristics of soils in the Arid Region of Turkey. Pkistan Journal of Biological Sciences 10(4): 528 – 536.

[14] IUSS Working Group WRB 2006. World Reference Base for Soil Resources 2006. Food and Agricultural Organization, Rome: World Soil Resources Reports.

[15] Iwara, A.I., F.O. Ogundele, U.W. Ibor, V.W. Arrey, and O.E. Okongor, 2011. Effect of vegetation adjoining Tourism Facilities on Soil properties in the tourism enclave of Cross-river state. Research Journal of Applied Science, 6(4): 276 – 281.

[16] Jackson, M.I., 1962. Soil Chemical Analysis. New York Prentice Hall Inc.

[17] Khare, N.D., S. Hesterberg, G. Beauchemin, and S. Wang, 2004. Xanes determination of absorbed phosphate distribution between ferrihydrate and boemite in mixtures. Soil Sci. Soc. Am. J. 68: 460 – 469.

[18] Lark, M. and H.C. Wheeler. 2000. Understanding and using yield maps and analytical tool for their interpretation. Outlook on Agriculture, 29 (1): 39 – 45.

[19] Mclean, E.O. 1965. Aluminium, In: C.A Black et al (Eds) Method of Soil Analysis, Part 2. 1st ed, Amer. Soc. of Agronomy Monogr. No 9 986- 994.

[20] Ndukwu, B.N., E.U. Onweremadu, U.N. Nkwopara, C.M. Ahukaemere. E.E. Ihem, and L.C. Agim, 2015. Variability of selected soil properties of a river slope in Amaigbo, Southeastern Nigeria. FUTO Journal Series, 1(2): 8 – 16.

[21] NIMET (Nigerian Meteorological Agency), Nigeria, 2014. Climate Weather and Water Information, for sustainable development and safety.

[22] Nkwopara, U.N., E.T Eshett, E.U. Onweremadu, C.C Opara, and J.O. Omeke, 2007. Physico-chemical properties of selected soils overlying three parent materials in the humid tropics. International Journal of Agriculture and Food Systems, 1(2): 159-164.

[23] Odunze., A.C., 2003. Northern Guinea Savana soils and rainfall properties for erosion control and fertility improvement. Journal of African soils/ African union (AU). Scientific Technical and Research Commission Lagos, 33: 73-82.

[24] Ogban, P.I and I.O. Ekerette, 2001. Physical and chemical properties of the coastal plain sands soils of South-Eastern Nigeria. Nigerian Journal of Soil and Environmental Research, 2: 6 -14.

[25] Ogukunle, A.O. 1993. Variation of some soil properties along two toposequence on quartile schist and banded gneiss in Southern Nigeria. Geoderma 30(4): 397 – 402.

[26] Onweremadu, E.U., F.O.R. Akamigbo, and C.A. Igwe, 2007. Lithosequential variability and relationship between erodibility and sodium concentration in soils of a rainforest. Research Journal of Forestry, 1(2): 73-79.

[27] Soil Survey Staff 2003. Keys to Soil Taxonomy 9th ed. United state Department of Agriculture.

[28] Soil Survey Staff 2006. Keys to Soil Taxonomy 10th ed. United state Department of Agriculture, Natural Resources Conservation Service. Washington D.C USA.

[29] Walkley, A and I.A. Black, 1934. An examination of Degtjareff Method for determining Soil organic matter and the proposed Modification of the chromic and titration method. Soil Sci. 37: 29 – 37.

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Water Use Efficiency of Selected Cowpea Cultivars (*Vigna unguiculata* (L.) Walp) Grown on Residual Soil Moisture in Northeast Nigeria

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Abstract**—** *A field experiment was carried out in the Fadama of Jere bowl to assess the water extraction and water use efficiency of two improved (IT 86D-719 and IT88D-867-11) and one local (Borno Brown) cowpea cultivars grown on residual soil moisture. The three cowpea cultivars and a control were laid out in a randomized complete block design and replicated three times. The result showed that yield and growth parameters were significantly (P< 0.05) different amongst the three cowpea cultivars. The improved cultivars gave significantly (P< 0.05) higher seed yields than the local cultivar. Cultivar IT 86D-719 had the highest seed yield of 893.0 kg ha^{-1} while the cultivar Borno Brown had the lowest seed yield of 675.3 kg ha^{-1}. On the other hand the cultivar Borno Brown had the highest 100 seed weight compared to the improved cultivars. The result also showed that water extraction in all the cultivars increased with depth, with maximum extraction occurring at the depth of 80-100 cm, suggesting that the lower soil layers were more effective in supplying water as the hydraulic conductivities of surface layers decreased. The water use efficiency of the two improved varieties of IT86D-719 (63.56 kg/m^3) and IT88D-867-11(70.06 kg/m^3), were higher compared to the local variety (45.69 kg/m^3). Borno brown and IT 88D-867-11 are good water extractors at field capacity but low extractors at moisture stress. IT 88D-867-11 displayed sign of higher extraction rate than IT 86D-719 at field capacity, but IT86D-719 displayed a higher extraction capacity at moisture stress (20WAS).*

Keywords**— Cowpea Cultivars, Soil Moisture, Northern Nigeria.***

I. INTRODUCTION

Cowpea production under residual moisture is currently gaining popularity in *Fadama* areas in Northern Nigeria where water is a major limiting factor. Wetlands are referred to as *Fadama* in the Hausa language, which is widely spoken throughout the West African Sahel (Oyebande, 2002) and similar environment elsewhere. Crop production in the *Fadama* depends not only on residual moisture but also on the ability of crops to extract the available soil water (Miller and Arstad, 1974). Jere bowl is one of the *Fadama* areas, a confluence catchment for Ngadda and Alau rivers in Borno State, which spans an area of 200 km^2. Rice is the dominant crop grown during the wet season, and is immediately followed with cowpea in the dry season under residual moisture cultivation. In this type of dual production system, the study of moisture dynamics becomes critical in the formulation of sound intervention strategies.

Cowpea is an important food and cash crop in Nigeria with an annual production of 4.33 million tonnes (CBN, 2005). This has placed Nigeria as the largest producer of cowpea globally. Nigeria, together with Niger and Chad Republics, accounts for about 70% of the global cowpea production (FAO, 2008). The benefit of cultivating cowpea includes fixation of atmospheric nitrogen to the soil as well as soil cover against land degradation. This is in addition to its rich protein which makes it the principal source of protein supply to the peasant communities and second only to meat in protein supply to the urban populace. Also, its haulms and pods serve as animal feeds (Grema and Hess, 1994).

In the past, cowpea was exclusively cultivated under rain fed conditions, but currently the residual moisture production shores up the deficit supply of rain-fed production and will help stabilize the price of cowpea out of season. Dry land crops grown during the cool harmatan season on the *Fadama* depend primarily on residual moisture. The extraction of the receding water depends on the amount stored in the profile, the ability of crops to extract the available soil water, and the rooting characteristics of the crops and their abilities to extract the available store soil water and in some cases, the upward

capillary movement from shallow ground water table (Miller and Arstad, 1974).

Several studies in dry land areas have shown that dry land crops grown after rice can extract substantial amounts of stored soil water (Angus *et al*., 1983; Klodpeng and Morris, 1984). Agriculture in dry land areas is very vulnerable to failure and the use of *Fadama* lands to complement upland farming becomes very vital (Kundiri, 1995). *Fadama* farming increases food security by serving as an alternative when rain fed crops fail and also expands production in the off season (Kundiri, 1995). In addition, *Fadama* lands are more contiguous niche than the rain fed production sites, which would ensure a more stable production.

Rainfall in the drier cowpea production region of the country is often unpredictably erratic, but within the *Fadama* areas, the farming systems can reliably utilize the seasonal flood water, shallow ground water for irrigation or residual soil moisture. Residual moisture agriculture is strictly reliant on moisture vis-à-vis the inherent moisture content, water use and the crop water use efficiency (De Tar, 2009). Thus, different crops and even different crop varieties are bound to display different water use efficiency as observed by Amato and Ritchie (2002) for maize, Abidoye (2004) for Soya bean, and Gui-Rui-Yu *et.al,* (2007) for tomato. Evidences exist in maize for varietal differences in water use efficiency, but there is paucity of research on the potential of growing cowpea and other short duration crop species in the *Fadama* area in north east Nigeria. Proper soil and water management practices are considered to be the key factors for sustainable crop production.

The clear understanding of soil water dynamics in the *Fadama* could suggest profitable direction for applied soil and water management research on efficient residual soil moisture utilization for cowpea production. The water table recession rates after rice harvest have obvious implications for cropping systems research in the *Fadama* as well as recession farming. In view of the limited water resources of the arid and semi-arid environments, it is considered desirable to assess the water extraction pattern and water use efficiency of cowpea grown using residual soil moisture.

II. MATERIALS AND METHODS

The study area

The study was carried out at Jere bowl located about 5.5 km north east of Maiduguri, the Borno state capital between latitudes 11º 51´and 12º 05´ N and longitudes 13º11´ and 13º 27´ E. The Jere bowl has an altitude of 305.5 meters above sea level, while the surrounding has an altitude that varies between 309.5 m and 311.5 m above sea level. The soils are sandy loam in texture with high organic matter content and generally high in fertility. The area has a semi-arid climate with a short unimodal rainy season that starts in June and ends in September and long dry season, starting around November and lasting till April/May. Average annual rainfall in Maiduguri is 568 mm and average maximum temperature of about of 34°c and minimum of 19.6°c is a common occurrence in the study area. The average relative humidity in the area is quite low especially during the dry season. The area receives a high radiation load (except during the cool Harmattan season from November to February) of 40.2% at 0900Z (10:00 am) and 26.1% at 1500Z (4:00 pm). Mean annual sunshine duration was 8.5 hrs/day with mean solar radiation of 14.2 ML.

Treatments, Experimental Design and Cultural Practice

The treatments comprised of four experimental plots planted to three cowpea cultivars (two improved IT86D-719, IT88D-867-11, and a local cultivar, Borno Brown), assigned to the three plots and a control plot with no cowpea planted in it. The three cowpea cultivars and the control were replicated three times, giving a total of twelve plots laid out in a randomized complete block design (RCBD) in experimental plots measuring 2 m × 3 m with an alley of 1 m between replicates. Cowpea seeds were sown at the rate of two seeds per hole at a spacing of 50 cm × 30 cm. No fertilizer application was made in line with farmers practice in the study area. Weed control was done manually using hand hoe as at when due. Insect pests control was done by spraying with karate (cypermethrin) 100g/ai at the rate of 1L/ha at 25, 45 and 55 days after sowing (DAS). Matured pods were harvested by hand picking when the pods were dried.

Collection and Preparation of Soil Samples

Composite soil samples (0-15 cm depth) were collected from the field for routine physico-chemical analysis prior to land preparation. The soil samples were air dried, ground and passed through a 2mm sieve and used for the analysis following standard analytical procedures. Particle size analysis was carried out using the hydrometer (Gee and Bourder, 1986). Soil bulk density at depths of 0-10 cm, 10-20 cm and 20-60 cm was determined by the undisturbed core sample method (Black and Hartage, 1986), while total porosity was calculated from the average bulk density value (0-60 cm depth) based on a particle density value of 2.65Mg m^{-3}. Soil pH was measured in 1:2.5 soil water suspension using glass electrode digital pH meter (model Kent Eil 7045/48) as described by Page *et al*.

(1982). Organic carbon and total nitrogen contents were determined by dichromate wet oxidation and regular macro-Kjeldhal methods, respectively while the available phosphorus was determined by Bray-1 method (Page *et al*. 1982). Exchangeable bases were determined using 1N neutral ammonium acetate (NH_4OA_C) saturation method (Page *et al*., 1982). Exchangeable calcium and magnesium were determined titrimetrically with 0.02N Na_2 EDTA, while the exchangeable potassium and sodium were determined with a flame photometer (model FGA 330C) at wavelengths of 767 and 589 nm, respectively.

Determination of Field Water Content

The field water content was determined gravimetrically at 20 cm depth intervals to a depth of 100 cm on each plot. Soil samples were collected at planting and subsequently at four weeks interval. The samples were brought to the laboratory, weighed and oven dried at105^o C for 24 hours and reweighed. The gravimetric moisture content was determined by the difference. Subsequently, the values were converted to volumetric moisture content by multiplying with the appropriate value of the bulk density.

Measurement of Plant Parameters

The plant parameters measured include total grain yield at harvest and root length measurements. The cowpea grains were harvested when the grains were fully matured. The mature pods were hand-picked per individual plot, threshed and weighed to obtain the yield per plot and subsequently converted to yield per hectare.

Soil core technique that permits quantitative analysis of the root system described by Raper and Barber (1970) was used to measure the root length density. Soil cores of 5.4 cm diameter and 10 cm height were removed for measurement of cowpea root distribution. Cores were taken at 5 cm from the cowpea row on a line perpendicular to a cowpea plant. The cores were sub-divided into segments of 0-10, 10-20, 20-40, 40-60 and 60-80 cm depth increments. Root samples, one per plot were collected per replication. Cores were collected at 50% flowering and at harvest. The soil root cores were placed in plastic bags and stored in the refrigerator until roots could be separated from the soil. Each sample was washed through three sets of sieves arranged in decreasing order of diameter (i.e. 4, 2 and 1 mm), then the roots remaining in the sieves were transferred into large Petri dishes with the aid of a tweezer and magnifying glass. Direct method of estimating root length (millimeter per unit volume of soil) as described by Reicosky *et al*. (1970) was used. The root samples in the large Petri dishes were placed over millimeter–graph paper. The roots were strengthened with tweezers, observations were made through a magnifying glass and the length of a given root segment was estimated to the nearest millimeter. The individual root lengths were summed up to give estimate of the total root length. Dead roots were not included in measurements of root length. Calculations of root lengths per unit volume of soil (RLD) were made at the end of the growing season.

Water Use Efficiency

The crop water use efficiency, defined as yield of plant produced per unit of water used was determined using the equation developed by Power (1983) for estimating water use efficiency as follows: WUE = $\frac{Y}{ET}$

Where, WUE is the crop water use efficiency, Y is the total yield per given area during the growing season and ET is the evapotranspiration. WUE is expressed as yield produced per unit volume of water (kg/m^3). Water use is restricted to that removed from soil by evaporation and transpiration excluding non-productive losses that might have occurred through deep drainage and surface run-off.

III. RESULTS AND DISCUSSION

Physico-Chemical Properties of the Soil

The selected properties of the soil of the experimental site are presented in Table 1. The soil has a sandy loam texture comprising 72.8, 10.0 and 17.2 % sand, silt and clay, respectively. The soil is moderately acidic with a pH value of 5.88 and electrical conductivity of 0.03 dSm^{-1}. The soil has low organic carbon and total nitrogen contents of 0.02 and 0.05 g kg^{-1}, respectively and low phosphorus value of 4.25 mg kg^{-1}. In general, the soil has low fertility status having exchangeable K, Ca, Mg and Na of 0.28, 1.00, 0.40 and 0.21 Cmolkg-^{1}, respectively. The bulk density at 0-10 cm was 1.83 Mg m^{-3} then decreased to 1.73 Mg m^{-3} at 10-20 cm depth and increased to 1.86 Mg m^{-3} at 20-60 cm depth.

Moisture Content at Different Sampling Depths

Results on soil moisture content at five sampling depths and six sampling periods were shown in Figure 2. The highest moisture content of 0.6267 (cm^3/cm^3) was consistently obtained from soil samples at 80-100 cm depth, while the lowest moisture content of 0.4967 (cm^3/cm^3) was recorded at 0-20 cm depth at sowing. The moisture contents at the end of the experiment were 0.3450 (cm^3/cm^3) and 0.0442 (cm^3/cm^3) at 80-100 cm depth and 0-20 cm depths, respectively.

Results at all sampling periods showed that soil moisture content significantly increased with each successive increase in sampling depth. The general trend was soil moisture content at 80-100 cm depth > 60-80 cm depth > 40-60 cm depth >20-40 cm depth > 0-20 cm depth. The result also indicated decrease in soil moisture over

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time, this corroborate with the result of Arya *et al*. (1975) who reported that since root growth is a continuing process and hydraulic properties of a drying soil change substantially, water depletion patterns are markedly time dependent.

Effects of Cowpea Cultivars on Moisture Content

There was significant difference in moisture content among cultivars at all sampling periods (Table 2). At all sampling periods, soil moisture content in plots cropped to IT88D-867-11 and Borno Brown were significantly ($P < 0.05$) lower than that of the uncropped plots. During the crop growth at 4 and 8 WAS there was no significant difference in soil moisture content between the uncropped field and that cropped to IT86D -719. Subsequent result from 12 and 16 WAS however revealed significantly lower soil moisture content in plots cropped to IT86D -719 than in the other cropped plots. Plots cropped to these cowpea cultivars also had significantly lower moisture content in comparison to that cropped to IT86D-719 at 4 WAS. In addition, plots cropped to IT88D-867-11 and IT86D-719 showed significantly higher soil moisture content compared to plots cropped to Borno Brown at 8 WAS. However, the terminal result at 16 WAS did not show significant difference in soil moisture content among all plots cropped to the cowpea cultivars.

The result generally suggests decline in soil moisture content over time as reported by Safir *et al*. (1972) who said that initially the hydraulic factors favour water uptake by roots in the surface layers. As the soil dries rhizosphere resistance to water flow increases more rapidly near the surface and a downward shift in the uptake pattern would be expected.

Grain yield of Cowpea Cultivars

The highest grain yield of 893 kg/ha was obtained from IT86D-719. This was followed by IT 88D-867-11 with mean yield of 846 kg/ha. There was no significant ($P >0.05$) difference between the yield of the two improved varieties. However, both varieties significantly ($P<0.05$) out-yielded the local cultivar, Borno Brown which recorded 675.3 kg/ha.

Root Length Density of Cowpea Cultivars at Different Depths

The root length density of the three cowpea cultivars investigated during the sixteen weeks of experimentation is presented on Table 5. The root length density at 50% flowering indicated that Borno Brown has the highest concentration compared to both IT86D-719 and IT88D-867-11. The root length density at 100% flowering followed similar pattern. The result revealed that there were no significant ($P>0.05$) differences among the cowpea varieties at flowering (RL 50%) and at harvest (RL 100%). In respect to the depth, there was significant difference for 60-80cm depth and 80-100cm at both RL 50% and RL100%. The highest concentration of root (0.183 g/cm^3) was obtained at 0-20 cm at 50% flowering, while lowest concentration of 0.027 (g/cm^3) was obtained at 80-100 cm depth. Similar root concentration pattern was obtained at 100% flowering. No significant difference was observed for interaction between variety and depth for root length density at 50%. However, there was highly significant ($P<0.05$) for variety and depth at 100%

Water Extraction Rate (Water Use) by Different Cultivars of Cowpea

Figure 1 shows the rate of water extraction by the roots of the three cowpea cultivars during 20 weeks growth periods. The initial extraction rate at 4 WAS was generally low for all cultivars, but increased with time. When cultivars were compared extraction rate was highest in Borno Brown (0.8 cm/day), followed by IT88D-867-11 (0.48cm/day) and then IT88D-719 (0.32cm/day). The result revealed similar extraction trend at 8 WAS, with extraction rate of 0.30, 0.31 and 0.18 cm/day, for Borno Brown, IT88D-867-11 and IT88D-719, respectively. The peak extraction rate for Borno Brown (0.80cm) and IT88D-867-11(0.48cm) occurred at 12 WAS as against 20 WAS for IT86D-719 (0.45cm/day).

The result at 12 WAS indicated substantially higher extraction rate for Borno Brown. For IT86D-719, the extraction rate increased throughout the growth cycle with peak extraction rate at 20 WAS. In contrast, for IT88D-867-11 and Borno Brown, an early increase in the extraction rate was followed by a sharp decrease later in the crop growth cycle to 0.33 cm and 0.37cm at 16 WAS and 0.48 cm and 0.38 cm at 20 WAS, respectively. Borno Brown and IT88D-867-11 were good water extractors at field capacity but low extractors at moisture stress. IT88D-867-11 displayed sign of higher extraction rate than IT86D-719 at field capacity. IT86D-719 displayed higher extraction capacity at moisture stress (20 WAS).

Water extraction generally increased with depth with highest extraction at 80-100 cm followed by 60-80 cm and then 40-60 cm in that order, indicating that lower soil layers became more effective in supplying water as the hydraulic conductivities of the surface layers decreased (Figure 2). Water extraction increased with depth and peaked at 12 WAS. For the 80-100 cm layers, the extraction rate was followed by a substantial decrease later in the drying cycle as shown in Figure 2.

The root water extraction efficiency of the 3 cowpea cultivars at flowering and at podding across depth

are presented in Figure 3 and 4, respectively. In general, the results indicated increase in water extraction efficiency with increase in depths (at lower depths). However, water extraction efficiency at podding almost doubled that at flowering.

IV. CONCLUSION

The study revealed that there was significant ($p<0.05$) difference in yield of the three cowpea cultivars. The IT 86D-719 produced the highest grain yield. However, there was no significant ($p>0.05$) differences in the yield between the two improved cultivars. Low initial rate of water extraction for all cultivars was observed, however, extraction rate increased with time with highest by IT 88D-867-11 > Borno brown > IT 86D-71. The peak extraction rate for Borno brown and IT 88D -867-11 occurred at 12 WAS as against 20 for IT 86D-719. Moisture content from the cropped and uncropped plots increased with increase in depth at 80-100 cm depth > 60-80 cm depth > 40-60cm depth >20-40 cm depth>0-20 cm depth. Decrease in soil moisture content over time from planting to harvest was also observed. Borno Brown and IT 88D-867-11 are good water extractors at field capacity, but low extractors at moisture stress. IT 88D-867-11 displayed sign of higher extraction rate than IT 86D-719 at field capacity, but IT86D-719 displayed a higher extraction capacity at moisture stress.

Table.1: Physico-chemical Properties of the Soil of the Experimental Site

CHARACTERISTICS	VALUES
Soil $pH_{1:2.5}$ (H_2O)	5.88
Electrical Conductivity (EC) dsm^{-1}	0.03
Organic Carbon (g kg^{-1})	0.20
Nitrogen (g kg^{-1})	0.05
C:N ratio	4.00
Phosphorus (mg kg^{-1})	4.20
Exchangeable bases (Cmol Kg^{-1})	
Na	0.21
K	0.28
Ca	1.00
Mg	0.40
Total exchangeable bases (Cmol Kg^{-1})	1.89
Particle size distribution (%)	
Sand	72.80
Silt	10.00
Clay	17.20
Texture	Sandy loam
Bulk Density (Mg m^{-3})	
0-10 cm	1.83
10-20 cm	1.73
20-60 cm	1.86
Total Porosity (%)	30.94

Table.2: Soil Moisture Content (cm^3/cm^3) of Cropped and Uncropped Plots at Sampling Intervals

Cultivars	At Planting	4 WAS	8 WAS	12 WAS	16 WAS	20 WAS
IT86D-719	0.57	0.51	0.38	0.22	0.17	0.11
IT88D-867-11	0.54	0.47	0.34	0.19	0.17	0.12
Borno Brown	0.54	0.47	0.34	0.22	0.18	0.12
Control	0.57	0.53	0.39	0.29	0.24	0.18
SE±	**0.0046**	**0.0077**	**0.0077**	**0.0072**	**0.0086**	**0.0078**
$LSD_{(0.05)}$	***	***	***	***	***	***

Table.3: Grain Yield for the Three Cowpea Cultivars

Cultivar	Grain yield (kg/ha)
IT 86D-719	893.00
IT 88D-867-11	846.00
Borno Brown	675.30
Mean	804.80
SE±	**26.50**
$LSD_{(0.05)}$	**104.10**

Table.4: Root Length Density as Affected by Cultivar and Depths

Treatment	Root length at 50%	Root length at 100%
Cultivar		
IT86D-719	0.079	0.061
IT88D- 867-11	0.070	0.068
Borno Brown	0.113	0.100
SE±	**0.0176**	**0.0074**
$LSD_{(0.05)}$	*	***
Depth (cm)		
0-20	0.183	0.151

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20-40	0.108	0.128
40-60	0.078	0.073
60-80	0.041	0.026
80-100	0.027	0.007
SE±	**0.0227**	**0.0095**
LSD$_{(0.05)}$	***	***
V×D	**NS**	***

Table.6: Water Use Efficiency of Three Cowpea Cultivars

Variety	**Water Use (cm/day)**	**Water Use Efficiency (kg/ha/cm)**
IT86D-719	14.93	63.56
IT88D-867-11	12.80	70.06
Borno brown	14.44	45.69
SE±	**1.103**	**10.936**
LSD$_{(0.05)}$	**4.331**	**42.940**

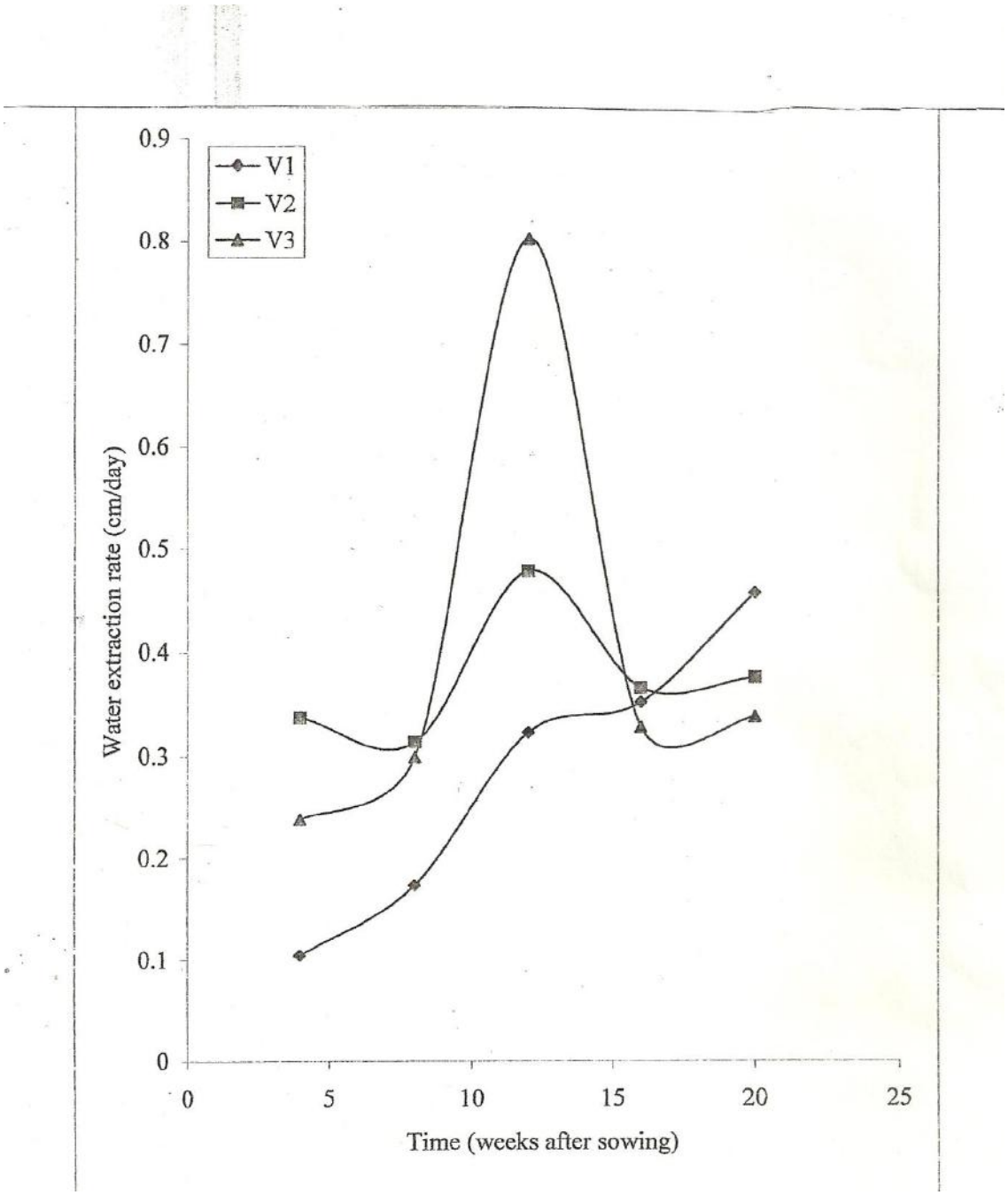

Fig.1: Rate of water extraction by cowpea cultivars at 4 weeks interval

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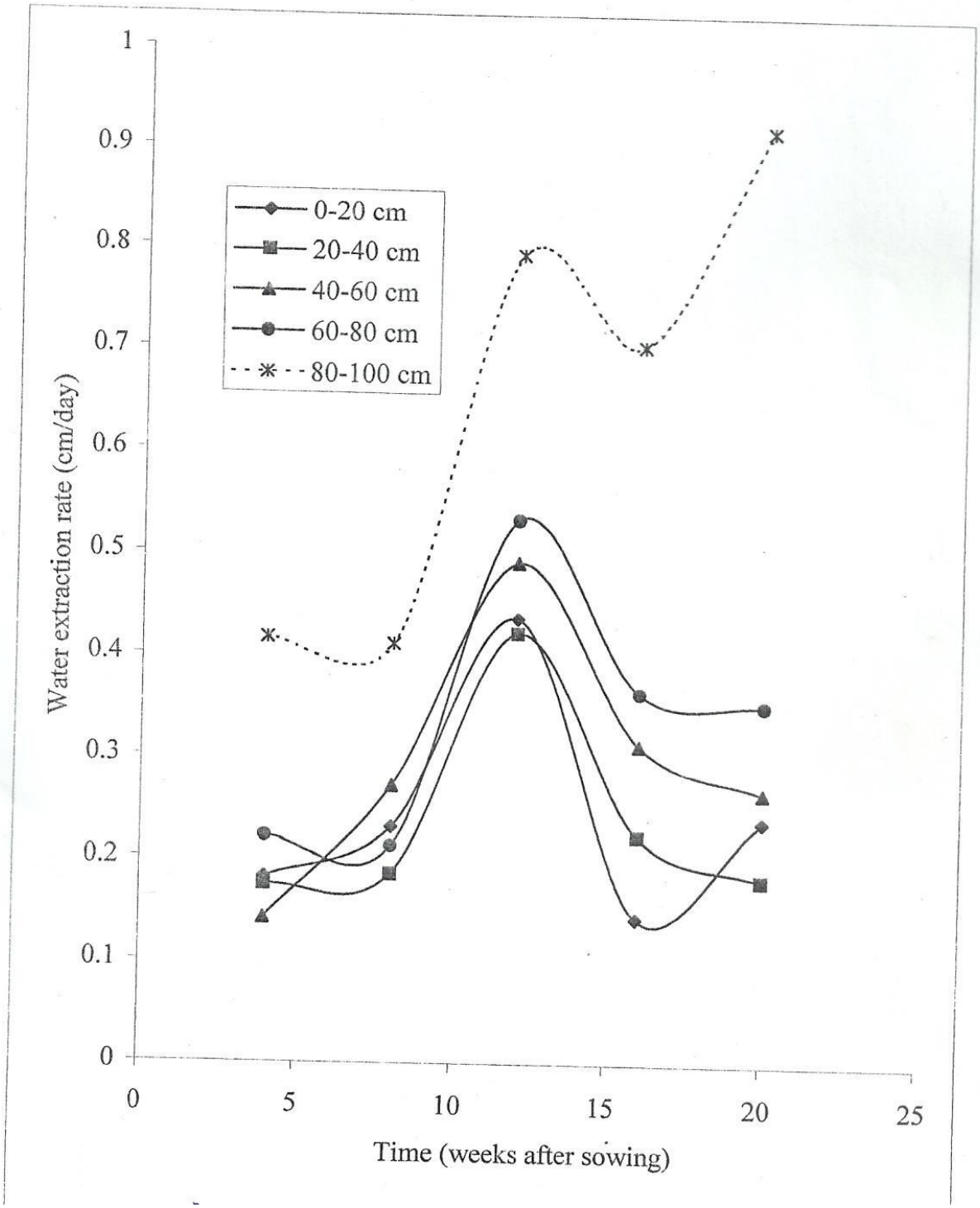

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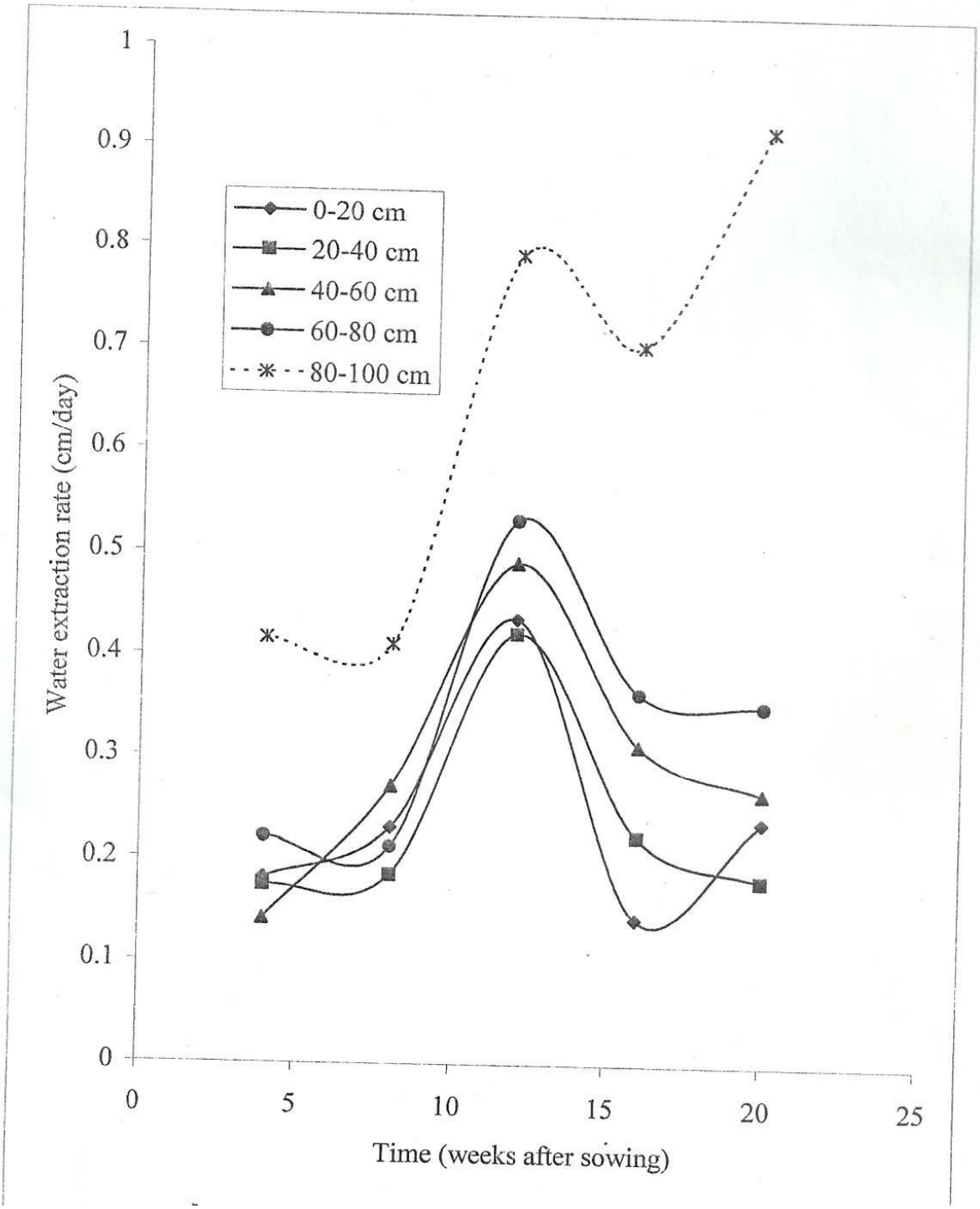

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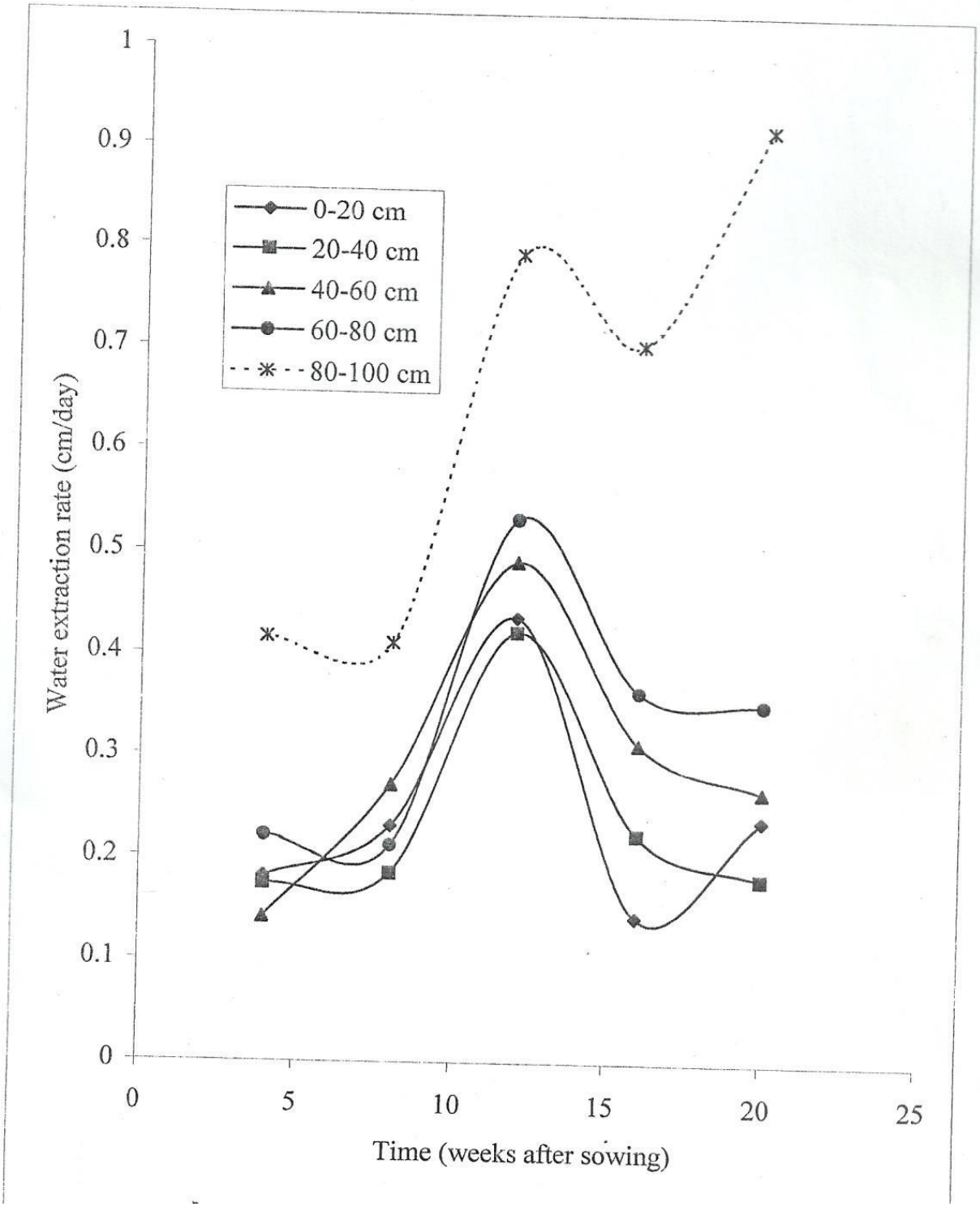

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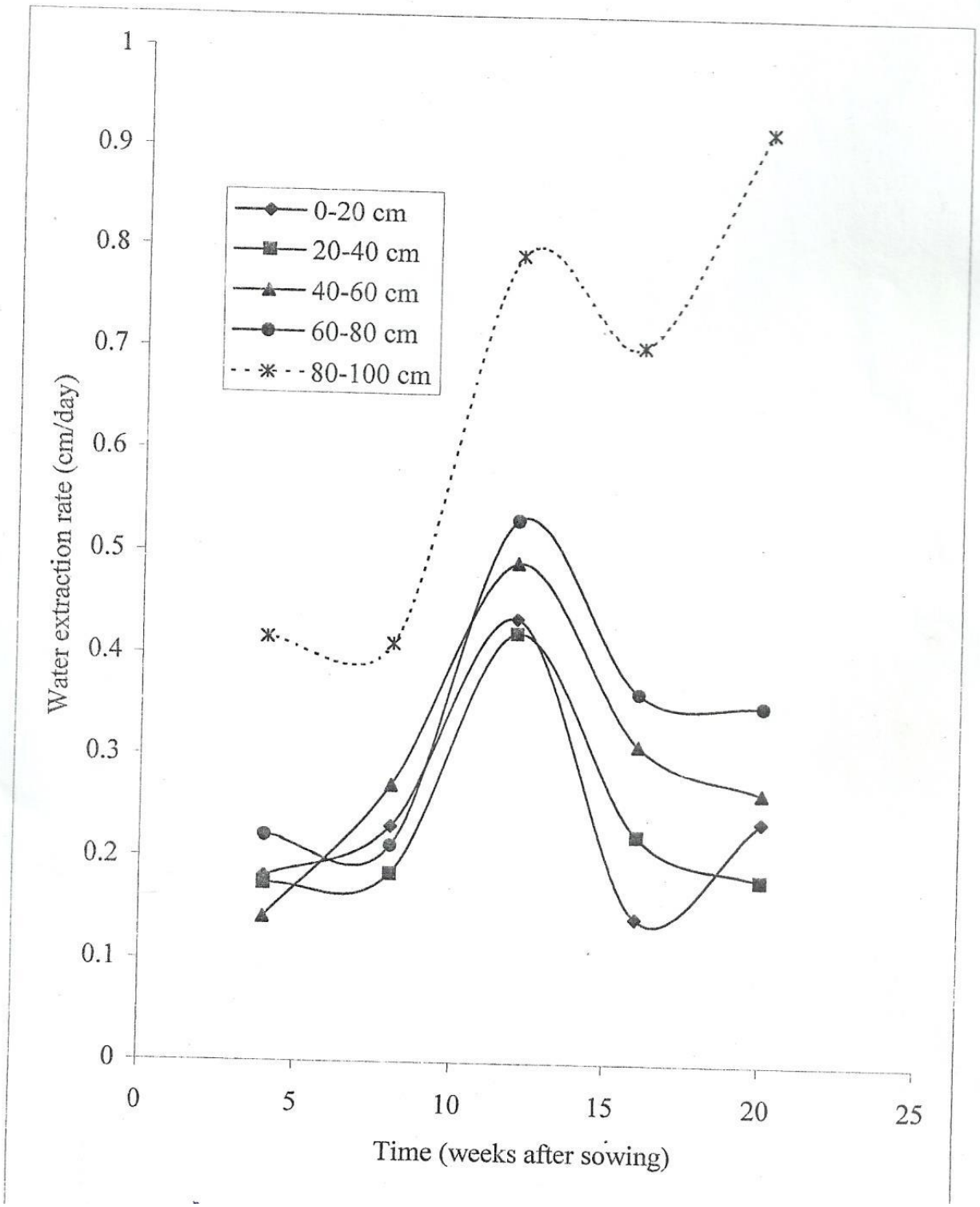

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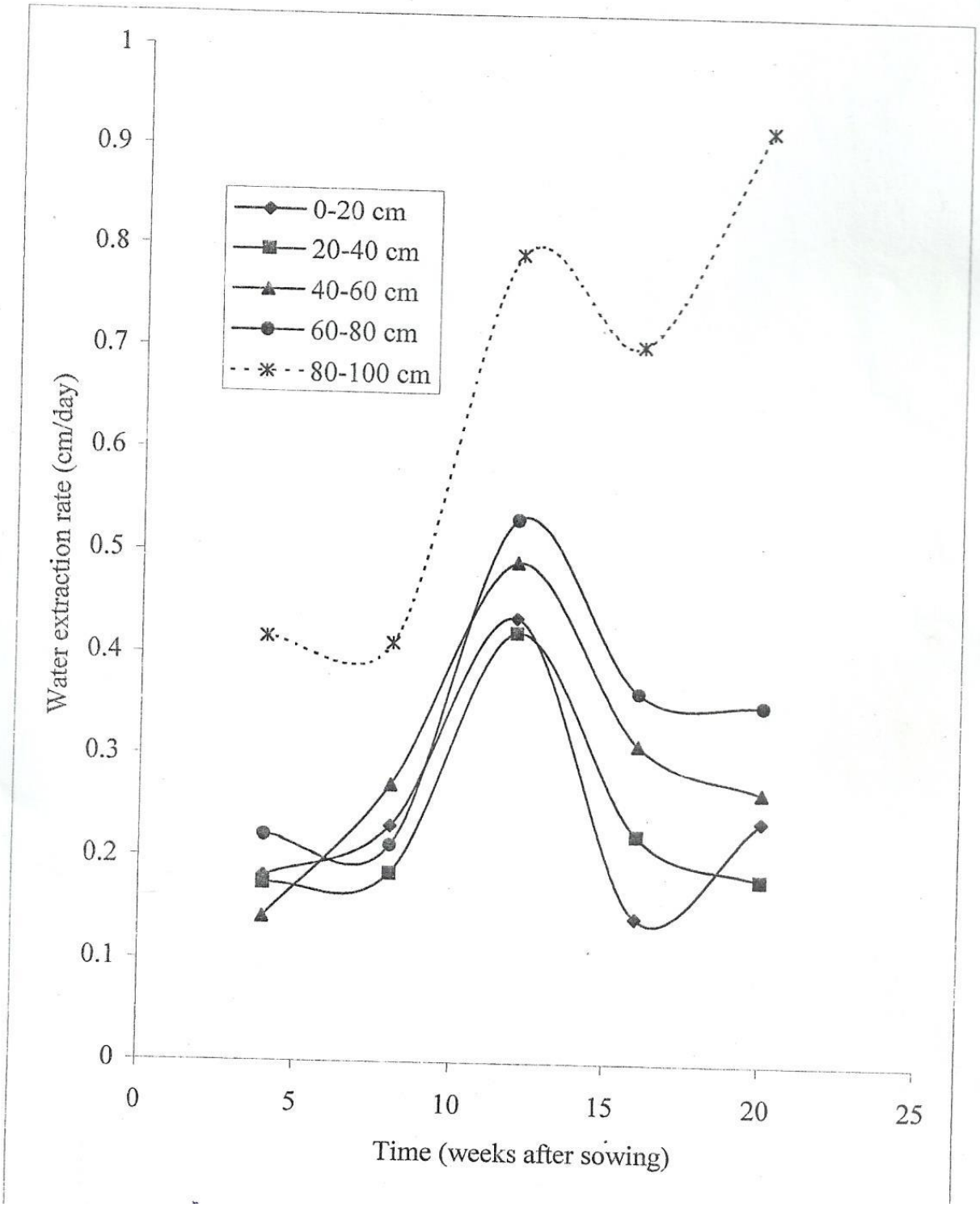

Fig.2: Rate of water extraction by cowpea cultivars at different depths

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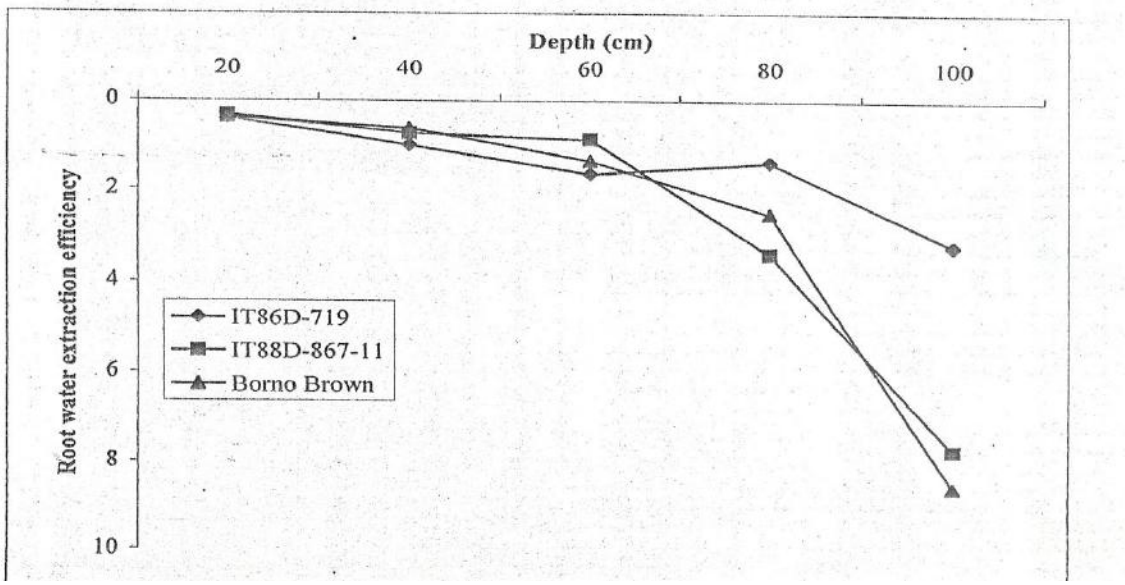

Fig.3: Root water extraction efficiency at flowering

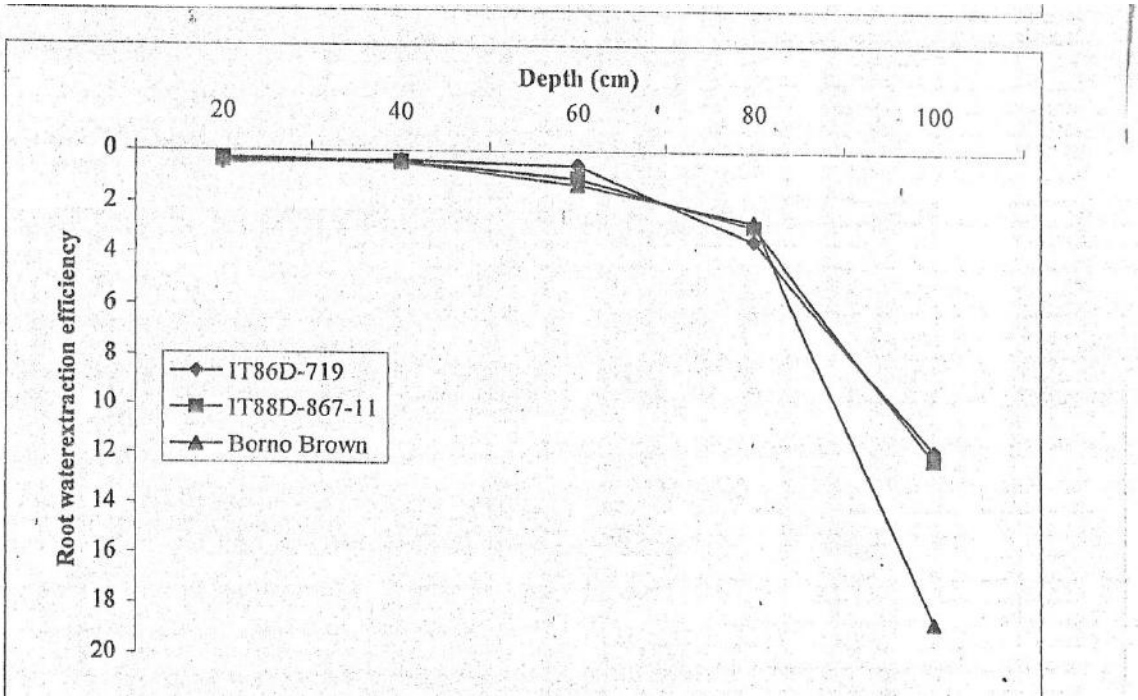

Fig.4: Root water extraction efficiency at podding

REFERENCES

[1] Abidoye T.O. (2004).Effects of soil moisture content on growth and yield of cowpea (vigna unguiculata (L)Walp.)B. Agric .Dissertation, University of Ilorin Nigeria.

[2] Angus, J.F.; Hasegawa,S. Hsiao, T.C. and Liboon, S.P. (1983). The Water Balance of Post Moonsonal Dry Land Crops. *Journal of Agricultural Science* (comb) 101: 699-710.

[3] Arya,L.M, Farrell, D.A and Blake, G.R. (1975) A Field study of soil water depletion patterns in presence

of Growing Soybean Roots: Determination of hydraulic Properties of the soil. *Soil Sci. Soc. Amer. Proc*., vol. 39, Pp 424-429.

[4] Blake, G.R. and Hartage, K.H. (1986). Bulk density. In: Klute, A. (ed). Methods of soil analysis, part 1, 2nd edition. Agronomy 9. Pp. 363- 375.

[5] Central Bank of Nigeria (2005). *Economic and Financial Review* Vol. 27, No.1

[6] De Tar, W.R. (2009) Crop Coefficients and Water Valley of California Agricultural Water Management. Vol. 96, Issue 1, pp. 53-66.

[7] FAO (2008).Food and Agriculture Organization, FAOSTAT http://faostat.fao org/site/567/default.aspx#ancor.

[8] Gee, G.W. and Bauder, J.W. (1986). Particle size analysis. In: Klute, A. (ed.). Method of soil analysis, part 1, physical and mineralogical properties. Agronomy monograph 9. ASA, Madison, Wisconsin. Pp. 383 – 411.

[9] Grema, A. K. and Hess, T.M. (1994). Water Balance and Water Use of Pearl Millet-Cowpea Intercrop in North East Nigeria. Agricultural Water Management 26:169-185.

[10] Gui-Rui Yu, JieZhwang, Keiichi Nakayama and Yan Jin (2007). Root Water Uptake and Profile Soil Water as Affected by Vertical Root Distribution Plant Ecology. 189:15-30.

[11] Klodpeng, T. and Morris, R.A. (1984). Drainage of a Tropaqualf before and after Extraction by a Crop. Soil Science Society of American Journal 48:632 -635.

[12] Kundiri, M.A. (1995) Soils, Land Management and Crop Suitability on the *Fadama* of North East Nigeria. Unpublished PhD Thesis, Cranfield University, UK.

[13] Miller, D.E. and Aarstad, J.S. (1974). Available Water as Related to Evapotranspiration rates and Deep Drainage. *Soil. Sci. Soc. Amer. Proc*. 35:131-134.

[14] Oyebande, L. (2002). Sustaining Wetland Eco-Systems in the West and Central African Sahel. Regional Hydrology: Bridging the Gap Between Research and Practice (Proceedings of the Fourth International Friend) Conference Held at Cape Town, South Africa TAHS Publication Number 274:365-372.

[15] Page, A.L; Miller, R.H. and Keeney, D.R. (1982). Methods of Soil Analysis Part 2, Second ed. Chemical and Microbiological Properties A.S.A; S.S.A. Madison Wisconsin.

[16] Raper, D.C. Jr. and Baber, S.A. (1970). Rooting Systems of SoyaBeansI. Difference in Root Morphology Among Varieties Agronomy Journal 62:581-584.

[17] Reicosky, D.C.,Millington, R.J and Peters, D.B. (1970). A Comparison of Three Methods for Estimating Root Length. Agronomy Journal 62:451-453.

[18] Richter, H. (1988). A Diagram for the Description of Water Relations in Plant Cells and Organs Journal of Experimental Botany 29: 1197-1203.

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Sorption of lead on variable charged soils as affected by temperature and time in three Provinces of China

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Abstract*—Effect of temperature and time on Lead (Pb) adsorption and affinity of the adsorbed Pb was evaluated in three acidic soils from China. The distribution coefficient increased slightly with increasing temperature up to 35 ^0C and then decreased at 45^0C.The increase of temperature was not favorable to sorption. The heat of reaction (ΔH) was positive (endothermic) at lower temperatures, while it was negative (exothermic) at higher temperatures. It was observed that there was an increase in the percent adsorption of Pb^{2+} within the first 8 min of the reaction and a decrease within 20 min of the reaction. The equilibrium was reached after 40min with 95.8 %, 87.4 % and 83.8 % for the Yellow brown soil (YBS), Latosol soil (LS) and Lateritic red soil (LRS) respectively. The analysis of images obtained by scanning electron microscope (SEM) with adsorbed Pb showed coverage of the surface with white layer (molecular cloud).*

***Keywords*—** *sorption, lead; variable charge soils; time; temperature.*

I. INTRODUCTION

Lead is a common contaminant of soil and considered to be a risk to human health. Lead may contaminate soil through vehicle exhausts, sewage-sludge biosolids, mining, and smelting [16] [1]. Toxicity from Pb-contaminated soils primarily occurs from direct ingestion. Symptoms of lead poisoning in human beings include irritability, poor muscle coordination, nerve damage, increased blood pressure, developmental delays etc. [20].

Heavy metal concentration in soil solution is of great importance for all ecological consideration because the plants are likely to take up the available metals from soil solution. The transport of metals within the soil or even to groundwater depends on the metal concentration of the solution phase [5]. It was suggested that the fate and transport of toxic metal ions in the environment are generally controlled by adsorption reactions, complexation etc. [21]. Studies on Pb (II) adsorption were performed on soils, clay minerals and oxides [8] [17][21] [2]. These studies showed that soil type, ionic strength, ion type, contact time affected the adsorption of heavy metals onto soils and clay minerals. Other factors such as liquid: solid ratio [14], solution pH, ionic strength [12] and temperature also affect sorption process [4] [11].

It is well known, tropical and subtropical regions are distributed with large areas of variable charge soils. These soils usually carry both positive and negative charges on their surfaces, therefore can adsorbed both anions and cations [23].

It has been observed that increasing the contact time

favor the adsorption of metal ions because the sorbed phase of metals changed from loosely bounded phase to strongly bounded phases with increasing contact time [9][15]. However, there are few studies and limited information on the effect of these operating variables on the adsorption and affinity of Pb onto variable charge soils .The process of adsorption, though widely applied, is still only partially understood [19].

This paper therefore investigated impact of reaction variables like contact time and temperature and on adsorption and affinity of Pb^{2+} onto three variable charge soils. The aim is to provide scientific information that will help in the management of lead contaminated soils.

II. MATERIALS AND METHODS

2.1 Soil samples and basic properties

Three representative variable charge soils were used in this study : Yellow brown soil (YBS), Alfisol in America Soil Taxonomy and Argosol in China Taxonomy; Latosol soil (LS) and Lateritic red soil, Oxisols in America Soil Taxonomy and Ferralosols in China Taxonomy，collected from Hubei, Hainan, and Guangxi provinces, respectively in China. These uncontaminated soils with contrasting properties were sampled at 0 – 20 cm depth. Composite samples of the soils were air-dried, ground, sieved through 2-mm mesh prior to use. Soil pH value was measured in de-ionized water at a soil: water ratio of 1:2.5. Cation exchange capacity (CEC) and organic matter content were determined by the methods described by Rhoades and Walkley – Black respectively [13]. Particle size distribution was determined using the pipette method. Amorphous iron (Fe) and aluminium (Al) oxides were determined by the oxalate extraction [10]. Crystalline Fe and Al oxides were determined by the oxalate – ascorbic acid extraction method of Shuman [18]. The mineralogical composition of the clay samples was determined with Cu-Ka radiation on X –ray diffractometer (D8 Bruker Advance X –ray diffractometer).

2.2. Effect of temperature on sorption

Weight of 1 g of soil samples were placed in 50 mL polyethylene bottles. Twenty-five mL of 0.01 KCl solution containing 100 mg L^{-1} Pb^{2+} was added. The solutions were adjusted to pH 5.5±0.1 with either 0.1 M NaOH or HCl. These were agitated at 230 rpm at different temperatures within the range of 5 – 45 ^{0}C, after which they were centrifuged and the supernatant solution taken for metal ion analysis using AAS. The amount adsorbed was calculated by the difference between the total applied Pb^{2+} and the amount of Pb^{2+} remaining in the equilibrium solution. The heat of reaction (△H) was determined using the formulae:

Heat of reaction = q/1000÷ Moles [3]

Where q= mass x specific heat capacity x change in temperature.

2.3. Effect of time on sorption

Weight of 1 g of soil samples were placed in 50 mL polyethylene bottles. 25 mL of 0.01 KCl solution containing 100 mg L^{-1} Pb^{2+} was added. The solutions pH were adjusted to 5.5±0.1 with either 0.1 M NaOH or HCl. These were agitated at 230 rpm at different times within the range of 5 – 80 min, after which they were centrifuged and the supernatant solution taken for metal ion analysis using AAS. The amount of metal ions adsorbed was calculated as above.

2.4. Scanning electron microscopy

After adsorption, the soil residues were air-dried for 7 days and gold coated under vacuum in a JFC – 1600 sputter coater (JEOL, Japan) for 20 min. The SEM images obtained by a JSM 6390 scanning electron microscope (JEOL, Japan)

2.5. Statistical analysis

All experiments were carried out in duplicate and only mean values are presented. All data were processed by Microsoft Excel, SAS and sigmaplot10.

III. RESULTS AND DISCUSSION

3.1. Physicochemical and mineralogical properties of soils

The results of the physicochemical properties showed that YBS had higher CEC and pH (27.4 cmol kg^{-1} and 5.2) respectively than the other soils, while LS had higher organic carbon and crystalline Fe_2O_3 and Al_2O_3 (13.1 g kg^{-1}, 84.0 g kg^{-1} and 5.2 g kg^{-1}) respectively than the other soils (Table 1). The particle size analysis showed that LS was clay, YBS was silt loam and LRS was clay loam. Mineralogical composition showed that LRS mainly consisted of kaolinite 60 %, and illite 40 % characterized by diffraction peaks at 0.719nm and 1nm, respectively. The LS consisted of kaolinite 75 %, hydroxyinterlayed vermiculite 15 % and goethite characterized by diffraction peaks at 0.71nm, 0.48nm and 0.27nm, respectively. Yellow brown soil consisted of illite 45 %, vermiculite 25 % and kaolinite 30 % characterized by diffraction peaks at 1nm, 1.396nm and 0.717nm, respectively (Fig.1). The YBS has higher adsorption capacity probably due to its higher CEC, pH and illite content (Table 1). This result is in agreement with previous reports suggesting that adsorption capacity of Pb^{2+} by soil has a significant relationship with CEC of soils [6][22]. Other properties that are associated with metal adsorption e.g. clay content and organic matter may also influence metal retention through their relation to CEC [22]. The result of particle size analysis agrees with [7] that oxisols with high Fe oxide content are rich in clay; however, the soils exhibit moisture characteristics of sands.

Table.1: Properties of the soils studied

soil	pH (H_2O)	O.M g kg^{-1}	CEC g kg^{-1}	Clay (<0.002mm) g kg^{-1}	Clay minerals	Crystal Fe g kg^{-1}	Crystal Al g kg^{-1}
YBS	5.2	10.8	27.4	256.5	I (45),V(25), K (30)	16.0	1.3
LS	4.5	13.1	15. 9	415.9	K(75), HIV(15), GE	84.0	5.2
LRS	4.1	9.9	21.9	361.2	K(80),GE(20)	21.0	2.1

I = illite, V = vermiculite, K = kaolinite, HIV =hydroxyinterlayed vermiculite, GE= goethite. Data in the parentheses are the contents (%) of the corresponding minerals.

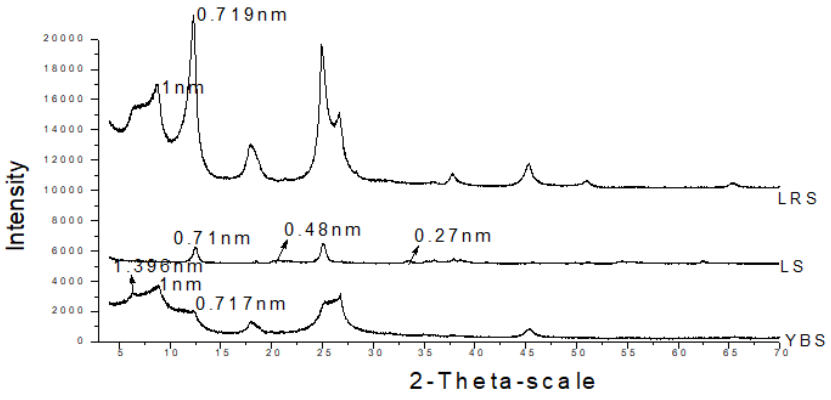

Fig.1: X-ray diffraction patterns of three soils, a. LRS; b. LS; c. YBS

3.2. Effect of temperature on sorption

In a series of adsorption experiments at temperature between 5 ^{0}C and 45 ^{0}C, the distribution coefficient (K_d) increased slightly with increasing temperature up to 35 ^{0}C and then decreased at 45 ^{0}C. The maximum increase of 365 mL/g was observed in YBS, while the maximum reduction

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of 608 mL/g was observed in LS (Fig.2). The heat of reaction (△H) was positive at lower temperature and negative at higher temperatures. At 5 ^{0}C, 15 ^{0}C and 25 ^{0}C, △H was 962 kJ mol^{-1},544 kJ mol^{-1}, and 126 kJ mol^{-1} respectively. At 35 ^{0}C, and 45 ^{0}C, △H was – 293 kJ mol^{-1} and -711 kJ mol^{-1} respectively (Table 2). To the best of our knowledge, this result has not been reported. The attraction of Pb^{2+} to soils increased slightly with increasing temperature up to 35 ^{0}C and then reduced at 45 ^{0}C. This could be because at (25 -35 ^{0}C) the temperature is warm and the environmental condition is conducive for rapid decomposition and mineralization or release of simple inorganic products such as sulfates, nitrates etc. which may lead to increase adsorption of Pb^{2+} onto soils, while at high temperature of 45 ^{0}C there was damage of active binding sites in the soils. The negative value for △H indicates that desorption is favoured as temperature increase [24] The distribution coefficient (K_d) of YBS is greater than other soils, indicating that Pb^{2+} has a greater affinity for YBS than other soils.

Table.2: Enthalpy change at different temperatures during Pb adsorption.

Temperature °C	Enthalpy change △H (kJ mol^{-1})
5	962
15	544
25	126
35	-293
45	-711

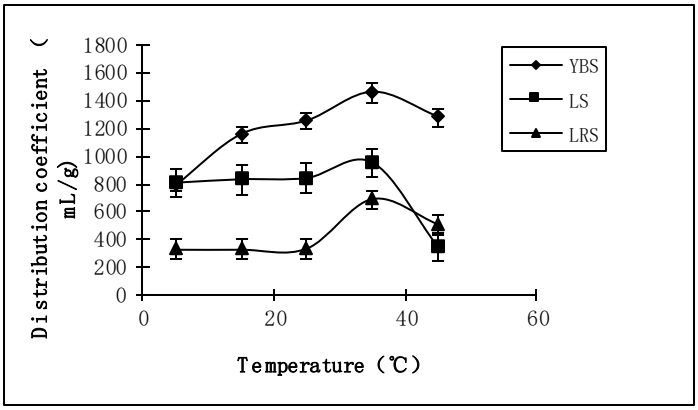

Fig.2: Distribution coefficient of Pb^{2+} at different temperatures

3.3. Effect of time on sorption

From Fig.3, it was observed that there was an increase in the percent adsorption of Pb^{2+} within the first 8 min of the reaction and a decrease within 20 min of the reaction. The equilibrium was reached after 40min with 95.8 %, 87.4 % and 83.8 % for the YBS, LS and LRS, respectively. In the experiments, 24 h was selected to achieve the adsorption equilibrium. The effect of time on adsorption of Pb ion is contrary to previous observation by [2] that adsorption of Pb^{2+} onto kaolinite clay increased with time and reaches equilibrium after 8 min and 20 min for P-modified and unmodified kaolinite respectively. The reason for this trend is not understood. Further investigation is warranted to explain this behavior, presumably associated with the interaction between Pb^{2+} and soils.

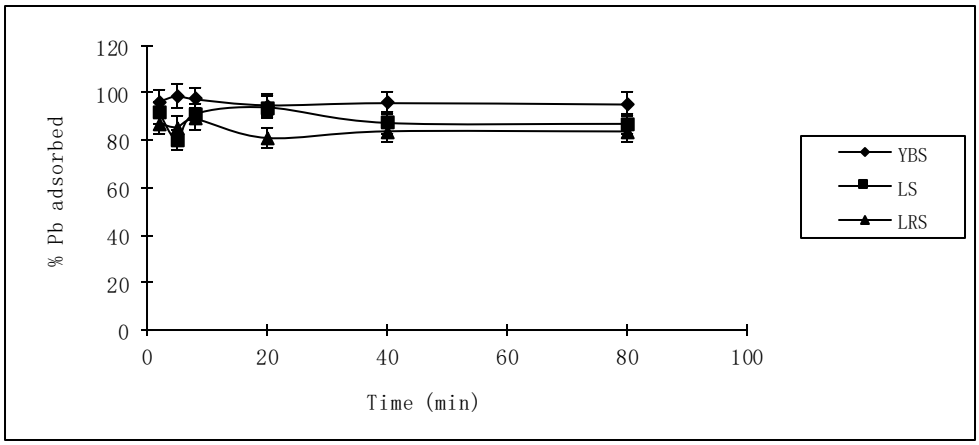

Fig.3: Percent Pb^{2+} adsorbed at different time intervals

3.4. Scanning electron microscopy analysis

The morphology of the soils with Pb^{2+} ion showed some important observations. Typical SEM photographs are shown in Fig.4 (a–f). Coverage of the surface of the soil due to adsorption of Pb^{2+} ion presumably leading to formation of layer of lead molecules over the soil surface. It is evident from the formation of white layer (molecular cloud). There was higher surface coverage of lead in YBS and LRS than LS. It is noticed that soils have bigger pore structures, 1 – 5µm and after adsorption, the pore size have been reduced to 0.4 – 0.6 µm where the lead has attached to it due to its surface chemistry. There was spherical like structures attached in bundles. This could be due to adsorption of Pb^{2+} ion on soils. We see beautiful surface adsorbed spherical shaped Pb particles on the soils.

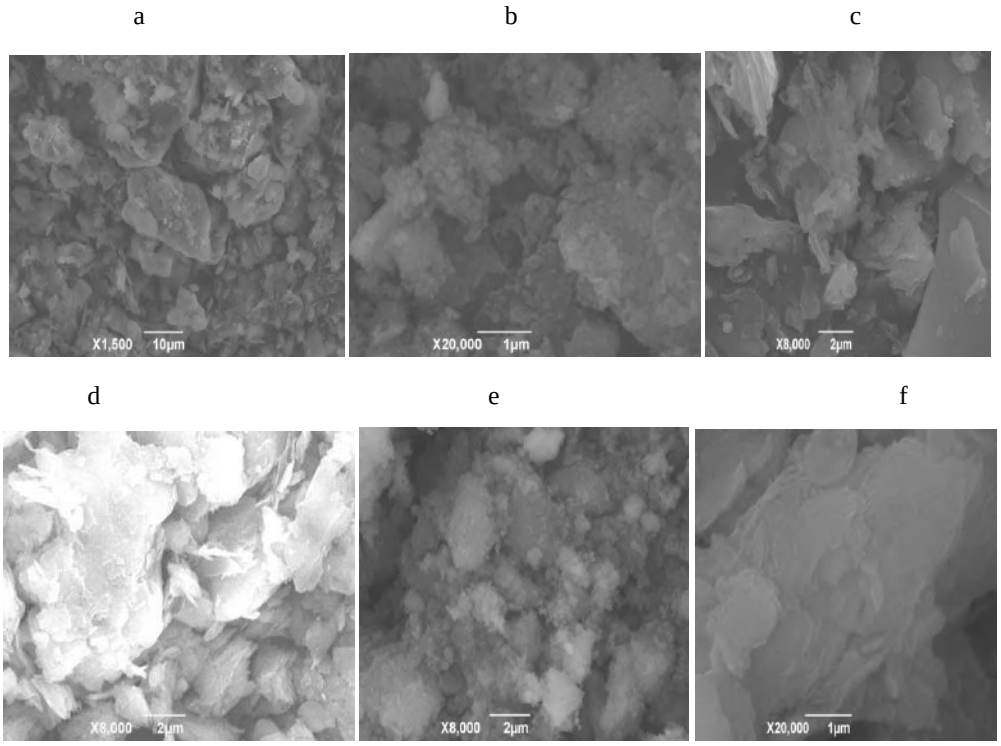

Fig.4: Scanning electron microscopy of soils without and with Pb a. LRS, b. LS, c YBS without Pb, d. LRS, e. LS, f. YBS with Pb

IV. CONCLUSIONS

At warm temperature the distribution coefficient (K_d) was highest in all the soils. Sorption of Pb^{2+} decreased with the increase in temperature. The Pb^{2+} has a greater affinity for YBS than other soils. Lead ion adsorption decreased with time in all the soils and decreased pore structures in studied soils.

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REFERENCES

[1] Abdel-saheb, I., Schwab, A.P., Banks, M,.K., and Hetrick, B.A., (1994). Chemical characterization of a heavy metal contaminated soil in southeast Kansas. Water Air Soil Pollut., 78: 73-82.

[2] Adebowale, K.O, Unuabonah, I.E., and Olu –Owolabi, B.I. (2006). The effect of some operating variables on

the adsorption of lead and cadmium ions on kaolinite clay. J. Hazardous materials B, 134: 130 – 139.

[3] AUS-e- TUTE n.d., (2011) June 1. Chemistry Tutorial : Heat (enthalpy) of Reaction. Retrieved 9 June 2011, http:// www. ausetute.com.au/heatreact.html.

[4] Bestas, N., Agim, B.A. and Kara, S. (2004). Kinetic and equilibrium studies in removing lead ions from aqueous solutions by natural sepiolite. J. Hazardous materials, 122: 115 – 122.

[5] Brummer, G.W., Gerth, J., and Herms, U. (1986). Heavy metal species, mobility and availability in soils. Z. Pflanzenernaehr. Bodenk., 149: 382 -398.

[6] Coles, C.A., and Yong, R.N. (2002). Aspects of kaolinite characterization and retention of Pb and Cd. Applied Clay Sci., 22: 39 -45.

[7] Foth, H.D. (1984). Fundamentals of Soil Science. 7th Ed. New York: John Wiley and Sons.

[8] Lee, S.Z., Cheng, L. Yang, H.H., Chen, C.M., and Liu, M.C. (1998). Adsorption characteristics of lead unto soils. J. Hazard. Mater., 63: 37 -49.

[9] Lim, T.T., Tay, J.H., and Teh, C.I. (2002). Contamination time effect on lead and cadmium fractionation in a tropical coastal clay. J. Environ. Qual., 31: 806 -812.

[10] McKeague, J.A., and Day, J.H. (1966). Dithionite – and oxalate – extracted Fe and Al as aids in differentiating various classes of soils. Can. J. Soil Sci. 46: 13-22.

[11] Nkwopara, U.N., Ihem, E., Agim, L. Uzoho, B., Onweremadu, E., Ahukaemere, C., and Hu, H. (2013). Effect of temperature and mechanism of Pb sorption on selected acid soils of Southern China. Acta Agronomica Nigeriana, 13(1-2): 43-56.

[12] Nkwopara, U.N., Emenyonu-chris, C.M., Ihem, E.E., Ndukwu, B.N.. Onweremadu, E.U., Ahukaemere, C.M. Egbuche,C.T. and Hu, H. (2015). Batch studies for the investigation of the mechanism of Pb sorption in selected acid soils of China. Agriculture, Forestry and Fisheries, 4(3-1): 11-20.

[13] Page, A.L., Miller, R.H. and Keeney, D.R. (1982). Methods of Soil Analysis: Part 2, Chemical and Microbiological Properties (2nd ed.). (Madison, WI : Soil Sci. Soc. Ame).

[14] Pulse, R.W., and Bohn, H.L. (1988). Sorption of cadmium, nickel and Zinc on kaolinite and montmorillonite suspensions. Soil Science society of America Journal, 52: 1289-1292.

[15] Qin, F., Shan, X., and Wei, B. (2004).. Effects of low – molecular – weight organic acids and residence time on desorption of Cu, Cd, and Pb from soils. Chemosphere, 57: 253 – 263.

[16] Schwab, A.P., He,, Y. and Banks, M.K. (2005). The influence of organic ligands on the retention of lead in soil. Chemospere, 61: 856 – 866.

[17] Sipos, P., Nemeth, T., Mohai, I., and Dodony, I.,(2005). Effect of soil composition on adsorption of lead as reflected by a study on a natural forest soil profile. Geoderma, 124: 363 - 374

[18] Shuman, L.M. (1982). Separating Soil Iron – and Manganese oxide fractions for microelement analysis. Soil Sci. Soc. Am. J., 46: 1099 – 1102.

[19] Unuabonah E.I., Olu–Owolabi, B.I., Adebowale, K.O., and Ofomaja, A.E. (2007). Adsorption of lead and cadmium ions from aqueous solutions by triphosphate – impregnated kaolinite clay. Colloids and surface A: Physicochemical and Engineering Aspects, 292: 202 – 211.

[20] USEPA (United State Environmental Protection Agency), (2000). Eliminating childhood lead poisoning: a federal strategy targeting lead paint hazards. Washington, DC: US Government Printing Office).

[21] Wang, S., Jun, H., . Li, J., and . Dong, Y., (2009). Influence of pH, soil humic/fulvic acid, ionic strength, foreign ions and addition sequence on adsorption of Pb (II) onto GMZ bentonite. J. Hazard. Mater., 167, 44 -51.

[22] Yang, J.Y., Yang, X.E. He, Z.L,. Li, T.Q., Shentu, J.L,.

and Stofella, P.J.(2006). Effects of pH, organic acids and inorganic ions on lead desorption from soils. Environ. Pollut., 143: 9 – 15.

[23] Yu, T.R. (1997). Chemistry of variable charge soils. New York: Oxford University Press.

[24] Zhang, J., Zeng, J., and He, M. (2009) Effect of temperature and surfactants on naphthalene and phenanthrene sorption by soil. Journal of environmental sciences 21: 667-674

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GC-MS analysis of bioactive compounds in methanolic extract of tubers of *Pueraria tuberosa* (Roxb. ex Willd.) DC. - Fabaceae

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Abstract—*The present experiment was designed to determine the bioactive constituents from tuber extracts of Pueraria tuberosa (Roxb. ex Willd.) DC. of the family Fabaceae. The medicinal value of a plant species is dependent upon its various phytochemical constituents. The chemical compositions of the methanolic extract of tubers of P. tuberosa were investigated using Gas chromatography-Mass spectrometry and about nineteen bioactive phytochemical compounds were identified. The prevailing compounds were 2, 3-Dimethylaziridine; 2-Cyclopenten-1-one, 2-hydroxy-; 2-Hydroxy-gamma-butyrolactone; 3-Methyl-1,2-cyclopentanedione; 2,5- Dimethyl-4-hydroxy-3 (2H) – furanone; Butane 2-methyl; Oxetane; Maltol; 1, 5-Anhydro-6-deoxyhexo-2,3-diulose; 2, 3-Dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-One; 5-Hydroxymethylfurfural, Phenol,2,6-dimethoxy; Dodecanoic Acid; Guanosine; Tetradecanoic acid; Myo-inositol; Hexadecanoic Acid; 9, 12-Octadecadienoic acid, methyl ester and Cis-vaccenic acid. This was the first report on identification of bioactive compounds from methanolic extract of tubers of P. tuberosa.*

Keywords— Pueraria tuberosa, methanolic extract, GC-MS analysis, bioactive compounds.

I. INTRODUCTION

From ancient times plants are best sources of bioactive compounds having interesting biological activities. Literature studies represent the medicinal plants as reservoir of effective chemotherapeutants, play a principal role in the maintenance of human health. Knowledge on the phytoconstituents of plants is highly desirable for disclosing the actual significance of folkloric remedies, Milne, (1993). The secondary metabolites of plants have a variety of structural arrangements and properties, De-Fatima *et al*., (2006). Phytochemicals of natural drugs have overlapping and complementary mechanism of action; hence thorough validation of natural drugs was prioritized and emphasized. Mass Spectrometry coupled with Gas Chromatography is normally used for the direct analysis of chemical constituents present in plant based medicine. GC-MS analysis is a highly commended analysis for non-polar components, fatty acids, volatile essential oil, lipids, Jie and Choi, (1991) and alkaloids, Betz *et al*., (1997).

The tubers of *P.tuberosa* are widely used in ayurveda and in ethanomedicine. It has been recommended for the treatment of menopausal syndrome, sexual debility, cardiovascular diseases, fertility disorders, hepatosplenomegaly and spermatorrhoea and has been used as antiaging, spermatogenic and immune booster, Amal *et al*., (2014). There has been tremendous progress in medicinal plant research which involve the isolation and identification of secondary metabolites of plants and their use as active principles in therapeutics, Mary *et al*., (2013). Literature studies indicate that no reports on GC-MS analysis *P. tuberosa* has so far been undertaken to provide enough data in favour of its traditional uses. As part of the endeavor for the study of therapeutic properties of *P. tuberosa* we herein reported the GC-MS analysis of methanol extract of the tubers.

II. MATERIALS AND METHODS

2.1 Collection of plant sample

The tubers (Plate 1) of the *P. tuberosa* (Roxb. ex Willd.) DC. were collected from Nelliyampathy forests of Palakkad district, Kerala state. The tubers were authenticated by Dr. P.S. Udayan, Sree Krishna College, Guruvayur and the voucher specimens were preserved for further reference.

2.2 Preparation of powder and extract

Collected tubers were thoroughly washed in running tap water for 10 minutes. These were cut into pieces and were air dried in shade so as to prevent

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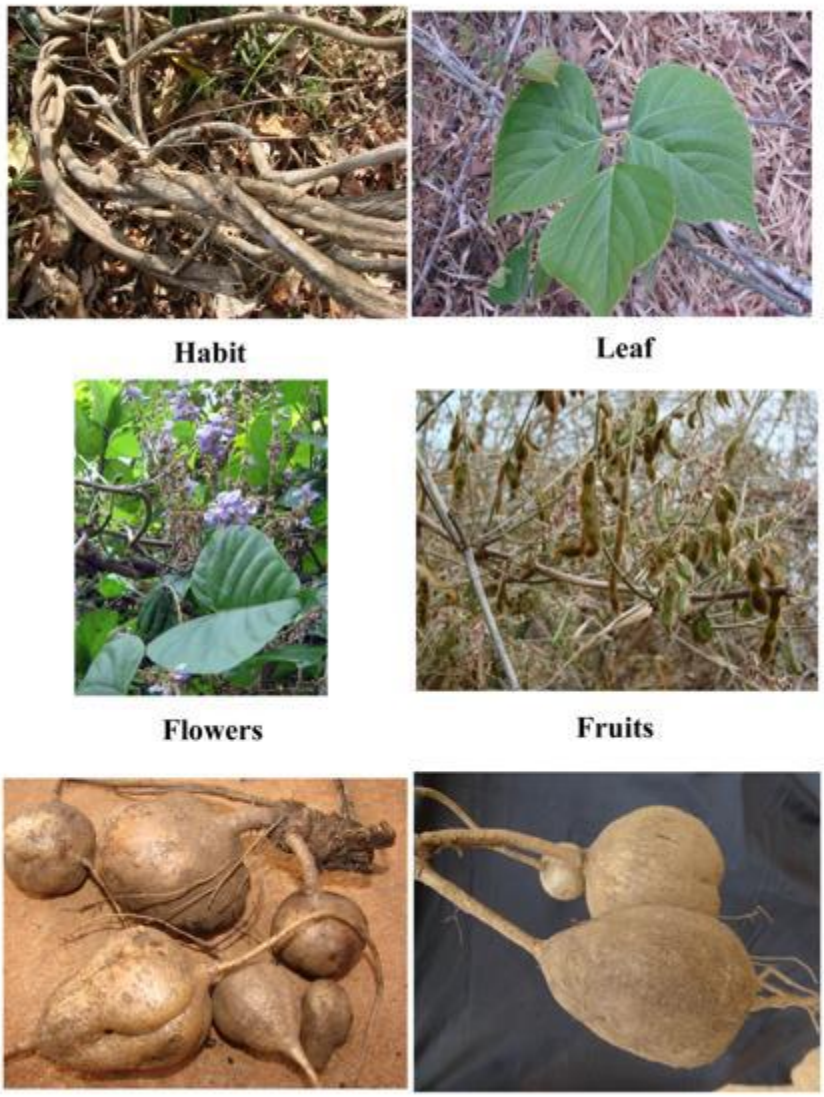
decomposition of active principle and made fine powder by using mechanical grinder. Then the powder was extracted using methanol as a solvent. Twenty gram of dried powder was weighed and subjected to extract successively with 200 ml methanol in soxhlet extractor. The extract was condensed and preserved in refrigerator in air tight bottles.

2.3 GC-MS analysis of bioactive compounds

The methanolic extracts obtained were subjected to GC-MS analysis for the determination of various bioactive volatile compounds in *P. tuberosa*. The analysis was carried out using Shimadzu Make QP-2010 with nonpolar 60 M RTX MS column, operating in electron impact mode at 70 eV. Helium was used as the carrier gas and an injection volume of 0.5µl was employed in split less mode at injection temperature 260ºC; ion-source temperature 200ºC. The oven temperature programming was set with a rate of 10 ºC with an initial oven temperature at 60º C and final temperature at 280º C, held for 8 minutes. The total running time for the sample was 25 minutes. The chemical constituents of the methanolic tuber extracts of plant samples were identified by comparing the retention times of peaks using NIST Library to relative retention indices. The relative percentage of each of the component in the extract was calculated by comparing its average peak area to the total areas.

III. RESULTS

PLATE 1
Pueraria tuberosa (Roxb. ex Willd.) DC.



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The results pertaining to GC-MS analysis leads to the identification of number of chemical constituents from the GC fractions of methanolic extract of tubers of *P. tuberosa*. Nineteen bioactive compounds were identified and their retention time (RT), % of peak area, molecular formula, molecular weight and biological activities are presented in Table 1 & 2 and Fig. 1. The prevailing compounds were 2, 3- Dimethylaziridine (1.96%); 2-Cyclopenten-1-one, 2-hydroxy-(2.84%);2-Hydroxy-gamma-butyrolactone(12.16%);3-Methyl-1,2-Cyclopentanedione (1.78%); 2, 5-Dimethyl-4-hydroxy-3 (2H)-furanone (3.24%); Butane 2-methyl (0.83%); Oxetane (12.37%); Maltol (7.50%); 1, 5-Anhydro-6-deoxyhexo-2, 3-diulose (3.28%); 2, 3-Dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-One (2.94%); 5-Hydroxymethylfurfural (19.82%); Phenol, 2,6-dimethoxy (0.92%); Dodecanoic Acid (3.39%); Guanosine (12.11%); Tetradecanoic acid (1.05%); Myo-inositol (3.36%); Hexadecanoic Acid (6.23%); 9, 12-Octadecadienoic acid, methyl ester (1.58%) and Cis-vaccenic acid (2.64%). Most abundant bioactive components among these were 5-Hydroxymethylfurfural (19.82%); Oxetane(12.37%); 2-Hydroxy-gamma-butyrolactone (12.16%) and Guanosine (12.11%).

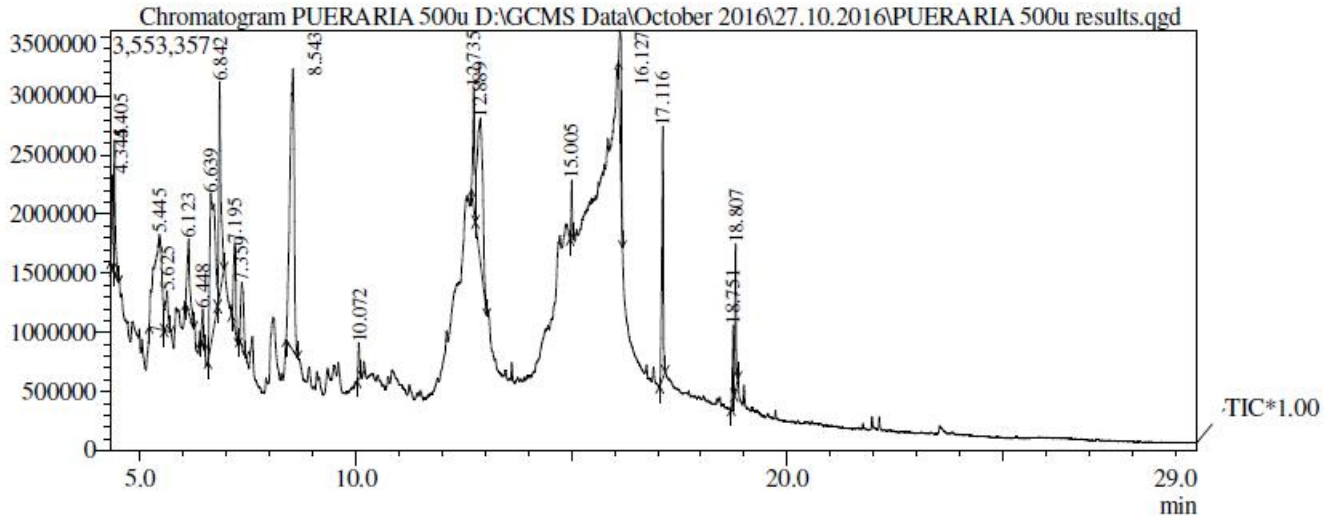

Fig.1: GC-MS Chromatogram of methanolic extract of tuber of P. tuberosa.

Table.1: Bioactive compounds detected from methanol extract of tubers of P. tuberosa.

Peak	Retention time	% of peak area	Compounds analysed	Nature of compounds	Molecular formula	Molecular weight
1	4.345	1.96	2,3-Dimethylaziridine	Heterocyclic compound	C_4H_9N	71.12
2	4.405	2.84	2- Cyclopenten-1-one, 2-hydroxy-	Organic compound	$C_6H_6O_2$	98.10
3	5.445	12.16	2-Hydroxy-gamma-butyrolactone	Lactone	$C_4H_6O_3$	102.09
4	5.625	1.78	3-Methyl-1,2-cyclopentanedione	Lactone	$C_6H_8O_2$	112.13
5	6.123	3.24	2,5- Dimethyl-4-hydroxy-3(2H)-furanone	Lactone	$C_6H_8O_3$	128.13
6	6.448	0.83	Butane 2-methyl	Alkane	C_5H_{12}	72.15
7	6.639	12.37	Oxetane	Heterocyclic	$C_{10}H_{16}O_3$	184.24
8	6.842	7.50	Maltol	Heterocyclic	$C_6H_6O_3$	126.11
9	7.195	3.28	1,5-Anhydro-6-deoxyhexo-2,3-diulose	Glucoside	$C_6H_8O_4$	144.0

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10	7.359	2.94	2,3-Dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-One	Unsaturated cyclic compound	$C_6H_8O_4$	144.13
11	8.543	19.82	5-Hydroxymethylfurfural	Heterocyclic compound	$C_6H_6O_3$	126.11
12	10.072	0.92	Phenol,2,6-dimethoxy	Organic compound	$C_9H_{12}O_3$	168.19
13	12.735	3.39	Dodecanoic Acid	Saturated Fatty acids	$C_{12}H_{24}O_2$	200.32
14	12.889	12.11	Guanosine	Purine nucleoside	$C_{10}H_{13}N_5O_5$	283.24
15	15.005	1.05	Tetradecanoic acid	Saturated Fatty acid	$C_{14}H_{28}O_2$	228.37
16	16.127	3.36	Myo- inositol	Vitamin like substance	$C_6H_{12}O_6$	180.16
17	17.116	6.23	Hexadecanoic Acid	Saturated Fatty acid	$C_{12}H_{32}O_2$	257.42
18	18.751	1.58	9,12-Octadecadienoic acid, methyl ester	Unsaturated fatty acid	$C_{19}H_{34}O_2$	294.47
19	18.807	2.64	Cis-vaccenic acid	Fatty acid	$C_{18}H_{34}O_2$	282.46

Table.2: Activity of Bioactive compounds identified in the methanol extract of tubers of P. tuberosa.

S. N.	Chemical constituents	Biological Activities	Literature cited
1	2,3-Dimethylaziridine	Antimicrobial and anticancer	Aleksandra *et al*., 2017
2	2- Cyclopenten-1-one, 2-hydroxy	Inducer of Heat Shock Protein 70 with Antiviral Activity	Antonio *et al*., 1996
3	2-Hydroxy-gamma-butyrolactone	Epigoitrin from4-hydroxy-γ-Butyrolactone presents antithyroid and antivirus activities	Wei and Chunhua, 2013
4	3-Methyl-1,2-cyclopentanedione	An effective anti-inflammatory agent	Jae *et al*., 2007
5	2,5- Dimethyl-4-hydroxy-3(2H)-furanone	Broad spectrum antimicrobial activities	Sung *et al*., 2007
6	Maltol	Flavor compound	Bhesh *et al*., 2013
7	1,5-Anhydro-6-deoxyhexo-2,3-diulose	Preservative	Prabu *et al*., 2013
8	5-Hydroxymethylfurfural	Antioxidant,Antiproliferative activity	Zhao *et al*., 2013
9	Phenol,2,6-dimethoxy	Antimicrobial, Antioxidant, Anti inflammatory	Salem *et al*., 2018
10	Dodecanoic Acid	Both antibacterial and antifungal	Belakhdar *et al*., 2015
11	Tetradecanoic acid	Both antibacterial and antifungal	Belakhdar *et al*., 2015
12	Hexadecanoic Acid	Antioxidant	Belakhdar *et al*., 2015

IV. DISCUSSION

Now a day, the study of bioactive components from medicinal plants and their activity has increased. The combination of GC (best separation technique) with MS (best identification technique) made GC-MS one of the ideal techniques for quantitative analysis of volatile and semi-volatile compounds, Grover and Patni, (2013). The identified compounds with more percentage like 5-Hydroxymethylfurfural (19.82%), Hexadecanoic acid, ethyl ester (Palmitic acid ester) (6.23%), 2-Hydroxy-gamma-butyrolactone (12.16%) showed a wide range of potent bioactivity. These phytochemicals are responsible for various pharmacological actions like antioxidants and antimicrobial activities, Tapiero *et al*., (2002). Among the nineteen compounds identified 7 showed Anti-microbial activity, 2 showed Anti-inflammatory, 1 showed Anti-cancer and 3 showed anti-oxidant and also showed activities such as antithyroid , inducer of heat shock protein 70, up-regulator of immunoglobulin synthesis, Bickerstaffe and Annison, (1970). The GC-MS analysis of methanolic extract of tuber of *Plectranthus rotundifolius* Spreng. showed the presence of forty different phytochemical compounds , among these Cis-Vaccenic acid was identified as an active phytocomponent, Manikandan *et al*., (2016).

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Sweetness enhancer maltol increases the creaminess and decreases the bitterness of food, Bhesh, (2013). Different kinds of dietary fat modify the risks of many chronic and acute inflammatory diseases by the differential regulation of gene expression and activation of macrophages Joo *et al*., (2001). Unsaturated fatty acids like 9, 12-Octadecadienoic acid, methyl ester, known as an omega-6 fatty acid are important for normal cell growth, to lower cholesterol levels of the blood, Igwe and Okwu, (2013) and to support the lubricating quality of skin, Okwu and Morah, (2006).

It has been reported that tuberosin, one of the active principles of *P. tuberosa* inhibits lipopolysaccharide (LPS) induced inflammatory changes in macrophages and directly scavenges various species of free radicals, Panday and Tripati, (2010). *P.tuberosa* showed significant dose dependent ulcer protective effect due to its antioxidant activities and it was comparable to the reference drug OMP, Sumalatha *et al*., (2010). In the present experiment different compounds displayed similar activity and the presence of various radical scavenging and anti-inflammatory compounds in the methanolic extract of *P.tuberosa* may be the responsible for its antioxidant properties.

V. CONCLUSION

The GC-MS studies carried out on methanol extract of tubers of *P. tuberosa* showed the presence of chemical components responsible for its potent medicinal activity. Further work regarding specific activity of various identified compound will provide more insight about the use of the tuber.

REFERENCES

[1] Aleksandra, K., Adam, M.P., Michał, R, Stanisław, L. and Paweł, St. A. 2017. Synthesis and Evaluation of Biological Activities of Aziridine Derivatives of Urea and Thiourea. *Molecules* 23(1): 45: 23010045.

[2] Amal, K.M., Subrata, P., Pratim, B. and Debdulal, B. 2014. *Pueraria tuberosa*: a review on its phytochemical and therapeutic potential. *J. Natural Product Res.* 28(23): 2111-2127.

[3] Antonio, R., Giuliano, E. and Gabriella, S.M. 1996. 2-Cyclopenten-1-one, a New Inducer of Heat Shock Protein 70 with Antiviral Activity. *J. Biol. Chem.* 271(50). 32192-32196.

[4] Bhesh B, Nidhi B and Pierre S. 2013. Hand book of food powders. A volume in Wood head publishing series in Food Science, Technology and Nutrition. Barringer S. pp. 625-640.

[5] Belakhdar, G, Benjouad, A. and Abdennebi, E.H. 2015. Determination of some bioactive chemical constituents from Thesium humile Vahl. *J. Mater. Environ. Sci.* 6(10): 2778-2783.

[6] Betz, J.M., Gay, M.L., Mossoba, M.M., Adams, S. and Portz, B.S. 1997. Chiral gas chromatographic determination of ephedrine type alkaloids in dietary supplements containing Má Huáng. *J. AOAC International*. 80: 303-315.

[7] Bickerstaffe, R. and Annison, E.F. 1970. Lipid metabolism in the per fused chicken liver. The uptake and metabolism of oleic acid, elaidic acid, *cis*-vaccenic acid, *trans*-vaccenic acid and stearic acid. *Biochem. J.* 118(3): 433-442

[8] De-Fatima, A., Modolo, L.V., Conegero, L.S., Pilli. R.A., Ferreira, C.V., Kohn, L.K., de-Carvalho, J.E. 2006. Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design. *Curr. Med. Chem.* 13: 3371-84.

[9] Grover, N and Patni, V. 2013. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L.) Kurz. Leaves. *Int. J. Phar. & Pharma. Sci*. 5(4):291-295.

[10] Igwe, O.U. and Okwu, D.E. 2013. GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the seeds of *Brachystegia eurycoma* (HARMS). *Asian J of Plant Science and Research* . 3(2): 47-54.

[11] Jae, H.C., Seo, Y.C., Ji, Y.K., Dae, H.K., Jae, W.L., Jae, S.C. and Hae, Y.C. 2007. 3-methyl-1,2-cyclopentanedione down-regulates age-related NF-kB signaling cascade. *J. Agric. & Food Chem.* 55(16): 6787-6792.

[12] Jie, M.S.F. and Choi, C.Y.C. 1991. *Characterization of picolinyl and methyl ester derivatives of isomeric thia fattyacids. J. Int. Fed. Clin. Chem.* 3: 122.

[13] Joo, Y.L., Kyung, H.S., Sang, H.R. and Daniel, H. 2001. Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through Toll-like Receipt or 4. *J. Biol. Chem.* 276(20): 16683–16689.

[14] Manikandan, S., Alagu, L.G.M. and Chandran, C. 2016. Phytochemical screening and evaluation of tuber extract of *Plectranthus rotundifolius* Spreng . by GC-MS and FT-IR spectrum analysis. *Int. J. Herb Med*. 4(2): 36-40.

[15] Mary Helen, P.A., Aswathy, M.R., Deepthi, K., Rathi, R.M., Joseph, J.J. and Sree, S.J. 2013. Phytochemical analysis and anticancer activity of leaf extract of

Mangifera indica (Kotttukonam Varika). *Int. J. Pharma. Sci. & Res.* 4(2): 819-824.

[16] Milne, A. 1993. Inhalational and local anesthetics reduce tactile and thermal responses in *Mimosa pudica Linn. Masui*. 1190-3. 7.

[17] Okwu, D.E. and Morah, F.N. I. 2006. The potentials of *Garcinia kola* seed as source for nutraceuticals. *J. Med. Arom. Plant Sci*. *28*: 605-11.

[18] Pandey N, Tripathi Y.B. 2010. Antioxidant activity of tuberosin isolated from *Pueraria tuberosa* Linn. *J. Inflamm. Res*. 7:47.

[19] Prabu. K.M., Samydurai, P., Subbaiyan, B. and Thangapandian, V. 2013. Phytochemical constituents and gas chromatography-mass spectrometry analysis of *Caralluma diffusa* (Wight) n. e. br. aerial part. *Int. J. Pharm. Pharm. Sci*. 5(3): 602- 605.

[20] Salem, S.A., Muhammad, A.K., Ehab, H.E., Megahed, H.A., Muhammad, F. and Hussein, M.M. 2018. Comparative phytochemical profiling of different soybean (*Glycine max* (L.) Merr.) genotypes using GC–MS. *Saudi J. Biol. Sci* . 25(1): 15-21.

[21] Sumalatha, G., Bahlul, Z.A., Chandu, B. and Mukkanti, K. 2010. Anti ulcerogenic & ulcer healing studies of aqueous extract of *Pueraria tuberosa* leaves on rats. *Int. J. Pharma & Biosciences*. 1(4): 657-661.

[22] Sung, W,S., Jung, H,J., Park, K., Kim, H.S., Lee, I.S. and Lee, D.G. 2007. 2, 5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF); antimicrobial compound with cell cycle arrest in nosocomial pathogens. *Life Sci*. 80(6): 586-91.

[23] Tapiero, H., Tew, K.D., Nguyen, B.G. and Mathé, G. 2002. Polyphenols, do they play a role in the prevention of human pathologies? *Biomed. Pharmacother*. 56: 200–207.

[24] Wei, Y. and Chunhua, Q. 2013. Synthesis of Epigoitrin from (*R*)- (+)-4-Hydroxy-γ-butyrolactone. *J. Heterocyclic Chem*. 50(6):1290-1293.

[25] Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z. and Chen, T. 2013. *In vitro* antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *J. Agric. Food Chem.* 61(44): 10604-11.

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Spatial Modeling of Cold Lava Flood Evacuation in Kali Putih, Magelang Regency, Using Network Analyst

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Abstract*—.The event of Mount Merapi's phreatic eruption, at least reminds us that the riverbank of Kali Putih which is located in Magelang Regency, Central Java Province has not been completely free from the threat of cold lava flood, especially during the rainy season. Therefore, a preventive action in relation to cold lava flood mitigation matters needs to be done. As part of the preventive action, a spatial modeling was carried out in Kali Putih to get an overview of the number and distribution of affected residential areas, the location and distribution of temporary evacuation sites (TES), the most effective number of final evacuation sites (FES), and various evacuation routes formed. Modeling began by calculating the level of vulnerability of cold lava in Sub Watershed of Kali Putih to get the most vulnerable areas for cold lava flood. 3D analyst and spatial analyst were used at this stage. The analysis was continued to calculate the number of affected settlements, using vector-based analysis. Furthermore, the determination of the number and distribution of TES, the number and distribution of FES, and determination of evacuation routes were carried out using Network Analyst. From this spatial modeling, the following results were obtained: 66 out of 179 residential areas were most likely affected by cold lava flood, 23 temporary evacuation sites (TES), and 7 final evacuation sites (FES), 57 evacuation routes from affected settlements to TES, and 22 evacuation routes from TES to FES*

Keywords*— *Evacuation, Cold lava flood, Network analyst, Spatial analyst, Spatial modeling.

I. INTRODUCTION

The cold lava flood as a result of Mount Merapi volcanic eruption In October 2010, has caused the mass evacuation of residents who live on the riverbanks of the rivers that flow on the slopes of Merapi. It was recorded about 10 million cubic volcanic materials mixed with rainwater flowing in 13 rivers that hailed at Mount Merapi, and caused the cold lava flood [28]. One of the areas that most affected by cold lava flood was the river bank of Kali Putih. Kali Putih that runs through 13 villages including Sirahan Village, Magelang Regency has ever overflowed and made Jumoyo Village, Seloboro Village, and Sirahan Village become ones of areas heavily affected by the cold lava flood.

The disaster principally brought impacts to the environment and people [24]. According to the National Disaster Mitigation Agency (BNPB), the eruptions and cold lava flood caused damages in the sectors of settlement, public infrastructure, economy and society [6]. There were more people living under poverty level so that social strata changed [6]. The residents of Sirahan Village lost their main livelihood as farmers because their farming lands and gardens were covered by the volcanic materials. Moreover, it was difficult for them to get funds to start businesses and recover their economy. The Magelang Regency Government as of 26 November 2010 - 16 March 2011 in more detail noted that losses due to lava disasters in this region include 2,082 displaced persons, 67 homes washed away, 262 houses were severely damaged, 32 houses were damaged and 47 were lightly damaged [33]. The amount of damage and losses caused by the eruption disaster was about Rp. 4.23 trillion [6].

Many experts and researchers predict [28][24][23][25] that the cold lava flood of Merapi will still occur in the future. It is reasonable since the large volume of cold lava that reaches tens of millions of cubic meters, at any time can turn into cold lava flood [28]. Therefore, mitigation in relation to cold lava flood must be immediately addressed. The mitigation phase is the series efforts to reduce disaster risk, either through physical development as well as awareness and capacity building in facing the threat of disaster. Disaster mitigation is an activity that acts as disaster risk reduction, or efforts made to reduce the victims of the disaster, both fatalities and property [21].

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The government has actually carried out several mitigation efforts in the Kali Putih area, such as building Sabo dams, raising embankments and relocating settlements to safe places. Unfortunately, the relocation effort of residents living on the banks of Kali Putih appears to have been rejected by local residents and chose to remain in their place of origin [21].

The reluctance of people living on the banks of Kali Putih to relocate, and the still high threat of cold lava flood, inevitably force the government and all parties concerned to implement a planned evacuation system. This effort is nothing but to suppress the number of damage and casualties that may arise due to cold lava flood disaster [34]. Provisions regarding this evacuation have actually been mandated in Article 45 paragraph 2 letter e, Law of the Republic of Indonesia (UURI) no. 24 Year 2007 on Disaster Management [31]. It stated in Article 45 paragraph 2, that the preparation of the evacuation site is a form of preparedness to ensure quick and precise efforts to deal with disasters.

This study will conduct spatial modeling of evacuation caused by cold lava flood in Kali Putih using Geographic Information System (GIS)-based Network Analyst [13]. The purpose of this study was to obtain an overview of the number and distribution of affected settlements, the number and distribution of temporary evacuation sites (TES), the number and distribution of final evacuation sites (FES), and the number and variation of evacuation routes formed, both routes from affected settlements to temporary evacuation sites (TES) and routes from TES to FES. This spatial modeling is expected to be a reference or even a guide for parties involved in disaster mitigation.

The reason for selecting Kali Putih as an object of analysis is due to the largest impact and losses caused by the cold lava flood was on the banks of the Kali Putih [33]. Previous researches with similar topic were carried out in year 2013[12] and 2016 [34] using different methods respectively, while research concerning the modeling about the vulnerable areas of cold lava flood in Kali Putih was carried out in year 2013 [17].

1.1. Evacuation

Evacuation is considered a way to prepare people when at risk from an impending hazard [29]. It is an important part of disaster management and is an effective way of minimizing loss of lives and property damage [20]. Evacuation is considered a process that constitutes hazard detection, issuance of warning, preparation to evacuate, movement to identified shelters through a network [27]. It is important that the preventive evacuation is well organized, efficient and will need a minimum of time in order to avoid casualties. An accurate estimate of the evacuation time is helpful in determining the start of the evacuation [32].

1.2. Disaster vulnerable areas

Refer to [27], hazard detection is required in evacuation management to ensure the extent of the affected area and the number of residents to be displaced, including the establishment of evacuation start point. A spatial analysis with the help of remote sensing and geographical information system technology so far is able to describe spatially the vulnerable areas of disaster, physically and socially [17], [8], [10]. With the spatial analysis, we can calculate the number of settlements and total inhabitant that live in the vulnerable areas that need to be displaced.

1.3. Evacuation Site

To optimize the routing problem, one has to know the destinations. To optimize the destination assignment, one has to know the minimal travel time, and hence route assignment to all destinations [16]. In Article 44 of UURI Number 24 Year 2007 [31], preparation to evacuate is considered as part of preparedness. It includes preparation of evacuation sites and determination of evacuate route. According to Regulation of The President of The Republic of Indonesia Number 70 Year 2014 About Spatial Plan For Merapi Mountain National Park [22], the disaster evacuation system shall be designated as an effort to move refugees from geological hazard areas to disaster-prone areas, facilitate the evacuation of refugees, and ensure the safety and basic needs of refugees during the occurrence of geological disasters in the area of Mount Merapi National Park. The disaster evacuation system shall consist of TES (Temporary Evacuation Site), FES (Final Evacuation Site), and evacuation line (evacuation route). The TES shall be stipulated by the following criteria: a. located in an accessible location by refugees and refugee vehicles; b. not in areas that endanger the safety of refugees, such as landslide-prone areas, high-voltage electrical grid areas, vulnerable areas of fallen trees, and river borders; c. adequate infrastructure and facilities are available with respect to security and accessibility; d. available communication networks; and e. evacuation signs are available. Meanwhile, The FES shall be stipulated by criteria: a. located outside of disaster prone areas in the rea of Merapi Mountain National Park; b. are located in locations accessible to refugees and refugee vehicles; c. not in areas that endanger the safety of refugees, such as landslide-prone areas, high-voltage electrical grid areas, vulnerable areas of fallen trees, and river borders; d. adequate infrastructure and facilities are available with respect to security and accessibility; e. available open space; f. available communications infrastructure and facilities; and g. Evacuation signs are available.

1.4. Evacuation route

Transportation planning during evacuation, from traffic demand generation, scheduling of movement, to network flow assignment towards identified shelters and to mention the reentry to households after the occurrence of

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disaster, is crucial for effective evacuation process [22]. From the point of view of the evacuee, evacuation is the whole process which include: a. organization of the departure; b. departure from home; c. travelling in the direction of a safe area; d. leaving the danger area through one of its exits; e. continuation of the journey to the destination in the safe area [32]. The evacuation route as referred to [22] shall be established to facilitate the evacuation of refugees from TES to FES. Furthermore, the evacuation route as referred to [22] shall be stipulated by criteria: a. is a road with a pavement that the refugee carrier can pass; b. available road markings: and c. evacuation signs are available. This indicates that the existing road network is a base for determination evacuation route.

1.5. Network analyst

Currently, implemented GIS network tools are dominated by routing functions [4][7][1][18]. The implementation of GIS-based networks for transport applications has increased dramatically in the past 10 years with the presence of an almost existing service center location in every place and direction of travel based through internet services such as Mapquest and Google Maps [9]. A routing is the act of selecting a course of travel, and it is arguably the most fundamental logistical operation in the network analysis. The most common objective in routing across networks is to minimize the cost of the route [1]. There are actually four fundamental operations that can be performed in network analysis, where all of which are derivatives of route finding algorithms. These functions are: a. finding a route between point locations; b. determining the service area for a facility; c. finding the closest facility across the network; and d. creating an origin–destination matrix 9]. The last version of ArcGIS Network analyst even added 3 other functions that are: a. vehicle routing problem; b. allocation to allocation; and c. time dependent [15].

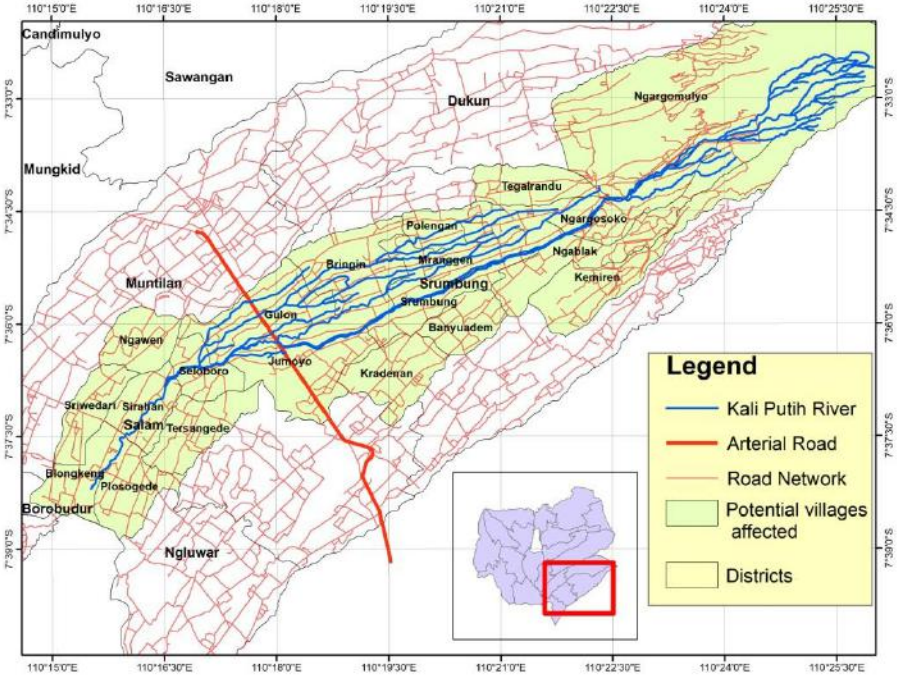

Fig.1: Study Area

II. METHODS

2.1. The Study area.

Kali Putih, geographically is located in between 110°15'0" E and 110°25'30" E and in between 7°20'0" S and 7°39'0" S. This river is part of Kali Putih sub-watershed which flows from the top of Mount Merapi to the southwest and passes through 13 villages (*see Fig. 1*).

2.2. Modeling Methods.

To achieve the objectives of this modeling, the study divided into four steps of analysis. The first step was to set the level of cold lava vulnerability, with the aim to produce a map of the distribution of prone areas of lava flood on the banks of Kali Putih. This step was continued to determine settlements affected by Kali Putih cold lava flood. The second step was to determine the number and the distribution of decent TES. The third step was determination the number and the distribution of reasonable FES. The fourth step was determination of the evacuation routes from the affected settlement spots to TES and from TES to FES.

2.2.1. The level of cold lava vulnerability and the affected settlements

Unlike the flood analysis, there is no default parameters for the level of vulnerability to cold lava flood analysis. Two previous research on the same topic and the same study area adopted different parameters, parameter

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classifications, and scoring system. The first research [19] used; a. slope; b. distance from river; c. rainfall intensity; d. land use; and e. rock hardness as the analysis parameters. The second [26] used: a. landform; b. slope; c. land use; d. distance from river; and e. rainfall intensity as the analysis parameters. Both were also different in defining parameters classifications, and there was no weighted for each parameter used. Therefore, in this study we set the parameters ourselves and assign weights to each parameter. The following are the table of scoring for each parameter used in this study, which include: a. slope; b. landscape morphology; c. land use; d. DEM curvature; and e. distance from river.

Table.1: Slope Classification and Score

Description	Slope (%)	Score
Very steep	>45	1
Steep	25-45	2
Rather steep	15-25	3
Sloping	8-15	4
Flat	0-8	5

Table. 2: DEM Curvature and Score

Curvature	Score
Convex	1
Concave	2

Table. 3: Classification and Score of Land use

Land use	Score
Forest, coastal area	1
Bare land, swamp, mixed farm, grassland	2
Garden, cropland	3
irrigated rice fields, rainfed rice fields, lake	4
River, settlement, buildings	5

Source: [19]

Table. 4: Classification and Score of Distance from River

Description	Distance from River (meter)	Score
Very far	>500	1
Far	250-500	2
Medium	100-250	3
Close	50 - 100	4
Very Close	< 50	5

Source: [19]

Table. 5: Parameter Weights

Parameter	Weights (%)
Land use	25
Slope	35
Curvature	25
Distance from River	15

The value of the vulnerability is determined by using the following equation:

$$K = \sum_{i=1}^{n} (W_i \times X_i) \quad (1)$$

Description:

K = vulnerability value

W_i = Weight for parameter i

X_i = Class score in parameter i

Score given to each parameter were determined in accordance with their contribution to the level of hazard posed by the flood event. Parameters with score 1 give the least effect on the level of vulnerability to cold lava flood. The greater the score, the greater the effect on the vulnerability to cold lava flood

A. Data used for the purposes of this analysis were a digital map of Rupabumi Indonesia on a 25,000 scale which was downloaded from http://tanahair.indonesia.go.id/portal-web. This map consists of layers; a. hypsography; b. administrative boundaries; c. hydrography; d built environment; e. transportation; and f. utility. For the reasons of updated information, a land use map was obtained from the result of the Ikonos 2010 image interpretation which was validated by field checking. A 30-Meter SRTM (Shuttle Radar Topography Mission) DEM downloaded from http://dwtkns.com/srtm30m/. The population data per village derived from the 2010 population census which was downloaded from https://www.bps.go.id/website/fileMenu/Penduduk-Indonesia-Menurut-Desa-2010.pdf [5].

The analysis method used to predict the level of cold lava flood vulnerability was the raster-based 3D analyst and spatial analyst. Both methods are extension packages that are in ArcGIS software. Layers such as: contours, rivers and villages boundaries prone to cold lava flood, DEM, and land use were used as input analysis.

Fig. 1 is a model builder that explains the steps taken to analyze the level of vulnerability of cold lava flood in Kali Putih.

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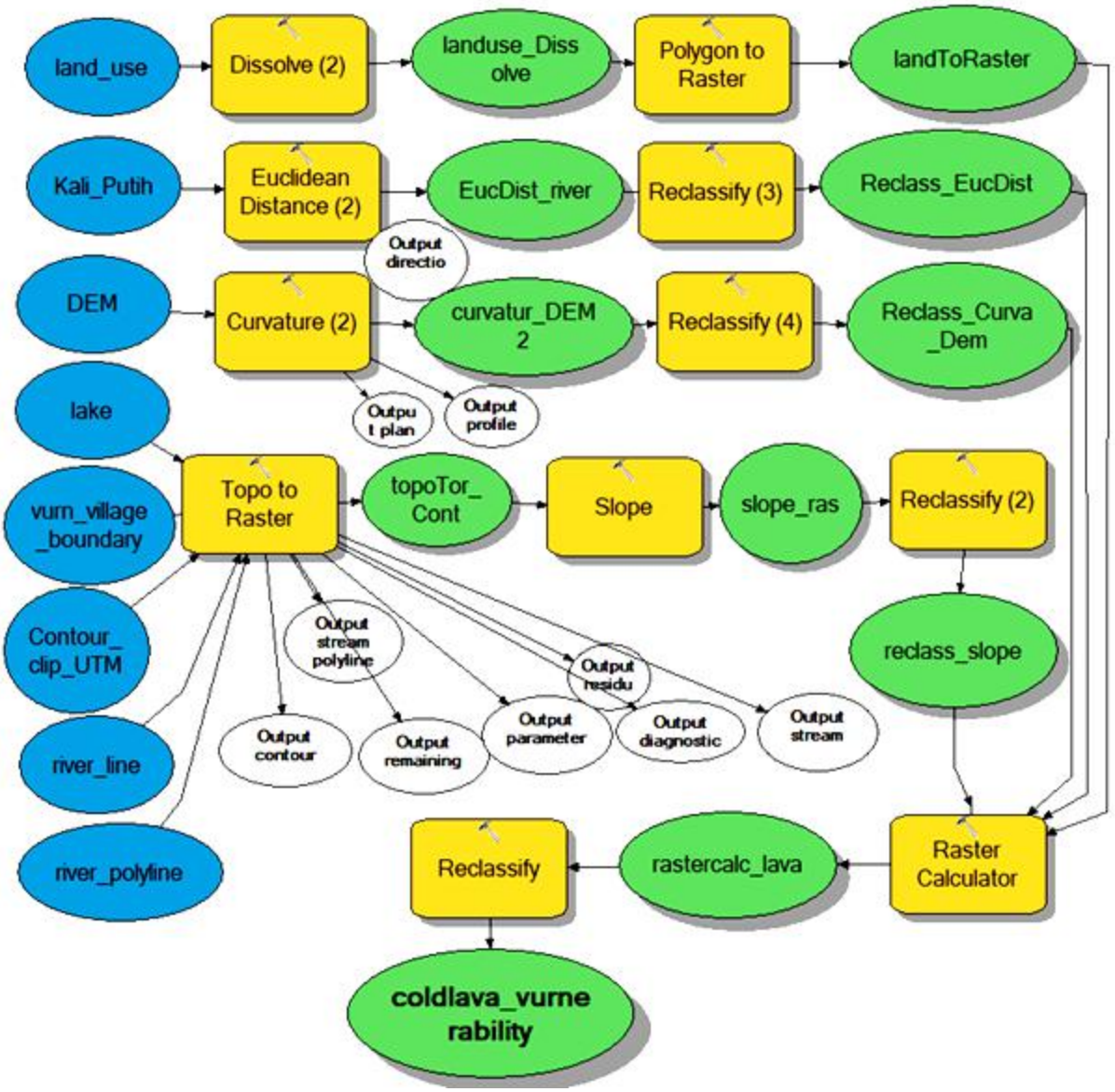

Fig. 2: Model builder for simulating the level of vulnerability to cold lava flood

As the main objective is to simulate evacuation routes, analysis of the level of vulnerability of cold lava flood was continued to obtain areas with the highest level of vulnerability to cold lava flood. These areas will be overlaid with residential areas to get residential areas affected by cold lava flood, both only part of the area or the entire area [27]. We assume that the population living in the affected areas is an object that must be evacuated, and the affected areas become the start points of evacuation.

For the purpose of getting the areas affected by cold lava, we conducted vector-based spatial analysis with input parameters: a. coldlava_vurnerability layer and b. settlement layer. Detailed steps of analysis can be seen in *Fig. 3*

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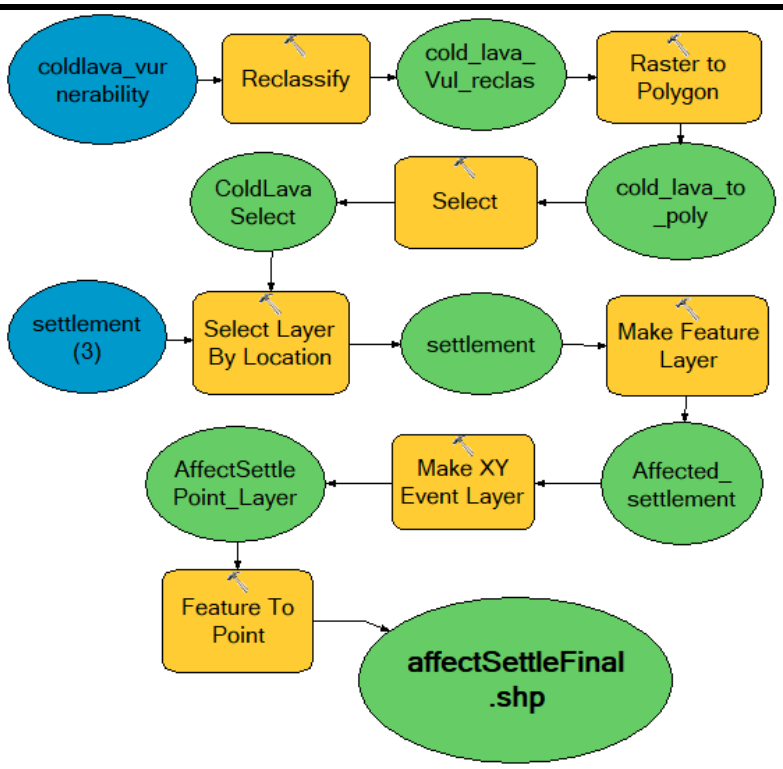

Fig. 3: Model builder of affected settlement detection

2.2.2. Determination of TES

According to National Board for Disaster Management, disaster emergency response command posts can occupy buildings or tents. Buildings or tents for disaster emergency command post occupies a strategic location with the following criteria: a. easily accessible by various parties involved in disaster emergency response activities; b. safe and free from disaster threats c. have adequate parking; d. land area of at least 500 m2 [2]. The determination of the TES basically follows the criteria set by the Regulations of The President of The Republic of Indonesia Number 70 Year 2014 About Spatial Plans For Merapi Mountain National Park [22]. In this case, we can assume that buildings such as schools, places of worship, village offices are representative places for TES. These buildings are spacious, easily accessible, and equipped with electricity, clean water, telecommunications.

For the sake of getting potential TES, we used vector-based analysis using layers: educational facilities, village offices, religious buildings, community health centers, affected settlements and vulnerable village boundaries as input parameters. The analysis was continued by using the New Closest Facility function to select the temporary evacuation sites which was considered the most appropriate in terms of distance. Layers used for this purpose were TES_TempEvacSite as a facility layer and affectSettleFinal as an incident layer.

The following Fig. 4 and Fig. 5 are the model builders that describe the step of analysis to determine the reasonable TES.

http://dx.doi.org/10.22161/ijeab/3.4.48 *ISSN: 2456-1878*

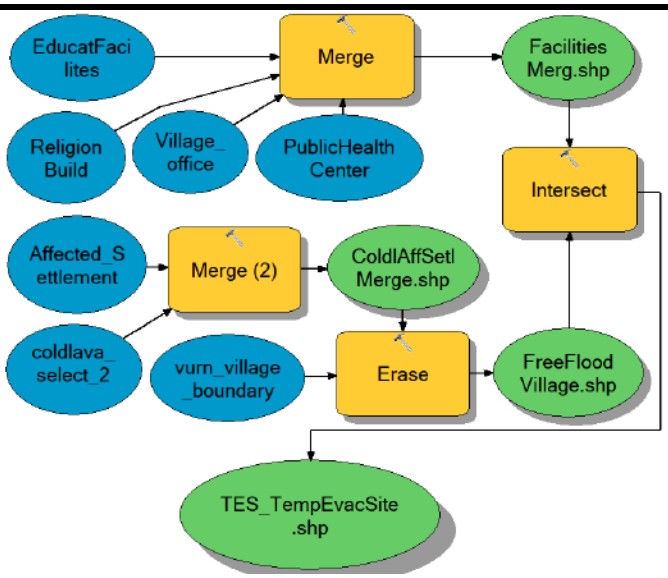

Fig. 4: Model builder of potential TES (temporary evacuation sites)

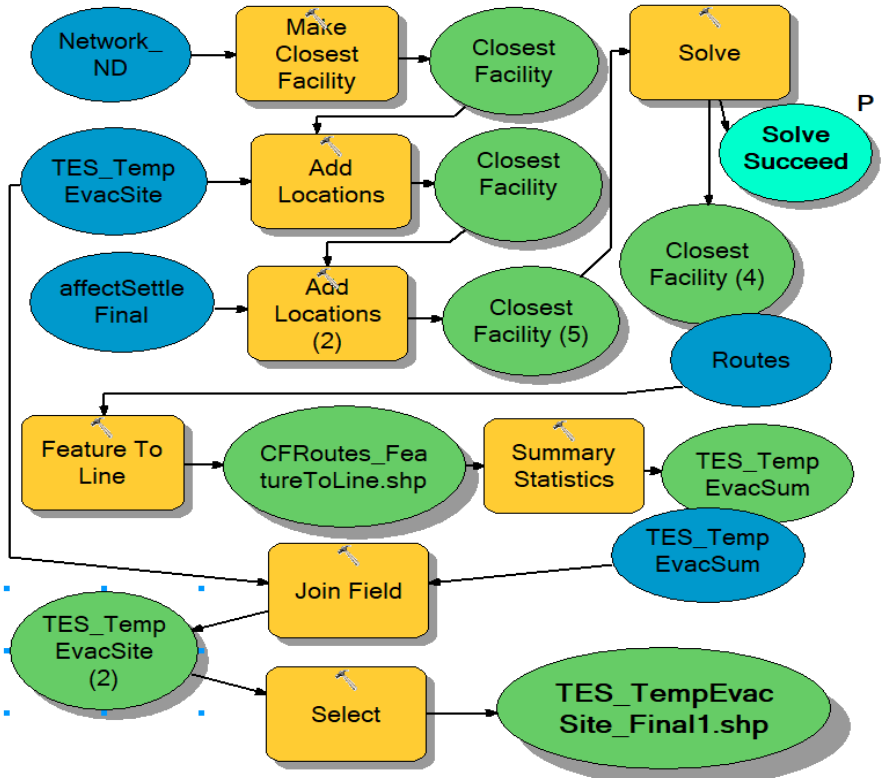

Fig. 5: Model builder of selected TES (temporary evacuation sites)

2.2.3. Determination of FES

The determination of FES also basically follows the criteria set by the Regulation of the President of the Republic of Indonesia Number 70 Year 2014 on Spatial Plans for Merapi Mountain National Park [22]. The regulation also states that FES must be at the district level. This means that FES must be located in the district capital, which is marked by the presence of a district office.

The policy that FES must be in the district capital is quite reasonable, because FES defined as a final gathering place for refugees that can also function as a temporary shelter in the event of a disaster, and definitely need support for health facilities, security and others. Health and safety facilities such as hospitals, public health centers and a police station are only available in the district capital. Some district capitals also have the district squares with an area of more than a football field, and are estimated to be sufficient to set up refugee tents.

In refer to the arguments above, we set the district office, hospital and public health center as the FES candidates. There are five districts where some of their villages are

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allegedly affected by cold lava flood. Those five districts are: Dukun District, Srumbung District, Muntilan District, Salam District, and Ngluwar District.

In this case, the capital of Srumbung District is considered unfit for FES because it is located on the edge of a very vulnerable area, and must be excluded from candidates for FES. As a substitute for the capital city of Srumbung district, school buildings and religion buildings were chosen to accommodate refugees from villages under Srumbung District.

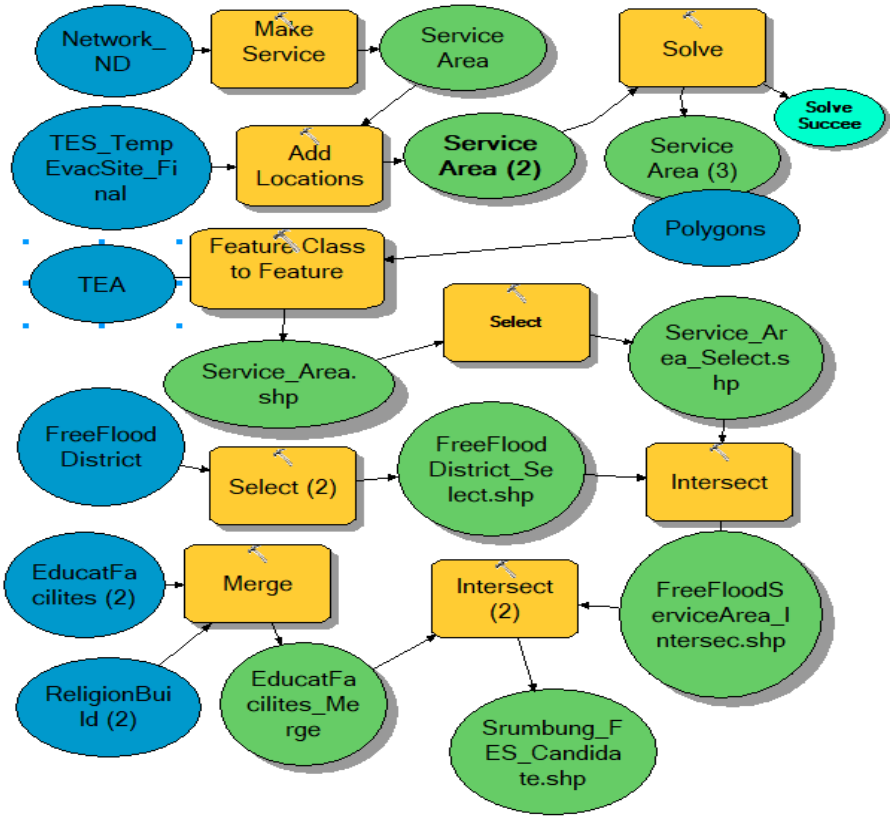

Fig. 6: Model builder of determining subsitute FES candidates of Srumbung District

The sum of all FES candidates including the substitute FES of the Srumbung District was carried out in stages as shown in Fig. 7

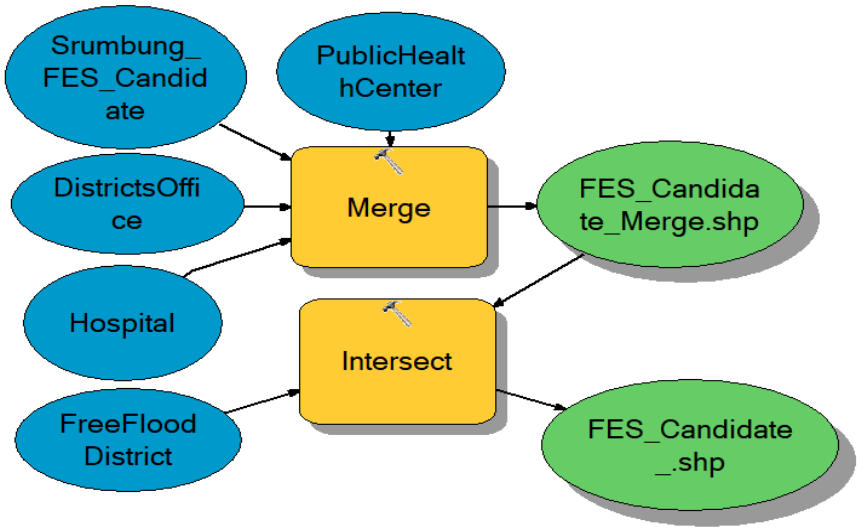

Fig. 7: Model builder of determining the total number of FES candidates for five districts

Determination of the number and location of selected FES was done with the help of the Location-Allocation function of Network Analyst, and continued with vector analysis to get the selected FES point layer. The following Fig. 8 is the steps to get the selected FES layer.

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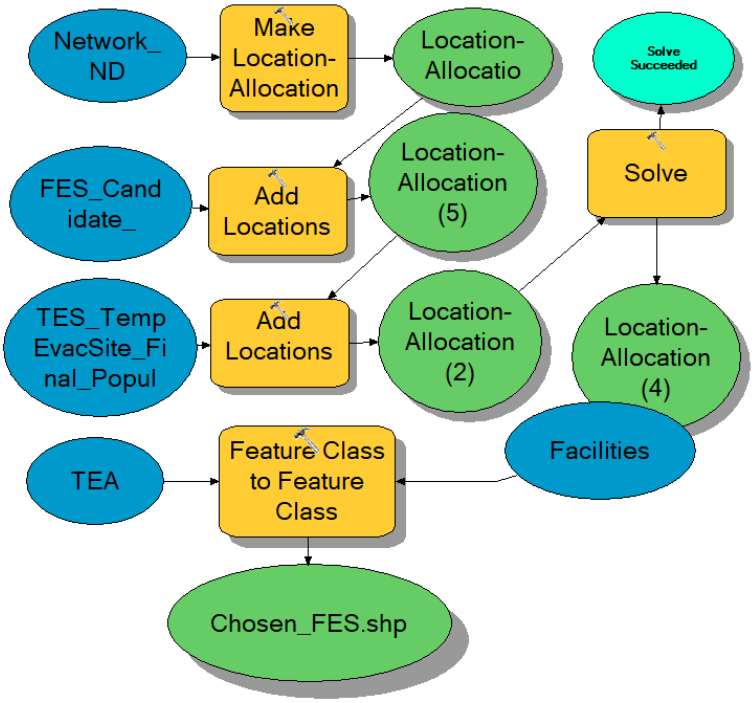

Fig. 8: Model builder of selected FES assignments

2.2.4. Modeling of Evacuation Routes from Affected Settlements to TES and from TES to FES

Determination of the evacuation routes is intended to provide direction to the refugees to reach the nearest evacuation sites from the settlement to TES and from TES to FES in case of the lava flood occurs, Determination of the evacuation routes from the affected settlement to the TES has actually been carried out in conjunction with the determination of the TES (Fig. 5).

Therefore, here only an analysis of the evacuation routes from TES to FES will be carried out. Fig. 9 below is a model builder that will be used to determine the evacuation routes from TES to FES.

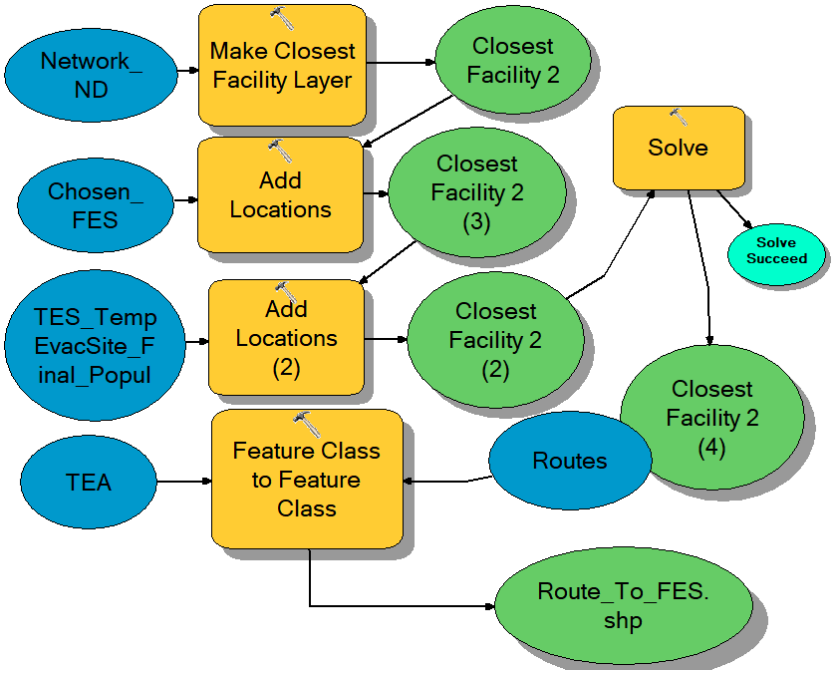

Fig. 9: Model builder of generation of various evacuation routes from TES to FES

III. RESULT AND DISCUSSION

3.1. The Level of Cold Lava Vulnerability and the Affected Settlements

Fig. 10 below is a map illustrates the level of vulnerability to the Kali Putih cold lava flood, resulting from 3d analysis and spatial analysis.

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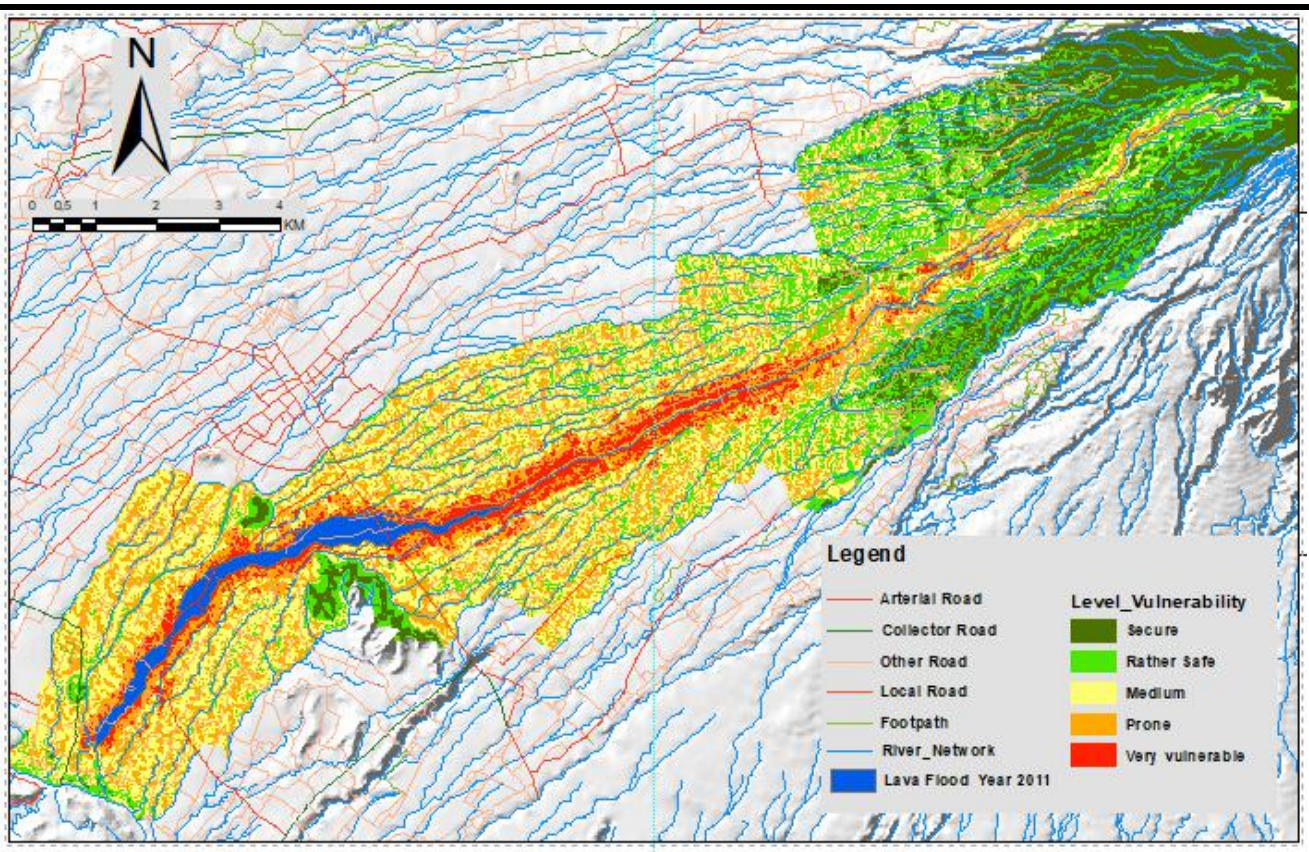

Fig. 10: Map of the vulnerability level to cold lava flood

It shows on the map that all villages in the study area are dominated by prone zones with an extent of about 4149.84 Ha or about 49.79 percent. Detail on the extent and percentage for each level of vulnerability can be seen in Fig. 11.

coldlava_vurnerability

OID	Value	Count	Degree_Vur	Area_Ha	Percentage
0	1	14961	Secure	598,44	7,18
1	2	27720	Rather Safe	1108,8	13,3
2	3	43162	Medium	1726,48	20,71
3	4	103746	Prone	4149,84	49,79
4	5	18795	Very Vulnerable	751,8	9,02

Fig. 11: Attribute table of layer Coldlava_vulnerability

We focus on the very vulnerable region, which is certain to be the earliest area overflowed by a cold lava flood if that happens. It can be seen in Fig. 11 that this level of vulnerability covers an area of 751.8 Ha. or 9.02 percent which spread along the banks of the Kali Putih. The estimated total area of 751.8 Ha seems too broad compared to the cold lava flood that occurred in 2011 which covered an area of 191.8 Ha. This may be related to the absence of rainfall intensity parameter, as well as the use of 30 meter SRTM DEM which is considered too rough to describe the curvature of the earth [14].

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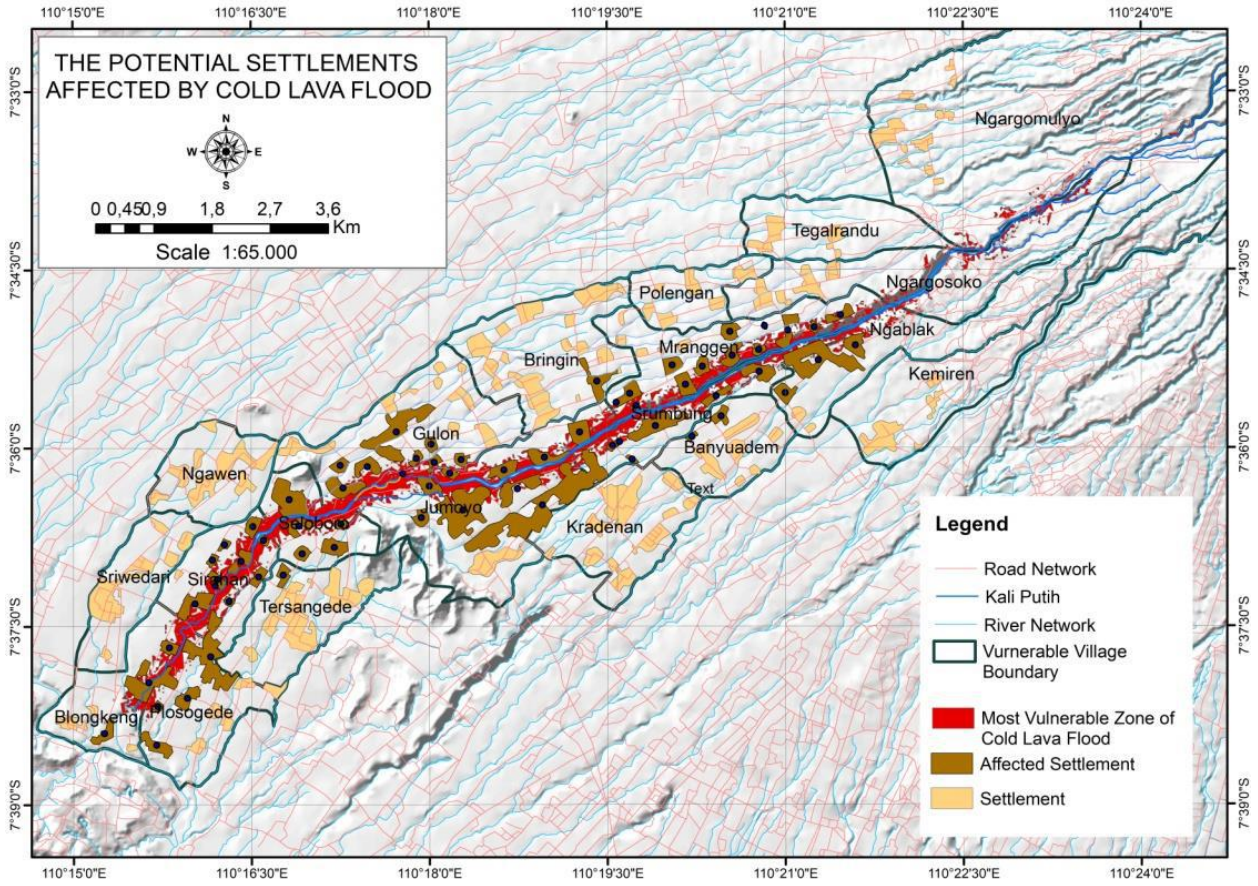

Fig. 12: Map of the potential settlements affected by cold lava flood

The use of a 30 meter DEM SRTM is actually simply to overcome the absence of elevation point data that should be available on the RBI map. As we know the elevation point data is an important component for building DEM with Topo To Raster. This elevation point can create more detailed curvature than curvature made from SRTM DEM. This may be the reason why the surface of the study area tends to look flat as shown on Fig. 10.

Apart from the extent of the very vulnerable area which might be debatable, this area seems to be in contact with a number of residential areas which are spread along the Kali Putih flow, as shown on Fig. 12.

Based on the result of affected settlement detection analysis (Fig. 3), if there is a cold lava flood in accordance with the modeling results, then 66 of 179 residential areas are very likely to be affected. All residents living in these areas must be moved. Furthermore, there will be 66 pick-up points for refugees who will be moved to temporary evacuation sites (TES).

It is unfortunate that there is no data explaining the population per residential area. Population data for each pick-up point is needed to determine the number and distribution of TES.

3.2. Determination of The Temporary Evacuation Sites (TES)

Fig. 13 is a map that draws the number and distribution of selected TES. This map is the result of analysis with stages as illustrated in Fig. 4 and 5. The stages of analysis as illustrated in Fig. 4 are intended to get potential TES candidates. At this stage, a TES_TempEvacSite layer containing the number and distribution of is obtained. There are 102 prospective TES scattered throughout the affected villages, consisting of village offices, schools, mosques and churches, and public health centers (see Fig. 14)

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Table

TES_TempEvacSite

FID_F	REMARK	VILLAGE	TES_CANDIDATE	DISTRICT	REGENCY
70	School1	Ngargomul	School1 - Ngargomulyo	Dukun	Magelang
162	School1	Kradenan	School1 - Kradenan	Srumbung	Magelang
146	School1	Kemiren	School1 - Kemiren	Srumbung	Magelang
163	School1	Jumoyo	School1 - Jumoyo	Salam	Magelang
125	School1	Bringin	School1 - Bringin	Srumbung	Magelang
27	School1	Blongkeng	School1 - Blongkeng	Ngluwar	Magelang
149	School1	Banyuadem	School1 - Banyuadem	Srumbung	Magelang
364	Other Worshi	Sriwedari	Other Worship1 - Sriwedar	Muntilan	Magelang
314	Mosque7	Ngawen	Mosque7 - Ngawen	Muntilan	Magelang
344	Mosque6	Sriwedari	Mosque6 - Sriwedari	Muntilan	Magelang
318	Mosque6	Ngawen	Mosque6 - Ngawen	Muntilan	Magelang
347	Mosque5	Sriwedari	Mosque5 - Sriwedari	Muntilan	Magelang
255	Mosque5	Polengan	Mosque5 - Polengan	Srumbung	Magelang

0 (0 out of 102 Selected)

TES_TempEvacSite

Catalog Table

Fig. 14: Attribute table containing part of the TES candidates

The stage of analysis as illustrated in Fig. 5 is a simulation in order to get the most representative TES in terms of travel distance. This simulation produces a TES_TempEvacSite_Final1.shp layer containing 23 TES that are considered feasible, with the distribution as illustrated in Fig. 13. Detailed information on the types of buildings used as TES can be seen in Table. 6.

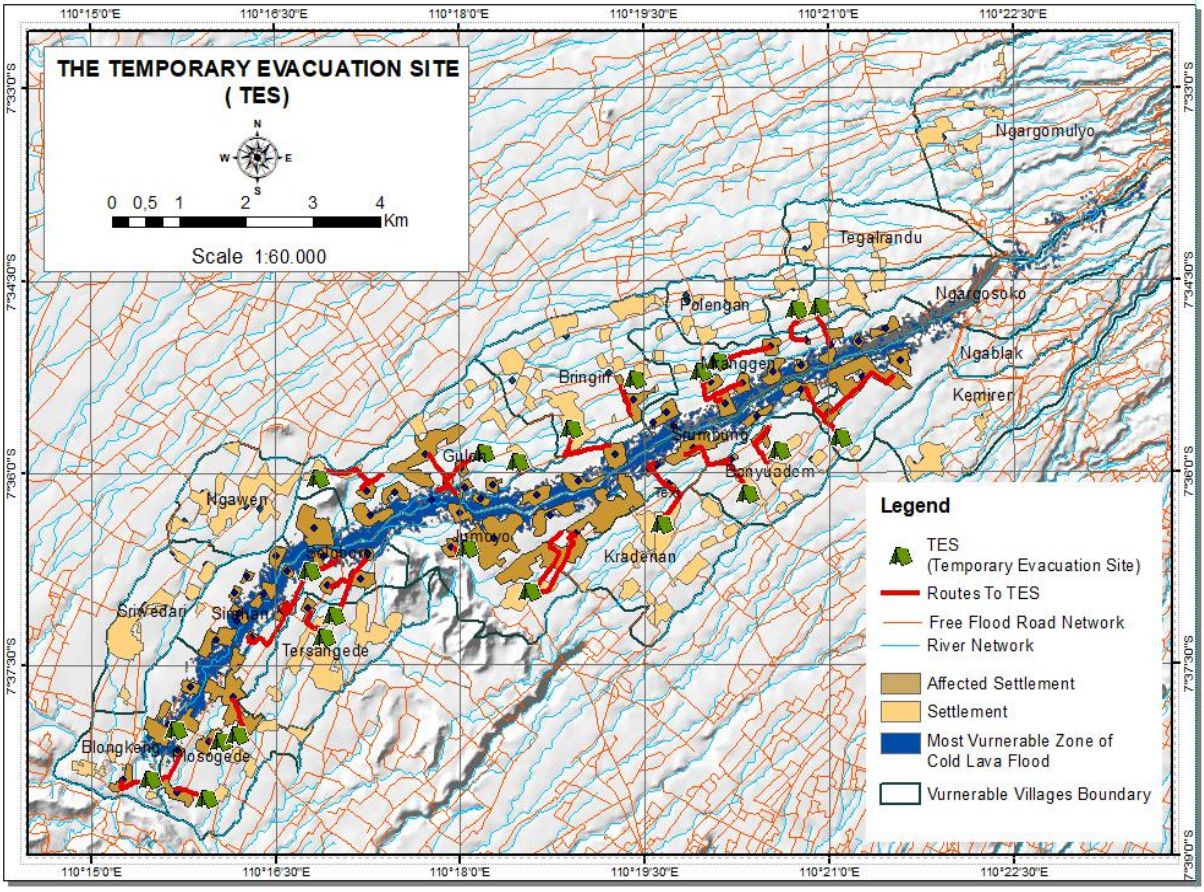

Fig. 13:. Map of the selected temporary evacuation sites (TES)

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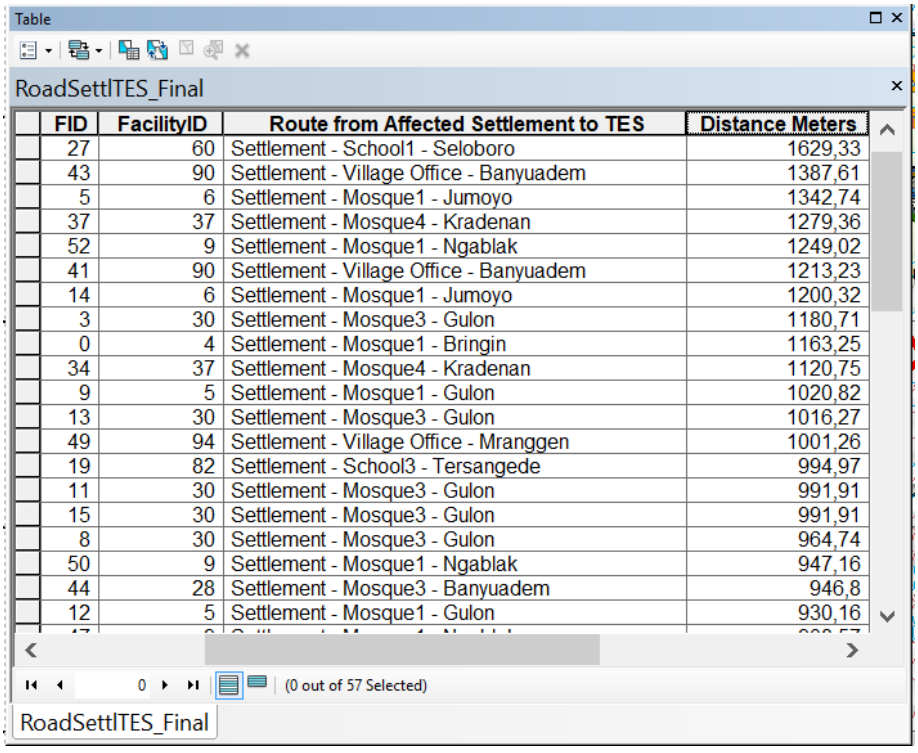
Table. 6: The Selected Temporary Evacuation Sites

No.	Temporary Evacuation Sites
1	School1 - Plosogede
2	School1 - Blongkeng
3	Village Office - Plosogede
4	School2 - Plosogede
5	School3 - Plosogede
6	Village Office - Tersangede
7	School3 - Tersangede
8	Mosque1 - Jumoyo
9	School1 - Seloboro
10	School1 - Jumoyo
11	Mosque4 - Kradenan
12	Village Office - Banyuadem
13	Mosque1 - Gulon
14	Mosque2 - Gulon
15	Mosque3 - Gulon
16	Mosque3 - Banyuadem
17	Mosque1 - Ngablak
18	Mosque1 - Bringin
19	School2 - Bringin
20	Village Office - Mranggen
21	School4 - Mranggen
22	Village Office - Ngargosoko
23	Mosque1 - Ngargosoko

The whole process of determining the TES analysis also results in the layer of Evacuation route from the affected settlements to the TES (see Fig. 13). The New Closest Facility used in this simulation produces 57 evacuation routes from the affected settlements to the TES. The farthest distance occurs on the route from the settlement to mosque 1 in Ngablak Village with a travel distance of 1839.56 meters.



Table

RoadSettlTES_Final

FID	FacilityID	Route from Affected Settlement to TES	Distance Meters
27	60	Settlement - School1 - Seloboro	1629,33
43	90	Settlement - Village Office - Banyuadem	1387,61
5	6	Settlement - Mosque1 - Jumoyo	1342,74
37	37	Settlement - Mosque4 - Kradenan	1279,36
52	9	Settlement - Mosque1 - Ngablak	1249,02
41	90	Settlement - Village Office - Banyuadem	1213,23
14	6	Settlement - Mosque1 - Jumoyo	1200,32
3	30	Settlement - Mosque3 - Gulon	1180,71
0	4	Settlement - Mosque1 - Bringin	1163,25
34	37	Settlement - Mosque4 - Kradenan	1120,75
9	5	Settlement - Mosque1 - Gulon	1020,82
13	30	Settlement - Mosque3 - Gulon	1016,27
49	94	Settlement - Village Office - Mranggen	1001,26
19	82	Settlement - School3 - Tersangede	994,97
11	30	Settlement - Mosque3 - Gulon	991,91
15	30	Settlement - Mosque3 - Gulon	991,91
8	30	Settlement - Mosque3 - Gulon	964,74
50	9	Settlement - Mosque1 - Ngablak	947,16
44	28	Settlement - Mosque3 - Banyuadem	946,8
12	5	Settlement - Mosque1 - Gulon	930,16

(0 out of 57 Selected)

RoadSettlTES_Final

Fig. 15: Part of the attribute table of evacuation routes from affected settlements to TES

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The shortest distance occurs on the route from the settlement to School3 in Plosogede Village with a travel distance only 0.9 meters. The average travel distance from the affected settlements to the TES is around 775 meters.

If you refer to the number of affected settlements that have to be evacuated as many as 66, then the number of routes should be 66. The fact that the number of evacuation routes is only 57 indicates that there are 9 affected settlements that cannot be evacuated due to the unreachable transportation network.

Argument due to the inaccessibility of the transportation network is basically understandable. In this network simulation, it was assumed that some of the roads in the very vulnerable area were blocked due to submerged cold lava flood. In this case we can argue that in order to avoid interruption of the evacuation route, an early warning system must be applied. With an early warning system, the evacuation process can be carried out before the cold lava flood hits the affected areas.

3.3. Determination of the Final Evacuation Sites (FES)

As mentioned above, the placement of FES in the capital of Srumbung District is certainly very risky and therefore the FES must be moved. In this case, the replacement FES must remain in the Srumbung District, and located at a distance between 2500 and 5000 meters from the TES.

Data processing with stages referring to Fig. 6 model builder, managed to get 9 prospective FES buildings, consisting of 1 school and 8 mosques. (Fig. 16)

Table

Srumbung_FES_Candidate1

FID	Shape *	REMARK	DISTRICT	Name	FromBreak	To
0	Point ZM	Mosque	Srumbung	2500 - 5000	2500	
1	Point ZM	School	Srumbung	2500 - 5000	2500	
2	Point ZM	Mosque	Srumbung	2500 - 5000	2500	
3	Point ZM	Mosque	Srumbung	2500 - 5000	2500	
4	Point ZM	Mosque	Srumbung	2500 - 5000	2500	
5	Point ZM	Mosque	Srumbung	2500 - 5000	2500	
6	Point ZM	Mosque	Srumbung	2500 - 5000	2500	
7	Point ZM	Mosque	Srumbung	2500 - 5000	2500	
8	Point ZM	Mosque	Srumbung	2500 - 5000	2500	

0 (0 out of 9 Selected)

Srumbung_FES_Candidate1

Fig. 16: Attribute table of Srumbung FES candidates

To get the total number of FES candidates for the five districts, data processing was continued by using the model builder in Figure 7. From this processing, 24 buildings for FES candidates were obtained. These buildings include: 1 Muntilan District office, 1 Dukun District office, 1 Ngluwar District office, 1 Salam District office, 1 Ngluwar District Public Health Center, 1 Salam District Public Health Center, 2 Salam District Public Health Centers, 2 Dukun District Hospitals, 1 Ngluwar District Hospital, 1 Srumbung District Hospital, 3 Muntilan District Hospital, 8 Srumbung District Mosques, 1 Srumbung District School. (Fig. 17)

Table

FES_Candidate_

FID	Shape	BUILDING	DISTRICT	REMDIS	FromBreak
0	Point Z	Hospital1	Ngluwar		0
1	Point Z	District Office	Ngluwar	District Office Ngluwar	0
2	Point Z	health center1	Ngluwar		0
3	Point Z	Mosque	Srumbung		2500
4	Point Z	School	Srumbung		2500
5	Point Z	District Office	Salam	District Office Salam	0
6	Point Z	health center2	Salam		0
7	Point Z	Mosque	Srumbung		2500
8	Point Z	Mosque	Srumbung		2500
9	Point Z	Mosque	Srumbung		2500
10	Point Z	Mosque	Srumbung		2500
11	Point Z	Mosque	Srumbung		2500
12	Point Z	health center4	Muntilan		0
13	Point Z	Hospital7	Muntilan		0

12 (0 out of 24 Selected)

FES_Candidate_

*Fig. 17: Part of the attribute table of FES_Candidate_*

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Fig. 19 below is a map that describes the distribution of FES that are considered most suitable for accommodating refugees from 22 TES. The Location-Allocation function with the Maximize_Attendance problem type used in this analysis shows that the determination of 7 FESs is considered reasonable. This amount is able to equally divide the number of TES that come to each FES as shown in Fig. 19. These selected FES buildings can be seen in Fig. 18.

Table

Chosen_FES_1

FID	Chosen	FES	DISTRICT	FacilityTy	We
0		Hospital1	Ngluwar	3	
1		Public Health Center1	Ngluwar	3	
2		Public Health Center2	Salam	3	
3		Mosque	Srumbung	3	
4		Hospital7	Muntilan	3	
5		Hospital5	Srumbung	3	
6		Mosque	Srumbung	3	

0 (0 out of 7 Selected)

Chosen_FES_1

Fig. 18: Attribute table of Chosen_FES

It can be seen on Fig. 18 that 3 FES located in Srumbung District, and none in Dukun District. The number of FES in Srumbung District seems to be related to the large number of residential areas affected in the Srumbung District. Other reasons related to the distance between the TES in Srumbung District and the number of FES that have been determined.
Referring to the number of TES that should have access to FES (23 TES), a TES located at School 1 in Jumoyo Village do not seem to have access to one of the FES. the problem seems to be similar to the case of 9 affected settlements that could not be evacuated due to lack of road access.

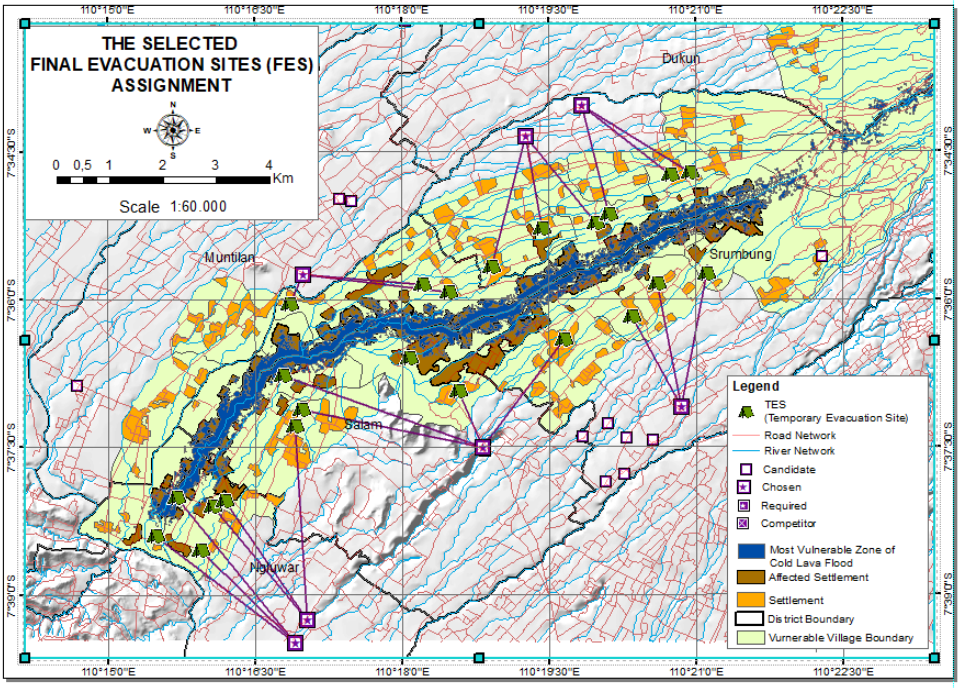

Fig. 19: Map of chosen FES based on Location Allocation function

Once again, the implementation of an early warning system seems to be a necessity in preventing the catastrophic cold lava flood in Kali Putih.

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3.4. Modeling Evacuation Route from Affected Settlements to TES and from TES to FES

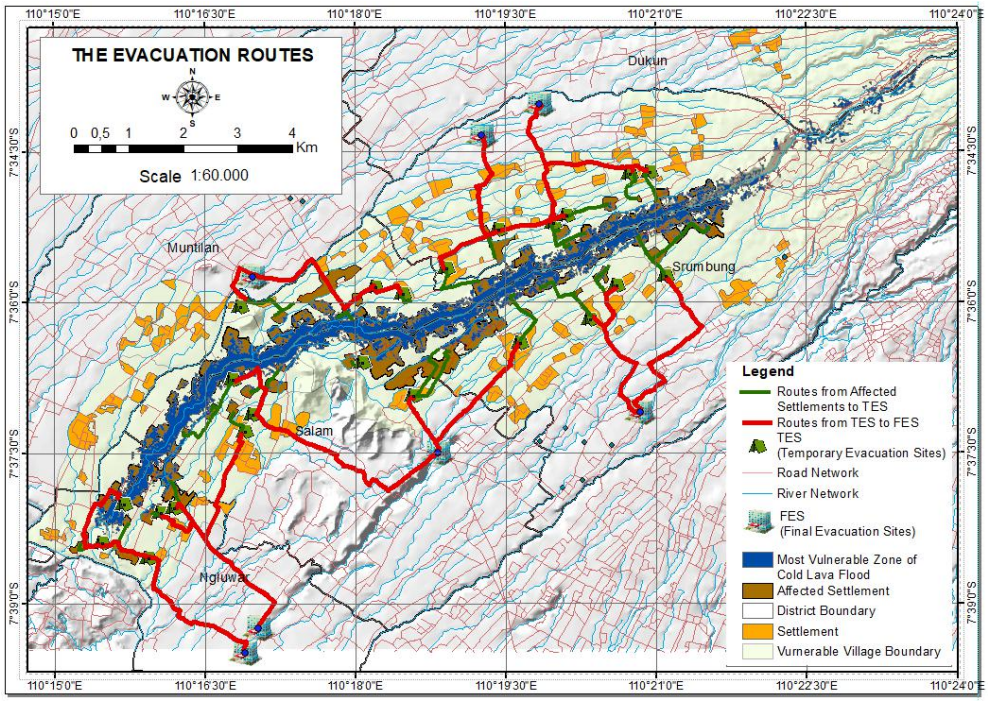

Fig. 20: Map of the evacuation routes from affected settlements to TES and from TES to FES

Figure 20 above is a map that describes the evacuation routes from affected settlements to TES and from TES to FES as a result of modeling evacuation. The modeling results of evacuation routes from affected settlements to TES have been discussed in the previous section.

It can be seen in Fig. 20 the number of evacuation routes and road networks that each route passes, both for evacuation routes from affected settlements to TES and from TES to FES. Some routes appear to pass through the same section of road. Using the same road section will cause traffic density in which can slow down the speed.

Details of evacuation routes from TES to FES including distance of travel for each route can be seen in Fig. 21,

Table

Route_To_FES

FID	FacilityID	Shape *	Route From TES to FES	Distance_Km
0	1	Polyline M	School1 - Plosogede - Hospital1	3,05
1	1	Polyline M	School1 - Blongkeng - Hospital1	4,04
2	2	Polyline M	Village Office - Plosogede - Public Health Center1	3,57
3	2	Polyline M	School2 - Plosogede - Public Health Center1	3,26
4	1	Polyline M	School3 - Plosogede - Hospital1	6,01
5	2	Polyline M	Village Office - Tersangede -Public Health Center1	4,57
6	3	Polyline M	School3 - Tersangede - Public Health Center2	4,3
7	3	Polyline M	Mosque1 - Jumoyo - Public Health Center2	1,26
8	3	Polyline M	School1 - Seloboro - Public Health Center2	5,69
9	3	Polyline M	Mosque4 - Kradenan - Public Health Center2	2,65
10	4	Polyline M	Village Office - Banyuadem - Mosque	2,45
11	5	Polyline M	Mosque1 - Gulon - Hospital7	1,03
12	5	Polyline M	Mosque2 - Gulon - Hospital7	3,73
13	5	Polyline M	Mosque3 - Gulon - Hospital7	3,03
14	4	Polyline M	Mosque3 - Banyuadem - Mosque	3,14
15	4	Polyline M	Mosque1 - Ngablak - Mosque	3,73
16	6	Polyline M	Mosque1 - Bringin - Hospital5	3,33
17	6	Polyline M	School2 - Bringin - Hospital5	2,37
18	6	Polyline M	Village Office - Mranggen - Hospital5	3,43
19	7	Polyline M	School4 - Mranggen - Mosque	2,76
20	7	Polyline M	Village Office - Ngargosoko - Mosque	3,05
21	7	Polyline M	Mosque1 - Ngargosoko - Mosque	3,28

(0 out of 22 Selected)

Route_To_FES

Fig. 21: Attribute table of Route _To_FES

In this evacuation modeling, the network data set is not equipped with a turn layer, one-way or two-way information, and travel time. Restricted turn, speed adjustment, one-way or two-way settings for each road section clearly affect distance and travel time. The absence of these three data in this modeling clearly influences the results of this modeling. Although in emergency cases such as evacuation of cold lava flood in Kali Putih, all traffic rules may be ignored.

IV. CONCLUSION

Modeling the evacuation of cold lava flood in Kali Putih using Network analyst proved to be able to visually and spatially describe the distribution of affected settlement locations, the distribution of temporary evacuation sites and the direction of refugees to the nearest temporary evacuation sites (TES). Network analyst is also able to simulate the most reasonable amount of FES that is clearly very helpful in the effectiveness and efficiency of managing the final evacuation site (FES).

In spatial modeling activities, completeness, accuracy and suitability of input data, so does the spatial logic capability of the operator is needed to get results that can be accepted scientifically and logically. The case of analyzing the level of vulnerability of cold lava flood in Kali Putih is an example, where due to lack of parameters and lack of appropriate data cause the results obtained are doubtful for accuracy, both scientifically and in spatial logic.

Modeling Evacuation of cold lava flood with network analysis is quite promising in terms of the ability to describe results spatially. Especially with the model builder automation system that makes the modeling process more interactive. Therefore, deepening needs to be continued by using all types of updated data needed, so that the results obtained can actually be applied in the field, and become guidelines for all parties related to the catastrophic cold lava flood in Kali Putih. Even not only the Kali Putih case, this modeling can also be applied to all cases of disaster evacuations.

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REFERENCES

[1] Abousaeidi, M., Fauzi R., Muhamad, R. (2016). Geographic information system (GIS) modeling approach to determine the fastest delivery routes. *Saudi Journal of Biological Sciences (2016) 23*, 555–564

[2] Badan Nasional Penanggulangan Bencana. (2010). *Peraturan Kepala Badan Nasional Penanggulangan Bencana Nomor 14 Tahun 2010 Tentang Pedoman Pembentukan Pos Komando Tanggap Darurat Bencana*. (State Document). Retrieved from https://www.scribd.com/doc/189278949/Perka-BNPB-14-2010-Pedomaokn-Pembentukan-Pos-Komando-Tanggap-Darurat-Bencana-2

[3] Bernadeth, M. L., LIM, H.Jr., PIANTANAKULCHAI, Mo. (2013) Factors affecting flood evacuation decision and its implication to transportation planning. *Journal of the Eastern Asia Society for Transportation Studies, Vol.10, 2013*, 163 – 177.

[4] Bhambulkar, A V. (2011). Municipal solid waste collection routes optimized with ArcGIS network analyst. *International Journal of Advance Engineering Science and Technologies. Vol No. 11, Issue No. 1*, 202 - 207

[5] Biro Pusat Statistik. *Jumlah Penduduk Indonesia menurut Desa (Hasil Sensus BPS)*. Retrieved on August 2018 from https://www.bps.go.id/website/fileMenu/Penduduk-Indonesia-Menurut-Desa-2010.pdf

[6] BNPB dan Bappenas. (2011). *Rencana aksi rehabilitasi dan rekonstruksi: Pasca bencana erupsi Gunung Merapi Provinsi D.I.Yogyakarta dan Provinsi Jawa Tengah tahun 2011 – 2013*. Retrieved on Juli 2018 from https://www.bnpb.go.id/rencana-aksi-rehabilitasi-dan-rekonstruksi-wilayah-pasca bencana-erupsi-gunung-merapi-di-prov-diy-dan-prov-jateng-tahun-2011-2013

[7] Bosonal T, Nordmark, I., Gebresenbet, G. & Ljungberg, D. (2013). GIS-based analysis of integrated food distribution network in local food supply chain. *International Journal of Business and Management; Vol. 8, No. 17; 2013 ISSN 1833-3850 E-ISSN 1833-8119*, 13 - 34

[8] Bukari, S. M., Ahmad, M. A., Wai, T. L, Kaamin, M., Alimin, N. (2015). Spatial analysis in determination of flood prone areas using geographic information system and analytical hierarchy process at Sungai Sembrong's Catchment. *Soft Soil Engineering International Conference 2015 (SEIC2015)*.

[9] Curtin, K. M. (2007). Network analysis in geographic information science: Review, assessment, and projections. *Cartography and Geographic Information Science, Vol. 34, No. 2, 2007*, 103-111

[10] Darwin, Kombaitan, B., Yudoko, G., Purboyo, H. (2018). Application of GIS on determination of

flood prone areas and critical arterial road network by using chaid method in Bandung area. *MATEC Web of Conferences 147, 02007 (2018)*. Retrieved from https://doi.org/10.1051/matecconf/ 201814702007

[11] Dinas Kesehatan Provinsi Jawa Tengah. (2015). *Buku alamat puskesmas dan rumah sakit Provinsi Jawa Tengah tahun 2014*. Retrieved August 2018 from https://www.dinkesjatengprov.go.id/

[12] Doma, M. S. A. & Purwanto, T. H. (2013). Penentuan jalur evakuasi dan dampak banjir lahar dingin Gunung Merapi, Jawa Tengah. *Jurnal Bumi Indonesia. Volume 2, Nomor 2, Tahun 2013*. 149-154

[13] ESRI. (2010). Network analyst tutorial. Copyright © 1995-2010. Esri All rights reserved. Retrieved from http://help.arcgis.com/en/arcgisdesktop/ 10.0/pdf/network-analyst-tutorial.pdf

[14] ESRI. (2016). ArcMap 10.3, curvature. Retrieved August 2018 from http://desktop.arcgis.com/en /arcmap/10.3/tools/3d-analyst-toolbox/curvature.htm

[15] Esri. 2018. Types of network analysis layers. Retrieved from http://desktop.arcgis.com/en/ arcmap/latest/extensions/network-analyst/types-of-network-analyses.htm

[16] Han, L., Yuan F., Chin, S. & Hwang, H. (2006). Global optimization of emergency evacuation assignments. *Interfaces, 36(6)*, 502-513

[17] Hidayat, F. & Rudiarto, I. (2013). Pemodelan resiko banjir lahar hujan pada alur Kali Putih Kabupaten Magelang. *Jurnal Teknik PWK Volume 2 Nomor 4 2013*. 895-904

[18] Kumar, P. & Kumar, D. (2016). Network analysis using GIS techniques: A case of Chandigarh City. *International Journal of Science and Research (IJSR) Volume 5 Issue 2, February 2016*, 409 – 411

[19] Larasati Z. R., Hariyanto, T., Kurniawan, A. (2017). Pemetaan daerah risiko banjir lahar berbasis SIG untuk menunjang kegiatan mitigasi bencana (Studi kasus: Gunung Semeru, Kab. Lumajang). *JURNAL TEKNIK ITS Vol. 6, No. 2, (2017)*, 2337-3520 (2301-928X Print)

[20] Na, L., Xueyan, S., Mingliang, Q. (2012). A bi-objective evacuation routing engineering model with Secondary Evacuation Expected Costs. *Systems Engineering Procedia 5 (2012)* 1 – 7

[21] Ni'am, L. (2014). Kepengaturan dan penolakan relokasi: Kasus warga Watugajah pascabencana Gunung Merapi tahun 2011-2013. *Jurnal Ilmu Sosial dan Ilmu Politik Volume 18, Nomor 1, Juli 2014*, 1-14.

[22] *Peraturan Presiden Republik Indonesia nomor 70 tahun 2014 tentang rencana tata ruang kawasan taman nasional Gunung Merapi*. (2014). Retrieved from https://www.hukumonline.com/pusatdata/ downloadfile/.../lt544f48950b14...

[23] Ramadhiyanti, D. W., Sokang, Y. E. S., Suciati, A. A., & Viola, H. (2012). Set of field notes of cold lava flood villages Sirahan (Hope for the future). Faculty of Psychology, UGM. Yogyakarta.

[24] Retnowatia, S., Ramadiyantia, D. W., Suciatia, A.A., Sokanga, Y. A,. Violaa, H. (2015). Hope intervention against depression in the survivors of cold lava flood from Merapi Mount. *Procedia - Social and Behavioral Sciences 165 (2015)* 170 – 178

[25] Rinawati, D. I., Sari, D. P., Handayani, N. U., and Siwi, B. R. (2018). Predicting the probability of Mount Merapi eruption using bayesian event tree_eruption forecasting. *MATEC Web of Conferences **154**, 01050 (2018)*. Retrieved from https://doi.org/10.1051/matecconf/ 201815401050

[26] Shinta D., Priyono, K. D., Sigit, A. A. (2015). Mitigasi bencana lahar hujan Gunung api Merapi berbasis sistem informasi geografis dan penginderaan jauh di Sub das Kali Putih Magelang. Fakultas Geografi Universitas Muhamadiyah. Surakarta.

[27] Stepanov, A. & Smith, J. (2009). Multi-objective evacuation routing in transportation networks. *European Journal of Operational Research, 1498*, 435 - 446.

[28] Surono, Jousset, P., Pallister, J., Boichu, M., Buongiorno, M.F., Budisantoso, A., Costa, F., Andreastuti, S., Prata, F., Schneider, D., Clarisse, L., Humaida, H., Sumarti, S., Bignami, C., Griswold, J., Carn, S., Oppenheimer, C. and Lavigne, F. (2012). The 2010 explosive eruption of Java's Merapi volcano – a '100-year ' event. *Journal of Volcanology and Geothermal Research, 241-242*. 121-135.

[29] Taylor, M. A. P & Freeman, S. K. (2010). A review of planning and operational models used for emergency evacuation situations in Australia. *Procedia Engineering 3 (2010)* 3–14

[30] Tempo.co. (2014). Rivers near Mt Merapi face cold lava flood. Retrieved from https://en.tempo.co/read/ news/2014/10/19/206615399/Rivers-near-Mt-Merapi-Face-Cold-Lava-Flood

[31] *Undang-Undang Republik Indonesia No. 24 Tahun 2007 Tentang Penanggulangan Bencana*. (2007). Retrieved from https://www.bnpb.go.id/ppid/file/ UU_24_2007.pdf.

[32] van Zuilekom, K.M., M.F.A.M. van Maarseveen, M.R. van der Doef. (2016). A decision support system for preventive evacuation of people. Retrieved from https://www.researchgate.net/ publication/261911710

http://dx.doi.org/10.22161/ijeab/3.4.48 *ISSN: 2456-1878*

[33] Wimbardana, R & Sagala, S. A. H. (2013). Kesiapan masyarakat terhadap bahaya lahar dingin Gunung Merapi. *Jurnal Bumi Lestari, Volume 13 No. 2, Agustus 2013*. 394-406

[34] Wiwaha, A. A., Mei, E. T. W. & Rachmawati, R. (2016). Perencanaan partisipatif jalur evakuasi dan titik kumpul Desa Ngargomulyo dalam upaya pengurangan resiko bencana Gunungapi Merapi. *Jurnal Perencanaan Wilayah dan Kota vol. 27, no. 1. April 2016*. 34-48.

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A Review of Solid Waste Management in Waste Bank Activity Problems

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***Abstract*—** *This paper presents a review of solid waste management problems, with a particular focus on the household waste management through community participation in waste bank activity problems. Waste, especially solid waste is a problem that will continue to exist. Waste is generated by human's effort to fulfil their needs of life. Solid waste that continues to accumulate in the environment will cause negative impact which can interfere with human life itself. One of the example is the outbreak of a disease that lowers the level of public health. The negative impact will affect our environment, social, and economy aspect. Until now, public awareness of the importance of processing waste is still very low. This is due to several factors such as economic conditions, education, and social attitudes of the society itself. Nowadays, every country in this world try to do solid waste management because it's effect to humanity. One of the efforts to assist in the case of solid waste management is by the existence of waste bank. Waste bank is expected to be a sustainable solution to overcome the existing waste problems in the society. Society can get several benefits from waste bank because not only reduce the existing solid waste but also can improve the economic quality of the society with the payment system. The payment system is to exchange waste from society with some payments. The factors that affect the sustainability of waste banks are Awareness, knowledge, equipment, support, and infrastructure.*

***Keywords*—community, solid waste management, sustainable, waste, waste bank**

I. INTRODUCTION

Waste is a problem that we must settle as early as possible. Waste is an inevitable product of society [1]. Solid wastes consist of all the solid wastes that comes from human and animal activities and discarded as useless or unwanted [2]. The increasing production of household waste is one of the effects of population growth, rising living standards, rapid development and urbanisation [3]. To avoid negative effect from waste, society can do waste prevention.

Waste prevention means eliminating or reducing the amount and/or the toxicity of waste, including recyclables. There ar excepstions for businesses, government agencies and other organisations. Waste prevention in that fields includes processes that: conserve supplies and inventory; eliminate, reduce and reuse products and packaging; deploy waste-reducing technology and equipment; use more durable, reusable, repairable and less toxic products and packaging; leave grass clippings on the lawn to naturally decompose; and reduce food and yard waste, including through on-site composting. Waste prevention for citizens also includes: buying products with the least amount of packaging; buying only the amount of a product that is needed; buying less harmful products; and reusing, donating or repairing items that might otherwise be discarded or recycled (it can appear in the form of a garage sale) [4].

Waste management is one of the public infrastructure to provide the goods or services, and it resembles the electricity, natural gas, and water sector goods and services [5]. Waste management system usually consist some activity like collection, transportation, pre-treatment, processing, and final abatement of residues. Waste must be collected separately based on their types. Waste transport can be to some local or regional pre-treatment facility, or directly to some regional or national processing facility, such as a waste incineration plant. In Indonesia, there are TPA (Tempat Pemroresan Akhir) as one of final processing facility. Local or regional pre-treatment may include compressing, sorting, separation, drying, storage and so on. But this treatment can change based on conditions of the country. Today, waste processing often yields some valuable product, such as electricity, compost or synthetic crude oil [6]. Solid Waste Management (SWM) is a major part of the social system [7]. SWM become part of social system as its benefit for the community.

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Waste Bank is one of several concepts in waste management model. Waste bank as waste management model in the form of waste management business by applying the principle of the 3-R (Reduce, Reuse, Recycle). The system implemented is a system to manage waste, to accommodate, to sort, and to distribute the waste to other waste treatment facility or to those who need it [8]. For example some company that use recycle material for their products.

This paper gives a review from available literature about waste management problems with a focus on the waste bank activity. The purpose of this paper is to guide the reader about waste management problems. Especially, to find factors affecting the continuity of the household waste management through community participation in waste bank activity so then it can provide solution to maintain the continutity of household waste management activities through community participation in waste bank activity.

Although it is generally agreed that wastes management services are essential services that must be provided in every society, nonetheless very little is known on what exactly constitute a waste. Knowing that the concept of waste is highly subjective as one man's wastes is a resource to another. Hence, it is important to have a clear guide as to what could be classed as waste. The present research therefore examines the concept of wastes and wastes management with a view to determining what waste is, how they are classified and managed.

According to the World Bank and USAID, municipalities in developing countries usually spend 20–50% of their available municipal budget on SWM, which often can only stretch to serve less than 50% of the population [9] [10]. Cost-effective techniques for minimising waste include public education (by the government and local or international environmental organization) and citizen encouragement to use and share the design of household recycling processes [11]. The success of household recycling programs strongly depends on citizens' participation in the source separation process. This process requires people to separate their houshold wastes and special products from their household wastes [12].

There are some aspect that influence household waste behaviours. Attitude, subjective norms, perceived behavioural control, moral obligation, self-identify, intention, action planning, and past behaviour significantly predicted household waste behaviours [13]. We can conclude citizens participation and their attitudes towards waste is the key to success in waste management. Citizens' attitudes are influenced not only by impacts, but also by a lack of credibility in waste managers, decision makers, decision processes, and control mechanisms for waste facility siting and operation. Without a clear or open decision making process, siting of undesirable facilities becomes an extremely difficult task. The decision transparency and information accessibility are key factors for public acceptance.

Nowadays, there are still many people who are less concerned about the importance of waste management and processing which is also a significant problem for solid waste management system.

II. SOLID WASTE MANAGEMENT

Humans generate solid wastes as by-product from all of their activities. Disposing of these solid wastes has become a big challenge, especially as population densities have grown over the year. Actually, waste deposits have been associated with human habitation since prehistoric times. Solid waste consists of a diversity of objects from a variety of sources [14]. Solid waste's source is depend on population or citizen's behaviour.

Household waste is one of the hardest sources of waste to manage effectively. It consists of a diverse range of materials (glass, metal, paper, plastic, organics) totally mixed together because it's benefits. MSW composition is also has several variety, both seasonally and geographically from country to country, and from urban to rural areas. On the contrary, commercial, industrial, and other solid wastes (except from household) tend to be more homogeneous, with larger quantities of each material. A system that can be devised to deal effectively with the materials in household waste should be possible for management of other sources of solid waste [1].

The production and disposal of large amounts of waste is still seen by many people to be a loss of the earth's resources. Putting waste into holes in the ground appears to be inefficient materials management. It needs to be remembered that although the earth is an open system regarding energy, it is essentially a closed system for materials. Energy and material is two different thing that has their own management. Whilst materials may be moved around, used, dispersed or concentrated, the total amount of the earth's elements or materials stays constant (with the exception of unstable radioactive elements). Thus although resources of 'raw materials' may be depleted or decreased, the total amount of each element present on Earth remains constant. In fact, we mus accept that the concentration of some useful materials is higher in landfills than in their original raw material ores. Such materials could be dug up at a later date [1]. Unfortunately, to dug up the useful materials from landfills cost more money.

Landfilling is one of waste management method by putting waste in holes in the ground. Landfilling could be considered as long-term storage of materials rather than actual disposal. Concerns over conservation of resources

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have led to calls for general reductions in the amount of waste generated (waste minimisation or waste reduction) and for ways to recover the materials and/or energy in the waste, so that they can be used again. Recovery of resources from waste should slow down the depletion of non-renewable resources, and help to lower the use of renewable resources to the rate of replenishment.

Waste management models can improve the basis for waste management's decisions [15]. One of the solution is modelling of waste generation. This is a useful way to anticipate the design of waste management strategies as a function of demographic changes and development. Modellng of waste generation can approximate future management needs, based on predictions according to social and economic changes. This process should lead to a more sustainable approach for waste management in terms of policy and waste practice alteration [16].

It is widely recognized that solid waste management is not only a technical problem but strongly influenced by political, legal, socio-cultural, environmental, and economic factors, as well as available resources. Moreover, these factors have interrelationships that are usually complex in the waste management system. This complexity depends on citizen's behaviour towards SWM. It is suggested that appropriate solutions to the complex waste management problems be sought from a system perspective, taking into account all of the above factors in the local area [17].

To increase people's acceptance level of an SWM facility, dialog with neighbors or public involvement in the planning stage has become popular. Nowadays, these procedures are widely discussed because it's benefit to increase people's acceptance of SWM facility. It is essential to understand people's concerns and concepts of SWM management facilities through communication. It is because people with different backgroun will have different way of thinking. This is also essential for better solid waste management practice [7].

As [18] mention, waste management takes place at the interface between the anthroposphere and the environment. The definition and objectives of waste management have changed over time and are still changing. The changing point in waste management based on people's behaviour. The first signs of organized waste management appeared when people started to collect garbage and remove it from their immediate living areas to haave comfortable living. This was an important step regarding hygiene and helped to prevent epidemics among the people. These practices were improved over the centuries. However, dramatic changes in the quantity and composition of wastes during the 20th century caused new problems. First, the emissions of the dumping sites (landfills) polluted groundwater because it produce leachate and produced greenhouse gases. Second, landfill space became scarce in densely populated areas. Even the concept of sanitary landfilling could not solve these problems in a long time. Today, waste management is an integrated concept of different practices and treatment options comprising prevention and collection strategies; separation steps for producing recyclables or for subsequent processing using biological, physical, chemical, and thermal treatment technologies; and different landfill types. People now have the opportunity and the duty to separate paper, glass, metals, biodegradables, plastics, hazardous wastes, and other materials into individual fractions. The goals of modern waste management are to:

1. Protect human health and the environment
2. Conserve resources such as materials, energy, and space
3. Treat wastes before disposal so that they do not need aftercare when finally stored in landfills

Solid waste management practices were initially developed to avoid the adverse effects on public health because the increasing amounts of solid waste being discarded to the environment without appropriate collection or disposal. Managing this waste more effectively is now a need and a duty that society has to address. In dealing with the waste, there are two fundamental requirements: less waste and an effective system for managing the waste still produced [1]. The Brundtland report of the United Nations, Our Common Future mentioned by WCED, 1987, clearly explained how sustainable development could only be achieved if society in general, and industry in particular, learned to produce 'more from less'; more goods and services from less of the world's resources (including energy), while generating less pollution and waste [1].

Those waste management strategies that focus on source reduction, resource recover, and reuse have proven to be more cost effective over the long run or in the near future. They are less damaging to the environment because they prevent or minimize waste generation at the source [19]. Based on [14], the methods of managing solid waste are as follows:

1. Source Reduction. Prevention of solid waste generation.
2. Recycling. Diversion of specific items from the solid waste stream for other uses (such as composting).
3. Combustion. Combustion of solid waste to reduce volume and in some cases to generate energy.
4. Landfilling. Disposal of solid waste by burial.

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III. RECYCLE BANK

Municipal solid waste (MSW) source separation is considered an effective solution of reducing waste disposal, enhancing recycling and reducing environmental damage caused by landfilling [20]. MSW source separation refers to the process of separating waste into several categories according to their different characteristics by the household, prior to further treatment [21]. Source separation systems involve higher investment costs compared to mixed waste management systems, because there are higher collection costs, additional workers needed, infrastructure adjustments, new equipment and collecting vehicles, Mechanical Biological Treatment (MBT) facilities, public education, etc. [22]. Solid waste source separation requires a complex system including the purchase of additional collection equipment as well as promotional activities for attracting public awareness [23]. Attracting public awareness can help because it can change people's perspective about waste separation's importance.

Materials are recovered from MSW by separate collection programs. There are two main options to separately collect materials: first is "the bring" and second is "the house-to-house kerbside collection systems". With the first method, putrescibles, recyclable materials (e.g. paper, plastic and glass bottles, cans and other metals, textiles, etc.) and residual waste (or ''residue'') are delivered to several collection banks sites. On the opposite, materials are collected door to door with the kerbside system. The separate collection centres (SCCs), where the citizens can deliver the recyclable fractions of MSW, allow to integrate the two collection modalities as well as to exploit the advantages of the two systems and minimize their defects [24]. Usually, they are fenced and manned areas, equipped to weigh and collect mainly recyclables. They can also be considered as educational centres because they are places where people can be informed about waste management and made aware of the separate collection program rules, actions for the reduction of waste production, improvement of waste management, etc [25].

For example in Bangladesh, small-scale picking, sorting, cleaning, trading and processing of inorganic recyclables is tolerated without official authorization of the authorities. There is a common understanding that the informal recycling sector helps reduce amounts of waste. The waste does not need to be transported and landfilled by the Municipalities. Estimates of total generated solid waste being recycled by the informal sector range from 4% to 15%. Informal recycling is also acknowledged in the national 3R strategy as an important source of income for the poor citizens. Despite the hazardous and unstable working conditions in the informal sector, such activities contribute to poverty reduction, which is also in the interest of society and the Government [26].

The formal recycling sector is driven by global resource scarcity and regional demand for recyclable materials to feed domestic and foreign industries. Policy incentives such as tax holidays for up to 5–10 years for all waste treatment and recycling plants help enhance opportunities from formalization for recycling enterprises. Formalized recycling companies however will still buy recyclables from informal traders to help their economic condition. Informal and formal sector is not a clear-cut one for their different. The level of formalization in the sector is progressive. Formalization is the process by which authorities register and authorize individual businesses that comply with rules and regulations. For small scale traders and recyclers, formalization means having to comply with norms and requirements of the authorities. The authorities such as registration of the business, environmental clearance, and authorization for the use of the land for shops and facilities. All these requirements are associated to high costs and long delays, which are clear disincentives for informal traders and recyclers to change their status quo where they are tolerated and do not need to comply with any formal regulations. Strictly enforcing current regulations on the informal sector is not of great interest to the local authorities as this would reduce the current recycled amounts which then again would additionally burden the waste collection and landfilling process and negatively impact on the living environment. The local industry, producing housewares, food and beverage packaging, or ready-made garments, are main buyers of recycled materials. Even though their quality is lower, these recycled materials are competitive on the market because they are cheaper than the virgin materials.

The international market for recycled materials, with a large demand from China, Japan and also India, is further triggering more recycling activities in Bangladesh [27]. Waste recycling should be operated in a free market and systems. They are free to choose which end market best suits their needs in terms of price and quality depending on material demand [28].

Based on the study, recycle bank or waste bank is one of the solution in waste management that can be applied because it has economy and social basic. Recycle bank aims to encourage recycling and environmentally-friendly habits. It brings people, businesses, and communities to achieve big scale impact by participating in household recycling and teaching how to live with more sustainable lifestyles.

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IV. WASTE BANK BENEFITS

The existence of a waste bank encourages the community activities on solid waste sorting and recycling. The customers of waste bank take an advantage of sorting and recycling solid waste, because they can gain money from selling recyclable waste [29]. It really help to build up their economic conditions.

There is a need to study the waste bank's economic viability because the studies related to the waste bank, especially because the waste bank's budget performances are very limited. Based on data collection, it seems that waste bank has contributed to reducing inorganic municipal solid waste disposed to landfills by more than 1.5% every year. The number of waste bank's members tends to increase every year. The increasing number of waste bank's members means that there is an increasing amount of community participation in separating waste at its source [30].

As [31] mentioned, two towns of peri-urban settlement in Thailand were investigated in case studies to compare eco-performance between the towns with and without implementation of the CBM program. MSW mass flows and MSW utilization records in 2013 were studied to examine climate co-benefits from waste utilization activities. The results indicated that waste banks in the CBM program can effectively prevent most of recyclables from entering landfills. The practice of waste bank recycling rate from the case study with CBM is 172.20 kg per member per year, which is about 926% higher than average CBMs with MSW recycling. The success of CBM can be attributed to its curbside collection service and fair and friendly pricing of recyclables. The study also found that if the town decided to prevent wastes from landfilling, carbon intensity of the MSW system would be 0.47 tons of CO2-eq per ton of collected MSW. The landfilling cost would be along 7.41 USD per ton of MSW as landfilling cost. Current MSW reutilization rate has achieved 9.68% of generated waste, can avoid 16.80% of GHG emission, together with a reduction in landfill costs of 11.57% with CBM program. Two scenarios of waste utilization in Thailand were explored and compared, in terms of which scenarios yielded the highest co-benefits. From the comparison result we can choose the best method to MSW utilization.

The study from [31], indicates that by involve local mechanism and community to develop with operational waste banks, the efficiency of collecting recycling wastes will be increase. A similar system can be applied to other communities in other countries. This has so many benefit for other countries because there are solution they can take for their country's future SWM.

Based on the study from [30], there are some advantages received by waste bank's members in transaction with waste bank than to the informal sectors. The advantages are:

1. Buying price of recyclable waste from waste bank is higher than the informal sectors.
2. Waste bank has a cooperative system (savings and loans).
3. Waste bank has a clear recording of each member's transactions.
4. Seventy kinds of recyclable waste can be accommodated by waste bank

The advantages make people more convenience in selling their recyclable waste to waste bank. The level of people's trust in dealing with waste bank is higher than with the informal sectors.

From the study above, we can know the benefit of waste bank as follow,

1. Reducing municipal solid waste disposed to landfill
2. Increase citizens economic viability

V. WASTE BANK CONTINUITY PROBLEM

To combat this ever-growing mountain of waste, policy-makers have embraced the 3Rs concept with special emphasis placed on recycling, albeit last on the 3Rs hierarchy. Though recycling has gained political momentum, such efforts must be made in concert with reduction strategies, as recycling alone is insufficient to cope with the environmental impacts of current consumption rates in a growing population [32]. Therefore, recycling is not a goal in and of itself, but rather a necessary response to societal consumption patterns [33].

A country's specific context conditions how waste management practices are established so far and can be further improved for the future. Specificities of policy instruments and market incentives for urban solid waste management in Bangladesh is one of the example of current policy in developed country. Based on this initial research centred on Bangladesh, a comparative framework in terms of policy instruments and market incentives can be developed as a further research project in order to enable mutual learning between Bangladesh and other countries currently facing, or having faced in the past, similar urban solid waste management challenges [27].

Apart from the incentive to push recyclable waste into the recycling channel, demand for recycled materials drives the market. The price paid for recycled materials is very important for the competitiveness of recycling initiatives. The capacity of the processing facilities (separating, sorting, etc.) in various countries also differs. Some facilities in countries with relatively more developed waste recycling system and advanced technology have a problem of over-capacity. UK is at risk of heavily over-

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investing in residual waste treatment infrastructure. If all of the facilities with planning consent are to be built, UK will have 5 million tonnes more capacity than it requires [28]. Countries like Germany and Sweden have already a problem with over-capacity, mostly in the sorting plants.

The waste management is a major issue in most of the developing countries in the world. India and China are two faster growing economies who also have similar problems in waste management, especially becauselarge amount of waste they produce. Moreover, with the population growth and the increasing GDP, the MSW generation rate is increasing proportionately. Both the countries investing a loads of funds in landfill sites, MSW handling and treatment, but the problem is not resolved. The main constraint is the awareness of the citizen and poor institutional initiative [34].

The current regulation system in developing country is not perfect, and the existing management system and the collection facilities do not fit the present requirements at all. Another problem is waste collection without separation, treatment facilities are limited, and the collected wastes dumped carelessly in open areas. Government, NGOs, CBOs and private sectors are working hard in this field but still the action is not enough. The main management strategies to remedy this should include amendment of current laws and regulations, improve current management systems and introduce classified collections to citizen [35].

Based on [36], most of Asian developing countries have solid waste generation problems. The main core problem are incompetent organization and limited budget allocation from the government that cause solid waste reduction is started from the source up to the landfill sites. Alternative solutions of solid waste management that can be adapted for Asian developing countries are social and technical approaches. Social approaches are changing the public behaviour by improving community through training, and encouraging partnerships with decentralized solid waste management. The technical approaches are reducing biodegradable solid waste at source, converting waste to energy, and using simple but effective technology. These approaches in social and technical aspect are expected to improve the sustainability of SWM in Asian developing countries.

Based on the research through in-depth interviews of key informants in the community groups that carry out the activities of waste banks in five locations in Bandung, it is noted that waste bank problem is similar to SWM problem. The main problem for their continuity in society is limited budget, current regulation system is not perfect, and the awareness of citizens is low.

VI. WASTE BANK CONTINUITY FACTOR

Waste bank continuity factor is similar with waste management continuity factor. Municipalities have failed to manage solid waste due to financial factors and their country's economiy condition. The huge expenditure needed to provide the service [37], the absence of financial support, limited resources, the unwillingness of the users to pay for the service [38], and absence of right use of economic instruments have hampered the delivery of proper waste management services. [37] indicated that the involvement of the private sector is a factor that could improve the efficiency of the system.

Waste management is the sole duty and responsibility of local authorities, and that the public is not expected to contribute [39]. However, public participation in waste management has important place to increase waste management efficiency. The operational efficiency of solid waste management depends upon the active participation of both the municipal agency and the citizens. Socio cultural aspects mentioned by some scholars include people participating in decision making [37], community awareness, and societal apathy for contributing in solutions [40].

Management deficiencies are often observed in the municipalities. Some researchers that have investigated the institutional factors that affect the system have come to the conclusion that local waste management authorities have a lack of organizational capacities especially in leadership factor and professional knowledge. The information about waste management system which available is very scanty from the public domain [41]. The extremely limited information is not complete or is scattered around various agencies concerned, therefore, it is extremely difficult to gain an insight into the complex problem of municipal solid waste management [42].

Waste workers are usually associated to low social status [39]. This situation's result is low motivation among the solid waste employees. Politicians give low priority to solid waste compared to other municipal activities [40] with the end result of limited trained and skilled personnel in the municipalities [37]. Major positive factors that improve the system are support from municipal authorities [43] and strategic plans for waste management that makes monitoring and evaluating annually for the system easier[44].

Waste management is also affected by the aspects that facilitate the effectivity of the system. The aspects are technical, environmental, financial, socio-cultural, institutional, and legal. Household's attitudes and behaviour related to separation of waste depends on active support and investment of a real estate company, community residential committees' involvement for public participation, and fee for collection service [45].

Household waste utilization and separation behaviour also depends on gender, peer influence, land size, location of household, and membership of environmental organization. Lack of knowledge of treatment systems by authorities is reported as one factor affecting the treatment of waste [41].

The availability of waste facilities affects waste disposal choice. The limited number of waste containers along with long distance to get the waste container will make people prefer to dumping the waste in open areas and roadsides. Lack of financial resources limiting the safe disposal of waste in better equipped and engineered landfills and absence of legislation are mentioned by [46]. [47] suggests that the quantity of solid waste generation is lower in countries with lower GDP. As mentioned, there are three most important components in relation to the separation of waste. These components are:

1. **Awareness**. The efficiency of waste separation depends on the awareness of citizens about the impacts of waste management systems in the city.
2. **Knowledge**. Decision makers at the municipality are prone to set up waste separation programs when they are familiar with new and appropriate technologies as well as good practices for the management of waste.
3. **Equipment**. The availability of equipment and machinery to manage and recycle waste seem to be key factors that promote separation of waste at the household level.
4. **Support**: Central and local government, service providers and service users' support to the system are key elements for the efficiency of the collection, transfer and transport of solid waste.
5. **Infrastructure.** In general, municipalities are responsible for the infrastructure and equipment needed for waste collection, transfer and transport. The improvement of the infrastructure affects positively the efficiency of the system

From in-depth interviews of key informants in the community groups that carry out the activities of waste banks in five locations in Bandung, awareness, knowledge, and support from local authority become major factors affecting the continuity of waste bank activities.

VII. CONCLUSION

Waste bank is one of the solution in waste management that can be applied to encourage recycling and environmentally-friendly habits. People, businesses, and communities are bring together to achieve big scale impact by participating in household recycling and teaching how to live more sustainable lifestyles. The main problem of waste bank continuity is limited budget, current regulation system is not perfect, and the awareness of citizens is low. The solution for waste bank continuity is to correct current waste management system based on several factor : awareness, knowledge, equipment, support, and infrastructure.

REFERENCES

[1] Dougall, F. R. M., White, P. R., Franke, M., Hindle, P. 2011. Integrated Solid Waste Management: A Life Cycle Inventory, Second Edition. UK : Blackwell Publishing Ltd Enayetullah, I., Sinha, A.H., Hossain, I., Islam, S., Khan, K.H., Banu, L.A., et al., 2006. Final Report on Composition of Plastic Waste and Market Assessment of the Plastic Recycling Sector in Dhaka City.

[2] Tchobanoglous, G., Theisen, H., dan Vigil, S. 1993. Integrated Solid Waste Management : Engineering Principles and Management Issues. Singapore : McGraw-Hill, Inc.

[3] Mahar, A., Malik, R.N, Qadir, A., Ahmed, T., Khan, Z., & Khan, M.A., 2007. Review and analysis of current solid waste management situation in urban areas of Pakistan. In: Paper presented at the Proceedings of the International Conference on Sustainable Solid Waste Management, Chennai, India.

[4] Sharp, V., Giorgi, S., Wilson, D.C., 2010b. Methods to monitor and evaluate household waste prevention. Waste Management Research 28, 269–280.

[5] Dijkema, G.P.J., M.A. Reuter, E.V Verhoef. 2000. A New Paradigm for WasteMmanagement. Journal of Waste Management 20, 633-638

[6] Utama, G. L., Kurnani, T.B.A., Sunardi, Balia, R.L. 2017. Reducing cheese-making by-product disposal through ethanol fermentation and the utilization of distillery waste for fertilizer. International J. of GEOMATE. Vol. 13(37), 103-107.

[7] Rahardyan, B., Matsuto, T., Kakuta, Y., Tanaka, N., 2004. Resident's concerns and attitudes towards Solid Waste Management facilities. Waste Management 24 (5), 437–451.

[8] Suwerda, B. 2012. Bank Sampah (Kajian Teori dan Penerapan). Pustaka Rihama. Yogyakarta

[9] Henry, R.K., Yongsheng, Z., Jun, D., 2006. Municipal solid waste management challenges in developing countries – Kenyan case study. Journal of Waste Management 26, 92–100.

[10] Memon, M.A. (2010). Integrated solid waste management based on the 3R approach. J Mat. Cyc.& Waste Manage., 12 (1), 30–40.

[11] De Feo, G., De Gisi, S., 2010. Public Opinion and Awareness Towards MSW and Separate Collection Programmes: A Sociological Procedure for Selecting

Areas and Citizens With a Low Level of Knowledge. Journal of Waste Management 30 (6), 958–976.

[12] Krook, J., Martensson, A., Eklund, M., 2007. Evaluating waste management strategies. A case of metal-contaminated waste wood. Resources, Conservation and Recycling 52, 103–118.

[13] Pakpour, A.H., I.M. Zeidi, M.M. Emamjomeh, S.Asefzadeh, H. Pearson, 2013. Household waste behaviours among a community sample in Iran: An application of the theory of planned behaviour. Journal of Waste Management 32, 980-986.

[14] Kangas, P. C. 2004. Ecological Engineering, Principles and Practice. Florida : Lewis Publishers.

[15] Eriksson, O., Olafsson, M., Ekvall, T., 2003. How model-based systems analysis can be improved for waste management planning. Waste Management and Research 21 (6), 488–500.

[16] Purcell, M., W.L. Magette, 2008. Prediction of Household and Commercial BMW generation according to socio-economic and other factors for the Dublin region. Journal of Waste Management 29, 1237-1250.

[17] Mashayekhi, A.N., 1993. Transition in the New York State solid waste system: a dynamic analysis. System Dynamics Review 9, 23–47.

[18] Brunner, P. H. andRechberger, H. 2004. Practical Handbook of Material Flow Analysis. Florida : Lewis Publishers.

[19] Cheremisinoff, N.P. 2003. Handbook of Solid Waste Management and Waste Minimization Technologies. USA : Elsevier Science.

[20] Chung, S.S., Poon, C.S., 2001. A comparison of waste reduction practices and new environmental paradigm of rural and urban Chinese citizens. Journal of Environmental Management 62, 3–19.

[21] Yang, L., Li, Z.S., Fu, H.Z., 2011. Model of municipal solid waste source separation activity: a case study of Beijing. Journal of the Air & Waste Management Association 61 (2), 157–163.

[22] Dodis, C., Kitis, K., Panagiotakopoulos, D., Aivaliotis, V., 2003. The impact of source separation on the cost of municipal solid waste management systems. In: Proceedings of the 8th International Conference on Environmental Science and Technology Lemnos Island, Greece, Full paper Vol. A, pp. 162–169.

[23] Lave, D., S. Nardiya. 2012. A cost evaluation method for transferring municipalities to solid waste source-separated system. Journal of Waste Management 33, 1064-1072.

[24] De Feo, G., De Gisi, S., 2010. Domestic separation and collection of municipal solid waste: opinion and awareness of citizens and workers. Sustainability 2 (5), 1297–1326.

[25] De Feo, G., 2014. Sociological survey in a municipality with a high level separate collection programme in an area of historic unpopularity. Waste Manage. 34 (8), 1369–1380.

[26] Gunsilius, E., Chaturvedi, B., Scheinberg, A., 2011. The economics of the informal sector in solid waste management⁄ CWGe Collaborative Working Group on solid waste management in low- and middle-income countries. GIZ – Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH.

[27] Matter, A., M. Ahsan., M. Marbach., C. Zurbrugg, 2014. Impact of policy and market incentives for solid waste recycling in Dhaka, Bangladesh. Journal of Waste Management 39, 321-328.

[28] Pro-Europe, 2009, PRO EUROPE Response to EUPR Press Release Increasing Plastics Waste Exported to Far East, <https://www.pro-e.org/files/PRO%20EUROPE%20response%20to%20EUPR%20press%20release%20on%20plastics%20waste%20export%20to%20Far%20East_010909x.pdf >. (accessed 07.07.18).

[29] Dhokhikah, Y., Trihadiningrum, Y., Sunaryo, S. 2015. Community Participation in Household Solid Waste Reduction in Surabaya, Indonesia. Resource, Conservation and Recycling, 102 : 153-162.

[30] Fauziah, R., Mardiyono, and Kawano, M. 2015. Budget Performance of Bank Sampah Malang (BSM): Current Reality and Future Subjects. Reformasi Vol. 5, No. 2.

[31] Challcharoenwattana, A. and Pharino, C. 2015. Co-Benefits of Household Waste Recycling for Local Community's Sustainable Waste Management in Thailand. Sustainability, 7 : 7417-7437; doi:10.3390/su7067417.

[32] Kollikkathara, N., Feng, H., Stern, E., 2009. A purview of waste management evolution: special emphasis on USA.

[33] Mueller, W. 2012. The effectiveness of recycling policy options: Waste diversion or just diversions? Journal of Waste Management 33,508-518

[34] Ghosh, S.K., 2015. Sustainable SWM in developing countries focusing on faster growing economies, India and China. International Conference on Solid Waste Management, 5IconSWM 2015, 176-184.

[35] Glawe, U., C. Visvanathan, M. Alamgir. 2012. Solid waste management in least developed asian countries – A comparative analysis. Waste Management 29, 974–985.

[36] Dhokhikah, Y., Y. Trihadiningrum, 2012. Solid Waste Management in Asian Developing Countries: Challenges and Opportunities. Journal of Applied

Environmental and Biological Sciences 2012, 2 (7), 329-335.

[37] Sharholy, M., Ahmad, K., Vaishya, R.C., Gupta, R.D., 2007. Municipal solid waste characteristics and management in Allahabad, India. Journal of Waste Management 27, 490–496.

[38] Sujauddin, M., Huda, M.S., Rafiqul Hoque, A.T.M., 2008. Household solid waste characteristics and management in Chittagong, Bangladesh. Journal of Waste Management 28, 1688–1695.

[39] Vidanaarachchi, C.K., Yuen, S.T.S., Pilapitiya, S., 2006. Municipal solid waste management in the Southern Province of Sri Lanka: problems, issues and challenges. Journal of Waste Management 26, 920–930.

[40] Moghadam, M.R.A., Mokhtarani, N., Mokhtarani, B., 2009. Municipal solid waste management in Rasht City. Iran Journal of Waste Management 29, 485–489.

[41] Chung, S., Lo, C., 2008. Local waste management constraints and waste administrators in China. Journal of Waste Management 28, 272–281.

[42] Seng, B., Kaneko, H., Hirayama, K., Katayama-Hirayama, K., 2010. Municipal solid waste management in Phnom Penh, capital city of Cambodia. Waste Management & Research 29, 491–500.

[43] Zurbrügg, C., Drescher, S., Rytz, I., Sinha, M., Enayetullah, I., 2005. Decentralised composting in Bangladesh, a win–win situation for all stakeholders. Resources, Conservation and Recycling 43, 281–292.

[44] Asase, M., Yanful, E.K., Mensah, M., Stanford, J., Amponsah, S., 2009. Comparison of municipal solid waste management systems in Canada and Ghana: a case study of the cities of London, Ontario, and Kumasi, Ghana. Journal of Waste Management 29, 2779–2786.

[45] Zhuang, Y., Wu, S.W., Wang, Y.L., Wu, W.Z., Chen, Y.X., 2008. Source separation of household waste: a case study in China. Journal of Waste Management 28, 2022–2030.

[46] Pokhrel, D., Viraraghavan, T., 2005. Municipal solid waste management in Nepal: practices and challenges. Journal of Waste Management 25, 555–562.

[47] Shekdar, A., 2009. Sustainable solid waste management: an integrated approach for Asian countries. Journal of Waste Management 29, 1438–1448.

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Making the best of a Human modified Habitat; an Assessment of Avian Distribution and Diversity in Federal College of Education (Technical) Gombe. Gombe State- Nigeria

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Abstract**—** *We assessed the abundance and diversity of avian species in two distinct habitats types; main campus area (human inhabited) and adjoining heavily degraded savannah grassland. By employing Jaccard/Tanimoto Coefficient of Similarity, we tested whether bird species assemblage will differ between the two habitats, while Shannon Weiner Diversity Index was used to determine the level of diversity between sites.*

Line transect assessment generated a total of 1035 individuals of 69 avian species from 53 genera and 32 families. The most diverse avian family was Estrildidae with nine (9) avian species, followed by Columbidae with six (6), while Falconidae, Nectriniidae, and Turdidae families had a record of four (4) species each. Five families (Ardeidae, Malaconotidae, Ploceidae, Silviidae, Sturnidae) and five families (Accipitridae, Bucerotidae, Capitonidae, Viduidaeand Psittacidae) followed with three (3) and (2) species respectively. Seventeen (17) families were each represented by a single species.

Jaccard/Tanimoto Coefficient revealed that species composition differed between the two habitats with a similarity coefficient of 66.7 %, while Shannon Weiner Diversity Index was 1.56 and 1.67 for human inhabited (HI) and degraded savannah (DS) habitats respectively. The proximity to a natural savannah habitat albeit degraded has positive implications for avian diversity in the study area. We recommend more exclusion of human activities such as fuel wood harvesting and land grab for farming as this has grave consequences for the thriving population of species that are sensitive to human presence and urbanization.

Keywords**— Avian species, Diversity, Habitat utilization, Disturbance. Abundance.**

I. INTRODUCTION

One of the most outstanding features of birds is their high mobility and ability to travel great distances even across oceans (Borrow and Demey, 2001). Birds occur in all habitats known to man. (Mann and Cheke, 2001); the ubiquitous nature of birds and their sensitivity to ecosystem change makes them a very important component of biodiversity, and as such; birds are often used as good indicators of the state of health of the environment (Pearce and Ferrier, 2001; Gregory et al., 2003; Krisanti et al., 2017). Birds reflect changes in other biodiversity (example other animals and plants) and are highly responsive to environmental perturbations; making them very useful in studies designed to address the effects of human and other environmental disturbances on community stability and ecosystem productivity (Ezealor, 2002; Gregory et al., 2009). Birds contribute substantially to the overall species richness of West African forests, currently recognized as biodiversity hotspots of global importance (Orme et al., 2005).

Species diversity is a community attribute that is directly related to ecosystem productivity and vegetation structure (Tilman, 1996). Research has shown that species diversity is directly linked with habitat structure(James and Warner, 1982) as well as patterns of distribution of resources within a given ecological setting (Pringle et al., 2010).

The pattern and distribution of species has serious implication for community productivity. For instance, Pringle et al. (2010) proved that the regular (even spacing) spatial pattern of termite mounds found in a homogeneous African savannah provided a guide for parallel spatial patterning in tree-dwelling, termite-eating animal communities. Their findings, which also confirm that the uniformity of these patterns at small spatial scales boosted productivity of the whole landscape; provide support for

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models linking spatial patterns with ecosystem processes and functioning (Memmott et al, 2004; Bakam et al., 2018).

In the same manner, we explored how habitat structure and resource availability in a human modified habitat will affect avian distribution, abundance and diversity (Odewumi et al., 2017). We tested whether species will partition resource use along a gradient of disturbance in the study area (Agbo et al., 2018). This was possible considering the fact that the campus is contiguous to a natural but patchy and degraded savannah landscape made of some remnant native tree species. Our experimental approach was guided by the fact that vegetation structure is the most proximate factor that determines the spatial distribution of species (James and Warner, 1982); and more specifically bird diversity, enhanced by the plant species composition (Manu et al., 2007; Manu et al., 2010).

The goal of this study was therefore to determine how well birds utilize human modified habitats as well as the factors that may be crucial for their persistence in this degraded landscape. Specific objectives were to;

i. Develop a comprehensive checklist of the area.
ii. Identify the most abundant species in the study area
iii. Determine whether species composition (diversity) will differ between the two sites.

II. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in The Federal College of Education (Technical Gombe), established in 1977. The college operated elsewhere for 17 years before moving to the present campus (permanent site)in 1996. The College is located along Ashaka road (Latitude 10˚ 18'.30" N, Longitude 11˚ 9'.30" E.) in Akko Local Government Area, Gombe.

The annual rainfall ranges from 850 to 1000 mm, with two distinct seasons; rainy and dry seasons. The rainy season starts from May to October and dry season from November to April. Average daily temperatures are 34º C in April and 27 º C in August. The relative humidity ranges from 70 to 80 % in August and decreases to about 15 to 20 % in December.

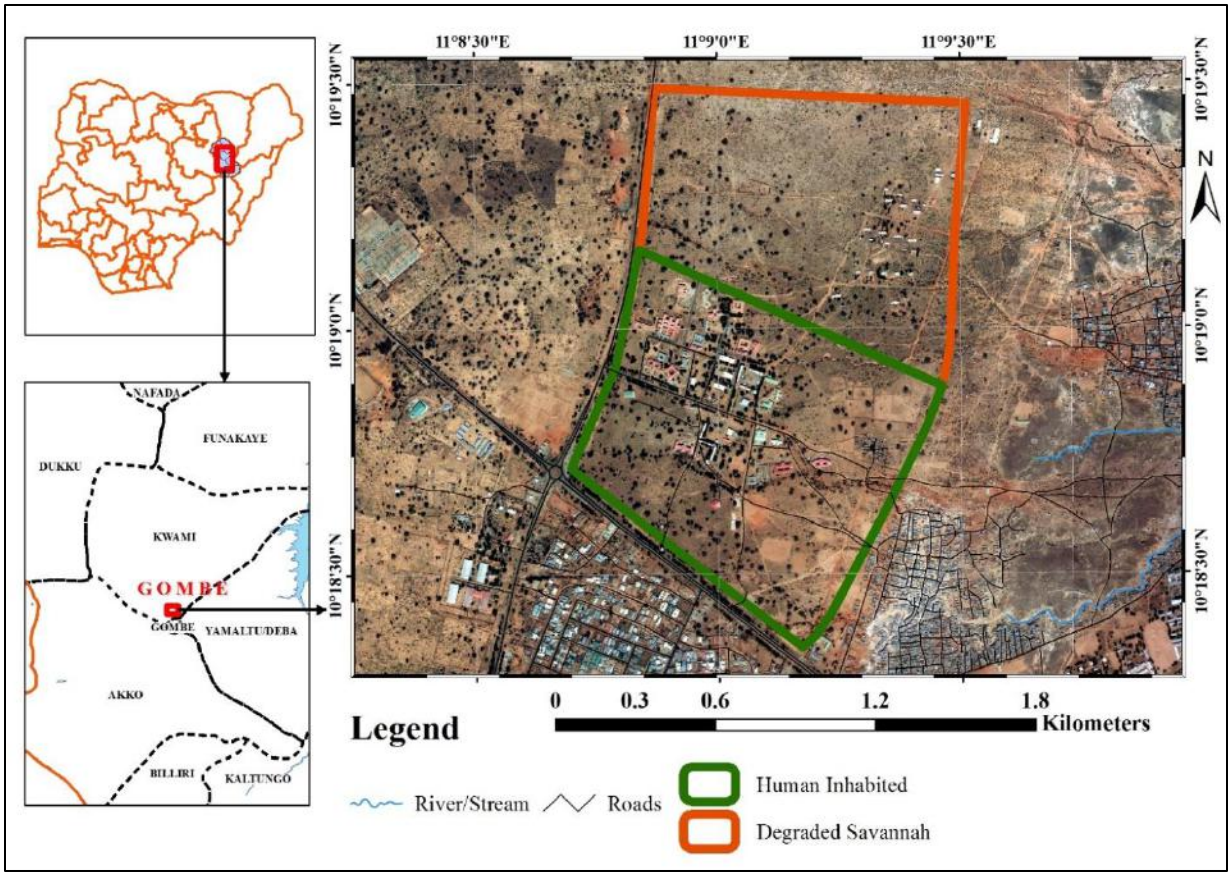

Fig.1: Map of study Area showing the two habitat types (human inhabited and degraded savannah).

The college lies within the Sudano–Sahelian Savannah vegetation typified by shrubs and sparsely distributed tree species. Regrettably, as is typical with most human modified habitats, the campus flora is now dominated by exotic and

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introduced tree species interspersed with a few remnant natives, the most prominent being *Parkia biglobosa* and *Tamarindus indica*. The college is divided into two unique habitats; the campus area hereinafter referred to as the human inhabited (HI) contiguous to a degraded savannah (DS) (Fig 1). The most common native tree species in the degraded savannah habitat was *Parkia biglobosa* while *Azadaricta indica* (Neem) was the most common tree species in the human occupied habitat.

2.2 Experimental Design

Line transect method (Bibly et al, 2000) was used to estimate and record bird species seen or heard within the study area. The campus was divided into two major habitat types; Degraded Savannah (DS) and Human inhabited (HI), with each habitat comprising of three transects. Each transect was located at a horizontal distance of 250 m apart to ensure that the same bird species was not recorded repeatedly in a given transect. Each transect covered a total distance of 2000 meters.

Transects were monitored twice each day in the morning and later in the evening. The morning session commenced at 6:30 am and lasted till about 9:30 am, while the evening sessions were conducted between the hours of 3:30 pm – 6:30 pm. During each transect survey we walked slowly along each transect and recorded bird species seen at least 50 m on either side of the transect or heard (Bibly et al, 2000). With the help of a pair of (Nikon sporter ® 8 x 42) binoculars we recorded the number seen and estimated the distance away from the transect. Each transect was repeated twice to optimize the record. The survey was conducted in 2016 during the end of the dry season and towards the onset of the rains.

Data generated from the survey was entered in excel spreadsheet version 2013 and explored before exporting same to SPSS. The statistical Package for Social Science (SPSS version 19.0 was used to analyze the data. Descriptive statistics was used to determine the frequency and numerical abundance of each avian species. Shannon Weiner Diversity index was employed to determine the species diversity and evenness in the study area and for each of the two habitats.

We calculated the level of similarity in species composition between the two habitats based on the Jaccard/Tanimoto Coefficient; which is one of the metrics deployed to compare the similarity and diversity of sample sets. It uses the ratio of the intersecting set to the union set as the measure of similarity or dissimilarity. Thus it equals to zero if there are no intersecting elements and equals to one if all elements intersect (common species to both sets). This was explored using the equation below;

$$T = \frac{N_c}{N_a + N_b - N_c}$$ Equation 1

where;

N_a- number of element in set A

N_b- number of elements in set B

N_c- number of elements in intersecting set

Shannon Wiener Diversity Index was used to estimate avian diversity of the study area. Effective number of species (Jost, 2006) was used to determine the pattern of distribution (even or uneven) of avian species. The closer the value of *Effective number* of species to the species richness (actual species count), the more even the distribution of the species and vice-versa.

Shannon Wiener Diversity Index was calculated using the formular below:

$H' - \sum_{i=1}^{s} p_i log_e p_i$

Equation 2

Where H' = Shannon Wiener Index

Pi = the proportion of individuals of species "i" in relation to the total population of all species.

Log_e = Natural logarithm of base e. To get the *effective number of species*, (the true value of diversity), we used the equation

$\exp\ (H') or \exp\ (-\sum_{i=1}^{s} p_i log_e p_i)$ Equation 3

III. RESULTS

A total of 1035 individuals of 69 avian species from 53 genera and 32families were recorded at the end of a four day transect survey with two days dedicated to each of the habitat types (Table 2). The most diverse avian family was the Estrildidae family with nine (9) avian species, followed by Columbidae with six (6), while Falconidae, Nectriniidae, and Turdidae families had a record of four (4) species each. Five families (Ardeidae, Malaconotidae, Ploceidae, Silviidae, Sturnidae) and five families (Accipitridae, Bucerotidae, Capitonidae, Viduadae and Psittacidae) followed with three (3) and (2) species respectively. However, 17 families were each represented by a single species (Table 1).

Laughing Dove *Streptopelia senegalensis* was the most abundant bird species with a total of 96 individuals sited in both habitats. Cattle egret *Bulbus ibis* and Vinaceous dove *Streptopelia vinacea* followed with 46 and 41 individuals respectively.

Jaccard/ Tanimoto coefficient of similarity revealed that the two habitats differed in species composition with a percentage difference of 33.3 %. Jaccard/Tanimoto

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coefficient was 0.6666 implying that the two habitats were 66.7 % similar in avian species composition. A total of 65 of the 69 species were recorded in the degraded savannah habitat (DS), while 50 species were recorded in the Human inhabited habitat (HI). Interestingly 19 and 4 species were unique to degraded savannah and Human occupied habitats respectively. However, 46 species were common to both habitats.

Shannon Weiner Diversity Index for Human occupied habitat was 1.56 with an effective number of diversity (true diversity) of 4.77. This was almost the same for degraded savannah with 1.67 and 5.32 for SWI and effective number of species respectively.

Investigations to determine the most common feeding guild in the study area revealed that 21 species were frugivorous, while 18 and 16 species were insectivorous and granivorous respectively (Fig. 2).

Table.1: Distribution of avian species across the 32 families recorded in the study area

S/n	Families	Number of species	Families	Number of species
1	Accipitridae	2	Malaconidae	3
2	Alcedinidae	1	Musophagidae	1
3	Ardeidae	3	Nectriniidae	4
4	Bucerotidae	2	Oriolidae	1
5	Capitonidae	2	Paridae	1
6	Charadridae	1	Passeridae	1
7	Ciconiidae	1	Phasiantidae	1
8	Cisticolidae	1	Picidae	1
9	Columbidae	6	Ploceidae	3
10	Coraciidae	1	Psittacidae	2
11	Corvidae	1	Pyconotidae	1
12	Cuculidae	1	Silviidae	3
13	Estrildidae	9	Sturnidae	3
14	Falconidae	4	Turnidae	4
15	Hirundiniidae	1	Viduadae	2
16	Laniidae	1	Zosteropidae	1

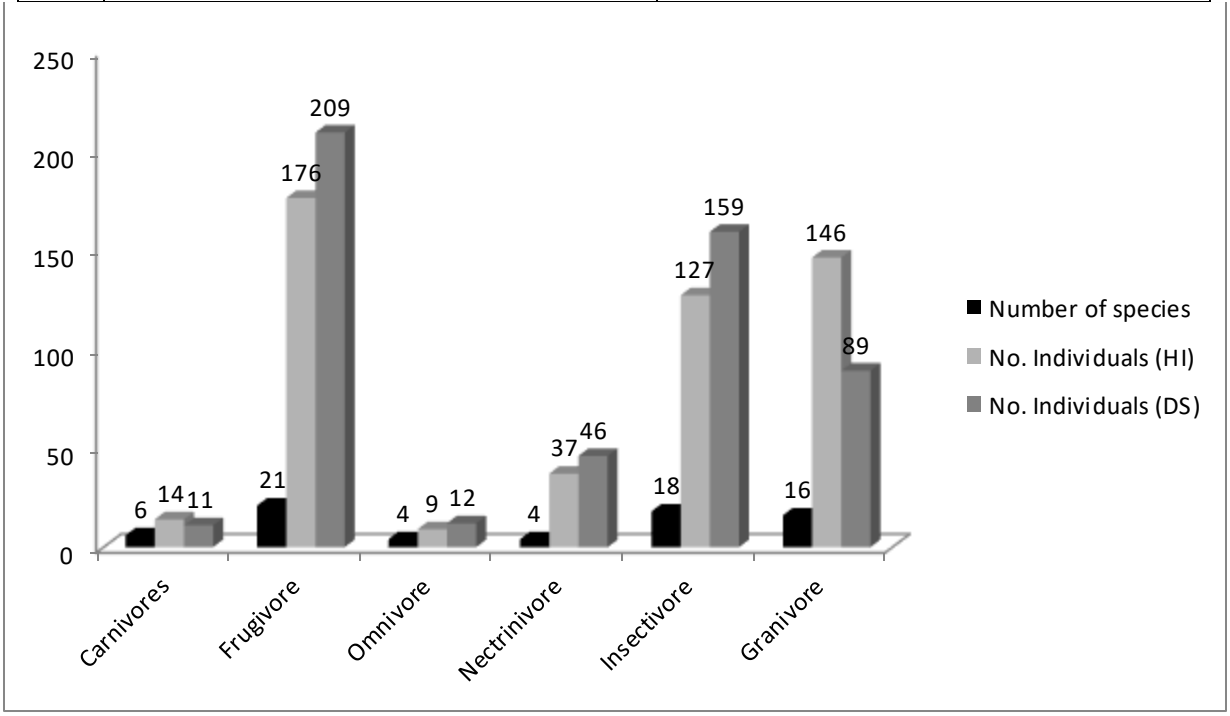

Fig.2: Distribution and abundance of avian species across feeding guilds between the two habitats; HI = Human inhabited, DS = Degraded savannah).

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Table.2: Checklist of avian species of the Federal College of Education (Technical) Gombe, Gombe State. √= present, - = absent

S/N	Species	Scientific name	Family	Human Inhabited	Degraded Savannah	Feeding Guild
1	Cattle Egret	*Bulbus ibis*	Ardeidae	√	√	Insectivore
2	Black headed heron	*Ardeame lanocephala*	Ardeidae	-	√	Insectivore
3	Grey heron	*Ardea cinerea*	Ardeidae	-	√	Insectivore
4	Abdim stork	*Ciconia abdimii*	Ciconiidae	√	√	Insectivore
5	Black headed lapwing	*Vanellus tetus*	Charadridae	√	√	Insectivore
6	Black shouldered kite	*Elanus caeruleus*	Accipitridae	√	√	Carnivore
7	Shikira	*Accipiter badius*	Accipitridae	√	√	Carnivore
8	Grey Kestrel	*Falco ardosiaceus*	Falconidae	√	√	Carnivore
9	Lanner falcon	*Falco biarmicus*	Falconidae	√	√	Carnivore
10	Fox Kestrel	*Falco alopex*	Falconidae	√	√	Carnivore
11	Common Kestrel	*Falco tinnunculus*	Falconidae	√	√	Carnivore
12	Double spur francolin	*Francolinus bicalcaratus*	Phasianidae	√	√	Omnivore
13	Black billed wood dove	*Turtur abyssinicus*	Columbidae	√	√	Frugivore
14	African Mourning dove	*Streptopelia decepiens*	Columbidae	-	√	Frugivore
15	Laughing Dove	*Streptopelia senegalensis*	Columbidae	-	√	Frugivore
16	Vinaceous Dove	*Streptopelia vinacea*	Columbidae	√	√	Frugivore
17	Bruce's Green Pigeon	*Treron waalia*	Columbidae	√	√	Frugivore
18	Speckled pigeon	*Columba guinea*	Columbidae	√	√	Frugivore
19	Rose ringed parakeet	*Psittacula krameri*	Psittacidae	-	√	Frugivore
20	Senegal parrot	*Poicephalus senegalus*	Psittacidae	√	√	Frugivore
21	Abyssinian roller	*Coracias abyssinicus*	Coraciidae	√	√	Insectivore
22	African Grey hornbill	*Tockus nasatus*	Bucerotidae	-	√	Frugivore
23	Red billed hornbill	*Tockus erythrorhynchus*	Bucerotidae	-	√	Frugivore
24	Bearded barbet	*Lybius dubius*	Capitonidae	√	√	Frugivore
25	Yellow fronted tinker bird	*Pogoniulus chrysoconus*	Capitonidae	√	√	Frugivore
26	Cardinal Woodpecker	*Dendropicos poecilolaemus*	Picidae	√	√	Frugivore
27	Ethiopian Swallow	*Hirundo aethiopica*	Hirundiniidae	√	√	Insectivore
28	Common Bulbul	*Pycnonotus barbatus*	Pycnonotidae	√	√	Insectivore
29	African Thrush	*Turdeus pelios*	Turdidae	√	√	Frugivore
30	Cliff chat	*Myrmeccocichla cinnamomeiventris*	Turdidae	-	√	Frugivore
31	Northern Ant eater chat	*Myrmecocichlaaethiops*	Turdidae	√	√	Insectivore
32	White Fronted black chat	*Myrmecocichla albifrons*	Turdidae	√	√	Insectivore
33	Senegal Eremomela	*Eremomela pussila*	Cisticolidae	√	√	Insectivore
34	Garden Warbler	*Silvia borin*	Silviidae	-	√	Insectivore
35	Grey backed Camaroptera	*Camaroptera brachyuran*	Silviidae	-	√	Insectivore
36	Tawny Flanked Prinia	*Prinia subflava*	Silviidae	-	√	Granivore
37	White shouldered black tit	*Parusleucomelas guineensis*	Paridae	-	√	Granivore
38	Beautiful sunbird	*Cinnyris pulchellus*	Nectriniidae	-	√	Omnivore
39	Copper Sunbird	*Cinnyris cupreus*	Nectriniidae	-	√	Nectarivore
40	Scarlet Chested sunbird	*Chalcomitra senegalensis*	Nectriniidae	√	√	Nectarivore
41	Variable Sunbird	*Cinnyris venustus*	Nectriniidae	√	√	Nectarivore
42	Yellow White eye	*Zosterops senegalensis*	Zosteropidae	√	√	Nectarivore
43	Yellow Bill shrike	*Corvinella corvina*	Laniidae	√	√	Insectivore
44	Black crown tchagra	*Tchagra senegalus*	Malaconotidae	√	√	Insectivore
45	Tropical boubou	*Laniarus turatii*	Malaconotidae	√	√	Frugivore

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46	Yellow crown Gonolek	*Laniarus barbarus*	Malaconotidae	√	√	Frugivore
47	Black headed oriole	*Oriolus brachyrhynchus*	Oriolidae	√	√	Frugivore
48	Pied crow	*Corvus albus*	Corvidae	-	√	Frugivore
49	Long tail glossy starling	*Lamprotonis caudatus*	Sturnidae	√	√	Omnivore
50	Purple glossy starling	*Lamprotornis purpureus*	Sturnidae	√	√	Granivore
51	Piapiac	*Ptilostomus afer*	Sturnidae	-	√	Granivore
52	Northern Grey headed Sparrow	*Passer griseus*	Passeridae	-	√	Granivore
53	Bush Petronia	*Petronia dentata*	Ploceidae	√	√	Granivore
54	Little Weaver	*Ploceus luteolus*	Ploceidae	-	√	Granivore
55	Village Weaver	*Ploceus cucullatus*	Ploceidae	√	-	Granivore
56	African Silver bill	*Eudice cantans*	Estrildidae	√	√	Granivore
57	Bronze Mannikin	*Spermetes cuccullatus*	Estrildidae	√	√	Granivore
58	Cinnamon Breasted-Rock Bunting	*Emberiza tahapisi*	Estrildidae	√	√	Granivore
59	Cut throat finch	*Amadina fasciata*	Estrildidae	√	√	Granivore
60	Red billed Fire Finch	*Lagonosticta senegala*	Estrildidae	-	√	Granivore
61	Orange cheeked waxbill	*Estrilda melpoda*	Estrildidae	√	√	Granivore
62	Black crown waxbill	*Estrilda nonnulacens*	Estrildidae	√	√	Granivore
63	Lavender waxbill	*Estrilda caerules*	Estrildidae	√	√	Granivore
64	Red Cheeked cordon bleu	*Uraeginthus bengalus*	Estrildidae	√	√	Granivore
65	Western Grey Plantain eater	*Crinifer piscator*	Musophagidae	√	√	Frugivore
66	Senegal Coucal	*Centropus senegalensis*	Cuculidae	√	√	Frugivore
67	Grey Headed Kingfisher	*Halcyon malimbica*	Alcedinidae	√	√	Omnivore
68	Village indigo bird	*Vidua chalybeate*	Viduidae	√	-	Granivore
69	Pintail Whydah	*Vidua macoura*	Viduidae	√	-	Granivore

IV. DISCUSSION

Many institutions of higher learning are adorned with ornamental as well as exotic and native tree species. Apart from the primary role of aesthetics, trees are biologically crucial in climate moderation, carbon sequestration, and mitigation of run-offs and floods during the rains. In addition plants help in air purification, shade provision/ wind break and reduction in noise pollution (Novak and Dwyer 2007).It is also a fact that plants inadvertently provide primary habitats for a vast number of life forms thereby promoting biodiversity.

Birds are ubiquitous and have learnt to utilize various habitats both natural and human modified (Borow and Demey, 2004), and as such,we tested whether species assemblage will differ between two distinct habitat types; a human occupiedand a degraded savannah habitat. Our thinking was predicated on the notion that habitat structure is a major predictor of habitat choice by birds as has been suggested by some studies (Nsor, 2006; Abalaka and Manu, 2007; Manu et al., 2007; Manu et al., 2010; Dami et al., 2014).

Our record of 69 avian species is in consonance with similar studies, example Agbo et al. (2018) who recorded 60 avian species in a similar landscape in Kaduna, Kaduna state. Moreover, our findings are in tandem with other surveys within the region of Gombe State where the authors reported species richness values similar to our present findings (Nsor and Adang, 2012; Adang et al, 2015a, Adang et al, 2015b). However, the scale of enquiry (survey duration) may be a limiting factor and a major bias if we were to run a comparative analysis of species richness among the various study sites. Nonetheless, our results indicate,a relatively higher species richness compared to previous studies given that the survey was conducted for just four days.

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Our quest to determine how well avian species make the best of a human modified and degraded savannah habitats was quite revealing; our results suggest that most of the birds in fact 66.7 % use both habitats freely although their distribution may favor one habitat over the other in terms of abundance. For example, the most abundant bird species Laughing Dove *Streptopelia senegalensis*, Cattle egret *Bulbus ibis* and Vinaceous dove *Streptopelia vinacea* were more abundant in the Human inhabited habitat than in the degraded savannah, alluding to the fact that perhaps becoming use to human presence was an adaptive advantage. However, it is interesting to note the possible interplay of resource distribution, competition, and habitat patchiness in driving certain individuals of some species to forage in specific habitats even at the risk of predation. This is in keeping with the source-sink theory and the meta-population concept (Hanski, 1994, Hanski et al., 1995).

Birds are known to occupy certain feeding guilds, with several species sharing the same food resources. While most studies on resource distribution focused on the spatio-temporal distribution, few have dwelled on the vertical distribution of avian food resources. However in a recent study, Bakam et al (2018) demonstrated how birds utilize resources along a vertical gradient. They authors asserted that the more structurally diverse a habitat is, the more likely it is to support diversity, which is in consonance with the works of Manu (2007). In this study, we recorded 21 frugivorous species occupying various heights in a vertically stratified niche arrangement which keeps them often above their zero elevation foraging counterparts –16 species of granivores, while 18 species of insectivores oscillated between different strata, often spending most of their time on the ground hunting for insects. Omnivorous species on the other hand occupied and fed along a vertical gradient while the birds of prey -6 species of carnivores (raptors) swoop down on their prey from the top stratum where they often perch for hours (Bakam et al., 2018).

This observed partition of resources reduces interspecies competition while facilitating species cooperation. Against this backdrop, it would not be out of place to say that based on the results of this study, that the relatively high level of diversity could be a direct benefit of habitat heterogeneity as reflected in the various feeding guilds highlighted above while also consolidating the notion that vertical stratification of resources is positively associated with avian species diversity and optimizes species richness in concert with other habitat and environmental parameters such as foliage volume and percentage vegetation cover (Karr and Roth, 1971; James and Warner, 1982).

Furthermore, the differences in species composition between the two habitats investigated in this study confirm the notion that a heterogeneous habitat supports more species diversity than a homogeneous one (Abalaka and Manu, 2007; Dami et al., 2014). The human inhabited habitat was found to be dominated by exotic and introduced plant species planted in a homogenous pattern (Pringle et al, 2010). This fact coupled with vehicular and human presence may be one of the reasons why more species were recorded in the degraded savannah habitat than the human inhabited one (Imong, 2007).

Moreover, most bird species are naturally elusive and avoid habitats that do not offer adequate cover; some of these species e.g. Bush Petronia *Petronia dentata*, Senegal Parrot *Poicephalus senegalus*, Tawny flanked Prinia *Prinia subflava*, Grey Backed Camaroptera *Camaroptera brachyuran*, Double spur Francolin *Poicephaluss enegalus* etc.,were found to occur only in the degraded savannah where some remnant shrubby patches offer cover. However because birds are highly mobile apart from the flightless ones, they often go beyond their comfort zones to human inhabited areas especially when the habitats offer some movement "corridors" or safe patches to facilitate movement between distinct habitats (Noss, 1991). This was the case with some species that are seldom seen in isolated human dominated landscape. Some of these human evading and habitat sensitive species (e.g. Yellow crown Gonolek *Laniarus barbarous*, Black crown Tchagra *Tchagra senegalus* and the Red-billed Hornbill *Lagonosticta senegala* were seen freely foraging in the human inhabited habitat in this study.

The aforementioned species could easily forage in both habitats because there was really no clear demarcation between the two habitats. Moreover, some portions of the human inhabited habitats tapered nicely into the degraded savannah contiguously (Noss, 1991). This observation emphasizes the need for landscape experts and environmentalist to design campuses and other public facilities such that natural patches of indigenous flora will be interspersed with buildings and introduced flora. This will go a long way to encourage diurnal movements of avian species between patches and on a broader scale more biodiversity.

V. CONCLUSION

The study identified certain anthropogenic activities that may be detrimental to avian species wellbeing and abundance in the study area if urgent actions are not taken. These include but not limited to; indiscriminate and

unregulated extraction of fuel wood, excessive conversion of remnant woodland to agricultural fields, unsustainable extraction of plants of ethno-botanical importance, unregulated movement of pedestrian and poachers into the college through multiple entry and exit routes.

We urge the college management to as a matter of urgencyblock all unauthorized entry and exit routes to check unsustainable harvest of fuel wood. More native tree species should be reintroduced to mute the invasive effect of exotic species and restore networks of interactions that have been broken with the exit of native key stone tree species. The campus has potential to be a major refuge for birds and other smaller invertebrate species if all stakeholders rejig their commitment to nature and their stewardship obligation to biodiversity.

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REFERENCES

[1] Abalaka, J. I., & Manu, S. A. (2007). Factors affecting forest bird diversity and recent avifaunal changes in the degrading Kagoro-Nindam forest reserves, Kaduna, Nigeria. *Ostrich*, 78(2), 233-238.

[2] Adang K.L., Nsor, C.A &Tela, M. (2015a). Survey of avifauna of Gombe State University, Gombe, Nigeria. *International Journal of Biology and Biological Sciences*. 4(2): 032-036.

[3] Adang K.L., Nsor, C.A &Tela, M. (2015b). Checklist of bird species at Dadin Kowa Dam, Gombe, Gombe State-Nigeria. *Global Advanced Research Journal of Agricultural sciences*, 4(5): 276-280

[4] Adang, K.L., Tanko, D., Kachi, J., and Abdulwahab, U. A. (2018). Bird Species richness and Diversity of Lokoja and Environs, Kogi state, Nigeria. *Proceedings of the 6th NSCB Biodiversity Conference*; UniUyo. Pp.117-124.

[5] Agbo, B. O., Bakam, H., Andong, F. A., Salihu, M. and Abdulhamid, H. (2018) Dawn and Dusk Observation of Bird Abundance and Diversity in the Federal College of Forestry Mechanization Afaka, Kaduna state.*Proceedings of the 6th NSCB Biodiversity Conference*; UniUyo. Pp.138-143

[6] Borrow, N and Demey, R. (2004). Birds of Western Africa. Christopher Helm Publisher. London.

[7] Bakam, H.;Zubairo, Gideon Philips and Agbo Boniface, O. (2018). Birds of General Hassan Usman Katsina Park in Relation to Vertical Stratification of Foliage, Kaduna, Nigeria. *Proceedings of the 6th NSCB Biodiversity Conference*; UniUyo. Pp.408- 415.

[8] Dami, F. D., Mwansat, G. S., & Shiiwua, A. M. (2014). The Effect of Fragmentation and Land Use Types on Bannerman's Weaver Ploceus bannermani (A Globally-Threatened Bird Species) on the Obudu Plateau, Southeast Nigeria. *Journal of Natural Sciences Research*, 4(19), 91-97.

[9] Ezealor, A.U. (2002). *Critical Sites for Biodiversity Conservation in Nigeria*. Nigerian Conservation Foundation, Lagos Nigeria 110p.

[10] Gregory, R. , Noble, D., Field, R., Marchant, J., Raven, M., & Gibbons, D. W. (2003). Using birds as indicators of biodiversity. *Ornis Hungarica*, 12(13), 11-24.

[11] Gregory, R. D., Willis, S. G., Jiguet, F., Vorisek, P., KLvanova, A., van Streien, A., . . . Green, R. E. (2009). An indicator of the impact of climatic change on European bird populations. *PloS one*, 4(3), e4678.

[12] Hanski, I (1994). A practical Model for for Metapopulation Dynamics. *The Journal of Animal Ecology*, Vol. 63, No. 1. (Jan., 1994), pp. 151-162.

[13] Hanski, I., Pakkala T., Kuussaari M, and Lei, G. (1995). Metapopulation persistence of an endangered butterfly in a fragmented landscape. - *Oikos* 72: 21-28.

[14] Imong, I. S. (2007). *Effects of Habitat Type and Anthropogenic Disturbance on the Density and Distribution of Birds in the Sankwala Mountains, Nigeria*, University of Jos. Masters Thesis.

[15] James, F.G., & Warner, N.O. (1982). Relationships between temperate forest bird communities and vegetation structure. *Ecology* 63: 159-171.

[16] Jost. L. 2006. Entropy and Diversity. *Oikos*, 113(2), 363-375

[17] Karr, J.R. & Roth, R. R. (1971). Vegetation structure and Avian diversity in several New World areas. *The American Naturalist* 105: 423-435.

[18] Krisanti, A.A., Choirunnafi, A., Septiana, N.O., Pratama, F.W., Amelia, F., Manjaswari, A., Septinigtyas, P.A., Wati, A.S., Satria, J.Y., Ani, I.L. Wibowo, T. and Sugiyarto (2017). The diversity of diurnal bird species on the Western Slope of Mount Lawu, Java, Indonesia. *Biodiveritas*, 18 (3): 1077-1083, DOI: 10.13057/biodiv/d180327.

[19] Manu, S., Peach, W., & Cresswell, W. (2007). The effects of edge, fragment size and degree of isolation on avian species richness in highly fragmented forest in West Africa. *Ibis*, 149(2), 287-297.

[20] Manu, S., Imong, I. S., & Cresswell, W. (2010). Bird species richness and diversity at montane Important Bird Area (IBA) sites in south-eastern Nigeria. *Bird Conservation International*, 20(3), 231-239.

[21] Noss, R. F. (1991). Landscape connectivity: different functions at different scales. Landscape linkages and biodiversity. Island Press, Washington, DC, USA, 27-39.

[22] Nowak, D.J. and Dwyer, J.F. (2007) "Understanding the benefits and costs of urban forest ecosystems," in *Urban and CommunityForestry in the Northeast*, J. E. Kuser, Ed., pp. 25–46, Springer Dordrecht, The Netherlands, 2nd edition, 2007.

[23] Nsor, C. A. (2006). *Habitat Utilization by ten bird species in Amurum forest Reserve, Jos, Plateau State-Nigeria*. University of Jos Masters Thesis.

[24] Nsor, C. A, Adang, K. L (2012). Guide to birds of Gombe State University, Gombe Nigeria. Pawo graphics and publishers ISBN: 978-978-922-747-1

[25] Odewumi, O.S., Okosobo, E.F. &Talabi, O. (2017). Diversity and Abundance of Avian Species of OwenaMultipurpose Dam, Ondo State, South West, Nigeria. *Journal of Biodiversity Bioprospecting and Development* 4: 163. Doi:10.4172/2376-0214.1000163.

[26] Orme, C.D.L., Davies, R. G., Burgess, M., Eigenbrod, F., Pickup, N., Olson, V. A., . . . Ridgely, R. S. (2005). Global hotspots of species richness are not congruent with endemism or threat. *Nature*, 436(7053), 1016-1019.

[27] Pauw, A., & Louw, K. (2012). Urbanization Drives a Reduction in Functional Diversity in a Guild of Nectar-feeding Birds. *Ecology and Society*, 17(2), 1-27.

[28] Pearce, J., & Ferrier, S. (2001). The practical value of modelling relative abundance of species for regional conservation planning: a case study. *Biological Conservation*, 98(1), 33-43.

[29] Pringle, R. M., Doak, D. F., Brody, A. K., Jocqué, R., & Palmer, T. M. (2010). Spatial pattern enhances ecosystem functioning in an African savanna. *PLoS biology*, 8(5), e1000377

[30] Memmott, J., Waser, N. M., & Price, M. V. (2004). Tolerance of Pollination Networks to Species Extinctions. *Proceedings: Biological Sciences*, 271(1557), 2605-2611.

[31] Tilman, D. (1996). Biodiversity: population versus ecosystemstability. *Ecology*, 77(2), 350-363.

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Isolation and Characterization of Plant Growth-Promoting Endophytic Diazotrophic Bacteria from Sri Lankan Rice Cultivars and Rapid Screening for their Effect on Plant Growth Promotion

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Abstract*-The present study was conducted to isolate and identify endophytic diazotrophic bacteria in two Sri Lankan rice (Oryza Sativa L.) varieties; Suwandel and Bg 358 and to evaluate their potential to promote rice plant growth. A total of 15 putative endophytic diazotrophic bacterial isolates were obtained from shoots and roots of Suwandel and Bg 358 rice varieties out of which 7 isolates were selected based on their ability to produce IAA and phosphate solubilization. According to the morphological characters and biochemical tests, these bacteria were identified belong to genera Bacillus (IN003, IN006, and IN007), Klebsiella (IN008 and IN018), Pantoea (IN009), and Enterobacter (IN015). All selected bacterial isolates produced IAA (7.1µmgl^{-1} to 30.9 µ mgl^{-1}) in the tryptophan supplemented medium. Five out of seven bacterial isolates (IN006, IN007, IN008, IN015, and IN018) were able to solubilize inorganic phosphate on Pikovskaya's agar medium. Rice seeds (Suwandel variety) treated with these endophytic diazotrophic bacteria with plant growth-promoting ability showed significantly enhanced shoot length, root length, shoot fresh weight, shoot dry weight and root fresh weight compared to the uninoculated control. Plant inoculation experiment indicated that Enterobacter sp. (IN015) was most effective in rice plant growth promotion among seven bacterial isolates tested. These results strongly suggest that endophytic diazotrophic bacteria characterized in this study could be successfully used to promote rice plant growth.*

Keywords*— *Endophytic diazotrophic bacteria, IAA production, Phosphate solubilization, Plant growth promotion, Rice.

I. INTRODUCTION

Rice (*Oryza sativa*) is the staple crop for more than half of the world's population [26] including Sri Lanka. With the increasing world population, the demand for rice is expected to increase and therefore there is an immense pressure for higher production to feed the largely growing world population. One of the major problems associated with the rice production is the use of a massive amount of chemical fertilizers which cause negative impacts on both human health and environment. Therefore, there is an urgent need to identify the less harmful substitutes to chemical fertilizers to increase rice production. Natural endophytic bacteria associated with the plants have become a promising alternative to chemical fertilizer due to their plant growth promoting abilities [36].

Endophytic bacteria lives inside the plant tissues without causing any harmful effect to the host plant [34] and endophytic bacteria involved in the biological nitrogen fixation process are known as endophytic diazotrophs [15]. These endophytes are most commonly originated from the rhizosphere and enter into the plant through the cracks at the point of lateral root emergence and root tips and then systematically spread throughout the plant colonizing various tissues including seeds, stem, roots, and leaves of the plant [26]. A diverse range of endophytic diazotrophic bacteria have been isolated from the surface sterilized stem, leaves, seeds, and roots of rice plant using nitrogen-free medium and most of these isolated bacteria belong to the genus *Pantoea*, *Klebsiella*, *Azospirillum*, *Enterobacter*, *Herbaspirillum*, *Serratia*, and *Bacillus* [5,6,8,13,25,41]. Thee endophytic bacteria can promote the growth, and yield of plants when applied to seed or crops due to their plant growth-promoting properties. The widely recognized mechanisms of plant

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growth promotion are the production of phytohormones, diazotrophic fixation of nitrogen, and solubilization of phosphate [26]. In addition to that these bacteria also have ability to produce siderophore to chelate various metals, including Fe, Zn, and Cu [1,4] and also suppress pathogens by producing inhibitory compounds [24,42].

Bacterial endophytes have been reported to produce various phytohormones including Indole-3-acetic acid (IAA), cytokinin and gibberellins [19].Among these IAA is one of the most vital hormones, mainly due to its function in lateral and adventitious root formation [11] and root elongation [18] According to the [39], bacterial species belong to genera *Pseudomonas*, *Pantoea*, *Bacillus*, *Klebisella*, *Enterobacter*, and *Serrartia* have ability to produce IAA. Earlier studies have found that endophytic bacteria with the ability to produce IAA can enhance the growth of plants by increasing plant height and biomass [37, 38]. Most of the soil phosphorous is in the form of insoluble phosphate and cannot be utilized by the plant and phosphorous deficiency in plants result in an inhibited stem and root development, poor flowering, lack of seed formation [16]. Endophytic bacteria can solubilize inorganic phosphate in soil by secreting organic acids and this in turn help to increase plant growth [17]. Endophytic bacteria have a potential to use as an inoculant to promote plant growth due to these plant growth-promoting properties. Previous study indicated that *Pantoea agglomerans* YS19 can enhanced the biomass of the rice seedlings after application which can be attributed to their nitrogen-fixing ability and phytohormone production [9]. A significant increase in the dry weight of leaf and root of rice plants has been recorded by rice plants inoculated with *Bacillus subtilis* [16].

Few studies have been conducted so far to identify the beneficial endophytic bacteria in Sri Lankan rice varieties. Therefore, the present study was carried out to isolate and identify endophytic diazotrophic bacteria with plant growth-promoting properties from roots and shoots of two Sri Lankan rice (*Oryza sativa* L.) varieties; Suwandel and Bg 358. Bacterial isolates were characterized on the basis of morphological and biochemical features. Furthermore, the effect of plant growth-promoting endophytic diazotrophic bacterial inoculation on the growth of rice seedlings was also evaluated to identify their potential to promote rice plant growth when applied to the plant.

II. MATERIALS AND METHODOLOGY

2.1 Collection of plant materials

The two rice (*Oryza Sativa* L.) varieties, Suwandel and Bg 358 were randomly collected in their heading stage from the soil pots in a greenhouse of Department of Botany, University of Kelaniya, Sri Lanka. The stock seeds of rice were provided by the Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka.

2.2 Isolation of endophytic diazotrophic bacteria

The endophytic bacteria from roots and shoots of two rice (*Oryza Sativa* L.) cultivars were isolated using nitrogen-free semi-solid media (Nfb). Plant tissue samples (0.5 g) were washed with running tap water and cut into 2-3 cm long sections with a sterile blade. Plant tissues were surface sterilized with 70% ethanol for 3min, subsequently with fresh NaOCl (v/v) for 5 min and finally with 70% ethanol for 30 s. Tissue samples were then washed five times with sterile distilled water. To confirm the success of the sterilization process, aliquots of 0.1 ml of distilled water from the final rinse were plated on nutrient agar (NA) plates and examined for contaminants after incubation at room temperature (29ºC) for 3 days. No contaminants were found indicating that the surface sterilization procedure was effective. Surface sterilized roots and shoots samples were homogenized separately in a sterilized mortar with 9.5 ml of 4% sucrose solution. Serial dilutions (10^{-4}, 10^{-6} and 10^{-8}) were prepared using a homogenous solution of tissue samples. Aliquots of 0.1 ml of these serial dilutions were used to inoculate into vials containing 5ml of the semi-solid N-free medium (Nfb) (Malic acid (5g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1g),$CaCl_2$ (0.02 g), 0.5% bromothymol blue in 0.2 N KOH (2ml), 1.64% Fe-EDTA solution(4 ml),vitamin solution (1 ml), micronutrient solution (2 ml), agar (1.9g) per liter, pH 6.8. The vitamin solution contained in 100 ml: biotin (10mg), pyridoxol-HCl (20mg) and the micronutrient solution consist of: $CuSO_4$ (0.4 g); $ZnSO_4.7H_2O$ (0.12 g); H_2BO_3 (1.4 g); $Na_2MoO_4.2H_2O$ (1.0 g) and $MnSO_4.H_2O$ (1.5 g) per liter) [21] and incubated for 4 to 6 days at 30 ºC. The growth of bacteria was observed by pellicle formation near the surface of the tube (qualitative evidence of bacterial ability to fix atmospheric nitrogen). The vials showing bacterial growth were used to inoculate plates of the same solid media with additional 20 mg l^{-1} of yeast extract [21]. After incubation at 30 ºC for 5 to 6 days, the single well separated and morphologically different bacterial colonies growing on the plates were randomly picked and transferred into fresh N-free solid media by streak plate method for purification. The transfer procedure mentioned above was carried out 3–4 times to isolate single colonies. The purified colonies were transferred into agar slants and stored in refrigerator at 4 ºC for further studies. For long-term storage at -20 ºC, the isolates were preserved in the 20% glycerol.

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2.3 Screening for plant growth-promoting characteristics of isolated bacteria

2.3.1 Quantitative analysis of Indole-3-Acetic Acid (IAA) production

The quantification of IAA production by bacterial isolates was determined by Salkowski's colorimetric test. The bacterial isolates were inoculated into the tubes containing Nfb medium supplemented with 0.5 gl^{-1} tryptophan. The bacterial cultures were harvested after 48 h of incubation at 30°C and centrifuged at 15000 rpm for 15 min. The supernatant (2 ml) was mixed with 3 ml of Salkowski's reagent and incubated 30 min in darkness at room temperature (29°C) for color development. IAA production was observed as the development of the light red color and the absorbance was measured at 530 nm using a spectrophotometer [30]. The concentration of IAA produced by each bacterial isolate was determined by using a standard curve prepared from commercial IAA solutions (0, 10, 20, 30,40,50,60,70,80,90 and 100 ppm). The test was repeated twice with three replicates for each and mean was calculated.

2.3.2 Plate assay for mineral phosphate-solubilizing ability

The ability of bacterial isolates to solubilize inorganic phosphate was tested on Pikovskaya's agar medium (Glucose (10 g), $Ca_3(PO_4)_2$ (5 g), $(NH_4)_2SO_4$ (0.5 g) and yeast extract(0.5 g), $MgSO_4.7H_20$ (0.2 g), NaCl (0.1 g), traces of $FeSO_4$(per liter), pH 7). Bacterial isolates were spot inoculated into the medium containing plates and incubated for 7 days at 30 °C [33]. The presence of clear zone around the bacterial colony was considered as an indicator for positive mineral phosphate solubilization.

2.4 Morphological and Biochemical characterization of bacteria

Endophytic diazotrophic bacteria with plant growth-promoting ability were identified according to Bergey's Manual of Systematic Bacteriology [2, 29]. The cultural characteristics of bacteria were determined on nutrient agar (NA) plates after 48 h of incubation. The cell shape was determined by microscope following gram staining. The Gram reaction was performed using 3% KOH [40]. Motility was examined on cultures grown in semi-solid nutrient agar (NA) medium. Bacterial isolates grown on nutrient agar (NA) were tested for the presence of catalase and oxidase within 24 h. Methyl Red (MR) and Voges-Proskauer (VP) tests were performed by inoculating MR-VP broth in a screw-capped tube, incubating for 24-48 h at 37°C and then 5 drops of methyl red solution was added for MR test and 5% (w/v) α–naphthol in absolute ethanol and 40% (w/v) KOH was added for VP test. The red color formation was taken as positive for MR and VP test. Lysine decarboxylase test was performed by inoculating lysine decarboxylase broth with bacterial cultures and incubated at 37°C for 24 h. The bacterial isolates that convert the color of the medium from purple to yellow after 24 h of incubation and ability to change back to purple from yellow after next 24 h of incubation were recorded as positive for the test. Starch hydrolysis was tested by inoculating bacterial cultures on starch agar plates. After incubation at 37°C for 2-4 days, plates were flooded with Gram's iodine solution. Bacterial isolates with the ability to produce clear zones around the colonies were taken as positive for the test. Gelatin hydrolysis was tested by inoculating Frazier's agar plates with bacterial cultures. After incubation at 37°C for 2 to 14 days plates, were flooded with 1% HCl solution. Gelatin hydrolyzing bacteria were identified by the clear zones around their colonies while non-hydrolyzing bacteria formed an opaque precipitate with HCl reagent. For citrate test, Simmons' citrate agar slants were inoculated with bacteria and incubated at 37°C for overnight. The bacterial isolates positive for the test. The bacterial isolates that positive for the test were changed the color of the media from green to blue. H_2S test was performed by inoculating tubes containing Kliger's iron agar with bacterial cultures by stabbing with inoculating needle and incubated at 37°C for 48 h. After incubation positive result was indicated by black precipitate formed at the lower portion of the tube. Nitrate reduction test was carried out by inoculating screwed capped tubes containing peptone water with 0.02% KNO_3 and 1ml of Sulfanilic acid. After incubation for 2-4 days at 37°C, 1 ml of Napthyamine was added. A red color developing within 5 min was taken as the positive reaction, while the absence of color indicated negative results. Negative results were confirmed using Zn dust. For the urease test, urea broth was inoculated and incubated at 37 °C for 48 h. The color change from yellow to pink was considered positive for the test. Acid production from D-glucose and lactose was tested by inoculating peptone broth containing tubes with bacterial cultures and incubated for 2-5 days at 37°C. The change of color from red to yellow was taken as positive for the test. Based on the results of specific biochemical tests all bacterial isolates were partially identified.

2.5 Preparation of bacterial culture for seed inoculation

Seven endophytic diazotrophic bacteria (IN003, IN006, IN007, IN008, IN009, IN015, and IN018) with plant growth-promoting ability were used to test their effect of inoculation on different growth parameters of rice. The Suwandel rice seeds were surface sterilized with 70% (v/v) ethanol for 30 sec and shaken in 1% (w/v) NaOCl

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solution for 5 min. Seeds were then washed three times with sterilized distilled water with shaking (15 min each). To ensure the sterilization efficiency, seeds were subjected to sterility check on nutrient agar media. For preparing bacterial cultures, each bacterial strain (IN003, IN006, IN007, IN008, IN009, IN015, and IN018) was cultured in 100 ml conical flasks containing 50 ml of nitrogen-free broth with 0.1% NH_4Cl on a rotary shaker at 100 rpm for 38 h at 32°C. Bacterial cells were collected via centrifugation at 8000 rpm for 10 min and the bacterial cell pellets were suspended in 10 ml of 66 mM phosphate buffer (pH 7.0). The surface sterilized seeds of rice cultivars were inoculated by soaking seeds in the respective bacterial culture for 6 h at 32°C. The treated seeds were spread on a sterilized petri dish and air dried in a sterilized environment for overnight at room temperature (29°C). The bacterial cell culture was standardized to 10^7CFU/ml via serial dilution before inoculation.

2.6 Screening the effect of bacterial seed treatment on growth of rice seedlings

To study the effect of rice seedling growth promotion by plant growth-promoting endophytic diazotrophic bacteria, 40 rice seeds inoculated with respective bacterial culture were placed in 7 sterilized transparent plastic containers (10 X 12 cm) containing moistened paper towel. Then the containers were sealed and incubated at room temperature (29°C). As a control treatment seeds inoculated with un-inoculated sterilized broth were established. Seeds were sprayed with distilled water to maintain moisture required for the germination. After 3 days, 40 germinated seedlings (inoculated) were transferred into hydroponic seed tray containing 800 ml full strength Hogland's nutrient solution (without N and P) along with 1 g of tricalcium phosphate while 40 germinated seedlings (un-inoculated) were transferred into hydroponic seed tray containing 800 ml of full strength Hogland's solution (with N and P) [23]. After 12 days, 10 rice plants were taken randomly and shoot lengths and root lengths were measured. The shoots and roots were separated, and fresh weights were measured. For determining dry weight shoots and roots of rice plants were kept in an oven at 60°C for 72 h to obtain a constant weight. The experiment was planned as a completely randomized design with 3 replications for each isolate.

III. RESULTS AND DISCUSSION

3.1 Isolation and Identification of isolated endophytic diazotrophic bacteria

Total of 15 putative endophytic diazotrophic bacterial isolates were obtained from shoots and roots of Suwandel and Bg 358 rice varieties. In this study screening for nitrogen fixing ability was done on N-free medium and all the isolates showed growth on the medium. However, diazotrophic capacity of isolated bacteria should be further confirmed by the presence of *nif H* gene or acetylene reduction assay. Seven diazotrophic bacterial isolates (IN003, IN006, IN007, IN008, IN009, IN015, and IN018) were selected for further studies based on their ability to produce IAA and phosphate solubilization. Among this 7 bacterial isolates, 4 isolates (IN003, IN006, INS008, and IN015) were obtained from Suwandel and 3isolates (IN007, IN009, and IN018) were obtained from Bg 358. The colony morphology of seven bacterial isolates was tested on nutrient agar (NA) medium and characteristics including shape, color, elevation, margin, and texture were studied (Table 1). Most of the colonies were circular to irregular and whitish or cream in color while yellow color is also observed. The margins of the colonies of isolated bacteria were found to be entire, undulate and irregular. The surface characteristics of bacterial isolates were found to be smooth and glistening. The cells were rod-shaped. Out of seven bacterial isolates, 4 were gram-negative while 3 isolates were gram-positive in reaction. All the isolates were motile except IN008 and IN018.Bacterial isolates were identified via different biochemical tests (Table 2) according to Bergey's Manual of Systematic Bacteriology [2, 29]. On the basis of morphological, and biochemical characteristics, 07 bacterial isolates were identified that belong to four different genera; *Bacillus* (IN003, IN006, and IN007), *Klebsiella* (IN008 and IN018), *Pantoea* (IN009), and *Enterobacter* (IN015). These bacteria were commonly isolated from various tissues of the rice plant. However, identification of bacterial strain to species level requires further molecular characterization. Therefore, the molecular characterization targeting 16S rDNA region needed to be done to confirm these results.

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Table.1.: Morphological characteristics of 07 plant growth-promoting endophytic diazotrophic bacteria on nitrogen-free(Nfb) media

Bacterial Strains	Colony characteristics on Nfb media						Cell shape	Gram's nature
	Size(mm)	Color	Shape	Elevation	Margin	Texture		
IN003	1-3	Cream	Circular	Flat	Entire	Smooth	Rods	+
IN006	2-4	Cream	Circular	Raised	Undulate	Smooth	Rods	+
IN007	1-2	White	Irregular	Raised	Undulate	Smooth	Rods	+
IN008	2-3	Light cream	Irregular	Convex	Entire	Smooth	Straight rods	-
IN009	2-3	Pale yellow	Circular	Convex	Entire	Smooth	Straight rods	-
IN015	2-3	White	Circular	Flat	Irregular	Glistening	Straight rods	-
IN018	3-4	Cream	Circular	Slightly raised	Entire	Glistening	Straight rods	-

(-): Positive; (+): Negative

Table 2: Biochemical characteristics of 07 plant growth-promoting endophytic diazotrophic bacteria

Bacterial strain	Motility	Catalase test	Oxidase test	MR Test	VP test	Lysine Decarboxylase test	Starch hydrolysis	Gelatin hydrolysis	Simmons Citrate Utilization	H_2S production	Nitrate Reduction	Urease Test	Acid from D- glucose	Acid from D-Lactose	Identified bacterial species
INS003	+	+	+	-	+	-	+	-	+	+	+	-	+	-	*Bacillus sp.*
INS006	+	+	-	+	-	-	+	-	+	-	-	-	+	-	*Bacillus megaterium*
INS007	+	+	-	+	-	-	+	+	-	-	-	-	+	-	*Bacillus pumilus*
INS008	-	+	-	-	-	+	-	-	+	-	+	+	-	-	*Klebsiella sp.*
INS009	+	+	-	-	-	-	-	+	+	-	+	-	-	+	*Pantoea sp.*
INS015	+	-	+	-	+	-	-	+	-	-	+	-	+	-	*Enterobacter sp.*
INS018	-	+	-	-	+	+	-	+	+	-	+	+	+	-	*Klebsiella sp.*

(-): Positive reaction; (+): Negative reaction

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3.2 Characterization of plant growth-promoting ability of putative endophytic diazotrophic bacteria

3.2.1 Production of IAA

All 7 diazotrophic endophytic bacterial isolates produced reddish pink color after addition of Salkowski's reagent indicating their ability to produce IAA through tryptophan. In the presence of tryptophan, isolated bacteria produce IAA with concentration ranging from 7.1 µg $ml^{-1}$to 30.9 µgml^{-1}(Table 3). The highest amount of IAA was produced by isolate IN015 (*Enterobacter* sp.) while lowest IAA production was recorded by isolate IN009 (*Pantoea* sp.). IAA produced by these bacteria has a favorable effect on plant growth promotion. The results of this study indicated that the amount of IAA produced by different bacterial species can be varied. Similar results had been reported by the [23]. This can be due to the several factors that affect the level of IAA production in bacteria such as the location of auxin biosynthesis genes in the genome, IAA biosynthetic pathways, culture conditions, growth stage of the bacteria and substrate availability [28].

Table.3: Concentration of IAA produced by 7 endophytic diazotrophic bacterial isolates

Bacterial strain	Concentration of [a] IAA(µg/ml)
[b]*Serratia marcescens*	20.2±0.78*
IN003	10.8±0.36
IN006	9.8±0.42
IN007	20.8±0.92
IN008	15.2±0.91
IN009	7.1±0.26
IN015	30.9±0.33
IN018	12.2±0.18

[a]IAA production was estimated on Nfb medium supplemented with tryptophan with absorbance at 540 nm.

[b]*Serratia marcescens* was used as positive control for IAA producer.

* Values are the Mean ± SE The experiment was repeated twice with three replicates for each isolate

3.2.2 Phosphate solubilization ability

Phosphate is one of the most important nutrients required for rice plant growth and development. In the soil, phosphate usually forms insoluble complexes, unavailable to plant. Therefore, the efficiency of uptake and use of phosphorous after fertilizer application is low [33]. Phosphate-solubilizing bacteria can increase the phosphorous availability to the plant by converting inorganic phosphorous into more available form [26]. These bacteria solubilize inorganic phosphate by producing organic acids which decrease the pH of the culture media [30, 34]. Among 7 bacterial isolates tested, only 5 bacterial isolates (IN006, IN007, IN008, IN015, and IN018) were showed inorganic phosphate-solubilizing ability by forming halo zones on Pikovskaya's agar plates after 7 days of incubation (Table 4). These bacterial isolates were identified as *Bacillus megaterium* (IN006), *Bacillus pumilus* (IN007) and genus *Klebsiella* (IN018 and IN008) and *Enterobacter* (IN015). Previous studies also indicated the phosphate-solubilizing ability of bacteria belong to genera *Bacillus*, *Enterobacter*, and *Klebsiella* [7, 10, 14, 16]. Moreover, all these five isolates which solubilized phosphate were also IAA producers.

Table.4: Phosphate solubilizing ability of 07 endophytic diazotrophic bacterial isolates

Bacterial Strain	Phosphate Solubilization
Pseudomonas aeruginosa[*]	+
IN003	-
IN006	+
IN007	+
IN008	+
IN009	-
IN015	+
IN018	+

The bacterial strains able to solubilize inorganic phosphate on Pikovskaya's agar (+); the bacterial strains unable to solubilize inorganic phosphate on Pikovskaya's agar **Pseudomonas aeruginosa* was used as a positive control

3.3 Plant growth-promoting parameters

Most of the bacterial isolates have a significant effect on the shoot length, root length, shoot fresh weight, shoot dry weight and root fresh weight of the rice seedlings compared to the un-inoculated control (Table 4; Fig 1 A, B). All the bacterial isolates significantly ($p \leq 0.05$) enhanced the shoot length of the rice seedlings compared to untreated control and maximum shoot length (23.33±0.34 cm) was observed in seedlings treated with *Enterobacter* sp. (IN015). However, inoculation with *Bacillus megaterium* (IN006) significantly ($p \leq 0.05$) reduced the root length compared to un-inoculated control whereas inoculation with *Enterobacter* sp. (IN015) resulted in longest root length (8.76±0.05cm) (Table 4; Fig 1 A). Inoculation with all bacterial isolates enhanced shoot fresh weight and dry weight, but there was no significant difference in the shoot dry weights between

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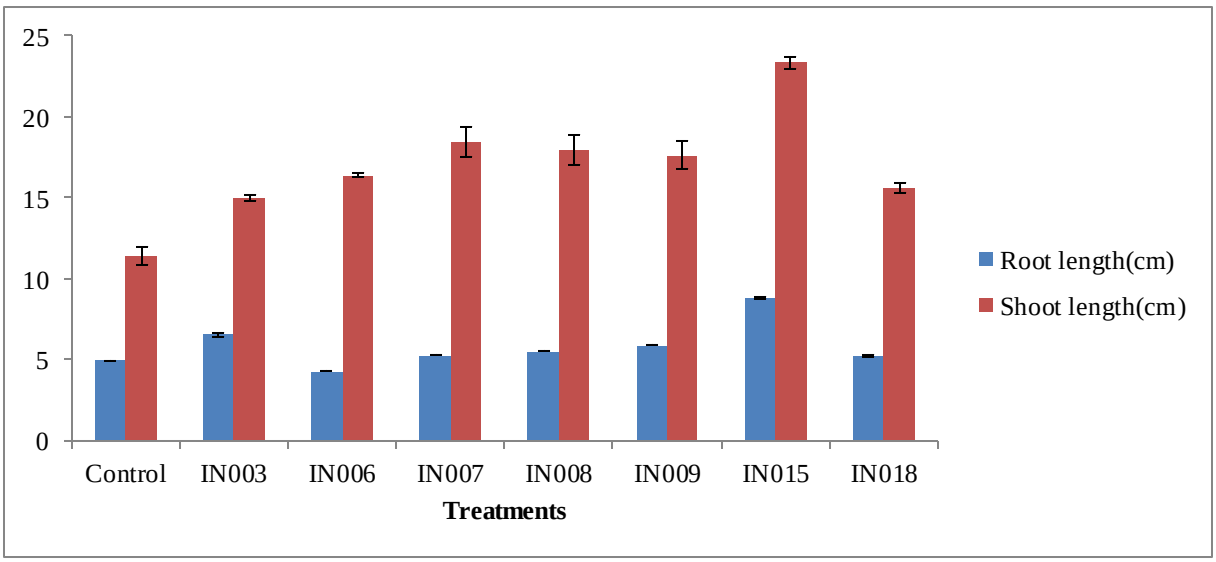
the plants treated with bacterial isolates except *Enterobacter* sp.(IN015) (Table 4; Fig 1 B). Treatment with all bacterial isolates increased the root fresh weight and dry weight of inoculated plants and significantly ($p \leq 0.05$) high root fresh weight and dry weight were recorded in plants inoculated with *Enterobacter* sp. (IN015) compared to the control and other bacterial isolates. The results of this study indicated that among all the other bacterial isolates, *Enterobacter* sp. (IN015) was more effective in rice plant growth promotion. Previous studies have also reported that certain *Enterobacter* sp. as effective plant growth promoters since they possess multiple plant growth-promoting activities [3, 20, 30]. Rice seedlings growth promotion could be attributed to the multiple plant growth-promoting properties of inoculated bacteria such as IAA production, nitrogen fixation, and phosphate solubilization. In the hydroponic test system, inoculated plants were not supplied with N or soluble phosphorous which are major nutrients required for rice plant growth. Therefore, N and P requirement of the plant could be supplied only by the inoculated bacteria with their ability to fix nitrogen and solubilize inorganic phosphate. All seven bacterial isolates used in the plant inoculation experiment have the ability to produce IAA which is an important mechanism of plant growth promotion. IAA promotes the growth of the plant by increasing the number and size of adventitious and lateral roots and thereby facilitates the nutrient uptake by the plant [22].

Table 5: Effect of bacterial isolates on growth parameters of rice seedlings

Treatments	Shoot Length (cm)	Root Length (cm)	Shoot fresh weight (mg/plant)	Root fresh weight (mg/plant)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)
Control	11.37±0.52^{d}	4.92±0.03^{f}	0.34±0.01 ^{f}	0.27±0.02 ^{d}	0.20±0.01^{c}	0.14±0.03 ^{c}
IN003	14.97±0.15^{c}	6.51±0.10^{b}	0.58±0.02 ^{e}	0.33±0.01^{cd}	0.49±0.03^{b}	0.19±0.01^{bc}
IN006	16.40±0.15^{bc}	4.26±0.04^{g}	0.66±0.01 ^{d}	0.39±0.02^{bc}	0.47±0.02^{b}	0.18±0.01^{bc}
IN007	18.43±0.93^{bc}	5.25±0.04^{e}	0.79±0.01 ^{b}	0.40±0.01^{bc}	0.59±0.05^{b}	0.22±0.01^{bc}
IN008	17.93±0.95^{bc}	5.50±0.04^{d}	0.65±0.01 ^{d}	0.42±0.02^{bc}	0.54±0.03^{b}	0.22±0.03^{bc}
IN009	17.63±0.87^{bc}	5.87±0.02^{c}	0.76±0.01^{bc}	0.39±0.02^{bc}	0.59±0.01^{b}	0.18±0.01^{bc}
IN015	23.33±0.34^{a}	8.76±0.05^{a}	0.99±0,02 ^{a}	0.57±0.04 ^{a}	0.81±0.01^{a}	0.37±0.03^{a}
IN018	14.90±0.35^{bc}	5.20±0.04^{e}	0.72±0.01 ^{c}	0.45±0.01 ^{b}	0.62±0.04^{b}	0.24±0.02^{b}

Values followed by the same letter are not significantly different as determined by Tukey's mean comparison test ($p \leq 0.05$). The statistics were performed separately for the data in each column (means of three replicates, ± standard error).

A



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B

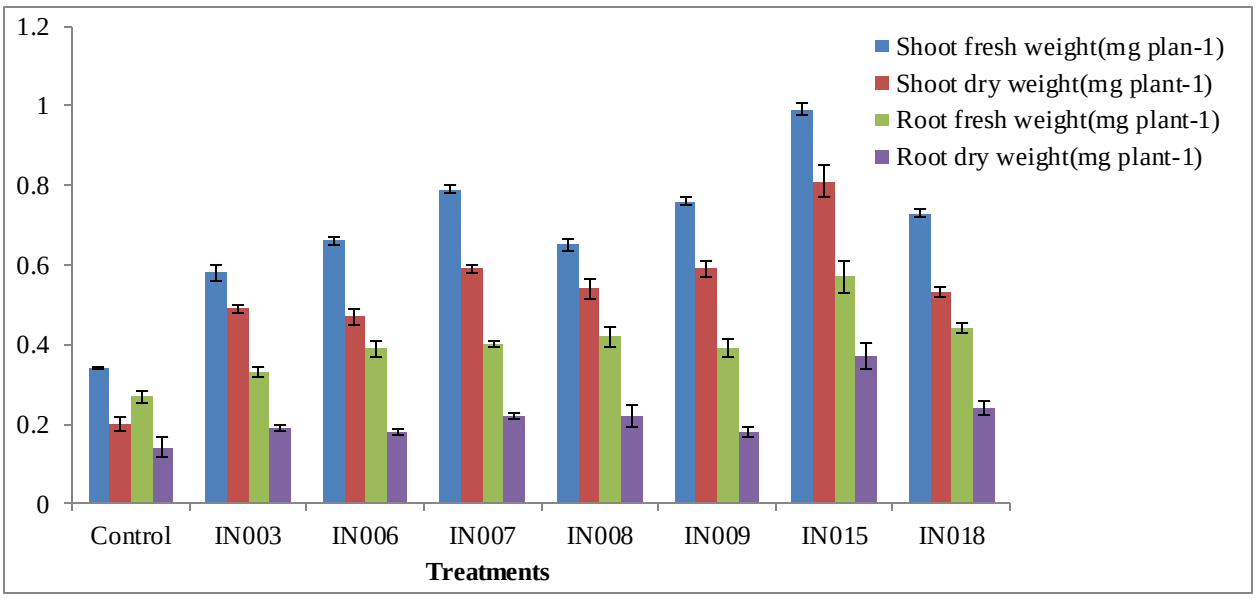

Fig 1: Effect of Plant growth-promoting endophytic diazotrophic bacteria inoculation on shoot and root length (A) and shoot and root fresh weight and dry weight (B) of rice seedling (Suwandel variety) growing in hydroponics. Error bars are SE from three replicates per same treatment.

IV. CONCLUSION

The current study indicated that endophytic bacteria isolated from the shoots and roots of Suwandel and Bg 358 has beneficial effects on plant growth promotion through IAA production, phosphate solubilization, and nitrogen fixation. Seven bacterial isolates belong to different species produced IAA and fix nitrogen in the nitrogen-free media while 5 out of 7 bacterial isolates showed phosphate solubilizing activity. Rapid screening of their ability to promote rice plant growth was carried out using a hydroponic system and the results of this study clearly demonstrated the positive effect of these bacterial inoculations on rice plant growth. However, plant inoculation experiment was carried out under laboratory conditions. Therefore, results obtained in this study may not reproduce exactly under the field conditions. Further studies under field conditions are recommended to identify the real potential of these bacteria to promote rice plant growth. Multiple plant growth-promoting abilities and positive effect on plant growth parameters in the plant inoculation experiment suggest that the bacteria isolated in this study have the potential to develop as biofertilizer to promote rice plant growth. *Enterobacter* sp. (IN015) showed most effective in rice plant growth promotion compared to other bacterial isolates. However, more research is needed to understand the interaction between endophytic bacteria and rice plant to fully exploit their potential as biofertilizers.

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REFERENCES

[1] Carrillo-Castaneda G., Munoz J.J., Peralta-Videa J.R., Gomez E. and Gardea-Torresdey J.L. (2005). Modulation of uptake and translocation of iron and copper from root to shoot in common bean by siderophore-producing microorganisms. *Journal of Plant Nutrition*. 28, 1853–1865.Retrieved from https://www.tandfonline.com/doi/full/10.1080/01904160500251340

[2] D.J. Brenner, N.R. Krieg and J.T. Sraley, Bergey's Manual of Systematic Bacteriology.2nd ed. vol 2, Springer; New York, pp 665-719

[3] Deepa C.K., Dastager S.G. and Pande A. (2010). Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea *Vignaunguiculata* L. Walp. seedling growth. *World Journal of Microbiology and Biotechnology*. 26,1233-1240. Retrieved from https://link.springer.com/article/10.1007/s11274-009-0293-y

[4] Dimkpa C., Merten D., Svato˘s A., B¨uchel G. and Kothe E. (2009). Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower (*Helianthus annuus*), respectively. *Journal of Applied Microbiology*. 107, 1687–96. Retrieved from

https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2672.2009.04355.x

[5] Elbeltagy A. K., Nishioka K., Suzuki H., Sato T., Sato Y., Morisaki H., Mitsui H. and Minamisawa K. (2000). Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Science Plant Nutrition*. 46,617-629. Retrieved from http://dx.doi.org/10.1080/00380768.2000.10409127

[6] Elbeltagy A., Nishioka K., Sato T., Suzuki H., Ye B., Hamada T., Isawa T., Mitsui H. and Minamisawa K. (2001). Endophytic colonization and in planta nitrogen fixation by a *Herbaspirillum* sp. isolated from wild rice species. *Applied Environmental Microbiology*. 67,5285-5293. Retrieved from https://aem.asm.org/content/67/11/5285

[7] El-komy H. M. A. (2005). Coimmobilization of *Azospirillumlipoferum* and *Bacillus megaterium* for Successful Phosphorus and Nitrogen Nutrition of Wheat Plants. *Food Technology and Biotechnol*ogy.43, 19-27. Retrieved from https://hrcak.srce.hr/110336

[8] Engelhard M., Hurek T. and Reinhold-Hurek B. (2001). Preferential occurrence of diazotrophic endophytes, *Azoarcus* spp., in wild rice species and land races of *Oryza sativa* in comparison with modern races. *Environmental Microbiology*. 2, 131-141. Retrieved from https://doi.org/10.1046/j.1462-2920.2000.00078.x

[9] Feng Y., Shen D. and Song W. (2006). Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. *Journal of Applied Microbiology*. 100,938-945.Retrieved from https://doi.org/10.1111/j.1365-2672.2006.02843.x

[10] Frey-Klett P., Chavatte M., Clausse M. L., Courrier S., Le R. C., Raaijmakers J., Martinotti M.G., Pierrat J.C. and Garbaye J. (2005). Ecto-mycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent *pseudomonas*. *New Phytologist*.165,317–28. Retrieved from https://doi.org/10.1111/j.1469-8137.2004.01212.x

[11] Gaspar T., Kevers C., Penel C., Greppin H., Reid D.M. and Thorpe T.A. (1996). Plant hormones and plant growth regulators in plant tissue culture. *In vitro Plant Cell Dev Biol*. 32,272–89.

[12] Glick B., Patten C., Holguin G. and Penrose D. (1999). Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial Col. London ,p 267

[13] Gyaneshwar P., James E.K., Mathan N., Reddy P.M., Reinhold-Hurek B. and Ladha J. (2001). Endophytic colonization of rice by a diazotrophic strain *Serratia marcescens*. *Journal of Bacteriology*. 183, 2634-2645. Retrieved from https://jb.asm.org/content/183/8/2634

[14] Hameeda B., Harini G., Rupela O.P., Wani S.P., Reddy G. (2008). Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. *Microbiological Research*. 163,234–42. Retrieved from https://www.sciencedirect.com/science/article/pii/S0944501306000589

[15] Hongrittipun P., Youpensuk S. and Rerkasem B. (2014). Screening of nitrogen fixing endophytic bacteria in *Oryza sativa* L. *Journal of Agricultural Science*. 6(6), 1020-1032. Retrieved from http://dx.doi.org/10.5539/jas.v6n6p66

[16] Ji S.H., Gururani M. A. and Chun S. (2014). Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiological research*. 169,83-98.Retrieved from https://www.sciencedirect.com/science/article/pii/S094450131300089X

[17] Joe M.M., Devaraj S., Benson A. and Sa T. (2016). Isolation of phosphate solubilizing endophytic bacteria from *Phyllanthusamarus* Schum & Thonn: Evaluation of plant growth promotion and antioxidant activity under salt stress. *Journal of Applied Research and Medicinal and Aromatic Plants*. 3(2), 71-77. Retrieved from https://www.sciencedirect.com/science/article/pii/S2214786116300080

[18] J.P.W. Young, Molecular phylogeny of rhizobia and their relatives, vol. 17. London, 1993, pp. 87-592.

[19] Kandel S.L., Joubert P.M. and Doty S.L. (2017). Bacterial Endophyte Colonization and Distribution within Plants. *Microorganisms*. 5(4), 77. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5748586/

[20] Kampfer P., Ruppel S. and Remus R. (2005). *Enterobacter radicincitans* sp. nov., a plant growth promoting species of the family *Enterobacteriaceae*. *Systematic Applied Microbiol*ogy. 28,213-221. Retrieved from http://europepmc.org/abstract/med/15900968

[21] Kirchhof G., Reis V.M., Baldani J. I., Eckert B., Dobereiner J. and Hartmann A. (1997). Occurrence, physiological and molecular analysis of endophytic diazotrophic bacteria in gramineous energy plants. *Plant and Soil*.194, 47. Retrieved from https://link.springer.com/article/10.1023/A:1004217904546

[22] Lins M.R.D.R., Fontes, J.M., De-vasconcelos N.M., Santos D.M.S., Ferreira O. E., De-Azevedo J.L., De-Araujo J.M. and Lima G.M.S.(2014). Plant growth promoting potential of endophytic bacteria from cashew leaves. *African journal of Biotechnology*.

13(33), 3360-3365. Retrieved from https://www.ajol.info/index.php/ajb/article/view/122003

[23] Majeed A., Abbasi M. K., Hameed S., Imran A. and Rahim N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology*.6, 1-8. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25852661

[24] Manjula K., Kishore G.K. and Podile A.R. (2004).Whole cells of *Bacillussubtilis* AF1 proved more effective than cell-free and chitinase-based formulations in biological control of citrus fruit rot and ground nut rust. *Canadian Journal of Microbiology*. 50,37–744. Retrieved from https://www.ingentaconnect.com/content/cndscipub/cjm/2004/00000050/00000009/art00011

[25] Mano H., Tanaka F., Nakamura C., Kaga H. and Morisaki H. (2007). Culturable endophytic bacterial flora of the maturing leaves and roots of rice plants (*Oryza sativa*) cultivated in a paddy field. *Microbes and Environments*. 22,175-185. Retrieved from https://www.jstage.jst.go.jp/article/jsme2/22/2/22_2_175/_article

[26] Mano H. and Morisaki H. (2008). Endophytic bacteria in the rice plant. *Microbes and Environment*. 2,109-117. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21558696

[27] Mehta S. and Nautiyal SC. (2001). An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Current Microbiology*. 43,51–56. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11375664

[28] Montanez A., Blanco A.R., Barlocco C., Beracochea M. and Sicardi M. (2012). Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects *in vitro*. *Applied soil ecology*. 58,21-28. Retrieved from https://www.sciencedirect.com/science/article/pii/S0929139312000418

[29] P.D.Vos, G.M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F.A.Rainey, K. Schleifer and W.B. Whitman, Bergey's Manual of Systematic Bacteriology, 2nd ed.,vol 3. Springer:New York, pp. 20-35

[30] Patten C. L. and Glick B. R. (2002). Role of *Pseudomonas putida*indole-acetic acid in root development of the host plant system. *Applied Environmental Microbiology*. 68, 3795-3801. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC124051/

[31] Puente M.E., Bashan Y., Li C.Y. and Lebsky V.K. (2004). Microbial populations and activities in the rhizoplane of rock-weathering desert plants.I.Root colonization and weathering of igneous rocks. Plant Biology 6, 629–642. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15375735

[32] Ramesh A., Sharma S.K., Sharma M.P., Yadav N. and Joshi O.P. (2014). Plant growth-promoting traits in *Enterobacter cloacae* subsp.*dissolvens* MDSR9 isolated from soybean rhizosphere and its impact on growth and nutrition of soybean and wheat upon inoculation. *Agricultural Research*. 3(1),53-66. Retrieved from https://link.springer.com/article/10.1007/s40003-014-0100-3

[33] Rodriguez H. and Fraga R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*. 17,319-339. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14538133

[34] Ryan R.P., Germaine K., Franks A., Ryan J.D. and Dowling N.D. (2008). Bacterial endophytes: recent developments and applications. *Microbiology Letter*. 278,1-9. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18034833

[35] Sahin F., Cakmakci R. and Kanta F. (2004).Sugar beet and barley yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. *Plant Soil*. 265,123–129. Retrieved from https://link.springer.com/article/10.1007/s11104-005-0334-8

[36] Saikia S.P. and Jain V. (2007). Biological nitrogen fixation with legumes: an achievable target or a dogma. *Current Science*. 92(3), 1204-1218. Retrieved from https://pdfs.semanticscholar.org/8bcd/db7c0a55fad679ccdae2f7135aaf27d7a230.pdf

[37] Santoyo G., Moreno-Hagelsieb G., Orozco-Mosqueda M. C. and Glick B.R. (2016). Plant growth-promoting bacterial endophytes. *Microbiology Research*. 183, 92–99. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26805622

[38] Shi Y., Lou K. and Li C. (2009). Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. *Biology and Fertility of Soils*. 45(6), 645–653. Retrieved from https://link.springer.com/article/10.1007/s00374-009-0376-9

[39] Spaepen S., Vanderleyden J. and Remas R. (2007). Indole-3- acetic acid in microbial and microorganisms plant signaling. *FEMS Microbiology Reviews*. 31,425-448. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17509086

[40] Suslow T. V., Schroth M. N. and Isaka M. (1982).Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without attaining. *Phytopathology*. 72, 917-918. Retrieved from

http://dx.doi.org/10.22161/ijeab/3.4.51 *ISSN: 2456-1878*

https://www.apsnet.org/publications/phytopathology/backissues/Documents/1982Abstracts/Phyto72_917.html

[41] Verma S.C., Ladha J.K. and Tripathi A.K. (2001). Evaluation of plant growth promoting and colonization ability of endophyticdiazotrophs from deep water rice. *Journal of biotechnology*. 91,127-141. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11566385

[42] Zhang Z. and Yuen G.Y.(2000).The role of chitinase production by *Stenotrophomonas maltophilia* strain C3 in biological control of *Bipolariss orokiniana*. *Phytopathology* 90, 384–389. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18944588

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Relevance of Industrial Wastes from *Jatropha curcas* L. Seed in Agricultural Biotechnology

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***Abstract*—** *This paper investigated the usefulness of seed cake and husk as industrial wastesgenerated from Jatrophacurcas in biotechnology. Seeds were dehulled, milled and processed for biodiesel production at department of Chemical and Biological Engineering, University at Buffalo, New York, USA.Proximate analysis of the wastes generated (seed husk and cake) was carried out the National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria. All concentrations were determined using the Microwave Plasma Atomic Emission Spectrophotometer Agilent 4200 (MP-AES) model. Statistical analysis was done on the Minitab software 16.The cake contained 35% moisture, 17% ash, 40% lipid, 70% protein and 78% carbohydrate.However, fibre content in husk (27%) was higher than in cake (16%). The husk also contained fairly high amount of carbohydrate (47.8%).Phosphorus was high in both husk and cake (17.1% and 22.3% respectively). The cake contained higher quantities of micronutrients than the husk with significant differences (t=2.243, p=0.05). The seed cake and husks are rich organic sources of manure as they contain basic nutrients needed by plants to grow. The high amount of protein and carbohydrate makes them perfect candidates as animal feeds when detoxified. From all indicators except in fibre and potassium contents, the seed cake is better than the seed husk. The combined strength and properties of both seed husk and cake should be exploited in Agricultural biotechnology. The two wastes are relevant in both crop and animal production when used as organic manure and animal feeds respectively.*

***Keywords*— Agricultural biotechnology, Animal feeds, Industrial waste, Jatrophacurcas, Organic manure.**

I. INTRODUCTION

Jatrophacurcas L. (family Euphorbiaceae) has recently gained global attention as potential source of biodiesel production(Akogwu, 2011; Nanda *et al*., 2015). The quest for biofuel as environmentally friendly and renewable energy source is in line with UN goal on climate and the need to ensure safer biosphere that is free from pollutants generated from fossil fuel combustion(Aguoru*et al*., 2015). Many advanced countries are now cultivating useful plants in large scale for oil and biodiesel production. *Jatrophacurcas*is particularly of interest today because of its availability, adaptability, ease of cultivation, non-interference with food production and non-edibility (Ouattara*et al*., 2018). For this reason, heaps of wastes are generated from the seeds after industrial processing, especially the cake and husks. Burning the wastes would add more to the global CO_2 level resulting in enhanced greenhouse effect and climate change(Rathore *et al*., 2016). Degradation of the recalcitrant husk may take long time thus polluting the environment. Biotechnological conversion of biowastes into useful products is now a welcome development.The aim of this research was to establish the relevance of wastes generated from *Jatrophacurcas* seed during biodiesel production. The report focused on the proximate analysis and nutritional composition of the seed cake and husks and their potential applications.

II. MATERIALS AND METHODS

For the purpose oil and biodiesel production, seeds of *Jatrophacurcas* were collected from 9 Local Government Areas in Benue State. Seeds were dehulled, milled and processed at department of Chemical and Biological Engineering, University at Buffalo, New York, USA.

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Proximate analysis of the wastes generated including seed husk and cake was carried out the National Research Institute for Chemical Technology (NARICT) Zaria, Kaduna State, Nigeria. Nutritive components, macro and micro-elements were investigated following standard protocols (Adinurani *et al*., 2015; Sanchez-Arreola *et al*., 2015). All concentrations were determined in part per million (ppm) using the Microwave Plasma Atomic Emission Spectrophotometer Agilent 4200 (MP-AES) model. Statistical analysis was done on the Minitab software 16.

III. RESULTS AND DISCUSSION

Table 1presents seven nutritional composition in the industrial waste investigated in *Jatropha*seed. The cake contained 35% moisture, 17% ash, 40% lipid, 70% protein and 78% carbohydrate. These components are relatively of higher values in cake than husk. However, fibre content in husk (27%) was higher than in cake (16%). The husk also contained fairly high amount of carbohydrate (47.8%). Box plot (Figure 1) revealed significant differences in the nutritional values of the two wastes from *Jatropha* seeds (t=4.092, P=0.002).Figure 2 shows the macronutrients investigated. Phosphorus was high in both husk and cake (17.1% and 22.3% respectively). The potassium content of husk (21.2%) doubled that of cake (11%) but the latter contained more sodium (20.4%). Among the five micro-elements studied (Table 2), the iron content recorded the highest values (3.12ppm in husk, 6.66ppm in cake). Other elements >1ppm were zinc (1.98ppm in cake) and lead (1.62ppm in cake). The cake contained higher quantities of micronutrients than the husk with significant differences (t=2.243, p=0.05). Arsenic was present in equal proportion (0.36ppm). With the exception of copper present in minute quantity in seed husk (0.059 ppm), other micronutrients were present in high quantity which appeared to confer toxicity to the seed.

Table 1: Comparative Nutritional Compositions of*Jatrophacurcas*Seed Husk and Cake
Jatropha seed cake and husk(t=4.092, P=0.002)

	Moisture (%)	**Ash (%)**	**Lipid (%)**	**Fiber (%)**	**Protein (%)**	**Carbohydrate (%)**
Husk	11.8	6	3.2	27	5	47.8
Cake	35	17	40	16	70	78

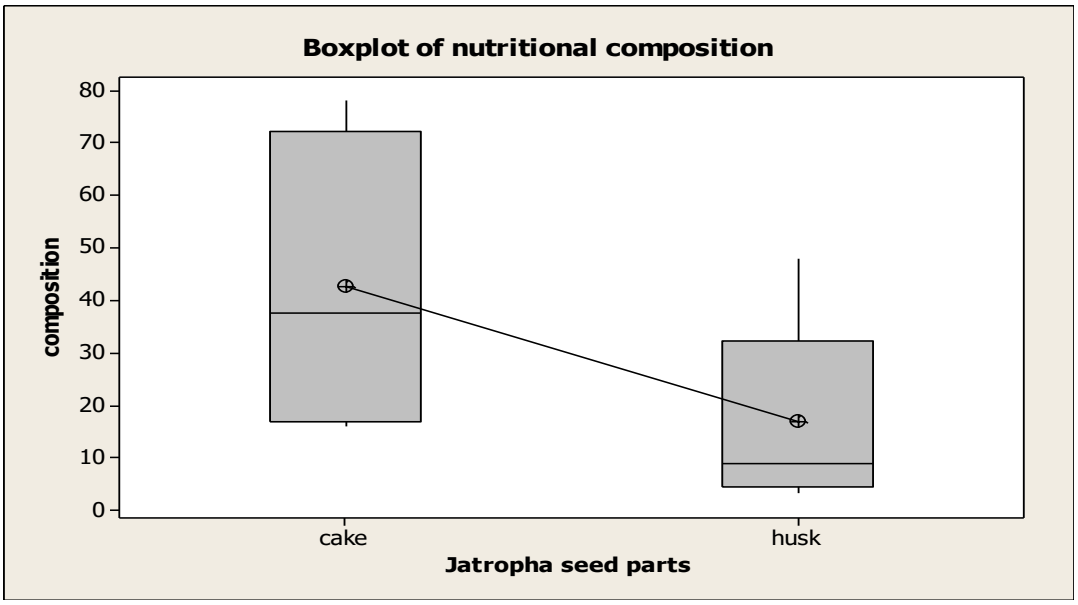

Fig.1: Box plot showing significant differences in nutrient composition in

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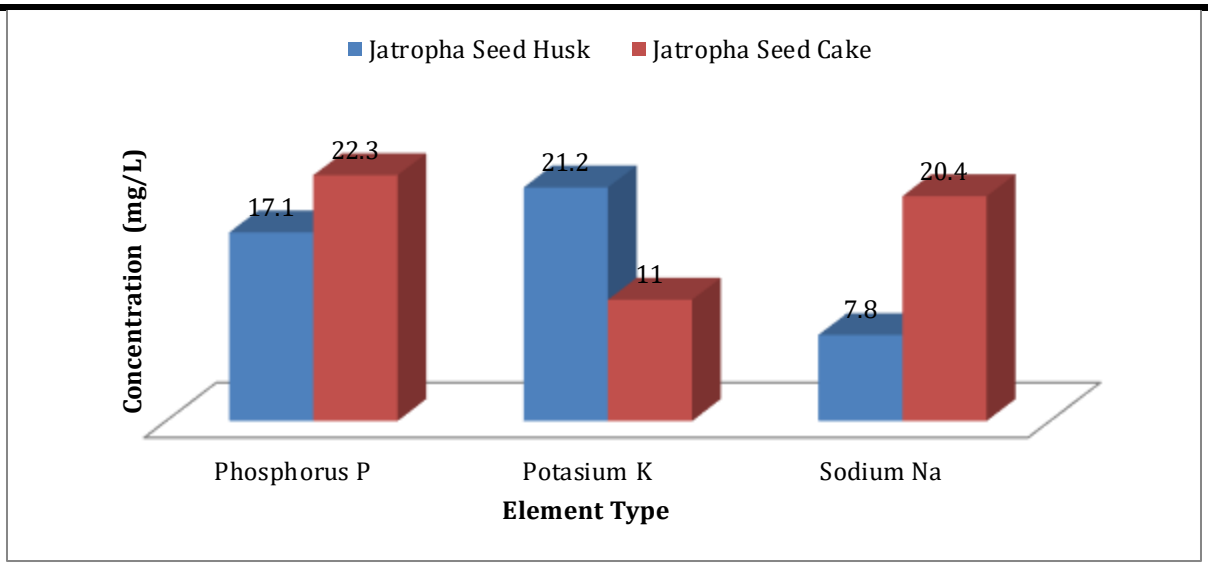

Fig.2: Macronutrients in Jatrophacurcas L Seed Husk and Cake

Table.2: Micro-elements in Jatrophacurcas Seed Husk and Cake

Element (ppm)	*Jatrophacurcas* Seed Husk	*Jatrophacurcas* Seed Cake
Zinc (Zn)	0.225	**1.980**
Arsenic (As)	0.360	0.360
Copper (Cu)	0.059	0.260
Iron (Fe)	**3.124**	**6.664**
Lead (Pb)	0.154	**1.618**

(t=2.243, p=0.05)

The seed cake after proximate analysis was found to have very high percentage of protein. This protein content obtained from the present report is far higher than similar report on *Jatropha* seed cake from India that contained 50% protein (Nepal *et al*., 2018). This difference may be attributed to the fact the seeds collected from Benue State is of higher qualitywhich maight be due to the soil fertility and higher amount of rainfall that characterize the climate in the study area (Aye and Haruna, 2018). This view agrees with similar findings that high rate of rainfall and soil fertility are required for better yield and optimum nutritive content in the plant (Matos *et al*., 2018), although Jatropha as a member of the Euphorbiaceae family can survive under low rainfall.

The outcome of this work strongly recommends *Jatropha* seed cake and husk asgood source of protein for animal feeds provided the protein can be rendered toxin free (Nepal *et al*., 2018). The present report agrees with earlier findings that *Jatropha*seed cake is an excellent source of protein but it contains some anti-nutritional factors (ANF) that can act as toxins and thus negatively affect the growth and health status of animals (Krome*et al*., 2018; Zhao *et al*., 2018). For instance, the concentrations of arsenic and lead as poisonous heavy metals are quite high in this report.Although toxin content can limit the consumption of *Jatrophacurcas* seed cake, detoxified *Jatrophacurcas* protein isolate (DJPI) may be a better option in combating malnutrition most especially in Africa (Musa, *et al*., 2018;Phulia*et al*., 2018).

Apart from high protein content, the cake also contains very high amount of carbohydrates (78%) of carbohydrate, little amount of lipids, crude fibre and ash. The quantity of iron is quite impressive. Hence, it is recommended as an excellent animal feed if detoxified. Detoxification should target the removal of poisonous lead and arsenic. The agrowaste can also serve as a source of organic manure. This is supported by the high amount of macronutrients such as phosphorus, potassium and sodium present in the seed cake and husk. The present study aligns with the work of Nepal *et a*l. (2018) where basic nutrients were reported in the seed cake and husk. In the proximate analysis and mineral composition of seeds of *Jatrophacurcas* from Pankshin Local Government Area of Plateau State (Magu*et al*. 2018), the percentages of micro, macronutrients and minerals contained in the cake and husk were smaller compared to the present findings.

From the above findings, the agricultural benefits of *Jatropha*plant are enormous. The plant can also be intercropped with many cash crops such as coffee, sugar, fruits and vegetables with the *Jatrophacurcas*offering both fertilizer and protection against livestock. *Jatrophacurcas* needs at least 60 mm of rain annually to thrive however it can survive three years of drought by dropping its leaves. *Jatropha*is excellent at preventing soil erosion, and the leaves it drops act as a wonderful soil enriching mulch (Baumer*et al*., 2018).The present report is in line with the work of Aguoruand Okibe(2015)where potential environmental pollutants were bioconverted into useful industrial products of global economic value.It also aligns with previous studies where *Jatropha* seeds had significant effects in the production of *Clariasgariepinus* fingerlings (Musa *et al*., 2008).

In conclusion, the seed cake and husks as agrowaste are rich organic sources of manure as it contains basic nutrients needed by plants to grow. The high amount of protein and carbohydrate makes the agrowaste perfect candidates as animal feeds when detoxified.From all indicators except in fibre and potassium contents, the seed cake is better than the seed husk. Thecombined strength and properties of both seed husk and cake as highlighted above should be exploited in Agricultural biotechnology. The two wastes are relevant in both crop and animal production when used as organic manure and animal feeds respectively.

REFERENCES

[1] Adinurani, P. G., Hendroko, S. R., Nindita, A., Wahono, S., Maizirwan, M., Sasmito, A., *et al*. (2015). Chaterization of *Jatrophacurcas* Linn. Capsule Husk as Feedstock for Anaerobic Digestion. *Energy Procedia*, *65*, 264-273.

[2] Aguoru, C. U., Azua, E.T. Olasan, O.J.(2015)Approaches to minimizing and overcoming current biodiversity loss. *British Journal of Environmental Sciences*,3(3):12-26

[3] Aguoru, C. U., Okibe, P. O.(2015). Content and composition of lipid produced by chlorella vulgaris for biodiesel production. *Advances in Life Science and Technology*, 36:96-100

[4] Akogwu, R. D. (2011). The viability of biodiesel production in Nigeria using *Jatropha curcas* as a feedstock. Msc Thesis. Department of Energy and Environmental Management. University of Abertay Dundee UK.

[5] Aye, G. C. and Haruna, R. F. (2018). Effect of Climate Change on Crop Productivity and Prices in Benue State, Nigeria: Implications for Food Security. *Establishing Food Security and Alternatives to International Trade in Emerging Economies*, 244:268.

[6] Baumert, S., Khamzina, A., & Vlek, P. L. (2018). Greenhouse gas and energy balance of Jatropha biofuel production systems of Burkina Faso. *Energy for Sustainable Development*, *42*, 14:23.

[7] Krome, C., Jauncey, K., Lohaus, G., & Focken, U. (2018). Phytate analysis and phytase application in Jatropha curcas kernel meal for use in aquaculture feeds. *Aquaculture, Aquarium, Conservation & Legislation*, *11*(3), 690:700.

[8] Magu Thomas Odey, Louis Hitler, Nzeata-Ibe Nelson . (2018) Proximate Analysis and Mineral Composition of *Jatrophacurcas* Seeds Obtained from Pankshin Local Government Area of Plateau State of Nigeria, *J Phys Chem Biophys*, 8:1

[9] Matos, F. S., Ciappina, A. L., Rocha, E. C., and Almeida, L. M. (2018). Factors that influence in Jatropha curcas L. latex production. *Bragantia*, *77*(1), 74:82.

[10] Musa, S. O., Tiamiyu, L. O., Solomon, S. G., Ayuba, V. O., & Okomoda, V. T. (2018). Nutritional value of hydrothermally processed *Jatropha curcas* kernel and its effect on growth and hematological parameters of *Clarias gariepinus* fingerlings (Burchell, 1822). *Aquaculture Reports*, *10*, 32:38.

[11] Nanda, S., Azargohar, R., Dalai, A. K., and Kozinski, J. A. (2015). An assessment on the sustainability of lignocellulosic biomass for biorefining. *Renewable and Sustainable Energy Reviews*, *50*, 925-941.

[12] Nepal, S., Kumar, V., Makkar, H.P.S. (2018). Comparative nutritional value of *Jatrophacurcas* protein isolate and soy protein isolate in common carp*Fish Physiology andBiochemical Journal*, 44: 143.

[13] Ouattara, B., Diédhiou, I., Belko, N., & Cissé, N. (2018). Growth and Transpiration of Jatropha curcas L. Seedlings under Natural Atmospheric Vapour Pressure Deficit and Progressive Soil Drying in Semi-Arid Climate. *Agricultural Sciences*, *9*(06), 639.

[14] Phulia, V., Sardar, P., Sahu, N. P., Sanap, B. N., Shamna, N., Fawole, F. J. and Gupta, S. (2018). Effect ofDetoxification Methods on Anti-nutritional Factors and Proximate Composition of Defatted *Jatropha curcas* Kernel Meal. *Animal Nutrition and Feed Technology*, *18*(1), 67:77.

[15] Rathore, D., Nizami, A.-S., Singh, A., and Pant, D. (2016). Key issues in estimating energy and greenhouse gas savings of biofuels: challenges and perspectives. *Biofuel Research Journal*, *3*(2), 380-393.

http://dx.doi.org/10.22161/ijeab/3.4.52 *ISSN: 2456-1878*

[16] Sanchez-Arreola, E., Martin-Torres, G., Lozada-Ramírez, J. D., Hernández, L. R., Bandala-González, E. R., and Bach, H. (2015). Biodiesel production and de-oiled seed cake nutritional values of a Mexican edible *Jatrophacurcas*. *Renewable Energy*, *76*, 143:147.

[17] Zhao, Y., Wang, Y., Wang, H., Wu, Y., Makkar, H. P. and Liu, J. (2018). Nutritional value of detoxified Jatropha curcas seed cake protein isolates using rats as an animal model. *Animal Nutrition*.

http://dx.doi.org/10.22161/ijeab/3.4.53 ISSN: 2456-1878

Concepts and Characteristics of Complex Systems and Final Energy Usage

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Abstract**—** *The growth, development and urban densification are related to consumption and energy dependence. In relation to the consumption of electric energy, such as cities and their regions with predominance of residential, commercial and more vulnerable services. It is vital the understanding of the various interactions between people and energy of utmost importance for 21st century cities and their spatial behavior and distribution. Cities can be understood as Urban Energy Systems - SEU, which represent the combined processes of consumption and energy acquisition as a supply of demands of an urban population. Systems, represented by cities, regions, sub-districts and districts are like space units, infrastructure facilities consisting of homes, buildings, schools, business centers, large shopping centers and streets that connect these space units. The energy issue is closely related to a spatial occupation and distribution of cities. Therefore, this paper explores this conceptual discussion, based on the theoretical and philosophical development of socio-ecological systems, comes with the main objective of serving as a tool for subsidizing urban energy planning and a proposal of public policies for a reduction of urban energy vulnerability.*

Keywords**— urban energy systems, urban energy vulnerability, electricity, spatial analysis, public policies.**

I. INTRODUCTION

Comprehending the several interactions between people and energy is the main point to understand 21st century cities. In these cities, the so-called Urban Energy Systems (UES) are consolidated and represent the combined processes of consumption and acquisition of energy in order to meet the demands of an urban population, as defined by Jaccard (2006).

The UES include very diverse demands: heating and cooling of buildings, lighting services in both public and private areas, transportation and communication services, electric power for devices and others (RUTTER & KEIRSTEAD, 2012).

In a society more and more dependent on, and connected to, electric services, the safety of the electricity distribution system is a determining factor for social well-being and maintenance of productive processes in cities and, due to its importance, it becomes a recurrent item in debates about public policies.

In order to contextualize societies' energy safety, some definitions are borrowed from other scientific areas, such as the concepts of vulnerability, resilience, and adaptability. There is an effort to uniform these different concepts; according to Gallopín (2006), this happens because they involve different areas of knowledge that are often unrelated as Evolutionary Biology, Ecology, Cultural Studies (interdisciplinary field of investigation – Sociology, Anthropology, Philosophy, Literature), Computer Science and Engineering.

Thus, this article presents general and applied concepts in the context of energy safety of strongly urbanized areas from the point of view of urban energy systems. The conceptualization will consolidate the understanding and definition of urban energy vulnerability and, for example, could guide public policies in order to construct models for understanding energy systems in urban areas, which shall be greatly useful in planning and managing said systems.

In order to do this, general concepts such as vulnerability, resilience and adaptability will be explored, including contributions from several areas of knowledge on the theme. Classic concepts from the energy science will also be presented and they will support such discussions, such as: energy, electric energy, primary and secondary sources, and final uses. These concepts will be connected and applied to concepts such as socio-ecological systems, complex systems, urban energy systems, energy safety, global continuity performance (DGC), frequency and duration of failures (FEC, DEC), and others.

Then, these definitions will be extended into the context of urban energy system, resulting in the central point of this study: urban energy vulnerability and energy adaptability.

II. CITIES AND SYSTEMS

2.1 Cities

Many definitions presented for cities are based on historic, social, and political aspects. In this regard, some concepts by referenced authors are presented, pertinent to this research.

In Brazil, according to the Brazilian Institute of Geography and Statistics (IBGE, 2010), about 84.36% of

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the population lives in cities. In these urban spaces, the exchange of goods and services, culture and knowledge among habitants is outstanding via the energetic conditions fundamental to such habitants of urban life.

Cities are considered the great geographical expression of this century, that is, they are the consolidation of human society and economic, political, and social relations determine their growth. The urban space is shaped by the interdependencies among cities, which influences local environmental and energetic quality.

In this century, cities became, mainly, urban public spaces, scenery for major social, political and environmental issues, conflicts, inequality, and unbalance, where lies injustice and exclusion.

The low-income population who dwells in urban areas is directly affected by the fact that public authorities do not ensure their basic survival rights and by the deficiency in public policies that meet social and environmental demands.

This scenery is aggravated when one realizes the irrational use of natural resources, the inadequate infrastructure constructions and urban installations that impact the environment irreversibly and have their main effect as the deterioration of life quality in cities.

According to Lefebvre (2001), the urban space has a "conceived" character, that is, a homogenous space, abstractly, the place for social relations, for experience exchange; the space lived / perceived of representations and daily practices. At the same time, this abstract space works as an instrument, as control and as management for the State that it works as a controlling instrument for those owners of economic and political power.

Moreover, the author approaches the urban space as the result of a historical process of consolidation of cities that went through processes of industrialization and urbanization, a qualitative change, resulting of the way of life and daily practices of their population. The urban space must be considered the lived space and its place to understand urbanization.

When one analyses the meaning of the terms urban and urbanization under the critical perspective, one understands that their definitions go beyond the concept of cities and are defined from the condensation of social and spatial processes that allowed capitalism to go on and to reproduce its essential production relations and its own survival. Moreover, the urban space, for capitalism, is the conditioning and regulating agent of the socio-spatial contradictions.

In this line of thought, cities have different uses and can be articulated and fragmented at the same time; such uses define their function. Cities are defined by the areas inside the urban space, such as downtown, residential neighborhoods of marginalized social classes, popular districts, periphery – that is, a set of distinct areas in terms of form and social content.

The urban space also assumes a symbolic dimension in which several relations are reproduced in daily life, feelings of belonging to a place where individuals coexist, as well as social practices, beliefs, and values created over the consolidation of societies.

According to Serpa (2008, p.305), the space is what modifies the connections among spaces and facilitates flows. The integration allowed by globalization establishes that the participation in an integral spatiality, both of places and of flow, depends on the place's accessibility and on people in the technical-scientific-informational environment.

The levels of accessibility define the relations among urban space and other localities. The development of a place is related to several present infrastructure, as well as to economic activities established in the built space.

Understanding the space involves several meanings, receives different elements in a way that any and every definition is not a permanent conception; it is flexible and allows changes. The space has elements defined from their function in the maintenance of socio-spatial dynamics. Among them the infrastructures, which can be explained by the human work materialized and spatialized in the shape of buildings, automobiles, energy, plantations, and others.

Santos (2001, p. 60) believes that "the space denotes the result of constant interactions between 'fixed' and 'mobile'", namely, between materiality and immateriality. The fixed can be understood as elements built by human actions and equipped with intentionality; therefore, they have functions (means of transport, energy, capital, information, communication, knowledge).

Thus, each fixed spatial element is interconnected to a succession of interactions and local and distant interdependencies, with economic, historic, social, and cultural relevance. The fixed can be denominated territorial fixed, since they are built in space, have address, and have localization – they can be georeferenced.

On the other hand, the mobiles give meaning to life and economic activities over the historical processes and are considered the direct and indirect result of actions and cross or settle themselves in the fixed, modifying their meaning and value and, at the same time, modifying themselves (SANTOS, p. 15,1988).

The concepts presented here will be necessary for better understanding the cities, considering their relations and their multi themes and scales, namely, cities, and their sets and components must be considered as systems with energy interaction vectors of different sizes and meanings.

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2.2 Systems

Socio-ecological

Adguer (2006) says that the concept of a Socio-Ecological System reflects the idea that human actions and social structures are integrated to nature and there is no distinction between social and natural systems.

Complex

The Complexity Sciences appropriated characteristics of natural systems aiming at representing the artificial ones, as close to reality as possible.

III. GENERAL CONCEPTS RELATED TO ENERGY SCIENCES

The basic, general concepts necessary to understand this research refer to the energy sciences theme and shall substantiate the discussions presented here.

The energy relations of an area, usually a State, are presented in the energy balance. The energy balance refers to the study of the current landscape and the projections of energy inputs, production, consumption, and outputs in a defined spatial unit.

The energy balance's general structure is composed by primary energy, transformation, secondary energy, and final consumption.

Energetic compounds derived directly from nature, such as oil, natural gas, mineral coal, firewood, sugarcane products, plant and animal residues, uranium, hydraulic energy, solar energy, and wind energy form the **primary energy**. Primary sources are classified as renewable and non-renewable sources.

Secondary energy refers to energetic products resulting from the transformations centers, which forward such energy to several consumption sectors or to another transformation center. As examples, one can mention diesel oil, liquefied petroleum gas (LPG), naphtha, kerosene, and other residues derived from petroleum.

In order to the generated and transformed energy reach the consumer, it must go through the transmission and distribution system.

The distribution scenery in Brazil presents 63 energy providers plus authorized companies. In the state of São Paulo, there are 7 to 8 distributors and, in the capital, Eletropaulo provides the energy.

3.1. Energy supply and final consumption

Energy supply is the amount of energy made available to be consumed (final consumption). The amount of energy available to be consumed in a determined spatial unit or consumer group is defined as energy supplied.

According to BEN (2016), in 2013, the final consumption of electricity corresponded to 18% in the whole world, behind only of oil consumption, with 39.9%.

According to data obtained by BEN (2016), production of energy in the state of São Paulo decreased 4.2% in 2015 when compared to the previous year. In 2014, energy production was 65.409 GWh and, in 2015, it was 62.654 GWh (BEN, 2016, p. 150).

3.2. Energy sources

The main energy sources presented in the energy balances and existing in the country are oil, natural gas, electric energy, mineral coal, wind energy, biodiesel, and sugarcane products.

Brazil's electric matrix is composed by the following primary sources: wind, solar, hydroelectric, nuclear, thermal, sugarcane bagasse, and firewood sources.

Brazil has an original electric matrix that is predominantly renewable, specially the hydraulic generation, which corresponds to 64% of the internal supply. Renewable sources represent 75% of the internal energy supply in Brazil (BEN, 2016, p. 17).

3.3. Consumer economic sectors

The final consumption of energy encompasses all sectors of economic activity, which converge primary and secondary energies. Final consumption is the sum of energy and non-energy consumptions. Energy consumption is represented by sectors such as residential, commercial, public buildings, agribusiness, transport sector, industrial, and non-identified consumption.

The final consumption of energy is presented in national and state energy balances by the following sectors: energy, residential commercial, public, agribusiness, transportation, and industrial.

Among all sectors, the residential sector, when added to others (except industry and transportation), represents over 50% of global energy consumption. In 2013, of 1677 106 tep, 56.2% derived from sectors other than the industrial and the transportation sectors (BEN, 2016).

The residential sector is supplied, predominantly, by electricity, with almost 46% of participation; in 2015, it corresponded to almost 11.300 tep (BEN, 2016). Both firewood and petroleum liquefied gas (PLG) are still significant sources in the sector and together they represent over 50% of the total of the sector's participation, while natural gas represents little over 1%.

The chart presented by BEN (2016), concerning the final consumption of the residential sector during the analyzed period shows that energy consumption grows to the detriment of firewood. This behavior certainly corresponds to the correlation between economic development and consumption by energy sources in the residential sector. That is to say that as a region develops, its final consumption enhances, tending firstly to be of energy consumption.

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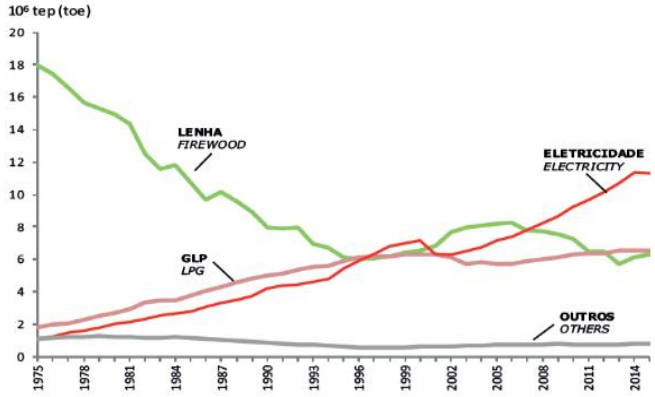

Fig.1: Final consumption of energy in the residential sector (1975-2014)

Source: BEN, 2016.

In 2015, the residential sector presented the second largest electricity consumption of the country, representing a little more than 21% of participation, with 131 TWh in the year (BEM, 2016, p.40).

In 2015, the state of São Paulo registered the highest energy consumption in residencies compared to other states, corresponding to 38.212 GWh (BEN, 2016). Thus, it can be observed the state's expressiveness in residential consumption in comparison to the rest of the country.

IV. APPLIED CONCEPTS

The concepts applied to this topic concern the theme of this research and are presented in the following structure: Urban Energy Systems, Energy Safety, Threat, Risk, Fragility, Resilience, Adaptability, Transformability, and Vulnerability.

4.1. Urban Energy Systems - UES

Urban Energy Systems (UES) can be defined based on the concept of Socio-Ecological System (SES), which is characterized as a system that includes the social (human) and biophysical subsystems in mutual interaction (GALLOPÍN et al., 1991). The SES, as well as the UES, can be applied in different scales, from local communities and their environment to global systems formed by all human communities and the biosphere (GALLOPÍN, 2006).

Based on the concept of socio-ecological systems, one can consider the energy context interconnected (mainly the question of electricity and transportation) and it is formed by social and biophysical subsystems. The UES refers to a complex system that is influenced by environmental, ecological, social, and market factors.

According to Gruble et al. (2012), the term "Urban Systems" is used for the urban phenomenon of a functional perspective, as well as a traditional territorial or administrative perspective. Thus, according to the author, urban energy systems encompass all components related to consumption and provision of energy services associated to functional urban systems, regardless of location, uses, and energy conversions.

Considering the natural tendency of economically developing countries to reach urbanization and consumption levels of developed countries, global challenges concerning access to clean energy and energy safety services have to take into account the limitations and opportunities of urban energy systems in local scale.

According to Walker et al. (2004), the dynamic stability of human and natural systems emerges from the complementarity and understanding of the following features: resilience, adaptability, and transformability. Adaptability can be understood as the capacity of managing the system's resilience, and it may employ managing tools in order to minimize the vulnerability of urban energy systems.

4.2. Energy Safety

According to Winzer (2011), reaching energy safety is among every nation's most important goals. In order to reach such goal without conflicts among countries (keeping in mind that energy systems do not follow political frontiers), the term energy safety must be well defined and clearly measured.

During the revision of the literature on the theme, it is possible to observe that there is no consensus, neither on the academics, nor on the legislators' part when it comes to defining "energy safety" [see in Sovacool (2011), the 45 definitions employed for the term]. However, the present research presents some definitions that were considered important, as seen in the consulted literature.

The international Energy Association (IEA, 2016), for example, defines energy safety as the continuous availability of energy sources for a fair price, so to balance economic development and environmental aspects. In this definition, it is clear the understanding that energy safety

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is similar to energy supply safety, which is common among other authors (e.g. Löschel et al., 2008; Kruyt et al., 2009; Australian Government, 2011; Winzer, 2011).

Parag (2014) criticizes the interpretation adopted by several academics and governments because it does not take into consideration, among other things, the active role that final consumers have over urban energy systems, above all in times of discussions about low-carbon economies. According to the author, energy safety must be approached as energy services safety in order to incorporate the complex nature of urban energy systems: the interaction among energy infrastructures, final uses, and behavioral, social, and cultural aspects of energy use.

When incorporating such aspects in what concerns energy, Jansen (2009) defines energy services safety as "the measure in which population in a certain area (country or region) can have access to energy services of adequate quality for a fair and competitive price".

At the same time, the IEA defines energy safety considering the equilibrium of economic development and environmental aspects for a fair price. Jansen (2009) focuses on the population with access to energy services with quality and fair price, but does not mention environmental aspects.

It is perceivable that all definitions are concerned with the challenge of offering energy services with quality and fair prices, even if not all authors consider environmental questions. Thus, the focus is shifted to consumers who desire quality services that meet their current and future demands. This is an intrinsic need of consumer goods orienting the identification of direct and indirect variables.

4.3. Threat and risk

The Chertoff Group (2014) defines threat as the potential capacity or pretension to cause damages and it is related to a probability (or potentiality) of certain damage occurring, being a non-null variable. However, Turner et al. (2003) argue that threats are dangers to a system that include disturbance, stresses, and stressors (source of stress).

In addition, Sovacool (2001) understands that threats can be defined from the system's scales, dividing them into three categories: macro, micro, and *meso* (middle or intermediate). Macro threats are those that impinge on the global system; micro threats impinge on local scale; and *meso* threats are located between the global and local scales.

Urban energy systems are limited by economic, technical, social, political, and environmental questions, which can represent threats. In the point of view of energy safety, it is understood that these threats are caused by the existence of a factor over the energy supply chain.

The idea, common to all these definitions of energy safety, can be described as "the absence of protection or adaptability to threats caused by an impact on the energy supply chain".

The concept of risk can be defined as the possibility that consequences from any event or action damage aspects valued by humans (Kates & Kasperson, 1983; Hohenemser, Kates, & Slovic, 1983). For example, the falling of branches on energy distribution cables causing power failure. The Chertoff Group (2014) presents risk as the intersection of three aspects: threat, vulnerability, and consequences.

4.4. Fragility

Fragility can be defined as propensity to deterioration or rupture of a system, being opposed, then, to system resistance and tenacity. This term is present in socio-ecological systems, economic sciences, urban systems, resistance of materials, and others.

Fragility is categorized by the consequences that the deterioration or rupture can cause upon certain impact. Fragility is evaluated in the face of a threat or risk. It is only active if said impact promotes alteration, deterioration, or rupture to a system.

Klemkosky (2013) defined the economic systems as fragile. Such fragility can cause economic impacts, as in Japan and in the United States, and usually generate periods of low economic growth.

On the other hand, Commins (2011), who studies the fragility of the African urban system, states that fragility manifests itself in a context of crisis of governmental deterioration and prolonged political conflicts in the urban scope. Fragile governments lose their capacity of providing basic and safety services to their citizens.

In urban areas, fragility is intensified by urban energy systems that are mostly fed – or, in most cases, exclusively fed – by the National Interconnected System (SIN) and by local distributors. The UES' fragility is also intensified because it refers to a system strongly dependent on a single source, electric power, with emphasis on demands and spaces, such as building set with data servers, elevators, and air-cooling central systems. Such spaces become more fragile the less diversified the energy sources that supply them.

In the case of a building set, the fragility of the system can be managed with complimentary sources or energy efficiency, as with cogeneration, i.e., diversification measures that decrease the dependence on SIN and that can minimize the building system's fragility.

4.5. Resilience

According to Folke et al. (2010), the resilience

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concept was originally introduced in socio-ecological systems by Holling (1973) as a concept for understanding the capacity that ecosystems have of persisting in their original state even when subject to disturbances.

Yet Walker et al. (2004) defines resilience as the capacity that a system has of absorbing disturbances and reorganizing itself while retaining the same function, structure, and identity. In this sense, one can point out some policies implemented in the city of São Paulo to decrease traffic and that, at time, culminated in unexpected behaviors. The first example was the creation of bus corridors, which could encourage people to use public transport instead of private, with the added bonus of spending less time in traffic.

This measure can promote the change towards the use of public transport in detriment of private transport or it can have no effect in the behavior of users. The choice of continuing to use private cars can increase travel time when the same route can be travelled in public transport. In this case, one can see resilience in the user who, instead of time, is prioritizing comfort and adapting to his new and longer stay in traffic during his dislocations – here, the user experiences the so-called "adaptability". However, if the user chooses the public transport, he can spend less time travelling, but naturally relinquishes certain comfort – this change of habits represents transformability.

An application of such concepts of socio-ecological systems can be seen in the concepts of mechanics of materials presented in Figure 2. A material subjected to axial loading will suffer deformations. If these deformations do not surpass the limit called "elastic region", the deformations will not be permanent and the material will come back to its original state. If the deformations go beyond the elastic region and reach the plastic region, then they will become permanent.

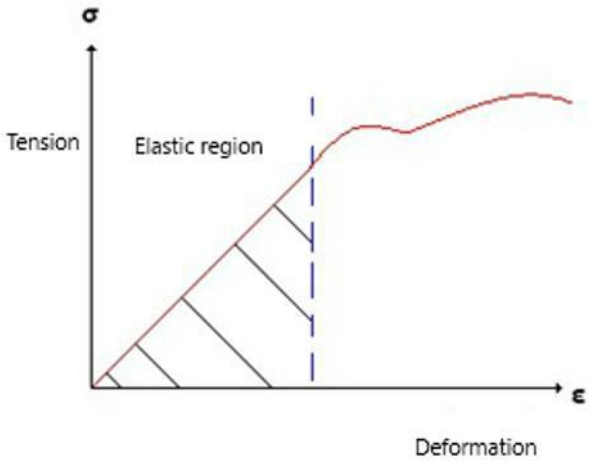

Fig.2: Elastic region of deformations on a material

In this context, *resilience* can be defined as the material's elastic region, in which deformations are dissolved and it will return to its initial state. The maximum energy necessary to the material overcomes the elastic region and reach the plastic region can be defined as *resistance*. The *precariousness* refers to the current deformation of the system in relation to the maximum deformation of the elastic area and it is, then, a function of time. Lastly, *panarchy* happens when the characteristics described in this system are altered by sudden and unexpected changes in variables external to the system.

4.6. Adaptability and Transformability

Adaptability has been discussed in several scientific areas, for example, in the energy systems. Authors Grubb e Minh Ha Duong (1995) say that energy systems and technologies adapt themselves to external pressures.

Walker et al. (2004) defines adaptability in the context of socio-ecological systems, such as the capacity that human agents have of influencing a system's resilience, changing its latitude, resistance, or precariousness. Going to back to the example of the bus corridors, their creation can be considered a measured that influenced resilience, increasing the system's latitude, resistance, and preciousness.

When going through a process of adaptability, a system can have its resilience limit pulled closer or pushed far away from its current state (alteration in its latitude). It can have an increase in its difficulty of reaching latitude (alterations in its resilience) or even have its current state moved, i.e., not in the latitude direction (alterations in the precariousness level).

Carpenten e Brock (2008) distinguish the term adaptability from transformability and state that, even though changes occur in the internal demands and forces external to the system, it can adapt to maintain certain processes and not transform itself into a fundamentally new system.

Transformability occurs when a current system is

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unsustainable, when the resilience zone has been surpassed, and a fundamentally new system is formed. Folke et al. (2010) considers the concept a capacity of crossing limits reaching new paths of development.

Based on these definitions, it is possible to believe that the city of São Paulo and its metropolitan region may be facing this moment of energy transformability concerning transportations. And it can also be experiencing the so-called adaptability concerning its demand for electricity.

4.7. Vulnerability

According to Calvo e Dercon (2005), the term vulnerability comes from the Latin "*Vulverare*", which expresses the idea of being hurt and suffering damages, associated to dangers and threats and not to general uncertainties.

Gallopín (2006) evaluated that this term is usually understood as the susceptibility of a system to a potential damage or transformation when subjected to disturbances or environmental pressure, instead of a real damage measure. When we compare that to the urban vulnerability, it comes from the fragmentation and segregation of the urban space.

The concept of vulnerability is not a consensus among the studies on the theme and it varies in several areas of study. A revision of the definitions of the concept of vulnerability can be found in Figueiredo et al. (2010).

The system's vulnerability, as defined by Doorman et al. (2006), is the system's insufficient capacity of bearing an unwanted situation. The unwanted situation is considered any unexpected externality that may disorganize the current shape and is, then, understood as a threat or risk.

V. URBAN ENERGY VULNERABILITY: CONNECTING THE CONCEPT

Energy systems' failures can affect the final user more or less and this intensity is called vulnerability. The availability of energy is connected to the wellbeing and safety of the population, from simpler cases as a consumer's food stock, the trajectory of an elevator in a building, the thermal comfort created by air conditioning, to the more complex case of the operation of a hospital.

The implantation of a network that favors the distributed generation, with micro generators in urban centers, can contribute to the reduction of the UES' vulnerability, since the exposition of a generation center to a threat does not compromise the operation of other centers.

In this context, a mathematical formula is proposed in order to evaluate the vulnerability according to the probability of failure of the electric system, taking into consideration the population the can be eventually affected.

$$V = q. \sum P$$

In which:

V –Vulnerability;

q –Probability of failure of electric system (FEC, DEC);

P –Population of evaluated area.

It is understood that vulnerability is a function between threat and damage intensity, thus being a portion of the relation of fragility, not considering consequence.

$$V = f[A, ID]$$

In which:

V –Vulnerability;

A –Threat;

ID – Damage intensity. Refers to portion of the fragility relation.

The threat represents the risk of a certain part of the population being without electric power or public transportation, for example. The treat can be defined as part of the study of FEC and DEC (frequency and length of system failures), which can express a threat when its linear and non-linear projection is made.

The threat considers the rupture or deformation *per se* and the probability of its occurrence, that is, the threat is the variation on axis X, referring to the sceneries 1 and 2.

$$A = [\Delta x, P]$$

In which:

A –Threat;

–Variation on axis X;

P –Probability of occurrence of threat.

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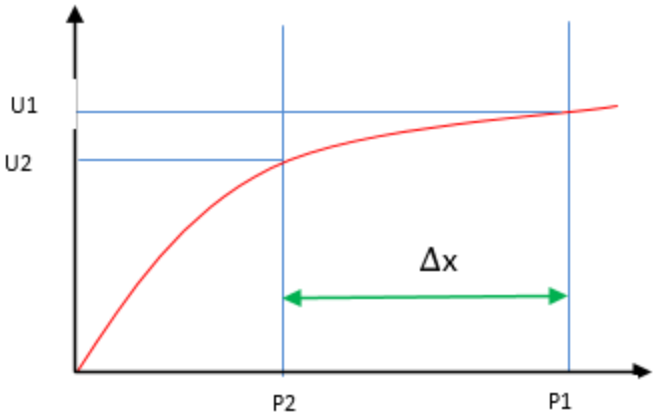

Fig.3: Utility's resilient region.

The intensity of damage can be measured through the hours of congestion caused by it, for example. All examples are vulnerability measures expressed by utility, rather, the marginal utility strongly discussed and applied in economic theory.

$$U' = \frac{dU}{dx}$$

ID = [U , U']

The marginal utility, here expressed by U', represents one of the manners of measuring the satisfaction, or the consumption vector of goods and services, represented from the measure of how much satisfaction increased as one unit of X increases.

The concept of disutility encourages the discussion, in the environmental area, of negative aspects. For example, the higher the goods consumption, the greater the need for raw material and the greater the environmental impacts generation by its extraction, i.e., each increase in marginal utility increases a marginal disutility in the same proportion until equilibrium, where the marginal utility increase does not represent more increase in marginal disutility.

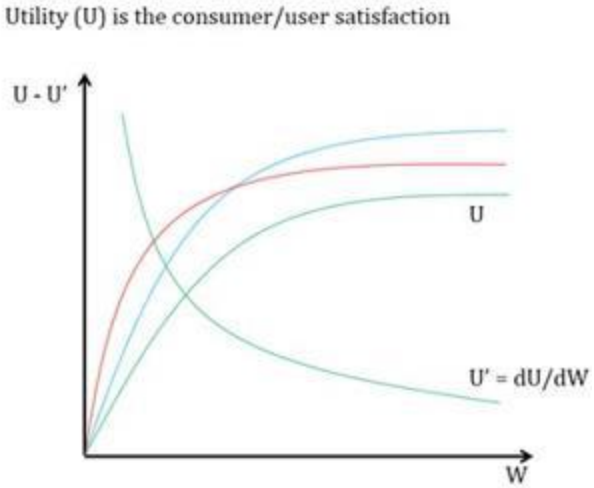

Fig.4: User's marginal utility curve (U).

What is proposed here is the use of the concept of urban vulnerability to evaluate the neighborhood effect in a city, which is defined as the sum of each scenery' utility and its respective probability of happening.

This concept is proposed as a means of measuring the vulnerability because it allows evaluating in an integrated manner each region's individual vulnerability, taking into account the integration of such regions and the influence of energy flows in the system's behavior as a whole.

The utility curve is determined in function of the energy flows among regions and determines the way in which other regions are capable of servicing the population of regions where failures occur.

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The importance of this concept is paramount for evaluating the present scenery and how the technological changes in the energy model can influence the utility curve positively.

VI. FINAL CONSIDERATIONS

Due to the strong energy dependency of the great urban centers, it is necessary to create managing tools and to implement new, integrative technological innovations using several energy vectors, aiming at efficiency and system safety.

Thus, a reductionist approach focused on isolated elements would not be enough to understand the processes in an urban energy system. Then, this conceptual discussion, based on the theoretical and philosophical development of socio-ecological systems, has the main goal of promoting the urban energy planning and proposing public policies to decrease urban energy vulnerability.

In this regard, based on the concepts discussed here, mainly that of the Urban Energy Vulnerability and the territorial space's importance and complexity, the city of São Paulo is seen as an ideal area for a case study in future studies. Therefore, it must be analyzed in detail in what concerns its formation and energy-space-time relations, presenting its background and relations with the energy and electric demands.

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REFERENCES

[1] AMARO, J. J. V. Sociedades complexas e risco ecológico – Epistemologia e meio ambiente na atual teoria de sistemas. Núcleo de Pesquisa e estudos em educação ambiental e transdisciplinaridade, Universidade Federal de Goiás (UFG). Vol. 3, p. 47–59, 2013.

[2] ARIAS, D. V. Gestión de riesgosenel Sector energético.Apresentação do curso Capev - Capacitación Energética Virtual. Organização Latino-Americana de Energia (Olade), 2012.

[3] ARNOLD, M.; BARTH, V. Open Inovation in Urban Energy Systems.Energy Efficiency, 5:351-364, 2012.

[4] CALVO, C.; DERCON, S. Measuring individual vulnerability. Department of economics. Discussion paper series. University of Oxford, Number 229, 2005.

[5] CARPENTER, S. R.; W.A. BROCK. Adaptive capacity and traps. Ecology and Society 13 (2):40, 2008. Available at: <http://www.ecologyandsociety.org/vol13/>. Access on: 04/nov/2013.

[6] COMMINS, S. Urban Fragility and Security in Africa. Africa Security Brief, Nº 12. Africa Center for Strategic Studies. <http://africacenter.org/wp-content/uploads/2011/04/ASB-12_Final-forWeb.pdf>. Access on: 27/nov/2013.

[7] FIGUEIREDO, M. C. B. Análise da Vulnerabilidade Ambiental, 2010. Embrapa - Empresa Brasileira de Pesquisa Agropecuária. Ministério da Agricultura, Pecuária e Abastecimento. Available at: <http://www.cnpat.embrapa.br/cnpat/cd/jss/acervo/Dc_127.pdf>. Access on: 27/nov/2013.

[8] FOLKE, C. ET AL. Resilience Thinking: Integrating Resilience, Adaptability and Transformability. Ecology and Society 15(4): 20. 2010. Available at: <https://www.ivey.uwo.ca:444/cmsmedia/222506/Resilience_thinking_IntegratingResilience__Adaptability_and_Transformability.pdf/>. Access on: 04/nov/2013.

[9] GALLOPÍN, G. C. Las dimension es humanas del cambio mundial: vinculación de losprocesosmundiales y locales. Revista Internacional de CienciasSociales. pp. 130, 707–718, 1991.

[10] GALLOPÍN, G. C. Linkages between vulnerability, resilience, and adaptive capacity. Global Environmental Change, 16, pp. 293-303, 2006.

[11] GRUBB, M. E MINH HA DUONG, T. C. The economics of changing course: Implications of adaptability and inertia for optimal climate policy. Energy Policy. V. 23, pp. 417-431, 1995.

[12] HIDALDO, B.D.; As divisões territoriais do município de São Paulo: uma proposta de classificação por meio da análise dos Distritos. Monograph. São Paulo, p. 116, 2013.

[13] JANSEN, J.C. Energy Services security: concepts and metrics, expert paper submitted as input to the ongoing IAEA project: Selecting and Defining Integrated Indicators for Nuclear Energy, Energy Research Centre of the Netherlands, 2009. Available at http://www.ecn.nl/docs/library/report/2009/e09080.pdf. Access on: 25/mai/2016.

[14] HOHENEMSER, C., KATES, R. W., & SLOVIC. The nature of technological hazard. Science, pp. 220, 378–384, 1983.

[15] KATES, R. W., & KASPERSON, J. X. Comparative risk analysis of technological hazards. A review. Proceedings of the National Academy of Sciences, pp. 80, 7027, 1983.
[16] KLEMKOSKY, R. Financial system fragility. Business Horizons. Vol. 56, pp. 675–683, 2013.
[17] Institute for Energy and Transport. European Comission. Available at:
[18] <http://ses.jrc.ec.europa.eu/complex-system-science-smart-grids>. Access on: 27/nov/2013.
[19] KRUYT, B., D. VAN VUUREN, H. DE VRIES & H. GROENENBERG. Indicators for energy security, Energy Policy 37(6), pp. 2166-2181, 2009.
[20] LEFEBVRE, HENRI. 2001. O direito à Cidade. Tradução Rubens Eduardo Frias. São Paulo, Centauro, 2001.
[21] PIMENTEL, D. et al. Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture, Ecosystems & Environment. Vol. 84, p. 1-20, 2001.
[22] RUTTER, P.; KEIRSTEAD, J. A brief history and the possible future of urban energy systems. Energy Policy, pp. 50, 72-80, 2012.
[23] SANTOS, M. A Natureza do Espaço. Técnica e Tempo. Razão e Emoção. 2nd ed. São Paulo, Hucitec, l997.
[24] SERPA, A., org. Apresentação. In: Espaços culturais: vivências, imaginações e apresentações [online]. Salvador: EDUFBA, 2008. pp. 9-11. ISBN 978-85-232-1189-9. Available from Scielo Books O direito à Cidade. Transl. Rubens Eduardo Frias. São Paulo, Centauro, 2001.
[25] SOVACOOL, B.K. (ed). The Routledge Handbook of Energy Security (New York: Taylor & Francis), 2011.
[26] TURNER II., B.L., KASPERSON, R.E., MATSON, P.A., MCCARTHY, J.J., CORELL, R.W., CHRISTENSEN, L., ECKLEY, N., KASPERSON, J.X., LUERS, A., MARTELLO, M.L., POLSKY, C., PULSIPHER, A., SCHILLER, A. A framework for vulnerability analysis in sustainability science. Proceedings of the National Academy of Sciences of the United States of America 100 (14), pp. 8074–8079, 2003.
[27] WALKER, B., H., ET AL. Resilience, adaptability and transformability in social–ecological systems. Ecology and Society 9 (2):5, 2004. Available at: <http://www.ecologyandsociety.org/vol9/iss2/art5>. Access on: 04/nov/2013.