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Editorial Board

FOREWORD

I am pleased to put into the hands of readers Volume-2; Issue-5:Sept-Oct2017 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: Nov, 2017

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Distribution and Seasonal Occurrence of major insect pests of cotton in Uganda

Dennis Gayi[1], Geoffrey Lubbadde[1], Moses biruma[1], Samuel Echaku[1], Emmanuel Ejiet[2], Denis Ocen[1]

[1]National Semi-Arid Resources Research Institute – NaSARRI, P.O. 56, Soroti
[2]Cotton Development Organisation, P.O. Box, Kampala
Correspondence: Gayi Dennis, National Semi-arid Resources Research Institute, Serere, Uganda. Tel: +256757893439, Email:

Abstract— *Both surveys and field expriments were conducted to study the distribution and seasonal fluctuation of major cotton pests in Uganda. The surveys were carried out in 13 cotton growing districts including; Wakiso, Mpigi, Mukono (Central region), Kamuli, Jinja (Eastern region), Amuria, Amolator, Pallisa, Serere (North-eastern region), Kasese, Nakasongola, Masindi and Kiryandongo (Western region), with laboratory rearing and the field experiment conducted at the national semi arid resources research institute (NaSARRI).Twenty cotton fields were selected from each district in four sub counties, 5 from each sub county giving a total of 260 fields for the study in 2013 and 2014 respectively. Each selected field was visited 3 times in each cropping season i.e. once at vegetative stage, once at Squaring stage and once at boll growth.*

For 2014 season. Cotton variety BPA2002, was sown on 16th, June, 2014 in plots, measuring 40 x 60 m^2 at 4 seeds/hole and soil depth of 2.5 cm. Plant to plant and row to row distances were kept at 30 cm and 75 cm, respectively. Recommended agronomic practices with no pesticide application were applied throughout the cotton season. Densities of insect pests were recorded on ten days interval on randomly selected leaves from top, middle and bottom of the 30 plants selected. The insects were recorded from 2nd week of July till 4th week of November.

On each sampling date, bollworm larval densities, Aphid counts, mite counts were evaluated on 20 randomly selected cotton plants per field in both 2013 and 2014 seasons respectively. Boll worm larvae collected were placed in plastic containers, reared in the laboratory until adult emergence, counted and added to the sweep net catches. For aphids and mite records were taken from upper, middle and lower leaves of randomly selected twenty plants (Shah et al., 2015). Adult boll worm moths, stainers and white flies were also collected using a sweepnet, where each field was divided diagonally and in the early morning and late evening insects were collected by sweeping through the field, the catches collected, separated by species, starved to death and then placed in alcohol for further morphological identification.

Data was analyzed by ANOVA using general linear model procedures at 5% level of significance. Meteorological parameters like temperature and relative humidity during the study period were recorded. Correlation analysis was made to study the relationship between weather parameters and incidence of major insect pests of cotton by following standard statistical methods

For the survey, high aphid occurrences were observed in Kasese, Serere, Wakiso and Mpigi districts respectively, Stainers (Mukono ,Serere, Pallisa and Kasese districts),Mites showed higher occurrences in all the districts surveyed with highest counts of bollworms observed in Kasese and Pallisa districts and for the seasonal fluctuation study, mites (Tetranychus urticae), peaked (38.4 individuals per plant), in the first week of September, Aphids (38 individuals per plant) in the last week of August, White flies (35 individuals per plant) in the last week of August, Jassids (17.5 individuals per plant) in the second week of October, and stainers peaked (10.5 individuals per plant) in the last week of September.

In conclusion the districts surveyed appeared to be hot spots for a particular pest and farmers should now employ integrated pest management in order to reduce the pest pressure in their regions. This should be employed with proper timing of when a particular pest becomes a threat to the cotton crop based on the seasonal fluctuation data obtained.

Keywords— insect pests, cotton, uganda

I. INTRODUCTION

Cotton plays a vital role in Uganda's economy and ranks the third in terms of traditional export crops (UBOS 2015).

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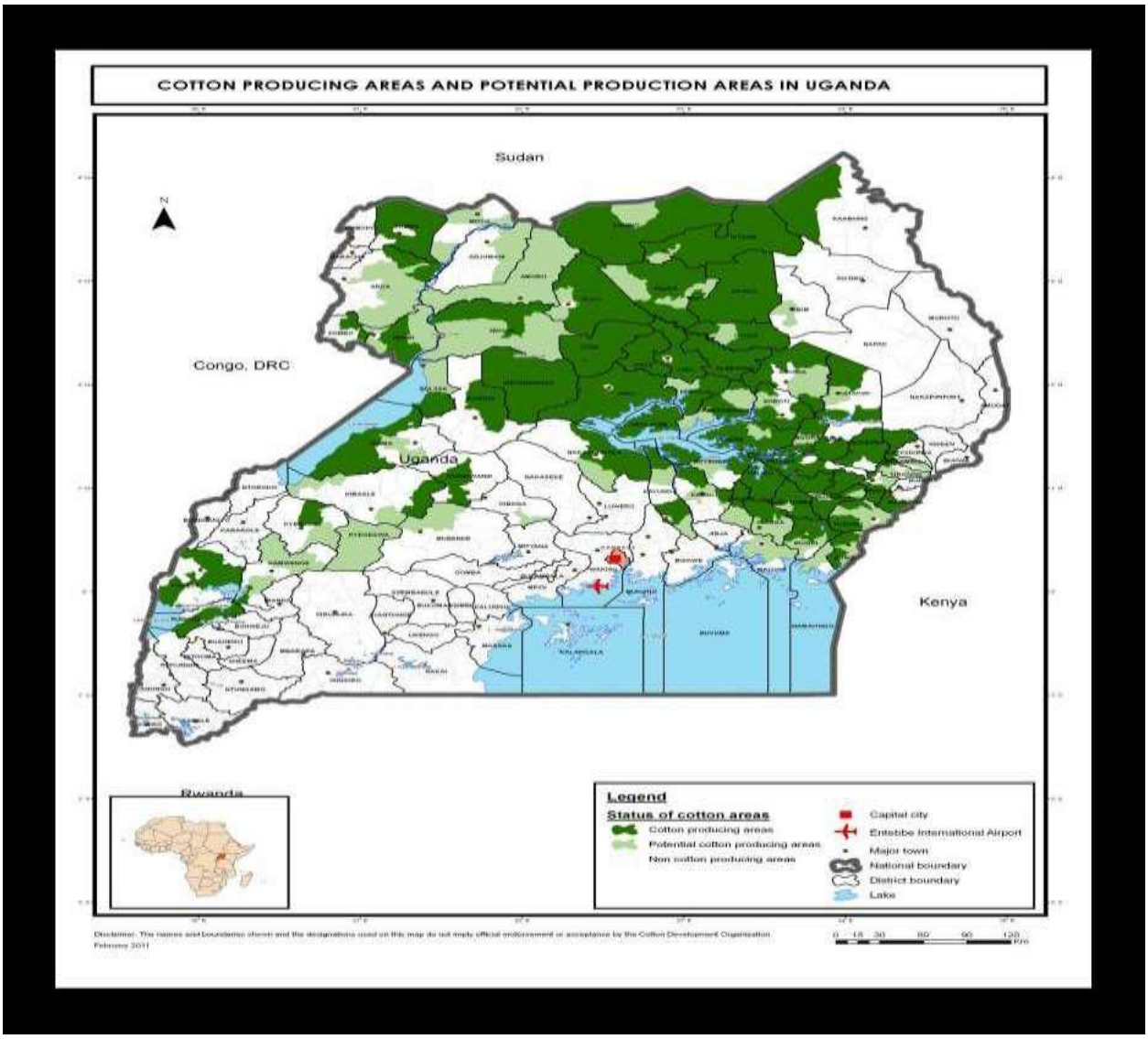
It is grown in all regions of the country with most production concentrated in the Northern, Eastern and South western regions whose sandy and loamy soils are suitable for cotton production (CDO annual report 2013).



Source: Cotton Development Organisation (CDO) final Uganda Brochure October 2011

It is a perennial semi-shrub grown as an annual crop in Uganda with production periods as shown in table 1.

Table.1: Periods of cotton planting and Harvesting In Uganda

Regions	Planting period	Harvesting Period
Northern	May-June	December-February
Eastern	May-June	December-February
Western	July-August	January-March
Mid-Western	July-August	January-March
Central	July-August	January-March
West Nile	June-July	January-March

Source; Cotton Development Organization (CDO) annual report 2011

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Gossypium hirsutum (L) is attacked by a range of both early and late season insect pests during its growth cycle including; the bollworm complex; American bollworm, *Helicoverpa armigera*, spotted bollworm, *Earias insulana*; pink bollworm, *Pectinophora gossypiella*, Stainers *Dysdercus cingulatus* Fabricius, Mites, *Tetranychus urticae* Koch (The yellow tea mite and red spider mite); Aphids, *Aphis gossypii*, Whitefly, *Bemisia tabaci* and Jassid, *Amrasca biguttula*; (Byabagambi *et al*, 2013).

These pests cause considerable yield losses estimated at 80% (Shahid *et al* 2015) worldwide with up to 100% loss registered elsewhere in Africa (Abate *et al* 2000). At present information on extent of spread by these insect pests of cotton in Uganda is scanty.

More still, Mujurizi J *et al*, 2007, reported that cotton farmers in Uganda spray cotton fields with high dosages of pesticides at a time when most pollinators are visiting the cotton flowers leading to high mortality of forager honey bees.

However information on seasonal fluctuation of these pests due to variations in climatic conditions in Uganda is also lacking. Hence, an attempt has been made to establish the distribution of major cotton pests and an insight on their spread in Uganda which will give an idea about peak periods of their activity and may be helpful in developing real time pest management strategies in cotton production systems.

II. MATERIALS AND METHODS

Study sites

Survey studies were carried out in 2013 and 2014 cotton growing seasons respectively in 13 cotton growing districts of Uganda including; Wakiso, Mpigi, Mukono (Central region), Kamuli, Jinja (Eastern region), Amuria, Amolator, Pallisa, Serere (North-eastern region), Kasese, Nakasongola, Masindi and Kiryandongo (Western region). These districts are well known for commercial cotton production.

Generally these districts are characterized by bimodal distribution of rain fall, with peaks in April and September and an annual average precipitation of about 1500mm. The short and long rainy seasons last from mid-March to end of June and from early August to end of November, respectively. A short dry spell of about four weeks occurs in July. The major dry spell begins in the last week of November and lasts until mid-March with an annual average temperature of 25˚C.

Since the predominance of cotton pests varies with the crop growth cycle (Shahid *et al., 2012*), surveys were conducted for one season each year in all the 13 districts while supporting laboratory and field studies were carried out at the National Semi-Arid Resources Research Institute (NaSARRI).These districts were selected basing on information provided by cotton development organisation (CDO) which indicated that these districts are among the main cotton growing districts in Uganda with organized farmer groups.

Experimental design

The study sites were selected basing on field sizes, average between 0.5-5 ha. Twenty cotton fields were selected from each district in four sub counties, 5 from each sub county giving a total of 260 fields for the study in 2013 and 2014 respectively. Each selected field was visited 3 times in each cropping season i.e. once at vegetative stage, once at Squaring stage and once at boll growth.

For the field experiment, the population fluctuation of insect pests of cotton were evaluated at the national semi-arid resources research institute (NaSARRI) for 2014 season. Cotton variety BPA2002, was sown on 16th, June, 2014 in plots, measuring 40 x 60 m^2. Cotton was sown at 4 seeds/hole at soil depth of 2.5 cm. Plant to plant and row to row distances were kept at 30 cm and 75 cm, respectively. Recommended agronomic practices were applied throughout the growing period of the crop. No pesticides were applied to the crop. Densities of insect pests were recorded on ten days interval on randomly selected leaves from top, middle and bottom of the 30 plants selected. For counting the insect pests, the leaf was gently held at the petiole by thumb and fore finger and turned until the entire underside of Leaf was clearly visible. Data were recorded at morning hours (8-10 am) because at that time winged pests were Sluggish and could easily be counted. The insects were recorded from 2nd week of July till 4th week of November. The data recorded was subjected to mean values of individual plants for ten days interval

Data collection

Assessment of pests occurrence on cotton

On each sampling date, bollworm larval densities, Aphid counts, mite counts were evaluated on 20 randomly selected cotton plants per field in both 2013 and 2014 seasons respectively. Boll worm larvae collected were placed in plastic containers, reared in the laboratory until adult emergence, counted and added to the sweep net catches. For aphids and mite records were taken from upper, middle and lower leaves of randomly selected twenty plants (Shah *et al.,* 2015). Adult boll worm moths, stainers and white flies were also collected using a sweepnet, where each field was

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divided diagonally and in the early morning and late evening insects were collected by sweeping through the field, the catches collected, separated by species, starved to death and then placed in alcohol for further morphological identification.

Data analysis

Data was analyzed by ANOVA using general linear model procedures at 5% level of significance. Meteorological parameters like temperature and relative humidity during the study period were recorded. Correlation analysis was made to study the relationship between weather parameters and incidence of major insect pests of cotton by following standard statistical methods.

III. RESULTS AND DISCUSSION

A) Survey on occurrence of cotton pests in major cotton growing districts

Generally 2014 season had significantly higher pest infestation and occurrence than 2013 (Figure2-5) season.

Aphid Occurrence

Comparison of aphid infestation and occurrence in the 13 districts surveyed showed significantly higher counts in Kasese and Serere and Wakiso districts in both 2013 and 2014 seasons at ($P<0.05$). Mpigi district had the highest aphid infestation in 2014 than any other district with lower infestation in 2013 season. The district of Pallisa, Masindi, Mukono, Kamuli, Jinja, Nakasongola, Kiryandongo, Amuria and Amolator showed relatively higher aphid counts albeit at varying levels (Figure 2).

The highest aphid occurrence observed could be due to the fact that cotton farmers in Serere, Kasese, Mpigi, Wakiso and Pallisa mainly grow cotton in a monocroping system which eased host searching ability and distribution of the aphids than the rest of the districts surveyed which had cotton intercropped with mainly legumes and vegetable crops or a vegetable crop within the vicinity of the cotton field. The results of this study support findings by Chabi *et al* (2005) and Kalidas *et al* (2012) who found intercropping to reduce the host searching ability of pests than in the monocrops for field cropping systems

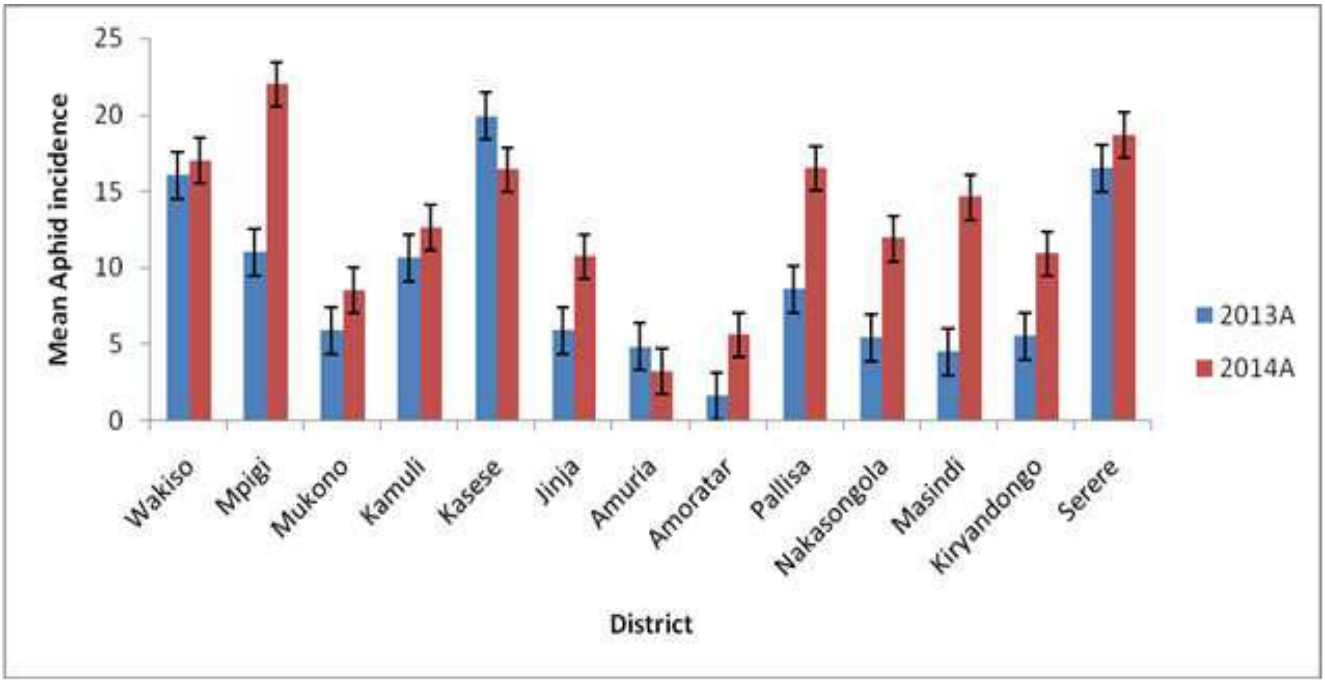

Fig.2: Mean Aphid occurrence in cotton agro ecologies in 2013 and 2014

Cotton stainer occurrence

Like aphids, 2014 season showed higher stainer infestation compared to 2013 season (Figure 3). Highly significant differences were observed ($P<0.05$) with highest stainer counts in Mukono, Serere, Pallisa and Kasese districts respectively. Higher occurrences were observed in Kamuli, Jinja, Amuria, Amolator, Masindi and Kiryandongo with no significant differences between the two cropping seasons. Nakasongola, Mpigi and Wakiso districts showed lower stainer counts in both 2013 and 2014 respectively. Cotton stainers in Uganda were known to feed on cotton, hibiscus and Okra (B. sekamatte *et al* 2003). This spread and level of occurrence in the surveyed districts could be an indicator that the pest now has a wider host range hence the higher

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counts observed in all the 13 districts surveyed since in the study cotton was planted both in monocropped and intercropped systems respectively.

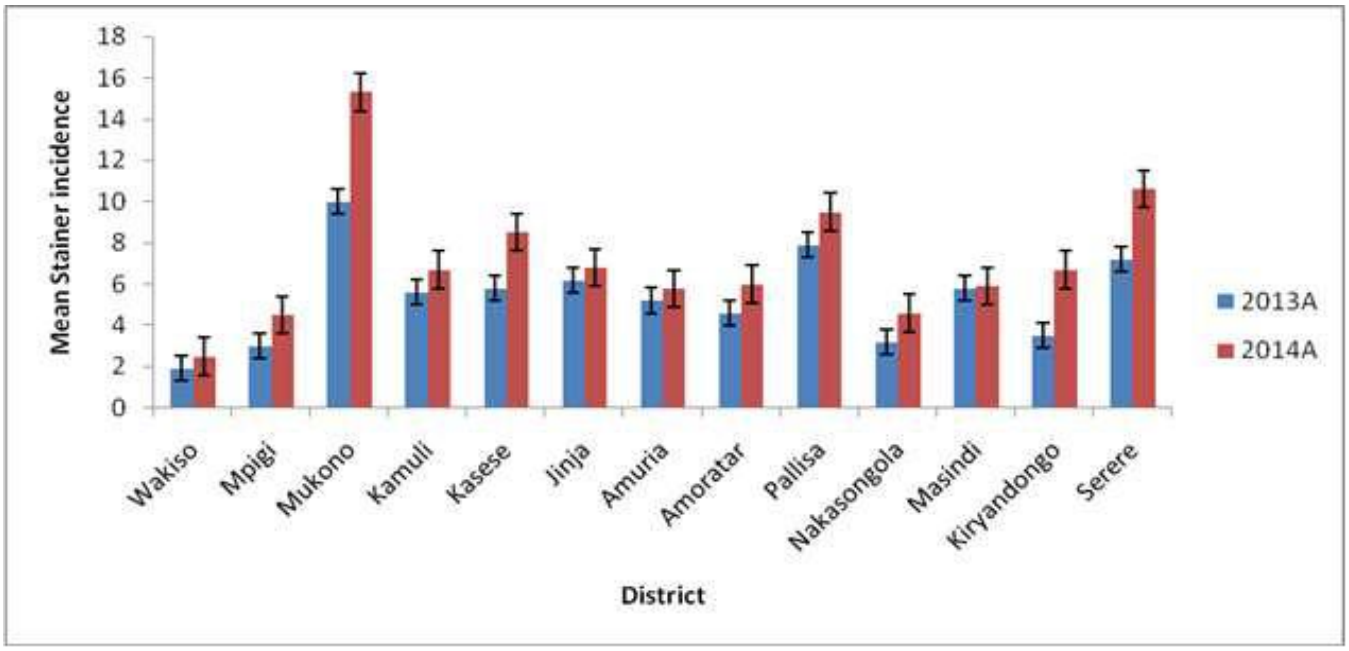

Fig.3: Mean cotton stainer occurrence in cotton agro ecologies in 2013 and 2014.

Mites Occurrence

For mites, the highest occurrence was recorded in Wakiso, Kasese, Amolator, Mukono, Pallisa, Masindi, Serere and Mpigi districts respectively (Figure 4). Unlike the other pests surveyed in the study, both 2013 and 2014 seasons showed higher mite occurrence which differed for each district with no significant differences between the cropping seasons ($P<0.05$). The survey results showed that mites were distributed throughout the 13 cotton growing districts with very high occurrences. The highest occurrence for the mite pest observed in the 13 districts surveyed supports findings by Hui Lu *et al* (2012) who found that although this pest has a low dispersive capacity, its spread increased with the rapid exchange and development of planting materials. This situation was also true for the districts surveyed where there was free exchange of planting materials for all crops between farmers hence the higher occurrences observed during the survey.

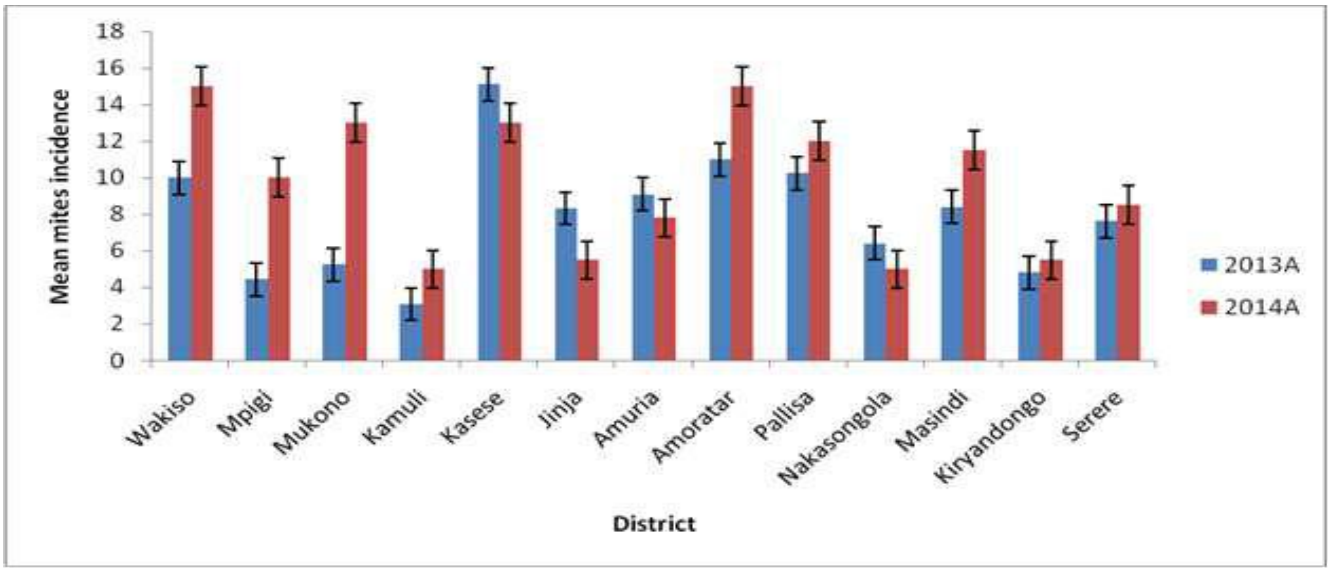

Fig.4: Mean mites occurrence in cotton agro ecologies in 2013 and 2014

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Cotton bollworm occurrence

Bollworm infestation and occurrence showed significant differences with highest counts recorded in Kasese and Pallisa districts (Figure 5). Serere, Nakasongola, Kiryandongo, Masindi, Amolator and Amuria districts showed higher bollworm occurrences with no significant difference between the two cropping seasons($P<0.05$). Tahvanainen and Root (1972) stipulated that intercropping lowers pest densities by reducing immigration into the crop or increases emigration from the field.

The observed difference in cotton bollworm populations in the surveyed field could be due to the fact that Kasese, Pallisa and Serere grow cotton as a sole crop. Another reason could be the continuous use of pesticide in Kasese and Pallisa and Serere which might have led to bollworms developing resistance to pesticide use hence the observed difference during the survey compared to other districts surveyed where pesticide use is minimal. The results of this study support findings by Kranthi *et al* (2005) who found bollworms to develop resistance to pesticides very fast and thus high population build up in cotton pesticide growing districts of India.

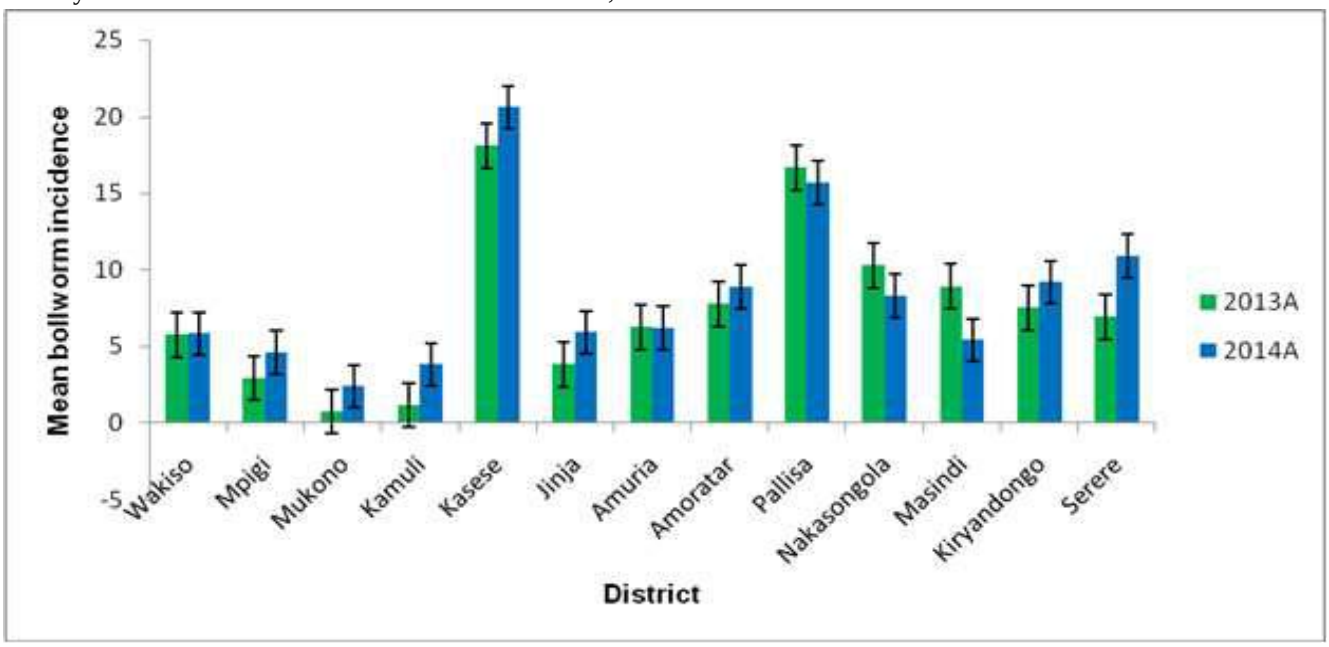

Fig.5: Mean bollworm occurrence in cotton agro ecologies in 2013 and 2014.

b) Pest population density counts during the cotton crop growth cycle in the field experiment

Results of observations on population fluctuations of sucking cotton pests made at various growth stages of the cotton crop at NaSARRI in 2014 are presented in Table 2 and 3.

Mites Infestation

Mite infestation begun as early as July in the season and increased rapidly till it peaked (38.4 individual mites per plant) on September 01st.2014 (Table 2). In general infestation was low and below ETL before end of August, then the population peaked in September with higher infestation up to late November and declined thereafter. The data on correlation between mite infestation and weather parameters showed that temperature had a positive relationship with mite population (0.59) and a negative relationship with relative humidity(-0.133) (Table 3).

Results of our study are supported by the findings of Shahid *et al* (2012) who reported that cotton mite populations were favoured by higher temperatures. The results described in Table 3 depicted that increasing temperature favoured the mite population growth with relative humidity exerting a negative correlation with the mite population. These research findings are in agreement with findings of Khan & Ullah (1994), who observed a negative relationship between population buildup of *Tetranychus urticae* and the mean relative humidity and crop growth reduction due to mite infestation was greater in early than in late infested crops.

Aphids Infestation

Aphid incidence was observed at varying intensities ranging from 0.52-39.2 individuals per plant with a mean of 17.95 aphids per plant. However, peak incidence was observed from 01st.08.2014 up to 01st.11.2014 and declined thereafter (Table 2). Aphids exhibited a positive correlation with

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temperature (0.256) and a negative response to relative humidity (-0.59) (Table 3). The present findings are in conformity with the findings of Ashfaq *et al* (2011) who reported population of *A.gossypii* throughout the cotton growth period.

White flies infestation

Like aphids, whiteflies population also varied significantly ranging from 0.4-35.0 individuals per plant with a mean of 15.-6 whiteflies per plant (Table 2). Unlike the aphids, the whiteflies infestation begun low early in the season (whole month of July), population peaked in the last week of August 2014 (35 whiteflies per plant) and remained consistently higher throughout the growing period. Whiteflies populations were positively correlated with temperature (0.312) and relative humidity (0.42) (Table 3). Findings of our study were in agreement with Shahid *et al* (2012) and Seif (1980) who also got similar results.

Jassids Population

The highest jassid population was observed in the second week of October with the peak population (17.5 Jassids per plant) recorded on 20th.10.2014. In general jassid population remained above ETL from 30th.08.2014 onwards (Table 2). Correlation studies (Table 3) showed that relative humidity exerted positive pressure (0.60) on jassid population with temperature exerting a negative relationship (-0.262). Our findings are in agreement with findings of Shahid *et al* (2012) and partially in agreement with findings of Khan & Ullah (1994). However the slight variations from Khan and Ullah (1994) could be due to difference in materials and methods used.

Cotton stainer Population

Cotton stainers infestation started in the 3rd week of august 20th.08.2014 and gradually increased up to peak value of 10.5 stainers per plant in the last week of September 30th.09.2014 (Table 2). On average mean cotton stainers per plant were 4.70 and the population steadily increased up to 30th.12.2014. Incidence of stainer population showed a negative relationship with temperature and relative humidity (-0.322,-0.193) respectively (Table 3).our study confirmed findings by David S *et al* 1965 who reported cotton stainers (*Dysdercus fasciatus* Sign.) being hygrosensitive to both dry and moist air, but not to intermediate relative humidities.

Table.2: Fluctuation of sucking insect pest population on cotton in 2014 season

Months	Mites	Aphids	Whiteflies	Jassids	Stainers
1-Jul	0.69	0.85	0.4	0	0
10-Jul	5.25	9.80	1.45	0	0
20-Jul	7.42	8.70	5.32	0	0
30-Jul	8.36	26.2	5	0	0
1-Aug	14.5	39.2	6.64	0.96	0
10-Aug	20.9	23.1	6.43	0.75	2.3
20-Aug	19	30.2	22.3	2.2	3.3
30-Aug	26.9	38	35	5.2	4.5
1-Sep	38.4	35.1	10.8	6.08	5.3
10-Sep	13.3	20.4	3.5	9.6	8.6
20-Sep	20.6	18.4	11.5	10.5	8.5
30-Sep	25.5	21.5	22.5	5.3	10.5
1-Oct	28.4	26.5	18.9	12.1	7.2
10-Oct	35.3	29.6	20.6	17.5	5.4
20-Oct	25.5	24.1	18.2	14.2	9.3
30-Oct	6.5	19.2	17.5	8.6	8.9
1-Nov	10.3	36.3	11.6	10.3	5.5
10-Nov	23.1	7.2	20.3	6.1	6.8
20-Nov	30.3	8.5	22.1	3.1	4.3
30-Nov	5.5	4.1	11.2	1.8	9.3
1-Dec	0.64	2.3	10.9	6.8	5.8

10-Dec	0.28	0.56	17.6	3.55	5.6
20-Dec	0	0.35	18.4	4.7	3.3
30-Dec	0	0.52	12.3	1.75	2.5
Mean	15.3	17.95	15.6	5.24	4.70
P(0.05)	0.56	0.002	0.002	0.004	0.006

Table.3: Correlation between Temperature, Relative humidity and insect pest population on cotton in 2014 season

Parameters	**Temp.°C**	**RH%**
Mites	0.59*	-0.133
Aphids	0.256**	-0.59*
Whiteflies	0.312**	0.42
Jassids	-0.262**	0.60*
Stainers	-0.322**	-0.193

* Correlation is significant at $P \leq 0.05$; ** Correlation is significant at $P \leq 0.01$

IV. CONCLUSION

In the present research work a general investigation of the distribution of major cotton pests has been conducted, especially on the occurrence and the potential geographical distribution areas of these pests in Uganda with districts showing higher occurrences as hotspots for a particular pest ie, in our study, high aphid occurrences were observed in Kasese, Serere, Wakiso and Mpigi districts respectively, Stainers(Mukono ,Serere, Pallisa and Kasese districts),Mites showed higher occurrences in all the districts surveyed with highest counts of bollworms observed in Kasese and Pallisa districts.

Similarly, for the seasonal fluctuation study, mites (*Tetranychus urticae*), peaked (38.4 individuals per plant), in the first week of September, Aphids (38 individuals per plant) in the last week of August, White flies (35 individuals per plant) in the last week of August, Jassids (17.5 individuals per plant) in the second week of October, and stainers peaked (10.5 individuals per plant) in the last week of September.

These findings are of great relevance by providing base line data on the status of major cotton pests in terms of species distribution and abundance in cotton agro ecological zones of Uganda and provides a better understanding of how insect pest population dynamics and seasonal weather variation mechanism can fine-tune pest management strategies and response to pest attacks for adopting their IPM strategies. This study also focused on ecological principles that are cheap and sustainable, relying on need/timing for control of insect pests of the cotton crop

V. RECOMMENDATIONS

The districts surveyed appeared to be hot spots for a particular pest and farmers should now employ integrated pest management in order to reduce the pest pressure in their regions. This should be employed with proper timing of when a particular pest becomes a threat to the cotton crop based on the seasonal fluctuation data obtained.

We also recommend that in order to control major *cotton pests*, effectively, we should investigate their biological characteristics and ecology. Further still use of molecular methods to determine levels of genetic diversity and population genetic structure, which will contribute to the elucidation of the population structure and history of these pest should be investigated.

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Radioprotective action of venom of honey bee Apis mellifera Caucasica

Topchiyeva Shafiga, Babayev Elmar

Institute of Zoology of NAS of Azerbaijan, Baku
Adress: AZ1073,Baku, Sabail, Abbasgulu Abbaszadeh,115

Abstract**—** *The paper presents experimental data on the influence of the product of the activity of the honey bee Apis mellifera Caucasica on the life span of experimental animals irradiated with small doses of gamma radiation.*

The aim of the studies was to study the radioprotective effect of the pre-introduced zootoxin Apis mellifera Caucasica with a single gamma irradiation of 60Co mice at doses of 1.3, 5, 7, 10 Gy at irradiation dose rates of 1 Gr / min.

Injection of venom followed by gamma irradiation of 60Co at a dose of D = 1, 3, 5 and 7 Gy at an irradiation dose rate of 1 Gy / min increased the life span of the experimental groups of mice ranging from 45% to 56 % and from 52% to 67%, respectively.

An increase in the lifespan of experimental animals exposed to radiation with the preliminary introduction of the venom of the honey bee Apis mellifera Caucasica was revealed.

Keywords— honey bee, Apis mellifera Caucasica, venom, radioprotective action.

I. INTRODUCTION

The problem of anti-radiation protection is becoming increasingly important in connection with the expansion of the use of ionizing radiation sources in various areas of human activity.

Important tasks of modern pharmacology are the search and development of new effective medicines for the prevention and treatment of various diseases, the study of the mechanisms of their action in animal experiments and the scientific rationale for rational schemes for their application. One of the most severe pathologies, requiring intensive pharmacotherapy and prevention, are radiation injuries arising from acute external radiation exposure [1, 2]

At present, radioprotectors are the most studied and highly effective medical means of radiation protection [10, 15]. However, their use is limited to the periods of use (exclusively up to radiation exposure), often by a small therapeutic breadth and, as a consequence, enough high toxicity in optimal radioprotective doses. Preparations of cytokines and growth factors are considered as the most promising pharmacological agents for early therapy of radiation pathology [3, 4, 5, 6].

Prevention of adverse effects of irradiation in hazardous doses to humans is achieved through the use of preventive anti-radiation means of radioprotectors.

However, existing radioprotectors do not always meet the requirements for efficacy and tolerability [7, 8].

In this regard, both in our country and abroad, the search for new radioprotectors from various classes of chemical compounds, as well as compounds of natural origin, continues.

During the study of the radioprotective effect of anticoagulants, it was found that heparin in the recommended radio-protective dose of 250 U / kg raises the survival rate of irradiated animals with respect to control by 40-50%.

As the dose decreases by 1 and 2 orders of magnitude, the effectiveness of the drug decreases and when administered at a dose of 2.5 U / kg, the survival gain was only 10%. However, the subsequent reduction in the dose of heparin to 0.25 U / kg was again accompanied by an increase in its radioprotective effect:

Survival increased to 40-50%, in peripheral blood not only the number of neutrophilic leukocytes, but also the content of cationic proteins in them (according to the lysosomal cation test) significantly increased [9].

No less active is the study of the radioprotective action of zootoxins and preparations of animal origin. In the research of A.S. Koryagina (2006) the radioprotective effect of honey bee venom was studied in detail. For this purpose, rats were injected intraperitoneally with a bee venom at a dose of 0.1 μg / kg for 7 days at a frequency of 1 time per day.

A single total gamma irradiation (60Co) at a dose of 3 Gy (dose rate of 1 Gy / min) was performed 7 days after the end of injections in the first series of experiments, after 14 days in the second series, 21 days later in the third series and 28 Days in the fourth series of experiments. The radioprotective effects of the bee venom were most pronounced in the first 3 weeks after the end of the injections of zootoxin. During this period, bee venom significantly increased the total number of surviving bone marrow cells. On the 28th day after the injection of

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venom, there was a significant decrease in its anti-radiation activity, although it did not disappear completely. The author believes that the radioprotective effect of bee venom is associated with the formation of a nonspecific adaptation reaction [12].

Thus, analyzing the sources of modern literature, it should be noted that domestic and foreign scientists have studied the radioprotective properties of drugs from various pharmacological groups, but currently the search for radioprotectors is actively continuing. All this is due to the relatively low radio-protective efficacy of the substances studied, the toxic properties of radioprotectors and the inability to use them for a long time. However, it is necessary to note the prospects for solving this issue, which may be due to the combined use of radioprotective drugs from various pharmacological groups.

Bee venom Apis mellifera is a complex mixture of enzymes and polypeptides with low molecular weight, including enzymes phospholipase A2, hyaluronidase, phosphomonoesterase, acid esterase, D-glucosidase, lysophospholipase, A-galactosidase, α-acetylaminodeoxy glucosidase and arylamidase

It has been revealed that the effect of bee venom on the body shows a decrease in the protein content in the blood serum, which is due to the effect of poison on vascular permeability.

The lethal dose of bee venom for humans is 1.4 mg per 1 kg of body weight. The lethal outcome comes most often from the paralysis of the respiratory center [13, 14].

Because of anticoagulant and anti-inflammatory properties, bee venom is mainly used to treat many inflammatory diseases such as arthritis, bursitis, herpes zoster, joint disease and rheumatoid arthritis Lyme disease, multiple sclerosis and osteoarthritis [16, 17, 18, 19, 20, 21, 22],

Proceeding from a few studies on the effect of bee venom on the life expectancy of experimental animals exposed to radiation a number of issues remain unexplored and is of great scientific and practical interest.

Analyzing the literature data on the degree of study of the venom of honey bees, the aim of the studies was to study the radioprotective effect of the pre-introduced zootoxin Apis mellifera Caucasica with a single gamma irradiation of 60Co mice at doses of 1.3, 5, 7, 10 Gy at irradiation dose rates of 1 Gr / min.

II. MATERIAL AND METHODS

The material of the study was ecologically pure whole poison collected from bees from apiaries, located on ecologically clean territory of Azerbaijan and venom irradiated with small doses of gamma radiation. After taking, venom was stored in a desiccator over a couple of calcium chloride. Water solutions of venom were prepared immediately before the experiment. The choice of doses that cause the development of the adaptation reaction of stable activation is due to the corresponding literary data (Koryagin, Erofeeva, 2004). For mice, these doses are 8-10 mg / kg of bee venom. In studies of A.Koryagin (2006), when studying the radioprotective effect of honey bee venom in experiments on rats, bee venom was injected at a dose of 0.1 μg / kg [11].

Proceeding from the above, the bee venom dissolved in physiological solution in a dose of 0.1 μg / kg, 0.2 μg / kg, 0.5 μg / kg, 1.0 μg / kg and 2.0 μg / kg was injected to animals of control groups to study the radioprotective action of the bee venom. A single total γ-irradiation of 60Co mice was carried out in doses of D = 1, 2, 3, 5, 7 Gy at irradiation dose rate of 1 Gy / min. The experiments were carried out in 5 series of experiments in vitro.

III. THE RESULTS OF THE RESEARCH AND THEIR DISCUSSION

We have considered the prevention of radiation damage to experimental animals by venom of the honey bee that occurs when external mice are irradiated.

Table.1: The survival of mice with intraperitoneal injection of bee venom followed by a single γ-irradiation of 60Co

The dose of irradiation, Gy	Groups of experimental mice (venom dose in μkg / kg)				
	control	0.01	0.02	0.04	0.05
	Life expectancy in days				
1	40	45	49	53	58
3	35	37	42	46	49
5	23	26	27	31	35
7	18	21	24	27	28

In order to study the radioprotective effect of the honey bee venom, a control group of mice was irradiated at D = 1, 3, 5, and 7 Gy. Experimental groups of 2-3-month-old white mongrel mice with a total body weight of 18-22

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grams were first intraperitoneally injected with bee venom at a dose of 0.01, 0.02, 0.04 and 0.05 mg / kg of body weight, 3 days at a frequency of once a day. Then, the first experimental group of mice was subjected to a single gamma irradiation of 60Co in a dose of D = 1 Gy at a dose rate of 1 Gy / min after 3 days, the second experimental group of mice after 3 days subjected to a single gamma irradiation of 60Co in a dose of D = 3 Gy at a radiation dose rate of 1 Gy / min. The third experimental group of mice was subjected to a single γ-irradiation of 60Co in a dose of D = 5 Gy at a dose rate of 1 Gy / min 3 days after irradiation, and the fourth experimental group of mice, 3 days after irradiation, subjected to single 60Co γ-irradiation at a dose of D = 7 Gy radiation at a dose rate of 1 Gy / min, the fifth experimental group of mice 3 days later were subjected to single 60Co γ-irradiation at a dose of D = 10 Gy radiation at a dose rate of 1 Gy / min.

The survival of mice with intraperitoneal injection of bee venom at a dose of 0.01, 0.02, 0.04 and 0.05 mg / kg of body weight followed by a single γ-irradiation of 60Co in a dose of D = 1 Gy at a dose rate of 1 Gy / min is given in Table 1.

The following 5, 6, 7, and 8 groups of experimental mice were injected intramuscularly with bee venom at a dose of 0.1 mg / kg body weight followed by a single γ-irradiation of 60Co irradiation at a dose of D = 1 Gy at dose rate of 1 Gy / min.

The survival of mice with intramuscular injection of bee venom at a dose of 0.01, 0.02, 0.04, and 0.05 mg / kg of body weight followed by a single γ-irradiation of 60Co at a dose of 1, 3, 5 and 7 Gy at an irradiation dose rate of 1 Gy / min (table 2).

Table.2: The survival of mice with intramuscular injection of bee venom followed by a single γ-irradiation of 60Co

The dose of irradiation, Gy	Groups of experimental mice (venom dose in μkg / kg)				
	control	0.01	0.02	0.04	0.05
	Life expectancy in days				
1	40	47	51	55	61
3	35	38	44	47	50
5	23	27	29	33	36
7	18	23	26	28	30

The following 9, 10, 11, 12, and 13 groups of experimental mice were injected intraperitoneally with bee venom at a dose of 0.01, 0.02, 0.04 and 0.05 mg / kg of body weight after a day, subjected to a single gamma irradiation of 60Co at a dose of D = 1, 3, 5 and 7 Gy at an irradiation dose rate of 1 Gy / min.

Table.3: The survival of mice with intraperitoneal injection of bee venom followed by a single γ-irradiation of 60Co

The dose of irradiation, Gy	Groups of experimental mice (venom dose in μkg / kg)				
	control	0.01	0.02	0.04	0.05
	Life expectancy in days				
1	40	42	45	48	52
3	35	36	39	43	48
5	23	25	26	29	33
7	18	19	21	23	25

The survival rate of mice with intraperitoneal injection of bee venom at a dose of 0.01, 0.02, 0.04 and 0.05 mg / kg of body weight, followed by (after one day) by a single gamma irradiation of 60Co at a dose of D = 1, 3, 5 and 7 Gy at an irradiation dose rate of 1 Gy / Min is shown in table 3.

The following 14, 15, 16, and 17 experimental groups were intramuscularly injected with bee venom at a dose of 0.01, 0.02, 0.04 and 0.05 mg / kg of body weight after a day, subjected to single γ-irradiation of 60Co at a dose of D = 1, 3, 5 and 7 Gy at Dose rate of 1 Gy / min.

The survival of mice with intramuscular injection of bee venom at a dose of 0.01, 0.02, 0.04 and 0.05 mg / kg of body weight with a single (after a day) γ-irradiation of 60Co in a dose of D = 1, 3, 5 and 7 Gy at a dose rate of 1 Gy / Min are given in Table 4.

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Table.4: The survival of mice with intramuscular injection of bee venom followed by a single γ-irradiation of 60Co

The dose of irradiation, Gy	Groups of experimental mice (venom dose in μkg / kg)				
	control	0.01	0.02	0.04	0.05
	Life expectancy in days				
1	40	43	46	49	54
3	35	38	40	45	49
5	23	26	27	30	35
7	18	20	23	25	27

An increase in the total number of surviving animals was noted in all the groups studied, but the nature of the change in the lifespan of mice differs both from the method of administration and from the time of administration of the poison after gamma irradiation of 60Co.

In experiments on mice with intraperitoneal or intramuscular fractional injection of venom followed by a single γ-irradiation of 60Co at a dose of A = 1, 3, 5 and 7 Gy, an increase in the lifespan of experimental groups of mice was noted at an irradiation dose rate of 1 Gy / min.

The survival rate of the experimental groups of mice, in relation to the control group, ranged from 45% to 56% and from 52% to 67%, respectively.

Survival of the experimental groups of mice, with respect to the control group with a single intraperitoneal or intramuscular injection, increased within the range of 30% to 39% and 35% to 50%, respectively, 24 hours after the injection of venom.

We believe that the radioprotective effect of bee venom is associated with the formation of a nonspecific adaptation reaction.

Thus, we detected a radioprotective effect of the honey bee venom, which manifests itself in an increase in the lifespan of experimental animals subjected to γ-irradiation of 60Co.

For the first time it was revealed that the injection of venom is accompanied by a prolonged radioresistance, reducing the effect of ionizing radiation on the life span of mice under conditions of a single gamma irradiation.

Consecutive introduction of bee venom to radiation exposure and in the early periods after irradiation allows to increase the survival time of experimental animals subjected to irradiation.

Investigation of the radioprotective effect of the course injection of small doses of bee venom under conditions of a single fractionated gamma irradiation makes it possible to broaden the notion of nonspecific radioresistance and suggests the possibility of creating new preparations on the basis of biologically active substances of animal origin that enhance the radioresistance of the organism.

The practical significance of using bee venom in small doses is that they can be considered as a drug of choice in conditions of fractionated and, possibly, chronic exposure to ionizing radiation.

Radioresistance, which develops in the body in response to multiple injections of bee venom, can successfully protect the body from fractionated gamma irradiation.

IV. CONCLUSIONS

1. The increase in the lifespan of mice differs from the method of injection, and the time of injection of the venom after γ-irradiation.
2. Intraperitoneal or intramuscular fractional injection of venom followed by a single gamma irradiation of 60Co at a dose of D = 1, 3, 5 and 7 Gy at an irradiation dose rate of 1 Gy / min increased the life span of the experimental groups of mice ranging from 45% to 56 % And from 52% to 67%.
3. At a single (after 24 hours) intraperitoneal or intramuscular injection of the venom, the life span of the experimental groups of mice increased from 30% to 39% and from 35% to 50%, respectively.

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Study of the Monthly and Annual Behavior of Temperature and its Impact on Climate Change in Iraq for the Period (1982-2012)

Dr. Osama T. Al-Taai[1], Jamal S. A. Al-Rukabie[2], Iqbal H. Abdalkareem[3]

[1]Department of Atmospheric Sciences, College of Science, University of Al-Mustansiriyah, Baghdad, Iraq
[2]Department of Natural Sciences, Collage of Basic Education, University of Sumer, Thi-Qar, Iraq
[3]Envirnomental Research and Studies Center, University of Babylon, Babylon, Iraq

Abstract— *Temperature in Iraq is an important meteorological factors because of its great impact on the daily life of human in terms of health, work and others. This research aims at identifying and studying the climate change in the study stations and during the specific period of time and its future impact on the climate of Iraq. This study analyzes the behavior of monthly and annual temperature data obtained from the (ECMWF) for selected stations from Iraq (Mosul, Baghdad and Basrah), which represent (Northern, Middle and Southern) Iraq, respectively, for the period of one and thirty years (1982-2012) and found the relationship between the temperature with period of study from 1982 to 2012 using some statistical methods (SLR and Rsqr), The lowest monthly average of temperature was in DEC and the highest monthly average of temperature was in JUL in all stations of the study, and found that the lowest annual average of temperature was in 1992 and the highest annual average of temperature in 2010 and for all stations, and found that there is a change climate in the month of MAY of Spring and the month of SEP of the autumn with the summer months (JUN, JUL and AUG), and found that there is a clear increase in the annual average temperature during the study period, where the Rsqr for Mosul station was (R^2=0.4), Baghdad station was (R^2=0.5) and Basrah station was (R^2=0.4), with the possibility of dividing study stations (Mosul, Baghdad and Basrah) into three regions climate in terms of total annual average of temperature (low, high, and very high) respectively, and Predictability of future drought in Iraq.*

Keywords— Temperature, Climate change, ECMWF, Meteorological factors, Iraq.

I. INTRODUCTION

Temperature can be defined as a form of energy. It is one of the most important components of the climate. It has a direct impact on human activity, clothing, housing and food, as well as other elements of the vital system. Temperature affects most climate elements such as atmospheric pressure, wind, evaporation, and relative humidity. Climate scientists have recently been interested in the subject of changing the climate of the Earth where scientists have tried to determine the nature of climate change and its reasons. The change is the shift from one case to another. It is different from the oscillation (which is about the rate of the situation and for a short period). The case rate continues for decades, and climate change as defined by the Intergovernmental Panel on Climate Change (IPCC) is a climate change that can be determined by the use of statistical tests, for example the change in the average and that this change continues for a long period of decades [1]. There is also another definition of climate change that is directly or indirectly attributable to human activity, which changes the composition of the atmosphere. It should be noted that the atmosphere consists of the two groups of the gas group and the non-gas group [2]. The reason why scientists are interested in changing the Earth's climate is the obvious effect on natural phenomena and its implications for human activity. The cause of climate change is due to natural internal processes or to external cosmic effects (solar radiation processes and solar energy) or continuous human changes in the composition of the atmosphere [3].

- **Causes of Climate Change**

The causes are divided into two groups: natural causes and human causes. Some scientist's divide them into two groups of external causes, namely, astronomical and the group of internal causes, namely natural or human, or both [4] [5]:

a) Natural causes include theories:
1. Displacement of continents theory.
2. Volcanic dust theory.
3. Solar Spots theory.

b) Human causes include theories:
1. Carbon dioxide theory.
2. Human dust theory.
3. Air pollution theory.

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- **Geographical Distribution of Temperature**

The main features of temperature distribution in summer and winter are [6]:

1. The world's hottest regions for the summer are the tropics, where the average temperature exceeds 30ºC in some areas. Tropical regions are below 25ºC
2. The temperature of the tropics remains high during the winter as in the summer, while the temperature of the tropics falls below 15ºC.
3. Equivalent heat lines are generally parallel to the circuits for special displays during the summer, in the northern half where the effect of overlapping between land and water is less.
4. The temperature generally decreases as we move towards the Polar Regions, and the rate of decline rapidly during the winter and slow during the summer.
5. The coldest places in winter are located in the middle of the continents away from marine influences.
6. There is no counterpart to this extreme heat in the southern half because of the high proportion of water surfaces.

The most important factors affecting the geographical distribution of temperature on the surface of the earth can be summarized as follows [7] [8]:

1. Latitude angle.
2. Distribution of land and water.
3. Terrain.
4. Marine currents.
5. Prevailing winds.
6. Declinational angle.

- **Daily Temperature Path**

The days that are free of the succession of air mass and fronts of the airways are characterized by a regular course of temperature. The temperature starts to rise since the sun rises and continues to rise to the back two hours or more depending on the location of the place and its proximity to the sea. In the afternoon, the temperature will decrease steadily until it reaches its lowest level before the sunrise the next day, as in "Fig. 1". The daily path of the temperature is a clear reflection of the daily path of the solar radiation that starts from the sun rising, until it reaches its maximum noon time, and then takes the decrease thereafter, until it stops completely at sunset. The day-to-day path of solar radiation and the daily path of temperature are not exactly the same. The daily path of temperature remains too far away from the daily path of solar radiation. Some of the time in marine areas is two hours. The daily temperature trajectory can be explained by the daily path of solar radiation, as the temperature is the result of the thermal balance of the surface of the earth and the air near it.

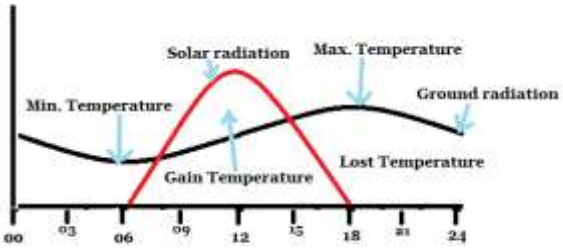

Fig.1: Daily temperature path [9].

- **Daily Fluctuations in Temperature**

The daily path of the temperature does not follow the daily path of solar radiation, especially during the winter. As the control of the weather a number of factors, including the succession of air mass and fronts airways. Therefore, the daily path of the temperature may be completely obscured and instead shows fluctuations in the temperature of one of them or shorten depending on the direction of wind and movement fronts, and so on. In fact, sudden fluctuations in temperature associated with the rotation of air fronts are more pronounced and have a greater impact on human health and activity than the regular daily course of temperature, especially as these fluctuations may be very acute.

- **Annual Temperature Path**

The annual path follows the annual path temperature of solar radiation, as in the daily path, the annual path of temperature remains far behind the annual path of radiation. The length of time in which it is delayed varies depending on whether the area is continental or marine. Ranging from one month or less in continental areas, and nearly two months in marine areas [9].

II. METHODOLOGY

1. The Statistical Using

- **Simple Linear Regression (SLR)**

Simple linear regression is the study of the relationship between two variables just to get to the linear relationship (i.e. a straight line equation) between these two variables, a parametric test, which assumes that the data are distributed normally distributed and to find out the gradient value is calculated slope of the regression through the linear equation of the following [10]:

$$\bar{Y} = a + b\bar{X} \qquad (1)$$

$$b = \frac{\sum_{i=1}^{n}(X_i - \bar{X}) - (Y_i - \bar{Y})}{\sum_{i=1}^{n}(X_i - \bar{X})^2} \qquad (2)$$

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Where a: Steady decline or part of the cross axis ($\bar{Y}$) to the equation of the straight line (equation 1), b: Slope of the regression and found a slope straight line.

- **Rsqr**

R^2 is the coefficient of determination, the most common measure of how well a regression model describes the data. The closer R2 is to one, the better the independent variables predict the dependent variable.

R^2 equals zero when the values of the independent variable does not allow any prediction of the dependent variables, and equals one when you can perfectly predict the dependent variables from the independent variables [11].

2. The Data Source and Study Stations

Were used the data for monthly averages of temperature from The European Centre for Medium-Range Weather Forecasts (ECMWF) for a period of thirty one years (1982-2012) [12]. Were calculated Temperature values of three different stations Mosul, Baghdad, and Basrah representing the northern, central and southern regions of Iraq respectively, these stations different in terms of climate change, terrain and altitude from sea surface level (see "Fig. 2" and "Table 1").

Table.1: The latitude, longitude and altitude of the study stations in Iraq [13].

Stations	Latitude (ºN)	Longitude (ºE)	Altitude (meter)
Mosul	36.19	43.09	223
Baghdad	33.14	44.14	34
Basrah	30.34	47.47	2

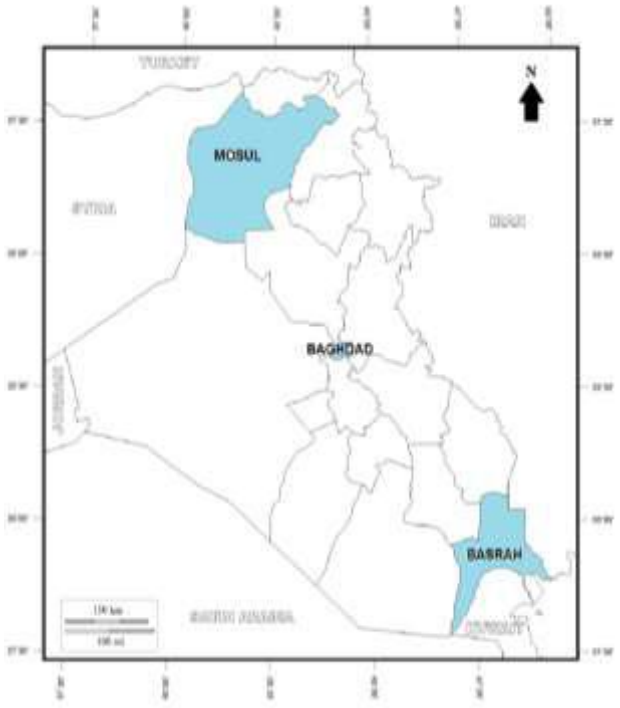

Fig.2: Iraq map, showing stations of the study [14].

III. THE RESULTS AND DISCUSSION

In the "Fig. 3", which shows the analysis of the monthly average temperature data during the study period (1982-2012) of the Mosul, Baghdad and Basrah study stations, where Mosul station was found to have the lowest temperature followed by Baghdad station and the highest temperature recorded at Basrah station during the study years. The highest temperature was in JUL and AUG and the lowest temperature was in DEC and JAN in all the stations of the study.

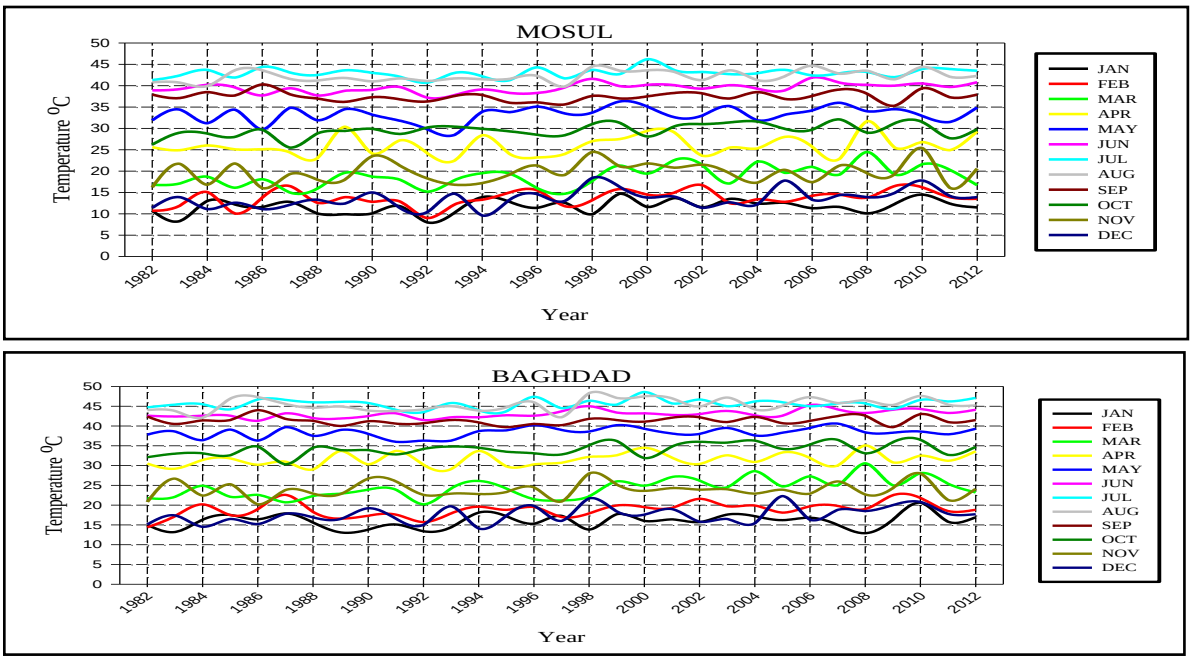

Fig.3: The monthly averages of temperature during the years (1982-2012) for stations (Mosul, Baghdad and Basrah)

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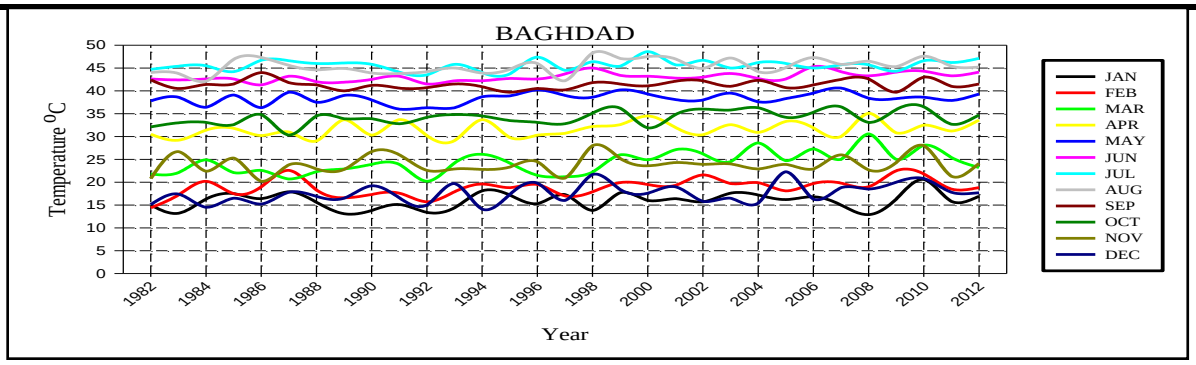

Followed the Fig.3

In the "Fig. 4", which shows the behavior of the monthly average temperature during the period of study one-thirty years (1982-2012) of stations Mosul, Baghdad and Basrah, where found in the Mosul station that the lowest temperature recorded in JAN 11.8ºC and highest temperature was recorded in JUL 43ºC.

At Baghdad Station, the lowest temperature was recorded in JAN 16ºC and the highest temperature recorded in JUL 45.6ºC. At Basrah station, the lowest temperature was recorded in JAN 17.9ºC and the highest temperature recorded in JUL 46.6ºC during the study period.

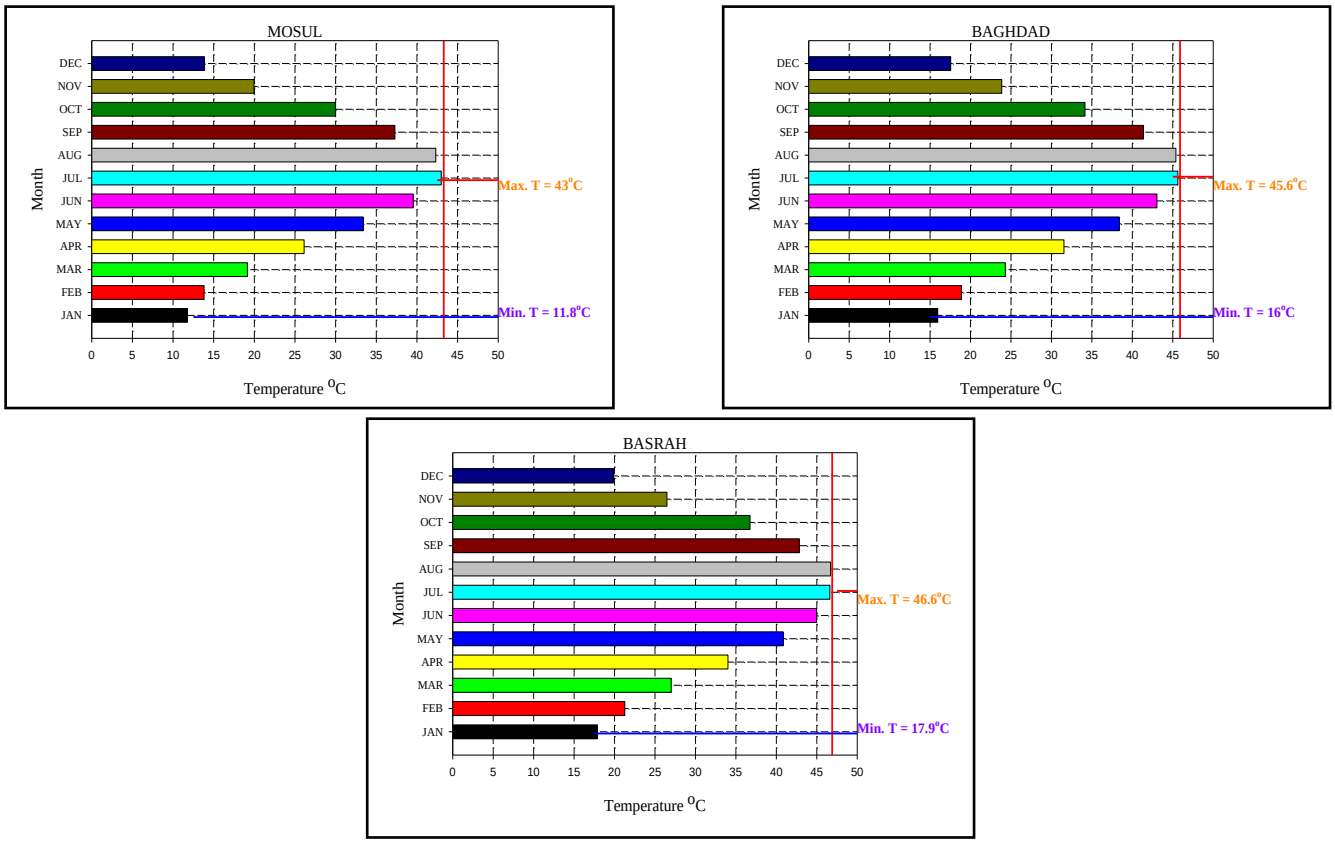

Fig.4: The total monthly average of temperature during one and thirty years from 1982 to 2012 for stations (Mosul, Baghdad and Basrah)

In the "Fig. 5", which shows the behavior of the annual average temperature during the study period one-thirty years (1982-2012) and the study stations Mosul, Baghdad and Basrah where it was found that the lowest temperature recorded in 1992 in all the study stations Mosul, Baghdad and Basrah, 25ºC, 29.8ºC and 32ºC respectively. The highest temperature recorded in 2010 at all study stations Mosul, Baghdad and Basrah, where it was 29.6ºC, 34ºC and 35.8ºC respectively.

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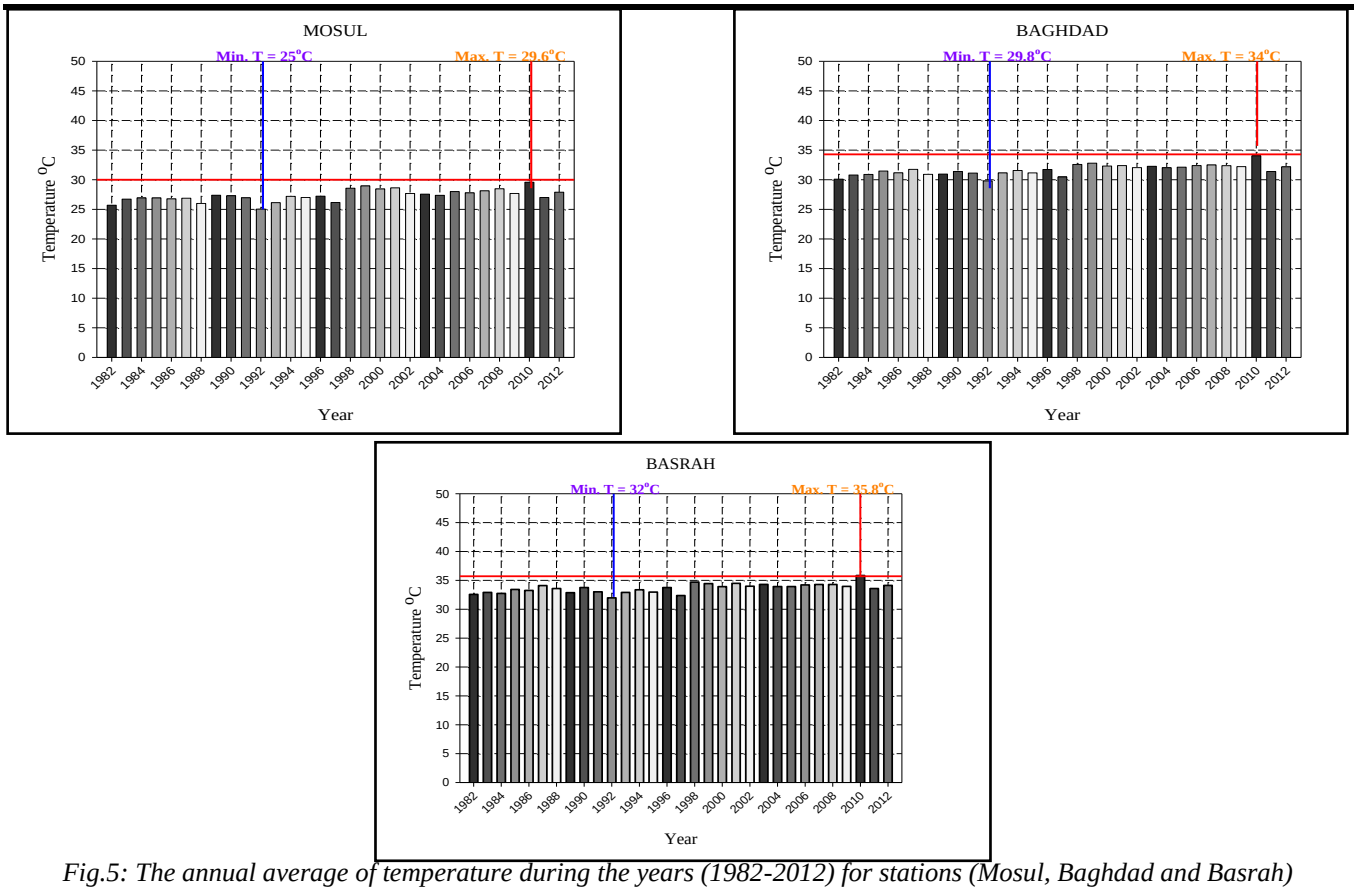

Fig.5: The annual average of temperature during the years (1982-2012) for stations (Mosul, Baghdad and Basrah)

In the "Fig. 6, 7, and 8", showing the temperature during the months of the seasons of the years of study (1982-2012) in stations Mosul, Baghdad and Basrah. The lowest temperature in the seasons was found in winter months (DEC, JAN and FEB) and the highest temperature in the seasons in the summer months (JUN, JUL and AUG), that because winter solstice occurred in 21/DEC and the summer solstice occurred in 21/JUN at all study stations. In spring months (MAR, APR and MAY) and autumn months (SEP, OCT and NOV), temperatures were moderate and varied due to the spring equinox at 21/MAR and the autumnal equinox at 21/SEP at all study stations. The results obtained and the analysis of temperature data showed that there is a high probability of significant climate changes in the study stations in particular and in all regions of Iraq in general in terms of high temperature in MAY of the spring, where temperatures recorded more than 40°C in stations Basrah and Baghdad, as well as recorded a large rise in temperature in SEP of the autumn of more than 42°C in the stations of Basrah and Baghdad, and this indicates the convergence of temperature in these months (MAY and SEP) of temperature in the summer months in the stations of Baghdad and Basrah.

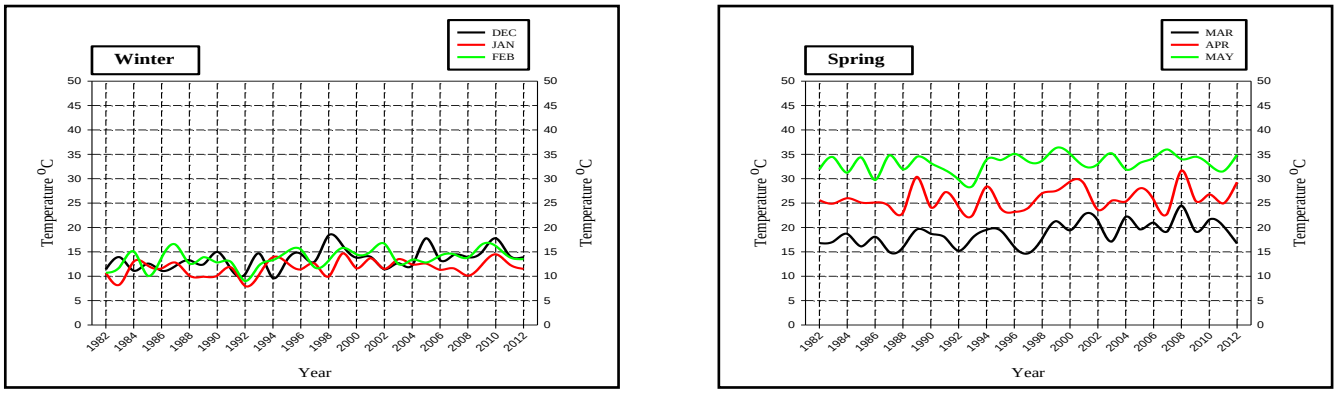

Fig.6: The monthly average of temperature during the seasons for the years (1982-2012) in Mosul station

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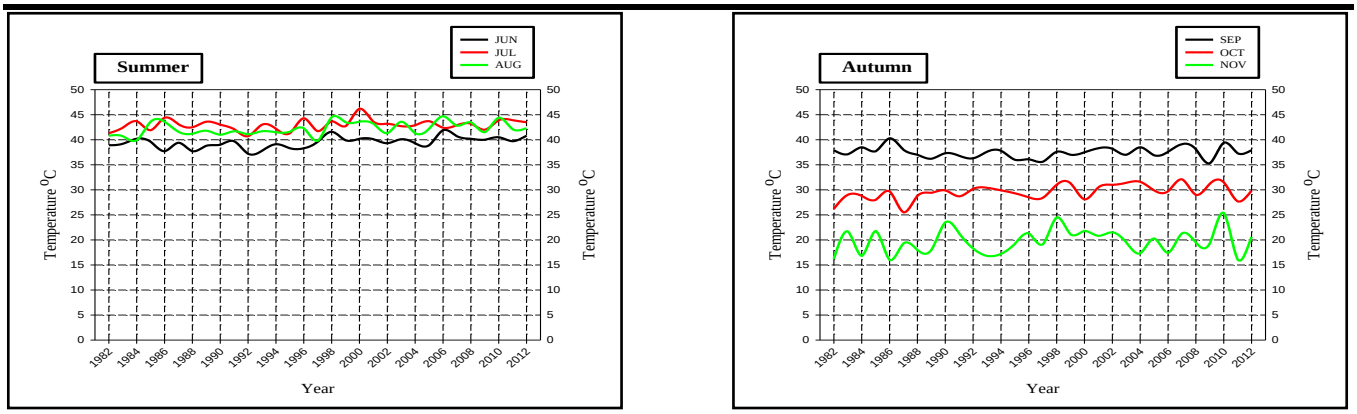

Followed the Fig.6

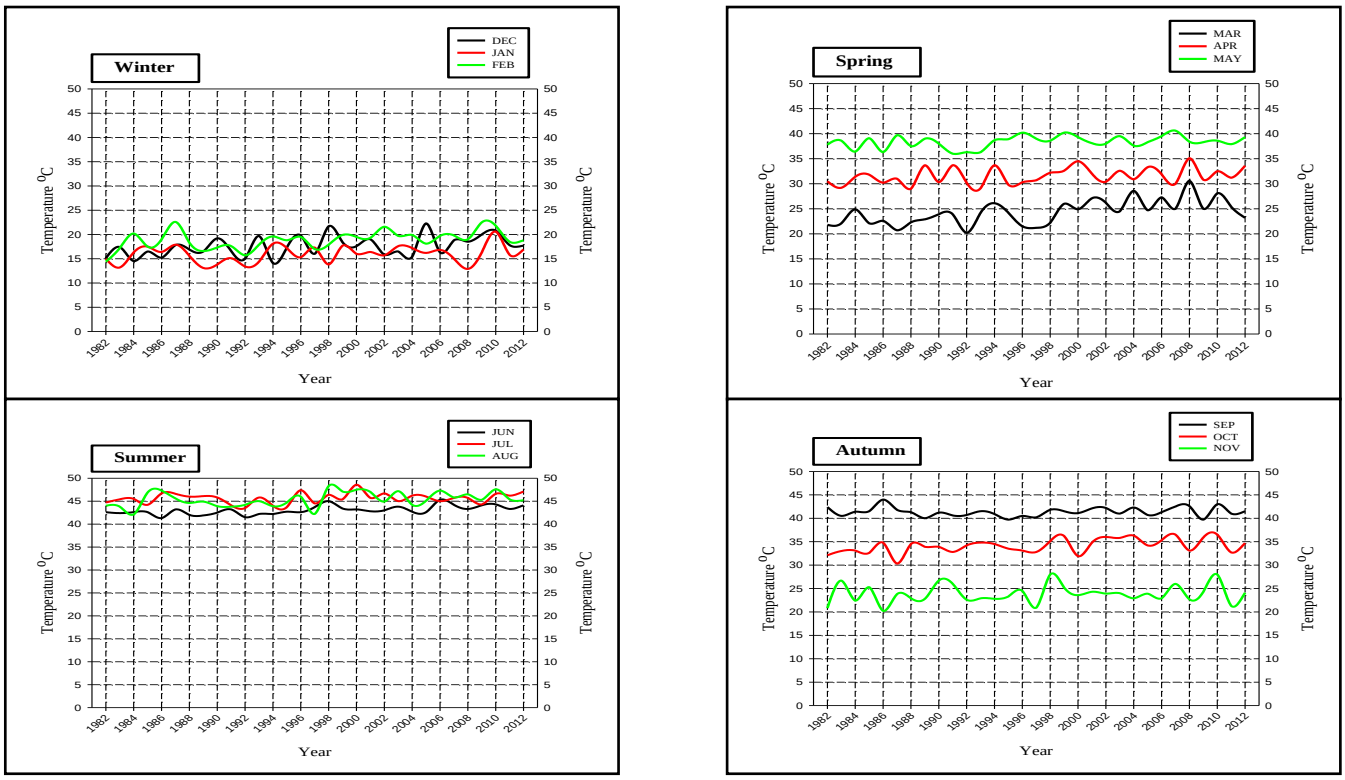

Fig.7: The monthly average of temperature during the seasons for the years (1982-2012) in Baghdad station

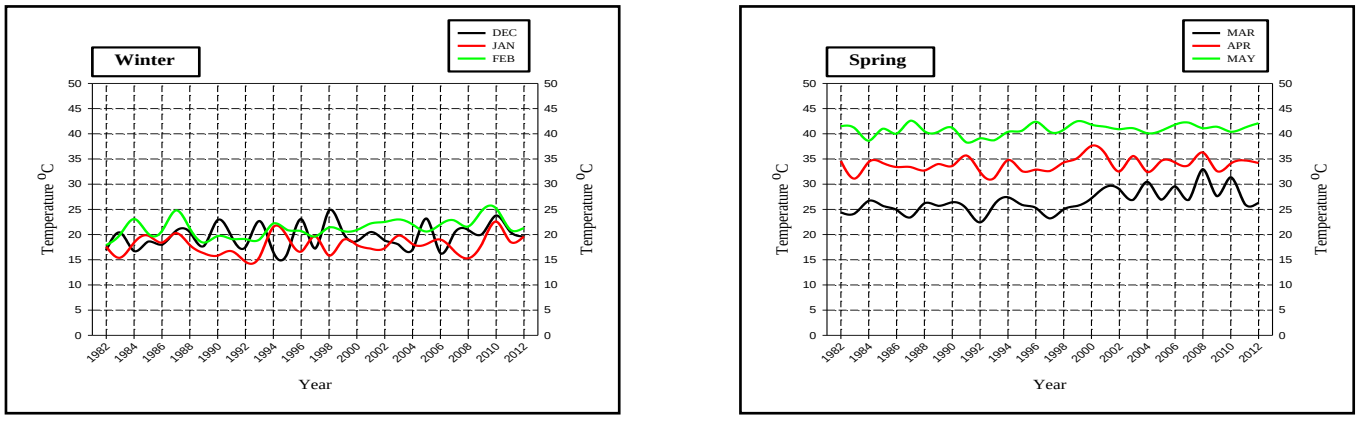

Fig.8: The monthly average of temperature during the seasons for the years (1982-2012) in Basrah station

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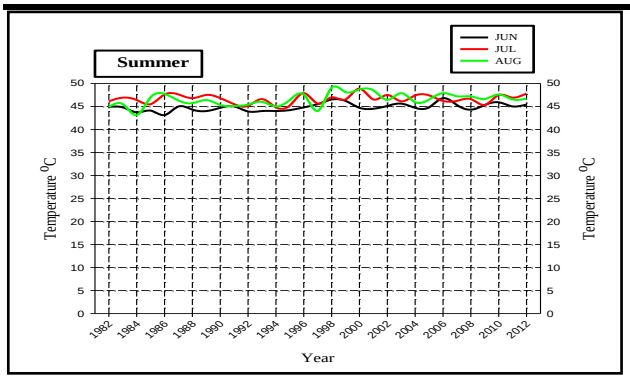
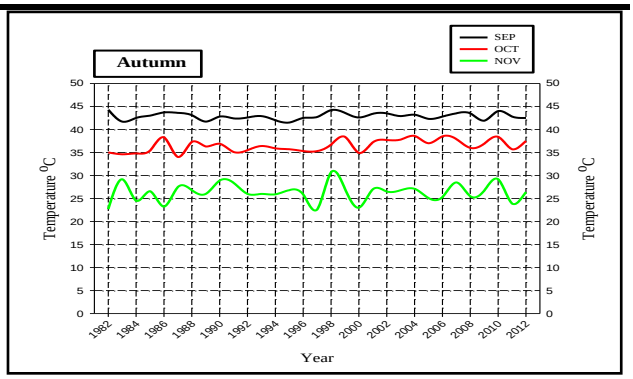
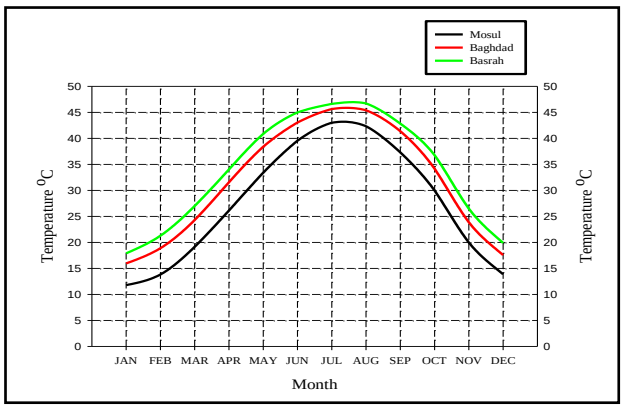




Followed the Fig.8

In the "Fig. 9", shows a comparison between the monthly and annual average of thirty-one years from 1982 to 2012 for stations Mosul, Baghdad and Basrah. The lowest monthly average of temperature was at Mosul station and the highest monthly average of temperature was at Basrah station, The lowest annual average of temperature was found at the Mosul station (northern Iraq) and the highest annual average of temperature was at the Basrah station (southern Iraq).



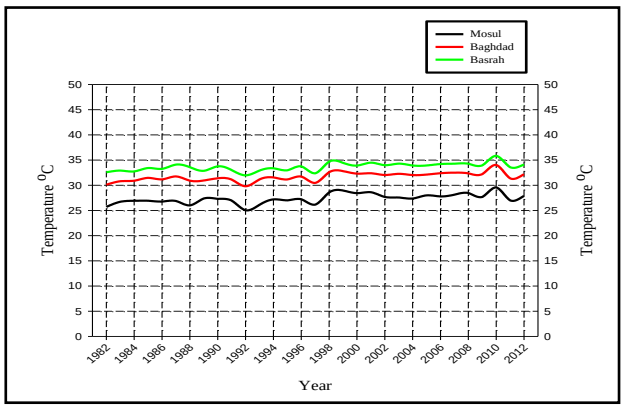
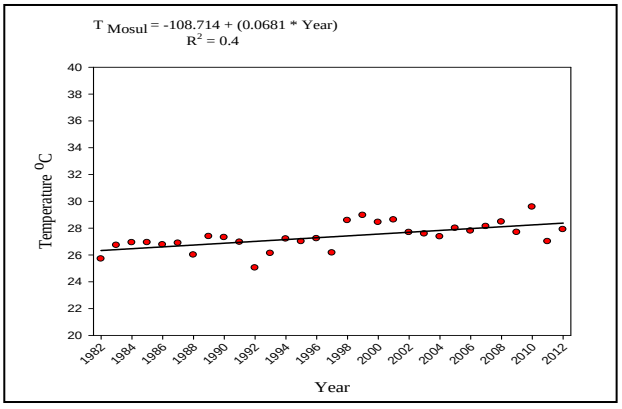


Fig.9: Behavior of the monthly and annual average temperature for the years (1982-2012) for study stations

In the "Fig. 10", which shows the shape of the positive linear relationship between the temperature average with the years of study from 1982 to 2012 (thirty-one years) where there was a clear increase in temperature over time and for all the study stations Mosul, Baghdad and Basrah and this indicates the existence of climate change in due to the increase in pollutants, the lack of annual rainfall, lack of vegetation and many other factors, which in turn lead to the prediction of future drought, where the Rsqr for Mosul station was (R^2=0.4), Baghdad station was (R^2=0.5) and Basrah station was (R^2=0.4).



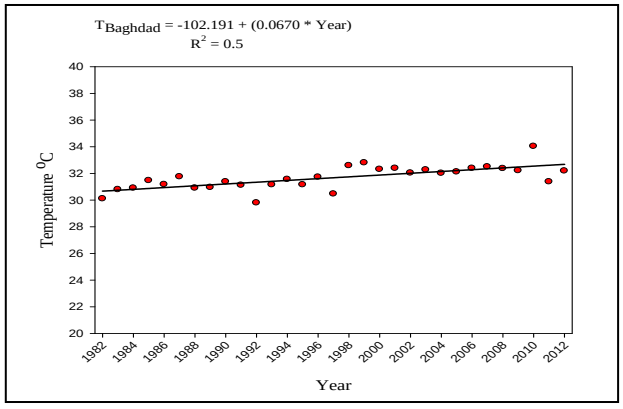


Fig.10: The relationship between the annual averages of temperature with the years (1982-2012) for stations (Mosul, Baghdad and Basrah)

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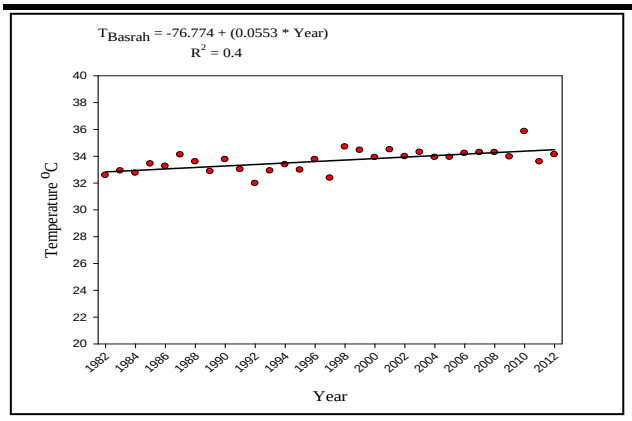

Followed the Fig.10

In the "Fig. 11", shows the comparison of annual temperature data for three successive decades (1982-1992), (1992-2002), and (2002-2012) for three different stations in Iraq Mosul, Baghdad, and Basrah.

Which represent North, Middle and South Iraq, respectively, where it was found that the highest annual average of temperature was in the last decade (2002-2012), where the annual average of temperature to more than 32°C and the lowest annual average of temperature was higher than 25°C in the Basrah station. The results have been reached the possibility of dividing study stations into three different climatic regions in terms of change and difference in temperature where it was found that the lowest total annual average of temperature was in the Mosul station was 27.4°C, the highest total annual average of temperature was in the station of Baghdad was 31.7°C, and the very highest total annual average of temperature was in the station of Basrah was 33.7°C, Iraq and the world, where the highest annual average of temperature at Basrah station as shown in "Fig. 12".

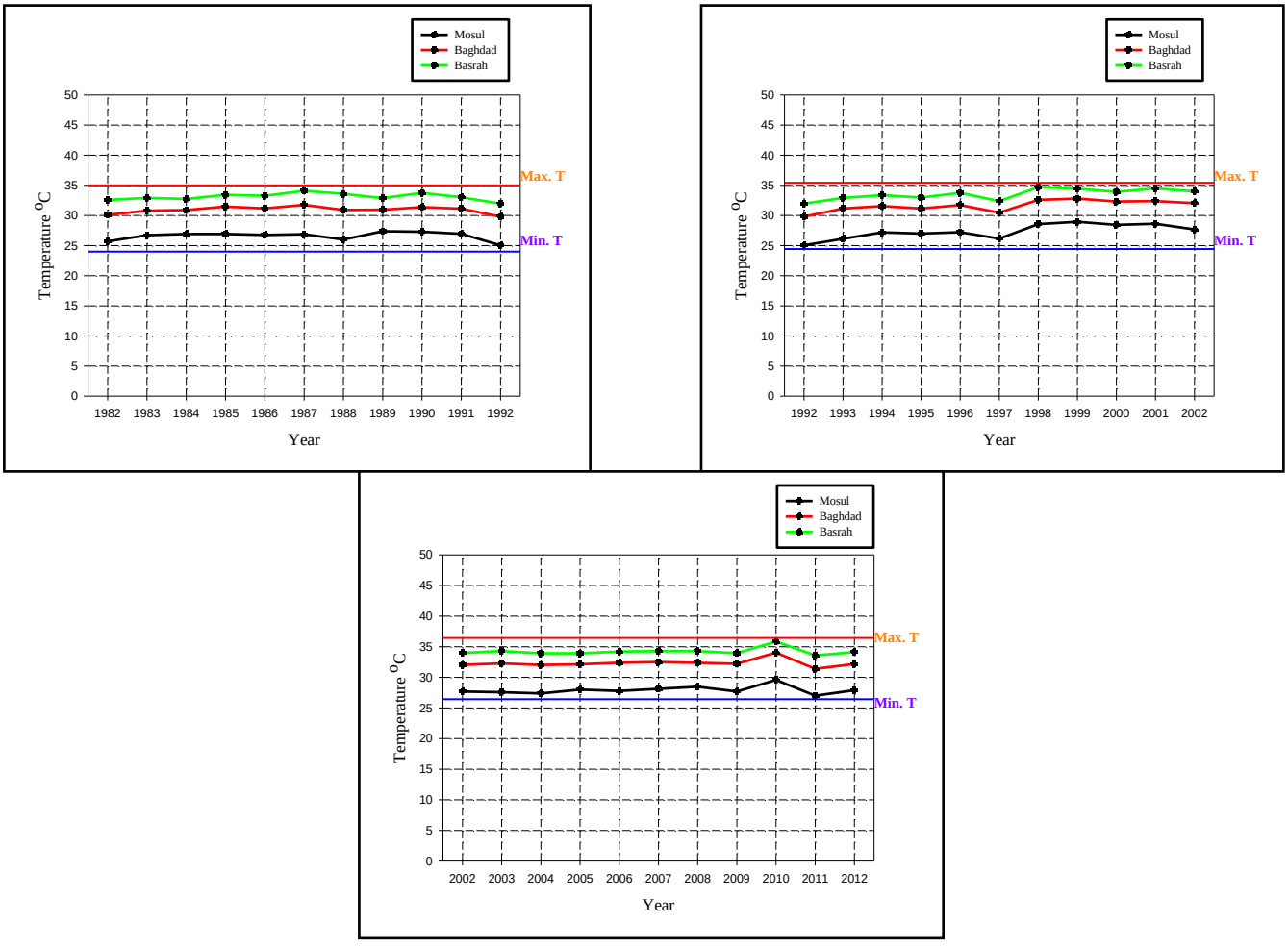

Fig.11: Highest and lowest annual average of temperature for three consecutive decades (1982-1992), (1992-2002) and (2002-2012) for stations (Mosul, Baghdad and Basrah)

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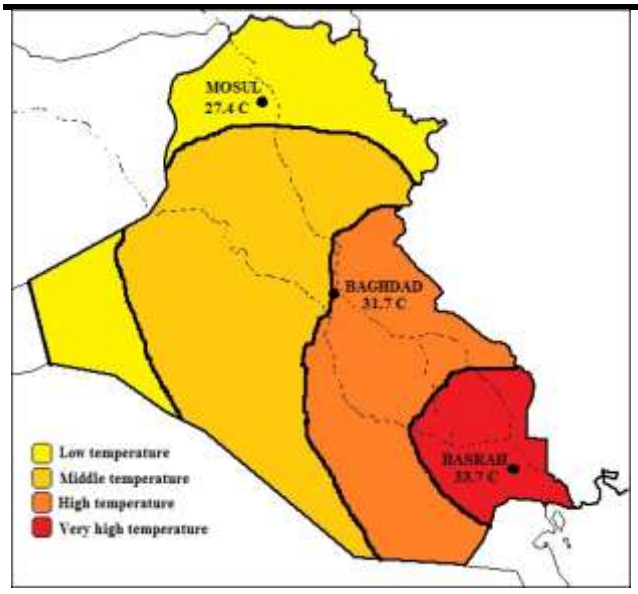

Fig.12: The total annual average of temperature during one and thirty years from 1982 to 2012 for stations (Mosul, Baghdad and Basrah) in Iraq

IV. CONCLUSIONS

- Mosul station is ranked first in the low monthly and annual temperature, followed by Baghdad station and the highest monthly and annual temperature of the station in Basrah.
- The lowest monthly average of temperature in JAN and the highest monthly average of temperature in JUL and all the study stations (Mosul, Baghdad and Basrah).
- The lowest monthly average of temperature at Mosul station was 11.8ºC and the highest monthly average of temperature at Basrah station was 46.6ºC during the study period.
- The lowest annual average of temperature in 1992 and the highest annual average of temperature in 2010 and for all study stations (Mosul, Baghdad, Basrah).
- Recorded the lowest annual average of temperature at Mosul station 25ºC and the highest monthly average of temperature at Basrah station 35.8ºC during the study period.
- The lowest temperature in the seasons was during the winter months and the highest temperature during the summer months and for all stations.
- The lowest annual average of temperature during the decade (1982-1992) and the highest annual average of temperature during the decade (2002-2012) and for all study stations.
- There is a clear increase in the monthly and annual average of temperature during the study years.
- There is a possibility of climate change in the stations of the study because of a clear increase in temperature in MAY of spring and SEP of autumn and the convergence of temperatures from the summer months.
- The possibility of dividing study stations into three climatic regions in terms of differences and variations in total annual average of temperature.
- Predictability of future Drought due to increasing averages temperature in Iraq.

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Exploration and Identification of Spermatophyta Plants Division that are potentially can be used for Medicine at Evergreen Forest *taman Nasional Baluran Indonesia*

Joko Waluyo, Dwi Wahyuni, Pujiastuti, Nuri, Wiwien Suqih Utami

Program Studi P. Biologi, Jurusan P.MIPA, FKIP Universitas Jember

***Abstract*—** *Indonesia is a country that has so many various floras. Nowadays Indonesia has more than 300.000 kinds of floras. More than 8000 kinds of plant belongto medicinal plants.WHO (World Health Organization) has stated about 80% of the population is still dependent on medicinal plants. Based on theAgriculture Ministry of Indonesia, the potential of medicine sales from 2010 to 2015 continues to increase. This is caused by the demand for medicine in 2010 reached 10 trillion rupiah. In 2015 is estimated to reach 20 trillion. Based on the Ministry of Agriculture (2007), traditional medicinal plants are not worth as much as the value of medicinal drugs, but the high value in demand for traditional medicine increases the value of traditional medicine sales from 2 trillion rupiah in 2003 to 7.2 trillion rupiah.The high number of needs is not equalwith the production capacity of medicinal plants. This is showedif Indonesia still importing medicinal materials with considerable value whereas in Indonesia hasso many medicinal materials especially from tropical forests of Indonesia.The absence of utilization of Indonesia's tropical forests is one of the factorsto do the research entitledExplorationand Identification of Spermatophyte Plants DivisionThat Are Potentially Can Be Usefor Medicineat Evergreen Forest Taman NasionalBaluran Indonesia. This Research conducted by using transect line method along the 100 meters enter the forest from the edge of the forest. The results from the exploration are found 22 types of medicinal plants which are included in 12 families and all of the medicinal plants can be use formedicine. The parts of the plants that can be utilized as a medicine are roots, leaves, flowers and even bark. From the parts of the medicinal plant leafs are the most potential parts for medicine. There are some various ways in utilizing medicinal plants starting with boiled, chewed, crushed and even mixed with other ingredients.*

***Keywords*—identification, Medicinal plants, Spermatophyta Divison, Evergreen Forest.**

I. INTRODUCTION

Indonesia is a country that has so many various floras. Nowadays Indonesia has more than 300.000 kinds of flora. More than 8000 kinds of plant are medicinal plantsand has utilized by the people as a traditional medicine (Rahmawati, 2004). WHO (World Health Organization) has stated about 80% of the population is still dependent on medicinal plantsthat can be roots, wood, rhizomes or other plant parts. According to the National Socioeconomic Survey in 2001, 57.7% of Indonesians had self-treatment without medical assistance and 31.7% of Indonesians using traditional medicine. And the other used other medicine.The meaning of Medicinal plants is a natural resource that can be used to treat a disease, herbal medicine or cosmetics.Medicinal plants has minimum side effect for our body. It is useful for medical field and it increases the utilization in commercial field.Based on the Ministry of Agriculture of Indonesia, the potential ofmedicine sales until 2015 reached 20 trillion rupiah in the domestic market and 16 trillion rupiah in export markets. The growth of agroindustry market of medicinal plants in 2010 reached 10 trillion rupiah and increased up to 11 trillion rupiah in 2011 and in 2012 to 12.35 trillion rupiah. This number indicates an increase in the number of demand of medicinal plants from year to year. The demand for this medicinal plant comes from traditional medicinal plants and modern medicine.

Based on LIPI (2003) in the Ministry of Agriculture (2007), although traditional medicine demand is not as high as

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modern medicine demand, but the increase of traditional medicinal plants demand in the country has increased in high amount. This can be seen in the data shown in 2003; the demand of traditional medicinal plants from 2 trillion rupiah increase up to 7.2 trillion rupiah in 2010. The high demand of medicinal plants causes the value of trade increasing. It is predicted to continue increasing. It can be a good chance for Indonesia to develop agro-industry in foreign markets. Based on WHO data, 80% of the world's population depends on traditional medicine and 20% of world's population use modern medicine. The modern medicines that marketed in the world made from medicinal plants in the tropics (KLH, 2014).

The high number of needs is not equalwith the production capacity of medicinal plants. This is showedif Indonesia still importing medicinal materials from another country with considerable value. The number of budget that has spent on medicinefrom abroad US $ 160 million every year LIPI (2003) in the Ministry of Agriculture (2007). Whereas the potential trade from medicinal plants originating from Indonesia's forest areas, especially tropical forests is estimated to reach US $ 1 trillion (Kompas, 2010). Based on that statement there are still many medicinal plants from the tropical forest area which has many types of flora and vast area that can be utilized to plant medicinal plant and preserving the potential plants as medicine. Therefore, a study conducted in one of Indonesia's tropical forests entitled "Exploration and Identification of Spermatophyte Plants Division That Are Potentially Can Be Use for Medicine at Evergreen Forest Taman NasionalBaluran Indonesia".

II. RESEARCH PROCEDURE

a. Research Design

The design of this research conducted by using transects line. The transect method is a method which is done by drawing the transect line by using the rope and then doing the research on the sample which passed by the transect line. The location of the transect line was located at Hm 81 to Hm 91 located on the road that runs from Batangan-Bekol.The sampling area was divided in to 6 spots 3 spots are on the left of the forest and the other 3 spots are on the right side of the forest.Each spot was chosen randomly.Rope was used as a tool to draw a transect line along 100 meters from the edge of the forest. From these area along 100 meters in transect lines the writers did the observation about Spermatophytaplants which included observation of plant habitus, photographing plant samples, and plant samplingwhich used for herbaria production purpose.

b. Plant Sampling

The Spermatophytadivison plant sampling was done in Evergreen forest Taman NasionalBaluranbetween Hm 81 to Hm 91 road from Batangan to Bekol. As a methodThe plants sampling was done by using rope 100-meter in lengthfor transect line starting from the edge of forest into the forest.In that transect line, the observation of Spermatophyta plant was done includes observation of plant habitus and plant sampling for herbariumpurposes for further identification. It also photographed samples of plants to see the parts of plants more detail. The Samples are taken during the dry season

c. Plants Identification

The plants identification is done by describing plants from exploration result which includes morphological observations of stem organs, leaves and reproductive apparatus includes flowers and fruits. From the result of description the writer identify the plant by matching with the identification book to obtain the species name of the plant. Plants that have not been identified willsend to LIPI Purwodadi - Pasuruan for further identification. After recognizing the name of the species of the plant from exploration result then conducted a study of literature to determine the efficacy of medicine produced from these plants.

d. Herbarium Production

Herbarium production is purposed for preservation of plant samples from Evergreen Forest Baluran Indonesia National Park to avoid damage at the time of identification in LIPI Purwodadi-Pasuruan. There are the steps that should do:

- Taking a plant samples' parts completely from the root until flower. If the plant belongs to tree habitus you can use branch which has leaf and flower as herbarium sample.
- Pack the plant samples by using paper
- Give a label for each plant samples.
- Put in toherbarium press then tied with raffia rope.

III. RESULT AND DISCUSSION

Based on the results of research conducted by researcherat Evergreen Forest Baluran Indonesia National Park that used plot method, the researcher found 22 species of Spermatophyta plants which are listed in the table as follows.

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Tabel.1: The list of plants found in Evergreen Forest Baluran Indonesia National Park.

No	Species	Family	Local Name	Benefit
	(1)	**(2)**	**(3)**	**(4)**
1	*Aglaia*sp.	Meliaceae	-	Anthelmintic, Can be use as medicine for Malaria, dysentery (Tukiran*et al*, 2008). Anticancer (Ahmad *et al.*, 2010).
2	*Aglaia argentea*Blume.	Meliaceae	Langsat	Digestion, cure colon cancer, fever, malaria and insect bites (Nugroho, 2015)
3	*Asystasianemorum*Ness.	Achantaceae	Kembanggeni	Treat cough and chest pain (Hidayat, 2015: 202). Treat ulcers and fever (Singh, 2006: 86).
4	*Azima sarmentosa*Blume.	Salvadoraceae	Sokdoy	Anti scorpion's poison(Uawonggul, 2005).
5	*Biden pilosa*L.	Asteraceae	Ketul	Treating bladder, kidney, abdominal pain, urinary infection, hepatitis, and rheumatism (Bartolome*et al.*, 2013).
6	*Capparis*sp.	Capparidaceae	-	Treating paralysis, rheumatism, abdominal pain, skin diseases, spleen, kidney, liver disease, and prevent scorpion stings (Rivera, 2013).
7	*Capparis micracantha*DC.	Capparidaceae	kencuran	Treat cancer and tuberculosis(Fernquest, 2012)
8	*Clerodendrum inerme*(L.)	Lamiaceae	Gambir	Treat poisoning, itching and rheumatism(Van Valkenburg, J.L.C.H*et al.*, 2015).
9	*Cordia oblique* Willd.	Boraginaceae	Kendal, nunang	Treating diarrhea, fever, dysentery, headache, stomach pain, cough medicine, and skin diseases such as ringworm (Van Valkenburg, J.L.C.H*et al*., 2015).
10	*Coripha utan*Lam.	Arecaceae	Gebang	Treat diarrhea, cough, dysentery and injuries(Nasution, 2015).
11	*Desmodium gangeticum*(L.) DC.	Leguminosaceae	Daunpicah	Treat ulcers and burns, treat diarrhea and dysentery, asthma, tuberculosis, and treat flatulence (Singh. 2015).
12	*Gloriosasuperba*L.	Colchicaceae	Kembangsungsang	Treat gout, diuretics, rheumatism (Winarno*et al.*, 2010). To treat skin diseases, skin, cardiovascular.
13	*Kleinhovia hospita*Linn.	Sterculiaceae	Timanga	Treat liver cancer and decrease cholesterol (Imaniyah, 2014).
14	*Lantana camara*L.	Verbenaceae	tembelekan	Treating asthma, gonorrhea, ulcers, deman, tuberculosis, rheumatism, and swelling(Yuliani, 2013).
15	*Neonauclea calycina*	Rubiaceae	Anggerit	Treat bone fractures and kidney

	Merr.			disease (Silalahi, 2015).
16	*Randiadumetorum*Lam.	Rubiaceae	Madana	Heals wounds, tumors, worms, skin diseases, and antibacterial activity (Ghosh*et al.*, 1983).
17	*Randiaspinosa*(Thunb.)	Rubiaceae	Timuntahil	Treating diarrheal diseases, inflammation, tumors, ulcers, dysentery, and stomach (Singh, 2010)
18	*Schleicheraoleosa*(Lour.)	Sapindaceae	Kesambi	Treat eczema, scabies, cancers and inflammation of the ear(Okan, 2015).
19	*Streblus asper*(Lour.)	Moraceae	Serut	Treat fever, dysentery, toothache, stomachache, and urinary disorders (Taweechaisupapong, 2015)
20	*Strychnos lucida*Lam.	Leguminaceae	Bidaralaut	Treat rheumatism, stomachache, ulcers, ringworm, inflammation of the skin purulent, overcoming blood sugar and anti-inflammatory (Gusmailina, 2015).
21	*Synedrellanudiflora*(Linn.) Gaertn.	Asteraceae	Legetan	Heals bleeding wounds, headache, earache, stomachache, and rheumatism (Sumi*et al.*, 2011).
22	*Thunbergiafragrans*Roxb.	Achantaceae	White lady	Treating external wounds (Samuel *et al.*, 2010).

The plants which were found consisted of 22 species and 15 genus that are Meliaceae, *Salvadoraceae, Capparidaceae, Rubiaceae, Lamiaceae, Boraginaceae, Arecaceae, Leguminosaceae, Sapindaceae, Moraceae,Sterculiaceae, Verbenaceae, Achantaceae, Asteraceae, Cholcicaceae.* All species which obtained belongs to trees, there are rarely found plants which belong toshrubs.This is because the Evergreen Forest is dominated by trees. when sampling time the weather is so hot and dry but the plants is still survive in extreme environments. It is about 22 plants found that belong to herbal medicine because eachplants content a materials that can cure or prevent disease. The medicinal material can be derived from its leaves, roots, flowers and even bark for example Aglaiaargentea plant whose bark is used to treat from insect bites. From all of the plant samples the parts which are the most commonly use for medicinal materials are leafs. There are some various ways in utilizing medicinal plantssuch as boiled, chewed, crushed and even mixed with other ingredientsso there is a mixture between the two materials and it is made the benefits more complementary.

IV. CONCLUSION

1. The plants which were found consisted of 22 species and 15 genusthat are Meliaceae, *Salvadoraceae, Capparidaceae, Rubiaceae, Lamiaceae, Boraginaceae, Arecaceae, Leguminosaceae, Sapindaceae, Moraceae, Sterculiaceae, Verbenaceae, Achantaceae, Asteraceae, Cholcicaceae*.
2. The medicinal material can be derived from its leaves, roots, flowers and even bark.
3. From all of the plant samples the parts which are the most commonly use for medicinal materials are leafs. There are some various ways in utilizing medicinal plantssuch as boiled, chewed, crushed and even mixed with other ingredients.

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Comparison of Different Models in Estimating Standard Evapotranspiration in Lampung Province, Indonesia

Tumiar K Manik[1], Purba Sanjaya[2], R.A. Bustomi Rosadi[3]

[1,2] Department of Agrotechnology, Faculty of Agriculture, Lampung University, Indonesia
[3]Department of Agriculture Engineering, Faculty of Agriculture, Lampung University, Indonesia

Abstract— *Evapotranspiration (ET) is the loss of water to the atmosphere by combined processes of evaporation from soil and plant surfaces and transpiration from plants. Since various factors affect ET, including weather, crops and soil parameters; numerous equations have been developed to quantify standard ET. The equations vary in data requirements from very simple, empirically based or simplified equations to complex, more physically based equations. This study used six methods in estimating standard evapotranspiration using data from September 2011–August 2012 from Climate Station at Masgar (05º10'20" S, 105º10' 49"E, 50 m dpl) Lampung, Indonesia. The six models are: Hargreaves-Samani 1985 (H/S), FAO 24 Radiation (24RD), FAO 24 Blaney-Criddle (24BC), FAO 24 Pan Evaporation (24PAN), Linacre (Lina), and Makkink (Makk). The results were analyzed using statistics methods in error indicators, which are: Root Mean Square Error(RMSE), Mean Absolute Error (MAE), and Logaritmic Root Mean Square Error(LOG RMSE), while the closeness among the models was analyzed using Index Agreement (IA). Direct measurement had been done using lysimeters (3x2x1) m. The study concluded that Makkink model is the suitable simple model that should be chosen in Lampung lowland area to calculate ET_o when climate data is limited, besides the recommended FAO 56 Penman Monteith.*

Keyword— Evapotranspiration, Standard Evapotranspiration, FAO 56 PM, Makkink Model.

I. INTRODUCTION

Evaporation is the main component in hydrology cicle, therefore, accurate estimation of evaporation rate is important for water management and eventually for agriculture production. However, it is difficult to measure evaporation rate directly since evaporation affected by various factors.

Evaporation is affected by climate factors such as solar radiation, air temperature and humidity and wind velocity; by crops type and environment and by soil condition and management (Temesken, Davidov and Frame, 2005). Since those factors are linked to each other and change in time and space, it is difficult to develop equation for estimating evaporation rate for various crops on different condition. Therefore, a scheme called reference evapotranspiration was developed.

Reference ET is defined as ''the rate of evapotranspiration from an extensive area of 0.08–0.15 m high, uniform, actively growing, green grass that completely shades the soil and is provided with unlimited water and nutrients'' (Bakhtiari et al., 2011). More recently, Allen et al. (1998) elaborated on the concept of ET_o, referring to an ideal 0.12 m high crop with a fixed surface resistance of 70 s m^{-1} and an albedo of 0.23. Several equations has been developed to estimate the reference evapotranspiration; some of that were derived based on physical processes of the evapotranspiration but mostly are empirical based on ststistical relationship between evapotranspiration and one or more climate variables (Berengena and Gavilan, 2005). Approaching methods to estimate evapotranspiration rate was developed increasingly in the last 30 years such as based on air temperature measurement (Hargraeves and Sumani, 1985), based on solar radiation Priestly and Taylor method (Priestly and Taylor, 1972) and based on combination of radiation balance and air moisture aerodynamic movement (Penman, 1948).

Penman method has been improved several times such as Penmann method modified by Monteith and known as Penman-Monteith equation (Monteith, 1965) approach method in FAO 24 version (Doorenbos and Pruitt, 1977), FAO 56 modification (Allen, 1998) and recently Matt-Shuttleworth approaching method (2009).Those estimations

have been derived and/or calibrated from the direct field measurement of ET using various grasses of alfalfas on a variety of lysimeter designs, climates, and management conditions.

Some studies showed that Penmann-Monteith model gave accurate estimation that FAO and other organizations

recommended this model to estimate reference evapotranspiration for calculating crops water requirement (Itenfisul.et.al., 2003; Berengena dan Gavilan, 2005). Even though Penman-Monteith model is accepted as accurate estimation, for using in local condition, it is necessary to validate the model to whether it estimates close enough to the direct measurement on the local climate station. Berengena dan Gavilan (2005) examined different methods in estimating evapotranspiration rate in Southern Spain, an area with strong advection. The result showed that Penman method modified with local wind function gave the best estimation compared with direct measurement in lysimeter following with Penman-Monteith FAO 56 version. Steduto et al. (2003) examine FAO 56 method in Southern Italia with Mediteranian semi-arid climate. The results showed that FAO 56 method was the best in estimating the evapotranspiration; however, tended to be over estimated in winter time when the evapotrasnpiration was low and under estimated in summer time when the evapotraspiration was high. Temesgen et al. (2005) also examined FAO 56 in California, USA and the result showed good correlations with evapotranspiration rate in 37 climate stations in this area.

Direct measurement of evapotranspiration is calculated using lysimeters; however, only small number of climate stations is equiped with a lysimeter; as an alternative, the measurement is done using evaporation pan known as Class A pan. A study by Fontenot (2004) showed that reference evapotranspiration measurement by Class A pan was not fit to the estimation by Penman-Monteith. The result from Class A pan should be corrected by a coefficient. Using Cuenca and Snyder method; Xing et al.(2008) obtained that pan coefficient for Canada maritim climate was between 0.78 – 0.94. Generally the result from Pan A was lower than the estimation result from Penman-Monteith or Priestley-Taylor method.

Research about comparing different models has been done in some countries. Chen et al. (2005) used 7 estimating models in four provinces of Taiwan and found that Makkink and Hargreaves-Samani models were the best models in estimating ET_o when compared to FAO 56 PM. Chowhury et al. (2010) also found that in India, Makkink model had the closest estimation to FAO 56 PM with a little underestimated result.

Xu and Chen (2005) did similar study in Germany with comparing 7 models and found that Granger-Gray and Makkink models were the best models for the area. In North China, Scneider et al. (2007) compared 4 models with direct observation and concluded that Hargreaves-Samani and Makkink models were the best models in estimating ET_o even better than FAO 56 PM. Jacobs et al. (2004) conducted research on estimating ET_o in Florida, using remote sensing method with data from GOES. The results showed that FAO 56 PM is the best model with R^2= 0.92; however, this result was not much different with estimated results from Makkink model which gave R^2= 0.90.

Various methods in estimating evapotranspiration also has been applied in Indonesia. Usman (2004) compared Thornthwaite, Blaney-Cridle, Samani-Hargreaves, Prestley-Taylor, Jansen-Haise, Penman and Penman-Monteith methods in five climate stations in West Java; the results showed that in general Priestley-Taylor in average gave the highest annual evapotranspiration rate, while the lowest was obtained by Blaney-Criddle method. It also showed that estimation using Penman Monteith method in general gave higher rate than Pan A measurement.

Lampung Province (103^0 40' – 105^0 50' E; and between: 6^0 45' – 3^0 45' S;35.288,35 km^2) is located at Southeast tip of Sumatra Island, Indonesia. Lampung climate is characterized by monsoonal rain distribution and local characteristics. Rain season in general is from October to March with the peak on January/February and dry season is from April to September . Monthly rainfall ranges from 50 – 200 mm and annual rainfall ranges from 1200 mm (lowland area) to 2500 mm (highland area). Lampung economic is dominated by agriculture products mainly coffee, chocolate, rubber and sugarcane. Lampung is also considered as main area for cash crops such as paddy, soybean and Maize. Therefore, finding good and reliable method in estimating crops water requirement is necessary for better agriculture management.

The objective of this research was to find a closest model to the FAO 56 PM model by comparing six different models in estimating standard evapotranspiration in Lampung area, Indonesia.

II. METHODS

This study used six methods in evaluating potential evapotranspiration using data from September 2011 to Agustus 2012 from Climate Station at Masgar (05º10'20" S, 105º10' 49"E, 50 m dpl) in Lampung, Indonesia.

The six models are: Hargreaves-Samani 1985 (H/S), FAO 24 *Radiation* (24RD), FAO 24 Blaney-Criddle (24BC), FAO 24 *Pan Evaporation* (24PAN), Linacre (Lina), dan Makkink (Makk). The results from those models were compared to FAO Penman-Monteith (56PM) as the standard model.

To evaluate the relation between models, the results were analyzed using statistics methods in error indicators, which are: *Root Mean Square Error* (RMSE), *Mean Absolute Error* (MAE), dan *LogaritmicRoot Mean Square*

Error (LOG RMSE), while the closeness among the models was analyzed using Index Agreement (IA).
Finally, the results were compared to the direct measurement using 3 lysimeters (3x2x1 m) planted with *Sporobulus diander* grass.

2.1 Description of Models

2.1.1 Hargreaves-Samani 1985 (H/S) (Hargreaves and Samani, 1985)
The equation of this model is:

$$ET_o = 0.0023(T_{mean} + 17.8)(T_{max} - T_{\min})^{0.5} R_a \quad (1)$$

with ET_o is standard evapotranspiration (mm/day), T_{mean} is daily mean temperature (°C), T_{max} is maximum temperature(°C), $T_{min}$is minimum temperature, dan R_a is radiation on top of the atmosfer (MJ/m^2/day).

2.1.2 FAO 24 *Radiation* (24RD) (Doorenbos and Pruitt, 1977)
The equation of this model is:

$$ET_o = a + b\left[\frac{\Delta}{\Delta + \gamma} R_s\right] \dots\dots (2)$$

ET_o is standard evapotranspiration (mm/day), Δvapor pressure curve (kPa/°C),γ is psychrometric constant (kPa/°C), $R_s$is solar radiation(MJ/m^2/day), a and b conversion factor with $a = -0.3$ mm/day and $b$derived from the equation:

$$b = 1.066 - 0.13 \times 10^{-2} RH_{mean} + 0.045 U_d - 0.20 \times 10^{-3} RH_{mean} U_d - 0.1315 \times 10^{-4} {RH_{mean}}^2 - 0.11 \times 10^{-2} {U_d}^2 \dots\dots (3)$$

$RH_{mean}$is daily relative humidity (%) and$U_d$is average wind velocity at 2 m height (m/s)

2.1.3 FAO 24 Blaney-Criddle (24BC) (Jensen et al., 1990)
The equation for this model is:

$$ET_o = a + bf \dots\dots (4)$$

$$f = p(0.46T + 8.13) \dots\dots (5)$$

$$a = 0.004 RH_{min} - \frac{n}{N} - 1.41 \dots\dots (6)$$

$$b = 0.908 - 0.00483 RH_{min} + 0.7949 \frac{n}{N} + 0.768[ln(U_d + 1]^2 - 0.0038 RH_{min} \frac{n}{N} - 0.000443 RH_{min} U_d + 0.281\left[ln(\frac{n}{N} + 1)\right] \dots\dots (7)$$

$$-0.0097[ln(U_d + 1][ln(RH_{min} + 1]^2 \left[ln(\frac{n}{N} + 1)\right]$$

ET_o is standard evapotranspiration (mm/day), P is percentage of day length, T is daily average temperature (˚C), RH is minimum relative humidity (%), n/N is ratio of possible actual day, Ud is wind speed at 2 m (m/s)

2.1.4 FAO 24 *Pan Evaporation* (24PAN) Doorenbos and Pruitt (1977)
The equation of this model is

$$ET_o = K_p \times E_{pan} \dots\dots (8)$$

$$K_p = 0.108 - 0.028 u_2 + 0.0422 \ln(FET) + 0.1434 \ln(RH_{mean}) - 0.000631[\ln(FET)]^2 \ln(RH_{mean}) \dots\dots (9)$$

ET_o is standard evapotranspiration (mm/day), K_p is pan coefficient, E_{pan} is class A Pan evaporation (mm/day), u_2 is average wind speed (m/s), RH_{mean} relative humidity (%), dan FET is distance between pan and green crops (m).

2.1.5 Linacre (LINA) (Linacre, 1977)

The equation of this model is:

$$ET_o = \frac{\left(\frac{500 T_m}{100 - A}\right) + 15(T - T_d)}{(80 - T)} \dots\dots (10)$$

$$T_m = T + 0.006h \dots\dots (11)$$

ET_o is standard evapotranspiration (mm/day)T is mean temperature (°C), A is latitude of the climate station (°), T_m is elevation of climate station (m), dan T_d is average dew point temperature (°C). T_d equation is:

$$T_d = \left(\frac{f}{100}\right)^{\frac{1}{8}} (112 + 0.9T) + 0.1T - 112 \dots\dots (12)$$

is average dew point temperature (°C), T is mean temperature (°C), dan f is average daily relative humidity (%).

2.1.6 Makkink (Makk) (Makkink, 1957).
The equation of this model is:

$$ET_o = 0.61 \frac{\Delta}{\Delta + \gamma} \frac{R_s}{2.45} - 0.12 \dots\dots (13)$$

ET_o is average dew point temperature (°C). R_s is solar radiation (MJ/m^2/day), Δ is vapor pressure curve (kPa/°C), and γ is psychrometric constant (kPa/°C).

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2.1.7 FAO 56 PM (56PM) (Allen et al., 1998)
The equation of this model is

$$ET_o = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T+273} U_2(e_{s-}e_a)}{\Delta + \gamma(1 + 0.34\, U_2)} \ldots\ldots (14)$$

ET_o is standard evapotranspiration (mm/day), Rn is netto radiation on crops surface (MJ/m^2/day),G is continuous heat flux to soil depth (MJ/m^2/day),T is daily temperature at 2 m (^{o}C),u_2 is wind speed at 2 m (m/s),e_s is vapor pressure (kPa),e_a is actual vapor pressure (kPa), Δvapor pressure curve (kPa/^{o}C),and γ is psychrometric constant (kPa/^{o}C).

In this study the ET_o estimation from FAO 56 Penman-Monteith model as the standard model was calculated using CROPWAT. CROPWAT is a ccomputer program recommended by FAO based on FAO 56 Penman-Monteith model (Allen *et al*., 1998)

2.1.8 Indicators

The error indicators equation used to evaluate the model follows Wilmorth (1982):

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(ET_{osi} - ET_{omi})^2} \ldots\ldots (15)$$

$$MAE = \frac{1}{N}\sum_{i=1}^{N}|ET_{osi} - ET_{omi}| \ldots\ldots (16)$$

$$LOG = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\log ET_{osi} - \log ET_{omi})^2} \ldots\ldots (17)$$

$$I.A = 1 - \frac{\sum(ET_{osi} - ET_{omi})^2}{\sum[|ET_{osi}'| + \|ET_{omi}'\|]^2} \ldots\ldots (18)$$

$$ET'_{osi} = ET_{osi} - \overline{ET_{omi}} \ldots\ldots (19)$$

$$ET'_{omi} = ET_{omi} - \overline{ET_{omi}} \ldots\ldots (20)$$

With ET_{osi} is Penman-Monteith standard evapotranspiration as the standard model
-i, dan ET_{omi} is others evapotranspirasi models (i).

Table.1: Climate parameters needed by each estimation model

No	Model	Climate data needed by each model							
		E_{pan}	T	R_s	R_n	RH	P	U_2	R_a
1	56PM		√	√	√	√	√	√	√
2	H/S		√						√
3	24RD		√	√		√		√	
4	24 BC		√		√	√	√	√	
5	24PAN	√				√		√	
6	Makk		√	√					
7	LINA		√						

Notes: E_{pan} : Evaporation pan (mm/day); T: average, maximum and minimum temperature (^{o}C); R_s: solar radiation(MJ/m^2/day); R_n: nett radiation (MJ/m^2/day); RH: relative humidity (%); P: day length (%); U_2: wind velocity (m/s); R_a: radiation at the top of the atmosphere (MJ/m^2/day)

Fig.1: The lysimeters

III. RESULTS AND DISCUSSIONS

3.1. Statistical analysis

The statistical analysis from daily evapotranspiration data including the error indicator of each model compare to the FAO 56 PM as the standard is presented in Table 2-5.

Table.2: RMSE value among the ET_0 estimating models

RMSE							
	56PM	Makk	24BC	24PAN	24RD	H/S	Lina
56PM	0	0,34	1,30	0,75	0,69	1,35	0,88

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Makk	0,34	0	1,61	0,48	0,49	1,52	1,12
24BC	1,30	1,61	0	*1,99*	1,92	1,12	0,79
24PAN	0,75	0,48	*1,99*	0	*0,33*	1,93	1,54
24RD	0,69	0,49	1,92	*0,33*	0	1,98	1,54
H/S	1,35	1,52	1,12	1,93	1,98	0	0,59
Lina	0,88	1,12	0,79	1,54	1,54	0,59	0

Comparison of error indicator (RMSE) between models using monthly data was presented in Table 2. Based on the comparison among the six models, the error indicator RMSE ranged from 0.33-1.99 which means that ET_o difference among the models was 0.32 mm to 1.99 mm/day. This is not a small number since 1 mm/day ET in 1 ha area is equivalent with water loss of 10,000 liter/day or 3.6 million liter/year.

Using Lampung climate data, the lowest RMSE was found between FAO 24 *Radiation* and FAO 24 *Pan Evaporation* while the highest RMSE was found between model FAO 24 *Pan Evaporation* and FAO 24 Blaney-Criddle.

Tabel.3: MAE value of estimated monthly evapo- transpiration data among the models

MAE							
	56PM	Makk	24BC	24PAN	24RD	H/S	Lina
56PM	0	0,28	1,06	0,62	0,67	1,28	0,86
Makk	0,28	0	1,28	0,40	0,45	1,50	1,08
24BC	1,06	1,28	0	1,69	1,74	0,95	0,68
24PAN	0,62	0,40	1,69	0	0,25	1,90	1,48
24RD	0,67	0,45	1,74	0,25	0	1,95	1,53
H/S	1,28	1,50	0,95	1,90	1,95	0	0,47
Lina	0,86	1,08	0,68	1,48	1,53	0,47	0

The second error indicator (MAE) is presented in Table 3. Similar results with RMSE were found in error indicators for both MAE and log RMSE (Table 4). Makkink model was the model which is closest to FAO 56 Penman Monteith.

Table.4: LOG RMSE among the estimating models of ET_0

LOG RMSE					
	56PM	Makk	24BC	24PAN	24RD
56PM	0	0,04	0,13	0,11	0,10
Makk	*0,04*	0	0,17	0,08	0,07
24BC	0,13	0,17	0	0,23	0,22
24PAN	0,11	0,08	0,23	0	0,06
24RD	0,10	0,07	0,22	0,06	0
H/S	0,14	0,16	0,11	0,23	0,23
Lina	0,10	0,13	0,08	0,20	0,19

MAE between FAO 56 Penman-Monteith and other models ranges from 0.28 mm/day (Makkink) up to 1.28 mm/day (Hargreaves-Samani 1985) and LOG RMSE ranges from 0.04 mm/day (Makkink) to 0.14mm/day (Hargreaves-Samani).

Table 5 showed the results of Index of Agreement (IA). Consistently, Makkink model gave the best results with IA 0.78 followed by Linarch (0.42) and FAO Pan Evaporation (0.42)

Tabel.5: Index of Agreement among the models

I.A							
	PM	MK	BC	Pan	24 RD	HS	Ln
PM	1	0,78	0,09	0,42	0,55	0,26	0,42
MK	0,78	1	-0,35	0,80	0,81	0,10	0,10
BC	0,09	-0,35	1	-0,40	-0,22	0,79	0,85
Pan	0,42	0,80	-0,40	1	0,95	-0,03	-0,08
24 RD	0,55	0,81	-0,22	0,95	1	-0,03	-0,01
HS	0,26	0,10	0,79	-0,03	-0,03	1	0,93
Ln	0,42	0,10	0,85	-0,08	-0,01	0,93	1

The resume of statistical analysis from daily evapo transpiration data including the error indicator of each model compare to the FAO 56 PM as the standard was presented in Table 6.

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Table.6: Statistical of daily evapotranspiration data from each model

	Evapotranspiration Model						
	24	BC	HS	Mk	Lin	Pan	FAO
Average	2,851	4,607	4,821	3,306	4,387	2,925	3,533
STD	0,485	1,419	0,561	0,370	0,569	1,009	0,774
RMSE	0,884	1,392	1,481	0,683	1,065	1,283	
MAE	0,749	1,107	1,294	0,571	0,890	1,037	
LOG RMSE	0,117	0,130	0,170	0,088	0,131	0,239	
I.A	0,439	0,565	-0,403	0,595	0,263	0,363	

Table 6 showed that among the models, Makink model consistantly had the smallest RMSE, MAE and Log RMSE compared to PM model and had the highest agreement. On the other hand HS model had the biggest RMSE, MAE and log RMSE with the lowest agreement. Therefore, for Lampung, estimation ET model with the closest estimation to FAO 56 Penman-Monteith was Makkink model. It can be concluded that Makkink model was the suitable simple model that should be chosen in Lampung to calculate ET_o besides the recommended one, FAO 56 Penman Monteith, especially when the climate data is limited.

So far the estimating model that broadly used is FAO 24 PAN which is based on observation on Pan A evaporation pan. In this study this model did not give a good estimation compared to the FAO 56 PM model (RMSE 0.75; MAE 0.62; Log RMSE 0.11 and IA 0.42). In comparing 24 PAN model to 56 PM, using 3 years data in 2 stations in Lampung, Manik et al. (2012) found that the coefficient correlation between those two models are low (r=0.3 for Branti Station and 0.5 for Masgar station).

Daily modeled ET_o results from each model in 1 year is presented in Figure 2. Most of the models had similar trends with FAO 56 PM but with different estimation. Some models underestimated FAO 56 PM (Makkink, FAO 24 Radiation and FAO 24 Pan Evaporation) while some overestimated (Blanney Criddle, Hargreaves-Samani 1985 and Linarch). Makkink model had good estimation to FAO 56 PM in wet months October-March, and slightly underestimated in dry months March – October.

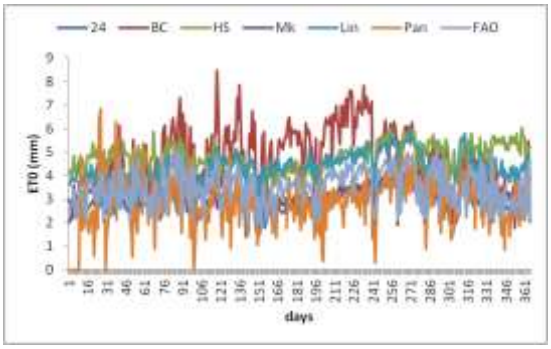

Fig.2: Daily estimation of evapotranspiration from different models

In general, Makkink model is a function of R_s (solar radiation MJ/m^2/day), Δ(slope of vapour pressure), and γ(psychrometric constant). Makkink is a simple model since γ is 66,1 (kPa/^{o}C), while R_s and Δ could be calculated using following equations (Allen, et al., 1998):

$$R_s = K_{Rs}\sqrt{(T_{max} - T_{min})}R_a \dots\dots (21)$$

$$\Delta = \frac{4098\left[0{,}6108\ exp\left(\frac{17{,}27\,T}{T+237{,}3}\right)\right]}{(T+237{,}3)^2} \dots\dots (22)$$

K_{Rs} is a coefficient, 0.16 for interior land area and 0.19 for coastal area, R_a is top solar radiation (MJ/m^2/day). Basically, this model can be calculated using only maximum and minimum temperature (^{o}C) which is more available in most research stations.

Irmak, Allen and Whitty (2003) conducted a research using daily measured weather data for a 23-year (1978–2000) in North-Central Florida to examine twenty one ET_0 methods (excluding the FAO 56-PM; 10 combination methods, 4 radiation methods, 5 temperature methods and 2 pan evaporation methods) and the results showed that the performance of all radiation methods including Makkink was poorer than that of all combination methods except the Stephens-Stewart method, which performed better than the original PM combination method. Makkink methods had a similar standard error of estimate for all months with the Stephens-Stewart method, but the method significantly underestimated ET_0 throughout the year.

The tendency to underestimate high evaporative demand in Makkink model has been found in most of previous studies e.g. in Korea (Chen, et al., 2005), in Germany (Xu and Chen, 2005) in Jordan (Mohawesh, 2011) in Iran (Bakhtiari et al., 2011); those results might be related to the ignorance of the significant influence of wind speed on ET_0. Regardless of that, Makkink model was considered as a good option model and the closest to Penman Monteith method in India (Haldar, Kumar and Sehgal, 2005) and in Hungary (Racz, et al., 2013).

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3.2. Comparing Makkink model with direct measurement

Direct evapotranspiration measurement was done using lysimeters. Measuring water input and out put together with soil water content on the lysimeters was a challenge. During this research the measurements were repeated several times, however due to technical problems they were not always done in the same day. Therefore, the results (Table 7 and 8) were considered as an average number.

In average from Table 7 and 8, evapotranspiration rate during the research was 3.8 +1.11 = 4.91 mm/day, higher than estimated Makkink (3.306 mm) and FAO (3.533 mm) (Table 6). In general, the average and accumulation of observed Pan A gave slightly higher evapotranspirasion compared to estimated Makkink. However, observed Pan A had much wider standard deviation; Makkink model gave more flat result in daily estimation while Pan A was more fluctuative.

Table.7: Water input and output to the lysimeters

Time of Observation (n)	Rainfal (R) (mm)	Irrigation (I) (mm)	Percolation(P) (mm)	R+I-P (mm)
1	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00
5	3.30	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00
7	43.00	0.00	0.00	0.00
8	9.40	0.00	0.00	0.00
9	26.50	0.00	0.00	0.00
10	1.00	0.00	0.00	0.00
11	12.50	0.00	2.70	9.80
12	0.00	0.00	4.17	-4.17
13	0.00	0.00	1.62	-1.62
14	0.00	0.00	0.76	-0.76
15	0.00	10.00	0.38	9.62
16	0.00	15.00	0.24	14.76
17	2.00	0.00	1.21	0.79
18	9.00	0.00	1.19	7.82
19	0.00	0.00	2.07	-2.07
20	0.00	0.00	0.66	-0.66
21	0.00	0.00	0.26	-0.26
22	5.00	0.00	0.00	0.00
23	2.70	0.00	0.18	2.52
24	5.00	0.00	0.03	4.97
25	0.50	0.00	0.02	0.48
26	0.00	15.00	0.00	15.00
27	0.00	0.00	0.00	0.00
28	0.00	10.00	0.00	10.00
29	3.00	0.00	2.30	0.70
30	3.50	0.00	1.75	1.75
31	0.00	0.00	0.00	0.00
32	5.50	0.00	0.00	5.50
33	0.00	0.00	0.23	-0.23

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Time of Observation (n)	Rainfal (R) (mm)	Irrigation (I) (mm)	Percolation(P) (mm)	R+I-P (mm)
34	3.40	0.00	0.00	3.40
35	0.50	0.00	0.17	0.33
36	3.20	0.00	0.02	3.18
37	0.00	11.67	0.02	11.65
38	0.00	53.33	0.00	53.33
39	0.00	0.00	3.00	-3.00
40	2.60	0.00	1.53	1.08
41	0.50	0.00	0.73	-0.23
42	3.20	0.00	0.00	0.00
43	0.00	0.00	0.44	-0.44
44	0.00	0.00	0.00	0.00
45	0.00	0.00	0.08	-0.08
46	0.00	0.00	0.11	-0.11
47	24.00	0.00	0.00	24.00
48	0.00	0.00	0.71	-0.71
49	0.00	0.00	0.00	0.00
50	0.60	0.00	0.00	0.60
51	0.00	0.00	0.00	0.00
52	0.00	0.00	0.00	0.00
53	1.00	0.00	0.00	1.00
54	0.00	0.00	0.00	0.00
55	29.50	0.00	0.00	29.50
56	44.90	0.00	0.00	44.9
57	0.00	0.00	0.00	0.00
58	0.00	0.00	0.00	0.00
59	22.00	0.00	0.00	22.00
60	8.00	0.00	0.00	8.00
61	0.40	0.00	0.00	0.40
			Average	3.80

Table.8: Soil water content of the lysimeters

Number of Observation (n)	Soil Tension (kΩ)	Volumetric Soil water content (%)	Daily Δ S (mm)	Cumulative Δ S (mm)	Number of Observation (n)	Soil Tension (kΩ)	Volumetric Soil water content (%)	Daily Δ S (mm)	Cumulative Δ S (mm)
1	122	43.96			13	171	39.20	-0.41	-11.90
2	113	45.04	2.70	2.70	14	168.33	39.42	0.55	-11.35
3	131	42.96	-5.21	-2.51	15	179	38.56	-2.17	-13.51
4	143.33	41.69	-3.17	-5.68	16	176.67	38.74	0.46	-13.05
5	146.67	41.37	-0.81	-6.49	17	188.33	37.84	-2.25	-15.30
6	157.33	40.38	-2.47	-8.96	18	203.33	36.76	-2.70	-18.01
7	160	40.14	-0.59	-9.56	19	236.67	34.62	-5.35	-23.36
8	154	40.68	1.35	-8.21	20	280	32.25	-5.93	-29.28
9	153.67	40.71	0.08	-8.14	21	246.67	34.04	4.47	-24.82
10	152.67	40.80	0.23	-7.91	22	249	33.90	-0.33	-25.15
11	148.33	41.21	1.02	-6.89	23	251.67	33.75	-0.38	-25.52
12	169	39.37	-4.60	-11.49				average	-1.11

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B. Using Pan A evaporation

Table.8: Estimation of ET_0 comparing Pan A with The Makink

	April		May		June	
	Pan A	Makink	Pan A	Makink	Pan A	Makink
Average Sum Standard Deviation	3.96 118.72 2.15	3.39 101.80 0.25	3.40 105.50 1.61	3.45 106.99 0.36	4.19 121.38 2.01	3.40 98.66 0.30

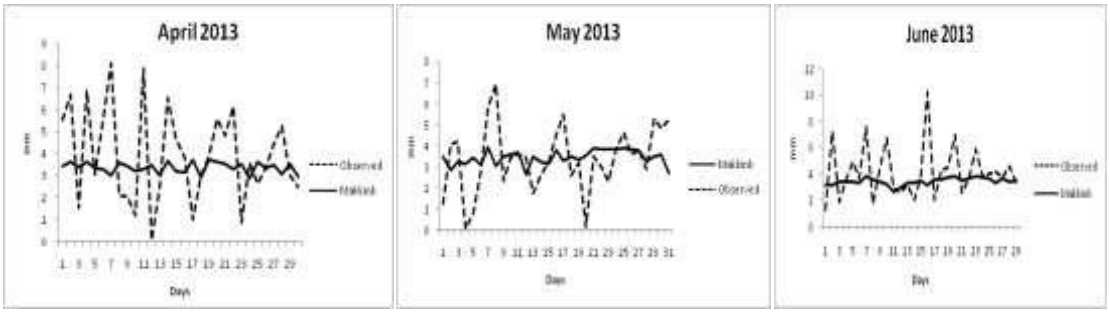

Fig.3: Daily evapotranspiration from observed Pan A compared to estimated Makkink.

IV. CONCLUSION

This study concluded that Makkink model is a simple model that can be chosen in Lampung as an alternative to estimate standard evapotranspiration in an area with limited climate data needed to apply FAO 56 PM, with a note that Makkink tended to be underestimated during dry months. Estimation of evapotranspiration using models are sufficient for averaged and accumulated result from some period of time, not for daily or single measuremnt.

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Anthracnose Disease of Walnut- A Review

Mudasir Hassan, Khurshid Ahmad

Department of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar Campus, 191 121, Jammu & Kashmir, India

Abstract**—** *Walnut (Juglans regia) an important commercial dry fruit crop, is attacked by several diseases causing economic damage and amongst them walnut anthracnose caused by Marssonina juglandis (Lib.) Magnus has posed a serious threat to this crop in India and abroad. Walnut anthracnose results in reduction in quantitative parameters such as size, mass and actual crop of nuts, failure in metabolic processes in leaves and change in biochemical indices. Premature loss of leaves results in poorly-filled, low-quality, and darkened kernels. The disease initially appears on leaves as brown to black coloured circular to irregularly circular spots. These spots eventually enlarge and coalesce into large necrotic areas. Later on these infected leaves turn yellow and drop prematurely. Infection of anthracnose disease on leaves occurred at relative humidity above 95 per cent and severity of infection was not influenced by temperature between 10-32^{o}C but was significantly reduced below 10^{o}C. Anthracnose of walnut has been reported to be caused by Marssonina juglandis (Lib.) Magnus,with Gnomonia leptostyla (Fr.) Ces. and de Not as its perfect stage reported that acervuli produced by fungus appeared early in the season as small black specks on the lower surface of diseased leaves. The pathogen(G. leptostyla) reportedly perpetuates primarily on infected leaf debris, and ascospores produced in perithecia act as the primary inoculum during spring. Burying (ploughing in) the fallen leaves in autumn and winter ,pruning of infected twigs and branches and adequate nitrogen fertilization has been recommended for the management of walnut anthracnose as well as under planting walnut saplings with annual and perennial legumes has been shown to increase foliage nitrogen content. Different formulations of mancozeb, dithianon, flusilazole and copper fungicides controlled anthracnose disease.*

Keywords**— Walnut, Anthracnose, Marssonina juglandis, Perpetuates, Management.**

I. INTRODUCTION

Walnut (*Juglans regia* L.) is economically an important dry fruit crop which belongs to family *Juglandaceae*. It originated in Iran from where it was distributed throughout the world (Arora, 1985). It is mainly grown in china, USA and Iran, whereas India stands seventh in production accounting upto 2.14 per cent of the world walnut production (Anonymous, 2010). In India, walnut is grown in Jammu and Kashmir, Arunachal Pradesh, Himachal Pradesh and Uttarakhand. In J&K, Walnut is grown in Badrawah, Poonch, Kupwara, Baramulla, Bandipora, Ganderbal, Budgam, Srinagar, Anantnag and other hilly areas occupying an area of 83,219 ha with an annual production of 20,873 tonnes (Anonymous, 2012). Jammu and Kashmir State has attained a special place in the international trade of walnuts contributing about 98 per cent of the total production in India (Sharma, 2012). Its cultivation plays a significant role in the economic profile of the farmers living in hilly and backward areas, where economic condition of the people is extremely fragile (Anonymous, 2012).

Walnut fruit is consumed as a dry fruit and is also used for preparation of bakery products, confectionary and oils. Walnut shells are used in glue and plastics as well as in dusting and solution making for cleaning and polishing surfaces (Bal, 2006). Walnut wood and even its leaves are usable in wood and veneer industry, dying, pharmaceutical and food industries (Zamani *et al.*, 2011). Among all nuts, walnut fruit is rich in protein, oils including omega-3 fatty acids, vitamins and minerals with excellent flavor and rich source of energy (Rana *et al.*, 2007). Its alpha linolenic acid has substantial cardio protective effects as it increases the ratio of high-density lipoprotein cholesterol to total cholesterol, reducing inflammation and improving arterial function (Hu *et al.*, 1999; Diousse *et al.*, 2001; Patel, 2005). It contains 'melatonin' an antioxidant produced by pineal gland and responsible for inducting and regulating sleeps (Reiter *et al.*, 2005). It also reduces the incidence of cancer and, delays neurodegenerative diseases of aging (McGranahan and Leslie, 2012).

Among the major biotic factors, the important fungal diseases include walnut anthracnose (*Marssonina juglandis* (Lib.) Magnus), root and crown rot [*Phytopthora cactorum* (Lebert and Cohn) Schrot], branch wilt [*Hendersonula*

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toruloidea Nattras], ringspot [*Ascochyta juglandis* Blotshauser.), downy leaf spot [*Microstroma juglandis* (Berenger) sacc.)], heart rot [*Polyporus squamosa* Huds. ex Fr.) Fr.], powdery mildew [*Phyllactinia quttata* (Wallr. ex Fr.) Lev.;*Microsphaera extensa* (Cke and Peck)] besides stem canker and die back diseases caused by fungi like *Cytosperma leucosperma* (Pers. ex Fr.) Fr., *Nectria galligena* (Bres.), *Fusarium solani* (Mart. Sacc), etc (Sharma and Sharma, 1999; Anonymous, 2013). Amongst them, anthracnose is the wide spread foliar disease of *Juglans* spp. and the fungus attacks leaves, nuts and shoots of the current season growth (Berry, 1977; Belisario *et al.*, 2008). Symptoms develop on the on leaves and fruits as irregular necrotic areas that are often surrounded by small chlorotic halos. The disease causes premature defoliation, slows down plant growth, reduces quantity and quality of nut crops, thereby resulting in huge economic loss in the walnut cultivation regions of the world (David, 1997; Belisario *et al.*, 2001; Van Sambeek, 2003; Kalkisim, 2012).

Occurrence and economic importance

Walnut anthracnose or black spot/blotch has been reportedly considered as most serious fungal disease of black walnut (*J. nigra* L.) and Persian or English walnut (*J. regia* L.) as well as other species of genus *Juglans* throughout the walnut growing regions including North and South America, Europe, Iran and other Asian countries (Behdad, 1991; Belisario, 2002; Belisario *et al.*, 2008; Salahi *et al.*, 2009). In India, Kaul (1962) reported the occurrence of walnut anthracnose disease for the first time from Kashmir valley. Hassan (1979) reported the occurrence of walnut anthracnose in Iraq while as, Werner (1994) reported from poland. It is indigenous to North America (Todhunter and Beineke, 1984), economically important in the main production areas of Italy and Hungary (Belisario, 1992; Pinter*et al.*, 2001) and most widespread and dangerous disease in Bulgaria (Tsanov and Roshev, 1976; Kalkism, 2012). Saremi and Amiri (2010) reported that this disease caused 60-80% yield losses in quality and quantity in Iran. Walnut anthracnose results in reduction in quantitative parameters such as size, mass and actual crop of nuts, failure in metabolic processes in leaves and change in biochemical indices (Shirnina and Kotljarova, 2000). Premature loss of leaves results in poorly-filled, low-quality, and darkened kernels (Black and Neely, 1978;Zamani*et al.*, 2011). Walnut anthracnose infection results in reduction in nut yield which varied from cultivar to cultivar (Pinter *et al.*, 2001; kalkism, 2012). Early infection on nuts results in premature fruit drop (Worste and Beineke, 2001).

Symptamotology

The symptoms of the walnut anthracnose are mainly observed on current year leaves, twigs, fruits and rarely on shoots. The disease initially appears on leaves as brown to black coloured circular to irregularly circular spots. These spots eventually enlarge and coalesce into large necrotic areas. Later on these infected leaves turn yellow and drop prematurely however severe infection and leaf drop usually occurs late in the season (Black and Neely, 1976;Todhunter and Beineke, 1984; Belisario, 2002; Kalkism, 2012). Berry and Frederick (1997) observed symptoms on leaves, fruits and branches which appear as dark brown spots, more or less circular, usually bordered by a yellow ring. In later stage, spots merge to form large dead areas which usually results in leaf defoliation. Saremi and Amiri(2010) observed the characteristics variation in the anthracnose diseased spot during leaf development. Spot shape and area varied from several mm to several cm, from oval to round shape and often surrounded by a yellow halo. They further observed that the infection of leaves was severe in late summer and some infected trees became defoliated. The disease also affected fruits and nut meat since nut from diseased trees showed dark and shriveled meat and necrotic spots.

Zamani *et al.* (2011) observed that the walnut anthracnose caused by*M. juglandis*may appear on green outer layer of fruits in the form of circular black or brown stains while as the disease spots on leaves appear as dark brown, more or less circular spots bordered by a yellow ring which vary from 1/16 to 5/16 inch in diameter. These individual spots later on coalesce and form large necrotic areas. Leaf infection usually results in defoliation but sometimes the infected leaflets remain attached to the tree for much of the growing season. This fungus also appears on thinner branches in the form of oval lesions or irregular circles with brown color tending to grey and with reddish brown peripherals. They further reported that at the middle of season, black points appear on the upper part of the infected leaves bearing reproductive organ of fungi. These organs produce bicellular spindle-shaped conidia somewhat tending to limber (embowed) shape. Worste and Beineke (2011) also observed that the symptoms of walnut anthracnose develop on current year leaves, nuts and stem as irregular necrotic areas, which are usually less than 5mm in diameter, and are often surrounded by small chlorotic halos.

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Causal pathogen

Anthracnose of walnut has been reported to be caused by *Marssonina juglandis* (Lib.) Magnus,with *Gnomonia leptostyla* (Fr.) Ces. and de Not as its perfect stage (Sogonov *et al.*, 2008; Dastjerd and Hassani, 2009; Anonymous, 2013). Sharma and Sharma (1999) reported that acervuli produced by fungus appeared early in the season as small black specks on the lower surface of diseased leaves. Conidiophores were hyaline, short, simple, elliptical, and one celled packed together in a small layer bearing conidia at the tips. The conidia were variously shaped being straight, ovoid, falcate or with only one end rounded and the other pointed. There was one septa, and two cells are unequal with prominent oil globules and measured 15- 26 × 2-5 µm. They further reported that brown coloured perithecia developed on fallen leaves which were immersed in the leaf tissues while the beak protrudes considerably on to the leaf surface. These were amphigenous, solitary, scattered, globose, reddish brown with long cylindric beak. The beak measuring 140-170 µm and 25-40 µm in breadth, while the globose base has a diameter of 120-125 µm. The inner cavity of the perithecia was lined with club shaped to fusiode asci while as the asci were hyaline aparaphysate, 8 spored, measuring 56-62 × 14-16 µm. The ascospores were hyaline, fusiode, straight to slightly curved septate and measured 15-19×4-5 µm.

Saremi and Amiri (2010) isolated *M. juglandis* on potato dextrose agar (PDA), corn meal agar (CMA) and nutrient agar (NA) fromleaves, fruits and foliage. They also observed that isolated fungus produces minute black fruiting bodies called acervuli in which conidia were colorless, usually crescent-shaped, and divided by a cross-wall into two approximately equal cells. Perithecium with 380- 450 µm in length and 150-260µm breadth neck like structure with 19.5-24.5 × 7.2-8 µm ascospore size. Salahi *et al.*(2007) isolated as a streak single spore on oat meal agar while Jamshidi *et al.*(2012) isolated *M. juglandis* from the leaf discs bearing acervuli by transferring germinating macroconidia from 2% water agar to 39% potato dextrose agar added with 7g/litre of oatmeal. Dastjerdi *et al.* (2009) isolated *G. leptostyla* (anamorph: *M. juglandis*) on oat meal agar and corn meal agar media. Kalkism (2012) isolated *G. leptostyla* on potato dextrose agar (PDA) from the walnut (*Juglans regia* L.) leaves showing typical symptoms and identified as *G. leptostyla* according to optimal growth of *G.leptostyla* occurred at 22ºC and pH 5.4.

Physiological studies

Fayret and Parguey (1976) reported that production of ascospores of *G. leptostyla* occoured at 10ºC. Perithecia remain immature at 20ºC in which the multinucleate pro-sporophyte grows normally and produces a syncytium from which the ascosporophyte originates, but differentiation of the ascogenic phase is heat inhibited. They further observed that the cold is necessary for the evolution of initial dicaryotic cells, which are the true carpospore cells. These cells and the ascosporophytic phase have special physiologic requirements as regards temperature. Matteoni and Neely (1979) reported that growth of *M. juglandis* was maximum at 22 ºC and at pH 5.4 whileas sporulation was maximum at 26ºC with pH 6.8 on oatmeal agar and the optimum temperature for germination of ascospores and conidia were 26ºC and 24ºC, respectively. Microconidia were not produced at temperature less than 10ºC and did not germinate. They further observed that the light reduced vegetative growth, but promoted production of conidia and acervuli. Production of fertile perithecia were observed *in vitro* after incubating crosses of 2 mating types in darkness at 10ºC for up to 3 months. Belisario *et al.*(2008) while investigating the mean diameter of colonies grown *in vitro* at 22ºC and sporulation of 191 isolates of *G. leptostyla* grouped by site of collection, were compared observed that the isolates that grew significantly more slowly were from sites with colder early springs and higher altitudes. Acervular conidiomata were abundantly produced by all isolates at 22ºC in darkness after 21 days, while productions of protoperithecia were noticed within 2 months by most isolates, under the same conditions. Production of conidiomata was observed at 20 and 25ºC while as after 2 months, protoperithecia were present in most isolates at 20ºC, very few at 25 and 15ºC, and no production was recorded at 10 and 30ºC after 2 months. Fertile perithecia with asci and ascospores were produced, after 3 months at 10ºC in darkness. They further noticed that the ascocarp diameter, width of asci and length of ascopores of *in vitro*produced perithecia were larger than those of perithecia produced in nature. The latter showed a neck length longer than *in vitro*-produced perithecia. Salahi *et al.*(2009) reported that oat meal agar to be the best artificial media for growth and asexual reproduction of the *M. juglandis.* Dastjerdi *et al.*(2009) noticed that the isolates of *G. leptostyla* produced acervular type of conidiomata at 21°C temperature, under photo-period of 16-hours light : 8-hours darkness) after 18-21 days of incubation period. Jamshidi (2011) reported that colonies exposed to light produced acervuli faster and in a denser way on optimum pH of 5.8. He further reported that fertile perithecia with asci and

ascospores were produced *in vitro* after 75 to 90 days at 4°C in darkness. Slow growths of different *Marssonina* spp. have also been reported by several workers. Zhao *et al.* (2010) reported that for rapid mycelial growth and sporulation of *Diplocarpon mali*, potato and carrot dextrose broth (PCDB) and potato and carrot sucrose broth (PCSB) were most favorable. The optimum temperature for mycelial growth and conidial production was 25 ^{o}C. Active mycelial growth occurred at pH 5-7, and pH 5-8 was favorable for sporulation.Galea*et al.*(1986) reported that lettuce anthracnose pathogen, *M. panattoniana*, grows at a wide temperature range at 3-26°C and pH 4-5.2. Colony growth was best on potato dextrose agar (PDA) at 20 ^{O}C and pH 5.2. Wolcan (1985) reported that sporulation of *Diplocarpon earliana* causing leaf scrotch of strawberry was best on malt extract agar with peptone and yeast, at 20 ± 2°C.

Pathogenicity

In order to prove the pathogenic nature of *Marssonina* spp. Different workers have adopted different methods. Figueiredo and Hennen (1995) proved the pathogenicity of *M. salicicola* in the laboratory by artificial inoculation with a conidial suspension brushed on to healthy detached young leaves of *Salix babylonica* and left in the greenhouse. When maintained in wet conditions, leaf symptoms were evident 12 days after inoculation, and threads of conidia coming out from acervuli were produced 15 days later. Nadroo (2006) proved the pathogenic nature of *M. coronaria* by spraying the conidial suspension on one year old budded potted plant of red delicious apple cultivar. Cline and Neely (1983) proved the pathogenic nature of the of *Gnomonia leptostyla* byspraying conidial suspension on mature leaves of *Juglans nigra*and observed macroscopic lesions at 240 h and acervuli formation after 240 h. Dastjerdi *et al.* (2009) proved the pathogenicity of *M. juglandis* by spraying the conidial suspension on mature, fully expanded leaflets. Macroscopic brown spots were observed on leaves 16 days after inoculation while acervuli were observed after 24 days while as Neely (1986) proved the pathogenic nature of *G. leptostyla* by spraying the conidial suspension on mature leaves of walnut seedlings and noticed the development of lesions on leaf surface after approximately two weeks.

Epidemiological studies

Black and Neely (1976) observed that infection of anthracnose disease on leaves occurred at relative humidity above 95 per cent and severity of infection was not influenced by temperature between 10-32^{o}C but was significantly reduced below 10^{o}C. Rosnev and Naidenov (1986) also reported that *M. juglandis* requires temperature of 15-30°C, frequent precipitation, and humidity over 65 per cent. Matteoni and Neely (1977) observed that incubation period was directly related to infection frequency. Infection was more severe on older leaves and 10 times more frequent with adaxial inoculation.

Vonica (1970) reported that conidia reportedly cause secondary infections and intensify the disease during summer. Low temperature, rainfall and high relative humidity at the start of growth promoted infection by the pathogen (Andrievskii and Rikhter, 1976; Jamshidi and Salahi, 2009). Kessler (1984) reported that, after initial lesions arising from infections by ascospores in the month of May, the lesion numbers increased through early summer as a result of secondary leaf infection by conidia. He further observed that disease development was maximum in late July and early August, when most defoliation of previously infected leaflets occurred. Hashemi (2005) noticed that relative humidity of 80 percent for 24 hours and temperature in the range of 10-20°C under windy conditions resulted in more than 80 per cent of ascospores released, whereas high humidity and wind during growing season were necessary for occurrence of secondary infections and dispersal of conidia. Belisario *et al.*(2001) reported that increase in disease incidence during late August to ending September could be related to the increasing number of leaflets bearing fertile acervuli for secondary infections, leading to progressive senescence of leaves.

Perpetuation

Different *Marssonina* spp. attacking different crops overwinter by different means either by forming sexual fruiting bodies on overwintering leaves, shoots and fruit debris or as conidia. Vucinic (1977) reported the formation of apothecia of*M. brunnea*at the end of winter on fallen leaves while Sokolova (1975) observed maturation by late march to early april. Harad *et al.* (1974) reported the formation of apothecium of *M. coronaria* on overwintering apple leaves infected with blotch, containing ascospores which served as primary inoculums in spring. Anselmi (1979) reported that *M. salicicola* infecting *Salix babylonica*overwinters by means of small stromata on the edges of cankers or as conidia in the acervuli on branches. Milicevic*et al.*(2002) reported that *Diplocarpon earliana* (anamorph *M. fragariae*), the causal organism of leaf red spot or leaf scorch of strawberry, overwinters in the form of mycelium and produces two types of fruiting bodies (apothecia and acervuli) in early spring. Primary infections are caused by ascospores and conidiospores from the

acervuli while as secondary infections are caused only by conidiospores.

The pathogen(*G. leptostyla*) reportedly overwinters primarily on infected leaf debris, and ascospores produced in perithecia act as the primary inoculum during spring (Vonica, 1970; Black and Neely, 1978; Dimova and Arnaudov, 2008). Veghelyi and Penzes (1990) observed that the fungus overwintered in infected leaves and occasionally in the epicarp of the fruit. Asci were formed by the end of February and ascospores developed during March. Incubation period took 3-5 weeks. After the appearance of the first symptoms, the development of conidia and secondary infections occurred almost continuously until late autumn.

Jamshidi*et al. (*2009) reported the perithecia from fallen leaves. Perithecia had one beak on leaves and up to four beaks on culture media. Perithecium in homothallic isolates had significantly higher diameter and longer beaks than non-homothallic isolates. Saremi and amiri (2010) reported that the fungus commonly overwintered in fallen walnut leaves, infected during the preceding summer in walnut orchards

Disease management

Cultural as well as chemical management has been suggested by various research workers for the management of walnut anthracnose.

Cultural practices

Burying (ploughing in) the fallen leaves in autumn and winter to a depth of 10-15 cm, pruning of infected twigs and branches and adequate nitrogen fertilization has been recommended for the management of walnut anthracnose(Rosnev and Tsanova, 1980; Neely, 1981; Pscheidt and Ocamb, 2014).Zakhov (1980) recommended rouging and planting healthy material. Shevchenko (1981) advocated that the best control in rugged terrain where the chemical control is difficult is the selection of local immune forms of *Juglans regia* and production of hybrids with *J. nigra*, *J. cinerea* etc. Kessler (1985) found that an over winter cover of autumn olive leaves reduced the number of ascospores, the primary inoculum, discharged from infected fallen walnut leaves. He further observed nitrogen fixed and released from autumn olive increases the walnut foliage nitrogen content and reduces walnut susceptibility to secondary infections initiated by conidia released from the primary infections. Neely (1986) suggested that the incidence or severity of anthracnose can be altered through site modification and increase in foliage nitrogen content. Van Sambeek *et al.* (2003) reported that under planting herbaceous legumes in walnut plantations could potentially reduce the severity of anthracnose either in response to increased soil nitrogen or by disrupting ascospore dispersal, while as under planting walnut saplings with annual and perennial legumes has been shown to increase foliage nitrogen content. Saremi and Amiri (2010) recommended that eradication of walnut plant residue, especially fallen leaves is very beneficial in reducing disease. Kalkasim (2012) recommended leaf extracts of *Corcus mas* and *Morus nigra* against *M. juglandis*. Neely (1986) suggested that the incidence or severity of anthracnose can be altered through site modification and increase in foliage nitrogen content.

Resistance of different cultivars

Black and Neely (1978) while artificially inoculating *juglans* species with *G. leptostyla* conidia observed that the Hinds (*J. hindsii*) and Arizona (*J. major*) walnuts were more susceptible than black walnut (*J. nigra*). Little walnut (*J. microcarpa*), Japanese walnut (*J. ailantifolia*), butternut (*J. cinerea*), heartnut *(J. ailantifolia var. cordiformis*), and assorted hybrids, were less susceptible than black walnut while as the clones of English walnut (*J. regia*) showed the greatest range in susceptibility. Dimova (2007) reported that Izvor-10 is medium susceptible and the index of infesting could reach up to 52.5 per cent in the leaves and 34.5 per cent in the fruit. Belisaro *et al.*(2008) observed *J. sieboldiana* and *J. cinerea* to be highly resistant and both *J. nigra* and *J. hindsii* to be highly susceptible to the disease while as *J. regia* showed an intermediate response of susceptibility to anthracnose. Salahi and Jamshidi (2009) reported that Z67 and K73 cultivars showed more resistance in comparison with others and Z67 was the most resistant one. The cultivars Ser, Vina, Hartley, Ronde de Montignac, Lara and Franquett had moderate to weak resistance and the cultivars Z63, Z60 and Pedro were all susceptible to the disease. While evaluating 15 walnut cultivars for susceptibility to *G. leptostyla* infection, Arnaudov and Gandev (2009) recorded that only one cultivar "Chandler" was resistant and five cultivars were slightly susceptible, whereas rest were either susceptible or highly susceptible (cvs. "Sheinovo", "zvor 10" and "Slivenski") or very highly susceptible (cvs. "Alososzentivani" and "Seer") with disease intensity ranging from 4.9 to 62.4 percent.

Chemical management

To manage the disease caused by various *Marssonina spp.* in different host crops, the use of chemicals has been suggested by various workers. Rimfeldt (1979) suggested

that effective control of *M. salicicola* can be achieved with copper oxychloride at 2500ppm, captafol at 1800ppm, and benomyl at 600 ppm while as Anselmi, (1979) recommended Benomyl, maneb and mancozeb against *M. salicicola* infecting *Salix babylonica*. Sharma (2000) recommended protective sprays with broad spectrum fungicides, mancozeb (0.3%) and carbendazim (0.05%) reduced the disease significantly. Further he observed that benomyl, thiophanate methyl, propineb, chlorothalonil, dithianon and ziram were also effective in controlling the disease.

Milicevic *et al.*(2002) suggested that fungicide Folicur M 50 WP (tebuconazole + tolylfluanid) and Kidan SC (iprodione) were the most effective against the leaf spot disease of strawberries (*M. fragaria*). Devappa*et al.(*2006) suggested that chlorothalanil (0.2%), mancozeb (0.2%), hexaconazole (0.1%), propiconazole (0.1%), carbendazim (0.1%) copper oxychloride (0.3%) were highly effective in controlling the black spot of rose (*Diplocarpon rosae*). Thakur and Nirupma (2010) recommended that Indofil M-45 (0.3%), Antracol (0.3%), Indofil Z-78 (0.3%), Bavistin (0.05%), Kocide (0.3%), Tohfa (0.075%) and Copter (0.3%) against premature leaf fall of apple caused by (*M. coronaria*).

Vonica (1970) reported that (3-6 treatments per year) of zineb, dodine, zinc-metiram, phaltan, PMC, maneb, captan and thiram gave effective control of walnut anthracnose while as Reznikova (1977) observed that 2-6 sprays of combined treatment with Bordeaux and urea gave the best control than treatments with 1% Bordeaux alone. Various workers reported that fungitoxicants like carbendazim, benomyl, DNOC, dodine, cupric oxide, zineb, maneb and chlorothalonil proved to be effective against *M. juglandis* and significantly reduced the disease (Berry, 1977; Kleiner and Bulatova, 1978; Zamani *et al.*, 2011). Neely (1977) reported that soil application of benomyl, reduced the the incidence and severity of the anthracnose of black walnut for several years. Movsesyan (1978) recommended spraying with 0.5% copper oxychloride and 0.4% zineb during the growing period for control of walnut anthracnose. Rosnev and Tsanova (1980) recommended that Bordeaux mixture 1%, Dithane M-45 0.3%, and Dithane cupromixin 0.6% against walnut anthracnose while as Zakhov (1980) recommended that spraying with Bordeaux, at 2% during winter and 1% before flowering and once after flowering against *G. leptostyla*. While testing various fungitoxicants on 8-year old walnut trees in Poland, Cimanowski *et al.*(1991) observed that different formulations of mancozeb, dithianon, flusilazole and copper fungicides controlled anthracnose and leaf spot (*Xanthomonas campestris* pv. *juglandis*). Nakova and Dimova (2003) while investigating the effects of 22 fungicides on the mycelial growth and ascospore germination of *G. leptostylain vitro* observed that the inhibition of both parameters was greatest with the contact fungicides Kuprozine Super (copper oxychloride), Dithane (mancozeb) and Ronilan (vinclozolin), and with the systemic fungicides Corzate (simoxanile), Rubigan (fenarimol), Topsin M (thiophanate-methyl), Anvil (hexaconazole) and Fundasol (benomyl). In field tests conducted in Bulgaria, the efficacy of Dithane, Corzate, Topsin M, Anvil and Fundasol against *G. leptostyla* in walnut was evaluated. The most effective were Anvil and Fundasol (control of more than 90%), followed by Topsin M (control of more than 80%).Zamani *et al.*(2011) advocated that application of Bordeaux solution in winter and copper fungitoxicants in early spring could be highly effective for controlling the walnut anthracnose.

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Perceived Effect of Climate Variability on Arable Crop Production in Bayelsa State, Nigeria

Okringbo I. J.[1], Ibe M. N.[2], Oduehie T. C.[3]

[1]Department of Rural Sociology and Agricultural Extension, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

[2]Department of Agricultural Extension, Federal of Technology, Owerri

[3]National Agricultural Extension and Research Liaison Services, Ahmadu Bello University Zaria. South-East Zonal Office Umudike

Abstract— *The study examined the perceived effect of climate variability on arable production in of Bayelsa State, Nigeria. Primary data were collected using structured interview guide administered to 120 farmers. Purposive random sampling technique was used to select twelve communities and two agricultural zones. Data collected were analyzed using descriptive such as mean while, inferential statistics was used to test the null hypothesis. The findings showed that the perceived effect of climate variability on cassava in regards to poor yield, damage and breaking of plants due to windstorm. The hypothesis test showed that the mean of perceived effect of climate variability on arable crop production in Nembe agricultural zone was (3.6530) while that of Yenagoa Agricultural Zone was (3.3272). The* Z_{-cal} *(6.747) was much higher than* Z_{-tab} *(2.02). The study concluded that the food security status of rural farmers is threatened. Hence, it was recommended that farmers should form cooperative societies in order to cope with high cost of agricultural production and government should reduce tax on farm input purchased by farmers.*

Keywords— Climate, variability, farmers, cassava, yam, cocoyam, production.

I. INTRODUCTION

Agricultural production, be it crops, livestock, fishery and the like has been a dominant issue of discussion in national economic development of this country. However despite government campaigns and slogans, farm production has not kept pace with food demand. Most food crops produced in the country come from the efforts of the small-scale resource poor farmers who depend largely on traditional farming systems for their agricultural inputs [1]. The re-current food crisis in Nigeria is partly due to high rate of population growth over the food production level and erratic amounts of food crops produced from year to year. Some of the reasons that can be adduced to this; is high prone of the country to serious environmental hazards from low rainfall, extreme temperature, acid rainfall, gas flaring, oil spillage, deforestation, continuous cropping and unhindered desert encroachment [2].

Arable crops such as cassava, yam and cocoyam are the chief sources of dietary food energy for the majority of the people living in the lowland tropics, and much of the sub-humid tropics of West and Central Africa [3]. Therefore, their production and utilization must be given prime attention in food policy. Even though farmers have not yet attained the desired technical efficiency in their production as a result of weak access to external inputs such as fertilizers and herbicides [4], the wide scale adoption of high yielding varieties and the resulting increase in yield have shifted the problem of the arable crops sector from supply (production) to demand issues, such as finding new uses and markets for cassava, yam and cocoyam. The government of Nigeria considers a transition from the present status of usage to the level of industrial raw material and livestock feed as a development goal that can spur growth with increase in employment [5].

However, agriculture is still the main source of food and employer of labour employing about 60-70 per cent of the population [6]. It is a significant sector of the economy and the source of raw materials used in the processing industries as well as a source of foreign exchange earnings for the country [7]. Since agriculture in Nigeria is mostly rain-fed, it follows therefore that any variability in climate is bound to impact its productivity in particular and other socio-economic activities in the country. The impact could, however, be measured in terms of effects on crop growth, availability of soil water, soil erosion, incident of pest and diseases, sea level rises and decrease in soil fertility [8]. In view of the above fact, this study was designed to assess the

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effect of climate variability on arable crop production in Bayelsa state, Nigeria

The specific objectives were to;

i. to ascertain the perceived effect of climate variability on arable crop production in the study area
ii. to identify the manifestation of climate variability observed in the farmers environment in the study area.
iii. to examine the constraints to climate variability adaptation strategies in the study area.

Hypothesis

HO_1: There is no significant difference in perceived effects of climate variability on arable crop production in two agricultural zones (comprise Nembe and Yenagoa) in the study area.

II. METHODOLOGY

The study was conducted in Nembe and Yenagoa agricultural zones in Bayelsa State. Bayelsa State comprises eight Local Government Areas, namely: Yenagoa, Kolokuma/Opukuma, Nemebe, Sagbama, Southern Ijaw, Brass, Ogbia and Ekeremor Local Government Areas. The State is geographically located within latitude 04^0 15' North, 05^0 22' West and 06^0 45 East. It shares boundaries with Delta State on the North, River State on the East and the Atlantic Ocean on the West and South. Bayelsa State lies in the heaviest rainfall area in Nigeria, with heavy rain forest and short dry season from November to March [9]. Purposive sampling technique was used to selected climate change prone Local Government Areas and twelve communities were selected within the three Local Government Areas of the State. The three LGAs are: Nembe, Ogbia and Yenagoa while the communities are: Oloibiri, Otuoke, Otusega, Oruma, Akenfa-Epie, Bessein, Okorama, Tombia, Ogbolomabiri, Bassambiri, Adukiri and Igbeta-Ewoama. Ten rural farmers were randomly selected from each of the communities, which gave a sample size of 120 respondents.

Objective 1, 2 and 3 was analyzed with descriptive statistics such as frequency distribution, percentage and mean counts. The null hypothesis was tested using paired sample z-test technique. The choice for Z-test in the study is because n >30. The Z-statistic is given as:

$$Z_{cal} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S^2\bar{X}_1}{n_1} + \frac{S^2\bar{X}_2}{n_2}}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where,

$\bar{X}_1$ = mean score response of perceived effect of climate variability on arable crop (Cassava Yam and Cocoyam) production that were made available by farmers in Nembe agricultural zone.

$\bar{X}_2$ = mean score response of perceived effect of climate variability on arable crop (Cassava Yam and Cocoyam) production that were made available by farmers in Yenagoa agricultural zone.

$S^2\bar{X}_1$ = variance of the response of perceived effect of climate variability on arable crop (Cassava Yam and Cocoyam) production that were made available by farmers in Nembe agricultural zone.

$S^2\bar{X}_2$ = variance of the response of perceived effect of climate variability on arable crop (Cassava Yam and Cocoyam) production that were made available by farmers in Yenagoa agricultural zone.

n_1 = sampled number of arable crop (Cassava Yam and Cocoyam) farmers in Nembe agricultural zone.

n_2= sampled number of arable crop (Cassava Yam and Cocoyam) farmers in Nembe agricultural zone.

III. RESULT AND DISCUSSION

TABLE 1: FREQUENCY COUNT AND PERCENTAGE ON THE PERCEIVED EFFECT OF CLIMATE VARIABILITY ON CASSAVA PRODUCTION

		HE		ME		LE		Mean
S/N	Items	Freq.	%	Freq.	%	Freq.	%	$\bar{x}$
1	Poor crop yield	96	80.0	22	18.3	2	1.7	2.78
2	Washing away of soil surface applied with fertilizer	73	60.8	38	31.7	9	7.5	2.53
3	Frequent leaching of nutrient	36	30.0	51	42.5	33	27.5	2.03
4	Disease incidence	111	92.5	9	7.5			2.93
5	Frequent pest attack	44	36.7	58	48.3	18	15.0	2.22
6	Damage/breaking of plants, due to windstorm	79	65.8	21	17.5	20	16.7	2.49

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7	So much labour demand on the farm	67	55.8	38	31.7	15	12.5	2.43
8	Increase in cost of production	93	77.5	17	14.2	10	8.2	2.69
9	Post-harvest losses	71	59.2	40	33.3	9	7.5	2.52
10	Loss of improved planting materials	76	63.3	32	26.7	12	10.0	2.53

Source: Field Survey, 2016. Note: HE = High Effect; ME= Moderate Effect, and LE= Low Effect

Table 1: reveal the perceived effect of climate variability on cassava production were measured in the highlighted items: poor yield ($\bar{x}$=2.78); washing away of soil surface applied with fertilizer ($\bar{x}$=2.53), frequent leaching of nutrient($\bar{x}$=2.03), disease incidence ($\bar{x}$=2.93), frequent pest attack ($\bar{x}$=2.22), damage/breaking of plants due to windstorm ($\bar{x}$=2.49), so much labour demand on the farm ($\bar{x}$=2.43), increase in cost of production ($\bar{x}$=2.69), post-harvest losses ($\bar{x}$=2.52) and loss of improved planting materials ($\bar{x}$=2.53).

The finding is in line with [10] who noted that cassava is a hardy crop that could have significant potential to adapt to climate variability. According to [11] who also revealed that cassava actually responded negatively to enhanced CO_2 and that the crop's cyanide concentrations increased with greater CO_2. Variability in climatic conditions has already affected the production of some staple crop, and future climate variability threatens to exacerbate this [12]. Farmer suffers great losses from the negative impact of climate variability amounting between 36 and 44% of the farm produce. The damages represent losses between 42 and 60% of agricultural GDP in the region [13].

TABLE.2: FREQUENCY COUNT AND PERCENTAGE ON THE PERCEIVED EFFECT OF CLIMATE VARIABILITY ON COCOYAM PRODUCTION

		HE		**ME**		**LE**		**Mean**
S/N	Items	Freq.	%	Freq.	%	Freq.	%	$\bar{x}$
1	Poor crop yield	60	50.0	41	34.2	19	15.8	2.34
2	Washing away of soil surfaces fertilizer applied	53	44.2	39	32.5	28	23.3	2.21
3	Frequency of nutrient leaching	33	27.5	49	40.8	38	31.7	1.96
4	Disease incidence	75	62.5	31	25.8	14	11.7	2.51
5	Frequent pest attack	44	36.7	58	48.3	18	15.0	2.22
6	Damage/breaking of plants, due to windstorm	79	65.8	21	17.5	20	16.7	2.54
7	So much labour demand on the farm	59	49.2	34	28.3	27	22.5	2.27
8	Increase in cost of production	98	81.7	12	10.0	10	8.3	2.73
9	Post-harvest losses	43	35.8	52	43.3	25	20.8	2.15
10	Loss of improved planting materials	70	58.3	31	25.8	19	15.8	2.43

Source: Field Survey, 2016. Note: HE = High Effect; ME= Moderate Effect, and LE= Low Effect

Table 2: reveal the perceived effect of climate variability on cocoyam production were measured in the highlighted items: poor yield ($\bar{x}$=2.34); washing away of soil surfaces fertilizer applied ($\bar{x}$=2.21), frequency of nutrient leaching ($\bar{x}$=1.96), disease incidence ($\bar{x}$=2.51), frequent pest attack ($\bar{x}$=2.22), damage/breaking of plants, due to windstorm ($\bar{x}$=2.54), so much labour demand on the farm ($\bar{x}$=2.27), increase in cost of production ($\bar{x}$=2.73), post-harvest losses ($\bar{x}$=2.15) and loss of improved planting materials ($\bar{x}$ =2.43). This implies that the negative impact of climate variability have resulted to poor crop yield and the inappropriate usage of modern and local adaptation strategies developed by farmers have led to low yield. In line with the finding of [14] and [15] asserted that local farmers with low adaptive capacity are thought to be more vulnerable to adverse effects of climate variability. Post-harvest losses at the farm level account for a substantial amount of food deficit [16].

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TABLE.3: FREQUENCY COUNT AND PERCENTAGE ON THE PERCEIVED EFFECT OF CLIMATE VARIABILITY ON YAM PRODUCTION

		HE		**ME**		**LE**		**Mean**
S/N	Items	Freq.	%	Freq.	%	Freq.	%	$\bar{x}$
1	Poor crop yield	69	57.5	37	30.8	14	11.7	2.51
2	Washing away of soil surfaces fertilizer applied	47	39.2	40	33.3	33	27.5	2.12
3	Frequency of nutrient leaching	33	27.5	49	40.8	38	31.7	1.92
4	Disease incidence	61	50.8	35	29.2	24	20.0	2.31
5	Frequent pest attack	39	32.5	58	48.3	23	19.2	2.13
6	Damage/breaking of plants, due to windstorm	82	68.3	22	18.3	16	13.3	2.58
7	So much labour demand on the farm	65	54.2	30	25.0	25	20.8	2.33
8	Increase in cost of production	97	80.8	14	11.7	9	7.5	2.73
9	Post-harvest losses	52	43.3	46	38.3	22	18.3	2.25
10	Loss of improved planting materials	78	65.0	28	23.3	14	11.6	2.53

Source: Field Survey, 2016. Note: HE = High Effect; ME= Moderate Effect, and LE= Low Effect

Table 3: show the effect of climate variability on yam production were measured in the highlighted items: poor yield ($\bar{x}$=2.51); washing away of soil surface fertilizer applied ($\bar{x}$=2.12), frequency of nutrient leaching ($\bar{x}$ =1.92), disease incidence ($\bar{x}$=2.31), frequent pest attack ($\bar{x}$=2.13), damage/breaking of plants, due to windstorm ($\bar{x}$ =2.58), so much labour demand on the farm ($\bar{x}$=2.33), increase in cost production ($\bar{x}$=2.73), post-harvest losses ($\bar{x}$=2.25) and loss of improved planting materials ($\bar{x}$ =2.53). This implies that the objective of Integrated Pest Management (IPM) "to maintain good productivity level as well as to reduce risks on human health and the environment" is defected. This finding is in line with [17] as temperature increases and rainfall pattern becomes more unpredictable, crop yields drop significantly and extreme weather events such as thunderstorms, heavy winds and floods devastate farmlands and can lead to arable crop failure. Pests and diseases migrate in response to climate changes and variations.

TABLE.4: FREQUENCY COUNT AND PERCENTAGE ON THE OBSERVED MANIFESTATION OF CLIMATE VARIABILITY IN THE FARMERS' ENVIRONMENT

		High		**Moderate**		**Low**		**Mean**
S/N	Items	Freq.	%	Freq.	%	Freq.	%	$\bar{x}$
1	Rate of rainfall	81	67.5	31	25.8	8	6.7	2.61
2	Occurrence of Erosion	43	35.8	60	50.0	17	14.2	2.22
3	Flooding of farm land	57	47.5	40	33.3	23	19.2	2.28
4	Lodging of crops	40	33.3	42	35.0	38	31.7	2.18
5	Deposit of unwanted debris in farms	38	31.7	45	37.5	36	30.0	2.00
6	Formation of hardpan in soil surface	25	20.8	50	41.7	45	37.5	1.83
7	Drying soil surface	25	20.8	42	35.0	53	44.2	1.77
8	Long hotness of the weather	91	75.8	26	21.7	3	2.5	2.73
9	Rise in sea level	38	31.7	61	50.8	21	17.5	2.14

Source: Field Survey, 2016.

The result in Table 4 reveal the frequency count as well as the percentage of the respondents on the manifestation of climate variability observed by farmers in their environment. From the table, farmers observed the manifestation of climate variability in their environment in regards to the rate of rainfall ($\bar{x}$=2.61). In line with the finding [18]; [19] stated that even if there is sufficient rainfall, it irregularity can affect yields adversely if rain fail to arrive during the crucial growing stage of the crops. And also[20] noted that if rainfall pattern is low it will lead to

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low yield of crop, stunted growth of crop, ease spread of pest and disease attack on crops, drying of seedling after germination and ineffectiveness of agricultural chemicals.

The occurrence of erosion observed by the respondents in their farming environment is ($\bar{x}$=2.22) and flooding of farm land was observed by farmer at ($\bar{x}$=2.28). The result therefore implies that farmers are restrained on the kind of agricultural activity to practice. Lodging of crop was observed by farmers in their farming environment to have manifested to be ($\bar{x}$=2.18). This implies that plant are exposed to pest and disease attack and there will also be reduction in yield. In line with the finding [21] stated that in a high-yielding environment, lodging is the most important constraining factor on yield for most arable crops. Deposition of unwanted debris on their farming environment ($\bar{x}$=2.00). The climatic variation will directly and indirectly affect the livelihoods of fish farmers in those environments as well as their immediate families and their dependents.

Formation of hardpan in the soil surface ($\bar{x}$=1.83), drying soil surface ($\bar{x}$=1.77) and long hotness of the weather was observed by farmer in their farming environment ($\bar{x}$=2.73). The finding therefore, implies that the long hotness of the weather have exposed the crop to drought, whereby causing food insecurity, reduction yield quality and farmers intend to expend less time in their farms. The finding is in line with [22] noted that extreme temperature tends to affect the life cycle of fish and livestock from their physiological, morphological, reproductive, migratory and behavioral responses.

Farmer observed rise in sea level in their environment ($\bar{x}$=2.14). The finding implies that rise in sea level tends to increase the vulnerability to climate variability by farmers. This study further stress the assertion of [22] who stated that Nigeria is vulnerable to the potential negative impacts of climate variability through the rise in annual mean temperature, declining rainfall, increasing frequency and intensity of floods, and variability in rainfall seasons. All these will contribute to negative impacts of artisanal fisheries of the country.

TABLE.5: DISTRIBUTION OF RESPONDENTS ACCORDING TO THE CONSTRAINTS TO CLIMATE VARIABILITY ADAPTATION STRATEGIES

S/N	Items	Frequency	Percentage	Ranking
1	Poverty	49	40.8	1st
2	Lack of technology	24	20	2nd
3	Technology dissemination	22	18.3	3rd
4	Information and skill	6	5.0	6th
5	Lack of infrastructure i.e. road water and electricity	8	6.7	4th
6	Un-favoring Land tenure	4	3.3	7th
7	Gender issues	7	5.8	5th
	Total	**120**	**100**	

Source: Field Survey, 2016

Tables 5 show the constraints to climate variability adaptation strategies in the study area. The result indicate that (40.8%) was constrained by poverty and it have exacerbated rural farmer economic condition towards adopting new adaptation strategies to curb climate variability in other to improve their household food security level. This finding in line with [23] who asserted that adaptation and adoption of new technology costs money, and because poor communities have less diverse and more restricted entitlements, they lack the empowerment to adapt, locking them into a vulnerable situation. This therefore implies that farmers should be provided with resources to adopt the new technology.

Table 5 revealed that (19.2%) rural farmer were constraint by lack of appropriate technology such as saline tolerant varieties and genetic improved varieties to curb climate variability. This may have seriously impeded community's ability to implement adaptation strategies by limiting the range of possible response and interventions. (19.2%) of the respondents revealed that inadequate technological dissemination by extension and research institution have also contributed to the constraint faced by farmer in other curb the menace of climate variability. In line with the

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finding [24] noted that a community's level of technology and it ability to adapt new technology are important determinants of adaptive capacity. Awareness and sensitization are important to curb climate variability. Information and skill (5.0%) of the respondents revealed that they are constrained with the right information and skill to curb climate variability.

Lack of infrastructures such (6.7%) as a constraint to adaptation strategies to climate variability. Poor physical and social infrastructure such as water management structures transport, marketing, storage and processing structures which can enhance farmer to adapt new strategies are not available to them. The findings further revealed that (3.3%) of the respondents were constraint with land tenure issues. In line with the finding [25] asserted that land tenure is a prerequisite to investments in climate variability adaptation related to land and water management. Finally gender issue had (5.8%) as a constraint to climate variability adaptation strategies. Inadequate integration of gender issues comprises the sustainability within the study area.

TABLE.4:

Z- test analysis result showing the significant difference in perceived effects of climate variability on arable crop production in two agricultural zones (comprise Nembe and Yenagoa) in the study area

Group	N	$\bar{x}$	Std	Std Error	P-Level	Z_{cal}	Z_{tab}
Nembe	40	3.6530	0.29365	0.04643			
Yenagoa	40	3.3272	0.16324	0.02581	0.05	6.747**	2.02
Nembe – Yenagoa		0.3258	0.30534	0.04828			

Source: Field Survey, 2016 ****= significant at 5%. Decision: H_0 rejected**

The perceived effect of climate variability on arable crop production in Nembe and Yenagoa Agricultural Zones were statistically compared in table 4. The result showed the mean of perceived effect of climate variability on arable crop production in Nembe agricultural zone was (3.6530) while that of Yenagoa Agricultural Zone was (3.3272). The difference in mean of the perceived effect of climate variability on arable crop production between Nembe and Yenagoa Agricultural Zones was (0.3258). These were subjected to Z_{-test} analysis; and the result was statistically significant at 5% level as the Z_{-cal} (6.747) was much higher than Z_{-tab} (2.02) which showed that there was significant difference in the perceived effect of climate variability on arable crop production in Nembe and Yenagoa Agricultural Zones. The implication is that climate variability is a threat to arable crop production in Nembe and Yenagoa Agricultural Zones and other socio-economic development, agricultural production activities are generally more vulnerable to climate variability [26]. [27] predicted future economic losses and increased risk of hunger due to climate variability. It seems clear the combination of high climatic variability, poor infrastructure, economic poverty, excessive heat stress, acidic rainfall, excess rainfall, poor livestock health, reduced crop yields, low productivity and a range of other problems associated with climate variability will constitute important challenges for Africa countries Nigeria (inclusive) in particular [28]. Therefore, the null hypothesis which state that there was no significant difference in perceived effects of climate variability on arable crop production in two Agricultural Zones (comprise Nembe and Yenagoa) was accepted, while the alternative hypothesis was rejected.

IV. CONCLUSION

The study concluded the vagaries in climatic conditions have lead to decline in production of some staple crops such as cassava, cocoyam and yam and the vagaries in climate variations exacerbate their level food security status and poor crop yield, washing away of soil surfaces fertilizer applied, frequency of nutrient leaching post-harvest losses at the farm level account for a substantial amount of food deficit. The adoption of new technology costs money, and because poor communities have less diverse and more restricted entitlements, they lack the empowerment to adapt, locking them into a vulnerable situation. Hence, the study recommended that farmers should form cooperative societies in order to cope with high cost of agricultural production and government should reduce tax on farm input purchased by farmers.

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Effect of Different Levels of N.P.K. 15:15:15 Fertilizer Application on the Yield of Sweet Potato (Ipomea Batatas) in South-South Nigeria

Nmor E.I, Okobia Uche B.

Department of Agricultural Technology, and Department of Science and Technology, Delta State Polytechnic, Ozoro Nigeria

Abstract— *This research was carried in Delta state Polytechnic School of Agriculture teaching farm in Delta state Polytechnic Ozoro in Isoko North local government area of delta state, Nigeria. There are different levels of fertilizer application suggested by various authorities. The need to elevate the effect of different levels of fertilizer application on the yield of sweet potato necessitated the study. Eighty vines of sweet potatoes collected from nearby farm were planted in complete randomized block design which was replicated three times. At establishment, twenty vines were dressed with 4.6kg fertilizer, another twenty 3.0kg, another twenty 7.4kg while the remaining twenty served as control. The parameters collected were subjected to analysis of variance (ANOVA). The results shows that (table 2) potato treated with 7.4kg fertilizer had more leaves of 127, 145.3, 177.7, and 184.7 as against 63, 83.3, 105.3, 127, and 83, 108.3, 134.3, and 162.0 for 3kg and 4.6kg respectively whereas the control had 28.7, 40.2, 58.6, and 67.3. Table (3) shows that Potato 7.4kgfertilizer had better length of vine of 66.1, 69.8, 81.2, 96.9 and 64.8, 70.1, 95.1 and 113.2 for 3.0 and 4.6kg respectively.Table (4) shows that 7.4kg treatment had superior weight of tuber of 114.38 as against 53.17, 80.26 and 34.11 for 3kg, 4.6kg and control respectively. Conclusively, sweet potatoes treated with 7.4kg fertilizer performed better in terms of number of leaves, vine length and weight of tubers at harvest. However there was significant difference among the treatment. It is therefore recommended that 7.4kg should be applied per stand so as to improve yield of potato and profit of the farmers.*

Keywords— sweet potato, number of leaves, length of vine, weight of tuber and number of leaves.

I. INTRODUCTION

Sweet potato (Ipomeabatatas L) is a dicotyledonous plantthat belongs to the family conovulacea (Miller, 2008). The crop is grown in many countries globally but production primarily occurs in tropical and subtropical areas where it is important staple food in the diet of many people (Hijamas, 2001). Sweet potato is one of the most important root and tuber crops in sub-saharan Africa with both domestic and industrial uses and its nutritional value far exceed that of yam, cocoyam and cassava (Onwueme, 1997). Potatoes are used for varieties of purposesand not as vegetable for cooking at home it is likely that less than 50% of potatoes grown worldwide are consumed fresh and the rest processed into potato.

Food product and food ingredient for cattle, pigs, and chicken (Adamu, 2002 and Abdulrazea, 2004). In Nigeria it is prepared into potato chips. More so, the starch from potato is widely used by pharmaceutical textile, wood and paper industries as adhesive agent. (Gravel, 1999).Ojiako (2009) said sweet potatoes yield per hectare in Nigeria has declined. This however yield low could be attributed to poor field management by the farmer. The main objective of the study is to determine different level of fertilizer application on the yield of potato while the specific objectives are

Determine the number of leaves

Determine the number length of vine

Determine the number of branches

Determine the weight of tubers at harvests.

Sweet potato maybe adapted to grow on poor soils, as such most farmers do not apply appropriate dosage to their crops resulting in poor yield. Though inorganic fertilizer have been the conventional method of soil mineral inputs in sweet potato production.

According to Bureshet al (1997) and Palm et al (1997). It has generally been accepted that both organic and inorganic inputs are needed to increase crop production in west Africa. With the increasing pressure on farm and for infrastructure development, limited land is available for this crop.

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Soil fertilization is one of the main factors increasing the yield of plant (Kolodzie2006) it effects the accumulation, mineralization and humification of fertilizer added to the soil (Loginowet al 1991) and determine plant production potential.(Iyagand Arora, 1988) . the amount of fertilizers introduced into the soil, including mineral fertilizers affects the amount of mineral nitrogen available to the plant and the organic carbon content of the soil. (Bijsmaand Arora2000).

Mineral fertilization improves light textured soil physical properties and water .fertilization not only increases crop yield but also alter its quality and results in the higher buildup of nutrient in the yield (Vos, 1999).

Crop yield and mineral fertilizer efficacy depend on the content of available phosphorous, potassium and nitrogen in the soil (Strimumarand Ockerman1990). It has been found that nutrient present in fertilizers are more effective than the equivalent amount of these nutrient present in farmyard manure.

Therefore mineral fertilizer efficacy for potatoes was noticeable higher than that of organic fertilizer (Sheilet al, 1997). Depending on fertilizer forms, rates and nutrient ratios, the content of dry matter, starch, protein and other substance may either increase or decrease. Excessive nitrogen application reduces starch , dry matter and sugar content in tubers and go bad more rapidly during

This result from the fact that nitrogen promotes growth of potato vines and over the year the use of fertilizers application at different level had improved agriculture. Fertilizer should be applied to potato at 200kg/ha of NPK (Schipper, 2000)

It was also reported that fertilizer should be applied at the rate of 250kg/ha and this must be done before and after planting before the flower emerged (rice et al, 1994). Researchers also shows that the crop requires as much of 169kg/ha of po5 with increasing phosphorous requirement (Aliyuet al, 2003). Elliot (2002) said 180kg/ha should be used to dress potato .However 168kg/ha fertilizer could be used to maintain long term soil fertility. Soil fertility make crop grow faster and also improve healthy soil.

II. MATERIALS AND METHOD

The research was conducted in Delta state Polytechnic research farm in Ozoro in IsokoNorth Local Government, Area in Delta State,Nigeria. It is locatedwithin the rain forest zone of the mid-western Nigeria between latitude 5 30

It has an annual rainfall of between 250mm-3000mm and temperature range between 280c and 300c. Its attitudinal position is below 50 meters above the sea level (Ofunne, 1999). The soil of the studied area is moderately drained acidic loamy sand (Ogboi and Emakpor, 2006). Eighty sweet potato vine collected from a nearby farm and were planted into Randomized Complete Block Design which was replicated three times. At establishment some of the vines were treated with 3kg , others 4.6kg another 7.4kg while others served as control. The parameters measured are number of leaves, number of branches and weight of tubers at harvest. Data were collected at intervals of two weeks. Data collected were subjected to analysis of variance (ANOVA).

III. RESULTS

Table 1, show the result of pre-planting analysis of selected soil properties. The texture of the soil was loamy sandy and this may be attributed to the parent material (coastal plain bands). The pH of the soil was strongly acidic. Organic matter were generally low. Similarly cation exchange capacity was equal low.

Table shows the number of leaves of potato at 6 – 12 week after planting. The result revealed that potato treat with 7.kkg has more leaves of 97.0, 145.3, 177.7 and 184.7 as against 63.0, 88.3, 105.3 and 127.3 for 3.0kg treatment. 4.6kg treatment has 83.0, 108.3, 134.1 and 162.0 while the control has 28.7, 40.2, 38.6 and 67.3.

Table (2) revealed the mean length f vine. 7.4kg treatment superior length of vein of 76.8, 90.7, 113.2 and 135.6 as against 3.0kg treatment which has 56.3,69.8, 81.2 and 96.7. while 4.6kg treatment has 64.8, 70.1,95.1 and 113.2 as against control which had 27.6, 33.8, 45.1 and 56.1. table (3) shows that the weight of tuber at harvest. The result also shows that 7.4kg treatment had the highest mean weight of 114.38kg as against 53.17, 80.26 and 34.1, for 3.0kg and control treatment respectively.

Table.1: physical-chemical properties of the soil at inception of the experiment

Soil properties	**Value**
Sand %	**47**
Silt%	**14**
Clay %	**39**
texture	Sandy clay
Soil bulk density/cm^3	1.12
Infiltration rate cm^3/sec	1.9
Soil pH	5.9
Organic matter %	1.96
Total nitrogen%	1.96

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Available ppm	6.6
Exchangeable cation	**Mg/100g soil**
Na	1.30
Ca	0.50
Mg	0.65
H	0.55
Al	1.46

Table.2: Mean number of leaves at 6th -12th week after planting

Treatment	6	8	10	12
3.0kg	**63.0**	**88.3**	105.3	127.0
4.6kg	**83.0**	**108.3**	134.3	162.0
7.4kg	**127.0**	**145.3**	177.7	184.7
Control	**28.7**	**40.2**	58.6	67.3
Fcal	**5.1**	**0.9**	4.3	
Ftab	**0.05**			

Table.3: Mean length of vine (cm) at 6th -12th week after planting

Treatment	6	8	10	12
3.0kg	**66.1**	**69.8**	81.2	96.9
4.6kg	**64.8**	**70.1**	95.1	113.2
7.4kg	**76.8**	**90.7**	113.2	135.6
Control	**27.6**	**33.8**	45.1	56.1
Fcal	**8.1**	**2.8**	19.8	2.7
Ftab	**0.05**			

Table.4: Weight of potato tubers at harvest

Treatment	**Weight (kg)**
3.0kg	**53.17**
4.6kg	**80.26**
7.4kg	**114.38**
Control	**34.11**

IV. DISCUSIONS

Table (1) revealed that the texture of the studied area was loamy, sandy and this may be attributed to parent materials (coastal plain sand). This agrees with the finding of Anikwe(2000) who observed that coastal plain and usually gives rise to coarse sandy soil. The PH of the soil was acidic and the organic carbon and organicmatter were generally low (London, 1991). Similarly, carbon exchange capacity was low. This suggests that the soil is low in fertility. This could be attributed to increased pressure on land use for cropping, oil exploration and massive infrastructuraldevelopment and limited fallow period due to increased population (Agbim, 2000). Table (2) revealed the mean number of leaves of potato. It shows that 7.4kg treatment had highest mean number of leaves throughout the experimental period. This agreed with (005, 1999) who reported that crop yield and mineral fertilization depend on the content of available phosphorous, potassium and nitrogen in the soil. However there was significant difference among the various treatment used.

Table (3) shows the mean length of vine potato. It shows that 7.4kg treatment had the highest mean length of vine. This finding agreed with (rice and schipper, 2000). Who reported that fertilizer application at different levels had improved yield and soil nutrient status. Table (4) shows the weight of yield kg per treatment. It also shows that 7.4kg treatment had highest weight of tuber at harvest. This also agreed with (Strimumarand Ockerman, 1990) who stated that crop yield and mineral fertilization efficiency depend on the content of available phosphorous, potassium and nitrogen in the soil. It has been found also that nutrient present in fertilizers are more effective than its equivalent amount of these nutrient present in farmyard manure. Therefore fertilizer efficiency for potato was noticeably higher than that of organic manure (Shielet al, 1997).

V. CONCLUSION

The result revealed that potato treated with 7.4kg per stand performed better than those treated with 3.0kg, 1.6 and control in terms of number of leaves, length of vine at weight of tuber at harvest.

RECCOMENDATION

Since there was significant difference among the potato treated with 3.0kg, 7.4kg,4.6kg of fertilizer and control. It is therefore recommended that 7.4kg of NPK 15-15-15 fertilizer should be applied per stand so as to improve the yield of potato and the profit margin of potato and the profit margin of the potato farmers.

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3D Arbitrary Channel Fabrication for Lab on a Chip Applications using Chemical Decomposition

Jahan Zeb Gul[1], Jinhee Na[2], *Kyung Hyun Choi[1]

[1]Department of Mechatronics Engineering,

[1]Jeju National University, Jeju 690-756, South Korea

[2]Biophilic Ltd, 152, Juggunro, Youngin-si, Gyunggi-do, South Korea.

Abstract— *This article demonstrate a simple method to use of three-dimensionally (3D) printed molds that are chemically decomposable for rapid fabrication of complex and arbitrary microchannel geometries. These complex microchannel are unachievable through existing soft lithography techniques. The molds are printed directly from hand held 3D printing pen that can print in midair, making rapid prototyping of microfluidic devices possible in hours. PLA based copper filament is used to print the arbitrary channels. The printed channels are then placed inside PDMS and PDMS is cured. The cured sample is then immersed in chemical solution (Acetic Acid + Sodium Chloride+ Hydrogen peroxide), which decomposes the PLA based copper channel thus leaving an empty channel inside the PDMS block. This method enable precise control of various device geometries, such as the profile of the channel cross-section and variable channel diameters in a single device.*

Keywords— Micro Channel, Arbitrary, 3D Micro Channel, Lab on a Chip.

I. INTRODUCTION

Microfluidics is anunceasinglydeveloping field of great importance in drug discovery[1], physics[2], [3], biology[4], [5], biomedical research[6], and organs-on-chip[7]. The slightvolumes of liquid required fortrials, the behavior of fluids at the micro domain and the lab-on-chip method make microfluidics one of the interdisciplinary field par excellence[8]. For the fabrication of microfluidic devices, polydimethylsiloxane (PDMS) is the most general material in research laboratories because of its low cost, gas permeable and has refractive index of 1.4 (transparent)[9]–[15]. Conventional fabrication strategies for the fabrication of microfluidic micro channels are essentially two-dimensional (2D) which confine the geometries of micro channels within 2D planes. A master is designed first which is usually obtained by clean-room lithography of silicon wafers. PDMS is poured on the master, and after curing, the rubber is peeled off from the master and subsequently chemically bonded to another surface after activation with oxygen plasma or using chemical solutions. The main limitations with PDMS is that, the fabrication method of PDMS is considered too complex for many junior scientists without any experience in microfabrication[16]. There are a lot of alignment issues when multiple layers of PDMS are stacked and sealed using oxygen plasma treatment[17].

The ability to make 3D micro channels is complex and standard fabrication method cannot achieve precise and accurate results. 3D micro channels also adds functionality in devices such as micro valves and mixers. Another benefit of 3D structures is that it can increase the areal density of micro-components by vertical stacking, to replicate the complex microvasculature found in living creatures, and

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ease integration of electronics and optics.

Recently there has been progress towards employing 3Dprinting to fabricate microfluidic devices of desired complexities[18]–[22]. This method involves the creation of 3D objects using layer by layer deposition approach. 3D printing is successfully employed in tissue engineering to develop scaffolds based on hard polymericmaterials and hydrogels[23].However such techniques require the use of manual assembly for producing large scale structures. Thus the fabrication of 3D microfluidic channels with defined microarchitectural details in cost-effective, scalable manner remains a challenge. Another method involves, sacrificial mold or fugitive ink for the fabrication of PDMS microfluidic devices[18], [24]–[26]. Although the use of sacrificial mold is a step forward in simplifying the fabrication of microfluidic devices, it still requires either harsh condition like the use of high temperatures for creatingor removing, a template, applying heavy swelling for pulling out the template, or the use of complex mold fabrication.

Taken in sum, most fabrication processes generally involves 2D micro channels[27]–[30], but with some effort, can form 3D channels by bonding multiple layers. Bonding involves the need for challenging alignment of layers at micro-scale.Each of these fabrication technique adds additional steps, time, expertise and cost in the fabrication process. In addition, the bonded interface is typically a weak-point in the device that is prone to leakage. Moreover, embedding other functionalities such as sensors, valves and mixers is extremely hard or even impossible using these methods.

Here we present an easy two-step chemically decomposablePLA based copper filament removal method for achieving arbitrary 3Dmicrometric channels in a single block of PDMS. The PLA based copper filament is 3D printable. A microchannel structure is first 3D printed and then immersed in PDMS block and cured. The cured PDMS is then placed inside an organic solution of acetic acid, sodium chloride and hydrogen peroxide. The organic solution decompose the PLA based copper structure inside the PDMS block thus leaving a hollow 3D channel. The channel is then washed with water to remove impurities.Using the chemically scaffold-removal method, there is no need of lithography steps nor silicon masters, no need of bonding the PDMS on surfaces nor of repetitive procedures for obtaining multi- level channels, making the fabrication of microfluidic devices easy, low-cost and opening up the field for a plethora of scientists working in different areas.

II. EXPERIMENTAL

An easy two-step chemically decomposable 3D printed PLA based copper structure-removal method for achieving 3Dmicrometric channels in a single block of PDMS. Using the thiscopper removal method, there is no need of lithography steps nor silicon masters, no need of bonding the PDMS on surfaces nor of repetitive procedures for obtaining multi-level channels, making the fabrication of microfluidic devices easy, low-cost and opening up the field for a plethora of scientists working in different areas.

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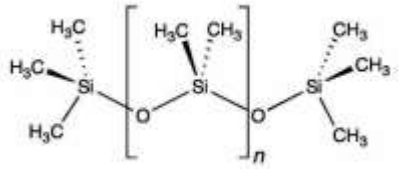
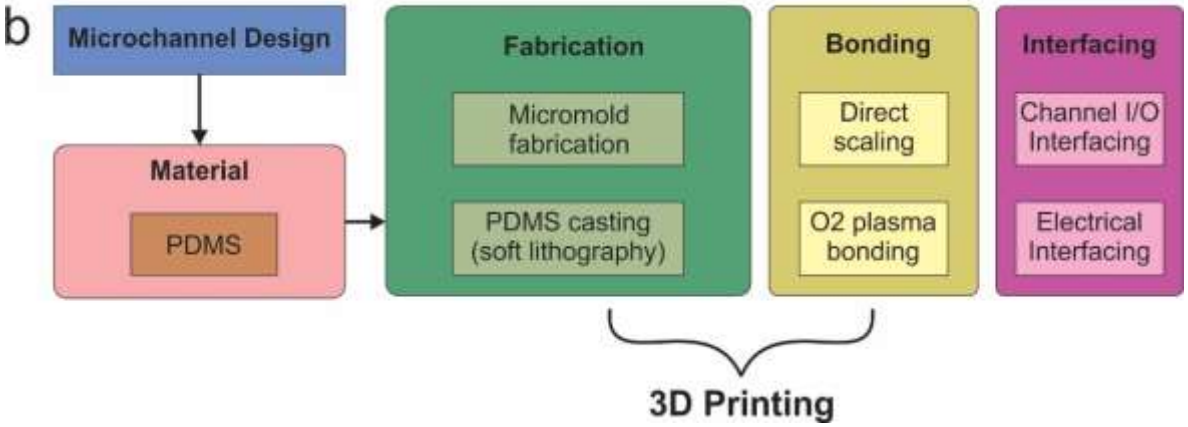
S.No.	Property	PDMS
1.	Mechanical behavior	Elastomeric
2.	Thermal range	~80 ^{0}C
3.	Acid/base resistance	Not Good
4.	Solvent resistance	Not Good
5.	Optical transmissivity (visible range)	Excellent
6.	Biocompatibility	Biocompatible



b



Fig,1(a) Properties of PDMS with its molecule (b) Illustration of microchannel design process with PDMS. The conventional casting and bonding steps are replaced with 3D printing.

PDMS is widely used material because of various properties shown in Fig.1(a). The chemical decomposition method replaces the conventional fabrication of microchannel using PDMS. The two steps i.e. molding and bonding is replaced with 3D printing here as shown in Fig. 1(b). The material used here is commercially available 3D printable PLA based copper filament. The technique is inspired by etching technique of printed circuit boards. The copper is decomposed in printed circuit board etching using acidic chemicals such as ferric chloride. Here in order to save the PDMS shape and microchannel architecture, a less sensitive organic compound of acetic acid, sodium chloride and hydro peroxide is used to decompose the copper. The decomposition time is long as compared to original decomposition chemical i.e. ferric chloride but there is no or negligible harm with organic solution.

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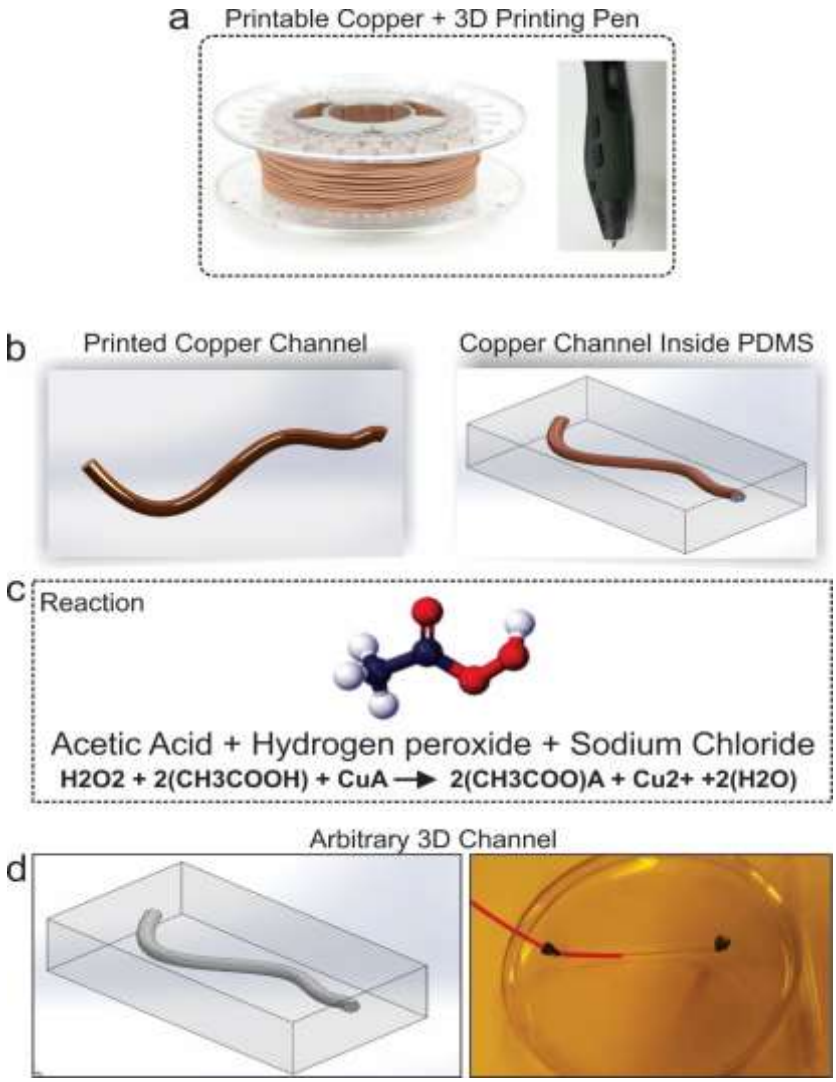

Fig.2: Illustration of micro channel fabrication using chemically decomposable and 3D printed PLA based copper. (a) The channel shape is 3D printed using hand held 3D pen. (b) An arbitrary 3D channel of copper filament. The copper channel is then set inside PDMS mold and PDMS is cured. (c) The PDMS mold is then immersed in organic solution of acetic acid, sodium chloride and hydrogen peroxide. (d) The decomposition of copper inside the PDMS results in a hollow channel inside PDMS. The actual photograph of fabricated channel is also shown.

III. RESULTS AND DISCUSSION

A cross-section of an emptied microchannel has an exact circular profile. The circular profile arises from the shape because of midair 3D printing of PLA based copper using a hand held 3D printing pen which consists of a conical polypropylene needle. It is possible to control the width of the features simply by varying the diameter of the nozzle and that the line widths are uniform (e.g. a needle with a 200 µm diameter produces lines with a standard deviation of 200 µm). If 3D printing of copper filament is done on a substrate or a FDM printer with stage, the cross section of an emptied micro channel in this case will be in semi-circular profile. The semi-circular profile is because of natural adoption of copper metal on straight surface. In this case, it is also possible to control the width by simply changing the diameter of the nozzle e.g. a nozzle with a 200µm diameter produces lines with a standard deviation of 7µm. The printed structure is the then cured inside PDMS

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using acetic acid, hydrogen peroxide and sodium chloride. The chemical reaction and materials are shown in Fig.2(a)(c).

Fig.3 shows the images of the fabricated micro channels inside PDMS using organic decomposition of PLA based copper. Two types of channels are fabricated. One a simple straight bridge shaped channel with single inlet/outlet and second is a complex helical structure with single inlet/outlet. The diameter of the both channels are 200 and 250µm respectively. The length of the bridge channel and helical channels are 6cm and 4cm respectively. A red color liquid is flown through these channels to verify the smooth flow. The SEM images shown in Fig. 3(b) is the cross sectional images of both channels. The images indicates a smooth channel with no or negligible harm to PDMS. A two inlet and one outlet helical mixer channel is then fabricated to proves the potential of various embedded concepts using the chemical decomposition method. The fabricated mixer channel shown in Fig. 3(c) is smooth and can mix two liquids if certain parameters are kept in control such as velocity of incoming liquids or viscosity of liquids etc.

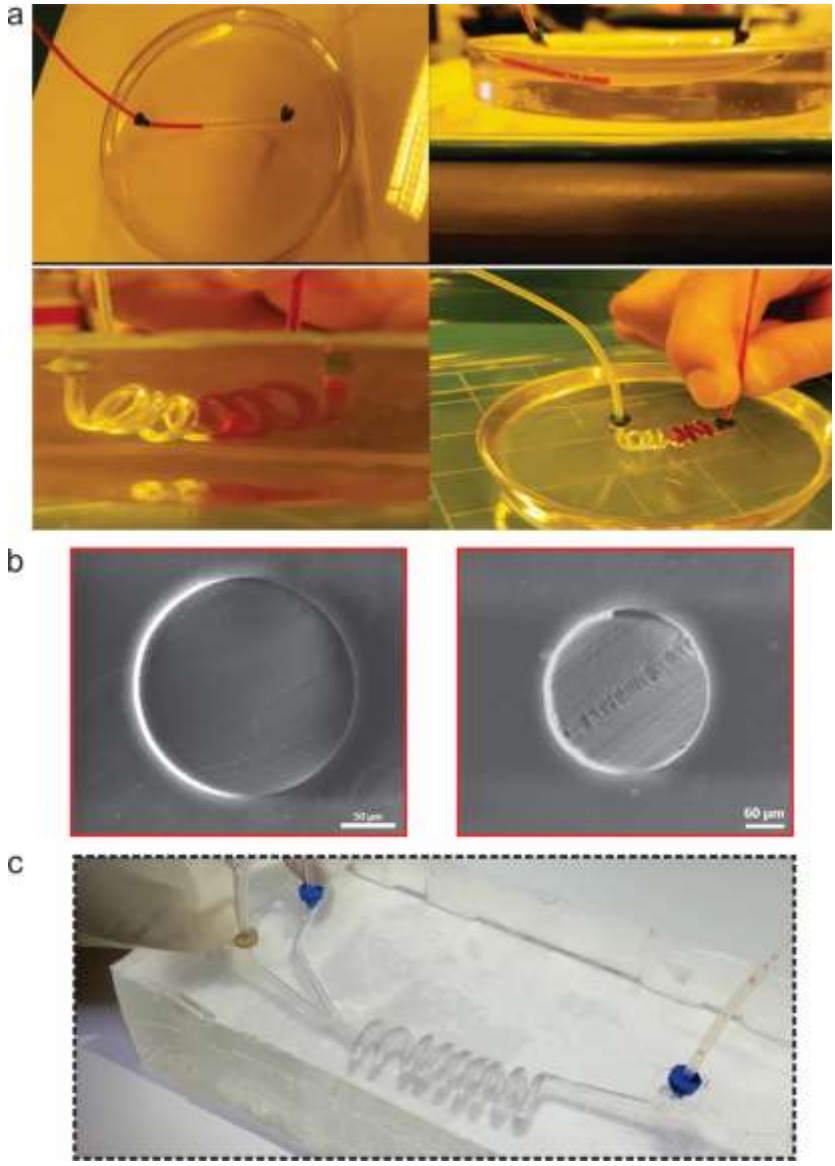

Fig.3 (a)Images of the fabricated micro channels inside PDMS using organic decomposition of PLA based copper. An arbitrary hanging bridge channel and a helical channel. (b) SEM Images of the cross section of a straight and helical channels (d) A two inlet one outlet helical mixer channel.

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Fig.4 shows results of the cost comparison and copper decomposition duration between the conventional copper etching material Ferric Chloride and Organic Compound (Acetic Acid, Sodium Chloride, and Hydrogen peroxide). The results shown in Fig.4 proves that conventional material i.e. Ferric Chloride can decompose the copper much faster as compared to organic compound but the cost is high. Moreover, it's not safe to use ferric chloride as main material to decompose the PLA based copper because it will damage the PDMS and overall purpose of the fabrication of complex micro channels will not met.

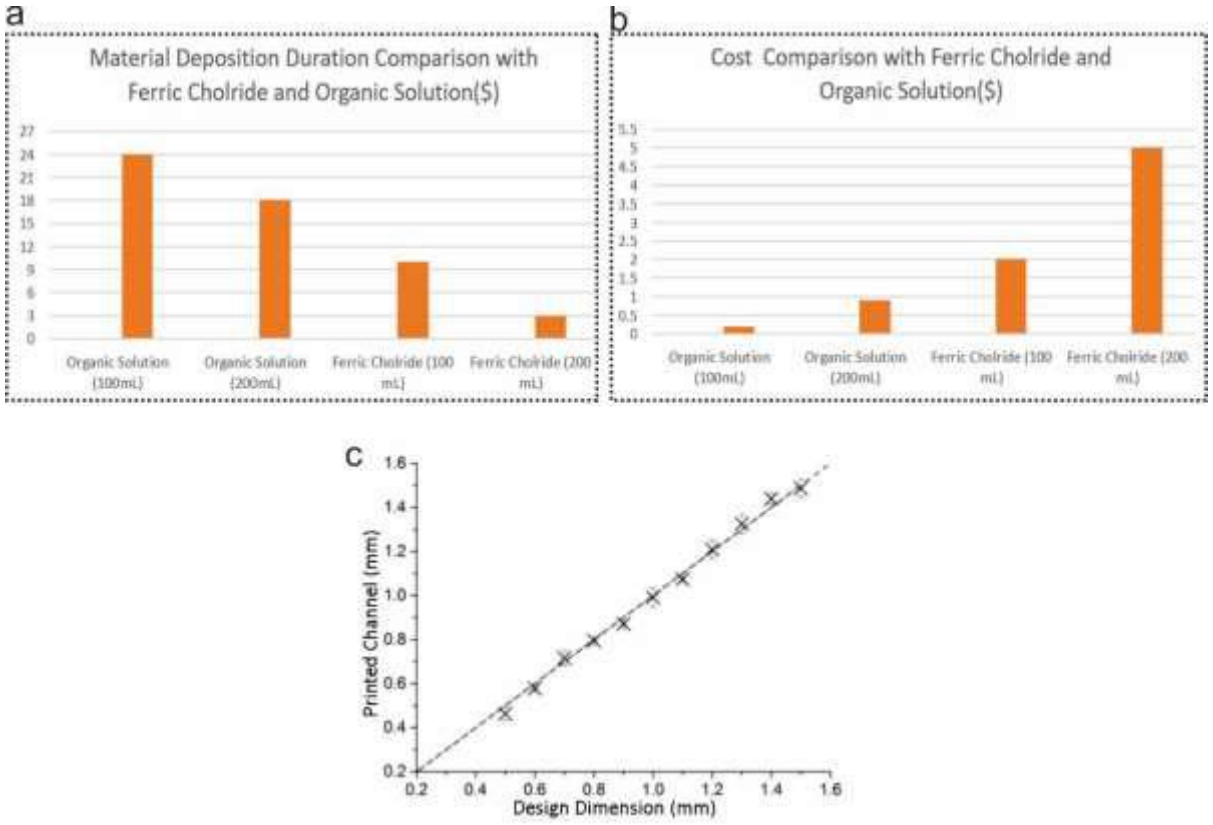

Fig.4: Results of time comparison and cost comparison with ferric chloride (a conventional etching material) with organic compound (acetic acid, hydrogen peroxide and sodium chloride). (a) Duration comparison results (b) Cost comparison results (c) The linear behavior of the fabricated channel w.r.t. model in software proves the efficiency and accuracy of fabrication method.

The consistency of dimensions of the fabricated microchannels in all directions, is shown in Fig. 4(c), although there is some unavoidable roughness resulting from the layer by layer nature of copper PLA printing as can be seen in Fig 3b however, the channel surface roughness was not considered for optimization in this work.

IV. CONCLUSION

Chemically decomposable 3D printed structures are used for the fabrication of PDMS channels demonstrating variable channel dimensions as small as 100s of micrometers in all-PDMS channels. The fabricated 3D arbitrary micro channel is expected to exactly mimic the human blood channels. These channels will be used in various applications such as LOC platforms. The fabrication of 3D arbitrary channels and investigation of fluidic motion inside them has laid a new foundation with promising applications in the area specially related to LOC, artificial organs, bio medical applications and implantable devices.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

J.Z.G. performed the experiments and also wrote the paper. The analysis of the results and the final revision of the paper was done by J.N and K.H.C.

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Enhancement of protease production by Bacillus sp. and Micrococcus varians induced by UV-mutagenesis

Chibani Hiba Rahman*, Fellahi Soltana, Chibani Abdelwaheb

Laboratory of Microbiology and Plant Biology, Faculty of Natural and Life Sciences, University of Mostaganem, Algeria.
*Corresponding author

Abstract— *Microbial proteases contribute nearly 40% of the total worldwide enzyme market. Hence, with the view of this significance, the main objective of the present study was to enhance protease production of two bacterial strains, Bcillus sp. and Micrococcus varians using UV mutagenesis. Induction of mutation in both strains was carried out at different exposure times: 0, 3, 6, 9, 12, 15, 18 and 21 min at a distance of 10 between UV source and treated bacteria.Two best protease producer mutants for the two bacterial strains (UV-9 for Bcillus sp.and UV-18 forMicrococcus varians) were selected based on the clearance zone diameter of mutant colonies on 1% skimmed milk agar plates. UV-9 mutant showed 1.4 fold higher protease activity than the wild type in solid and liquid medium. However UV-18 mutant was found to produce 2.5 fold increases over the wild type on agar plates and 2.1 fold enhancement in liquid-medium assay.The two mutants were very effective in feather keratin-degrading in less than two days, UV- 18 was more efficient than UV-9.*

Keywords— Bcillus sp., Micrococcus varians,protease, UV-mutagenesis.

I. INTRODUCTION

Proteases are group of enzymes which catalyze hydrolysis of peptide bonds in proteins. They are also called as peptidases or proteinases or proteolytic enzymes (Rao*et al.,* 1998).Among the various proteases, bacterial proteases are the most significant,compared with animal and fungal proteases and among bacteria, *Bacillus* sp. are the most important producers of extra-cellular proteases (Boominadhan*et al.,* 2009).

Proteases are among the most important industrial enzymes due to their biotechnological interests. They account for about 60% of the total worldwide sale of enzymes(Reddy *et al.,* 2008), and are widely used in several industries that include detergent, food, pharmaceutical, leather, diagnostics,meat processing, waste management and silver recovery (Gupta *et al.,* 2002;Chellappan*et al.,* 2006). These enzymes also have potential to contribute in the development of high value added products due to their characteristic nature of aided digestion (Glazer and Nikaido, 1995). Due to their increased economic importance, research is being carried out throughout the world to isolate hyperactive strains for the production of proteases (Gupta *et al.,* 2002).

Microbial strain improvement plays a key role in the commercial development of microbial fermentation processes. As a role, the wild strains usually produce limited quantities of the desired enzyme to be useful for commercial application (Glazer and Nikaido, 1995). Mutation induction and/or selection techniques, together with cloning and protein engineering strategies have been exploited to develop enzyme production (Schallmey*et al.,* 2004). Ultraviolet radiation is one of the well-known and most commonly used mutagen and it is also very easy to take effective safety precautions against it. It gives a high proposition of pyrimidine dimmers and includes all types of base pair substitutions (Javed*et al.,* 2013). The present study highlights a possible enhancement of extracellular protease production from two bacterial strains, *Bcillus sp.* and *Micrococcus varians* via UV-mutagenesis.

II. MATERIAL AND METHODS

Bacterial strains used

Two bacterial strains *Bacillus sp.* and *Micrococcus varians*- producing protease were employed. *Bacillus sp.* was isolated from compost, whereas *Micrococcus varians* was isolated from soil. They were selected because of their high proteolytic activities.

Qualitative assay of proteolytic activity

The ability to produce protease enzyme was checked by transferring a single isolated colonies of both bacterial strains (wild and mutants) on 1% of skimmed milk agar plates. Plates were incubated at 37° C for 24h. The diameter (in mm) of the clear hydrolysed zone around each bacterial colony (X) was divided by the diameter of the same colony (Y). The ratio (X/Y) was taken as an indication of protease activity.

Quantitative assay of proteolytic activity

Protease activity was assayed by measuring the tyrosine released in culture supernatant from the action of protease on casein substrate bya modified Anson's method (Yang and Huang, 1994).The cell free supernatant of overnight cultures was used for protease assay.The reaction mixture contains 1 ml of enzyme was added to 1 ml of casein solution (1% w/v in 50 Mm potassium phosphate buffer, pH 7.5) and the mixturewas incubated for 10 min at 37ºC. The reaction was terminatedby adding 2 ml of 10% trichloroacetic acid reagent, keptfor 30 min incubation at room temperature and then centrifugedfor 15 min at 10,000 rpm. Then 2 ml of filtrate was mixed with3 ml of 500 mM sodium carbonate solution and absorbance was measured at 280 nm. One unit of enzyme activity is definedas the amount of enzyme required to liberate 1 µmol of tyrosineper min under the defined assay conditions. Enzyme units were measured using tyrosine (0-100µmole) as standard.

Preparation of cell suspension

Cell suspension was prepared by transferring colonies from 24h Luria- Bertani Agar culture of both strains into a 100ml-Erlenmeyer flasks containing 20ml of LB broth under aseptic conditions. Flasks were placed in a shaker incubator at 37ºC, 160 rpm for 24 h. after reaching an optical density of about 1,5 at 600 nm (corresponding to approximately 10^9- 10^{10}UFC/ml), it was used as source of cell suspension for irradiation.

UV mutagenesis

Ultraviolet (UV) irradiation as a physical mutagenic agent was used to select mutants which produce more protease than their parent strain. Mutagenesis was carried out according to Justin *et al.,* (2001) using different exposure times. 5ml of bacterial suspensions prepared previously were placed into 10-cm diameter-petri dishes at a distance of 10 cm from the UV lamp (30-W germicidal lamp, 2540-2550Å) and exposed to UV radiation for 0, 3, 6, 9, 12, 15, 18 and 21 min. Portions of 0.5 ml of suitable dilutions of bacterial suspensionsstrains were spread on five LBplates and incubated at 37ºC for 24 hr.Colonies developed after incubation werecounted and transplanted onto slants forfurther studies. The survival percentage was estimated for each treatment.

Screening of higher-proteolytic mutants

Plates having between 0.1 and10 % of survival rate were selected for isolation of mutants (Hopwood *et al.,* 1985). The isolates were selected on the basis of macroscopic differential characteristics. According to Solaiman*et al.,* (2005), for isolation of high protease producing mutants after UV irradiation, developed colonies inoculated into skim milk agar medium and incubated at 37ºC for 24h. Depending on the zone of clearance, mutants of the two bacterial strains exhibiting maximum zone of hydrolysis as compared to the wild type were selected.

Feather-degrading capacity of wild and mutant isolates

The wild type and the best mutant of *Bacillus sp.* and *Micrococcus varians*were tested for their ability to degrade feather by culturing both of them in modified basal medium II supplemented with 1% of chicken feather. Chicken feathers collected (medium size white hens) were chopped to small fragments, washed with distilled water and dried overnight at 60ºC (Bernhardt *et al.,* 1978; Johnvelsy, 2002). Cultures were incubated for 3 days at 37ºCwith shaking at 160 rpm. The feather-degrading capacity was assessed according to the physical appearance of feather pieces observed by naked eyes. The bacterial strain with high keratinolytic activity is the strain that degrade feather- keratin in shorter time.

III. RESULTS AND DISCUSSION

UV mutagenesis

The cost of enzymes in a bioprocess can be reduced by introducing hyper-productive strains after suitable mutagenic treatments. Results in Table 1 and 2 showed that thesurvival percentages for both isolatesdecreased by increasing the time ofexposure. The percentage of survivals has been sharply decreased from 100% to 15.07 and 35.62 % after 3min of UV treatment for *Bacillus sp.* and *Micrococcus varians*respectively.Thenitreaches 0.001 and 0.02 % after 20 minof UV treatment for *Bacillus* and *Micrococcus* respectively. This is maybe explained by the short distance from the UV lamp (10 cm). Similar trend of decrease in survivability with increase in exposure time has also been reported by Solaiman*et al.,* (2005) in which the distance from the UV lamp was 10 cm and the percentage of survivals was 0.12% after 10 min of UV treatment.However higher percentage of survivals has been reported by other investigations where the distance was 20 cm(Javed*et al.,* 2013; Karn and Karn, 2014).

After UV treatment plates having survival rate between 0.1 and 10 % corresponding to exposure time of 6, 9, 12 min for *Bacillus* and 9, 12, 15 for *Micrococcus*, were selected for isolation and screening of overproducing mutants. Based onmorphology and colour differencesbetween colonies, 27 and 18 mutants for *Bacillus* and *Micrococcus* respectively were selected and transferred to skimmed milk agar plates to test proteolyticcapacities. In case of *Bacillussp.* and depending on their proteolytic activity (X/Y) only four UV-mutants (9, 12, 16 and 21) did exhibit higherproteolytic activity compared to the wild type

(Table 3). Among the four mutants the most efficient strain (UV-9) was selected for further studies.*Bacillussp.* mutant 9(UV-9) showed 1.4 fold higher protease activity than the wild type.Similar fold increase in protease production was obtained by Nadeem*et al.,* (2010). Concerning *Micrococcusvarians*results showed that the majority of mutants were efficient in protease production. The superior protease producing mutants were 2, 8, 13, 16, 17 and 18. The X/Y values ranged from 03 to 08 for the mutant 18, so the improvement of *Micrococcus*was better than that of *Bacillus*(Table 4).*Micrococcus.*mutant 18(UV-18) showed 2.66 fold higher protease activity than the wild strain and was chosen for further studies. Shikha and Darmwal(2007) reported 1.44 fold increase in alkaline protease production over the wild strain of *B. pantotheneticus* while Dutta and Banerjee (2006) obtained 2.5 fold increase in protease production by UV-mutant *Pseudomonas sp.* Rao*et al.,* (1998) reported that mutagenesis either by conventional methods or by recombinant-DNA technology play an important role in improving the yield of protease. *Bacillus sp.* and *Micrococcus varians*mutantsshowed variable responses to UV radiation for protease production. These variations are more probably due to the differences induced in their genetic background.

Protease activity of the selected mutants in submerged culture

The best protease producing mutants UV-9 and UV-18 were further evaluated trough shack flask enzyme production studies over their wild strains. Results obtained proved that there is correlation between hydrolysis zone diameter and the ability to produce protease enzyme for *Bacillus sp.*and UV-9 mutant. UV-9 mutant produced almost 1.4 the yield of the wild strain. On the other hand, in the case of *Micrococcusvarians*and its mutant UV-18 therewas no correlation between the clearance zone on plates and proteolytic activity in liquid assay (2.5 fold increase in solid assay whereas it was 2.1fold increase in liquid assay) (Table 5). Similar results were found by Solaiman*et al.,* (2005), where some potent mutants had great in protease production in plates and were not able give any proteolytic activity in shack flask.

Feather-degrading capacity of selected mutants

The two UV mutants, UV-9 and UV-18 were used for testing their ability in feather degrading. The results showed that both mutants were able to degrade feather over their wild type. The mutants grew and produced protease using chicken feather as a source of carbon, energy and nitrogen. UV-18 was more effective in keratin-degrading than UV-9 and chicken feather completely disappearedin less than two days using UV-18 mutant (Figure 1). The ability of microorganism to grow and produce appreciable levels of protease using several wastes could offer tremendous potential for development of biological methods for the hydrolysis of such products. The use of these natural residues, especially in countries where they are generated in abundance, could results in a sustainable reduction in cost of enzyme production (Wang *et al.*, 2008).

IV. CONCLUSION

The results of the present investigation revealed that among different UV- mutants of *Bacillus sp.*and *Micrococcusvarians* UV- 9 and UV-18 were selected as higher-proteolytic mutants. UV- 9 and UV-18 mutants were able to increase protease production in plates and in liquid assay reaching 2.5 fold higher productions than the wild type.Hence these mutants were very effective in feather keratin degrading in two days presenting a potential use in keratin recycling and can result in a sustainable reduction in the cost of enzyme production.

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Table.1: Survival data for Bacillus sp. after UV treatment at different exposure times.

Exposure time (min)	CFU/ml	Survival %
0	$65x10^8$	100
3	$98x10^7$	15.07
6	$22x10^7$	3.38
9	$47x10^6$	0.72
12	$113x10^5$	0.17
15	$59x10^5$	0.09
18	$39x10^4$	0.006
21	$104x10^3$	0.001

Table.2: Survival data for Micrococcus variansafter UV treatment at differentexposure times.

Exposure time (min)	CFU/ml	Survival %
0	$16x10^8$	100
3	$57x10^7$	35.62
6	$188x10^6$	11.75
9	$69x10^6$	4.31
12	$86x10^5$	0.53
15	$26x10^5$	0.16
18	$95x10^4$	0.05
21	$36x10^3$	0.02

Table.3: Protease production (X/Y) of Bacillus sp. mutants.

Mutant	Zone diameter X (mm)	Colony diameter Y (mm)	X/Y	Mutant	Zone diameter X (mm)	Colony diameter Y (mm)	X/Y
Wild	17	07	2.4	14	19	07	2.71
1	11	07	1.5	15	20	08	2.5
2	22	11	2	16	19	06	3.16
3	17	08	2.12	17	04	02	02
4	17	09	1.8	18	20	18	1.11
5	05	04	1.25	19	26	18	1.4
6	17	07	2.42	20	04	03	1.1
7	18	07	2.57	21	18	06	03
8	19	06	3.16	22	19	08	2.37
9	17	05	3.4	23	4.5	03	1.5
10	17	06	2.83	24	11	10	1.1
11	05	04	1.25	25	04	03	1.33
12	19	06	3.16	26	19	09	2.11
13	18	07	2.57	27	04	03	1.1

Table.4: Protease production (X/Y) of Micrococcus varians mutants.

Mutant	Zone diameter X (mm)	Colony diameter Y (mm)	X/Y	Mutant	Zone diameter X (mm)	Colony diameter Y (mm)	X/Y
Wild	09	03	03	10	21	04	5.25
1	20	04	05	11	19	04	4.75
2	18	03	06	12	19	04	4.75
3	20	04	05	13	19	03	6.33
4	09	03	03	14	19	04	4.75
5	08	04	02	15	18	05	3.6
6	20	05	04	16	18	03	06
7	19	04	4.75	17	19	03	6.33
8	18	03	06	18	24	03	08
9	22	04	5.5				

Table.5: Protease activity of the best Bacillus sp.and Micrococcus varians mutants.

Strain	Protease production (U/ml)	Protease activity (X/Y)
*Bacillus sp.*wild type	0.73	2.4
UV-9 mutant	1.02	3.4
*Micrococcus varians*wild type	0.65	03
UV- 18 mutant	1.37	08

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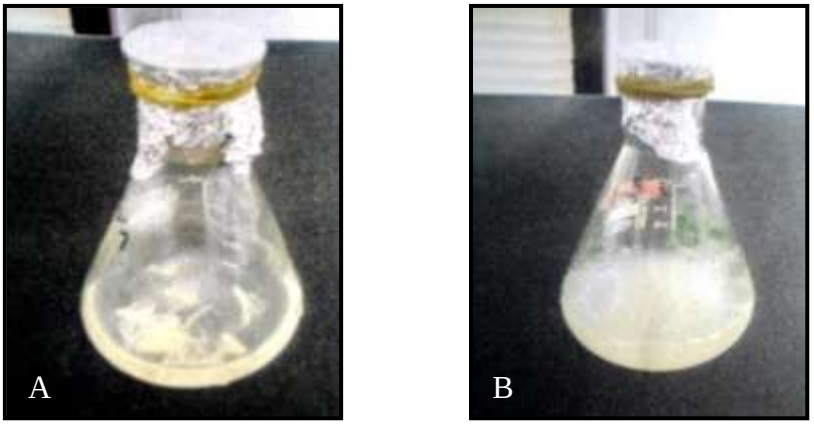

Fig.1: Chicken feather- degrading by Micrococcusvarians(A)and its mutant UV-18 (B).

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Ecology factor and Venom of snake Macrovipera lebetina obtusa

Sh.A. Topchiyeva, H.A. Abiyev, E.T.Babayev

Institute of Zoology of NAS of Azerbaijan, Baku
Address: AZ1073, Baku, Sabail, Abbasgulu Abbaszadeh, 115
e-mail.: shafiga.topchiyeva@mail.ru

***Abstract*—** *In this article presents experimental data, the basic composition of the venom of Macrovipera lebetina obtusa, captured from different regions of Azerbaijan, differing in degree of contamination by industrial emissions was studied. γ – radiospectrometric studies showed that the samples of venom also contain radionuclides as Ra228, Ra226, K40 and 137 Cs. It was established that the radiation dose (up to dose 1.35 kGy) for 3 minutes did not cause structural changes in the samples venom of vipera, but rather contribute to the stabilization of both toxicity and pharmacological activity while increasing the shelf life of aqueous solutions of vipera venom. At high doses (2.7, 4.05 and 5.4 kGy) γ-irradiation for 3 minutes there was a gradual decrease in toxicity (pharmacological activity of enzymes) of snake venom. We can assume that these data can be used in the identification of zootoxins and their metabolites, and these criteria can serve as a theoretical basis for the development of effective methods for diagnosis of poisoning zootoxins.*

***Keywords*—snake, venom, Macroipera lebetina obtusa, Ecology factor radionuclides, radiation dose.**

I. INTRODUCTİON

One of the global problems is the study of the influence of environmental factors on snake venoms [20, 21].

Relative density of venom of the cobra which's value corresponded 1.084 was experimentally defined. It was noticed, that reaction of snake venoms -is sour. Water solutions of snake venom are unstable and lost toxicity in some days after mixing. The toxicity of snake venoms (Echis carinatus, Vipera lebetina obtusa, Naja naja) in the physiological solution containing 50 % of glycerin, at storage in a refrigerator within 6 months did not get reduced In this research the influence of environmental factors on snake venoms was revealed. It should be noted that snake venom only after drying over calcium chloride vapors or after liofil drying, retains pharmacological and toxicological properties. Venom of a cobra, at storage on a cold in the soldered ampoule kept toxicity more than 20 years. Snake venoms are thermostable in the sour environment and can withstand heating up to 120°C without loss of activity [12,14,15].

Some chemical agents, such as potassium permanganate, chloroform, ethanol, methyl-blue, have a destructive effect on poisons. The activation of snake venoms occurs under the influence of some physical factors, for example, under the influence of ultraviolet irradiation and X-rays [12]. So, in 7 days after a venom irradiation of Filipina cobras by γ radiation (^{60}Co) in doses 0.25, 0.5, 1 mrad, toxicity of snake venom made 83, 66 and 43 %, accordingly from not irradiated venom [16, 17, 18].

On literary data snake venom in the dried up kind keeps pharmacological and toxic properties till 22 years and even more. At cultivation of venom by water by physiological solution or glycerin, toxic properties of snake venoms are not lost. Short-term influence of heat also does not render essential influence on toxicity of venom. Considerable influence on toxicity and quantity of allocated venom is rendered by various biological, ecological factors, and also chemical agents and some physical factors [22, 23, 24, 19].

Small doses of venom do not cause any clinical displays of a venoming and long ago was used at treatment of many serious illnesses.Venom of Vipera lebetina renders anti-inflammatory and analgeting action. It is considered possible to use of venoms of Vipera libetina, Echis carinatus and cobras in otolaryngology [12, 13].

Snake venoms are a great value for medicine and biology. Snake venoms are used at preparation of mono- and polyvalent whey. Venom of Vipera libetina is applied as blood stopping means in otoloringology at operations - during removing of glands, at nasal bleedings etc. [1, 4, 5, 7, 8]. Influence of ecological factors on a chemical compound of venom Caucasian Vipera lebetina obtusa was revealed. Elementary structure of venom of Vipera lebetina obtusa caught from the various areas of Azerbaijan polluted by technogenic emissions of the industrial enterprises was studied by the atom -absorption spectrophotometer method. In venom of Vipera lebetina obtusa the maintenance of heavy metals; Cr, Pb, Cd, Ni and Zn were defined [9,10,11]. Researches on revealing of influence of γ -radiation on toxicity and on pharmacological properties of venom of Vipera lebetina

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obtusa were carried out [2, 3].

Progressing environmental pollution by polutant leads in turn to gradual pauperisation of fauna and flora of the given areas of Azerbaijan. In addition, it directly affects the pharmacological and toxicological activity and, in turn, the chemical structure, biochemical and biophysical properties of the products of the biosynthesis of poisonous animals. 23 kinds of snakes live in territory of Azerbaijan, 4 kinds of them are venomous. Research of toxins of various kinds of snakes, and also huge attention to zootoxins is defined not only by inquiries of medical practice, but their studying and use represents the big interest for various branches of biology, ph Many questions on influence of -radiation and other ionising radiation on a live organism and venom of snakes remain opened. These questions are important for technology of radiating sterilisation of medical products on the basis of venom of snake.

Research objective was studying of influence of biotics and abiotics factors, on biochemical indicators, pharmacological and toxicological properties of venom of Transcaucasian vipera and an establishment of optimal doses of radiation sterilisation of venom.

II. MATERIAL AND METHODS

The material of the study was the whole venom of the Macrovipera lebetina obtusa collected in the area of the Azerbaijan. The venom was stored in a desiccator over a couple of calcium chloride.

The maintenance of heavy metals- polluters in venom of vipera caught from ecologically polluted sites of Absheron was defined by the atom-absorbtsion spectrophotometries method (AAS-300, Perkin-Elmer).

As a result, chemical compound changes and also influence of heavy metals on albuminous structure, fermentative activity and other biochemical indicators of venom of viperas was established.

Influences of intensity of electromagnetic radiation on albuminous structure, the maintenance of the general fiber and the change of toxicity of venom of snakes was revealed.ysiology, bioorganic chemistry, biophysics, toxicology and other areas of sciences.

Despite numerous studies on the study of snake venom, a number of questions of which is of great scientific and practical interest. Proceeding from the foregoing, the purpose of these studies was to Experimental researches by influence definition of pollution on biochemical characteristics and toxicity of venom of vipers was carried out.

III. RESULTS

We conducted summer field researches in vicinities of Baku, and also in different areas of Azerbaijan: Gobustan, Shamakhi, Kurdamir and Sabirabad. During the expedition catching of viperas was spent with milking venom, taking samples of soil and vegetation. Snakes after milking have been released in the nature, venom was placed in exicators, dried up for analysis carrying out on the maintenance of heavy metals by a method of atom-absorbtion spectrometry. A part of venom of snake was subjected to the analysis on the maintenance of radionuclides on installation Camberra.

We had carried out experimental measurements of the maintenance of radio nuclides in samples of venom of viperas, caught from districts of Azerbaijan with various degree of impurity. The spectrum defining activity of radionuclides in venom of snakes was drawn on spectrometer Camberra. The radiating background of radionuclides (uranium, caesium), defined in venom of snakes is presented in the table 1, and fig. 1, 2, 3. The radiating background of radionuclides in venom of snakes from various zones was identical.

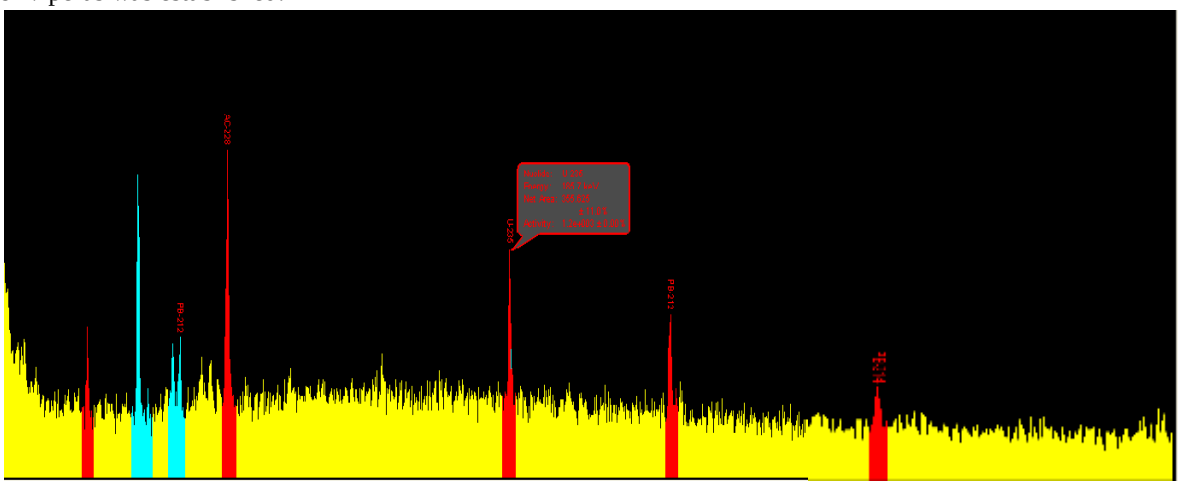

Fig.1. A spectrum of activity of radionuclides in venom of snakes, caught in territory of Gobustan and Absheron peninsula of Azerbaijan

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Table.1: Radiating activity of elements in investigated samples

The crystals were Venom	Sample 1	Sample 2	Sample 3
The name radionuclide	Ra^{226}	K^{40}	Ra^{226} = Pb^{212}, U^{235}, U^{238}
Energy radionuclide	186.2 keV	1460.8 keV	185.97 keV
Radiating activity	0.539663 Bk/g	1.44382 Bk/g	
Radiatsionno-chemical exit	3.28%	10.67%	5.26%
The peak area with a margin error	356; 10.98%	483 4.897%	77, 770
Mass	56 mg	60mg	55 mg
Specific activity	-	-	0.427, 0.26, 12.64 Bk/kg
Width of semiheight	0.936 keV	1.501 keV	

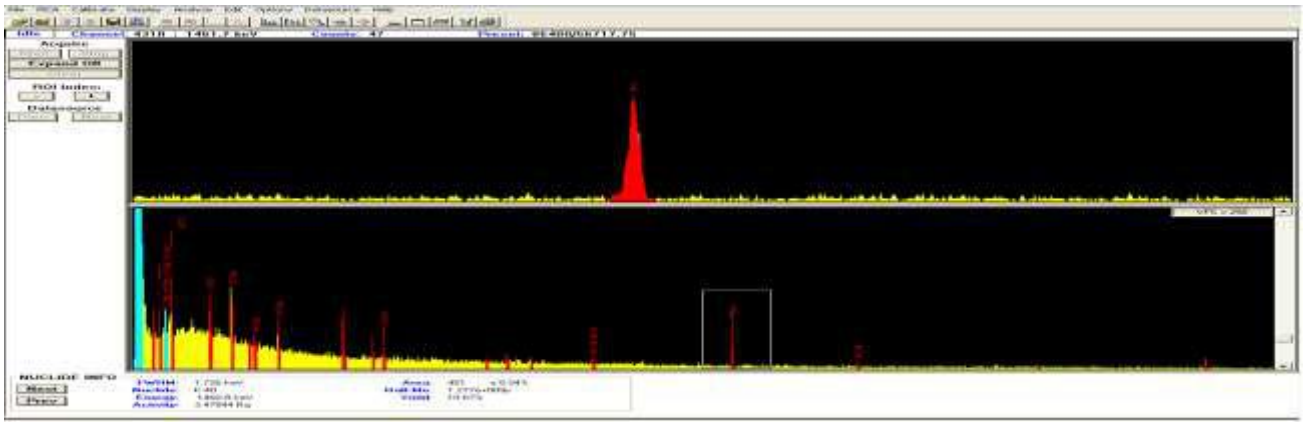

Fig.2: A spectrum of activity of radionuclides in the venom of snakes, caught in territory of Shamakhi of Azerbaijan

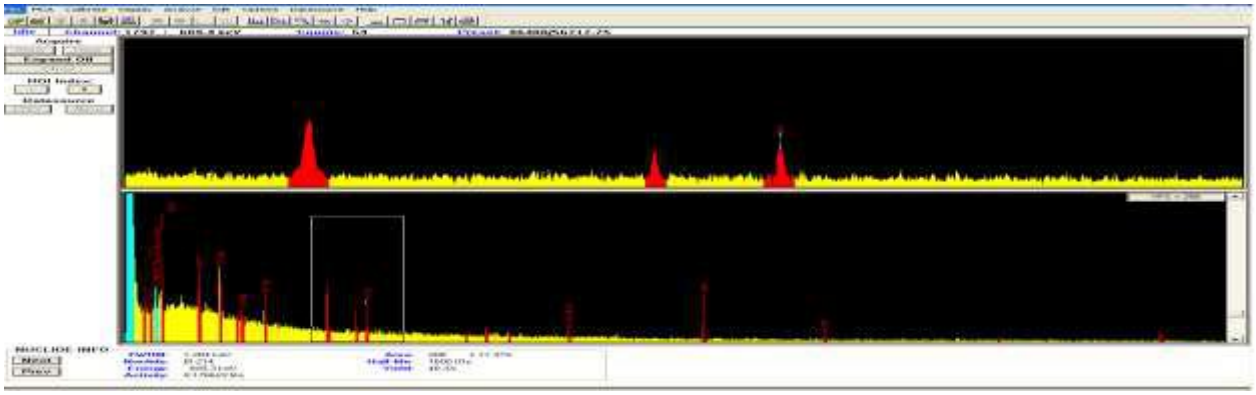

Fig.3: A spectrum of activity of radionuclides in the venom of snakes, caught in territory of Sabirabad and Kurdamir

Research on the influence of EMR on lifespan of envenomed mice were carried out in experiment on three-monthly not purebred white mice with weight of a body 22-25 g. Snake venom was injected into the mice intraperitoneally in a dose of weight of a body of 0.2 mg/g. We had used 70 mice in experiments. Mice were divided into two groups: control and experimental. Standard venom was injected i/p into control group of mice and the venom allocated from snakes, subjected to an irradiation at various modes was entered into experimental groups of mice. After envenoming of mice with vipera venom, oppression of condition was observed in all experimental animals, and they perished. However, lifespan fluctuated depending on the maintenance of the total protein and apparently from degree of toxicity of Venom. Studying of influence of electromagnetic radiation on toxicity of Venom Vipera lebetina obtusa was spent in experiment in several stages.

The subsequent groups of the mice subjected to electromagnetic radiation at high modes of an irradiation, were also divided into experimental and control groups. Simultaneously, vipera venom, allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field, (14000V) was

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injected in a dose of 0.2 mg/g to the first experimental group of animals.

Vipera venom, allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field (15000V) was injected into the second experimental group of animals.

Vipera venom, allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field (16000V)was injected into the third group of experimental animals. Vipera venom allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field (17000V) was injected into the fourth group of experimental mice. Vipera venom, allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field (18000V) was injected into the fifth group of experimental mice. Vipera venom, allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field (19000V) was injected into the sixth group of experimental mice. Vipera venom allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field (20000V) was injected into the seventh group of experimental mice. Though identical changes of the general condition were observed at experimental group of mice which was injected with vipera venom, allocated from snakes preliminary irradiated throughout 24 hours at high intensity of an electromagnetic field (14000V-20000V), however in lifespan of mice the tendency to insignificant increase in term of life was marked. Deterioration of the general condition at all groups of mice also was observed already later 5-10 minutes.

Breath increase, infringement of coordination of movement was marked after injection of venom of vipera, irradiated at high intensity, first minutes after injection of venom in all mice. After 20-30 minutes a condition of experimental groups of mice worsened. Mice had dyspnea, slackness, puffiness. Further the condition of mice gradually worsened, and mice perished. Lifespan of experimental groups of mice corresponded to 45-61 minutes. The identical phenomena has been noted at experimental animals after injection of venom, irradiated with electromagnetic radiation both at modes low, and at high intensity of an irradiation. However lifespan at mice of all groups varied from 25 till 66 minutes.

Thus, comparing the obtained data to results of references it is possible to ascertain that appreciable reduction of toxicity of vipera venom was marked at an irradiation of snake venom in small doses of radiation scale. With increase in a dose of an irradiation, change of both physical and chemical properties of vipera venom, and therefore its toxicity and pharmacological activity is most likely marked. It is necessary to specify the advantage of an irradiation of vipera venom with electromagnetic radiation in comparison with a radiating irradiation. Minor alteration in lifespan of experimental mice was marked at low EMR, and high intensity, in comparison with a radioactive irradiation. From the above-stated follows that application of EMR for sterilization of snake venom and its preparations is preferable rather than application for the similar purposes of radiating sterilization.

It is necessary to notice that the change of quantity of the total protein in snake venom was not marked at an irradiation of snakes by electromagnetic radiation of low intensity. Toxicity of venom also wasn't exposed to changes. At high intensity of radiation, insignificant fluctuations in the maintenance of the total protein and change of toxicity of snake venom were observed. Influence of electromagnetic radiation on the maintenance of the total protein of standard samples of vipera venom was observed. We carried out experimental measurements on studying of influence of electromagnetic radiation on the maintenance of the total protein of vipera venom. In experiments a generator of the microwave oven (with frequency of 10-460 MHz) was radiation source. The irradiation of vipera venom was spent at modes high (U-14000V) and low (U-7000V) intensity of an irradiation throughout 30 minutes, 1 and 2 hours. Thus, target capacities of a radiator -P (70 Vt) were applied. Definitions of the maintenance of total protein in the samples of venom subjected to influence of EMR was defined by a method of Lowry.

From the given tables it is visible that under the influence of electromagnetic radiation of low intensity (U - 1000-7000V) throughout 30 minutes, 1 and 2 hours, minor alterations in the maintenance of total protein of venom were marked. The maintenance of protein in investigated samples of Venom fluctuates within 96.50 ± 0.48 - 95.00±1.90mg/g. At influence of EMR of high intensity (U-14000-20000V) throughout 30 minutes, 1 and 2 hours, considerable changes in quantity of the total protein of venom were marked in comparision with samples of the venom, subjected influence of EMR of low intensity.

The quantity of protein in investigated samples of vipera venom, under influence of EMR of high intensity fluctuates in limits -96.10±3.64-90.00±3.79mg/g. Thus, on the basis of the carried out experimental researches it is possible to ascertain that under the influence of electromagnetic radiation of low intensity (U – 1000V-7000V), minor alterations in the maintenance of the total protein of venom were marked. At influence of EMR of high intensity (U-14000V-20000V) considerable changes were marked in quantity of the general fiber of Venom in comparison with samples of venom, subjected to EMR

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with low intensity of radiation. Apparently, these changes influence also on toxicity of snake venom. We drew absorption spectra in infra-red, visible and ultra-violet areas, standard venom and the samples of snake venom subjected selectively to influence of EMR of low intensity in limits U – 1000V-7000V and high intensity in limits U – 14000V-20000V.

Measurements by definition of the maintenance of radionuclides in samples of venom of vipera, caught in districts of Azerbaijan with various degrees of impurity, and also in samples of soils, vegetation and water was conducted on spectrometer Canberra. Time of experiment was 24 hour. The spectrum defining activity of radionuclides in snake venom was drawn on a spectrometer "CANBERRA" (table 2).

Table. 2: Influence of vipera venom (irradiated with small doses of γ-radiation) on lifespan of mice (a dose of venom entered i/p -0.2 mg/g)

γ-irradiation dose, κGy	Duration of an irradiation in hours	Lifespan of mice in minutes	
		Control	Experiment
1.35	0.5	28-36	28-36
2.7	1.0	28-36	45-60
4.05	1.5	28-36	60-70
5.4	2.0	28-36	70-90

Thus, comparing the obtained data with results of references it is possible to ascertain that at an irradiation of venom with small doses of gamma radiation, appreciable reduction of toxicity of zootoxin was marked. With increase in a dose of an irradiation, change in both physical and chemical properties of vipera venom and its toxicity and pharmacological activity, as a general rule, was marked.

Thus, changes of toxicity of vipera venom under the influence of γ-radiation were established experimentally on 50 white not purebred mice at doses 2.7, 4.05, 5.4 κGy. From the above-stated it is possible to ascertain that influence of small doses of γ-radiation on toxicity and on pharmacological properties of venom of Vipera lebetina obtusa was revealed. The regularities in reduction of toxicity of venom was revealed at an irradiation by small doses of γ-radiation. It was experimentally established that with increase in a dose of radiation, reduction of toxicity of venom is accordingly observed.Essential change of toxicity of vipera venom was not noted under the influence of γ-radiation at a dose 1.35 κGy. Changes of toxicity of Vipera venom under the influence of g-radiation were revealed at doses 2.7, 4.05, 5.4 κGy. The regularities in reduction of toxicity of venom at increase of a dose of γ- irradiation was established by radiation.

Lifespan at mice of 1st group didn't differ from experimental groups of mice while it varied from 45 till 90 minutes at mice of 2nd 3 and 4th experimental groups.

From the above-stated it is possible to ascertain that study of consequences of global anthropogenous pollution of biosphere and preservation of the environment are among the most actual and essential problems of the nowadays.

On the basis of the received data it is possible to ascertain that depending on degree of impurity of district where catching, milking of snakes, taking samples of vegetation and soils were conducted, fluctuations in the maintenance of heavy metals were marked. However, radiating activity of elements in snake venom and soils didn't change almost.

Thus, the influence of ecological factors (biotic, abiotic), on biochemical indicators, pharmacological and toxicological properties of Venom Caucasian Viperas was studied and optimum doses of radiating sterilization of Venom were established.

IV. CONCLUSIONS

1. The method atom-absorbtcnion spectrophotometry (AAS-300, Perkin-Elmer) in vipera venom, caught from ecologically polluted sites of Absheron, defined the maintenance of heavy metals-pollutants. As a result, changes in chemical compound and also influence of heavy metals on protein structure, fermentative activity and other biochemical indicators of vipera venom were established.
2. Influences of intensity of electromagnetic radiation on protein structure, the maintenance of the general fiber and change of toxicity of venom of snakes were revealed.
3. Influence of the metals-pollutants, ionising radiation and electromagnetic radiation on biochemical parametres of vipera venom, on fermentative activity and toxicity of venom were experimentally revealed. Doses of radiations were determined and revealed for sterilization of both snake venom, and its water solutions.

4. Influence of ecological factors (biotic, abiotic) on biochemical indicators, pharmacological and toxicological properties of Caucasian vipera Venom was studied and optimum doses of radiating sterilization of venom were determined.
5. The method of atom-absorbtion spectrophotometry (AAS-300, Perkin-Elmer) in vipera venom, caught from ecologically polluted sites of Absheron, defined the maintenance of heavy metals-pollutants.

Thus, in comparative aspect, the influence of ecological factors on venom of Transcaucasian viperas was revealed. Concentration of heavy metals in venom of vipera, caught in various biotopes of Azerbaijan, and also in vegetative and soil tests was defined. As a result, changes in chemical compound and also influence of heavy metals on protein structure, fermentative activity and other biochemical indicators of venom of viperas were established. The revealed values will be applied at preparation of preparations on the basis of venom of snakes. It will be in turn recommended for pharmaceutical industry by manufacture of injections on the basis of venom of snakes.

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Climate Change farm-level Adaptation Measures among Soybean Farmers in Benue state, Nigeria

Ikyoosu B. M, Ezihe J.A.C, Odoemenem I. U.

Department of Agricultural Economics, University of Agriculture Makurdi, Benue state. Nigeria

Abstract— *This study analyses climate change farm-level adaptation measure among soybean farmers in Benue state, Nigeria. The study used multistage sampling technique and primary data were collected from 217 soybean farmers. Objective (i) was realized using descriptive statistics, viz. percentages and frequencies. Objective (ii) was achieved using stochastic frontier model. Objective (iii) made use of multivariate discreet choice model (MNL). Objective (v) was realized using Factor Analysis model (FA). Results of the multinomial logit analysis showed that Age positively influenced the use of crop diversification at 5% significant. Household size had positive relationship with the choice of crop diversification as farm-level adaptation measures. Farm size had a negative effect on the choice of multiple crop varieties. The stochastic frontier analysis showed that farm size was highly significant at 1% level of probability among soybean farmers. The computed mean of technical efficiency estimate was 0.12 and 0.90. The technical inefficiency model showed that land fragmentation (i.e. multiple farm plots) is significant at 5%, off farm employment is significant at 1%, both organic and inorganic had 10% significant technical inefficiency. The factor analysis revealed that the major constraints to climate change and farm-level adaptation measures among the soybean farmers were public, institutional and technological constraints; land, traditional beliefs and farm distance constraints; high cost of inputs, small scale production and knowledge of cropping or building resilience constraints; The study, therefore, recommends, inter alia, proactive regulatory land use systems that will make soybean farmers to participate in cooperative membership, have access to extension services to enhance their investment in climate change farm-level adaptation measures that has a long-term effect. More also, Government and non-governmental organizations should help the farmers in the area of provision and/ or facilitate the provision of input-based farm-level adaptation measure in the study area. Again, intensive use of already proven adaptation measures at farm-level by the farmers at their present resource technology will make them to reduce technical inefficiencies in the study area.*

Keywords— Climate change, Farm-level, Adaptation, Measures, Soybean Farmers, Benue state.

I. INTRODUCTION

Research on climate change adaptation has been conducted by the IPCC, UNFCCC, United Nation Environmental Programme (UNEP), and several climate scientists. There are different definitions of adaptation, (Pielke, 1998, IPCC, 2007, and Smith, 1993), Defined adaptation as the adjustment in ecological, social, or economic systems in response to actual or expected climatic stimuli and their effects or impacts (IPCC, 2001).

The importance placed on adaptation is reflected in Article 10 of the Kyoto protocol where it "commits parties to promote and facilitate adaptation and deploy adaptation technologies to address climate change". Also Paris (2015) UNFCCC adopted version of the agreement charged parties; especially developing countries to pursue and redouble efforts to limit the temperature increase to 1.5^{o}c. The 1.5^{o}c goal will require zero emission sometime between 2030 and 2050. Appropriate adaptation can reduce the negative effect of climate change. The capacity to adapt to climate change depends on many non-climatic factors: level of economic development and investments, access to markets and insurance and political considerations (Lioubimsteva and Henebry, 2009).

Soybean, *Glycine max* (L Merr) the miracle seed is the world's most important oil seed legume which is produced in most part of middle belt of the country especially Benue state. Some of other states producing soybean in the country includes Kwara, Kogi, Oyo, Ondo, Osun, Nasarawa, Taraba, Niger, Bauchi, Kaduna. (Salunkhe ; Adsule, *et.al*., 1992). In 1986 Nigeria was the second largest producer of soybean in sub-Saharan Africa (SSA), with over 65,000 metric tons (MT) followed Zambia 36,000 tons. (Singh *et.al*., 1987). Presently Nigeria produces about 500,000 MT of soybean annually making it the largest producer of the crop on the African continent. Benue state is producing

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above 175,000 MT out of the total 500,000MT making it the highest producer of the crop in the country. Recent study has showed that due to changes in climate being experienced, soybean production in Benue state has dropped by 10% of total annual production in the state between 2006 to 2007 (Agada, 2014). Soybean is a versatile crop and one of the mandate crops in Benue state.

Nutritionally, the important of soybean in the diet is explicitly stated in the following areas: soybean is economical and effective in the control of diseases such as stroke, heart disease, cancer, ulcer, high blood pressure, diabetes and loss of body weight among people living with HIV/AIDS, etc due to it protein mineral content. However the rapid climatic changes and inadequate farm-level coping strategies is threatening the production and utilization of soybean in Benue state. Thus, the need to analyze climate change and farm-level adaptation measures among soybean farmers in Benue state.

1. describe the farm-level adaptation measures being practiced by soybean farmers in Benue state.
2. determine the effect of farm-level adaptation measures on farm output of soybean farmers.
3. assess the factors that are influencing the choice of farm-level adaptation measures by soybean farmers in Benue state.
4. identify the major constraints to climate change farm-level adaptation by soybean farmers in Benue state.

The following hypothesis were postulated and tested.

1. Farm-level adaptation measures have no significant effect on farm output of soybean farmers in the study area.
2. There are no significant factors influencing choice of farm-level adaptation measures by soybean farmers in the study area.

II. METHODOLOGY

Research Design

This study made use of public opinion survey to collect the needed data, well-structured questionnaires were used.

The Study Area

The study area was Benue state.

Geographically, Benue state is located in the middle belt of Nigeria with Makurdi as its capital and lies between latitude 8 and 10 N and between longitude 6 and 8 E, with a land mass of 6.595 million hectares (BNARDA 1998).

Benue state shares boundaries with Cameroun to the south, Nasarawa to the North, Taraba state to the East, Cross River to the South, Enugu and Kogi states to the South West and West respectively (Anonguku *et.al*., 2010). The state is also bordered on the North by 280km River Benue, and is traversed by 202km of River Katsina-Ala in the inland areas.

The state has a population of 4,253,641. By sex distribution the state has a population of 2,144,043 male and 2,109,598 females, making it the ninth most populous state in the country with about 80% of its population involved in agriculture and produces, rice, benniseed and maize. Others include sweet potato, millet and wide range of other crops viz. sugar cane oil palm, mango, citrus, bananas etc. The state has two distinct seasons, rainy and dry seasons. The rainy season stretches from April-october and the dry season from November-March. Annual rainfall varies from 1250mm. the hot season comes in mid April with temperatures between 32°C and 38°C.

Agriculturally, Benue state is segmented into three Agricultural zones of A, B and C. the major ethnic groups in the state include Tiv, Idoma, Igede, Etulo, Aakpa, Lukum, Hausa, Akwaya and Nyifon. Benue state has a Guinea savannah kind of vegetation characterized with scattered trees and coarse grasses. (BNARDA, 1998).

Administratively the state is divided into three zones namely, Eastern or A, Northern or B and Central or C zones by the Benue Agricultural and Rural Development Authority (BNARDA). The zonal headquarters of the three zones are Adikpo, Gboko and Otukpo respectively in that sequence. The state has a total land area of about 30,955 square kilometers and administratively it is divided into 23 Local Government Areas.

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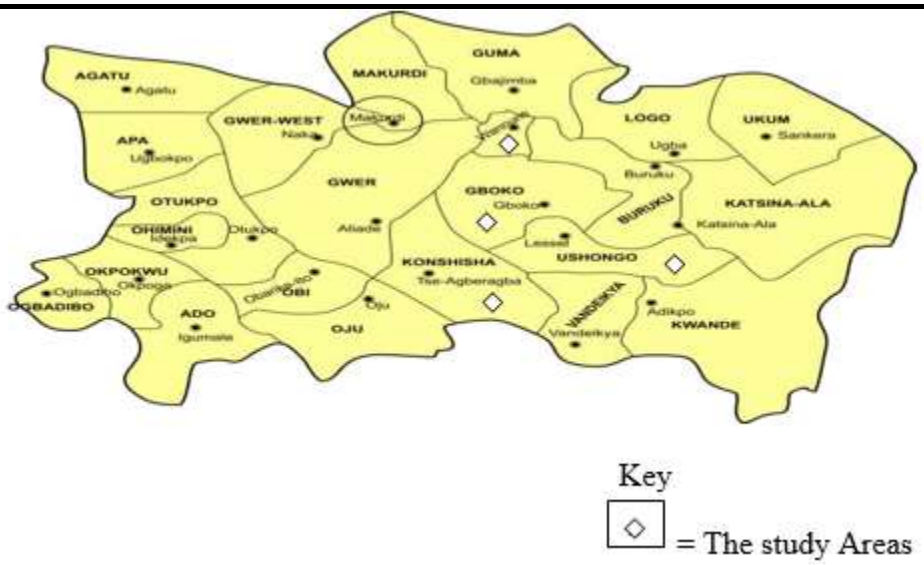

Key
◇ = The study Areas

Fig. 1: Map of Benue State Showing the Location of the Study Areas.

Source: www.Benuestatemap.com.

Population of the study.

The population for the study comprises of all soybean farmers in Benue state.

The data for the study was collected from 217 randomly selected soybean farmers in the study area due to high population of soybean farmers and high level of soybean cultivation.

Sample and Sampling Techniques.

The major soybean producing agricultural zone was purposively selected for the study. Northern and North-West agricultural zones consisting of two (2) Local Government Areas were randomly selected from each zone. Three communities were randomly selected from each local government area and three soybean farming villages were also selected from each community.

Five (5) households were randomly selected from each farming village.

Table 1: Summary of the study location and sample chosen

S/no	Zones	LGA	Communities	Sampling Frame	Respondents Sample 0.17%
1	Northern	Ushongo		-	-
			Utange	133	20
			Mbakuhwa	122	18
			Mbadede	166	25
2		Konshisha		-	-
			Iwarnyan	89	14
			Mbamar	78	12
			Iwarev	122	18
3	North West	Gboko		-	-
			Mbadeda	133	25
			Mbanev	122	18
			Tse-kucha	122	20
4		Tarkaa	-		-
			Mbanoughul	56	10
			Shitile	78	15
			Pipeline	89	20
	2	4	12	1320	217

Source: field survey (2016)

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Instrument of Data Collection.

Method of Data Collection.

Data for this study was collected from primary source.

Primary data was collected through the use of a well structured questionnaire, copies of which were administered to the selected 217 soybean farmers in the study area.

Primary data was collected on the adaptive measures for mitigating the effect of climate change, factors influencing the choice of adaptation measures, constraints to climate change adaptation measures in the study area.

Model Specification.

The data for this study was both descriptive and inferential statistics.

Objective (i) was realized using descriptive statistics, viz. percentages and frequencies. Objective (ii) was achieved using stochastic frontier model. Objective (iii) made use of multivariate discreet choice model (MNL). Objective while objective (iv) was analyzed using Factor Analysis model (FA).

Multivariate Discreet Choice Model.

The Multinomial Logit (MNL) model for climate change adaptation choice specifies the following relationship between the probability of choosing option *A*i and the set of explanatory variables X as (Greene, 2003):

$$\Pr(Yi = j) = \frac{e\beta j\ Xij}{1+\sum_{m=0}^{6} e^{\beta m\ Xij}}, j = 0,1,2,3, \ldots,6$$

Where β_j is a vector parameter that relates the socio-economic, farm and institutional characteristics X_i to the probability that Y_i= j. Because the probabilities of the six (6) main climate change adaptation strategies must sum to one, a convenient normalization rule is to set one of the parameter vectors, say β0, equal to zero (β0=0). The probabilities for the six (6) alternatives then become (Greene, 2000):

$$Pj \equiv \Pr(Yi = j) = \frac{e\beta i\ Xij}{1+\sum_{m=0}^{6} e^{\beta mXij}}, j = 1,2,3\ldots..6$$

$$P_0 \equiv \Pr(Yi=0) = \frac{1}{1+\sum_{m=1}^{6} e^{\beta m\ Xij}}$$

The estimated parameters of a multinomial logit system are more difficult to interpret than those in a bivariate (or binomial) choice model. Insight into the effect that the explanatory variables have on the climate change adaptation strategies decision can be captured by examining the derivative of the probabilities with respect to the kth element of the vector of explanatory variables. These derivatives are defined as (Greene, 2000):

$$\frac{\propto Pr(Yi=j)}{\propto Xik} = Pj[\beta jk - \sum_{m=0}^{6} Pr(Yi = m)\ \beta jk]\ j = 0,1,\ldots..6;\ k = 1,..,k$$

Clearly, neither the sign nor the magnitude of the marginal effects need bear any relationship to the sign of coefficients.

The Y_i is the probability of choosing a climate change adaptation strategy. The following are the main climate change adaptation strategies used among soybean farmers;

1. using different or multiple varieties of soybean
2. change in location of soybean farmlands/plots (i.e. land fragmentation/ land use planning)
3. change in timing of operations/ change in planting dates (i.e. multiple planting dates)
4. crop diversification (i.e. changes in crop mix)
5. diversification of source of household income to unrelated off-farm employment (off-farm employment opportunities)
6. Planting of cover crops (cover cropping).

X_i= socio-economic, farm-specific and institutional variables.

Socio-economic variables that were used partly as independent variables include:

Household size (X_1) = Number of individuals in the household.

Age (X_2) = Age of household head in years.

Education level of farmer (X_3) = number of years of schooling of household head.

Years of climate change awareness (X_4) = number of years of household head's awareness of climate change.

Marital status (X_5) = farmers marital status or his responsibility.

Gender (X_6) = sex category of household head (dummy1for male; 0 otherwise).

Farm-specific variables that were used partly as independent variables include:

Farm size (X_7) = measured in hectares.

Average distance from homestead to the farm(s) (X_7) = Average distance from homestead in kilometers.

Institutional variables that were used partly as independent variables include:

Access to extension services (X_8) = number of formal extension visit in the cropping season.

Membership of cooperation (X_{10}) = Number of membership of cooperative that the farmer belong to.

Access to credit facilities (X_{11}) = access to formal credit (dummy 1 for access to credit; 0 otherwise).

Stochastic Frontier Models

Stochastic Frontier Production Function

The data in this study was fitted into Cobb-Douglas and average production forms of stochastic frontier production function and the best form was selected through the use of generalized log-likelihood test after meeting the econometric requirements.

Cobb-Douglass production form:

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$1n\ Y_i = \beta_o + \Sigma\beta_i \ln (X_i) + (V_i - U_i)$

Where:

$\beta_0 - \beta_i$ = parameters estimates.

Σ is the sign of summation.

Y_i = the value of output in naira,

X_1=the total labour used in soybean production in mandays;

X_2=the total land area (farm size) used in soybean production in hectares;

X_3= the total quantity of fertilizer used in soybean production in kilogrammes;

X_4= the total value of other agrochemicals (i.e. pesticides and herbicides) used in soybean

Production in naira, and

X_5= the depreciated value of farm implements (i.e. hoes, cutlasses, watering can, etc.) in naira.

It was calculated using straight line method of calculating depreciation. That is, Depreciation is

$$\frac{Purchasing\ cost\ of\ the\ asset - Salvage\ value}{Life\ span\ of\ the\ asset\ in\ years}$$

The V_is are random errors that are assumed to be independent and identically distributed as N

(0,σv2) random variables; and the U_is are non-negative inefficiency effects that are assumed to be independently distributed among themselves and between the Vis such that U_i is defined by the truncation of the N (U_i, σ) distribution. Where U_i is defined by:

Inefficiency Effects Model

$U_i = \delta_o + \sum_{i=1}^{\infty} \delta i Z j i$

U_i = inefficiency effect; δ_i = coefficients of climate change adaptation strategies and socioeconomic factors.

Z_{ji} = climate change adaptation strategies and socio-economic factors (i.e. hypothesized efficiency changing variables).

Z_1= land fragmentation (number of farm plots used for soybean production as a result of change in climate);

Z_2= off-farm employment (income from unrelated employment in naira in order to adapt to climate change);

Z_3= inorganic fertilizer (in kg, 0 otherwise);

Z_4= organic fertilizer (in kg);

Z_5= tree planting date (number of trees per farm);

Z_6= multiple planting date (number of trees planted in a season);

Z_7= years of awareness of climate change, and

To choose the functional form that best describes the inefficiency effect, the following hypothesis will be tested;

H_0: $\gamma = \delta_0 = \delta_1 = ...\delta_7 = 0$, this hypothesis specifies that the inefficiency effects are not present in the model. If this hypothesis is accepted, then the soybean farmers are fully efficient. Then, the data will be better analyzed using average production function rather the frontier function, which assumes the presence of inefficiency in soybean production.

Test of the above hypothesis will be obtained by using the generalized likelihood-ratio statistic, which is defined by;

$\lambda = -2 \ln [L (H_0)/L (H_1)] = -2 \ln[L(H_o)-L(H_i)]$

Where L (H_0) is the value of the likelihood function for the average production function

(Model 1), in which the parameter restrictions specified by the null hypothesis, H_0 are imposed;

and L (H_1) is the value of the likelihood function for the general frontier model. If the null

hypothesis is true, then λ has approximately a Chi-square (or a mixed square) distributed with degrees of freedom equal to the difference between the parameters under H_1 and H_0, respectively; that is the number of parameters excluded in the model.

Factor Analysis Model.

Principal component analysis model was used in achieving objective (vii), which is

Specified as:

Y1 = a11X1 + a12X2 + * * *+ a1nXn

Y2 = a21X1 + a22X2 + * * * + a2nXn

Y3= a31X1 + a32X2 + * * * + a3nXn

* = *

* = *

* = *

Yn= an1X1 + an2X2 + * * * + annXn

Where:

Y1, Y2 …Yn = observed variables/constraints of soybean farmers on adoption of climate change adaptation strategies.

a1 – an = factor loadings or correlation coefficients.

X_1, X_2, … Xn = unobserved underlying factors constraining soybean farmers from adapting to climate change adaptation strategies were retained, the study selected factors with high factor loadings scores ± 0.4 or greater.

Data Analysis Techniques.

Descriptive statistics was used to analyze the objectives in this study especially,

Objective (i) and (ii) were analyzed using Descriptive Statistics such as Frequency, and Percentage.

Objective (iii) was analyzed using stochastic frontier analysis.

Objective (iv) was analyzed using Multinomial Logit Model MNL.

Objective (v) was analyzed using Factor Analysis Technique.

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Hypotheses (i) was tested using F-test and hypothesis (ii) were tested using t test as embedded in stochastic frontier models and multinomial logit, respectively.

III. RESULT AND DISCUSSION

3.1. Result and Discussions

Table 1 shows the numbers of questionnaire administered, completed, and returned. The analysis of data shall be restricted to the 204 questionnaires collected from respondents.

Table.1: questionnaire administered and returned

S/no	Items	Respondents	Percentages
1	**Number**	**217**	**100%**
2	**administered Number returned**	**204**	**96%**

Source: **Field questionnaire, (2016)**

3.2. Climate Change Adaptation Measures used by Soybean Farmers.

About 53.9% respondents used planting across slop as a crop management practice to adapt to climate change, multiple soybean varieties were used by about 50.5% of the respondents. About 51.0% respondents were using land fragmentation to cope with change in climate. Majority of 1.5% respondents were practicing fallow or alternative tillage system to cope with changing climate. Multiple planting dates were used by 77.0% respondents in the study area. About 36.8% respondents in the study area were involved in off farm employment to reduce the reduction in income cause by climate change. The respondents practicing cover cropping were about 12.3% to caution the effect of climate change on their farm. Majority of 93.6% respondents in the study area were applying inorganic fertilizer to cope with the reduction in output as a result of changes in climate. About 27.9% of the respondents were using organic fertilizer or manure to adapt to climate change. About 27.9% of the respondents were planting trees to adapt to climate change. About 12.3% respondents in the study area were practicing shading or sheltering as an adaptation measure on their farms, 52.5% of the respondents were changing farm size as an adaptation measure on their soybean farm.

Table.5: Distributions of Respondents based on the Farm-Level Adaptation Measures use by Soybean Farmers in Benue state.

S/No. Variables	Frequency	Percentage
1 Planting Across Slop	110	53.9
2 Multiple Soybean Varieties	103	50.5
3 Land Fragmentation	104	51.0
4 Fallow/Alternative Tillage	3	1.5
5 Multiple Planting Date	157	77.0
6 Irrigation Practice	-	-
7 Crop diversification	85	41.7
8 Off Farm Employment	75	36.8
9 Cover Cropping	25	12.3
10 Inorganic Fertilizer	191	93.6
11 Organic Fertilizer	56	27.5
12 Planting Trees	57	27.9
13 Shading/ Sheltering	25	12.3
14 Change in Farm Size	107	52.5

Source: Field Survey 2016

Table.6: Descriptive statistics distribution of respondents by the number of Farm-Level Adaptation measure used by Soybean Farmers.

Adaptation Number	Mean	Standard Deviation
Multiple Soybean type Number	1.09	1.126
Land Fragmentation	1.99	2.620
Multiple Planting Date Number	1.64	0.975

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Off Farm Income	37424.02	83030.874
Cover Cropping Number	166	18.905
Fertilizer Number in KG	149.88	90.505
Tree Planting Number	26.82	68.216
Change in Farm Size	1.25	1.414

Source: Field Survey 2016.

3.3. Effects of Farm-Level Adaptation Measures on Farm Output of Soybean Farmers in Benue State Nigeria.

This presents the results of the analysis of the farm-level adaptation measures that determine the influence of technical efficiency in soybean production in Benue state.

The explanatory variables (or factors) are important in this study because they have important policy implications. The following variable were hypothesized as farm-level adaptation measures and other farmers and farm specific variables, land fragmentation, (i.e. number of farm plots), off-farm income (₦) inorganic fertilizer used, organic manure, tree planting (no of trees), multiple planting dates and years of awareness of climate change. The results of the inefficiency models of soybean farmer in Benue state, Nigeria as showed in the table below. The following variable land fragmentation, inorganic fertilizer use, organic manure and multiple planting date had significant positive relationship with technical inefficiency while off-farm employment, years of awareness of climate change had significant inverse relationship with the technical inefficiency.

The positive coefficients simply imply that the variables have the effect of decreasing the level of technical efficiency. Any increase in the value of such variables would lead to an increase in the level of technical inefficiency. The inverse relationship implies that any increase in the value of the variable would lead to an increase technical efficiency.

3.4. Factors Influencing Technical Inefficiency are Discussed below.

1. Land fragmentation: the result shows that the coefficient for land fragmentation is positive and significant at 5% level of probability for all the respondents. For the positive significant coefficient, if implies that an increase in land fragmentation tends to increase level of the technical efficiency (i.e. decrease technical inefficiency). This finding agrees with the findings of Obwona (2000, 2006) and nearly similar with the finding of Otitoju (2008) of small-scale soybean production in Benue state, Nigeria which found out that increase in the number of fragmented land decreased technical efficiency.

2. Off-farm income or employment: the estimated coefficient of off-farm employment is positive and significant at 1% level of probability for the respondents in the study area. The positive relationship implies that as off-farm employment or income increases, the level of technical inefficiency tend to increase (i.e decrease technical efficiency). The positive relationship suggests that increases in non-farm activities are accompanied by a reallocation of time away from farm-related activities such as adoption of new technologies, intensification of other adaptation measures and gathering of technical information that is vital for enhancing production efficiency. The finding agrees with the finding of Abdulai and Huffman (2000) in which inefficiency increases with involvement in off-farm employment.

3. Inorganic and organic fertilizer use: the result showed that the coefficient for inorganic and organic fertilizer use is positive and are significant at 10% level of probability. The positive relationship for both inorganic and organic fertilizer use implies that as inorganic and organic fertilizer use increases, the level of technical inefficiency tend to decrease.

Table.7: Maximum likelihood Estimates (MLE) of the Stochastic frontier Production Function for Soybean Farmers in Benue state.

Beta(ß)	Variable	Coefficient	t-ratio
0	Constant	6.79	8.93*
1	Farm size	2.24	28.11*
2	Seed	-0.53	-0.33
3	Fertilizer	-0.007	-0.08

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4	Herbicide	2.01	2.45**
5	Labour	-0.03	-0.99
6	Depreciation	-0.02	-0.51
	Inefficiency Model		
Delta			
0	Constant	-0.22	-0.24
1	Land Fragmentation(No of Plots)	2.01	2.36**
2	Off-Farm Income(₦)	4.8557	2.69*
3	Inorganic Fertilizer(1,0)	1.19	1.38***
4	Organic Fertilizer(1,0)	6.65	1.38***
5	Tree Planting (No of Trees)	-0.009	-0.22
6	Multiple Planting Dates	-0.99	-0.02
7	Years of Awareness of Climate change	-0.29	-0.02
	Sigma Squared δ^2	1.84	4.09*
	Gamma γ	4.20	2.19**
	Log Likelihood Function		-0.316

Source: field survey 2016.
***, **, *** = t-ratio Significant at 1%, 5% & 10% level respectively**

3.5. Technical Efficiency Estimates for Soybean Farmers in Benue State.

The technical efficiency shows the ability of farmers to derive maximum output from the inputs used in soybean production. Given the results of the preferred models (Cobb-Douglas stochastic frontier models), the technical efficiency estimates are presented and discussed subsequently (Table 8).

The results show technical efficiency among the soybean farmers in the study area; the computed technical efficiency varies between 0.12 and 0.90 with a mean of 0.6975. This result of the mean efficiency (0.6975) is closely similar to the finding of Otitoju (2008) on small-scale soybean farmers in Benue State, Nigeria.

Table.8: Distribution of Technical Efficiency Estimate for Soybean Farmers in Benue state.

Efficiency Index	Frequency	Percentage
<=30	6	2.9
31-60	32	15.7
61-90	166	81.4
Total	**204**	**100**
Minimum Efficiency	-	0.12
Maximum Efficiency	-	0.90
Mean Efficiency	-	0.6975

Source: Field Survey 2016

3.6. Factors that Influence the choice of farm-level adaptation measures by soybean farmers in Benue state.

The estimate of the multinomial logit (MNL) model for this study was undertaken by normalizing one category, which is referred to as the reference category; in this analysis, the base category is fertilizer application.

The result of the multinomial logit (MNL) model indicate that different socio-economic factors like (Age, Education year, year of awareness of climate change, marital status, household size, gender) farm-specific variables (farm distance, farm size) and institutional variables (Extension visit, membership of cooperative, access credit) affect the farmer`s choice of the farm-level climate change adaptation measures in soybean production in Benue state, Nigeria.

The results of the parameter estimates (the estimated co-efficient) from the multinomial logit (MNL) model are presented in the table. The likelihood ratio test as indicated

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by Chi-Square statistics were highly significant at (82.39*), suggesting the model has a strong explanatory power.

Age is significantly and positively correlated to the probability of choosing crop diversification to fertilizer application farm-level climate change adaptation measures in the study area. This implies that as age of increase, soybean farmers have a long planning horizon and are more likely to choose crop diversification as farm-level climate change adaptation measure to be able to cope with climate change than the older counterparts.

This result disagrees with the work of Hassan and Nhemachena (2008) which found that age is inversely related to the probability of choosing Mono crop-livestock under irrigation. This also disagrees with the discovery by Bayard *et al*., (2006) that the age of farmers has a negative influence on the adoption of rock walls as soil management practice in Fort-Jacques in Haiti and on adoption of ibST in Connecticut Dairy farm (Foltz and Chang, 2001). It is assumed that the younger the farmer the likelihood that he/she is to adapt measure that will reduce the negative effect of climate change is more.

A unit increase in the age of soybean farmers would probably decrease respondent choice of crop diversification to fertilizer application farm-level adaptation measures by 0.139 (1.99) in the study area.

The result showed that there is a positive relationship between household size and the probability of choosing crop diversification to fertilizer application as farm-level adaptation measures in the study area. This implies that, the bigger soybean families are, the better they are able to choose crop diversification than fertilizer application by 0.177(1.97) significance as farm-level climate change adaptation measures in the study area. This result disagrees with the finding of Birungi and Hassan (2010) which found out that household size is negatively related to the adoption of fallow as land management technology in Uganda.

Farm distance to the residents of the soybean farmers` household is negatively related to the probability of choosing multiple crop varieties and crop diversification to fertilizer application as farm-level adaptation measures in the study area. It implies that the proximity of the farmers residents to the farm permit or gives farmers the opportunity to choose multiple crop varieties and crop diversification by -0.176(-1.65) and -0.219(-1.68) 10% significance to fertilizer application as farm-level adaptation measures in the study area. This result disagrees with the study of Birungi and Hassan (2010) that found out that distance for plot to farmers residence had positive relationship with adopting fallow, inorganic fertilizer as land management practices in Uganda.

Farm size has negative relationship with the probability of choosing multiple crop varieties to fertilizer application as farm-level adaptation measures in the study area. This means that household that own more plots or large farm size have higher probability of choosing farm-level adaptation measures than their counterparts with smaller farm land. This also implies that large hectares of land or farm size can influence farmers' decision to choose and use farm-level measures that will probably reduce the effects of climate change.

This finding agrees with the study of Birungi and Hassan, (2010) that larger land increases the probability of investment in land management.

Marital status is negatively related to the probability of respondents choosing crop diversification to fertilizer application as farm-level adaptation measure in the study area. This means that marital status of respondents would more likely influence their decision in choosing crop diversification to fertilizer application by -3.597 (-2.34) at 5% significance as farm-level adaptation measure in the study area.

Table.9: Parameter Estimates of the Multinomial Logit (mnl) Analysis of the Factors that Influence the Choice of Farm-Level Adaptation Measures by Soybean Farmers in Benue state.

Explanatory Variables	Coefficient(Z) MLT CRP V	Coefficient(Z) LAND FRAG	Coefficient(Z) MLT PLT D	Coefficient(Z) CRP DIV	Coefficient(Z) OFF F EMP	Coefficient(Z) COVER CRP
Age (yrs)	0.019(0.55)	0.005(0.19)	0.532(1.28)	0.136(1.99)**	-0.075(-1.25)	-16.411(-0.01)
Gender	0.623(0.85)	-0.454(0.93)	-0.714(-0.87)	-1.659(-1.56)	-0.316(-0.39)	91.332(0.01)
Edu year	0.009(0.16)	0.079(1.57)	0.038(0.51)	0.027(0.28)	0.063(0.62)	-7.269(-0.00)
Household size	-0.374(0.62)	0.022(0.46)	0.094(1.22)	0.177(1.97)**	0.035(0.37)	6.772(0.00)
Farm distance	-0.176(-1.65)***	-0.112(-1.38)	-0.134(-1.05)	-0.219(-1.68)***	-0.051(-0.41)	-26.182(-0.00)

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C/change awareness	0.002(0.04)	0.037(0.90)	0.021(0.36)	-0.026(-0.27)	0.095(1.14)	19.370(0.02)
Extension contact	-24.961(-0.00)	0.272(0.39)	-0.592(-0.45)	0.962(0.65)	-24.888(-0.00)	91.855(0.01)
Access credit	-0.220(-0.17)	-25.674(-0.00)	1.444(1.04)	-25.976(-0.00)	-25.548(-0.00)	26.205(0.00)
Farm size	-0.395(-2.58)**	0.010(0.09)	-0.148(-0.83)	-0.182(-0.71)	-0.327(-1.43)	-25.616(0.00)
Marital status	-0.613(-0.95)	-0.677(-1.37)	-0.634(-0.69)	-3.597(-2.34)**	0.514(0.56)	83.132(0.01)
M/ship of cooperative	0.419(0.62)	-0.438(-0.68)	-25.522(-0.00)	0.037(0.03)	-25.066(-0.00)	-27.521(-0.00)
constant	-0.815(-0.58)	0.994(-0.99)	-3.223(-2.01)	-4.591(-2.19)**	-0.350(-0.16)	241.854(0.01)
Number of observation			**204**			
			LR Chi^2= 82.39			
			PROB>Chi^2=0.0838			
			PSEUDO R^2=13.85			

Source: field survey, 2016

Note: MLTCRPV= multiple crop varieties, LAND FRAG= land fragmentation, MLTPLTD= multiple planting date, CRPDIV= crop diversification, OFFFEMP = off farm employment. COVERCRP = cover cropping.

* = Significant at 10%,** = significant at 5%, *** = significant at 1%.

Reference base: Fertilizer Application

3.8 Constraints to Climate Change Adaptation by Soybean Farmers in Benue State.

The constraints by the respondents (soybean farmers) limiting soybean farmers on climate change adaptation in Benue state.

Under factor **1** (Lack of access to weather information, public and private institutions and technological constraints) were; lack of access to weather forecast technologies (0.431), lack of or inadequate government policies to empower soybean farmers (0.513), lack of access to supporting institutional facilities (0.671), lack of access to and awareness about NGOs programmes on climate change adaptation(0.588) limited government irresponsiveness to climate change management (0.644), poor information on early warning system (0.468), poor access to climate change adaptation information (0.600) lack of / or inadequate extension programmes directed to meet the climate adaptation measures in soybean production (0.737) and poor agricultural extension delivery (0.554).

In the present information age, information problems could pose serious challenges to farmers' coping strategies as they may not be aware of recent developments regarding climate change adaptations and the necessary readjustments needed. The lack of adaptive capacity due to constraints on resources such as the lack of access to weather forecasts technologies and information creates serious gaps between the farmers and useful information that should help them in their farm work. Weather forecasts are supposed to guide farmers on climate variability so that they can make informed decisions and useful farm plans. However, the absence of this facility will undoubtedly make the farmers become ignorant of the weather and situations and hence become vulnerable to the impact of changes in the climate and weather. This result agrees with the findings of the study of Ozor *et al*. (2010) that identified lack of access to weather forecasts and government irresponsiveness to climate risk management as a major barrier to climate change adaptation among households in Southern Nigeria.

Under factor **2** (land, traditional beliefs, and farm distance constraints) the constraining variables or factors that loaded high were; poor access to and control of land
(0.756), high cost of farmland (0.731), inherited system of land ownership (0.679), traditional belief against adaptation (0.562), far distance of household to soybean farm to their homestead(0.502). Individual farmer in traditional and/ or rural societies and or communities do not usually have title to farmland but enjoy user rights, which could be withdrawn at any time by the custodian of the communal land. Benhin (2006) noted that farm size and land tenure status are some of the major determinants of speed of adoption of adaptation measures to climate change.

The variables or factors that loaded high under factor **3** (high cost of seed, fertilizer and other inputs, small-scale soybean production and knowledge of coping or to build resilience constraints) includes; high cost of improved soybean seed (-0.627), high cost of fertilizer and other

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inputs (0.537), small scale production of soybean farming household (0.496) and inadequate knowledge of how to cope or build resilience (0.767). Ozor *et al*., (2010) noted that high cost of farm input is a major constraint or barrier to climate change adaptation among farming households in southern Nigeria.

Table.10: Varimax Rotated Factors/ Variables Contraining Soybean Farmers on Climate Change Farm-Level Adaptation in Benue state, Nigeria.

		Components		
S/no	**Constraints**	**Factor 1**	**Factor 2**	**Factor 3**
1.	Lack of access to weather forecast technology	0.431		
2.	Lack of or inadequate government policies to empower soybean farmers	0.513		
3.	Lack of access to supporting institutional facilities	0.671		
4.	Lack of access to and awareness about NGOs programmes on climate change adaptation	0.588		
5.	Limited government irresponsiveness to climate change risk management	0.644		
6.	Poor information to early warning system	0.468		
7.	Poor access to climate change adaptation measure information	0.600		
8.	Lack of or inadequate extension programme directed to meet the climate change adaptation measures in soybean production	0.737		
9.	Poor agricultural extension delivery	0.554		
10.	High cost of farm land		0.731	
11.	Poor access to and control of land		0.756	
12.	Inherited system of land ownership		0.679	
13.	Traditional belief/ practice e.g. on the timing of planting		0.562	
14.	Far distance of household to soybean farms to their homesteads		0.502	
15.	High cost of improved soybean seed			-0.627
16.	High cost of fertilizer and other input			-0.537
17.	Some scale production of soybean farming household			0.496
18.	Inadequate knowledge of how to cope or build resilience			0.767
19.	Non availability of farm labour		0.574	0.460

*Factor 1 = Public, institutional and technological constraints, Factor 2 = Land, traditional belief and farm distance constraints, Factor 3 = high cost of inputs, small scale production and knowledge of copping or to build resilience constraints.
**Constraints that loaded under more than one factor.
Note: Factor loading of /0.40/ is used at 10% overlapping variance variables with factors loadings of less than /0.40/ not reported.
Source: computed from field data, 2016.

TEST FOR HYPOTHESES

H1. The significance of the Gamma (γ) parameter at 5% level of significant rejects the null hypothesis that farm-level adaptation measures have no significant effect on the farm output of soybean farmers due to the difference in their technical inefficiency effects were present and makes significant contribution to the farm output of soybean farmers.

H2. Result of the chi-square (χ^2) at 10% level of significant means the null hypothesis that there are no significant factors influencing choice of farm-level adaptation

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measures by soybean farmers in the study area is hereby rejected.

IV. CONCLUSION AND RECOMMENDATIONS

4.1 CONCLUSION

Rural farmers' inability to access information regarding change in climate is known to be a big challenge. With the use of different climate change adaptation strategies, the farmers are still underutilizing their present resources and this make them to be both technically inefficient. Right combination of different farm-level adaptation measures rather than using one of these measures through their wealth of experience and making judicious use of their resources at the present technology level will make them to be more efficient.

4.2 RECOMMENDATIONS

There is need for putting in place policies and programmes that will make the soybean farmers to be proactive in the use of resources and at the same time adapting to climate change. Particularly the following recommendations are proffered:

1. There is a need to make the soybean farmers participate in programmes that address adaptation policies in the country;
2. For soybean farmers to be more efficient technically, government and non-governmental organizations should help them in the provision of input-based farm-level adaptation measures (e.g. multiple crop varieties) so that their production can be enhanced in the face of changing climate;
3. The extension programme aspect of climate change adaptation measures policy in Benue state should focus much more on the bottom-up participatory approach so that the indigenous and the emerging adaptation measures and technologies can be focused in the various soybean producing zones in the state;
4. Government should focus on provision of functional credit facilities to help the soybean farmers in the area of climate change adaptation especially the input based ones and/or government should make the financial environment conducive for private players to act because government cannot do everything; and

Institutional reforms or innovation that can make soybean farmers to relate socially with their fellow farmers especially in the same area or vicinity should be encouraged, since farmer-to-farmer extension paradigm can promote innovation faster than other form of extension methods.

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Analysis of Yield Attributing Characters of Different Genotypes of Wheat in Rupandehi, Nepal

Gobinda Pandey[1]*, Laxmeshwar Yadav[1], Anand Tiwari[1], Hom Bahadur Khatri[1], Samsher Basnet[1], Kamal Bhattarai[1], Binod Gyawali[1], Nabin Rawal[2], Narayan Khatri[2]

*Corresponding author email: gobi8448@gmail.com

[1] Institute of Agriculture and Animal Science, Paklihawa, Rupandehi, Nepal

[2] National Wheat Research Program, Bhairahawa, Rupandehi, Nepal

***Abstract*—** *Field experiment was conducted at National Wheat Research Program, Bhairahawa, Rupandehi with the objective to identify high yielding superior wheat genotypes for Rupandehi district of Nepal during 2014. Experiment was laid out in one factorial Randomized completely block design with ten wheat genotypes including both released and promising; Annapurna 1, Annapurna 3, Pasang Lahmu, Bijaya, BL 3623, Bhirkuti, NL 297, BL 4316, BL 3978 and BL 4347 with three replications. The results showed that the grain yield of BL 3978 was found higher (4.03 t ha^{-1}) than other genotypes followed by BL 4347 (3.93t ha^{-1}). BL 3978 have also higher number of effective tillers m^{-2} and test weight. Among release varieties, NL 297 show higher yield (4 t ha^{-1}) followed by Bhirkuti (3.43 t ha^{-1}) and Bijaya (3.37 t ha^{-1}). From this experiment it can be concluded that BL 3978 was found promising among all genotypes however should be tested at on-farms before promoted for general cultivation in Rupandehi district of Nepal.*

Keywords- Genotypes, Wheat, Yield.

I. INTRODUCTION

Agriculture contributes on an average 33 percent to Gross Domestic Product and employs 65.7 percent of the labor force in Nepal [1]. Wheat is the third most important crop after rice and maize, but in terms of human consumption it ranks second. Wheat is grown in different agro-ecological zones and environments with different production potentials. It is cultivated on 745,823 hectares of land and has the production of 1,736,849 tones with average productivity of 2.32 ton hac^{-1} in Nepal [2]. Cereals crop share about 37 % to agricultural GDP, among this wheat share about 7.14 % [3]. It occupies 24% of total cereal area and contributes 20% of the total cereal production in Nepal [2]. Most of the wheat area (57.8%) and production (65.2%) occurred in terai region which occupy only 23% of the total land area of Nepal [2]. Improved varieties cover about 95.8% of the total wheat area whereas, 66.21% of total wheat crop area is grown under irrigated environment [2].

National Wheat Development Programme was established in 1972 to organize the research and development works on wheat as a commodity crop. Since then, there have been great achievements brought out by the consolidated efforts of wheat researchers, extension workers and farmers. So far there are 35 improved wheat cultivars and 90% of the wheat area is covered by modern wheat cultivars in Nepal [4]. Nepal Agriculture Research Council [5] mentioned that performance in wheat production in Nepal has increased remarkably due to wide spread cultivation of high yielding varieties since 1972. In fact Department of Agriculture had launched a "Grow More Wheat Campaign" in 1965/66 with the introduction of Mexican wheat varieties introduced via India. The new varieties of seed were launched since then and now occupy 96% in 2006/2007 [6]. There are altogether 30 varieties developed for different environment in Nepal [7]. During the last 38 years period from 1970/71 to 2007/08 the production of wheat in the Terai region increased from 81,600 Mt to 1,040,535 Mt [8]. One of the reasons for increase in wheat yield is the use of improved seeds. About 97% of seeds used in Nepal during 2007/ 2008 [9] was improved. With availability of the high yielding varieties as well as improved irrigation facilities in terai, wheat yield has increased more than three times in the terai. The low productivity of wheat in Nepal is mainly due to three reasons; low yielding varieties, low use of production inputs like seeds, fertilizer etc, and lack of irrigation and poor soil fertility management practices [10].

Therefore this study was conducted at National Wheat Research Program Bhairahawa, Rupandehi, Nepal in 2014/15 during winter seasons in order to identify high yielding superior wheat genotypes for Rupandehi district of Nepal.

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II. MATERIALS AND METHODS

Location, Climate and Weather Condition

A field experiment was conducted at NWRP (National Wheat Research Program) farm, Bhairahawa, Rupandehi which is located in the south part of Rupandehi and near the India border (Figure 1). This area is located at latitude 27° 30' 0" N and longitude 83° 27' 0" E. Weather and climate of this area is around 40^0C in summer season and 10^0C in winter season. Average Monthly Rainfall: 545.6 mm.

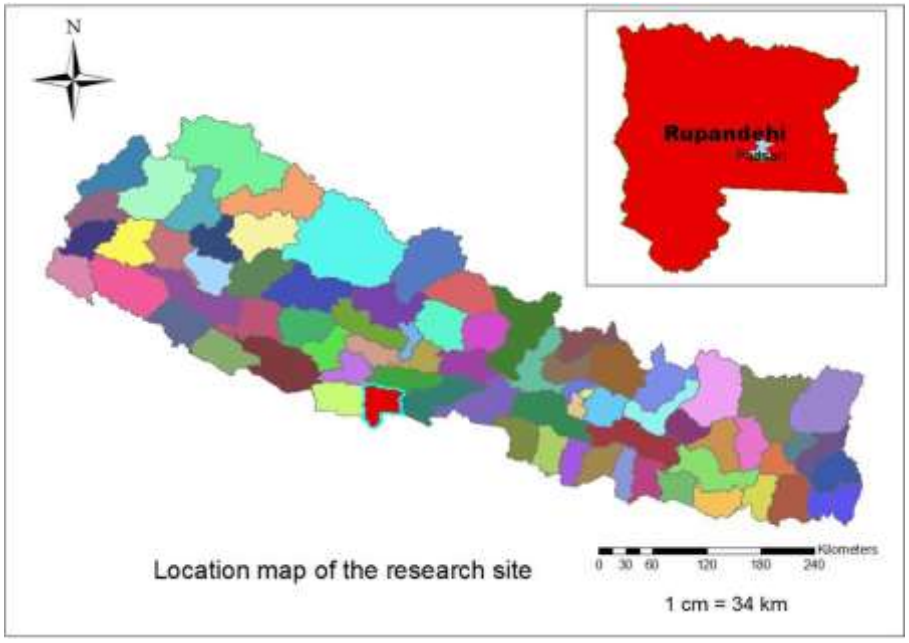

Fig.1: Map of Rupandehi district sowing research site (NWRP, Bhairahawa)

Design of the experimental plot and sowing

The experimental plots were laid out in one factor RCB design consisted of 10 wheat genotypes (Annapurna 1, Annapurna 3, Pasang Lahmu, Bijaya, BL 3623, Bhirkuti, NL 297, BL 4316, BL 3978 and BL 4347) with three replications. Each replication was separated by 2m tally, while the plot was separated by 1m. The size of the individual plot was 3m × 2m i.e. 6 m^2. Spacing between row to row was 25 cm and plant to plant is continuous, there was 12 rows of 2 m long. All wheat genotypes were sown on same date of 15 December of 2013 with seed rate of 120 kg ha^{-1}. Chemical fertilizer was applied @ 100:50:25 kg N:P_2O_5:K_20 kg ha^{-1}.Half dose of nitrogen, full dose of phosphorus (50 kg ha^{-1}) and potash (25 kg ha^{-1}) were applied as basal dose. Remaining half dose of nitrogen fertilizer was applied as top dress in two-split doses i.e. 1/4th at CRI stage after first irrigation and 1/4th at panicle initiation stage. Data collection based on plant height, spike length, effective tillers m-2, number of grains per spike, grain yield t ha-1, biomass yield t ha-1, harvest index and test weight. Statistical analysis was done using Microsoft Office Excel, and MSTAT-C Package program.

III. RESULTS AND DISCUSSION

Biometrical observation

Plant height

Result revealed that plant height was highly significantly influenced by different genotypes. Plant height was observed maximum on Pasang variety (111.1cm). Whereas minimum plant height was recorded on Bhirkuti (66.87 cm). Which was at par with BL 3623 (73.4 cm) (Table 1). The minimum plant height was due to varietal characters, lack of proper irrigation at CRI stage and soil condition. These results were in line with [11] who reported that the plant height was significantly different between genotypes.

Spike length

Spike length was highly significantly influenced by the different genotypes of wheat (Table 1). BL 3978 have more in length (11.47 cm) and it was at par with NL 297 (11.27 cm) and Pasang (11.2 cm). And the shortest spike length was observed in Bijaya (8.06 cm). Which was at par with Annapurna 1 (8.46 cm) (Table 1). These results were in line with [11] who reported that the spike length was significantly different between genotypes.

Effect of genotypes on yield attributing traits of wheat

Effective tillers per square meter

Among yield attributing components, productive tillers are very important because the final yield is mainly a function of the number tillers bearing spike per unit area. The effective tillers m^{-2} was highly influences by different genotypes significantly (Table 1). An average effective tiller m^{-2} was recorded in the experiment was 217.

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Among cultivars, BL 3978 showed higher effective tillers m^{-2} (285) (Table 1), which was followed by BL 4347 (270). Whereas lower effective tillers was given by Pasang (179), which was at par with Annapurna 1 (182) and BL 3623 (189). Significant difference in effective tillers among the cultivars might be due to their genotypic characteristic. These results were in line with [11] who reported that the productive tiller was significantly different between genotypes. Our finding was also confirmed by [12].

Number of total grains per spike

Genotypes highly influenced the number of grains per spike significantly (Table 1). The average number of grains per panicle was found 40. Higher number of grains spike-^{1} was found in Annapurna 1 (52) followed by Bhirkuti (46). Lowest number of grains spike^{-1} was found in BL 4316 (30) followed by BL 4347 (32) (Table 1). These results were in line with [11] who reported that the number of total grains per spike was significantly different between genotypes. Quite identical results were obtained by [13, 14, 15 and 16].

Thousand grains weight (Test weight)

Effect of genotype on thousand grains weight was highly significant (Table 1). Comparatively higher test weight was found in BL 3623 (40.7 g) followed by Bijaya (40.1 g). Annapurna 3 has minimum test weight (29.1 g) followed by Pasang (29.2 g) (Table 1). Higher test weight was found due to varietal characters as well as sufficient moisture during the growing period. [17] Also found that test weight was significantly influenced by the genotypes.

Table.1: Effect of genotypes on grain yield, biomass yield and harvest index of wheat at National Wheat Research Program, Bhairahawa, Rupandehi, 2014

Genotypes	Plant height (cm)	Spike length (cm)	Effective tillers m^{-2}	Total grains spike^{-1}	Test weight (g)
Annapurna 1	74.4^{cd}	8.467^{de}	182^{d}	52^{a}	32de
Annapurna 3	81^{bc}	8.667^{d}	200^{bcd}	41^{bcd}	29.1^{e}
Pasang	111.1^{a}	11.2^{a}	179^{d}	35^{cdef}	29.2^{e}
Bijaya	82.87^{b}	8.067^{e}	222^{b}	42^{bc}	40.1^{a}
BL 3623	73.4d^{e}	8.8^{d}	189^{cd}	44^{b}	40.7^{a}
Bhirkuti	66.87^{e}	8.933^{cd}	222^{b}	46^{ab}	35.1^{cd}
NL 297	74.07^{cd}	11.27^{a}	213^{bc}	34^{def}	36.03^{bc}
BL 4316	75.47^{cd}	10.0^{b}	209^{bc}	30^{f}	39.3^{ab}
BL 3978	85.73^{b}	11.47^{a}	285^{a}	39^{bcde}	37.9^{ab}
BL 4347	78.73^{bcd}	9.333^{c}	270^{a}	32^{ef}	31^{e}
F test	**	**	**	**	**
SEM	2.21	0.1643	8.347	2.293	1.05
LSD (0.05)	6.565	0.4882	24	6	3.129
Grand mean	80.36	9.6	217	40	35.07
CV (%)	4.76	2.95	6.65	10.04	5.2

Means followed by the common letter (s) within each column are not significantly different among each other based on DMRT at 5% level of significance. F: test: ** denotes highly significance at 1 % level

Effect of genotypes on yield and harvest index

Grain yield

Grain yield is determined by the yield attributing traits of the crop. The yield of the particular crop in a location is a combined effect of genetic makeup of the cultivar, growing environment and the crop management practices. Grain yield is a function of yield attributing traits, primarily productive tillers, numbers of grains per spike and thousand grains weight etc.

Grain yield was highly significantly influence by the genotypes (Table 2). Higher grains yield t ha^{-1} was obtain in BL 3978 (4.03 t ha^{-1}) followed by NL 297 (4.0 t ha^{-1}) and BL 4347 (3.93 t ha^{-1}) (Table 2). Lowest yield was observed in Annapurna 3 (2.33 t ha^{-1}) followed by the Annapurna 1 (2.77 t ha^{-1}) and Pasang (2.80 t ha^{-1}) (Table 2). Low yield was found in these varieties due to their genotypic characters because they were recommended variety for hilly region; they show low performance in terai region.

[18] also found that grain yield was significantly influence by the genotypes. He also found that Gautam produced significantly higher yield than Bhrikuti and BL1473 under both the tillage practices. However,

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Bhrikuti also produced significantly higher grain yield than BL1473 under conventional tillage.

Biomass yield

Biomass yield was found to be highly significantly influenced among all genotypes (Table 2). Maximum biomass yield was observed in Bhirkuti and BL 3978 (8.3 t ha^{-1}) followed by BL 4347 (8.13 t ha^{-1}) (Table 2). Low biomass yield was found in BL 3623 (4.83 t ha^{-1}). Low biomass was due to the low straw yield in BL 3623 (1.23 t ha^{-1}) but have higher grain yield (3.6 t ha^{-1}) (Table 2). [18] supported our above results for biomass yield, which was significantly influenced by different genotypes.

Harvest index

Harvest index was found to be highly significantly influenced among all genotypes (Table 2). Maximum Harvest index was observed in BL 3623 (0.74) (Table 2). Whereas low Harvest index was observed in Annapurna 3 (0.33) followed by Annapurna 1 (0.37). Low harvest index was due to these variety was recommended to hilly region; they show low performance in terai region. [12] also found that harvest index was significantly differs in all genotypes.

Table.2: Effect of genotypes on grain yield, biomass yield and harvest index of wheat at National Wheat Research Program, Bhairahawa, Rupandehi, 2014

Genotypes	Grain yield (t ha^{-1})	Biomass yield (t ha^{-1})	Harvest index (HI)
Annapurna 1	2.77^{c}	7.3^{de}	0.37^{i}
Annapurna 3	2.33^{d}	7.06^{def}	0.33^{j}
Pasang	2.80^{c}	6.7^{f}	0.41^{g}
Bijaya	3.43^{b}	7.8^{bc}	0.43^{f}
BL 3623	3.60^{b}	4.83^{g}	0.74^{a}
Bhirkuti	3.37^{b}	8.3^{a}	0.40^{h}
NL 297	4.0^{a}	7.5^{cd}	0.53^{c}
BL 4316	3.50^{b}	6.83^{ef}	0.54^{b}
BL 3978	4.03^{a}	8.3^{a}	0.47^{e}
BL 4347	3.93^{a}	8.13^{ab}	0.48^{d}
F test	**	**	**
SEM	0.089	0.15	0.0057
LSD (0.05)	0.266	0.447	0.0017
Grand mean	3.38	7.27	0.46
CV (%)	4.59	4.58	4.51

Means followed by the common letter (s) within each column are not significantly different among each other based on DMRT at 5% level of significance. F: test: ** denotes highly significance at 1 % level

Correlation regression studies

To assess the relationship between growth parameters, yield attributing traits and grain yield simple correlation coefficients were worked out. The number of effective tillers m^{-2} contribute approximately 44.8 % (R^2 = 0.488) on the grain yield. Whereas the remaining 55 % increase in grain yield may be due to other variables (Figure 2). Similarly, approximately 26 % (R^2 = 0.26) contribution by test weight on the grain yield and the left 74 % increase in the grain yield by the other variables except test weight (Figure 3). Whereas grain yield contributed about 36 % (R^2 = 36) towards increase in the harvest index (Figure 4). And the remaining 64% increase in the harvest index by the other variables rather than harvest index.

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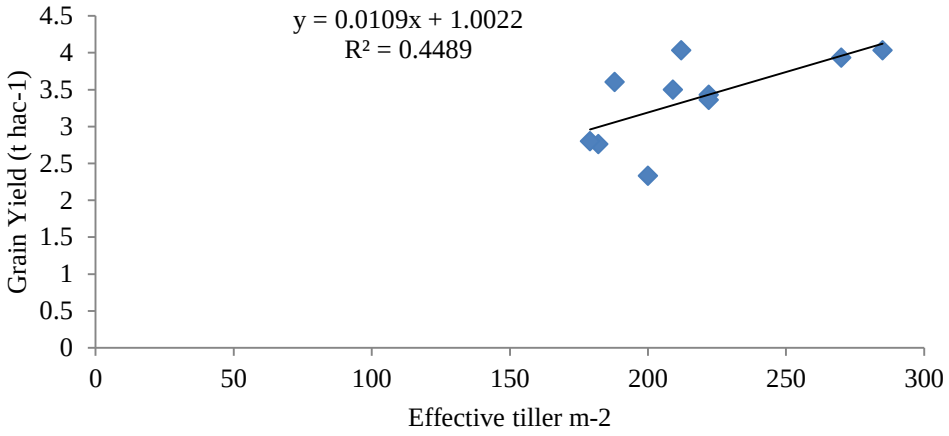

Fig.2: Relationship between grain yield and number of effective tillers per square meter of wheat at NWRP (Bhairahawa), Rupandehi, 2014

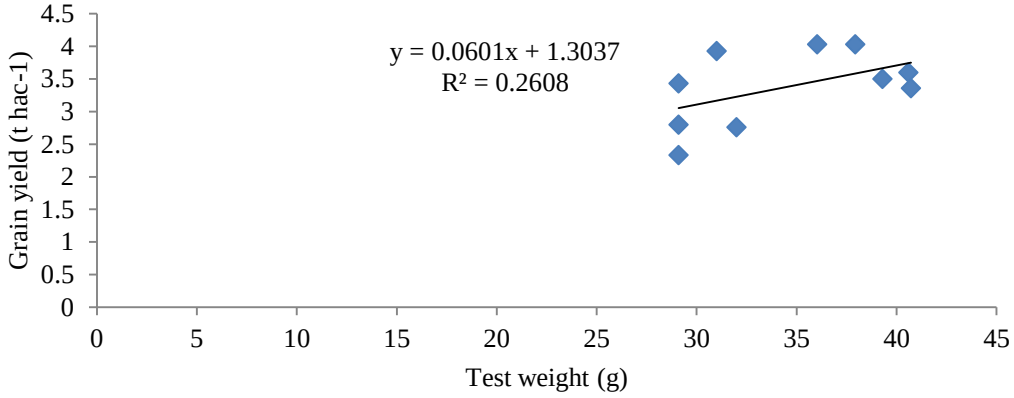

Fig.3: Relationship between grain yield and test weight (g) of wheat at NWRP Bhairahawa, Rupandehi, 2014

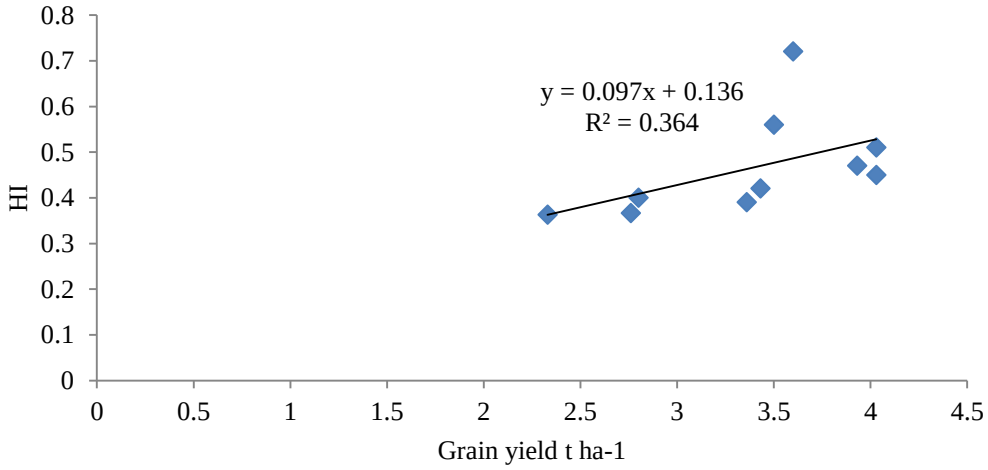

Fig.4: Relationship between grain yield and harvest index of wheat at NWRP Bhairahawa, Rupandehi, 2014

IV. CONCLUSION

The grain yield of BL 3978 was found higher than other genotypes followed by BL 4347. BL 3978 have also higher number of effective tillers m^{-2} also have higher test weight. Higher biomass yield also found in the BL 3978. Among release varieties NL 297 show higher yield followed by Bhirkuti and Bijaya. Bhirkuti show higher number of effective tillers m^{-2}. Grain per spike also found higher in Bhirkuti and Bijaya. From our experiment we concluded that BL 3978 is higher yielder among all

genotypes and NL 297 and Bhirkuti is found to high yielding varieties. However, at least three years of multi-location experiment will be needed to validate this research further.

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Diversity study of Drumstick (*Moringaoleifera* Lam.) using Microsatellite markers

Amao A.O.[1], Echeckwu C.A.[2], Aba D.A.[2], Katung M.D.[2], Odeseye A.O.[3]

[1]Forestry Research Institute of Nigeria, Jericho Hills, Ibadan, Nigeria.
[2]Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria
[3]Nigerian Institute of Science Laboratory Technology (NISLT),Samonda, Ibadan, Nigeria
Corresponding Author's email: funkebee2002@yahoo.com
Phone Number; +234 8180854553

Abstract— *The study of the magnitude of genetic diversity existing within thirty one accessions of Moringaoleifera collections made within and outside Nigeria was conducted using ten Randomised Amplified Polymorphic DNA and tenMicrosattellite markers.None of the RAPD showed amplification bands. Five out of the Microsattellites markersamplified, four primers MO1, MO10, MO15 and MO41 were polymorphic in nature while the marker MO6 produced only a monomorphic band.PIC value was highest for the primer MO41 with 0.75 followed by primer MO1 with 0.68 while, the lowest PIC value was recorded by the primer MO15 with 0.11.A total of 19 alleles were produced by the four primers and the number of alleles ranged from two to nine with an average of 4.75 alleles per primer. The maximum number allele frequency was generated by primer MO15 followed by MO10.The gene diversity varied from 0.12 to 0.78 with an average of 0.52, PIC content of the SSR primers ranged from 0.11 to 0.75 with an average of 0.48 with primers MO 41 followed closely by primer MO1 having maximum numbers of allele number, PIC and gene diversity. Hence, the primer pairs MO41and MO1 can be considered in future molecular studies of Moringaoleifera.The Cluster analysis was able to group the thirty one accessions into two main clusters with four sub clusters. Six of the accessions were found to be duplicated or closely related with one or two other accessions having 0.00 genetic distances between them. The clusters were having some accessions grouped based on same area of collection, however there still existed groupings that were not having link with area of collection.*

Keywords—Moringaoleifera, molecular diversity, SSR Markers, gene diversity, PIC value.

I. INTRODUCTION

Moringaoleifera commonly known as drumstick is the most widely cultivated species of Monogenetic family, Moringaceae (Fuglie, 2013). A total of 13 tropical and subtropical species of the Moringa genus are known out of which some species such as *M. arborea, M. borziana, M. longituba, M. rivae, M. ruspoliana,* and *M. stenopetala* are endangered (Stephenson and Fahey, 2004). *Moringaoleifera* L. is the only cultivated species in the Moringa genus (Sanchez *et al*., 2006). *Moringaoleifera* tree comprises of 4 different edible parts: leaves, pod, stem and root (Morton, 1991)which arewell known for their richness in proteins, minerals, and vitamins, the leaves of *M. oleifera*are used as a highly nutrient vegetable and as cattle fodder (Mughal *et al.*, 1999). In addition, the seed powder is used in water purification and the seed oil is acquired for edibles, lubrication, and cosmetics (Anwar and Bhanger, 2003). Genetic diversity has been described by Brown, (1983) as the amount of genetic variability among individuals of a variety or population of species resulting from many genetic differences between individuals and may manifest in differences in DNA sequence, in biochemical characteristics like protein structure, in physiological properties like abiotic stress resistance or growth rate, or in morphological characters such as flower colour or plant form. Genetic variation in plant is generally accepted to be structured in space and time (Rao and Hodgkin, 2001). Knowledge of population genetic diversity is one of the prerequisites for development of plant species conservation strategies there is a need for a highly reliable and precise method to detect the variation without any environmental effects. Variation among the provenances might be attributed to genetic differences caused by the adaptation of different provenances to diverse environmental conditions (Ginwal*et al*., 2005) and soil types (Elmagboul*et al.,* 2014).

Molecular techniques have been applied to increase the understanding of the distribution and extent of genetic diversity within and between species. Molecular markers detect genetic variation within genotypes of interest at the DNA level.They are not influenced by environments, nor by pleiotrophism, or episttic interactions (Kameswara, 2004). They offer numerous advantages over conventional, phenotype-based alternatives as they are

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stable and detectable in all tissues regardless of growth, differentiation, development, or defence status of the cell they save time and cost (Tanksley*et al*., 1989). Variability studies using molecular tools helps in identifying duplications within the collection, and the genetic linkage among the accessions which can be estimated and which are used in quantifying the genetic variability. Microsatellite or simple sequence repeats (SSR) markers are considered useful to these approaches, due to their effectiveness in genealogy analysis and in the assessment of genetic diversity among organisms (Narvel*et al*., 2000; Kuroda *et al.*, 2009). It is thus important to determine the nature and magnitude of the diversity existing among accessions of drumstick (*M. oleifera)*repository established in Forestry Research Institute of Nigeria to identify accessions that would be superior in terms of important characteristics using molecular primers to detect DNA polymorphism among collected accessions of drumstick and for selecting parents for further breeding program.Another focus is also to be able to improve the adaptability potential *Moringaoleifera* for future genetic diversity studies in Nigeria.

II. MATERIALS AND METHODS

The field study was conducted at the Forestry Research Institute of Nigeria, Jericho Ibadan South west, Nigeria, located on Longitude 0723 1^0N to 07 23 43^0 N and Latitude 03 51 20^0E to 0351 43^0 E, with West African Monsoon climatehaving dry and wet season. The location has a mean annual rainfall of approximately 1548.9mm within a period of 90 days. The mean maximum temperature is 31.9^0C minimum 24.2^0C. Mean daily relative humidity is about 71.9% (FRIN, 2015).The laboratory analysis was carried out at Nigerian Institute of Science Laboratory Technology (NISLT), Samonda, Ibadan, Oyo- State and International Institute of Tropical Agriculture (IITA) Ibadan.The plant materials used for the genetic diversity study were collected from each of the 31 accessions of *Moringaoleifera*six months after transplanting to the field. Leaf sample were carefully collected from a specifically randomly tagged plantin each plot of the 31 accessions. This was done at the tip of freshly growing branch early in the morning and the samples were refrigerated till they were ready for use. Genomic DNA samples were extracted using a RPN-8510 illustra DNA extraction kit Phytopure for plant DNA extraction. (Buckinghamshire, U.K).3g of young leaf tissue was ground with liquidnitrogen and to this powder 15 ml of preheated CTABbuffer (65°C) was added. It was then incubated at 65°Cin a water bath for one hour. After bringing the tubesto room temperature equal volume (15ml) ofchloroform: Isoamyl alcohol (24:1) was added and thecontents were mixed well for 10 minutes to form anemulsion. It was then centrifuged at 10,000 rpm for15 minutes at 15°C. The supernatant was transferredto a fresh tube and the chloroform: isoamyl alcoholstep was again repeated. The aqueous phase wastransferred to a new tube and equal volume of icecold isopropanol was added and incubated in afreezer overnight. The contents were thencentrifuged at 10,000 rpm for 20 minutes at 16°C.The pellet was now saved by discarding the solution. The pellet was washed with 70% ethanol by centrifuging the contents at 10,000 rpm for 10 minutes. The alcohol was discarded and the pellets air dried. The pellets were dissolved in 3 ml of double distilled water thereafter 1 µl of RNase was added and incubated at37 °C for 30 minutes.DNA was precipitated by adding 50 µl of 3M sodium acetate and 7.5 ml of 100% ethanol and the contents were again centrifuged at 10,000 rpm f or 10 minutes.Supernatant was discarded. The pellet was washedwith 70 % ethanol and air dried. It was finally dissolved in TE buffer (150 µl) and stored at - 20°C for long term use.The quantification of the DNA was carried out using a Nanodrop.. Ten Microsatellite primers were randomly selected from the list prepared by Wu and Yang (2010) for *Moringaoleifera*which were used for the genotyping. The PCR was carried out with an initial denaturing at 95 ^{0}C for 5 min, followed by 30 cycles of 94 ^{0}C for 30 s, primer- specific annealing temperature 55 to 61 ^{0}C for 30 s, 72 ^{0}C for 30 s and a final extension at 72 ^{0}C for 8 min and a hold at 4 ^{0}C. For enrichment of the fragments containing SSRs, the PCR products, with a size range of 200 to 1000 bp, were denatured at 95 ^{0}C for 5 min and were then hybridized with 5^{1}biotinylatedprobe (AG) in a 250-mL solution (4.16 · SSC and 0.07% SDS) at 48 ^{0}C for 2 hours. The mixture was incubated at room temperature for 30 min with constant gentle agitation.The amplified products were then electrophoresed in 8% polyacrylamide gels and the amplified fragments were visualized by silver staining as described by Bassam*et al.* (1991). Electrophoretic patterns were scored and checked with a 20-bp DNA ladder marker (Takara, Tokyo) used to estimate allele sizes. The gel pictures were recorded using Gel Documentation System. The SSR electrophoretic profile of each gel was transformed into a binary matrix of visible presence (1) and absence (0). The SSR data were subjected to analysis to determine the major Allele Frequency, Genetic Diversity and Polymorphic Information Content (PIC). Polymorphic Information Content (PIC) is a parameter that provides an estimate of the discriminatory power of molecular marker per primer and this was calculated using Power Maker 3.5 (Liu and Muse, 2005). Genetic distances across the accessions and neighbour joining trees were calculated using Power Maker 3.5.

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III. RESULTS

Primers Characteristics

10 RAPD primers and 10 Microsatellite SSR markers were used for the study, however none of the RAPD primers amplified at the electrophoresis stage. From the 10 Microsatellites SSR markers that were used 5 produced amplified bands which were scored and used in the assessment of the genetic diversity. Four out of the markers MO1, MO10, MO15 and MO41 as shown in Figures (1a, 1c, 1d and 1e) respectively were polymorphic in nature while the marker MO6 Fig 1b produced only a monomorphic band. Polymorphism Information Content (PIC) value was calculated for four polymorphic primers out of the five primers used in the analysis as given in the Table1. PIC value which estimates the quantity of information that can be obtained from a particular primer was highest for the primer MO41 with 0.75 followed by primer MO1 with 0.68 while, the lowest PIC value recorded by the primer MO15 with 0.11.The mean PIC value for 4 polymorphic primers was 0.481. Polymorphic Information Content (PIC) reveals the quantity of information that can be obtained from a particular primer. The polymorphic information content (PIC) ranged from 0.1134 for MO15 to 0.7519 for MO41 with an average of 0.4813.

The gene diversity ranged from 0.1207 for MO15 to 0.7825 for MO41 with average value of 0.5224. The major allele frequency calculated ranged from 0.3226 for MO41 to 0.9355 for MO15 with average of 0.5726. The four SSR markers produced 19 alleles and the number of alleles ranged from 2 to 9 with an average of 4.75 alleles per locus in the 31 accessions. The maximum number of amplified products was generated by primer MO41 with nine alleles followed by MO1 with 5 alleles. Temperature of amplification for the SSR markers ranged from 55^0C for MO 41 to 61^0 C for MO6. These were used in generating amplification profiles for the 31individual accessions of Drumstick.

Cluster Analysis from the SSR markers

The cluster analysis from the Molecular diversity using five Microsatellite markers generated a dendrogram which is presented in Fig 2 below. Molecular analysis using SSR markers was able to group the 31 accessions into two main clusters (1 and 2) separating at 0.04 and 0.13 coefficients. At 0.04 coefficients, cluster 1, there are two distinct accessions FRIN MOR12-27 and FRIN MOR12-31 that are clustered. The remaining 29 accessions are clustered at 0.13 coefficients. At 0.13 coefficients, there are two distinct sub clusters 2a and 2b. At 0.08 coefficients (Cluster 2a) six accessions clustered together and the remaining twenty three accessions were clustered at 0.06 coefficients (Cluster 2b). At 0.57 similarity index cluster 2 had two separate distinct clusters that grouped the remaining twenty nine accessions. At 0.43 the remaining twenty nine accessions were grouped into two sub clusters having 0.08 and 0.06 coefficients. At 0.08 coefficients six accessions were grouped with their genetic difference having FRIN MOR12-2 at 0.35, FRIN MOR12-10 at 0.31. At 0.17 genetic distances FRIN MOR12-15, FRIN MOR12-4, FRIN MOR12-26 and FRIN MOR12-13 are sharing same genetic composition. The other sub cluster (2bi) of 0.06 was sub clustered with the rest accessions at 0.39, at this coefficient, nine accessions are sub clustered. 29% (nine out of thirty-one) of the accessions under study clustered at this distributing them at different genetic distance. The cluster comprised of FRIN MOR12-1, FRIN MOR12-23, FRIN MOR12-18, FRIN MOR12-14, FRIN MOR12-19, FRIN MOR12-5, FRIN MOR12-8, FRIN MOR12-3 and FRIN MOR12-7 respectively. Under sub cluster 2b (ii), 45.2% (fourteen out of thirty one) the rest of the accessions were grouped together at 0.39. Cluster 2bi comprised of: FRIN MOR12-29, FRIN MOR12-24, FRIN MOR12-25, FRIN MOR12-30, FRIN MOR12-21, FRIN MOR12-28, FRIN MOR12-20, FRIN MOR12-9, FRIN MOR12-11, FRIN MOR12-6, FRIN MOR12-12, FRIN MOR12-16, FRIN MOR12-17 and FRIN MOR12-22. They were grouped at various genetic distances having 0.16 and 0.11 coefficient of genetic distance. At 0.16 six accessions: FRIN MOR12-29, FRIN MOR12-24, FRIN MOR12-25, FRIN MOR12-30, FRIN MOR12-21 and FRIN MOR12-28 were clustered together. The remaining eight accessions were clustered at coefficient 0.11 having 0.04 genetic distance level in-between them. Generally, the result from the dendrogram at 0.00 genetic distance showed some duplications among the accessions in FRIN MOR12- 15 and FRIN MOR12- 4; FRIN MOR12-23 and FRIN MOR12-18; FRIN MOR12-8, FRIN MOR12-3 and FRIN MOR12-7; FRIN MOR12-29,FRIN MOR12-24 and FRIN MOR12-25; FRIN MOR12-21 and FRIN MOR12-28 also FRIN MOR12-9, FRIN MOR12-11 and FRIN MOR12-6.

IV. DISCUSSIONS

The Microsatellite SSR markers analysis gave the polymorphic Information Content average value of approximately 0.5 in this study. This agrees with the results of Salvakumari and Ponnuswami (2015) in their genetic study on 34 ecotypes of *Moringaoleifera* using 20 SSR markers. A contrary result was obtained by Ganessan*et al.* (2014) who reported an average PIC value of 0.15. Saini *et al*. (2013) used RAPD, ISSR, Cytochrome P_{450} markers also for *Moringaoleifera* and reported high PIC values of 0.72, 0.81 and 0.68 respectively which are all higher compared to what was obtained in SSR marker. The average gene diversity value

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is 0.52 in this study also indicates a wide variability among the accessions. A contrary result was observed by Ganesan*et al*. (2014) study on *Moringaoleifera* using SSR markers who reported gene diversity range between 0.01 and 0.49 with an average of 0.18. The amplified alleles with an average value of 4.75 alleles per locus in the 31 accessions is also contrary to the report of Ganesan*et al.* (2014) of 35 alleles with an average of 1.84 per locus in diversity assessed in their study with 300 individuals of *Moringaoleifera*using SSR markers. Kuo (2002) reported 75 RAPD markers with an average of 6.98 bands per primer. This is much higher than SSR markers. Muluvi*et al.* (1999) also reported 59 bands per primer with AFLP. It is clearly seen that SSR markers produce the least number of bands. This is because they are locus specific and normally only two allelles are expected from each locus. The molecular analysis in this study show a relatively high polymorphism and gene diversity this indicates sufficient polymorphism exists within the present collection showing high level of variability and can be exploited for genetic linkage maps. With insufficient SSR markers the efficiency of the selected markers is also reflected. Primers MO1 and MO41 should be considered in future study for the genetic resource management in*Moringaoleifera*.

The cluster analysis of 31 M*oringaoleifera* accessions based on UPGMA suggested the formation of two main clusters with four sub clusters formed at different genetic distances. The clustered groups comprised of accessions from different ecological locations being grouped together, there is no clear geographical isolation of the accessions studied. This might be attributed to genetic component, breeding system and phenotypic similarities. The accessions were raised in a common environment and subjected to similar treatments which invariably might have reduced the effects of the environment on their phenotypic expression. The absence of clustering based on geographical location indicates that individuals from different locations are not significantly different genetically. This is similar to previous report on ninety seven accessions using SSR markers in India by Rajalakshmi*et al*. (2017); Ganesan*et al*. (2014) and Rufai*et al*. (2013) using RAPD markers. Clustering of individuals from the same population in different clusters indicates high genetic variation within population which may be attributed to the use of seed sources or breeding system which is in agreement with the fact that it is predominantly an out-crossed plant Mgendi*et al*. (2010). However, the results are contrary to the study of Muluvi*et al.* (1999) where clustering of accession was based on their geographic origin. The dendrogram from the cluster analysis after clearly showing the genetic diversities among the accessions went further to show that 15 of the accessions were duplicated or closely related based on the primers used in this study and the 15 accessions were now grouped into just 6 accessions. This duplication or close relationship could have resulted from low level of genetic distances between the concerned accessions or resulting from low frequency of the allele obtained with the five primers used in this study. The similarities may also be as a result of gene flow between adjacent populations or spread from cuttings and seeds used in planting although Muuluvi*et al*. (1999); Zenglu and Randal (2002) had reported grouping of genotypes based on geographic origin. It is inferred from the cluster analysis that Cluster 1 and cluster 2a are most differed at the molecular level and the accessions in the clusters can be employed in improvement programme of *Moringaoleifera*. The results from this research have shown that enough variability and genetic heritability exist in the studied characters among the evaluated 31 accessions of drumstick. These observations indicate great diversity exists between the accessions and also demonstrate that the selected primers are highly informative and useful for further studies on *Moringaoleifera* genetic diversity study and improvement programmes.

V. CONCLUSIONS

Genetic diversity of *Moringaoleifera*is effectively investigated using SSR or microsatellite markers, which allow a more complete coverage of the existent genetic variation. The genetic diversity of the investigated accessions is relatively high, distributed over two main clusters and four sub clusters, and exhibits a moderate level of association between genetic divergence and geographical origin of accessions. This species shows diversifications and may become a resource for the conservation and the selection of *Moringaoleifera*germplasm.

Table.1: Status of SSR markers used with respect to allele frequency, allele number, gene diversity and polymorphic information content (PIC)

Marker		Allele Frquency	SampleSize	No. of obs.	AlleleNo	Availability	GeneDiversity	PIC
MO1	**F TTGTCTGCCTCCTTTTGTCA R AACTGTCACCCTCCTATCCA**	0.3548	31	31	5.0000	1.0000	0.7305	0.6827
MO6	**F GCATAGCCACCTTTACTCCT**	-	31	31	-	1.0000	-	-

	R GACTTTTGAACTCCACCACC							
MO10	**F CTTTACACCTCAGTATCCCT**	0.6774	31	31	3.0000	1.0000	0.4558	0.3773
	R GTTCGGCTTATGTTCTCGTT							
MO15	**F CCCCTCTATTTCCATTTTCC**	0.9355	31	31	2.0000	1.0000	0.1207	0.1134
	R GCTCCATAAACCCTCTTGCT							
MO41	**R TAGTGGGTCCAAGACAAAGC**	0.3226	31	31	9.0000	1.0000	0.7825	0.7519
	F TGGGATTAGGGCATTAGAAA							
Mean		**0.5726**	**31**	**31**	**4.7500**	**1.0000**	**0.5224**	**0.4813**

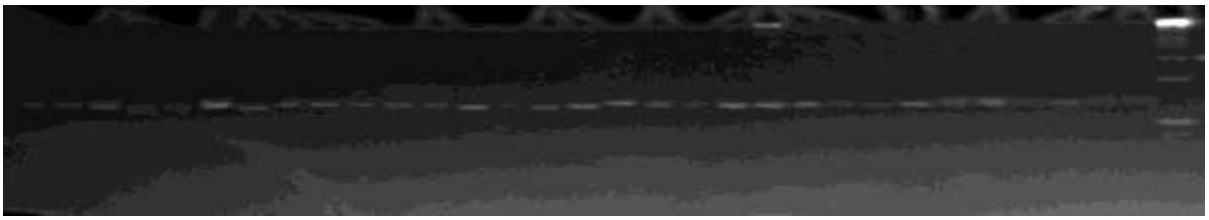

Fig.1a PRIMER 1(MO1) F TTGTCTGCCTCCTTTTGTCA
R AACTGTCACCCTCCTATCCA

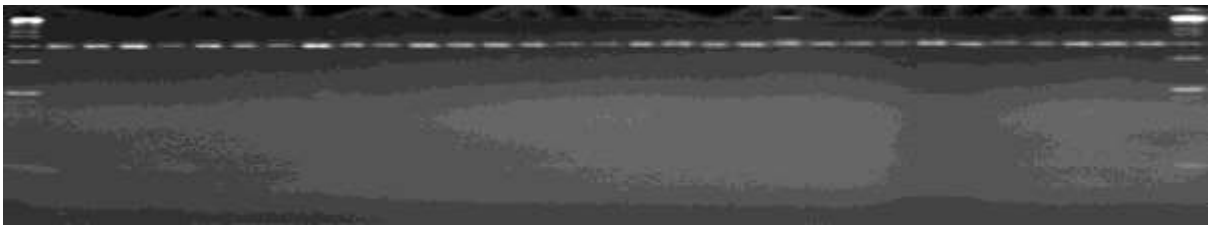

Fig.1b PRIMER2 (MO6) F GCATAGCCACCTTTACTCCT
R GACTTTTGAACTCCACCACC

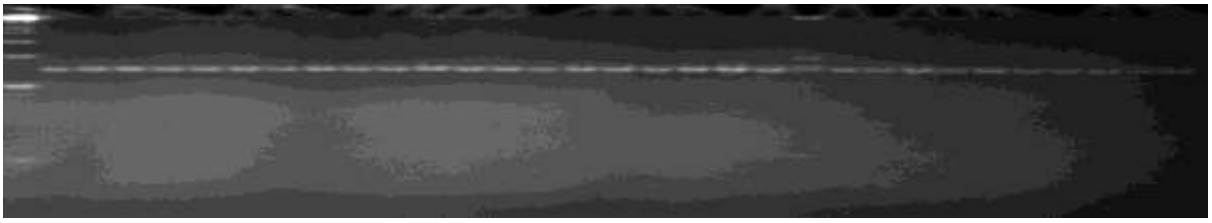

Fig.1c PRIMER 3 (MO10) F CTTTACACCTCAGTATCCCT
R GTTCGGCTTATGTTCTCGTT

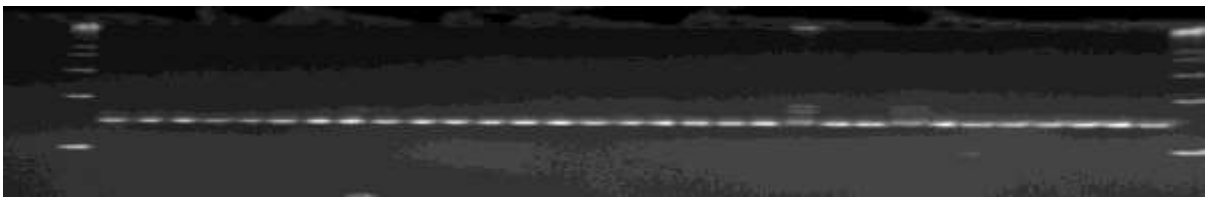

Fig.1d PRIMER4 (MO15) F CCCCTCTATTTCCATTTTCC
R GCTCCATAAACCCTCTTGCT

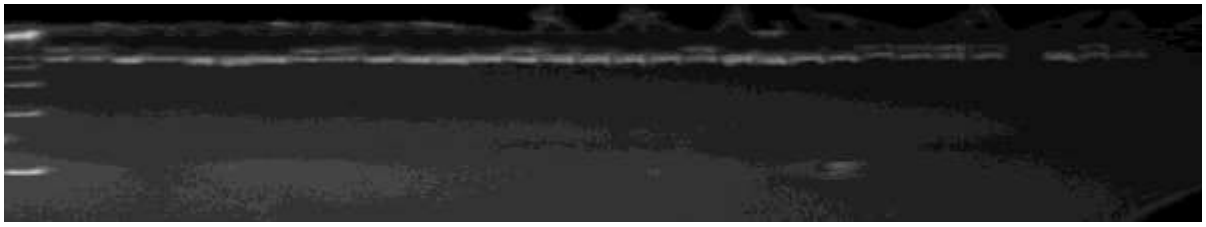

Fig.1e PRIMER5(MO41) R TAGTGGGTCCAAGACAAAGC

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F TGGGATTAGGGCATTAGAAA

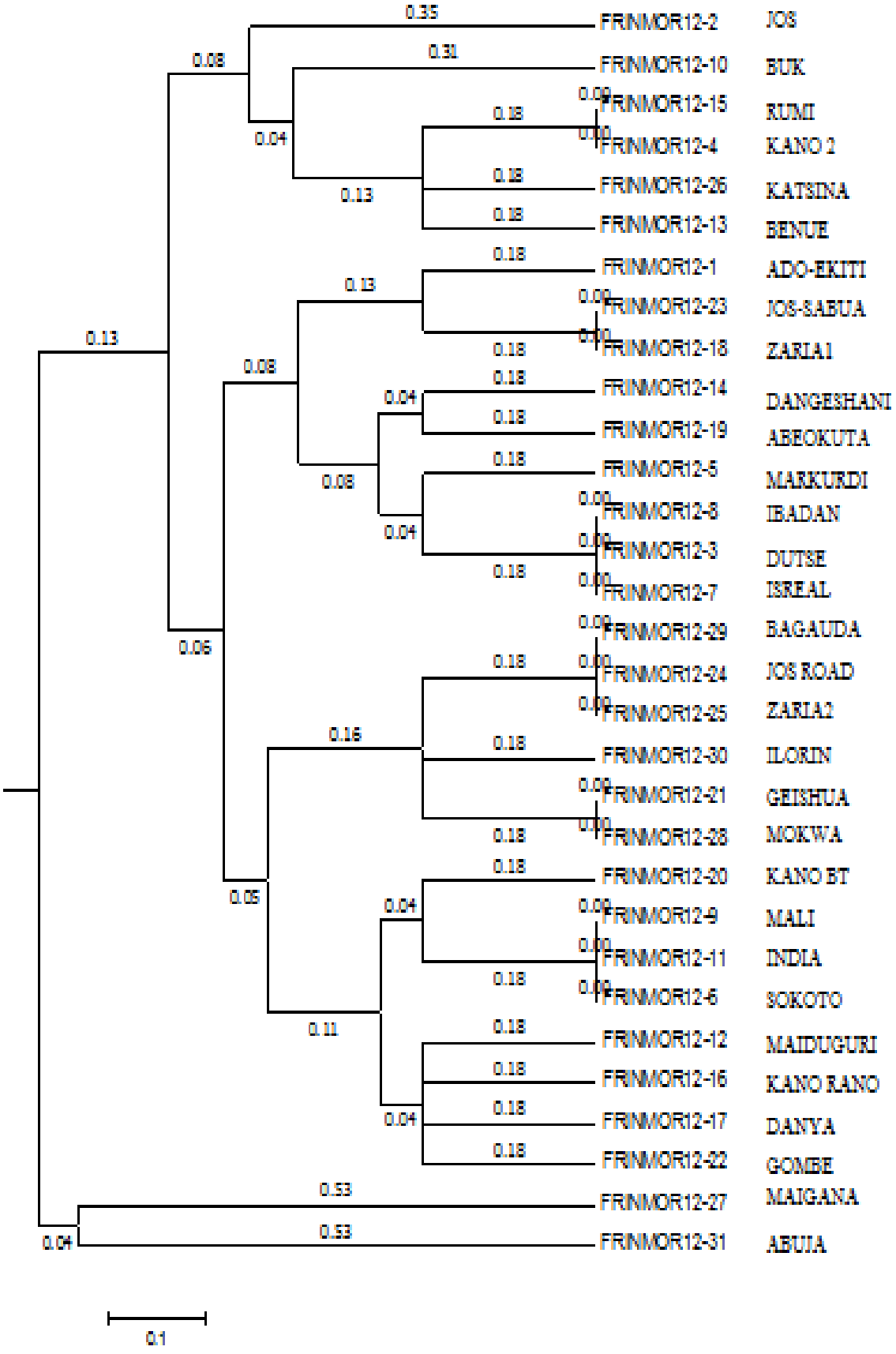

Fig.2: The Dendrogram generated from the cluster analysis

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Effect of pre-sowing Application of Nitrogen, Potassium and Sulfur and its relationship on Egyptian Cotton Productivity.

Amany A.El-Ashmouny[1], Kholoud A.El-Naqma[2], Azza A. El-Hendawy[3]

[1,3]Cotton Research Institute,A.R.C, Giza, Egypt
[2]Soils, Water and Environment, Res. Ins. A.R.C, Giza, Egypt

Abstract*—Fertilizer use in till systems must be aligned with a correct interpretation of soil chemical attributes and cotton demands. The objectives of this work were evaluate the effect of pre-sowing application of urea, potassium and sulfate on the yield of cotton and soil chemical attributes of till cotton (Gossypium barbadense L.) over two harvesting years. The experiment was arranged in complete randomized block design; the doses of an activation macronutrients were the quarter of recommended dose of them. We were applied the recommended doses of N, P,K on soil. The cotton plants in the experimental plots were manually harvested on October 25, 2015 and October 15, 2016. The soil samples were collected between cotton rows in all plots before sowing on March 27, 2015 and April 1, 2016 at depth of (0-30, 30-60 and 60-90 cm) for soil fertility analyses.

-The increasing doses of N,K,S induced a higher values when we addition mixture of urea , potassium and sulfate on plant height, no. of fruiting branches, no. of open bolls before harvest, boll weight, seed cotton yield/ kentar, lint % , micronaire values and pressely values.

-On the other hand, % earliness increased by control and reduced by addition the mixture of nutrients.

-The mixture (N+k+S) treatment had the highest values of available N and S in the first and second respectively compared with the control treatment.

-The addition of potassium Sulphate had gaven a highest values of available K in the first and second seasons respectively compared with the control treatment.

-The Mixture treatment had gaven a highest values of N:K which reflected on a Lint% and seed cotton yield/fed at the first and second season , respectively

-The control treatment had highest values of N: S ration which affected on seed cotton. It gave a lowest values of seed cotton yield/fed. But the treatment of mixture (N + K + S) gave moderate values of N:S ratio while it was gave a highest values of seed cotton yield / fed.

-The highest values of N concentration in fourth leaf (last mature leaf) due to treatment with N (urea) in the first and second seasons. But it had gaven a lowest yield compared with the control treatments.

-The highest values of K concentration led to K (potassium sulfate) treatment compared with the control in first and second seasons, respectively

-The highest values of N: K ratio was obtained with N (urea) treatment. It led to decrease in the seed cotton yield / fed but the highest values of yield / fed was obtained when added all mineral fertilizers as a mixture in soil when N: K was as a compared on control treatments.

- The second treatment which caused a highest in yield after mixture treatment was k (potassium sulphate) , it had gaven (9.01 & 9.64) yield/fed while N:K was (0.34 & 0.32) in the first and second seasons respectively.

***Keywords*—Gossypium barbadense, fertilizer management, Macronutrients, pre-sowing.**

I. INTRODUCTION

Cotton is an important crop worldwide, with a high aggregated value due to its many processed derivates and the high consumer demand. Fertilizer recommendation forcotton is based on soil and leaf analysis. However, it is necessary to interpret such results with respect to the field management history. Soil fertility evaluation aims to quantify the availability of soil nutrients in order to overcome deficiencies and promote the growth and development of plants (**CANTARUTTI et al, 2007**). Nitrogen is the nutrient most necessary to the cotton plant, an excess or deficiency can lead to losses in cotton yield and quality **(Rosolen and Van Mellis, 2010)**.The occurrence of K in stomata activity when the sun energy is used to combine co2and water to form sugars , the initial high- energy product is ATP. The ATP is then used as an

energy source for many other chemical reactions; potassium also plays a major role in the transport of water and nutrients throughout the plant in the xylen. When K supply is reduced trans location of nitrates phosphates, Calcium magnesium and Amino acids is depressed. The role of Sulfur deficiency in cotton has increased due to the decrease in the use of S-bearing fertilizer and reduced atmospheric S deposion.the application of it increased the lint % by 8:9 % and micronaire by 4:5 % when compared to control over growing seasons.

Therefore, this work aims to evaluate the effects of pre-sowing application of Urea, Potassium, Sulfate and their mixture on cotton yields and soil chemical attributes over two years.

II. MATERIALS AND METHODS

An experiment was performed at Sakha Agriculture Research station, Kafr El- Sheikh, Egypt to study the effect of addition some macronutrients before sowing cotton plants (Giza 94 cultivar).

This investigation included one experiment was carried out during 2015 and 2016 seasons as follows:-

1- Control (recommended dose) of P before sowing and splitting N during the growth period.
2- Addition Urea before sowing (46.5 % N) 15kg $N.fed^{-1}$.
3- Addition potassium Sulphate (48% K2O) 6 kg $k_2o.fed^{-1}$
4- Addition Micronic Sulfur (80 % S) 2 $kg.fed^{-1}$
5- Addition the mixture of (N+K+S).

Preceding crop was Egyptian clover in the two seasons.

Chemical analyses for the experimental field were done at Sakha agricultural Research Station. Soil samples air dried crushed, some physical and chemical properties were determined according to **Jackson, (1967)** and **Black et al. (1965)** some soil chemical and physical properties (Table 1).

Table.1:- Chemical properties of the top experimental soil at 2015 and 2016 seasons.

Seasons	Partical size distribert			Texture	pH	ECds/m	Cation meq/L				Anion meq/ l				N ppm	P ppm	K ppm
	Sand	silt	clay				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Co_3	Hco_3	Cl^{-}	$SO4^{—}$			
1st	11	38.3	50.7	**clayey**	8.15	2.6	7.8	6.6	8.1	3.5	-	3	17	6	35.39	7.7	235.1
2nd	11.2	38.1	50.7	**clayey**	8.15	2.7	8	6.6	8.3	3.7	-	3.1	18	5.9	33.14	7.23	220.0

Experiment plot consisted of five rows , 4 m along and 0.7 m width (plot area= 14m2). The seeds were sown on 10 and 12 April in first and second seasons, respectively. The treatment of macronutrients was applied before sowing the plants and after tillage system between rows with nitrogen at a rate of (15 KG/ fed) in the form of Urea (46.5%) for treatment 2 , potassium sulphate at a rate of (6 Kg K2O/ fed) in the form of potassium sulphate for treatment 3 , finally, sulfur at a rate of (2 kg S / fed) in the form of Micronic sulfer for treatment 4 . They all as an activation dose adding before sowing.

All plots were soil fertilized with nitrogen fertilizer at a rate of (60 kg N) in two equal doses, the first dose was applied after thinning , while the second one was applied before the second irrigation but the second treatment (N)only fertilized with nitrogen fertilizer at a rate of (15 kg N/fed) in three equal dose, the first dose was applied before sowing at a rate of (15 KG/ fed) in the form of Urea (46.5%) , while the second dose was applied after thinning at a rate of (22.5 kg N), at the last , the third dose was applied before the second irrigation at a rate of (22.5 kg N) as a complete dose. Phosphorus fertilizer was applied during soil preparation in the form of Calcium Super Phosphate (15.5 P2o5) at a rate of 100 KG/ fed, potassium fertilizer was applied before the beginning of flowering stage in the form potassine F.

Character studied:-

A) Growth characters:-

Samples of five guarded plants were taken at random from experimental plot at 120 days after sowing to estimate the following groth characters:-

1- Plant height (cm)
2- No. of fruiting branches

B) Yield and its components:-

At first pick, random sample of ten guarded plants was

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taken and labeled from each plot to determine the following characters:-

1- No. of open polls / plant.
2- Boll weight (g)
3- Seed index (100-seed weight)
4- Earliness % = seed cotton yield of the first pick / total seed cotton yield x 100
5- Lint %= weight of lint / plant / weight of seed cotton / plant x 100
6- Seed cotton yield / fed (Kentar , i.e 157.5 kg)

c) Fiber characters:-

Samples of lint were collected from each treatment at each replicate to determine the following characters:-

1- Fiber fineness (micronaire): it was determined by HVI.
2- Fiber streingth (pressely index) it was determined by HVI.

D) chimecal analysis :-

Available nitrogen of the soil was extracted by 1N potassium chloride and determined by Kjleldhl method (Jackson, 1967), phosphorus was extracted by 0.5N Sodium bicarbonate and colormitrically measured by spectrophotometer (Jackson, 1967). Plant samples (the fourth leaf as the first mature leaf) oven dried 700C and ground thoroughly, wet digested using sulphoric and perchloric acids mixture, total nitrogen and total phosphorus were determined according to Jackson (1967) N utilization rate was calculated according to the equation

N utili. = N uptake for treatment - N uptake for control / N upplied for treatment.

E) Statical analysis:-

The analysis of varience for complete randomized block design was carried out for each character in each season as out lined by **Snedecor and Cochran(1967)**. The differences between the means of different treatment were tested using (LSD) at 5% level of probability were used to compare between treatments means.

III. RESULTS AND DISCUSSION

It is very important to know that, the nutrients elements play the main role in plant life either vegetative growth, flowering stages as well as yield stages. Moreover, the macronutrients make an important role in increasing the productivity of cotton crop through activation of many structural, catalytically, electronical and vital processing in plant. Therefore, the results will be classified as follows:-

A) Growth characters:-

The effect of activation dose of some macronutrients on some vegetative growth and development parameters were studied at 120 days after sowing.

1- Plant height

Data recorded in table (2) showed that , plant height was significantly increased by the application of all tested macronutrients eigher separately or mixes as compared with untreated plants in 2015 season. Moreover, 2016 seson was not significant.

2- Number of fruiting branches / plant.

With regard to the number of fruiting branches / plant. The results in table (2) show that addition mixture of three macronutrients produced the highest number of fruiting branches / plant compared to other tested macroelements in two growing seasons.

Table.2: Effect of addition some macronutrients as an activation dose on plant height and no. of fruiting branches/ plant during 2015 and 2016 seasons.

No.of fruiting branches	Plant height	Characters Treatments
2015 season		
18.30 c	161.83 b	Control
18.80 bc	162.80 b	N
19.20 ab	163.21 b	K
18.67 c	162.26 b	S
19.47 a	167.36 a	Mix.
2016 season		
18.33 d	181.66	Control
19.66 bc	186.66	N
20.33 ab	173.33	K
19.00 cd	193.33	S
21.33 a	195.00	Mix

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B) Yield and its components:-

It is clear from data recorded in table (3) that, addition of some macronutrients had significant effect on cotton yield and its components.

1- No. of open bolls / plant.

It is clear from the data presented in the same table that, the application of nutrients significantly increased number of open bolls / plant compared with control plants in both seasons. The maximum values were (28.92 and 33.36) obtained from adding mixture of (N, K and S). In this respect, many investigators found that, no. of open bolls / plant was increased by addition the mixture of macronutrient.

2- Boll weight (g)

From the data in the table we found that, application addition of some macronutrients and their mixture tended to increase boll weight/ plant significantly in the first season. Moreover, mixture addition increased boll weight compared with other treatments.

3- Seed index (100-seed weight)

Results in the same table show not significant differences among addition some macronutrients for 100-seed weight in both seasons.

4- Earliness %

The values of earliness % were increased by addition sulfer nutrients and untreated plants. On the other hand, it can be noticed that the application of N and mixture gave the lowest values of earliness % in the first season.morover; the second season was not significant.

5- Lint %

Results in the table clear that, lint percentage was increased by addition K, S and mixture before sowing cotton plants compared to addition of N and untreated plants in the second season.

6- Seed cotton yield / faddan (Kentar)

Seed cotton yield was significantly increased with the tested microelements and their mixture compared to control in both seasons. Moreover, it can be noticed that, addition of mixture produced the highest values, i.e. 26.13 and 14.54 % more than control treatment in the first and second seasons, respectively. The highest increase in seed cotton yield / fed by addition mixture of (N, K and S) might be directly attributed to increase in yield components (no.of open bolls and boll weight).In this concern, **Twolde et al (2005)** found that mixture of some macronutrients increased seed cotton yield over control treatment about 12.5%.

Table.3: Effect of addition some macro elements and their mixture on yield and its components of cotton during 2015 and 2016 seasons.

Seed cotton yield / fed	Lint %	Earliness %	Seed index	Boll weight (g)	No.of open bolls	
2015						
7.73 d	41.00	68.05 a	12.37	3.06 b	24.89 c	Control
8.23 bc	40.30	62.78 b	12.29	3.24 a	25.67 c	N
9.01 ab	40.90	63.57 b	12.17	3.25 a	27.30 b	K
8.00 c	42.12	66.06 a	12.31	3.08 b	24.93 c	S
9.75 a	42.09	62.11b	12.00	3.31 a	28.92 a	Mixture
2016						
8.73 d	41.76 c	69.72	12.53	2.93	28.83 d	Control
9.50 bc	42.56 b	61.14	12.33	3.03	33.63 a	N
9.64 b	43.26 a	66.39	12.30	3.11	31.33 b	K
9.00 cd	43.13 ab	67.77	12.47	2.96	29.59 cd	S
10.0	43.37 a	64.51	12.16	3.12	33.63 a	Mixture

C) Technological characters of fibers.

It is clear from table (4) that the application addition of some macronutrients led to significant increase in all studied technological characters of fibers (fiber length and fiber fineness) as compared with that obtained from the control plants in both seasons.

Table.4: Effect of mixture of macronutrients on technological characters

Fiber fineness	Fiber strength	Fiber fineness	Fiber strength	
2016		2015		
4.2 b	10.2 a	4.1 b	9.8 ab	Control
4.5 a	9.8 b	4.5 a	9.6 b	N
4.5 a	10.1 a	4.5 a	9.9 a	K
4.4 a	9.4 c	4.3 ab	9.3 c	S
4.4 a	10.3 a	4.5 a	10.0 a	Mixture

The highest values of these characters were mostly obtained by potassium and mixture of macronutrients for fiber strength and fineness in the first and second seasons. In this respect, **Twolde et al. (2005)** found that fiber strength and fiber fineness were significantly increased by foliar application with some macro elements.

D) Chemical caracters:-

Table.5: Effect of some N, K, S and their mixture fertilization on Available N, K and S mg.kg^{-1} at the soil after harvest .

	Available N		Available K		Available S	
	1st	2nd	1st	2nd	1st	2nd
control	32.00e	31.7e	206.00e	201.3e	3.23e	2.92e
N	55.00b	51.7b	333.70c	327.70c	9.97d	8.30d
K	45.00d	43.7d	405.70a	402.0a	59.00c	54.67c
S	50.70c	48.7c	322.30d	313.7d	78.33b	73.33b
Mixture	60.30a	58.7a	342.70b	339.0b	83.33 a	78.33 a
LSD	**0.97**	**0.018**	**5.41**	**4.28**	**0.57**	**0.76**

The data obtained from Table (5) show that a significant effect of all treatments on available N , K & S after harvest . The mixture (N+k+ S) treatment had the highest values of available N and S (60.3 & 58.7) and (83.33 & 78.33) in the first and second Respectively compared with the control treatment . On the other hand the addition of potassium Sulphate had gaven a highest values of available K (405.7& 402.0) in the first and second seasons respectively compared with the control treatment. These results may be due to potassium sulphate and sulphur still in the soil because they less mobile in the soil . These results are agree with those obtained by **Tisdale et. al.(1990)** who found that , the K^+ ion is held around negatively charged soil colloids by electrostatic attraction. Cations held in this manner are easily displaced or exchanged when the soil is brought into contact with neutral salt solutions. The amount of potassium exchanged varies with the cation used in the measurement.

Also, They found that the inorganic forms are readily-soluble sulfate, adsorbed sulfate, insoluble sulfate coprecipitated with calcium carbonate, and reduce inorganic sulfur compounds. Scince plants obtained sulfur primarily from soil as dissolved sulfate, easily soluble sulfate plus adsorbed sulfate represent the readily available fraction of soil sulfur which is utilized by plants.

Table.6: The relationship between N:K , N:S ration at the soil after harvest and their association on lint% and seed cotton yield / fed.

	N:K		N:S		Lint%		Seed cotton yield / fed	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	0.155c	0.157	9.92a	10.86 a	41.00	41.76c	7.73 d	8.73 d
N (urea)	0.165b	0.158	5.52b	6.23b	40.30	42.56b	8.23 bc	9.50 bc
K (potassium sulphat)	0.111d	0.109	0.76 c	0.6.23 **c**	40.90	43.26a	9.01 ab	9.64 b
S (gypsum)	0.157c	0.155	0.65 c	0.664 c	42.09	43.13ab	8.00 c	9.00 cd
Mixture(N+k+S)	0.176a	0.173	0.724 c	0.750 c	42.12	43.37a	9.75 a	10.00 a
LSD	**2.01**	**0.005**	**0.57**	**0.76**				

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The data obtained from Table (6) show that a significant effect of all treatments on relations between N:K , N:S , Lint% and seed cotton yield/fed .Data observed that the Mixture treatment had gaven a highest values of N:K which reflected on a Lint% and seed cotton yield/fed . they were (42.12 & 43.37) for lint% and (9.75 & 10.00) for seed cotton yield/fed at the first and second season , respectively . On the other hand the control treatment had a highest values of N:S ration which affected on seed cotton. It gave a lowest values of seed cotton yield/fed. But the treatment of mixture (N + K + S) gave moderate values of N:S ratio while it was gave a highest values of seed cotton yield / fed. .

This may be due to the plants absorbed the elements in equilibrium amount and deficiency in one element limits the growth and yield. These results are agree with those obtained by **Mengel, et al. 1987**. Who show that in the soil , the concentration of N dissolved in the soil solution can change considerably over short period (leaced into deeper soil layers , Nitrification, taken up by plant root so the NO3- content of the soil solution is of major importance in plant nitrogen nutration.

Table.7: Relationship between applied N , K , S , their mixture on the ratio between N and K in the plant and its effect on seed cotton yield .

	N%		K%		N:K		Seed cotton yield / fed	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
control	0.90d	0.783d	1.18e	1.02d	0.76b	0.767b	7.73 d	8.73 d
N (urea)	2.13a	1.900a	1.63d	1.33d	1.30a	1.447a	8.23 bc	9.50 bc
K (potassium sulphat)	1.38bc	1.183b	4.03a	3.70a	0.34c	0.32c	9.01 ab	9.64 b
S (gypsum)	1.26c	0.990c	2.80c	2.467c	0.45bc	0.403c	8.00 c	9.00 cd
Mixture(N+k+S)	1.55b	1.217b	3.53b	3.10b	0.44bc	0.393c	9.75 a	10.00 a
LSD	**0.21**	**0.133**	**0.33**	**0.35**	**0.16**	**0.178**		

Data in Table (7) show that a significantly increased in N: K ration due to N fertilization. Data demonstrated that the highest values of N concentration in fourth leaf (last mature leaf) due to treatment with N (urea) (2.13 & 1.90%) in the first and second seasons . But it had given a lowest yield compared with the control treatments. These results agree with **Gormus et al., 2016**; who found that N deficiency decrease fiber length, lint % yield without any fertilizer with it . On the other hand, the highest values of K concentration led to K (potassium sulfate) treatment compared with the control in first and second seasons, respectively. It was (4.03 and 3.70%) respectively. These results due to potassium presence in the clay minerals and there are equilibrium between the three status (soluble , exchangeable and fixed) while N lost quickly from the soil. A gree with

(Ehsan Akhtar, et al., 2003; Xiaoli Tian et al., 2016) which they show that seed cotton yield increased with K fertilizers **.**

Also data show that a relation between N: K ratio and yield of cotton . The highest values of N: K ratio was obtained with N(urea) treatment which was (1.30 & 1.447) . It led to decrease in the seed cotton (8.23 & 9.5) yield / fed but the highest values of yield (9.75 & 10.00) yield / fed was obtained when added all mineral fertilizers as a mixture in soil when N:K was (0.44 & 0.393) as a compared on control treatments . the second treatment which caused a highest in yield was k (potassium sulphate) , it had gaven (9.01 & 9.64) yield/fed while N:K was (0.34 & 0.32) in the first and second seasons respectively, K is an important major nutrient in cotton production because it affects yield **(Bauer et al., 1998; cassman et al ., 1990; Girma et al., 2007; Mullins et al.,1997) .** In the finally studies show that addition of N , K & S fertilizers led to increase in yield and its components **(Nascimento et al.,2014; Gwathmey et al., 2012; Ashfaq et al., 2015; Nasseem et al., 1981; Makhdum et al., 2001; sawan et al., 2006 and Sawan 2014.**

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Extraction and Quantification of Carpaine from *Carica papaya* Leaves of Vietnam

Do Thi Hoa Vien[1], Tran Van Loc[2]

[1]School of Biotechnology and Food Technology, Hanoi University of Science and Technology, Hanoi, Vietnam
[2]Institute of Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam

Abstract*— *Our previous research indicated that carpaine and its derivative pseudocarpaine extracted from Carica papaya leaves had anti-cancer activity. In this study, we extracted the total alkaloid from Carica papaya leaves, then extracted carpaine and quantitative analyzed carpaine in the total alkaloid. Carica papaya leaves was crushed, and then extracted with EtOH to obtain the total extract. This extract was extracted with suitable solvent to obtain total alkaloid. Continued to extract the total alkaloid by using open column chromatography and crystallizing method to purify carpaine. The research result showed that the total alkaloid in Carica papaya leaves was 0.2% comparing with dried material. Quantitative analyze of purified carpaine by HPLC determined that carpaine was the main alkaloid with the content was 63% of the total alkaloid extracted from Carica papaya leaves.

Keywords*— *Alkaloid, Carica papaya leaves, carpaine, extract, purify, quantitative analyze.

I. INTRODUCTION

Carica papaya (CP) leaves have been used as folk remedies to treat cancer in Australia, Brazil and Vietnam *(H.W. Tietze, 1997)*. It is widely believed that *Carica papaya* L. (papayaceae family) originated from Central America, and then widely planted in tropical and subtropical countries. The major components in papaya plants have been known to consist of papain and chymopapain - two important proteolysis enzymes, carotenoids, alkaloids, monoterpenoids, flavonoids, glucosinolates, minerals, vitamins, etc... The distribution of these components is dependent on the parts of tree *(A.U. Ogan, 1971; A. Canidi, 2007; Do Tat Loi, 1999; Do Huy Bich, 2004)*.

CP leaves have been known as the by-products of the process of harvesting CP fruits. The use of papaya leaves as a folk medicine has been reported in several countries. For instance, aqueous extract of CP leaves have been used as a folk medicine to support in cancer treatment process in Vietnam for a long time ago. Similarly, aboriginal inhabitants of Gold Cost-Queensland in Australia also used papaya leaves (paw paw leaves) as folk remedy to treat lung cancer since 1962 *(H. Clark, 2010)*. A recent study by Otsuki et al. found that the fraction of papaya leaves extract with molecular weight less than 1,000 might inhibit the tumor cell growth on the 10 tested tumor cell lines and mediated Th1-type cytokines in human immune system. Interestingly, it has been found that aqueous papaya leaves extract is relatively safe to normal cells. Therefore, the use of papaya leaves extract in cancer treatment may help to avoid the unexpected effects for patients compared to other common therapeutics *(N. Otsuki et al., 2010)*.

Our previous research indicated that the total alkaloid, carpaine and pseudocarpaine extracted from *Carica papaya* leaves had toxic activity on four tested cancer cell lines: carcinoma cell KB, lung cancer cell LU-1, breast cancer cell MCF7 and leukemia cell HL-60 *(Do Thi Hoa Vien et al., 2013; Ho Thi Ha, 2014)*. Among them, carpaine showed the most powerful toxicity toward four of above cancer cell lines with IC_{50} from 1.13 to 2.94 µg/ml *(Ho Thi Ha, 2014)*.

This study extracted the total alkaloid and carpaine from papaya leaves. Therefore, quantified the carpaine in obtained total alkaloid to apply carpaine as well as total alkaloid from papaya leaves as anti-cancer therapy.

II. MATERIALS AND METHODS

Carica papaya leaves was collected at Dong Anh district – Hanoi, cleaned, dried at 50°C to humidity 7.5 – 9.5%, and then crushed to the size of 1 mm.

Crushed CP leaves was extracted by EtOH to obtain the total extract. Then used the suitable solvent to extract the total alkaloid.

Continued to extract the total alkaloid by using silica gel open column chromatography, solvent system of CH_2Cl_2/MeOH (with MeOH gradient from 0 to 20%), we obtained 5 fractions. Then, extracted third fraction as above (with CH_2Cl_2/MeOH = 95:5), we obtained CP2. Crystallized CP2 with CH_2Cl_2/n-hexane (rate 3:1), we obtain purified compound CP-pur. Evaporated the solvent using Rotavapor Buchi R-114 at 45-50°C.

Used MS and NMR method (^{1}H-NMR, ^{13}C- NMR, COSY, DEPT) to determine the mass and the structure of CP-pur.

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Quantitative analyzed of carpaine using LC/MS method with Alliance series 2695; detector PDA 2996 of Waters Company; column: Sunfire -C18 RP (4.6 x 150 mm), 5µm.

III. RESULTS

3.1. Extract of CP-pur compound from total alkaloid

Carica papaya leaves was crushed, and then extracted with EtOH to obtain the total extract. This extract was extracted with CH_2Cl_2 at acidic and alkaline condition to obtain total alkaloid.

Continued to extract 500mg total alkaloid by using open column chromatography (OCC): absorbent was silica gel (Merck, size: 0.40 – 0.63mm), solvent system was CH_2Cl_2/MeOH (with MeOH gradient from 0 to 20%). Qualitatively analyzed extracted fractions by thin layer chromatography (TLC): silica gel thin layer (Merck, $60GF_{254}$, thick: 0.2mm), using Dragendorff reagent to detect alkaloids. Based on the result of TLC qualitative analyze, we group to 5 main fractions F1, F2, F3, F4 and F5 with the mass was 20mg, 60mg, 260mg, 50mg and 25mg, respectively. Then, the third fraction F3 with the most of mass (260mg) was selected to continue to also extract by silica gel open column chromatography (with CH_2Cl_2/MeOH = 95:5), we obtained 190mg CP2. Crystallized 190mg CP2 with CH_2Cl_2/n-hexane (rate 3:1) to obtain purified compound CP-pur with the mass of 150mg. This process is showed in Fig. 1.

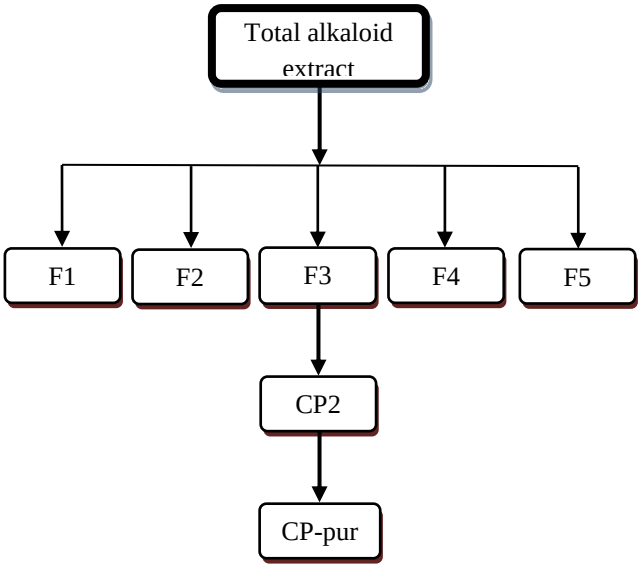

Fig. 1: The extracted and purified process of CP-pur

3.2 Determination of structure of CP-pur compound

ESI-MS spectre of CP-pur: positive ion; *m/z* 479 $[M+H]^+$ (Fig. 2)

^{1}H-NMR spectre of CP-pur (Fig. 3) showed the signals of 2 group of methyl doublet bond with carbon of 3rd grade (–CH_3) at δ_H 1.16 ppm (6H, d, J= 6.5 Hz, H-15, H-15'). The resonant signals of 2 protons were showed at δ_H 4.89 ppm (2H, br, s, H-12 and H-12') in the low magnetic area.

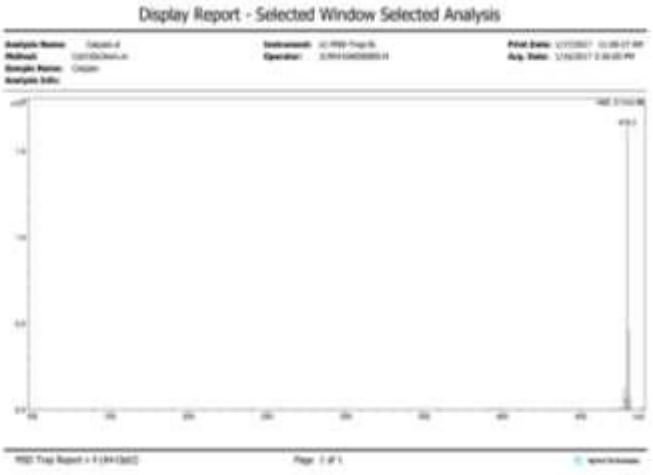

Fig. 2: ESI-MS spectre of CP-pur

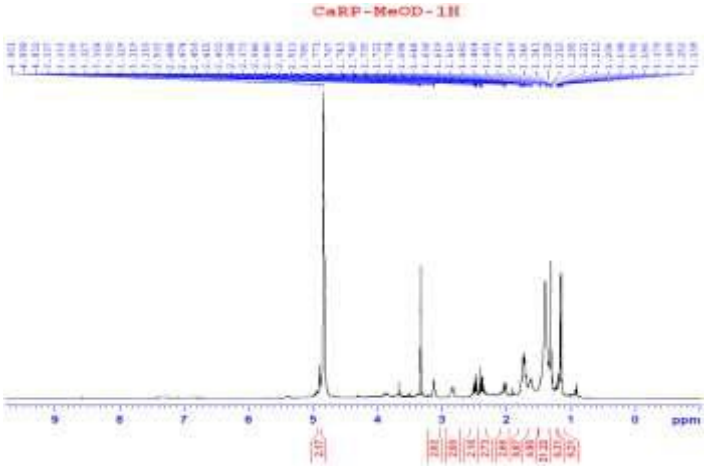

Fig. 3: ^{1}H-NMR spectra of CP-pur

^{13}C-NMR spectre (Fig.4) showed resonant signals of 14 carbons with 1 carbon of 4th grade at δ_c 174.8 ppm of ester group; 3 carbons of 3rd grade at δ_c 55.0, 57.6 and 70.9 ppm; 9 carbons of 2nd grade and 1 group of methyl at δ_c 17.4 ppm.

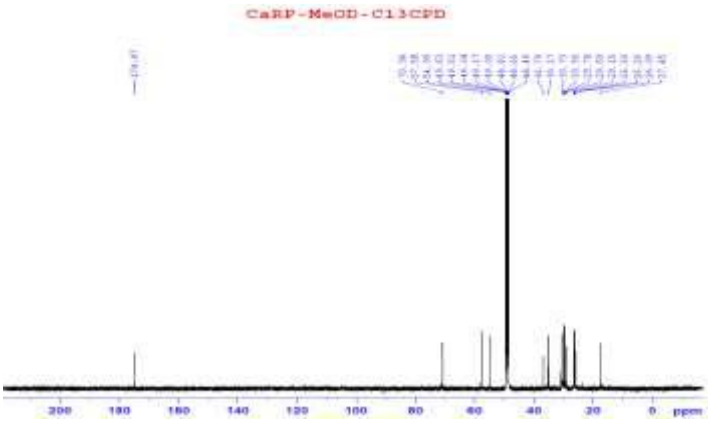

Fig.4: ^{13}C-NMR spectra of CP-pur

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Based on the data of MS and NMR spectra, and compared with reference *(Tasqiah Julianti, 2014; Taro Sato et al., 2003)*, we determined that CP-pur was an alkaloid with symmetric structure and it was carpaine. The spectra data NMR of CP-pur isolated from *Carica papaya* leaves and of published carpaine in reference *(Tasqiah Julianti, 2014)* were described as in Table 1.

The chemical structure of carpaine was described in Fig. 5.

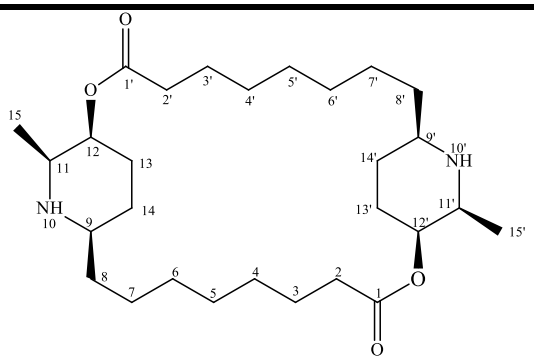

Fig. 5: Chemical structure of carpaine

Table 1: The NMR data of CP-pur and of carpaine

Location and groups		CP - pur (CD_3OD)		Published carpaine ($CDCl_3$)	
		δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C
1	C=O	-	174.8	-	172.8
2, 2’	CH_2	2.36-2.51 (4H, m)	35.2	2.34-2.50 (4H, m)	32.8
3, 3’	CH_2	1.60-1.70 (4H, m)	26.2	1.40-1.72 (4H, m)	23.3
4, 4’	CH_2	1.29-1.43 (4H, m)	29.6	1.16-1.42 (4H, m)	27.7
5, 5’	CH_2	1.29-1.43 (4H, m)	29.8	1.16-1.42 (4H, m)	27.8
6, 6’	CH_2	1.29-1.43 (4H, m)	30.6	1.16-1.42 (4H, m)	28.2
7, 7’	CH_2	1.29-1.43 (4H, m)	26.1	1.16-1.42 (4H, m)	23.1
8, 8’	CH_2	1.60-1,.70 (4H, m)	36.8	1.40-1.72 (4H, m)	24.1
9, 9’	CH	2.81-2.85 (2H, m)	57.6	2.75-2.80 (2H, m)	56.6
10, 10’	NH	-	-	-	
11, 11’	CH	3.10-3.14 (2H, m)	55.0	3.06-3.11 (2H, m)	53.8
12, 12’	CH	4.89 (2H, br, s)	70.9	4.84 (2H, br, s)	68.1
13, 13’	CH_2	2.05-2.10 (2H, m)	29.2	1.92-1.98 (2H, m)	26.6
14, 14’	CH_2	1.29-1.43 (4H, m)	26.5	1.16-1.42 (4H, m)	25.2
15, 15’	CH_3	1.16 (6H, d, J = 6,5 Hz)	17.4	1.09 (6H, d, J = 6,4 Hz)	15.8

Carpaine was isolated from papaya leaves in 1962 *(Tichý M. et al., 1962)*. Carpaine also was separated from the leaf, fruit and root of papaya *(Singh I.D, 1978; Pedro Chávez – Quintal et al., 2011)*. In Vietnam, carpaine also extracted from papaya leaves by Nguyen Tuong Van et al. *(Nguyen Tuong Van et al., 1983)* and by Ho Thi Ha *(Ho Thi Ha, 2014)*. However, not yet have publication about the content of carpaine in papaya leaf and also in other extract from papaya.

3.3. Quantitative analyse of carpaine

3.3.1. Etablish of standard curve

Prepared original solution of carpaine in MeOH with the concentration of 1mg/ml. Diluted the original solution to the solutions with concentration of 0.1, 0.2, 0.5 and 0.7 mg/ml. Then, loaded the aboves solution by LC/MS: mobile phase was acetonitrile and formic acid 0.1%; flowing speed was 1 ml/min; detector PDA at 205 nm. Gradient loading was presented as in Table 2.

Table 2: LC/MS gradient loading

Time (min)	Formic acid 0.1% (%)	Acetonitrile (%)
0 - 2	80	20
2 - 20	0	100
20 - 30	0	100
30 - 35	80	20

The results of HPLC loading were following as in Table 3.

Table 3: The results of LC/MS

Concentration (mg/ml)	Peak intensity	Peak intensity LT	RT (min)
0.1	5943201	9337168	17.8
0.2	16854336	15043641	17.8
0.5	37818224	32163058	17.8
0.7	39504112	43576003	17.8

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Followed the results in Table 3 to establish standard curve as in Fig.6.

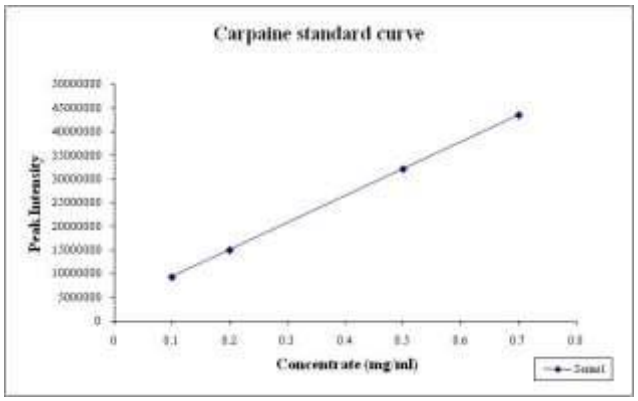

Fig. 6: Carpaine standard curve

3.3.2. Quantitative analyze of carpaine

Carried out the HPLC analyze of total alkaloid extract in the same conditions of standard curve establish. Then, quantitatively analyzed carpaine in the total alkaloid extract basing on standard curve of carpaine, we obtained the result in Table 4.

Table 4: The content of carpaine in the total alkaloid

Total alkaloid extract (mg)	Peak intensity	Carpaine (mg)	Carpaine (% in total alkaloid extract)
1	4E+07	0.63	63

The result on table 3 indicated that the content of carpaine in the total alkaloid extract was 63%.

IV. CONCLUSION

Used two times of silica gel open column chromatography (OCC) with gradient solvent system CH_2Cl_2/MeOH, then crystallized with CH_2Cl_2/n-hexane, we obtain carpaine from total alkaloid. The content of carpaine was 63% comparing with the total alkaloid extracting from *Carica papaya* leaves.

V. ACKNOWLEDGEMENTS

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Callus induction and plant regeneration via leaf segments of three accessions of African rice *(Oryza glaberrima*Stued.)

R. G. Diawuoh[1*], G. Y. P. Klu[2], H. M. Amoatey[2], S.A. Otu[1], K.Asare[1]

[1]Biotechnology and Nuclear Agriculture Research Institute, Ghana Atomic Energy Commission, P. O. Box LG, 80, Legon-Accra, Ghana

[2]Department of Nuclear Agriculture and Radiation Processing, Graduate School of Nuclear and Allied, Sciences, University of Ghana. P. O. Box AE 1, Atomic Energy- Accra, Ghana

Abstract— *A study conducted with the aim of developing a protocol for callus induction and plantlet regeneration in vitro from leaf segments of three accessions of African rice (O. glaberrimaSteud.) indigenous to Ghana. Leaf segments of the accessions namely, Guame, N/4 and SARI 1 were assessed for callus induction and plantlet regeneration ability on different concentrations of plant growth regulators, incorporated into Murashige and Skoog, (1962) (MS) basal medium. Frequency of callus induction which was achieved on MS medium supplemented with (0-10) mg/l 2,4-D differed significantly ($p \leq 0.05$) among the accessions, as well as among the levels of 2,4-dichlorophenoxyacetic acid (2,4-D) tested. Highest callus induction frequency was exhibited at a concentration of 6 mg/l 2,4-D for all three accessions.Sub-culturing of callus on regeneration medium, which consisted of MS supplemented with (1:0-5) mg/l NAA:BAP resulted in no plantlet regeneration in all tested accessions. Instead, prolific root formation was observed.*

Keywords— callus induction, Oryza glaberrima,picloram, plantlet regeneration and 2,4-D.

I. INTRODUCTION

Rice is the most important food crop in the world and feeds over half of the global population (Sasaki, 2005).Increased production of the commodity is necessary to meet the predicted demands of an ever increasing population. One option is to increase the area under rice cultivation, which is getting more difficult as more farmlands are being converted to residential areas in the developing world. The most viable option, therefore, is to increase productivity by advances in biotechnology (Bajaj and Mohanty, 2005).

Biotechnological techniques have been used to improve the existing cultivars, for the synthesis of novel plants and early release of high-yielding plants and plants resistant to various diseases, pests, stresses and temperature. The successful application of technology for crop improvement requires suitable *in vitro* plant regeneration methods. Callus, which is an unorganised, proliferative mass of differentiated plant cells, is one of such means by which crop improvement can be undertaken. The ability to regenerate plants from callus is influenced by physiological factors as well as the genotype of the plant (Henry *et al*., 1994). The regeneration of plants of some cereal crops such as bread wheat (Redway*et al*., 1990; Vasil*et al*., 1990), barley (Luhrs and Lorz, 1987),rice (Yamada *et al.*, 1986) and maize (Duncan *et al*., 1985), from callus have been documented.

In rice, there are reports on successful plant regeneration from explants such as coleoptile (Oinam and Kothari, 1995), root tips (Sticklen, 1991), immature embryos (Koetje*et al.*, 1989), leaf blades (Yan and Zhao, 1982), and other parts of *O. sativa*. However, protocol for callus induction and plant regeneration for *O. glaberrima* accessions have not been achieved. The objective of this study was to induce callus and regenerate plantlets *in vitro*in three different accessions of *O. glaberrima* by determining the hormone types and their concentrations suitable for inducing callus from their leaf segments as well as determining the hormone types and hormone concentrations /combinations for regenerating plants from leaf-derived calli of three accessions of *O. glaberrima.*

II. MATERIALS AND METHODS

2.1 Callus induction from leave segments of *O. glaberrima.*

Seeds of three *Oryza glaberrima* accessions namely N/4, Guame and SARI 1 were manually dehusked and were surface sterilized by immersing in 0.1% mercuric chloride ($HgCl_2$) andvigorously agitated for 2 minutes under the

laminar flow hood and thereafter rinsed with threechanges of sterile distilled water. The sterilized seeds were inoculated in test tubes containing 1.15ml of hormone-free Murashige and Skoog (MS) (1962) basal medium prepared from stock, supplemented with 30 g/l sucrose and 100 mg/l myo-inositol with pH 5.8 adjusted using 1M KOH prior to addition of 3.5 g/l phytagel and autoclaving at 121°C for 15 minutes at 15 psi. The cultures were kept in a growth room at a temperature of 21°C under a 16/8-hr (light/dark) photoperiod with light provided by white fluorescent tubes (T 5 fluorescent fitting, UK) at an intensity of 3000 lux. Seeds were allowed to germinate under these conditions to produce seedlings. Leaves obtained from four-days old *in vitro* germinated *O. glaberrima* seedlings were excised from base and cut into three pieces (referred to as segments 1-3, with 1 being the leaf base segment closest to the seed and 3 being the segment closest to the tip of the leaf) and inoculated in culture jars containing MS medium supplemented with different concentrations of picloram (0-10 mg/l) or 2,4-dichlophenoxyacetic acid (2,4-D) (0-10 mg/l) together with30 g/l sucrose and 100 mg/l myo-inositol. The pH was adjusted to 5.8 and 3.5 g/l phytageladded to the medium before autoclaving at 121°C for 15 minutes at 15 psi. The cultures were subsequently incubated in total darkness at 21°C for 12 weeks.

The experiment was set up as a completely randomized factorial design. The factors tested were three accessions of *O. glaberrima* by six concentrations of 2,4-D by six concentrations of picloram. Data were subjected to analysis of variance (ANOVA) based on 5 replications for percentage callus formed. The means were separated, where appropriate, at the 5% significance level using the least significant difference (LSD). Data were analyzed using Genstat statistical package.

2.2 Plant regeneration from leaf-derived callus of *O. glaberrima*

Calli obtained were sub-cultured on MS medium supplemented with napthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) in a ratio of (1:0-5) mg/l NAA:BAP followed by addition of 30 g/l sucrose and 100 mg/l myo-inositol. The pH was adjusted to 5.8 and 3.5 g/l phytageladded to the medium before autoclaving at 121°C for 15 minutes at 15 psi. The cultures were subsequently kept in a growth room at a temperature of 21°C under a 16/8-hr (light/dark) photoperiod with light provided by white fluorescent tubes (T 5 fluorescent fitting, UK) at an intensity of 3000 lux.

The experiment was set up as a completely randomized factorial design, (3x6). The factors tested were three accessions of *O. glaberrima* by six concentrationsof NAA:BAP.

III. RESULTS

3.1 Effect of concentration of 2,4-D or picloram on callus formation from leaf segments of *O. glaberrima*

Leaf segment explants of the three *O.glaberrima*accessions; N/4, Guame and SARI 1 cultured on MS medium supplemented with varying concentrations (0-10 mg/l) of picloram did not develop callus irrespective of the accession or concentration of picloram present in the medium. However there was development of callus from leaf segment explants cultured on the same MS medium but supplemented with varying concentrations of 2,4-D (0-10) mg/l , occurred after 12 weeks of culture.

Amongst the three portions of leaf segments inoculated, only segment 1 (leaf base) was tissue culture responsive (Fig.1(D)). Segments 2 (middle) and 3 (tip) did not form callus on any of the callus induction media tried. All calli obtained from this experiment were formed from the leaf bases (segment 1).

Colour of callus ranged from cream (Fig.1(E)) to pale yellow (Fig.1(F)) to brown with callus becoming intense in colour as concentration of auxin in medium increased, eventually becoming necrotic at 8mg/l (Fig.1(G)). Higher concentrations of 2,4-D caused necrosis of the initiated calli.

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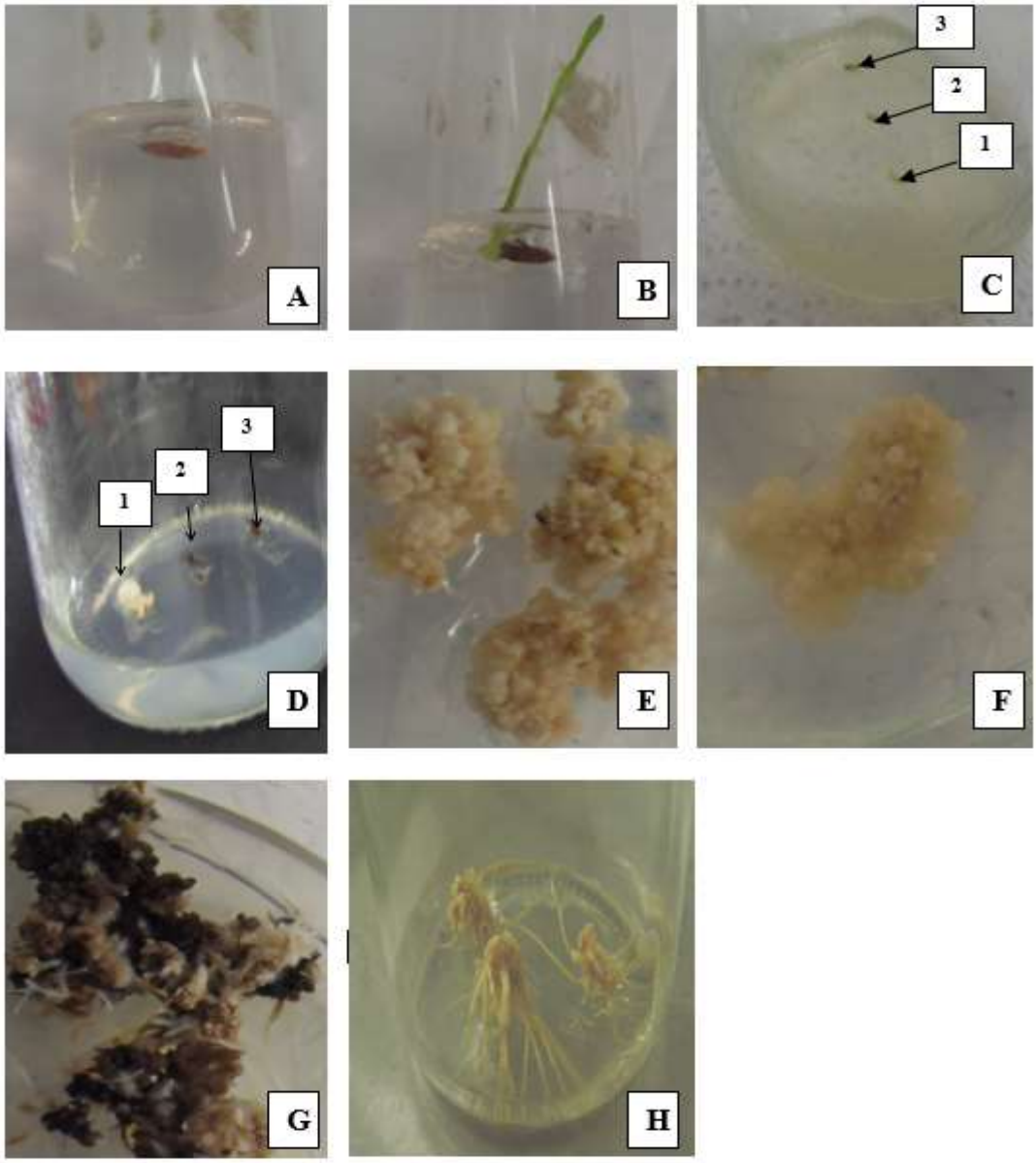

Fig.1 Callus induction from leaf segment explants of O. glaberrima seedling
(A) Dehusked mature seed inoculated on hormone-free medium; (B) 4 days old in vitro germinated O. glaberrima seedling; (C) Leaf segments inoculated on callus induction medium; (D) Callus formed from leaf base (segment 1); [(E-G) Callus formed from the leaf base (segment 1) showing different colours; (E) Cream (F) Pale yellow (G) Necrotic calli]; (H) Calli showing only root formation

Callus formation was observed in all the three rice accessions following addition of4 mg/l, 6 mg/l and 8 mg/l 2,4-D to the culture medium (Fig.2). The highest percentage callus formation was recorded at 6.0 mg/l 2,4-D, recorded in all the three rice accessions, and which proved significantly higher compared to percentage callus formation at other concentrations of 2,4-D used, in each case. Percentage callus formation following an increase in concentration of 2,4-D from 6 mg/l to 8 mg/l was not statistically different ($p \geq 0.05$) from a decrease in 2,4-D concentration from 6 mg/l to 4 mg/l (Fig.2).Callus formation however, depended on the concentration of the auxinin the culture medium. The percentage callus formation increased as the concentration of 2,4-D in the media increased for all the *O. glaberrima* accessions used.

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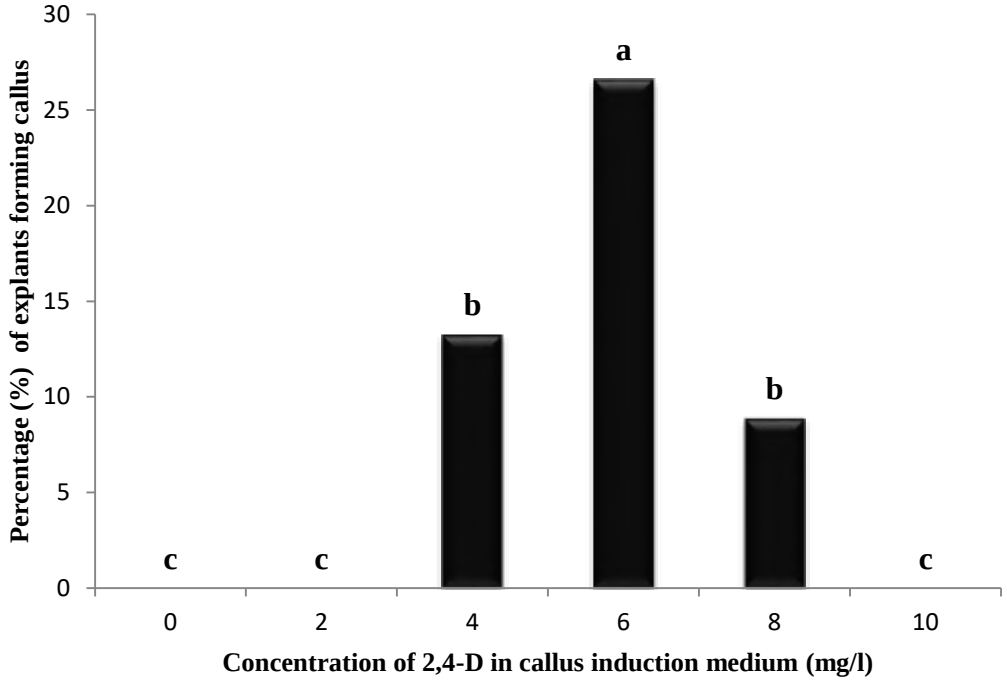

Fig.2: Effect of increasing concentration of 2,4-D in callus induction medium on percentage callus formation(Bars having the same letter are not significantly different (P≥0.05)).

3.2 Effect of accession of *O. glaberrima* on callus formation from leaf segments

Callus induction from *O. glaberrima* used for this study was found to be variable and accession dependent. Among the three accessions, N/4 recorded the highest number of explants forming callus (12.2%) from inoculated leaf segments, which was significantly ($p \leq 0.05$) higher than the other two accessions (Fig.3) Frequencies of callus induction from the leaf segments ofGuame and SARI 1 (6.7% and5.5% respectively) were notstatistically different from each other (Fig.3).The three accessions showed significant ($p \leq 0.05$) differences as regards frequency of callus formed.

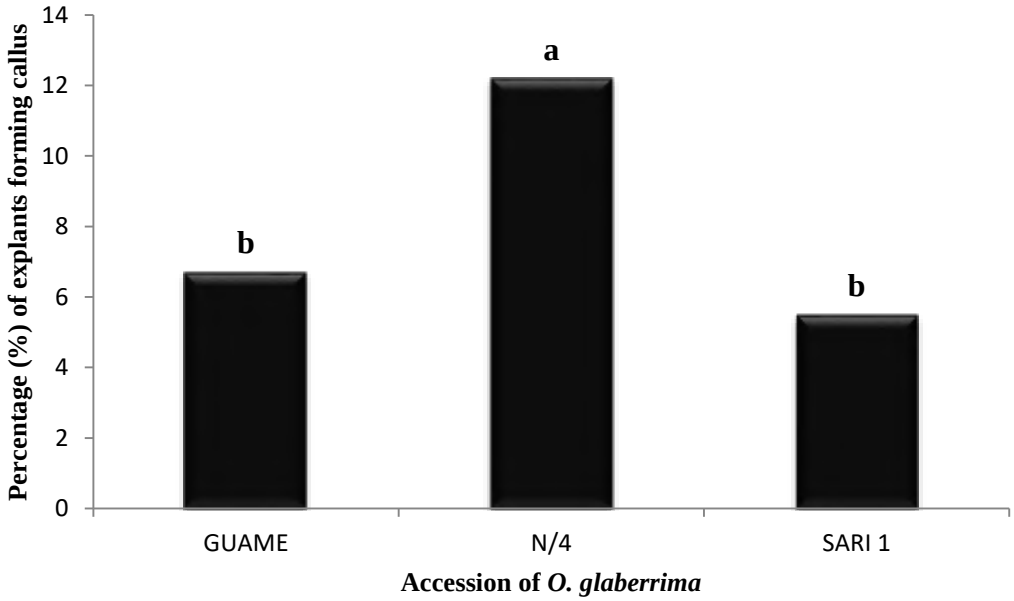

Fig.3: Effect of different accessions of O. glaberrima on percentage callus formation(Bars having the same letter are not significantly different (P≥0.05)).

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3.3 Interaction effect of *O. glaberrima* accession and level of 2,4-D in culture medium on callus formation from leaf segments

Generally, callus formation was low even with 2,4-D in the culture medium. However, some accessions performed better than others. Amongst the three *O. glaberrima* accessions, leaf segment explants from N/4 developed the most calli on a medium with 6.0 mg/l 2, 4-D, (40%), with Guame and SARI 1 both recording 20.0% (Fig.4) at the same concentration of 2,4-D. In all the three rice accessions, percentage callus formation increased with increasing concentration of 2,4-D from 4 mg/l to a peak at 6 mg/l (Fig.4) before dropping.. However, the increase was at different rates depending on the accession. The interaction between accession and concentration of 2,4-D in callus induction medium was however, not statistically significant (P≥0.05).

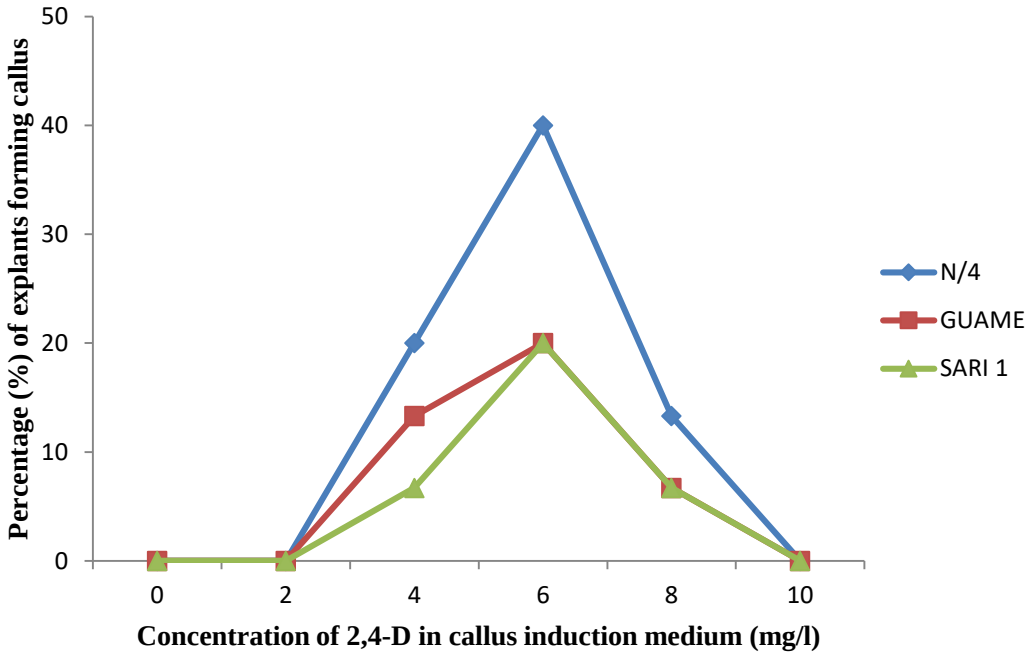

Fig.4: Effect of different accessions of O .glaberrima and increasing concentration of 2,4-D in callus induction medium on percentage callus formation

3.4 Effect of NAA and BAP on shoot and root formation from leaf-derived callus of *O. glaberrima*

Calli induced from leaves of the three *O. glaberrima* accessions were further cultured *in vitro* for assessment of shoot and root regeneration ability on MS medium supplemented with a single concentration of NAA(1 mg/l) and varying concentrations (0-5 mg/l) of BAP. Of the six regeneration media evaluated with different combinations of NAA and BAP, none was able to regenerate plantlets (shoots with roots). Instead media containing low levels (0-2 mg/l) of BAP led to prolific root formation from calli while media containing high levels (3-5 mg/l) of BAP led to necrosis of calli. There were no signs of plantlet regeneration from calli sub- cultured onto any of the regeneration media for any of the three *O. glaberrima* accessions.

IV. DISCUSSION

4.1 Effect of hormone type and hormone concentration on callus induction from leaf segments of *O. glaberrima*

Results from the experiment indicate that 2,4-D but not picloramcan induce callus from leaf base segments of *O. glaberrima*. Also, 2,4-D at concentrations of 4mg/l, 6mg/l and 8mg/l yielded calli for all three accessions of *O. glaberrima*tested with the best result recorded at 6mg/l, suggesting that the hormone 2,4-D plays a crucial role in callus induction as earlier reported by Chen *et al.*, (1991).This observation also concurs with previous studies by Ramesh *et al*., (2009), which showed that 2,4-D at 3.0 mg/l was effective in inducing callus from leaf base segments of *O. sativa*. It is also consistent with findings by Abe and Futsuhara, (1984) who induced embryogenic callus from the roots of *O. sativa* at a concentration of 2.0 mg/l of 2,4-D.

Furthermore, this study revealed that different portions of the leaf responded differently to callus induction with the leaf base segment showing the highest percentage callus induction in all the accessions used for the study. This is consistent with an earlier report by Ramesh *et al*.,(2009) which stated that induction of embryogenic calli from rice leaf is restricted to only the leaf base.

Another interesting observation from this study was the colour of calli obtained. Calli induced from media that had lower levels of 2,4-D were creamish. However, the colour intensified as the concentration of 2,4-D in the induction medium increased. In a related study in chick pea (*Cicerarietinum*L.), by Zaman*et al.,* (2010) calli was induced on MS medium supplemented with varying concentrations of 2,4-D. The colour of these calli were found to be creamish on media containing lower concentrations (0.5 and 1.0) mg/l of 2,4-D, but as concentration increased (1.5 and 2.0) mg/l, creamish brown colour of calli was observed and the highest concentrations (2.5 and 3.0) mg/l gave brownish calli. Zaman*et al.,* (2010) explained that browning of calli occurred if 2, 4-D concentration increases beyond the optimum. Browning of calli was also observed with increased concentration of 2,4-D beyond the optimum by Ramesh *et al.,* (2009), who worked on *indica* rice.

The results of this study also showed that the presence of 2,4-D in culture medium is vital for the induction of callus from leaves of *O. glaberrima*. Absence of 2,4-D resulted in no callus formation among the tested accessions. In most tissue culture experiments, a high auxin/cytokinin ratio is used for initiatingembryogenic callus formation compared to a low ratio for the regeneration of plantlets (Ge*et al.,* 2006). The exact molecular function of plant growth regulators in tissue culture is unclear. However, it may probably be involved in the reprogramming of the expression of embryogenic genes (Ge*et al.,* 2006). In the current study, absence of or very low concentrations of 2,4-D (0-2 mg/l) in the medium did not yield any callus. This observation might be because the concentration of 2,4-D was below level required to trigger cell proliferation. Similarly, at very high concentrations of 2,4-D (10 mg/l) callus failed to form. The inhibition of callus induction may be attributed to phyto-toxic effect of this synthetic auxin on *O. glaberrima.* Significant differences in callus induction were detected among the cultivars when different concentrations of 2,4- D were used.

4.2 Effect of accession of *O. glaberrima* on callus inductionfrom leaf segments

This study proved that the ability to induce callus was greatly influenced by the genotypeof O. glaberrima used. The accession N/4 formed the most calli compared to the other two accessions. This demonstrates that different accessions responded differently to callus formation and is in agreement with earlier reports by Gandonou*et al*. (2005). Significant differences observed in the rates of callus formation of the three accessions of *O. glaberrima* even when subjected to the same nutritional and culture conditions further indicates that callus induction potential is genotype dependent. The differences between the accessions in their response suggest that callus induction may be genetically controlled as observed by some earlier workers (Li *et al.,* 2007; Ozawa *et al.,* 2003; Taguchi-Shiobara*et al.,* 1997).

4.3 Effect of interaction of *O. glaberrima* accession and 2,4-D concentration on callus induction medium on callus formation from leaf segments

The present investigation revealed that all three *O. glaberrima* accessions, concentration of 2,4-D as well as their interaction largely affected callus induction. This observation is in agreement with findings by Pandey*et al.,* (1994), who reported that the success of *in vitro* culture largely depends on the nutritional media, growth regulators, genotype and on the interaction of genotype and medium. Similar reports were also made by earlier workers (Abe and Futsuhara, 1986; Guo and Cao, 1982).

4.4 Effect of NAA and BAP on plantlet regeneration from leaf-derived callus of *O. glaberrima*

Results from the currentinvestigation indicated that the response of the three accessions of *O. glaberrima*to a combination treatment of a single concentration of NAA and different levels of BAP on plantlet regeneration was very poor. The best results obtained from this combination were production of roots at low levels of BAP. This contradicts findings by Ramesh *et al.,* (2009), Ramesh and Gupta, (2005), Boissot*et al.,* (1990) and Abdullah *et al.,* (1986) who reported on the stimulatory effect of BAP in combination with NAA in facilitating plantlet regeneration in rice callus cultures. Differences in response may be due to different concentrations of hormone used.

The rationale behind combining NAA and BAP was to simultaneously regenerate shoots with roots so as to reduce duration of culture of plantlets. NAA is a synthetic auxin that stimulates cell division in the pericylce leading to the formation of lateral and adventitious roots (Taiz and Zeiger, 1998) and BAP is a first-generation synthetic cytokinin that elicits plant growth and development responses, by stimulating cell division (Raven *et al.,* 1999). The

combination of these two hormones obviously did not achieve the intended purpose.

V. CONCLUSIONS

In vitro culture holds potential for improving overall yield of *Oryza glaberrima*Steud., the traditional rice of African origin, with multiple adaptation to several biotic and abiotic stress factors. Based on the results this study, Murashige and Skoog (MS) basal medium supplemented with 30 g/l and 100 mg/l myo-inositol with pH adjusted to 5.8 and incorporated with the plant hormone (auxin) 2,4-diclorophenoxyacetic acid (2,4-D) at an optimal level of 6 mg/l provided a viable medium for induction of callus in three accessions of *O. glaberrima*, namely N/4, Guame and SARI 1, using leaf base segments as explants. Whole plantlet (shoot with roots) regeneration which was not successful in this study may be achieved through sub-culture of induced calli onto same MS-supplemented medium incorporated with varying combinations of the cytokinin benzy-aminopurine (BAP) and the auxinnaphthaleneacetic acid (NAA) to stimulate shoot and root development respectively.

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Age of Transplant and Row Spacing Effects on Growth, Yield and Yield Components of Chilli Pepper (*Capsicum annuum* L.)

M. E. Essilfie[1*], H. K. Dapaah[2], E. Boateng[1], R. J. Damoah[1]

[1*] Department of crop and soil sciences Education, Faculty of Agriculture Education, University of Education, Winneba P.O. Box 40, Mampong-Ashanti. maggifremp@yahoo.co.uk.

[2] University of Energy and Natural Resources. P.O.Box 214, Sunyani. hkdapaah@yahoo.com

Abstract**—** *Two field experiments were conducted for two years (2013 and 2014) at the Multipurpose crop nursery of the University of Education, Winneba, Mampong-campus from May to September to evaluate the effect of three transplanting ages (30, 37 and 44 days) and four row spacing (30 x 30 cm, 40 x 30 cm, 50 x 30 cm and 60 x 30cm) on growth, yield and yield components of pepper. The experimental design used was a 3 x 4 factorial in randomized complete block design with three replicates for both experiments. The results showed that 44 aged transplants had the highest yield during the 2013 cropping season, tallest plant in both cropping seasons, highest number of branches and canopy width for the entire 2014 cropping season. The 30 aged transplants differed significantly from the other aged transplants in number of fruits per plant and widest fruit diameter during the 2013 cropping season. The 40 x 30 cm row spacing had the tallest plant and highest number of branches from 8 to 10 weeks after transplanting in both cropping seasons. The 30 x 30 cm row spacing differed significantly from the 50 x 30 cm and 60 x 30 cm row spacing in fruit yield during the 2013 cropping season. The 60 x 30 cm row spacing had the longest fruit length and the heaviest fruit weight per plant during the 2013 cropping season. It is concluded that for high fruit yield, farmers are to transplant pepper seedlings at 44 days using 30 x 30 cm row spacing.*

Keywords**— *Age of transplant, row spacing, pepper, fruit yield, yield components.***

I. INTRODUCTION

Pepper *(Capsicum annuum*, L.) (Bell Pepper, Sweet Pepper, Cayenne or Round Chilli) is one of the two cultivated hot pepper species in Ghana, and occurs in two major fruit forms recognized locally as 'Kpakposhito' (bell pepper, small round- shaped fruits, hot) and 'Legon 18' (Cayenne red, bell- shaped fruits, hot). Pepper is very popular in all the agro-ecological zones of Ghana even though production is mainly under rain-fed conditions. Ghana ranked the 11th largest producer of pepper in the world and the 2nd largest producer in Africa with an estimated total production of 88,000 metric tons in 2011 which accounted for £96,397 FAOSTAT, (2011). It is estimated that pepper growers in Ghana are producing about 50% of the attainable yields MiDA, (2010). The low production may be attributed to inappropriate use of cultural practices. Cultural factors such as transplant age, geographical location of transplant production (Weston, 1988) and plant spacing (Stofella and Bryan, 1988) influenced pepper yield. Modern vegetable production practices emphasize the need to use optimum plant population attained with appropriate spacing Sayed and Hossein, (2010). Plant spacing can influence morphological development of pepper including reproduction characteristics. Competition for available water and mineral nutrients from the soil and light is greater at high plant population densities. Environmental factors, especially light intensity, stimulate the process of photosynthesis which, in turn, affects biomass production and is closely associated with plant growth rate Alabi *et al*., (2014). In plant densities studies, inter-plant competition is one of the most important stress affecting biomass production, crop yield and economic profitability Naser, *et al*., (2013). Even though pepper is popular in all the agro-ecological zones of Ghana, very little has been achieved in the improvement of the indigenous cultivars probably due to limited information on the use of appropriate cultural practices. The study was conducted to determine the effects of ages of transplanting and different row spacing on growth, yield and yield components of chilli pepper and with the results make a recommendation that will be a component improved practices for pepper cultivation.

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II. MATERIALS AND METHODS

2.1 Description of Study Area

Two field experiments were conducted at the Multipurpose crop nursery of the University of Education, Winneba, Mampong- Ashanti campus for two consecutive years (2013 and 2014) from May to September. The soil type is the savannah ochrosol formed from the Voltaian sandstone of the Afram plains. Texturally, the soil is friable with a thin layer of organic matter and is deep and brown-sandy loam and well-drained. It however, has a good water holding capacity. The soil has been classified by FAO / UNESCO legend as Chronic Luvisol and locally as the Bediesi series with a pH range of 4.0-6.5.

The weather conditions during the experimental periods show that differences in climatic factors (rainfall, temperature and relative humidity) were observed between cropping seasons. In the 2013 cropping season, the total monthly rainfall was 686.1 mm and it occurred from May to September with the peak in May and September (Table 1). The mean monthly temperature of the site for the 2013 cropping season ranged between 21.5 °C to 48.3 °C, with the highest daily of 48.3 °C occurring in July. The mean monthly relative humidity ranged from 63.0 to 97.0 % with the peak occurring in May, July and September. For the 2014 cropping season, the total monthly rainfall was 712.8 mm and it occurred from May to September with the peak in June and July (Table 1). The mean monthly temperature of the site for the 2014 cropping season ranged between 22.0 °C to 34.7 °C, with the highest daily of 34.7 °C occurring in May. The mean monthly relative humidity ranged from 61.0 to 96.0 % with the peak occurring between June and July.

2.2 Experimental Design and Planting

The experimental design used for both study was a 3 x 4 factorial arranged in randomized complete block design (RCBD) with three replicates made up of three transplanting ages (30, 37 and 44 days) and four row spacing (30 x 30cm, 40 x 30cm, 50 x 30cm and 60 x 30 cm) was assign to each block. The total field size of 33.6 m x 13.0 m (436 m^2) was cleared followed by ploughing and harrowing as there were no stumps. Each plot measured 1.2 m x 3.0 m, 1.6 m x 3.0 m, 2.0 x 3.0 m and 2.4 x 3.0 m based on the respective row spacing with 1.0 m left between each treatment plot. Cayenne variety of hot pepper was used. Each plot had 4 rows of 40 plants.

Table.1: Climate data for 2013 and 2014 Experimental periods

Month	Total monthly Rainfall (mm)		Mean monthly Relative humidity (%) (hours GMT)				Mean monthly temperature (^{O}C)			
			06.00	06.00	15.00	15.00	Minimum		Maximum	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
May	207.4	121.4	97	61	63	95	22.7	23.7	31.6	34.7
June	114.9	336.4	96	69	71	96	22.6	22.6	30.0	32.2
July	138.0	131.4	97	67	71	96	21.9	22.8	48.3	31.6
August	6.0	3.9	95	69	71	94	21.5	22.0	27.7	30.6
September	219.8	119.7	97	64	70	95	22.9	22.5	29.6	32.3
Total	686.1	712.8								

(Meteorological Department – Mampong -Ashanti, 2013, 2014)

2.3 Data Collection and Analysis

Number of leaves per plant was counted on three plants from the two central rows, plant height was measured on three plants, canopy width was measured on three plants, while three plants were randomly sampled for the dry matter accumulation. Data were collected at three weeks after transplanting and at two weeks interval. Number of plants harvested, number of fruits per plant, fruit weight per plant, total fruit yield and yield components including fruit diameter and fruit length were estimated from the two central rows. Data analysis was done using analysis of variance and Statistical Analysis System software, version

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9.0 (SAS, 2002). Least significant difference (LSD) was used to separate means at 5% level of probability.

III. RESULTS AND DISCUSSION

3.1 Vegetative Growth

3.1.1 *Plant height*

The plant height responded significantly to variation in ages of transplant during the two year cropping seasons. There was a significant difference between 44 aged transplants from 30 and 37 aged transplants in plant height from 2 to 6 weeks after transplanting (WAT) during the 2013 cropping season (Fig. 1). This contradicts with the findings of (Ibrahim *et al.*, 2013) that transplanting of pepper at younger age was better in performance, especially in height than those transplanted later. Plant height however, was not significantly affected by age of transplant from 8 to 10 WAT at the same cropping season. The 44 aged transplants differed significantly from 30 and 37 aged transplants in plant height for the entire 2014 cropping period. Increased plant height in 44 aged transplants might be that in younger seedlings there was less stored food needed for vegetative extension while the older transplants switched over to reproductive phase earlier and had little time for establishment. The length of seedlings at transplanting increased with increase in seedling age. Increased plant height in older transplants might also be attributed to higher biomass, especially the well developed and established root system which resulted into more uptake of water and nutrients from the soil leading to better cellular elongation. Similar trends have also been reported by Lee Jiwon *et al.*, (2001).

The 30 x 30 cm and 40 x 30 cm row spacing produced the tallest plant height for the entire 2013 cropping season (Fig. 1). The 40 cm x 30 cm row spacing however, produced the tallest plant from 6 to 10 WAT during the 2014 cropping season (Fig. 1). This shows that lower inter row spacing resulted in increased plant height. This might be due to maximum competition for light and air and probably in relation to lower competition for physical production resources (soil moisture and nutrients) which would enhance nutrient availability and efficient utilization of assimilates. This agrees with (Alabi *et al.*, 2014) that plants grow taller at narrower row spacing, and that taller plants were observed as plant population reduced. Peppers and other plants grown in denser population tend to be taller than those grown in less dense planting Stofella and Bryan, (1988).

3.1.2 *Number of leaves per plant*

The 37 aged transplants produced the highest number of leaves per plant for the entire 2013 cropping season (Fig. 2). There was a significant difference between 44 aged transplants from 30 and 37 aged transplants in number of leaves per plant from 2 to 6 WAT during the 2014 cropping season (Fig. 2). This might be due to older aged transplants used. Older transplants with sufficient number of true leaves might be responsible for manufacturing a sizable amount of photosynthates required to establish vigorous plant and complete its life cycle more comfortably YR Shukla, and Rajender, (2011). There was no significant difference between ages of transplant at 8 WAT at the same cropping season.

There was no significant difference between row spacing treatments in number of leaves per plant during the 2013 cropping season although differences exist between treatment means with 40 x 30 cm producing the highest number of leaves per plant from 4 to 8 WAT(Fig. 2). The 60 x 30 cm row spacing produced the least number of leaves per plant from 6 to 8 WAT at the same cropping period (Fig. 2). This is in contrast to (Islam *et al.*, 2011) that wider spacing gave high number of leaves per plant. The 40 x 30 cm row spacing produced the highest number of leaves per plant for the entire 2014 cropping season (Fig. 2). This might be due to the fact that plant density affected leaf formation and development in response to competition for available space for nutrient absorption which would influence plant vegetative growth and development. This agrees with the findings of (Rafiei, 2009; Albayrak *et al.*, 2011; Ciampitti and Vyn, 2011) that for most crops, plant density has a major influence on biomass.

3.1.3 *Number of branches*

The 37 aged transplants produced the highest number of branches from 8 to 10 WAT and the least with the 30 aged transplants during the 2013 cropping season (Fig. 3). This contradicts with the findings of (Ibrahim *et al.*, 2013) that pepper transplanted earlier gave higher number of branches than those transplanted later. There were no branches observed in the three different aged transplants at 2 WAT during the 2014 cropping season (Fig. 3). The 44 aged transplants produced the highest number of branches from 4 to 8 WAT at the same cropping season. The 30 and 44 aged transplants produced the same number of branches at 10 WAT at the same cropping period. The 37 aged transplants had the least number of branches from 8 to 10 WAT during the 2014 cropping season (Fig. 3). The poor performance of 30 and 37 aged transplants during 2013 and 2014 cropping seasons from 8 to 10 WAT respectively might be due to

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differences in plant morphology and its response to high temperature tolerance experienced during the later stage of plant development.

The 30 x 30 cm and 40 x 30 cm row spacing produced the highest and the same number of branches from 4 to 10 WAT during the 2013 cropping season (Fig. 3). There were no branches observed in the different row spacing treatments at 2 WAT during the 2014 cropping season (Fig. 3). The 30 x30 cm and 40 x 30 cm produced the highest and the same number of branches at 4 WAT at the same cropping period. The 30 x 30 cm row spacing had the highest number of branches at 6 WAT during 2014 cropping season. The 40 x 30 cm row spacing produced the highest number of branches from 8 to 10 WAT during the 2014 cropping season followed by the 30 x 30 cm row spacing (Fig. 3). This is an indication that when inter row spacing increase, the number of branches per plants per unit area becomes less. This contradicts with the findings of (Sarfo-Kantanka and Lawson, 1980) that plants develop fewer branches at narrower row spacing.

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3.1.4 *Canopy Width*

The 44 aged transplants produced the widest canopy width from 2 to 4 WAT during the 2013 cropping season (Fig. 4). The 37 and 44 aged transplants produced the widest and the same canopy width from 6 to 10 WAT at the same cropping period. This might be due to older aged transplants used and differences in plant morphology.

The 30 aged transplants had the least canopy width from 4 to 10 WAT during the 2013 cropping season (Fig. 4). The 44 aged transplants produced the widest canopy width for the entire 2014 cropping season (Fig. 4). The 30 aged transplants produced the least canopy width from 2 to 4 WAT and the 37 aged transplants from 6 to 10 WAT at the same cropping period (Fig. 4).

The 40 x 30 cm row spacing had the widest canopy width at 4 WAT and from 8 and 10 WAT during the 2013 cropping season and for the entire 2014 cropping period (Fig. 4). This might be due to differences in plant morphology and plant spacing. Plants with increased canopy width tend to have higher photosynthetic potential (NAR). The 50 x 30 cm row spacing had the lowest canopy width for the entire 2013 and 2014 cropping periods (Fig. 4). This might be due to differences in plant spacing and its effect on plant structure. Plant density can affect canopy architecture, light conversion efficiency and duration of vegetative growth. Therefore, optimising plant density, which could be defined by both the number of plants per unit area and the arrangement of plants on the ground, is a pre-requisite for obtaining higher biomass hence canopy width.

3.1.5 *Dry matter Accumulation*

The 44 aged transplants produced the highest dry matter accumulation for the entire 2013 cropping period followed by 37 aged transplants (Fig. 5). The increased dry matter accumulation in 44 aged transplants might be that at this stage, the stem had developed more branches, become woody and so steadily accounted for increasing part of the total dry matter produced. The 30 aged transplants had the least dry matter accumulation for the entire 2013 cropping period (Fig. 5). The 37 aged transplants produced the highest dry matter accumulation from 2 to 4 WAT and at 8 WAT for the 2014 cropping season (Fig. 5). The 44 aged transplants had the least dry matter accumulation from 2 to 4 WAT followed by the 30 aged transplants from 6 to 8 WAT at the same cropping period. This might be due to differences in transplanting ages of seedlings, plant morphology and climatic conditions. The plant dry matter accumulation increased linearly in the later stages of plant development. This is an indication that the large plant size starts to compete mainly for water, light and nutrients thereby resulted in higher dry matter accumulation in the older aged transplants.

Row spacing had no significant effect on dry matter accumulation at 2 WAT in both cropping seasons (Fig. 5). The pepper plants increased linearly in dry matter accumulation for the entire cropping period in both seasons. The 40 x30 cm produced the highest dry matter accumulation from 6 to 8 WAT during the 2013 cropping season. Higher dry matter yield might be due to differences in plant structure and row spacing. The 60 x 30 cm row spacing had the least dry matter accumulation for the entire 2013 cropping season (Fig. 5). However, the 60 x 30 cm row spacing had the highest dry matter accumulation from 4 to 8 WAT during the 2014 cropping season (Fig. 5). This might be due to the root and leaf dry matter yields on account of more branches and leaves produced and increase in stem dry weight. Plant density can affect dry matter production and ultimately, the economic productivity of a crop.

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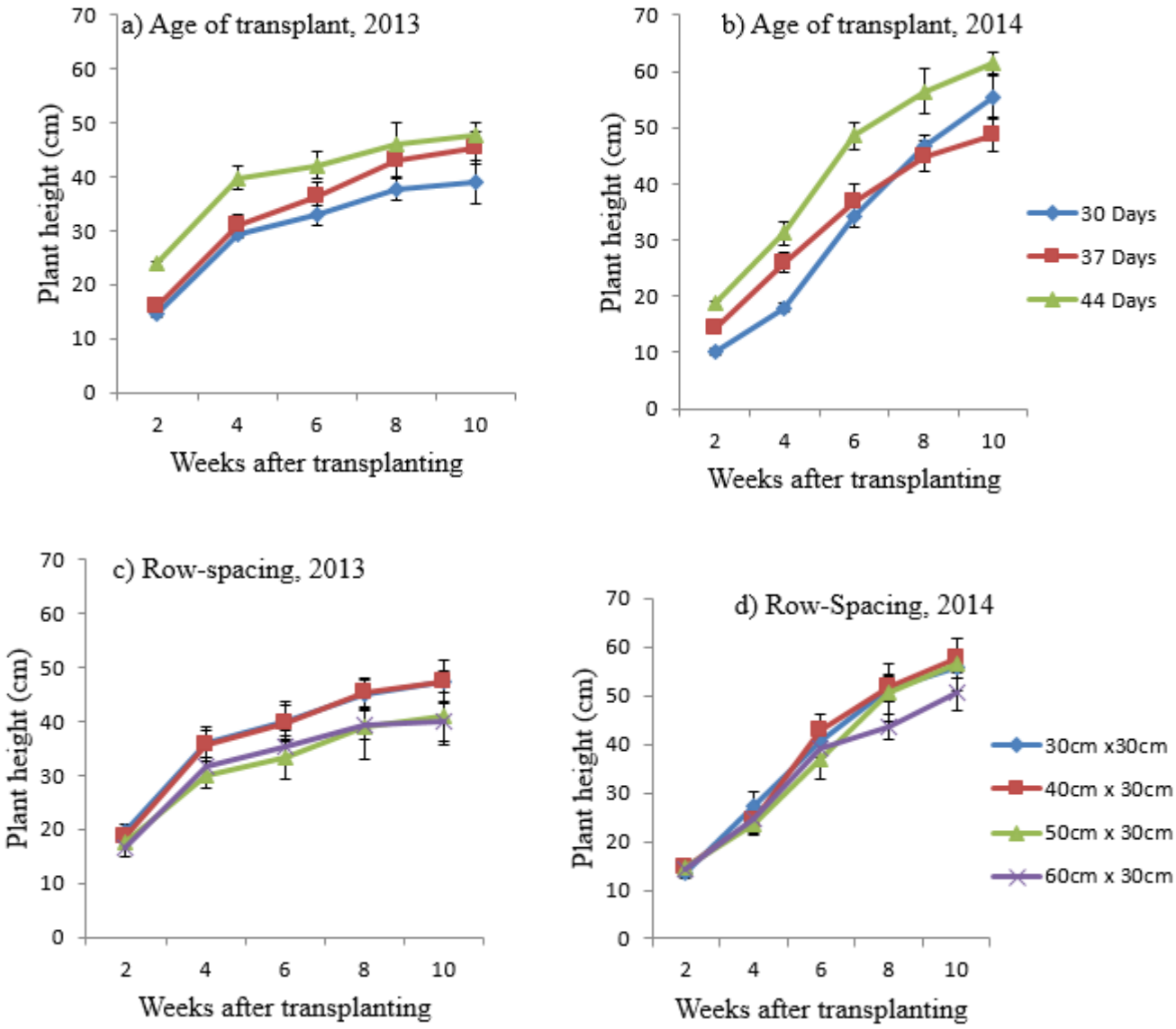

Fig.1: Effects of age of transplant and row-spacing on plant height of chilli pepper, 2013 and 2014

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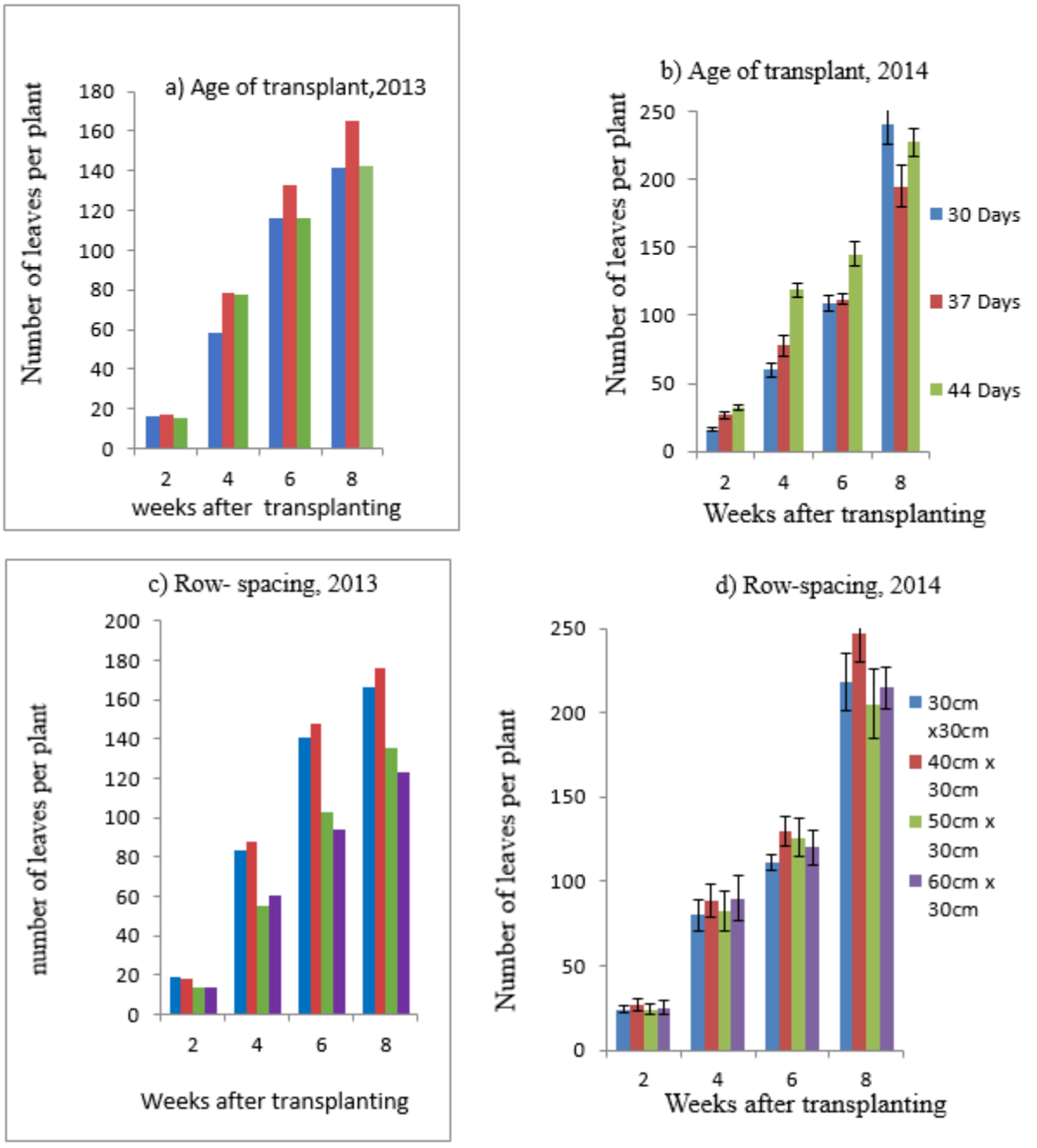

Fig.2: Effects of age of transplant and row-spacing on number leaves per plant of chilli pepper, 2013 and 2014

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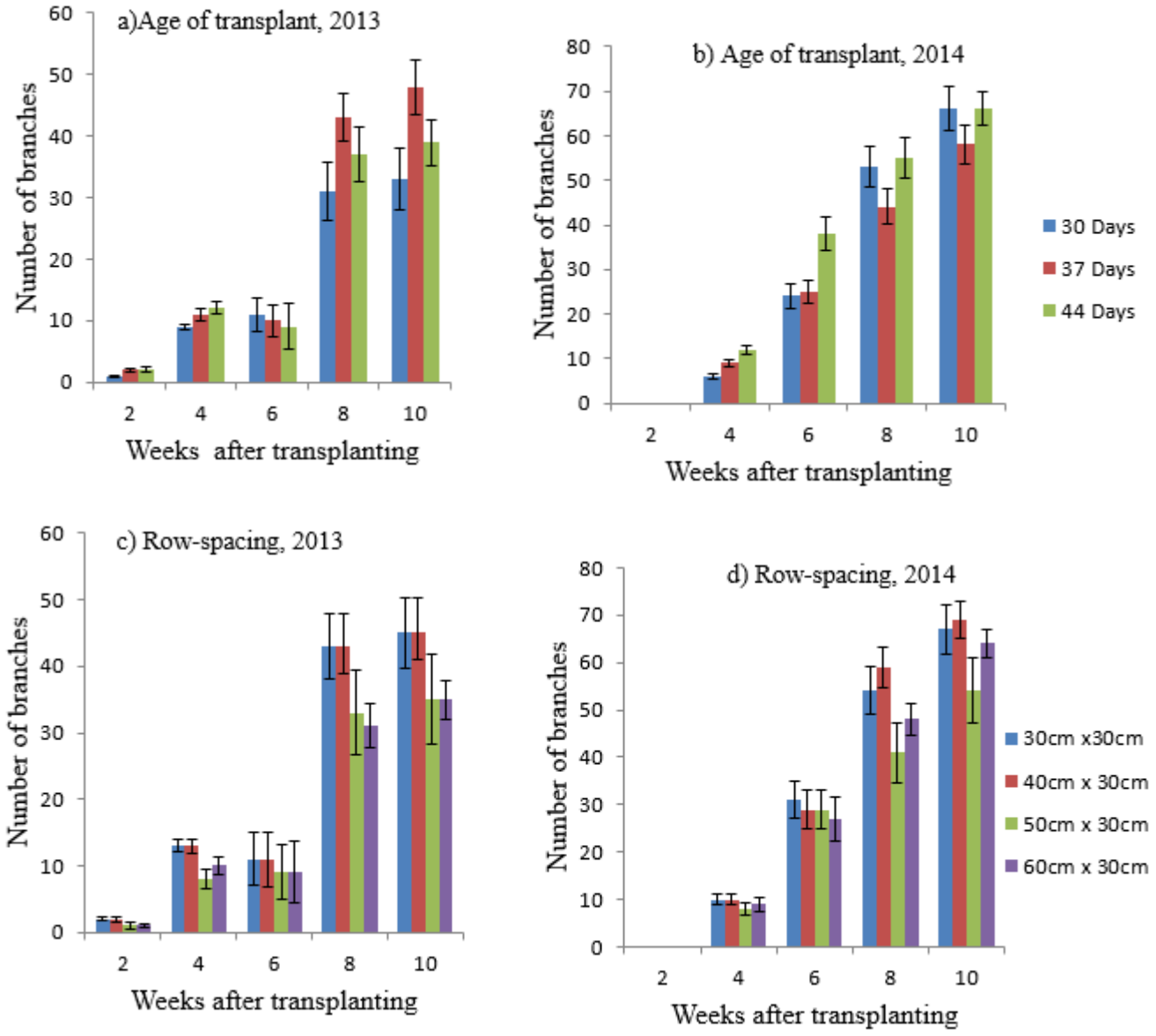

Fig.3: Effects of age of transplant and row-spacing on number of branches of chilli pepper, 2013 and 2014

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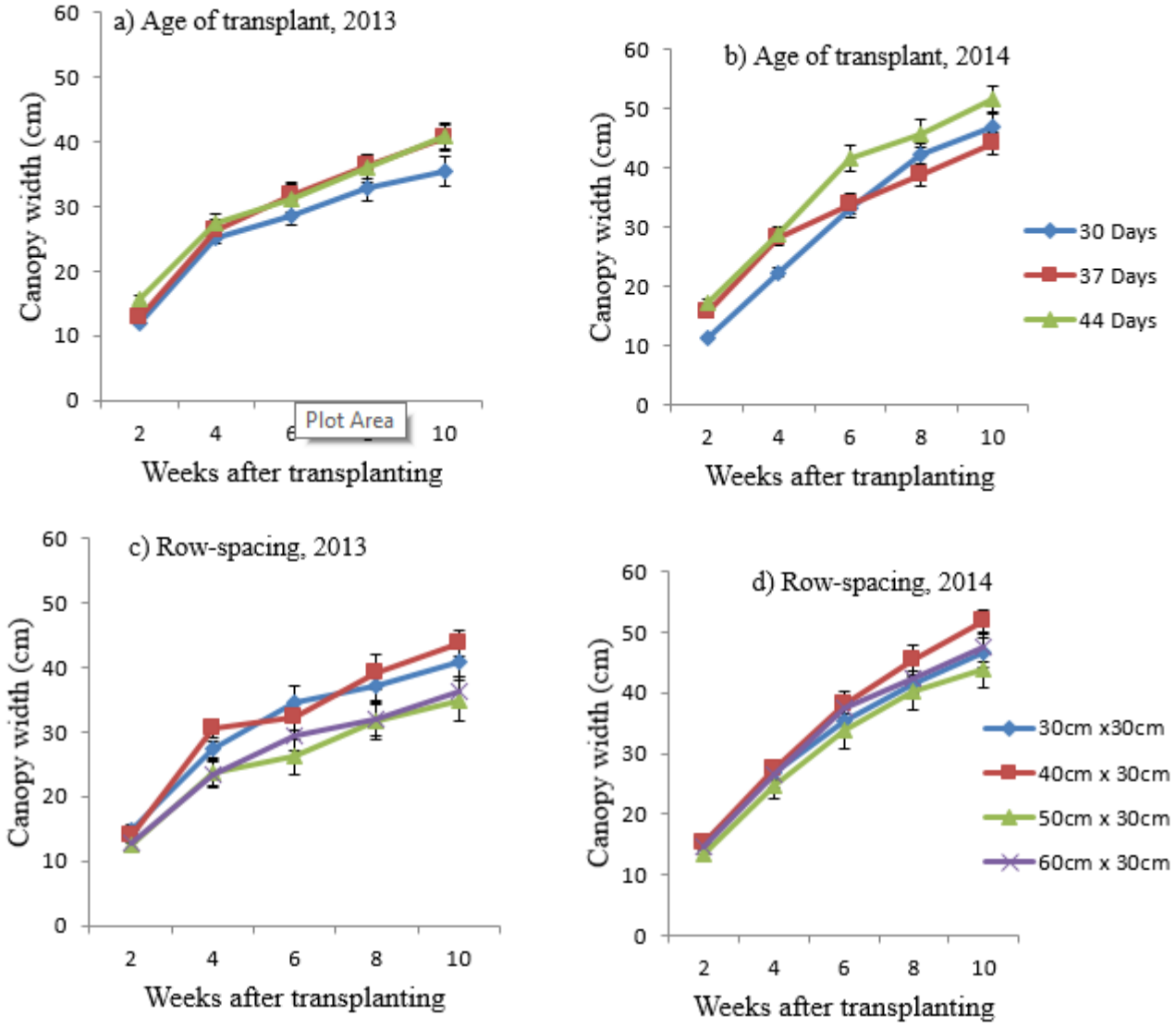

Fig.4: Effects of Age of transplant and row-spacing on canopy width of chilli pepper, 2013 and 2014

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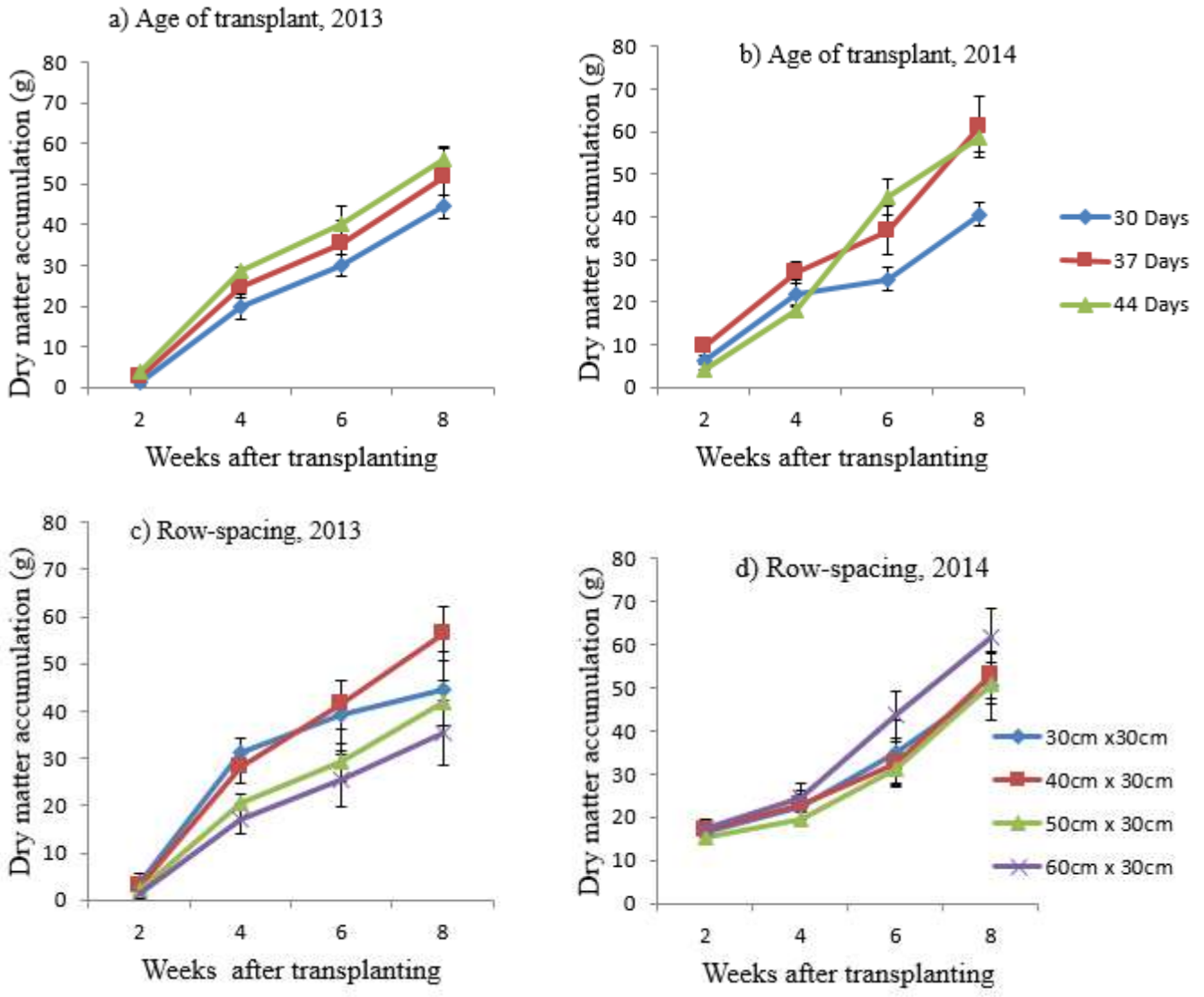

Fig.5: Effects of age of transplant and row-spacing on dry matter accumulation of chilli pepper, 2013 and 2014

3.2 Yield and yield components

3.2.1 *Number of Plants harvested*

There was no significant difference between ages of transplants in number of plants harvested during the 2013 cropping season. The 37 aged transplants differed significantly from the 30 aged transplants in number of plants harvested during the 2014 cropping season (Table 2). The 37 aged transplant had the highest number of plants harvested followed by the 44 aged transplants and the least by the 30 aged transplants during the 2014 cropping season (Table 2). High number of plants harvested in older aged transplants might be due to well established root system in older seedlings which was capable of causing enhanced water absorption and translocation along with nutrients from the rhizosphere. This agrees with the findings of (Safina-Naz *et al.*, 2006 ; Yr. Shukla and Rajender, 2011) that older transplants with sufficient roots and number of true leaves might be responsible for absorbing soil water and manufacturing a sizable amount of photosynthates required to establish vigorous plant and complete its life cycle more comfortably.

There was no significant difference between row spacing in number of plants harvested during the 2013 and 2014 cropping seasons (Table 2).

3.2.2 *Number of fruits per plant*

The 30 aged transplants differed significantly from the 37 aged transplants in number of fruits per plant during the 2013 cropping season (Table 2). This might be due to young seedlings transplanted. This agrees with the findings of

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(Adelana, 1983) who reported of highest number of fruits from younger transplants. Contrary to this (Renuka and Perera, 2002) found more fruits from older transplants. There was no significant difference between 37 and 44 aged transplants in number of fruits per plant at the same cropping period (Table 2). There was no significant difference between ages of transplants in number of fruits per plants during the 2014 cropping season although there were differences between treatment means. The 37 aged transplants produced the highest number of fruits per plant followed by the 44 aged transplants and the least with 30 aged transplants (Table 2). This might be due to the fact that in younger seedlings there was less storage of food needed for vegetative extension, whereas, older transplants were mature enough and limit vegetative extension. Moreover, middle aged seedlings on account of extended lateral branches produced maximum number of fruits per plant than younger or older ones. This is in conformity with the findings of (Yr. Shukla and Rajender, 2011) that middle aged transplants produced higher number of fruits per plant than the younger or older transplants. Highest number of fruits per plant by middle aged transplants was also reported by (Salik *et al*., 2000) in tomato.

Row spacing had no significant effect on number of fruits per plant in both cropping seasons although differences exist between treatment means. The 40 x 30 cm row spacing had the highest number of fruits per plant in both cropping seasons (Table 2). The high number of fruits per plant in 40 x 30 cm row spacing might be due to better vegetative growth in terms of plant height, number of leaves per plant and number of branches in both cropping seasons.

3.2.3 *Fruit weight per plant*

There was no significant difference between ages of transplants in fruits weight per plant in both cropping seasons although the 30 and 44 aged transplants had the highest fruit weight per plant during the 2013 and 2014 cropping seasons respectively (Table 2). The 60 x 30 cm row spacing differed significantly from 50 x 30 cm in fruit weight per plant during the 2013 cropping season (Table 2). The high fruit weight per plant in 60 x 30 cm row spacing might be due to differences in row spacing coupled with available soil mineral nutrients and high soil moisture content for the vegetative growth to the efficiency of photosynthesis which resulted into accumulation and translocation of photosynthates leading to efficient utilization of assimilates. There was no significant difference between row spacing in fruit weight per plant during the 2013 and 2014 cropping seasons (Table 2).

Table.2: Number of plants harvested, number of fruits per plant and fruit weight per plant as influenced by ages of transplant and row spacing during the 2013 and 2014 cropping seasons.

Treatment	**Number of plants harvested**		**Number of fruits per plant**		**Fruit weight per plant (g)**	
	2013	2014	2013	2014	2013	2014
Age of transplant						
30 Days	13.96	11.00	26.45	19.00	48.42	22.20
37 Days	13.03	20.00	22.87	26.00	38.07	24.90
44 Days	14.71	17.00	23.29	24.00	40.29	25.90
LSD (0.05)	NS	6.00*	2.96*	NS	NS	NS
Row spacing						
30cm x30cm	16.82	14.00	24.83	18.00	40.84	22.60
40cm x 30cm	16.30	19.00	25.51	27.00	43.44	22.00
50cm x 30cm	11.21	14.00	22.75	21.00	32.92	24.30
60cm x 30cm	11.27	17.00	23.73	25.00	51.83	28.40
LSD (0.05)	NS	NS	NS	NS	12.22*	NS
Age x row spacing interaction	NS	NS	NS	NS	NS	NS
CV (%)	31.1	24.87	30.2	20.48	28.5	34.2

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3.2.4 *Fruit Length*

Ages of transplant showed no significant effect in fruit length during the 2013 cropping season (Table 3). There was a significant difference between 44 aged transplants from 30 aged transplants in fruit length during the 2014 cropping season (Table 3). The longest fruit length in the 44 aged transplants might be due to initial high rainfall and temperature during the cropping period that resulted in vigorous plant growth, especially development of deep roots for the uptake of soil moisture and nutrients for proper development of fruits. The shortest fruit length in 30 aged transplants might be due to low dry matter accumulated and less storage of food in terms of solutes needed for cellular elongation of fruits. There was no significant difference between row spacing treatments in fruit length in both cropping seasons although differences exist between treatments. The 60 x 30 cm and 50 x 30 cm had the longest fruit length during the 2013 and 2014 cropping seasons respectively (Table 3).

3.2.5 *Fruit diameter*

The 30 aged transplants differed significantly from 44 aged transplants in fruit diameter during the 2013 cropping season (Table 3). This might be due to differences in age of transplant of seedlings. This contradicts those found by (McCraw and Greig, 1986; Weston, 1988) that pepper transplants of 8 and 11 weeks (older transplants) have a yield advantage for early fruit size. The 44 aged transplants differed significantly from 30 aged transplants in fruit diameter during the 2014 cropping season (Table 3). The widest fruit diameter in 44 aged transplants might be attributed to high or enhanced biomass, accumulation of resources and improved water relationship in the plants. This heightened meristematic activities that favoured the enlargement of fruit. This agrees with the findings of (Vavrina, 1998) that due to slow growth habit of pepper, older transplants (i.e > 4 to 6 weeks) may be advised. There was no significant difference between row spacing treatments in fruit diameter in both cropping seasons (Table 3).

3.2.6 *Fruit Yield*

There was no significant difference between ages of transplant in fruit yield during the 2013 cropping season although the older transplants had the highest fruit yield (Table 3). The 37 aged transplants differed significantly from the 30 and 44 aged transplants in fruit yield during the 2014 cropping season (Table 3). This might be due to enhanced initial plant growth due to high dry matter yield. Peppers require strong initial growth to promote earliness and abundant fruit set. The 30 aged transplants had the least fruit yield at the same cropping period (Table 3). This contradicts those found by (Ibrahim *et al.,* 2013) that higher fresh fruit yield was recorded in pepper transplanted earlier than those transplanted later. The 30 x 30 cm row spacing differed significantly from the 50 x 30 cm and 60 x 30 cm row spacing in fruit yield during the 2013 cropping season (Table 3). This might be due to differences in plant spacing and plant morphology. This agrees with the findings of (Rafiei, 2009; Albayrak *et al*., 2011; Ciampitti and Vyn, 2011) that for most crops, plant density has a major influence on crop yield and economic profitability. Yildiz and Abak (2003) attested that plant yield can be variable in high density according to branch numbers per plant and that more plant density in comparison with lower plant density will lead to higher yield. Similar findings was reported by (Alabi *et al.*, 2014) that total fruit yield per hectare increased with higher population densities. Some other studies showed that increasing yield will follow by increasing plant density Cavero *et al*., (2001); Nyambi *et al.,* (2004). Peppers and other plants grown in denser population tend to be taller and may set fruit higher on the plant than those grown in less dense planting Stofella and Bryan, (1988). There was no significant difference between row spacing treatments in fruit yield during the 2014 cropping season although the 40 x30 cm row spacing had the highest yield (Table 3). This implies that a further reduction in the row spacing would probably not be beneficial and so the 40 x 30 cm spacing appears optimum and should be recommended for hot pepper production.

Table.3: Fruit length, fruit diameter and fruit yield as influenced by ages of transplant and row spacing during the 2013 and 2014 cropping seasons.

Treatment	**Fruit length (cm)**		**Fruit diameter (cm)**		**Fruit yield (kg/ha)**	
	2013	2014	2013	2014	2013	2014
Age of transplant						
30 Days	7.68	4.14	0.99	0.49	2369.95	1028.0

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37 Days	7.12	6.06	0.90	0.69	2089.97	2116.0
44 Days	7.60	6.13	0.89	0.78	2667.59	1542.0
LSD (0.05)	NS	1.09*	0.09*	0.15 *	NS	369.3 *
Row spacing						
30cm x30cm	7.12	5.13	0.96	0.64	3749.23	1415.0
40cm x 30cm	7.22	5.60	0.94	0.66	2925.20	1843.0
50cm x 30cm	7.57	6.03	0.90	0.73	1295.62	1531.0
60cm x 30cm	7.96	5.02	0.92	0.60	1533.30	1459.0
LSD (0.05)	NS	NS	NS	NS	984.40*	NS
Age x row spacing interaction	NS	NS	NS	NS	NS	NS
CV (%)	24.5	23.9	25.6	26.9	40.7	27.9

IV. CONCLUSION

Farmers are encouraged to transplant chilli pepper seedlings at 44 days after planting and at a row spacing of 40 x 30 cm for taller plant, higher vegetative biomass and higher number of fruits per plant. For longer fruit, wider fruit diameter and higher fruit weight per plant, pepper farmers should transplant chilli pepper seedlings at 30 days after planting and at a row spacing of 60 x 30 cm. However, for taller plant, wider fruit diameter and higher fruit yield farmers are to transplant chilli pepper seedlings at 44 days after planting and at a row spacing of 30 x 30 cm.

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Mathematical Modeling of Sun and Solar Drying Kinetics of Fermented Cocoa Beans

Olabinjo O.O.[1*], Olajide J.O.[2], Olalusi A .P.[1]

[1]Department of Agricultural Engineering and Environmental Engineering, Federal University of Technology, Akure.
[2]Department of Food Science and Technology, LadokeAkintola University of Technology, Ogbomoso

***Abstract*—** *In this study, thin layer drying experiments were conducted to compute drying characteristics of fermented cocoa beans in open sun and indirect natural convection solar dryer. The drying experiments were conducted at the same time for comparison. Three different thin layers drying of the fermented beans were examined under field conditions for Akure, Nigeria. The drying process took place only in the falling rate period. The drying curves obtained from the experimental data were fitted to thirteen (13) different thin layer mathematical models. All the models were compared according to three evaluation parameters. These include coefficient of determination (R^2), Root mean square error (RMSE) and Chi-square (X^2).The results showed that increasing drying air temperature resulted to shorter drying times. The Vermal et al. model was found to be the most suitable for describing the drying curve of the convective indirect solar drying process of cocoa beans with R^2 = 0.9562, X^2=0.0069 and RMSE=0.0067; while, the Midilli and Kucuk model, best described the drying curve of fermented cocoa beans under open sun with R^2 = 0.9866, X^2=0.0024 and RMSE=0.0023.*

***Keywords*— Thin-layer drying, moisture content, modelling,cut test, pH, Cocoa beans.**

I. INTRODUCTION

Drying is one of the oldest methods of food preservation (Doymaz, 2007). Agricultural and other products have been dried by sun and wind in the open air for thousands of years. The purpose is either to preserve them for later use, as in the case with food; or as an integral part of the production process as with tobacco and cocoa beans. It is necessary that the traditional techniques be replaced with industrial drying methods. (Ertekin and Yaldiz, 2004).

Mathematical modelling and simulation of drying curves under different conditions is important to obtain an overall improvement of the quality of the final product. Simulation models of the drying process are used for developing new designs, improving existing drying systems, predicting the airflow over the product and for the control of the process. (Aghbashlo, *et al.*, 2008). Thin layer drying equations are used to estimate drying kineticsfor several products and also to generalize drying curves.

A critically important aspect of drying technology is mathematical modelling of the drying process. Modelling of drying process and kinetics is a tool for process control and necessary to choose suitable method of drying for specific product. Modelling is also essential for engineers to choose the most suitable climatic conditions in order to design appropriate drying equipment for perishable crops. The aim of this work is to study the drying process and select the most suitable model (in terms of fitting ability) to describe the thin-layer drying of cocoa beans. Although much information has been reported about modelling of thin layer drying (Togrul&Pehlivan, 2002) there is no information about modelling of thin layer drying of cocoa beans in Nigeria.

II. MATERIALS AND METHODS

2.1 Drying experiments

In this study, fresh healthy cocoa pods (Amenlonado variety) were procured from Oda village, Akure South Local Government, Akure Ondo State. The drying experiments were carried out using mobile solar dryer in the Department of Agricultural Engineering, Federal University of Technology, Akure. Plate 1 shows the schematic diagram of the solar dryer used for the experimental work which consists of a solar collector and a drying chamber. The samples were weighed using a digital balance with 0.01g sensitivity every 60 minutes throughout the drying process.

Three different thin samples of wet fermented cocoa beans were spread evenlyinto the solar dryer and in the open sun drier for the dehydration test. The experiment was replicated thrice and the mean value was used. Thermal drying method was used in the determination of moisture content of the samples.100g of sample were placed in oven at 105± 3^0C and allowed to dry to a constant weight for 24 hours (Lagha-Benamrouche, S. and Madani, K., 2013). The moisture content (MC) was calculated by expressing the weight loss upon drying a fraction of the initial weight of sample used. The moisture content of the seeds was determined by gravimetric method which determines the mass loss from the sample

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by drying to constant weight (ASABE STANDARDS, 1993 and AOAC, 2000).

$$DM(\%) = \frac{W_3 - W_0}{W_1 - W_0} * 100 \quad (1)$$

$$\%MC_{db} = 100 - DM\% \quad (2)$$

Where W_o is weight of empty crucible

$W_1$is weight of crucible plus sample before drying

$W_3$is weight of crucible plus sample after drying

DM is dry matter and MC_{db} is the cocoa beans moisture content (g water/g dry base, d.b).

Plate.1: Mobile Solar dryer

2.2 Mathematical modelling of drying process

Many researchers have worked on many thin layer models in the past and this study evaluate thirteen (13) of such models as shown in Table 1.

The moisture ratio, MR is given as follows:

$$MR = \frac{M - M_e}{M_o - M_e} \quad (3)$$

Where MR is the dimensionless moisture ratio or unaccomplished moisture content, M, M_e, M_o are moisture content (kg water/kg, dry matter) respectively.

The values of M_e are relatively small compared to those of M or M_o hence error involved in its simplification as negligible. (Aghbashlo,Kianmerhrk&Samini-Akhljahani, 2008), hence moisture ratio is calculated

$$MR = \frac{M}{M_e} or \frac{M}{M_o} \quad (4)$$

For drying model selection, drying data were fitted into thirteen well known thin layer drying models which are given in Table 1.

Table.1: Thin layer models used by some researchers and used in evaluating the drying kinetics of Cocoa beans.

S/N	Model name	Model equation	Refrences
1	Newton	MR=exp(-kt)	Upadhyay*et al.*, 2008
2	Page	MR=exp(-kt^n)	Saeed *et al.*, (2006)
3	Modified page	MR=exp[-(kt)^n]	Ceylan*et al.*, (2007)
4	Henderson and Pabis	$MR = aexp(-kt)$	Kashaninejadand Tabil(2004)
5	Logarithmic	$MR = aexp(-kt) + c$	Wang *et al.*, (2007)
6	Two-term	MR=aexp(-ko t)+bexp(-k1t)	Wang *et al.*, (2007)
7	Two-term exponential	$MR = aexp(-k_o t)bexp(-k_1 t)$	Tarigan*et al.*, (2007)
8	Wang and Singh	MR=1+at+ 〖bt〗 ^2	Wang and Singh (1978)
9	Diffusion approach	MR=aexp(-kt)+(1-a)exp(-kbt)	Wang *et al.*, (2007);
10	Modified Henderson and Pabis	MR=aexp(-kt)+bexp(-gt)+cexp(-ht)	Kaya *et al.*, (2007b)
11	Verma et al	MR=aexp(-kt(n))+bt	Doymaz, (2005b)
12	Midilli and Kucuk	$MR = aexp(-kt^n) + bt$	Midilli*et al.*, (2002)
13	Thomson	t=a lnMR+b(In(MR))^2	Thomson *et al.*, (1968)

Moisture ratio (MR) = dependent variable, Drying constant constant (k) = independent variable.

The goodness of fit was determined using three parameters; coefficient of determination (R^2), reduced chi-square (x^2) and the root mean square error (RMSE) using equations (4) - (6) as in Sacilik and Elicin (2008).The statistical analyses were carried out using SPSS 13.0 software and non-regression technique.

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a. Coefficient of determination (R^2)

$$R^2 = = \frac{\sum_{i=1}^{n}(MRi-MRpre,i)\ .\sum_{i=1}^{n}(MRi-MRexp,i)}{\sqrt{[\sum_{i=1}^{n}(MRi-MRpre,i)]}.\sqrt{[\sum_{i=1}^{n}(MRi-MRexp,i)]}} \quad (5)$$

Chi-square (x^2)

$$x^2 = \frac{\sum_{i=1}^{N}(MR_{exp.i}- MR_{pre.i})^2}{N-n} \quad (6)$$

b. Root mean square error (RMSE)

$$RMSE = \left[\frac{\sum_{i=1}^{N}(MR_{pre.i}-MR_{exp.i})^2}{N}\right]^{1/2} \quad (7)$$

Where $MR_{exp,i}$is ith experimentally observed moisture ratio, $MR_{pre,i}$is ith predicted moisture ratio, N is the number of observation, n is the number of model constants.

III. RESULTS AND DISCUSSIONS

3.1 Drying Kinetics of Fermented cocoa beans

From the experimental data, the moisture content (%wb) of fermented cocoa beans for the solar dryer and open sun drying at any time are represented in Figures 1-3. It was clearly evident from these curves that the drying rate of fermented cocoa beans in the solar dryer was faster than that of the open sun drying. The moisture content of the fermented cocoa beans reached 6.5% dry basis in 32hours of drying in the solar dryer, whereas the final moisture of the same product dried by open sun drying was only 9.87% dry basis thus moisture content was not enough for safe storage. When it was dried under open sun drying, the duration of dry was about two (2) sunshine days to bring it to the same moisture level.

This can be explained that the main factor influencing drying rate was the drying air temperature. Compared to open sun drying, solar dryer can generate higher air temperature and affected the significant increasing of evaporation rate of water and then result in lower final moisture content of drying samples. These results indicated that solar dryer was effective than open sun drying.

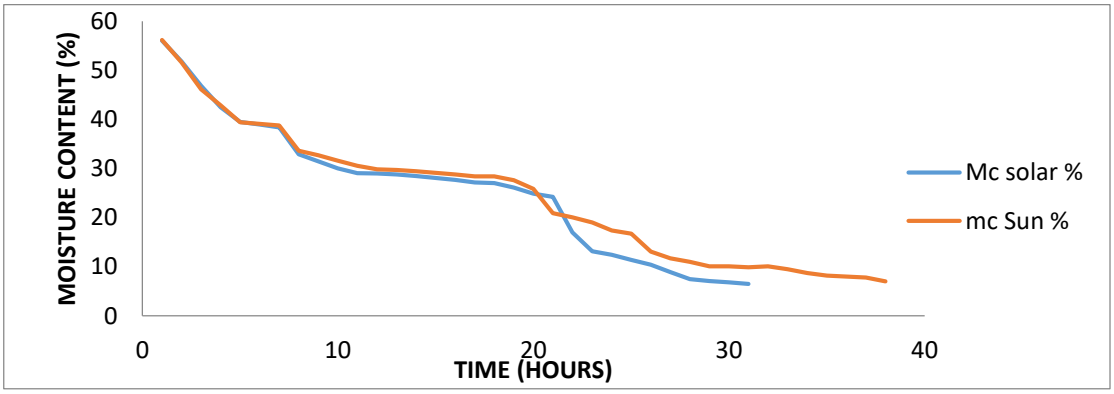

Fig.1: Variation of moisture content with drying time for fermented cocoa beans for 3.97g/cm²

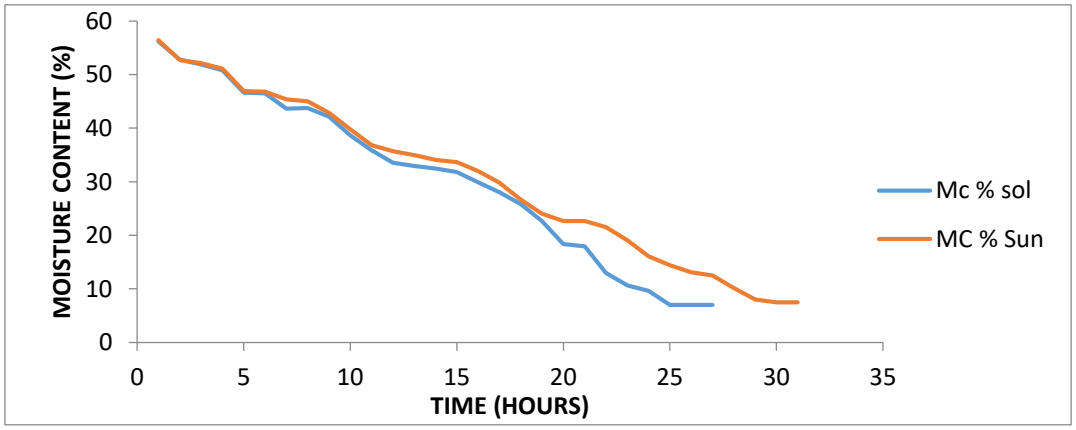

Fig.2: Variation of moisture content with drying time for fermented cocoa beans for 3.21g/cm²

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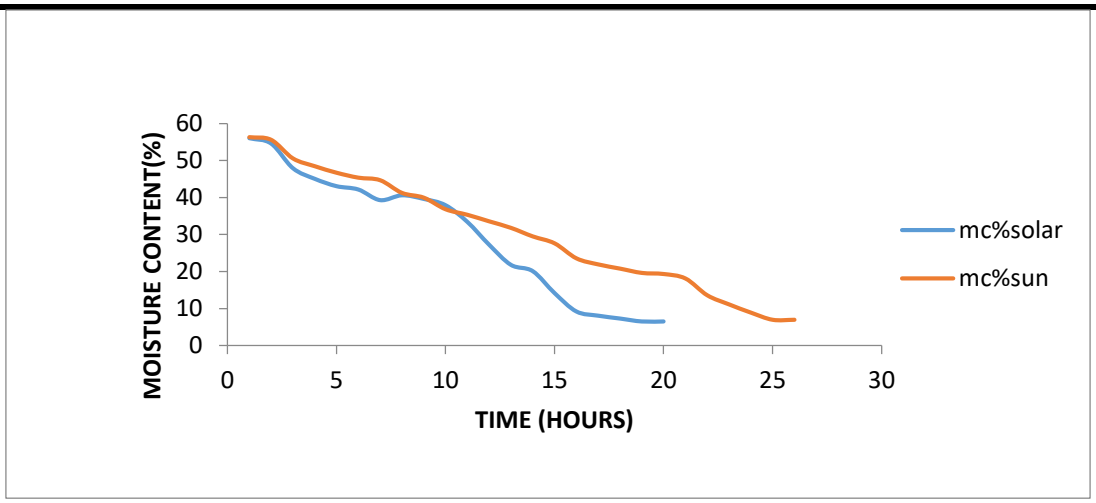

Fig.3: Variation of moisture content with drying time for fermented cocoa beans for 2.97g/cm²

3.3 Mathematical modelling

The moisture content data at different experimental modes were converted to the more useful moisture ratio expression, and curve fitting computations with drying time were performed with the thirteen (13) drying models presented by previous workers (Table 1).The results of the statistical analyses undertaken on these models for the natural convention solar drying and the natural sun drying are given in Table 2 and 3, respectively. The models were evaluated based on Coefficient of determination (R^2), Chi-square (x^2), and Root Mean Square Error (RMSE). All equations gave consistently high (R) values in the range of 0.91 -0.98.This indicates that all equations could satisfactorily describe the solar drying rates of fermented cocoa beans. RMSE ranged from 0.0067-1.6465, chi-square ranged from 0.0069-1.1000 and for solar drying while RMSE ranges from 0.0023-1.4672, and chi-square ranges from 0.0024-1.2700 for open sun drying. The result shows that for all thin layer drying models and conditions of solar drying ,the Verma*et al.* (1985) model gave the best fit with R^2 = 0.9562 , x^2= 0.0069 , and RMSE=0.0067 for solar drying . The Midilli model gave the best fit with R^2 = 0.9866, x^2= 0.0024, and RMSE = 0.0023 for open sun drying. The drying constants (k) and (l) and coefficients (a) and (n) values as well as the statistical parameters R^2, x^2, and RMSE are shown in Tables 2 and 3 for both solar drying and open sun drying. Validation of the Verma*et al* and Midilli and Kucuk models weremade by comparing the predicted moisture ratio with the experimented moisture ratio values from all the tests. The performance of the Verma*et al* model for the thin solar drying and the Midilli and Kucuk model for natural sun drying was illustrated in Figures 4 and 5.The predicted data is banded around the straight line which showed the suitability of the Verma*et al* and Midilli and Kucuk models in describing the drying behaviour of fermented cocoa beans in solar and open drying respectively.

Table.2: Modelling the drying process of fermented cocoa beans using solar dryer

MODELS	COEFFICIENT			R^2	x^2	RMSE
NEWTON	k=0.0186			0.9311	0.0134	0.013
PAGE	k=0.0383	n=0.8255		0.9351	0.0108	0.0105
MODIFIED PAGE	k=0.0192	n=0.8255		0.9351	0.0675	0.0654
HENDERSON &PABIS	k=0.0160	a=0.8748		0.9506	0.0085	0.0082
LOGARITHMIC	k=0.0057	a=1.5578	c=-0.7343 a=0.4523	0.9662	0.0088	0.0082
TWO TERM	ko=0.0162	k1=0.0160	b=0.4226	0.9506	0.0087	0.0081
TWO TERM MOD.	k=0.2539	a=0.0684		0.9398	0.0114	0.0107
WANG &SINGH	a=-0.0139	b=5.0149		0.9234	1.1	106465
APPRO OF DIF	k=1.1408	a=0.1712	b=0.0133	0.9562	0.2088	0.2021
MOD HENDER &PAB	k=0.0165	a=0.2746	b=0.2954	0.9506	0.009	0.0082
	c=0.3049	g=0.0160	h=0.0158			

VERMA ET AL	**k=1.1444**	**a=0.1705**	**g=0.0152**	**0.9562**	**0.0069**	**0.0067**
MIDILLI & KUCUK	k=0.2021	n=0.1734	a=1.0050, b=0.0049	0.9787	0.4659	0.4358
Thompson	a=-64.7773	b=-8.0810		0.9655	0.8923	0.9568

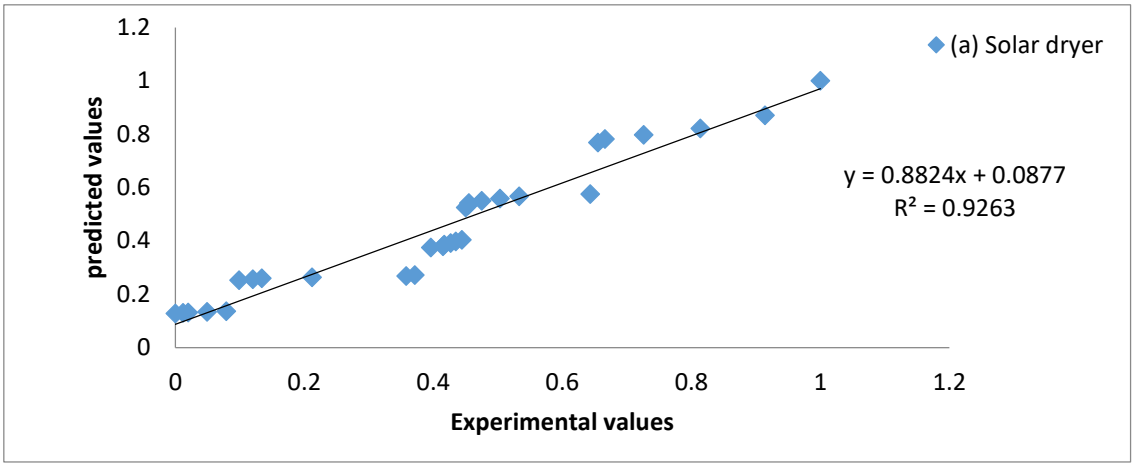

Fig.4: Comparison of experimental and predicted dimensionless moisture ratio for solar drying

Table.3: Modelling the drying process of fermented cocoa beans using open sun drying.

MODELS	**COEFFICIENT**			**R^2**	**X^2**	**RMSE**
NEWTON	k=0.0199			0.9423	0.0089	0.0086
PAGE	k=0.0710			0.9617	0.0063	0.0061
MODIFIED PAGE	k=0.0217	n=0.6905		0.9617	0.1376	0.1339
HENDERSON &PABIS	k=0.0160	a=0.8334		0.9741	0.0044	0.0042
LOGARITHMIC	k=0.0125	a=0.9083	c=-0.0955	0.977	0.0038	0.0037
TWO TERM	ko =0.0160	k1=0.0161	a=0.4507,b=0.3827	0.9741	0.0045	0.0042
TWO TERM MOD.	k=0.1656	a=0.1071		0.9572	0.007	0.0066
WANG &SINGH	a=-0.0144	b=5.3256		0.9149	1.27	1.1197
APPRO OF DIF	k=0.8487	a=0.2269	b=0.0173	0.9827	0.0032	0.0031
MOD HENDER &PAB	k=0.0159	a=0.2610	b=0.2814	0.9741	0.0048	0.0042
	c=0.2909	g=0.0161	h=0.0161			
VERMA ET AL	k=0.8584	a=0.0226	g=0.0147	0.9827	0.0135	0.0132
MIDILLI & KUCUK	**k=0.1921**	**n=0.3235**	**a=1.0040,b=-0.0023**	**0.9866**	**0.0024**	**0.0023**
THOMPSON	a=-59.2342	b=-4.0169		0.9827	0.3246	1.4672

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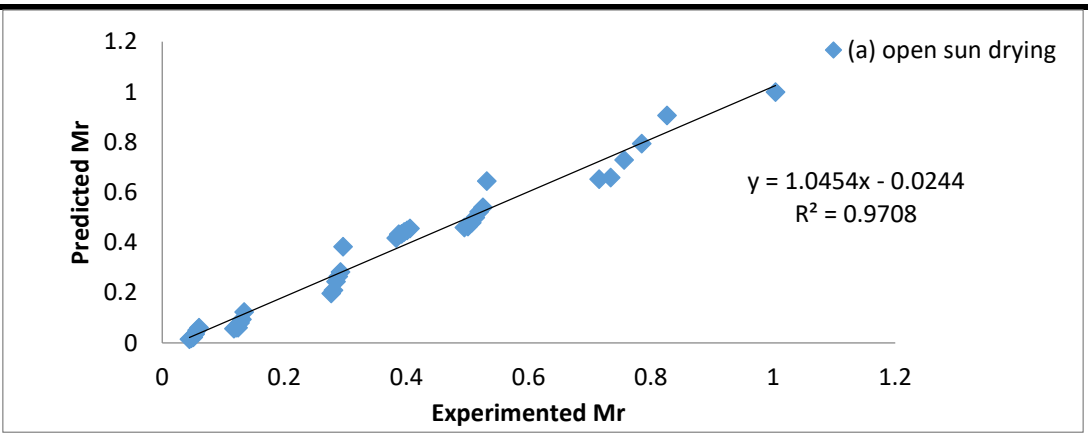

Fig.5: Comparison of experimental and predicted dimensionless moisture ratio for open sun drying

IV. CONCLUSION

The solar dryer proves useful for local farmer as it ensures high quality, good colour and flavour and reduces the drying time. It does not pollute the environment, requires minimal maintenance once it is installed and with good quality. In order to explain the drying behaviour and the mathematical models of fermented cocoa beans, thirteen models were applied to thin layer convective indirect solar dryer and open drying processes. The result showed that the Verma*et al* model was found to be the most suitable model for describing the drying curve of the convective indirect solar drying process of cocoa beans with R^2 =0.9562, x^2=0.0068, MBE=0.0383 and RMSE=0.0067 while, the Midilli and Kucuk model, best described the drying curve of fermented cocoa beans under open sun with R^2 = 0.9866, x^2=0.0024, MBE=0.0078 and RMSE=0.0023.

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Screening of different Rice entries against Rice Gall Midge, *Orseolia oryzae* (Wood-Mason)

Atanu Seni*, Bhima Sen Naik

Orissa University of Agriculture and Technology, AICRIP, RRTTS, Chiplima-768025, Sambalpur, Odisha

Abstract*—In order to develop rice cultivars for resistance to the gall midge, Orseolia oryzae (Wood-Mason), some rice entries were screened under natural field conditions at the Chiplima, OUAT, Odisha under All India Coordinated Rice Improvement Project during kharif 2016. Gall midge incidence as silver shoot was recorded on 30 and 50 days after transplanting and scoring was done.* H*ighest incidence of silver shoot was recorded in TN-1 (36.71% SS after 50 DAT) whereas 12 entries viz., W 1263, INRC 3021, Sudu Hondarawala, PTB 26, RP 4686-48-1-937, RMSG-11, WGL 1147, WGL 1127, WGL 1121, WGL 1131, WGL 1141, JGL 27058 were found resistant to gall midge damage. Based on the reaction of the different entries the presence of biotype 1 was identified.*

***Keywords*— Gall midge, Chiplima, rice entries, screening, field.**

I. INTRODUCTION

Rice is the most important cereal food crop of India covering about one-fourth of the total cropped area and providing food to about half of the Indian population. Introduction and wide adoption of high yielding varieties has led to severe incidence of different insect pests. Nearly 300 species of insect pests attack the rice crop at different stages and among them only 23 species cause notable damage [6]. Among them, Asian rice gall midge (GM), *Orseolia oryzae* (Wood-Mason) is one of the important insect which has been prevalent in almost all the rice growing states in India except the Western Uttar Pradesh, Uttaranchal, Punjab, Haryana and Hill states of Himachal Pradesh and Jammu and Kashmir [1]. GM causes an annual yield loss of 0.8% of the total production, amounting to US$80 million [5]. This is essentially a monsoon pest and causes damage wherever high humidity and moderate temperature prevail, even in dry seasons [3]. The external symptom of damage caused by gall midge is the production of a silvery-white, tubular leaf sheath gall called a *silver shoot* or *onion shoot*. This is due to the feeding and salivary secretion by the larvae which turn the growing shoot meristem into a gall [7]. This renders the tiller sterile and do not bear panicle [9]. Many management strategies viz., chemical, cultural, biological and planting of resistant cultivars that have resistance to insects are employed to reduce the damage caused by this insect-pest. Among them, the use of resistant rice varieties appears to offer the most effective component for incorporation into an integrated pest management strategy [11]. For this, breeding resistant varieties has been a viable, ecologically acceptable approach for managing this pest [5].

II. MATERIALS AND METHODS

The experiment was conducted in the experimental farm of Regional Research and Technology Transfer Station (OUAT), Chiplima, Sambalpur, Odisha, during *kharif*, 2016. The Station is situated at $20^0$21' N latitude and $80^0$55'E longitude in Dhankauda block of Sambalpur district at an altitude of 178.8 m above MSL. The climate of the area is warm/sub humid. Nursery of different rice entries were sown in the July and transplanting was done after 25 days of sowing at 15 cm x 15 cm hill spacing. All the agronomic practices were followed during crop growth period. Gall midge incidence as silver shoot was recorded on 30 and 50 days after transplanting and then percentage of silver shoot was worked out. The pest intensity was scored as per standard evaluation system, IRRI for gall midge.

Table.1: Standard evaluation system for rice gall midge

Scale	Damage (%)	Reaction
0	No damage	HR
1	<1%	R
3	1-5%	MR
5	6-10%	MS
7	11-25%	S
9	>25%	HS

III. RESULTS AND DISCUSSION

Rice gall midge is one of the major pestsof rice in Hirakud command area, Sambalpur, Odisha. Different rice entries obtained from ICAR-IIRR, Hyderabad were evaluated to

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find out the field resistance against rice gall midge. Rice gall midge is one of the major and regular pests of rice in Hirakud command area, Sambalpur, Odisha. The Sambalpur district in the west-central table land zone of Odisha is also considered to be the endemic pocket for gall midge in the state [2]. Different rice entries obtained from ICAR-IIRR, Hyderabad were evaluated to find out the field resistance against rice gall midge.

Among 164 entries screened against gall midge, the entries viz., W 1263, INRC 3021, Sudu Hondarawala, PTB 26, RP 4686-48-1-937, RMSG-11, WGL 1147, WGL 1127, WGL 1121, WGL 1131, WGL 1141, JGL 27058 were found resistant to gall midge damage. The entries viz., KAVYA, Aganni, INRC 15888, KAKAI (K 1417), SINNA SIVAPPU, PTB 12, PTB 32, TH BR 68, TH BR 69, TH BR 70, TH BR 71, TH BR 72, TH BR 74, TH BR 79, RMSG-7, RMSG-10, RP 5925-24, WGL 1143, WGL 1144, WGL 1145, WGL 1146, WGL 1118, WGL 1119, RP 2068-18-3-5, JGL 21789, JGL 21831, JGL 25154, JGL 25969, JGL 27015, JGL 27020, JGL 27056, JGL 27075, JGL 27361, JGL 27371, KNM 1592, KNM 1598, KNM 1621, KNM 2251, KNM 2275, KNM 2266, KNM 13595, KNM 1623, KNM 1638, JGL 3828, and WGL 505 were found to be moderately resistant to gall midge incidence. The entries viz., Phalguna, Dukong 1, RP 2333-156-8, Abhaya, CAUR-1, RP5923, COGR-2, IC 462362, IC 577588, ACC 4740, ACC 5403, IC 576897, IC 577224, IIRR-Bio-SB-6, ARC 15570C, VELLAI ILANKALAYAN, WGL32100, JGL 24520, JGL 25947, JGL 25960, JGL 25964, JGL 26989, JGL 27072, KNM 1717, KNM 1728, WGL 667, WGL 705, WGL 767 and TN 1 were found highly susceptible and the remaining entries were found susceptible to gall midge damage. Based on the reaction pattern of resistant-susceptible-susceptible (R-S-S) the prevalence of biotype 1 was identified at Sambalpur.

Table.2: Reaction of different rice entries against rice gall midge

Entry No.	Name of entry	Silver shoot (SS)		Reaction
		30 DAT	50 DAT	
1	KAVYA	1.37	1.96	MR
2	W 1263	0.57	0.00	R
3	ARC 6605	3.92	7.04	MS
4	PHALGUNA	15.23	32.03	HS
5	ARC 5984	6.11	18.08	S
6	DUKONG 1	13.10	35.48	HS
7	RP 2333-156-8	15.00	25.87	HS
8	MADHURI L 9	2.50	5.71	MS
9	BG 380-2	16.55	27.70	HS
10	MR 1523	12.12	25.00	S
11	RP 2068-18-3-5	6.54	13.79	S
12	ABHAYA	16.50	31.02	HS
13	INRC 3021	0.50	0.00	R
14	AGANNI	0.00	1.21	MR
15	INRC 15888	1.84	2.36	MR
16	B 95-1	6.63	2.90	MS
17	TN1	17.62	36.71	HS
18	CAUR-1	31.16	39.74	HS
19	RP5923	30.84	43.48	HS
20	COGR-2	25.17	31.87	HS
21	IC 462248	17.12	16.00	S
22	IC 462362	46.15	46.36	HS
23	IC 463334	12.41	1.34	S
24	IC 463414	17.33	19.05	S
25	IC 463393	14.10	19.14	S
26	IC 462447	18.32	14.81	S

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27	IC 463987	21.99	14.67	S
28	IC 577588	28.66	50.00	HS
29	ACC 3643	18.92	13.64	S
30	ACC 4656	13.48	15.98	S
31	ACC 4740	41.62	34.54	HS
32	ACC 5403	33.77	37.35	HS
33	IC 545528	16.18	20.74	S
34	IC 576897	26.72	38.58	HS
35	IC 462336	21.38	16.40	S
36	IC 545441	20.42	16.22	S
37	IC 459646	14.39	11.18	S
38	IC 450029	20.23	10.61	S
39	IC 462336	19.12	19.40	S
40	IC 577224	48.78	43.27	HS
41	IC 466408	14.38	6.99	MS
42	IIRR-Bio-SB-6	47.67	30.84	HS
43	AC 4236	17.16	7.14	S
44	ARC 10676	21.53	3.87	S
45	ARC 10840	17.19	20.16	S
46	ARC 11220	12.82	15.85	S
47	ARC 11281	20.11	17.46	S
48	ARC 14636	19.28	19.77	S
49	ARC 14771	25.73	17.39	S
50	ARC 15570C	25.74	37.91	HS
51	ARC 5754	14.93	18.71	S
52	ARC 5956	14.53	7.03	S
53	ARC 5981	9.83	17.65	S
54	ASD 7	9.20	3.88	MS
55	KAKAI (K 1417)	2.21	0.71	MR
56	SINNA SIVAPPU	2.10	0.00	MR
57	SUDU HONDARAWALA	1.09	0.00	R
58	PTB 12	3.10	0.80	MR
59	VELLAI ILANKALAYAN	26.42	25.42	HS
60	ARC 6248	11.30	20.62	S
61	CVL (CHINA)	4.32	8.39	MS
62	ARC 6031-B	6.90	1.57	MS
63	PTB 26	0.66	0.74	R
64	PTB 32	3.73	0.00	MR
65	IC 332045	11.73	1.55	S
66	IC 466451	7.87	0.00	MS
67	TH BR 68	2.48	0.68	MR
68	TH BR 69	0.72	1.77	MR
69	TH BR 70	4.11	0.00	MR
70	TH BR 71	3.55	0.00	MR
71	TH BR 72	3.83	0.00	MR
72	TH BR 74	5.48	0.82	MR
73	TH BR 79	5.77	3.85	MR

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74	RP4686-48-1-937	0.60	0.00	R
75	RMSG-2	13.69	15.75	S
76	RMSG-5	20.61	22.47	S
77	RMSG-6	6.90	7.58	MS
78	RMSG-7	2.03	0.60	MR
79	RMSG-10	1.37	0.00	MR
80	RMSG-11	1.08	0.00	R
81	RP 5925-24	1.55	0.00	MR
82	WGL 1143	1.44	0.83	MR
83	WGL 1144	1.80	0.00	MR
84	WGL 1145	1.28	0.00	MR
85	WGL 1146	1.12	0.00	MR
86	WGL 1147	0.00	0.72	R
87	WGL1118	1.29	0.00	MR
88	WGL1119	1.69	0.00	MR
89	WGL1121	0.00	0.00	HR
90	WGL1127	0.47	0.00	R
91	WGL1131	1.12	0.00	R
92	WGL1141	1.10	0.00	R
93	Tellahamsa	21.08	12.42	S
94	WGL32100	32.94	19.23	HS
95	RP1	7.14	0.76	MS
100	RP5587-B-B-B-32	23.87	15.38	S
101	RP 2068-18-3-5	2.14	0.00	MR
102	JGL 20644	7.95	20	S
103	JGL 21789	2.84	4.20	MR
104	JGL 21831	3.94	4.88	MR
105	JGL 24344	7.38	15.95	S
106	JGL 24520	27.27	37.59	HS
107	JGL 25154	0.72	2.38	MR
108	JGL 25925	3.05	12.93	S
109	JGL 25947	17.39	44.03	HS
110	JGL 25958	13.70	21.69	S
111	JGL 25960	20.11	40.56	HS
112	JGL 25964	15.43	40.61	HS
113	JGL 25969	5.13	4.86	MR
114	JGL 25975	13.33	17.17	S
115	JGL 25998	10.28	23.53	S
116	JGL 26772	4.32	9.35	MS
117	JGL 26960	11.34	21.60	S
118	JGL 26989	18.01	48.57	HS
119	JGL 27015	1.17	3.01	MR
120	JGL 27020	2.53	0.74	MR
121	JGL 27056	1.79	4.76	MR
122	JGL 27058	0.00	0.83	R
123	JGL 27063	1.76	7.63	MS
124	JGL 27072	13.04	35.76	HS

125	JGL 27075	4.47	3.76	MR
126	JGL 27143	5.38	24.38	S
127	JGL 27353	6.56	6.29	MS
128	JGL 27356	2.72	5.92	MS
129	JGL 27361	2.72	2.91	MR
130	JGL 27371	0.00	1.63	MR
131	JGL 27391	11.59	25.00	S
132	KNM 1592	0.66	4.07	MR
133	KNM1598	1.23	4.14	MR
134	KNM 1600	2.50	6.21	MS
135	KNM 1610	4.60	10.06	MS
136	KNM 1638	4.62	8.90	MS
137	KNM 1616	3.29	8.61	MS
138	KNM 1621	3.92	4.39	MR
139	KNM 1625	1.72	6.47	MS
140	KNM 1632	1.23	8.39	MS
141	KNM 1717	19.12	44.44	HS
142	KNM 1722	1.32	5.66	MS
143	KNM1724	0.67	7.50	MS
144	KNM1728	7.14	25.55	HS
145	KNM 1730	10.81	17.69	S
146	KNM 2213	11.92	24.14	S
147	KNM 2251	3.21	5.04	MR
148	KNM 2275	2.16	0.00	MR
149	KNM 2266	2.65	4.03	MR
150	JGL 13595	0.00	1.64	MR
151	JGL 3828	0.68	4.92	MR
152	KNM 1623	2.31	4.93	MR
153	KNM 1638	4.35	2.61	MR
154	WGL-401	1.29	5.76	MS
155	WGL-505	1.84	3.27	MR
156	WGL-667	28.77	35.41	HS
157	WGL-705	27.39	50.63	HS
158	WGL-767	16.38	34.09	HS
159	WGL-810	15.32	16.23	S
160	WGL-819	13.13	9.68	MS
161	WGL-825	14.39	8.11	MS
162	WGL-938	16.15	4.60	S
163	WGL-1062*	13.29	5.95	S
164	JGL1118	14.02	2.63	S

R–Resistant, MS–Moderately susceptible, S–Susceptible, HS–Highly susceptible

After extensive testing of host-plant differentials it is found that biotype 1 cannot damage entries containing resistance genes derived from either Eswarakora or Siam 29, biotype 2 can damage the Eswarakora group but is unable to damage the Siam 29 group, whereas biotype 3 can damage the Siam 29 group but not the Eswarakora group [4]. Although geographic distributions of different gall midge biotypes is complex. After testing of different rice entries it is found that gall midge biotype 1 is present in Sambalpur. Previously it is also reported that populations of Hyderabad,

Warangal, and Maruteru in Andhra Pradesh, Sambalpur in Odisha, and Raipur in Chhattisgarh qualified to be biotype 1, populations of Cuttack and Bhubaneswar in Odisha and at Mangalore in Karnataka, in Goa and at Sakoli in Maharashtra qualified to be biotype 2. GM populations at Ranchi in Bihar and Wangbal in Manipur had biotype 3 characteristics [4]. North coastal districts of Srikakulam and Vizianagaram in Andhra Pradesh and the Bhandara (Sakoli) region of Maharashtra qualified to be biotype 4 populations. Moncompu area of Kerala qualified to be biotype 5. In Manipur where biotype 3 had prevailed earlier another biotype emerged. The standard set of host-plant differentials confirmed the existence of yet another new biotype, designated as biotype 6 [7].

Many resistant and moderately resistant varieties are being cultivated by the farming community in Odisha to reduce gall midge damage considerably. These include Heera, Kalinga-II, Neela, Tara, Khandagiri, Udaya, Daya, Gouri, Pratap, Shakti, Phalguna, Meher, Birupa, Bhanja, Pratiksha and Samanta for medium lands and Samalei, Manika and Urbashi for lowlands [2]. Sumathi and Manickam, [10] tested different rice accession in field condition at Rice Research Station, Tirur, Tamilnadu during 2009 and found that the cultures viz., RP 4683-29-2-645, RP 4683-30-1-648, RP 4686-49-1- 943, RP 4687-52-2-1197, RP 4688-53-2-1258, RP 4688-53-2-1259, JGL 17025, JGL 17183, JGL 17187, JGL 17189, KAVYA, JGL 17190, JGL 17196, JGL 17198, JGL 17211 and JGL 17221 were recorded nil gall midge damage and found to be resistant in field screening.

IV. CONCLUSION

The genotypes W 1263, INRC 3021, Sudu Hondarawala, PTB 26, RP4686-48-1-937, RMSG-11, WGL 1147, WGL 1127, WGL 1121, WGL 1131, WGL 1141, JGL 27058 exhibited resistance against gall midge so, they can be developed as varieties or can be used in breeding programme as a source of gall midge resistance.

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Morphological and physiological variation among different isolates of *Alternaria spp.* from Rapeseed-Mustard

Rufaida Monowara[1], Nazmoon Naher Tonu[1,2*], Fatema Begum[1], Md. Masud Karim [2], Nazneen Sultana[1]

[1]Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh
[2] Saskatoon Research and Development Centre, AAFC, 107 Science Place, SK, Canada

***Abstract*—** *To find out the Morphological variation on growth and sporulation of Alternaria species of Alternaria leaf blight of mustard from 10 representative geographical locations of Bangladesh, this experiment was conducted at Plant Pathology Laboratory, Oilseed Research center, Bangladesh Agricultural Research Inistitute (BARI), Joydevpur, Gazipur, Bangladesh. All the isolates showed high level of variability in in-vitro in respect of radial mycial growth, colony colour, sub surface colour, colony shape, colony texture, zonation (surface and sub surface), length and width of conidia, beak length and number of septa. The maximum and minimum radial mycial growth was recorded 90 mm in isolate NAT_{Ab} and 83.67 mm in isolate GAZ_{Ab}, respectively at 14 days after incubation. Significant variation in conidial length, width, beak and no. of conidia observed in all isolates. The length of conidia ranged from 41.56 to 117.54µm with 3 to 11 transverse and 0 to 3 vertical septa. The width and beak length varied from 10.34 to 23.12 µm and 16.78 to 72.65 µm ,respectively. Surface colour were olivacious green to black and circular shaped colonies were observed in all isolates on PDA medium. Colony texture were cottony to velvety. Subsurface colour varied from light brown to black and pinkish. Zonation found in some isolates and some did not produce on both surface and subsurface. All conidia were murifrom and light brown to deep brown in colour. Potato Carrot Dextrose Agar medium (PCDA) and 25 ° C temperature were found optimum for different isolates for mycelial growth and sporulation.*

***Keywords*—Alternaria brassicae, mustard, morphology, physiology, culture media, variability.**

I. INTRODUCTION

Rapeseed-Mustard (*Brassica spp.*) is the principal oil-producing crop of Bangladesh yielding 77.51% [4] of total oilseed production from 60.3% of the total area coverage. This crop is cultivated, at present, in about 802882 acres. The production is about 359452 lac metric tons oil [4]. The average yield of mustard is 447 Kg/ha. Total production and per hectare seed yield of this crop may be increased by using high yielding variety (HYV) and improved production technologies.

Rapeseed-mustard is cultivated almost all over the world. It is grown in tropical as well as temperate agro climatic zones and are the best adapted to areas having a relatively cool, moist climate during the growing season. [18]. Alternaria leaf blight caused by *Alternaria brassicae* is one of the major diseases of mustard [21,29,15,1, 10,37, 7]. This disease reduces mustard yield up to 47% [30] in India. It is a prominent disease in India, Australia, Canada, Africa, England, Germany, France, Sri Lanka, Spain and Sweden, all most all around the world [12].

Around the initial site of host leaf *Alternaria* morphologically produces a series of concentric rings [2]. *A. brassicae* is a necrotrophic pathogen produce lesion on leaves, stem and siliquae which affect seed quantity, quality by reducing oil content, seed size and seed colour [8]. This disease may cause significant losses in both temperate and tropical Brassica crops [20].

The major aspects of biology of an organism are the morphological and physiological characters of an individual within a species. Although, it is not frequent in asexually produced individual of the progeny. Variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes [21]. Anamorph form of this pathogen shows great variability in morphology, physiology and pathogenicity. Several researchers have reported existence

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of variability based on morphology, sporulation, growth and cultural characteristics.

We know, every pathogen species has numerous biotypes, races or pathotype with specific genes in the respective host plants [36]. Proper understanding in the variation of pathogen population is highly crucial in the process of breeding for resistance against a particular disease.

Considering the above fact this research was undertaken to Find out the morphological and physiological variation among different isolates of Alternaria *spp*.

II. MATERIALS AND METHODS

The experiment was conducted in the Plant Pathology Laboratory, Oil Seed Research Center, Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur, Bangladesh during the period from July 2015 to March 2016.

2.1 Collection of leaf sample

Mustard leaves having typical symptoms were collected from 10 mustard growing districts of Bangladesh namely Dhaka, Rajshahi, Natore, Naogaon, Bogra, Lalmonirhat, Gazipur. Rangpur, Pabna and Mymenshingh.

2.2 Designation of collected isolates

The collected isolates were designed as DHA_{Ab}, GAZ_{Ab}, MYM_{Ab} based on their collected location. For example an isolate collected from Dhaka and recognized as first three letters of the area and Ab indicate *Alternaria brassicae* (Table 1).

Table.1: Designation of collected isolates of Alternaria Brassicae

District/Thana	Isolates designation	Village/Place
Dhaka (SAU)	DHA_{Ab}	Agronomy field
Gazipur (BARI)	GAZ_{Ab}	Oil Research field
Mymensingh (BAU)	MYM_{Ab}	Horticulture field
Pabna	PAB_{Ab}	Bhabarhat
Rangpur	RAN_{Ab}	Tillalpara
Natore	NAT_{Ab}	Dayarampur
Naogaon	NAO_{Ab}	Kamalpara
Lalmonirhat	LAL_{Ab}	Benupara
Bogra	BOG_{Ab}	Munail
Rajshahi	RAJ_{Ab}	Khorkhori

SAU = Sher-e- Bangla Agricultural University
BAU = Bangladesh Agricultural University
BARI = Bangladesh Agricultural Research Institute

2.3 Preparation of PDA medium

Potato dextrose agar (PDA) were prepared by 200gm potato extract, 1000ml distilled water, 17 gm agar. 20gm dextrose in a conical flask and autoclaved at 121 c under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile Petri plates.

2.4 Preparation of CDA

Carrot dextrose agar were prepared by 200gm carrot extract, 1000ml distilled water, 17 gm agar, 20gm dextrose in a conical flask and autoclaved at 121 c under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile Petri plates.

2.5 Preparation of Potato-Carrot Dextrose Agar (PCDA)

The combination of Potato-Carrot dextrose agar prepared by 100ml potato+100ml carrot extract, 1000ml distilled water, 17 gm agar, 20 gm dextrose in a conical flask and autoclaved at 121 c under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile petriplates.

2.6 Isolation and Identification of *Alternaria* spp.

The pathogen was isolated by tissue planting method and incubated at 25±1˚ C for 7 days. After incubation the fungus mycelia were examined under stereomicroscope (Model: Motic, SMZ-168) & compound microscope (Model: Omano, OMTM-85) for identification of the pathogen. The fungus was identified following the keys of Eills[9] .

2.7 Purification and preservation of the pathogen

The pure culture of *A. brassicae* from the PDA was transferred to PDA slants and allowed to grow at 25$\pm$ 1^0C for 7 days. After incubation PDA slants were preserved in refrigerator at 4^0C for further study.

2.8 Colony characters of *Alternaria* spp.

Colony characters in terms of surface colour, colony shape, colony texture, zonation (surface and subsurface) and subsurface colour were studied.

2.9 Morphological variability of *Alternaria* spp.

All the isolates were studied for morphological variations. In terms of conidia color, shape, size, septation, was observed on PDA medium.

2.10 Effect of culture media on growth, spore production and time of sporulation

Mycelial discs of 7 days old culture of *Alternaria* spp. isolates were transferred to the center of PDA, CDA and PCDA and incubated at 25˚C and 22±1˚C and data were recorded on growth, spore production and time of sporulation. 3 replications were maintained for each isolates in a completely randomized design. The colony diameter was recorded on 2, 4, 6, 8, 10, 12, 14 days after inoculation.

2.11 Data Analysis

For cultural, morphological and the treatment means the data were statistically analyzed by Duncan's Multiple Range test (DMRT) with significance level at 5% [13]. The package used for analysis was MSTAT-C version -88, developed by Michigan State University, Agricultural University of Norway [11].

III. RESULTS

3.1 Colony characters of isolates of *Alternaria* spp. on PDA

Variation was observed in colony characters of 10 isolates of *A. brassicae* like surface colour, shape, texture, zonation and subsurface colour are presented Table 2 and Figure 1.

3.2 Morphological variation of conidia of different isolates of *Alternaria* spp.

3.2.1 Size of conidia of *Alternaria* spp. on PDA

Remarkable variation was observed in length, breadth and beck size of conidia of different isolates of *A. brassicae* on PDA (Table 3). The length of conidia of different isolates varied from 41.56µm to 117.54µm. The maximum mean length was recorded at MYM_{Ab} 113.1µm. The minimum length was recorded at isolates GAZ_{Ab} 63.63µm.

The breadth of conidia of different isolates varied from 10.34 µm to 23.12 µm. The maximum mean breadth was recorded at PAB_{Ab} 17.36 µm. The minimum mean breadth was recorded at LAL_{Ab} 20.29 µm.The beak of conidia of different isolates varied from 16.78 µm to 72.65 µm. The maximum mean beak was recorded at PAB_{Ab}43.26 µm. The minimum mean beak was recorded at GAZ_{Ab} 24.84 µm.

3.2.2 Conidial characteristics of *Alternaria* spp. on PDA

All isolates were muriform. Colour of isolates of *A. brassicae* varied from light brown to deep brown (Table 4). Isolates NAT_{Ab} and GAZ_{Ab} show light brown colour, DHA_{Ab}, MYM_{Ab}, LAL_{Ab}, BOG_{Ab} and RAJ_{Ab} show brown

Table.3: Size of conidia of different isolates of Alternaria spp. on PDA

Isolate	Length(µm)[1]	Breadth(µm)[1]	Beak(µm)[1]
DHA_{Ab}	88.55 d	18.12 b	28.09 e
GAZ_{Ab}	63.63 e	18.09 b	24.84 e
MYM_{Ab}	113.1 a	18.56 ab	38.76 bc
PAB_{Ab}	103.4 bc	17.36 b	43.26 a
RAN_{Ab}	99.33 bc	17.90 b	37.19 c
NAT_{Ab}	90.03 d	17.87 b	37.93 c
NAO_{Ab}	87.73 d	20.17 a	25.98 e
LAL_{Ab}	101.5 bc	20.29 a	38.95 bc
BOG_{Ab}	97.61 c	18.23 b	41.89 ab
RAJ_{Ab}	104.7 b	19.12 ab	33.23 d
LSD (0.05)	6.82	1.82	3.47
CV (%)	4.22	5.75	5.82

[1]Mean of 15 replications for each isolates

Table.2: Colony Characters of different isolates of Alternaria spp. on PDA

Isolates	Colour		Texture	Colony shape	Zonation	
	Surface	Subsurface			Surface	Subsurface
DHA_{Ab}	Olivacious green	Black center with white surroundings	Cottony	Circular	No zonation	Zonation
GAZ_{Ab}	Olivacious green	Black center with pinkish surroundings	Cottony	Circular	Zonation	Zonation
MYM_{Ab}	Black	Brownish	Velvety	Circular	No zonation	Zonation
PAB_{Ab}	Olivacious green	Black center with pinkish surroundings	Cottony	Circular	No zonation	Zonation
RAN_{Ab}	Black	Black	Cottony	Circular	No zonation	No zonation
NAT_{Ab}	Black	Black center with	Cottony	Circular	Zonation	No zonation

		white surroundings				
NAO_{Ab}	Olivacious green	Greenish black	Velvety	Circular	Zonation	No zonation
LAL_{Ab}	Olivacious green	Light brown	Velvety	Circular	No zonation	Zonation
BOG_{Ab}	Black	Black	Cottony	Circular	No zonation	No zonation
RAJ_{Ab}	Olivacious green	Brownish green with pinkish surroundings	Cottony	Circular	Zonation	Zonation

Table.4: Conidial characteristics of Alternaria spp.

Isolates			**Septation (Range)**	
	From	**Colour**	Horizontal	Vertical
DHA_{Ab}	Muriform	Brown	5-9	0-1
GAZ_{Ab}	Muriform	Light brown	3-7	0-2
MYM_{Ab}	Muriform	Brown	5-7	2-3
PAB_{Ab}	Muriform	Deep Brown	5-7	1-2
RAN_{Ab}	Muriform	Deep Brown	5-7	2-3
NAT_{Ab}	Muriform	Light brown	7-11	2-3
NAO_{Ab}	Muriform	Deep Brown	5-8	1-3
LAL_{Ab}	Muriform	Brown	7-9	1-3
BOG_{Ab}	Muriform	Brown	7-11	0-3
RAJ_{Ab}	Muriform	Brown	5-9	1-2

colour. PAB_{Ab}. RAN_{Ab} and NAO_{Ab} show deep brown in colour. (Figure 2)

Variation in septation observed in isolates of *A.brassicae.* The horizontal septation varied from 3-7 to 7-11. The vertical septation varied from 0-1to 2-3. The maximum horizontal septation observed isolates BOG_{Ab} (7-11) and minimum septation observed in isolates GAZ_{Ab}.The maximum vertical (2-3) septation observed in isolates MYM_{Ab}, RAN_{Ab} and NAT_{Ab}. The minimum vertical(0-1) septation observed in isolate DHA_{Ab}.

3.3 Cultural variability of *Alternaria brassicae*

3.3.1 Radial mycelial growth of 10 isolates of *Alternaria* spp. on PDA

Radial mycelial growth of different isolates of *Alternaria* spp. significantly varied on PDA (Table 5 and Plate 1). After 2 days of inoculation the maximum radial mycelial growth of *A. brassicae* (30.50 mm) was observed in DHA_{Ab}, followed by PAB_{Ab} (27.00 mm). The minimum radial mycelial growth (14.67 mm) was recorded in NAO_{Ab} which was statistically similar to GAZ_{Ab} (17.33 mm).

After 4^{th} day, 6^{th} day, 8^{th} day, 10^{th} day and 12^{th} day of inoculation the maximum radial mycelial growth of *Alternaria* spp. were recorded in DHA_{Ab} which were 48.33 mm, 64.00 mm, 79.93 mm, 89.33 mm and 90.00 mm, respectively and the minimum radial mycelial growth were recorded in NAO_{Ab} which was 33.50 mm.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in DHA_{Ab} which was (90.00 mm), followed by PAB_{Ab} (88.33 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} which was (76.67 mm) which was statistically similar to MYM_{Ab} (79.67 mm).

3.3.2 Radial mycelial growth of 10 isolates of *Alternaria* spp. on CDA

After 2 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. 29.67 mm was measured in NAT_{Ab}, followed by RAJ_{Ab} (28.67 mm). The minimum radial mycelial growth was recorded in GAZ_{Ab} (17.67 mm) which was statistically similar to MYM_{Ab} (19.17 mm).

After 4^{th} days, 6^{th} days, 8^{th} days, 10^{th} day and 12^{th} days of inoculation the maximum radial mycelial growth of *A. brassicae* were measured in NAT_{Ab} which was 49.17 mm, 65.33 mm, 82.33 mm, 87.00 mm and 90.00 mm respectively and the minimum radial mycelial growth were recorded in GAZ_{Ab} 26.33 mm, 42.33 mm, 54.33 mm, 73.33 mm and 77.67 mm respectively.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in NAT_{Ab} **(**90.00 mm), which was statistically similar to RAJ_{Ab} which was 89.33 mm. The minimum radial mycelial growth was recorded in GAZ_{Ab} (83.67mm) which was statistically similar to MYM_{Ab} (85.33 mm).

3.3.3 Radial mycelial growth of 10 isolates of *Alternaria* spp. on PCDA

After 2 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in RAJ_{Ab} which was **(**33.17 mm) which was statically similar to RAN_{Ab} (31.83 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} which was (20.33 mm) which was statistically similar to MYM_{Ab} (23.33 mm).

Table.5: Radial mycelial growth of different isolates of Alternaria spp. at different days after incubation onPDA

Isolate	*2th Day*	*4th Day*	*6th Day*	*8th Day*	*10th Day*	*12th Day*	*14th Day*
DHAAb	*30.50 a*	*48.33 a*	*64.00 a*	*79.93 a*	*89.33 a*	*90.00 a*	*90.00 a*
GAZAb	*17.33 f*	*38.00 d*	*51.67 def*	*69.00 bc*	*76.33 cd*	*79.33 bcd*	*80.33 cd*
MYMAb	*20.33 e*	*41.83 bcd*	*50.50 ef*	*67.67 c*	*73.00 de*	*77.00 cd*	*79.67 cd*
PABAb	*27.00 b*	*42.33 bc*	*59.37 abc*	*73.33 abc*	*81.33 bc*	*82.67 abc*	*83.00 abcd*
RANAb	*23.33 d*	*45.67 ab*	*55.67 cd*	*70.57 bc*	*75.50 cd*	*78.33 bcd*	*82.67 bcd*
NATAb	*17.67 f*	*40.67 cd*	*56.33 bcd*	*73.67 abc*	*79.13 bcd*	*84.67 abc*	*85.00 abc*
NAOAb	*14.67 g*	*33.50 e*	*47.33 f*	*58.33 d*	*67.00 e*	*73.33 d*	*76.67 d*
LALAb	*21.00 e*	*41.67 cd*	*54.67 cde*	*76.67 ab*	*84.67 ab*	*82.67 abc*	*83.00 abcd*
BOGAb	*25.67 bc*	*43.50 bc*	*58.50 bc*	*75.83 ab*	*80.33 bcd*	*83.33 abc*	*85.00 abc*
RAJAb	*24.33 cd*	*40.83 cd*	*61.00 ab*	*74.93 abc*	*81.00 bc*	*86.33 ab*	*88.33 ab*
LSD (0.05)	*1.97*	*3.87*	*5.17*	*7.77*	*7.93*	*8.54*	*7.10*
CV (%)	*5.21*	*5.46*	*5.42*	*6.34*	*5.91*	*6.14*	*5.00*

Table.6: Radial mycelial growth of different isolates of Alternaria spp. at different days after incubation on CDA

Isolate	**2th Day**	**4th Day**	**6th Day**	**8th Day**	**10th Day**	**12th Day**	**14th Day**
DHAAb	**25.83 c**	**42.67 b-d**	**53.67 cd**	**64.83 ef**	**77.67 bc**	**80.00 de**	**86.67 ab**
GAZAb	**17.67 e**	**26.33 f**	**42.33 f**	**54.33 g**	**73.33 c**	**77.67 e**	**83.67 b**
MYMAb	**19.17 e**	**33.17 e**	**48.00 e**	**61.50 f**	**82.00 ab**	**83.67 b-d**	**85.33 ab**
PABAb	**26.67 bc**	**43.00 b-d**	**58.67 b-d**	**72.67 b-d**	**81.67 ab**	**85.33 a-d**	**87.33 ab**
RANAb	**22.00 d**	**38.83 d**	**53.17 de**	**69.77 de**	**81.67 ab**	**82.67 b-e**	**88.33 ab**
NATAb	**29.67 a**	**49.17 a**	**65.33 a**	**82.33 a**	**87.00 a**	**90.00 a**	**90.00 a**
NAOAb	**26.50 bc**	**42.67 b-d**	**58.83 bc**	**70.67 c-e**	**75.67 bc**	**85.33 a-d**	**87.67 ab**
LALAb	**26.00 c**	**42.00 cd**	**60.67 ab**	**75.33 b-d**	**76.67 bc**	**81.33 c-e**	**86.33 ab**
BOGAb	**27.33 a-c**	**46.50 ab**	**61.67 ab**	**76.33 a-c**	**82.33 ab**	**86.67 a-c**	**88.67 ab**
RAJAb	**28.67 ab**	**44.33 bc**	**63.33 ab**	**78.33 ab**	**85.33 a**	**88.33 ab**	**89.33 ab**
LSD (0.05)	**2.36**	**4.35**	**5.55**	**6.49**	**7.63**	**5.82**	**6.28**
CV (%)	**5.5**	**6.24**	**5.76**	**5.4**	**5.58**	**4.06**	**4.22**

Table.7: Radial mycelial growth of different isolates of Alternaria spp. at different days after incubation on PCDA

Isolate	**2th Day**	**4th Day**	**6th Day**	**8th Day**	**10th Day**	**12th Day**	**14th Day**
DHAAb	26.67 bc	45.20 cd	61.33 b	75.67 ab	85.00 ab	88.00 a-c	89.00 ab
GAZAb	26.67 bc	45.20 cd	62.30 ab	78.27 a	84.20 ab	87.67 a-c	88.00 ab
MYMAb	23.33 cd	42.33 de	52.33 c	69.33 bc	77.00 bc	81.67 c	85.33 c

PABAb	29.67 ab	50.00 a-c	63.50 ab	78.33 a	85.33 ab	88.67 ab	89.33 ab
RANAb	31.83 a	46.33 b-d	63.00 ab	76.00 a	83.33 bc	86.33 a	89.67 ab
NATAb	24.33 c	39.67 e	64.83 ab	76.10 a	84.83 ab	89.67 ab	90.00 a
NAOAb	20.33 d	33.83 f	48.33 c	64.67 c	70.67 c	74.67 d	79.00 d
LALAb	31.50 a	46.33 b-d	65.33 ab	78.67 a	83.00 ab	85.33 a-c	87.33 bc
BOGAb	31.00 a	50.67 ab	66.33 ab	79.00 a	84.33 ab	88.67 ab	90.00 a
RAJAb	33.17 a	54.83 a	68.17 a	80.27 a	88.33 a	90.00 a	90.00 a
LSD (0.05)	3.62	5.19	6.05	6.64	8.86	6.41	2.66
CV (%)	7.65	6.71	5.77	5.16	6.27	4.39	1.78

After 4th days, 6th days, 8th days, 10th days and 12th days of inoculation the maximum radial mycelial growth of *A. brassicae* 54.83 mm, 68.17 mm, 80.27 mm, 88.33 mm and 90.00 mm were measured in RAJ_{Ab} and the minimum radial mycelial growth were recorded in NAO_{Ab} 33.83 mm, 48.33 mm, 64.67mm, 70.67 mm and 74.67 mm.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* spp. was measured in RAJ_{Ab} 90.00 mm, which was statistically similar to BOG_{Ab} and NAT_{Ab}(90.00 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} (79.00 mm) proceeded by MYM_{Ab} (85.33 mm).

IV. DISCUSSION

A laboratory examination was carried out at Plant Pathology Laboratory of Oil seed Research Center, BARI, Joydevpur, Gazipur to find out morphological and physiological variation among ten different isolates of *Alternaria* spp. isolated from mustard leaf having typical symptoms of Alternaria blight.

Leaves of mustard having typical symptoms were collected from ten different location of Bangladesh and causal organisims were isolated on PDA medium. All the isolates produced light brown to deep brown murifrom conidia with beak. This finding was supported by previous findings [18] . They were also found murifrom conidia which were brownish black. Some researcher worked with *Alternaria* spp. and found murifrom, obclavate conidia with brownish black [28].

All 10 isolates showed variations in respect of their cultural and morphological characteristics on different media. In respect of cultural characteristics, the isolates of *Alternaria* spp. showed variation in mycial growth, colony color, shape, textures, subsurface color, zonation conidia production and sporulation time.

Remarkable effect of different culture media on radial mycelia growth was observed in *Alternaria* spp..Significant variation was found in colony color of *A. brassicae* on PDA medium. Most of the colony color of the isolates were olivacious green to black. The results are partially agreement with [33] who found that the colony color of *A. mali* isolated from apple was light to dark olivacious with greenish or brownish tinge. In case of *A.alternata* isolated from ribben plants colony colour black to olivaceous-black or grayish colour on PDA medium was found [24]. Thirty two isolates of *A brassicicola* for colony color and radial growth were observed by [6]. Colony colour of *A. brassicicola* varied from olive green to dark olivacious black on PDA.

All the isolates of *Alternaria* spp. colony had circular shaped. The results are in agreement with [38] were identified its morphological and cultural characters of *A.brassicae* isolates from four different locations, colonies of all the isolates were circular in shape.The colony shape of *A.solani* isolated from tomato plants were found circular margin with smooth surfaced colony[31]. The entire isolates colony had cottony and velvety texture on PDA medium. The results are in agreement with [3] examined 308 isolates of *Alternaria* spp. colonies generally had a cottony texture on group 4. Alternaria blotch, causal organism *A. mali*, colonies varied in their cultural behavior ranging from velvety to cottony [33]. Remarkable variation was observed on spore production and sporulation time on different media and temperature. Potato Carrot Media are found suitable for spore production and sporulation time for maximum isolate followed by CDA and PDA. This result was supported by the [23] found potato carrot broth are suitable for sporulation and spore production *A. brassicae*. Variation were found in mycial growth, sporulation in different nutrient media like Potato Dextrose Agar, Cauliflower Agar medium and Carrot Potato Agar good for 32 isolates of *A. brassicae*[33].

Variations were observed in accordance with length, breadth and beck on different isolates of *A. brassicae* on PDA media. The length of conidia of different isolates

varied from 41.56µm to 117.54µm. The breadth of conidia of different isolates varied from 10.34 µm to 23.12 µm. The beck of conidia of different isolates varied from 16.78 µm to 72.65 µm. The horizontal septation varied from 3-7 to 7-11. The vertical station varied from 0-1to 2-3. This result are partially supported with [28] define *A. brassicae* length of conidia varied from 96 µm -114 µm, breadth varied from 17 µm -24 µm and beck length varied from 45 µm-65 µm and transverse and longitudinal septation varied from 10-11 and 0-6 respectively. 322 isolates of *A. brassicae* variation was recorded among conidial length, breadth and beak length which range of 51.4-481.2 µm, 6.9-36.0 µm and 16.3 - 266.9 µm respectively [16]. Average numbers of horizontal septa were 9.7, vertical septa were 0.8. The horizontal septation of 5 different isolates of *A.brassicae* varied from 4-13 and vertical from 0-6[33]. 23 isolates of *A. brassicae* were collected and found maximum length of conidia ranged from 150 - 122 µm with 8 - 9 transverse and 2 vertical septation [27] Eight isolates of *A. solani* were examined [25] and found average conidial size (L×B) was 42.18×15.18 µm and beck size was 13.10µm. In ten isolates of *A. macrospora* size of conidia ranged from 20.81-56.23 x 9.2- 27.10 µm with 1 - 6 transverse and 0 - 4 longitudinal septa were found [14]. ten isolates collected by[26] of *A. alternata* the length and width of conidia were varied from 30.99 -42.47 µm and 11.90-17.37 µm respectively. All isolates produced both beaked and unbeaked conidia. The beak length of conidia varied from 18.7-23.81 µm. Alternaria blotch, causal organism *A. mali* 21 isolates of *A. mali* were collected from different locations. Average conidial size ranged from 21.36 to 31.74 x 8.34 to 14.48 µm. Among the isolates of *A.mali* size of conidia 19–50 µm ×5–9 µm in nature and 20–59 µm ×8–13 µm in culture, with 3–8 transverse septa and usually no longitudinal septa or only 1 longitudinal septa were found [33].

V. CONCLUSION

Rapeseed Mustard (*Brassica spp.*) is the principal oil-producing crop of Bangladesh and Alternaria leaf blight caused by *Alternaria brassicae*, is one of the major disease of rapeseed mustard. This research was conducted to find out existence of physiological races of *Alternaria* spp. causing Alternaria leaf blight of mustard on the basis of cultural and morphological aspects. The experiment was laid out in the completely randomized design with three replications. Ten isolates of *Alternaria* spp. were collected from ten different mustard growing districts of Bangladesh. Three different media and two different temperatures were used to measure growth and development of *Alternaria* spp..

All the 10 isolates showed variation in the terms of cultural and morphological characteristics. Among three different culture media, potato carrot agar medium at 25˚C showed the best performance in the terms of radial mycelial growth.

Colour of the colonies of *Alternaria* spp. showed variation among ten isolates. Olivacious green to black color colony developed on PDA medium. All the isolates produced Circular colony and the texture were cottony to velvety. All isolates showed compact type compactness. Variation also observed between surface and sub surface colour. Surface colour varied from light brown to deep brown. Subsurface colour varied from light brown to black and pinkish. Zonation was present both surface and subsurface in some isolates and some isolates showed no zonation on both side.

Effect of media on sporulation significantly differed among the isolates. The highest number of conidia production was recorded 48.17 to 59.79 × 10^6/ml was counted RAJ_{Ab}on Potato Carrot media at 25˚C temperature. Of all the isolates of *Alternaria* spp. with maximum in isolate RAJ_{Ab} and minimum in NAO_{Ab}. Temperature showed an influence on sporulation. (Data not shown).

Effect of media on sporulation time differed significantly among the isolates. The minimum days (4 days) required for sporulation in PCDA followed by CDA.

Remarkable variation among different *Alternaria* spp. isolates were observed in length, breadth and beak size of the conidia. The conidial length varied within a range of 41.56µm to 117.54µm and the breadths were varied from 10.34 µm to 23.12 µm. All isolates were muriform and deep brown to light brown in colour with a beak length of 16.78 µm to 72.65 µm.

On the basis of the above results and discussion it can be summarized that- variability exists in the pathogen of alternaria leaf blight caused by *Alternaria* spp. prevailing in the rapeseed mustard growing areas of Bangladesh. Potato Carrot agar medium and 25˚C temperature were appeared to be the best medium and temperature respectively for the mycelial growth and sporulation of this fungal pathogen. More research should be conducted on molecular characterization of this isolates to find out the phylogenetic relationship.

VI. ACKNOWLEDGEMENTS

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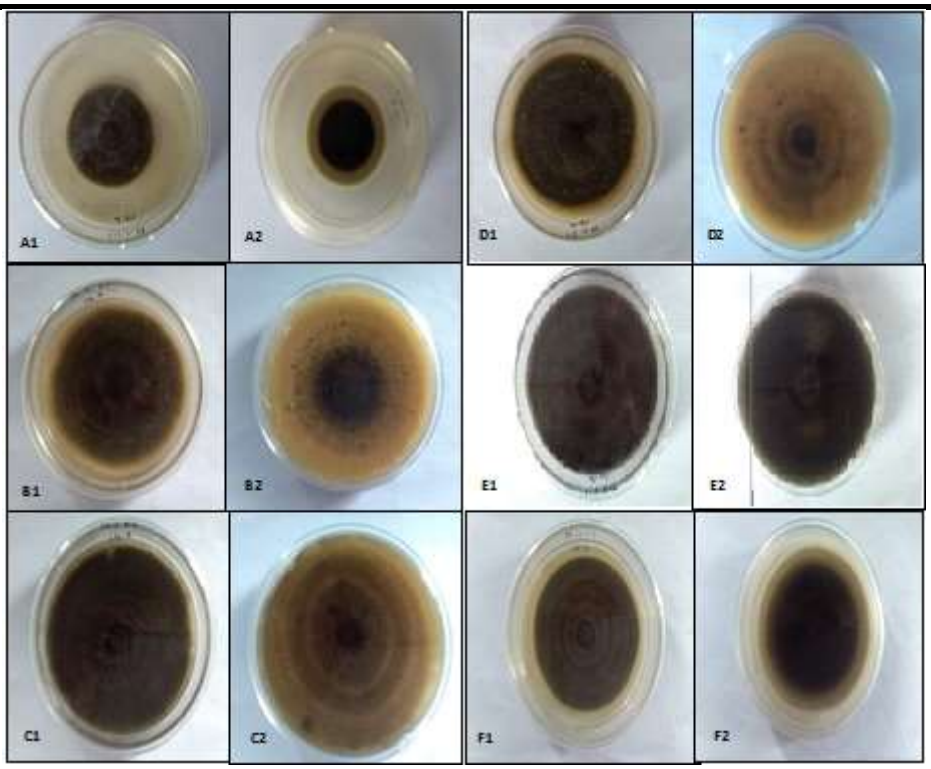

Plate.1: Colony charecters of different isolates of A. Brassicae on PDA media
1. Surface 2. Sub-surface
A. DHA_{Ab} B. GAZ_{Ab} C. MYM_{Ab} D. PAB_{Ab} E. RAN_{Ab} F. NAT_{Ab}

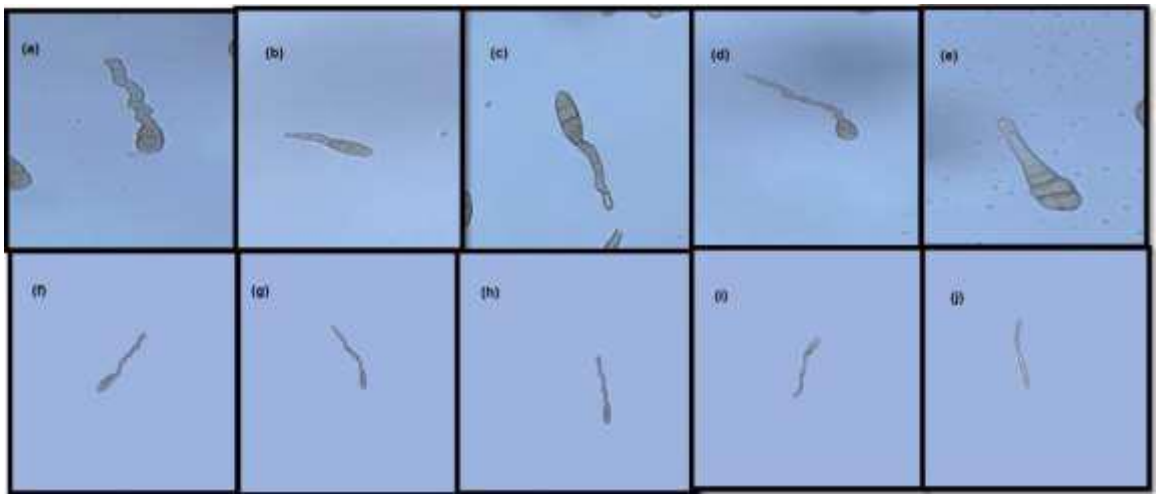

Plate.2: Conidial characteristics of A.brassicae A. DHA_{Ab} B. GAZ_{Ab} C. MYM_{Ab}
D. PAB_{Ab} E. RAN_{Ab} F. NAT_{Ab} G. NAO_{Ab} H. LAL_{Ab} I. BOG_{Ab} J. RAJ_{Ab}

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Diabetes Mellitus Type 2: A New Sour-Milk Product for Prevention and Treatment

U.A. Zhumabayev[1], R.S. Naimanbayeva[2], N.O.Ibragimova[3], O.U.Agabek[4], AU Issayeva[5]*

[1,2,4] International Kazakh-Turkish University named after Ahmed Yasavi, Turkestan, Kazakhstan
[3]Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan
[5]M.Auezov South-Kazakhstan State University, Shymkent city, Kazakhstan

Abstract— *Based on camel milk, a new multicomponent, specialized fermented dairy bio-product "Inulakt-Fito" was developed for the prevention and treatment of type 2 diabetes mellitus. Its expressed hypoglycemic, antioxidant effect was established in experimental alloxan diabetes.*

Keywords— sour-milk bioproduct, diabetes mellitus, antioxidant protection.

I. INTRODUCTION

Disease of diabetes is one of the most serious in modern endocrinology. According to ethnoecological research, type 2 diabetes mellitus at the early stages can be cured completely with the help of phytotherapeutic drugs (Ashcroft F.M. and Rorsman P., 2004, Abdel-Zaher A.O. et al., 2005, Andrade-Cetto A. et al., 2005, Eidi A. et al., 2006). Now several hundred food and medicinal plants are known that can reduce blood glucose levels (Esmaeili M.A. and Yazdanparast R., 2004, Arambewela L.S. et al., 2005, El-Demerdash F.M. et al., 2005). However, the mechanism of the sugar-reducing effect of medicinal plants has not yet been elucidated (Johnson L. et al., 2006). One of the reasons for the complexity of managing patients with diabetes mellitus is the unsatisfactory correction of high glucose in the blood with exogenous insulin and other antidiabetic drugs, which requires finding additional ways to optimize the level of glycemia (Knowler W.C. et al., 2002, Ashcroft F.M. and Rorsman P., 2004, Wild S. et al., 2006). For this purpose, not only medicines are often used, but also various natural compounds for which the effect on carbohydrate metabolism is shown. The huge variety of plant material, the need to take into account its complex effects, the individual approach to the state of health of a diabetic person, the presence of possible contraindications or complications - all this requires careful testing first in model experiments. So, it is extremely important to search for and preclinical trials of plant material with subsequent clinical studies to create valid recommendations for the pharmacological industry.

In sour-milk products, many of the nutrients of milk become more accessible: so proteolytic enzymes of milk microflora, partially break down proteins, which increases the completeness and speed of their assimilation (Mal G. et al., 2007, Shabo Y. et al., 2008, Abbas S, et al., 2013). Whey milk proteins of camel milk are considered biologically active substances and some of them possess anti-carcinogenic, antioxidant and immunostimulating properties (Al Haj O.A. et al.,2010, Yadav A. K. et al., 2015).

The most promising direction for inclusion in the diet of sweeteners as a substitute for sugar is the use of the products of processing stevia plants (*Stevia Rebaudiana Bertoni*), a natural sweetener of non-carbohydrate nature, possessing unique therapeutic and prophylactic and improving properties.

On the basis of the foregoing, to date, relevant are: research, development and creation of a new sour-milk biopreparation, in combination with medicinal extracts, which in the future is a unique therapeutic and prophylactic biopreparation that have no analogues in the world's dairy and pharmaceutical industries.

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II. MATERIAL AND METHODS

The experiment was performed on sexually mature rats weighing 180-210 g. of both sexes, grown in a vivarium with a standard diet at the Scientific Research Institute of Fundamental and Applied Medicine named after B. Atchabarov of the Kazakh National Medical University named after S.D. Asfendiyarov. Control animals were grown under the usual diet regime, without the administration of alloxan. The maintenance, care of animals and deducing of them from experiment were carried out according to (Frode T.S. and Medeiros Y.S., 2008).

Induction of diabetes in the animals studied was caused by intraperitoneal injection of a 5% solution of alloxan monohydrate (AL) at a rate of 100 mg / kg of the animal's weight in a 0.9% solution of NaCl (Methodical recommendations, 1986). All laboratory animals were

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previously starved for 24 hours, while access to water was not restricted. The Inulakt-Fito sour milk product was injected intragastrically at the experimental therapeutic dose of 1000 mg / kg once a day throughout the experiment. The indices of the group of animals receiving the Inulakt-Fito bio-product were compared to the group of animals treated with metformin at a dose of 500 mg / kg. The studies were performed 7, 14 and 28 days after the start of alloxan administration. The level of glucose in the blood was determined by the Bionime Rightest GM300. Also in the blood serum standard biochemical methods were used to study lipid and nitrogen metabolism using a set of Lahema reagents (Czech Republic). Catalase activity was determined according to a previously reported method(Abei H., 1974, Qujeq D. and Rezvani T., 2007.), the content of malonic dialdehyde in blood serum - according to the method (Kuntz E. and Kuntz H.D., 2006)

The statistical processing of the results was carried out using the computer program SPSS (Statistical Package for the Social Sciences).

III. RESULTS AND DISCUSSION

It is known that in the pathogenesis of diabetes mellitus one of the key links is the activation of processes of free radical oxidation: an imbalance occurs between pro-oxidants and antioxidants, leading to an excess of free radicals and the accumulation of products of free radical oxidation. The constant background of impaired prooxidant-antioxidant balance in the body in diabetes mellitus is one of the causes of death of pancreatic ß-cells and structural functional units of other organs, which causes the development of multiple organ dysfunction (Zhao Y.F. et al., 2005, Masiello, P., 2006). The mechanism of diabetic action of alloxan is also associated with its damaging effect through the formation of free radicals. In particular, it was shown that alloxane deposited in ß-cells due to its interaction with zinc generates the formation of O2-, OH-, H_2O_2. Formed free radicals and peroxide enter into a chain reaction of interaction with molecules of fatty acids of cell membranes, destroying them (Federiuk, I.F. et al., 2004) . The natural consequence of the decrease in the physiological action of insulin, due to its deficiency due to the destruction of a large number of β-cells of the pancreas, is hyperglycemia(*Table 1*).

Table.1: Influence of Inulakt-Fito on biochemical blood indices in alloxan diabetes in experimental rats

Indicators	**Groups of animals (n = 36)**			
	Intact	Control (Alloxan)	Experimental 1 (Alloxan + nulakt-Fito)	Experimental 2 (alloxan+ metformin)
7th day				
Glucose, mM / l	6,01 ± 0,33	9,63 ± 0,10	6,81 ± 0,58*	7,52 ± 0,09*
ALT, mE/ L	131,3 ±42,5	191,1±79 ,2	162,4±12 ,6 *	170,0±30,2 *
АСТ, мЕ/л	618,5 ±179, 0	1076,6±4 46,5	915,1±10 2,3*	936,6±93,2 *
Cholesterol, mM / l	0,63± 0,03	2,73±0,1 4	1,43±0,1 6*	1,45±0,06*
Urea, mM/ l	4,43± 0,07	12,63±1, 09	7,91±0,6 5*	6,92±0,68*
Creatinine, mmol / l	73,15 ± 1,22	173,27±9 ,50	129,02±5 ,20*	113,17±7,4 5*
14th day				
Glucose, mM / l	6,01 ± 0,33	12,40 ± 0,14	8,06 ± 0,08*	9,92 ± 0,09*
ALT, mE/ L	131,3 ±42,5	175,2±69 ,6	142,9±11 ,2*	154,7±29,3 *
АСТ, мЕ/л	618,5 ±179, 0	966,0±44 4,6	814,4±98 ,6*	852,3±86,1 *
Cholesterol, mM / l	0,63± 0,03	2,36±,25	1,38±0,1 1*	1,30±0,2*
Urea, mM/ l	4,43± 0,07	10,65±1, 7	5,42±0,7 *	4,53±0,36*
Creatinine, mmol / l	73,15 ± 1,22	149,35±7 ,60	86,36±4, 83*	92,6±5,3*
28th day				
Glucose, mM / l	6,48± 0,64	21,9 ± 0,22	9,85 ± 0,10*	13,9 ± 0,14*
ALT, mE/ L	131,3 ±42,5	171,3±10 0,3	132,8±9, 6*	143,8±22,9 *
АСТ, мЕ/л	618,5 ±179, 0	778,6±37 5,9	757,3±80 ,6*	770,1±92,3 *
Cholesterol, mM / l	0,63± 0,03	1,99± 0,67	1,13± 0,08*	1,15± 0,13*
Urea, mM/ l	4,43± 0,07	8,90± 0,53	3,18± 0,18*	3,56± 0,15*
Creatinine, mmol / l	73,15 ± 1,22	130,16± 8,50	77,60± 4,12*	83,80± 4,50*
NOTE - * Hereinafter, the difference is significant compared to the control group at P≤ 0.05				

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The administration of alloxan to rats is accompanied by a significant increase in the level of glucose in the blood, an increase in the cholesterol, creatinine and urea in the blood serum, which indicates a violation of carbohydrate and lipid metabolism, as well as a decrease in the functional state of the liver and kidneys.

Course introduction of rats with alloxan diabetes "Inulakt-Fito" was accompanied by normalization of carbohydrate metabolism. In particular, by the 7th day of observation, the blood glucose content under the effect of the tested bioproduct decreased by 41%, serum cholesterol concentration was reduced by 90%, urea by 59%, creatinine by 34%, alanine aminotransferase (ALT) - by 15%, aspartate aminotransferase (AST) - by 15%. By the 14th day of observation, the tendency towards normalization of biochemical parameters of blood persisted. Thus, the glucose level in the blood decreased compared to the parameters in the control by 23%. By the 28th day of observation, the blood glucose content under the action of the tested bioproduct was 41% lower than in the control group, and normalization of lipid and nitrogen metabolism was also observed in experimental diabetes mellitus in rats. The drug metformin also had a beneficial effect on the course of alloxan diabetes, but in a less pronounced degree than Inulakt-Fito.

Table 2 presents data characterizing the influence of Inulakt-Fito on the state of lipid peroxidation (LPO) indices and antioxidant protection in conditions of damage to the pancreas. As can be seen from *Table 2*, the introduction of this diabetogen has caused the development of "oxidative stress", characterized by an increase in the intensity of LPO, as evidenced by a significant increase in the level of malonic dialdehyde (MDA) and a decrease in the activity of antioxidant protection - there was a significant inhibition of catalase activity.

Thus, with alloxan diabetes in experimental rats on the 7th day in serum, the content of malonic dialdehyde is significantly increased 2.8 times, in the pancreas 56%. By the 14th day after the administration of alloxan, the content of lipid peroxidation products in blood of animals with alloxan diabetes increased, the content of malonic dialdehyde was 3.1 times, in the pancreas - by 78%. By 28 days with alloxan diabetes, respectively, quadrupled by 60% compared with the indices in animals of the intact group. Along with the activation of lipid peroxidation processes in alloxan diabetes in rats, the antioxidant defense of the organism decreases. In particular, reduced catalase activity of blood serum in animals of the control group by the 7th day of observation is 1.5 times, by the 14th day - by 1.7 times, by 28 days by 2.2 times. The application of Inulakt-Fito resulted in a significant improvement in the state of all the studied indicators. In particular, the MDA content in the blood serum decreased by 1.8 times in 7 days compared to the control. After 14 days, -58%, 28 days after the start of alloxan administration to rats in the blood serum, the MDA content was 72% higher than in the animals in the control group. The same regularity was noted in the evaluation of data on the content of lipid peroxidation products in the pancreas.

Table.2: Influence of Inulakt-Fito on LPO processes and blood catalase activity in alloxan diabetes in experimental rats

Indicators	**Groups of animals (n = 36)**			
	Intact	Control (Alloxan)	Experimental 1 (Alloxan + Inulakt-Fito)	Experimental 2 (alloxan + metformin)
7 th day				
MDA in serum, µM / ml min	2,35 ± 0,10	6,72 ± 0,33	4,52 ± 0,52*	5,72 ± 0,48*
MDA in the pancreas, nM / g	5,53 ± 0,60	8,62 ± 0,80	6,00 ± 0,60*	7,20 ± 0,20*
Catalase, mcd / l	21,32 ± 2,01	14,00 ± 1,32	18,2 ± 1,81*	16,52 ± 1,63*
14 th day				
MDA in serum, µM / ml min	3,27 ± 0,15	10,28 ± 0,11	3,95 ± 0,55*	4,92 ± 0,65*
MDA in the pancreas, nM / g	5,53 ± 0,60	9,85 ± 0,10	8,00 ± 0,80*	8,85 ± 0,90*
Catalase, mcd / l	21,32 ± 2,01	12,01 ± 0,80	18,62 ± 1,81*	15,41 ± 1,54*
28 th day				
MDA in serum, µM / ml min	3,85 ± 0,40	15,4 ± 0,16	4,86 ± 0,50*	7,47 ± 0,75*
MDA in the pancreas	5,55 ±	8,90 ± 0,90	6,02 ± 0,60*	7,80 ± 0,80*

, nM / g	0,60			
Catalase , mcd / l	2 1,35 ± 2,01	9,6 ± 0,85	17,08 ± 0,2*	13,2 ± 0,13*
NOTE - * Hereinafter, the difference is significant compared to the control group at P≤ 0.05				

When the animals were injected with Inulakt-Fito, the activity of blood catalase increased according to the observation periods by 35, 55 and 78%, as compared to the values in the control. The metformin comparison drug also inhibited lipid peroxidation processes, but to a lesser extent.

IV. CONCLUSION

As a result of studies on the basis of shubat (camel milk) in combination with 5 sugar-reducing medicinal extracts, a new multi-component, specialized fermented dairy bio-product "Inulakt-Fito" (conditional name) was designed for the prevention and treatment of type 2 diabetes mellitus. The pharmacotherapeutic effect of Inulakt-Fito on the parameters characterizing the course of experimental diabetes was established. This is due to its hypoglycemic and antioxidant properties and depends on the complex effects of its biologically active substances. The data obtained make it possible to consider the use of Inulakt-Fito in the complex treatment of type 2 diabetes.

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Antimicrobial and antioxidant activities of salt stress callus of Brinjal *(Solanum melongena* L.)

K. Kalimuthu[1*], A.Vanitha[1], Vajjiram Chinnadurai[1], R. Prabakaran[2]

[1]Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, India.
K_kalimuthu@rediffmail.com, avanithagreen@gmail.com, chinnaa.v1987@gmail.com.
[1]Department of Botany, PSG College of Arts and Science, Coimbatore–641014, India.
raju.prabakaran@gmail.com.

Abstract— *Ethanolic and methanolic salt stress callus extracts of Solanum melongena L. were tested for in vitro antimicrobial and free radical scavenging assayssuch as DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS$^+$(2,2'Azinobis (3-ethyl benzo-thizoline-6-sulfonic acid) . In both the extracts the zone of inhibition is higher in Escherichia coli, Klebsiella pneumonia, Staphylococcus aureusand Streptococcus pyogenesat 90 µl concentration against the control. The antifungal activity of these extracts also the zone of inhibition is higher at 90 µl concentration against the control. The DPPH activity of different concentration of solvent extracts (1 mg/ml to 5 mg/ml) along with standard ascorbic acid among the five different concentration (50 µg/ml to 250 µg/ml) of extracts tested, the higher percentage of inhibition was observed in 250 µg/ml of methanol extract followed by ethanolic extract against the standard ascorbic acid. In ABTS$^+$ activity the absorbance was increased with the increasing concentrations of both methanolic and ethanolic callus extracts.*

Keywords—Antioxidant, , Inhibition, In vitro, Stress tolerant callus, Solanum melongena.

I. INTRODUCTION

Plants have been an important source of medicine, mainly on traditional remedies for thousands of years [1]. The bioactive substances present in plants have wide range of biological functions including antioxidant and antimicrobial activities [2,3]. Environmental stresses strongly influence plant growth and development. Through biotechnology tools, the production of virus free plants, salinity tolerance, herbicide resistance, frost resistant is passible [4]. These stress agents influence on biosynthesis of secondary metabolites and resulting in considerable fluctuations in quality and quantity. The composition of secondary product may vary within the same plant. Generally, the efficacy of the plant depend on the combined effect of plant metabolites rather than the few fractions separated from the plant. This leads to select a plant *S. melongena* L. for the changes in biochemical activities under salt stress.

Brinjal or Eggplant (*Solanum melongena* L.) is an important vegetable crop belong to the family Solanaceae. The family contain 75 genera and around 2000 species. In India, it is represented by 21 genera and 70 species. Brinjal is an herbaceous perennial plant but cultivated as annual. In popular medicine, brinjal is indicated for the treatment of several diseases, including diabetes, arthritis, asthma and bronchitis. In addition, that brinjal extracts have a significant effect in reducing blood and liver cholesterol in humans [5,6]and adult rats [7]. Nasunin, a major component of anthocyanin pigment of brinjal, has been shown to inhibit lipid peroxidation [8]. Free radical scavenging and iron chelating activities of nasunin were demonstrated by electron spin resonance [9]. Furthermore, anti-mutagenic activity of pheophytin components from brinjal fruit extracts acting against several chemical mutagens was reported [10]. The unripe fruit of brinjal is primarily used as a cooking vegetable for the various dishes in different regions of the World. It has much potential as raw material in pickle making and dehydration industries [5,6].

Understanding the importance of salt tolerance in crop plants such as brinjal, the present work is to focus on the production of salinity tolerance brinjal callus through *in vitro* culture technology and to analyze antioxidant and antimicrobial activity of ethanol and methanol extracts of *in vitro* salt callus.

II. MATERIALS AND METHODS

2.1 Preparation of salt callus extracts

The leaf derived 40 days old 20 gram of powdered brinjal salt callus was successively extracted using 50 ml of ethanol and methanol by using the Soxhlet extractor for 8-10 hrs [11]. The extract was filtered through Whatmann No.1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent. The respective extracts were concentrated in vacuum (Rota vapor) and

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the residues from the ethanol and methanol extracts were weighed and stored in sealed vials in a freezer until tested.

2.2 Antimicrobial activity

2.2.1 Test microorganisms

The test organisms used were clinical isolates *viz., Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*and the human fungal pathogens like *Candida albicans* and *Trichoderma viride*, which were obtained from Department of Microbiology, Hindusthan College of Arts and Science Coimbatore. The bacterial and the fungal cultures were maintained on nutrient agar medium at 37˚C and potato dextrose agar (PDA) medium at 28˚ C respectively.

2.2.2 Preparation of Inoculum

The gram positive bacteria *Streptococcus pyogenes*, *Staphylococcus aureus* and gram negative bacteria *E. coli* and *Klebsiella pneumoniae* were pre-cultured in nutrient broth overnight in a rotary shaker at 37° C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculums *Candida albicans*, *Trichoderma viride* were prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ml.

2.2.3 Antibacterial Activity [12]

The *in vitro* salt callus extracts of *S. melongena* were tested by the well diffusion method. Different concentration of the extracts (30, 60, and 90 µg/ml) was prepared by reconstituting with ethanol and methanol. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Streptomycin (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm.

2.2.4 Antifungal Activity [13]

The antifungal activity of ethanol and methanol *in vitro* salt callus were tested by well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 20, 40 and 60µg/ml concentrations of the extracts were placed on test organism-seeded plates. Streptocycline (10 µg well 1) used as positive control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

2.3 Anti-oxidant activity

2.3.1 DPPH Radical Scavenging Activity

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at wavelength 517 nm [14]. Various concentrations of ethanol and methanol extracts of the sample (0.52.5 mg/ml) were mixed with 1.0 ml of ethanolic and methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture were shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as control. The percentage of inhibition in DPPH radical scavenging activity was calculated as follows

$$\% \text{ Inhibition} = A_0 - A_1/A_0 \text{ X } 100.$$

2.3.2 $ABTS^+$ Radical scavenging activity

$ABTS^+$ decolourisation assay involves the generation of the $ABTS^+$ chromophore by the oxidation of $ABTS^+$ with potassium persulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the leaf extracts on $ABTS^+$ radical cation was measured at 734 nm[15].

$ABTS^+$ solution: Equal volume of 7 mM of $ABTS^+$ was mixed with 2.45 mM potassium persulphate and the mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. $ABTS^+$ solution was diluted to an absorbance of 0.7 ± 0.05 with ethanol and methanol 734 nm. The reaction was initiated by the addition of 1.0 ml of diluted $ABTS^+$ to 10 µl of different concentrations (50 - 250 µg / ml) of leaf extract and also to 10 µl of ethanol and methanol as control. Ascorbic acid was used as positive control. The absorbance was read at 734 nm after 6 minutes and the percentage inhibitions were calculated. The inhibition was calculated according to the equation,

$$I = A_0 - A_1/A_0 \text{ x } 100,$$

Where, A_0 is absorbance of control reaction, A_1 is absorbance of test compound.

III. RESULTS

3.1 Antimicrobial activity

The antimicrobial activity of ethanolic and methanolic extracts of *S. melongena* salt callus against various microbial strains with respect to various concentrations (30 – 90µg/ml) were presented in the table 1. In *S. melongena* salt callus ethanolic extract, the zone of inhibition of test concentrations were compared with standard concentration of control (Streptocycline 10 µg/ml). Plate. 1A, B, C, and D shows significant result of different concentration of extract and the control. Among the four different bacteria used (*E.coli,K. pneumonia,S.*

aureus, S. pyogenes) in the case of *E.coli* the zone of inhibition is higher (13.60±0.20 mm) in 90 µg/ml concentration (Plate. 1A) against the control (08.50±0.17 mm10µg/ml), followed by 60 µg/ml concentration (09.66±0.17 mm). In the case of *S. aureus* the zone of inhibition is higher in 90 µg/ml concentration (12.70±0.17 mm) against its control (10mm/ 10µg/ml) (Plate. 1C) followed by 60 µg/ml concentration (07.66±0.14 mm). In *K. pneumonia* also the maximum inhibition zone (11.83±0.12 mm) was observed in 90 µg/ml concentration followed by 60 µg/ml (06.56±0.26 mm) (Plate. 1B) against the control (07.50±0.23 mm). At 90 µg/ml of concentration the zone of inhibition is higher (09.56±0.14 mm) followed by 60 µg/ml concentration (06.56±0.20 mm) in *S. pyogenes* against control (10.53±0.20 mm) (Plate. 1D, Table 1).

In methanolic extract among the four different bacteria used, in the case of *S. aureus* the zone of inhibition is higher in 90 µg/ml concentration (12.63±0.14 mm) against its control (10mm/ 10µg/ml) (Plate. 2C) followed by 60 µg/ml concentration (07.50±0.20 mm). In the case of *E.coli* the zone of inhibition is higher (12.50±0.17 mm) in 90 µg/ml concentration (Plate. 2A) against the control (8.73±0.08 mm1 µg/ml), followed by 60 µg/ml concentration (8.46±0.14 mm). In *K. pneumonia* also the maximum inhibition zone (12.13±0.26 mm) was observed in 90 µg/ml concentration followed by 60 µg/ml (8.53±0.12 mm) (Plate. 2B) against the control (8.80±0.11 mm) respectively. At 90 µg/ml of concentration the zone of inhibition is higher (11.23±0.23 mm) followed by (8.63±0.17 mm) at 60 µg/ml in *S. pyogenes* against control (10.63±0.14 mm) (Plate. 2D, Table 1).

The human fungal pathogens like *Candida albicans* and *Trichoderma viride*, the zone of inhibition was observed in ethanolic extracts of salt callus compared with standard drug and presented in Table 1. Among the two different fungal used, in the case of *C. albicans* the zone of inhibition is higher (8.63±0.21 mm) in 90 µg/ml concentration (Plate. 1E) against the control (8.63±0.12 mm1 µg/ml), followed by 60 µg/ml concentration (5.66±0.24 mm). In the case *T. viride* the zone of inhibition is higher in 90 µg/ml concentration (6.80±0.15 mm) against its control (8.70±0.15 mm/ 10µg/ml) (Plate. 1F) followed by 60 µg/ml concentration (5.56±0.08 mm).

In methanolic extracts *C. albicans* the zone of inhibition is higher (8.73±0.21 mm) in 90 µg/ml concentration (Plate.2E, Table 1) against the control (8.43±0.23 mm10µg/ml), followed by 60 µg/ml concentration (5.56±0.26 mm). In the case *T. viride* the zone of inhibition is higher in 90 µg/ml concentration (8.56±0.24 mm) against its control (7.76±0.18 mm/ 10µg/ml) (Plate. 2F) followed by 60 µg/ml concentration (4.53±0.21 mm).

3.2 Antioxidant activity

3.2.1DPPH activity

The antioxidant activities of *Solanum melongena* ethanolic and methanolic NaCl salt derived callus extracts were assessed by using DPPH and ABTS activity. The DPPH activity of different concentration of solvent extracts (1 mg/ml to 5 mg/ml) along with standard ascorbic acid is presented in the table 2. Among the five different concentration (50 µg/ml to 250 µg/ml) of extracts tested, the higher percentage of inhibition (52.25 ± 0.41) was observed in 250 µg/ml of methanolic extract followed by ethanolic extract (50.30 ± 0.28), against the standard ascorbic acid (61.53 ± 0.11) (Fig.1). The minimum DPPH activity 6.34 ± 0.09 and 8.12 ± 0.40 was noticed in 50 µg/ml concentration of ethanolic and methanolic extract respectively. The dose titration curves allowed determination of IC_{50} for the ethanolic and methanolic callus extracts towards DPPH scavenging activity. The extracts demonstrated dose dependent DPPH scavenging activity effects with IC_{50} values 248.50 µg/ml, 239.23 µg/ml and 203.15 µg/ml in ethanolic, methanolic extracts and standard ascorbic acid respectively (Fig. 1). The result showed that the both ethanolic and methanolic extracts possess almost similar potent scavenging activity of the stable free radical DPPH.

3.2.2 $ABTS^+$ Scavenging Assay

$ABTS^+$ activity of ethanolic and methanolic extracts of *S. melongena* NaCl salt stressed callus were assayed by using five different concentrations (50 µg/ml to 250 µg/ml). The result of the percentage of inhibition solvent extracts is presented in table 3 and figure 4. The absorbance was increased with the increasing concentrations of both methanolic and ethanolic callus extracts. In this study more inhibition (59.25± 0.49 and 54.90 ± 0.54) was observed in the concentration of 250 µg/ml of methanolic and ethanolic extracts respectively. It is followed by 200µg/ml concentration with 51.48 ± 0.69 and 45.32 ± 0.14 inhibition in methanolic and ethanolic extracts respectively, where as in standard ascorbic acid the absorbance was 72.10 ± 0.47 in 250 µg/ml concentration and 59.10 ± 0.20 in 200 µg/ml concentration (Table 3, Fig. 2). The IC_{50} values of ethanolic and methanolic extracts determined by the values presented in fig 2. IC_{50} values of ethanolic, methanolic and standard ascorbic acid is 227.68 µg/ml, 210.97µg/ml and 169.20µg/ml respectively (Fig. 2). The inhibition value of methanolic and ethanolic salt induced callus extract was almost equal.

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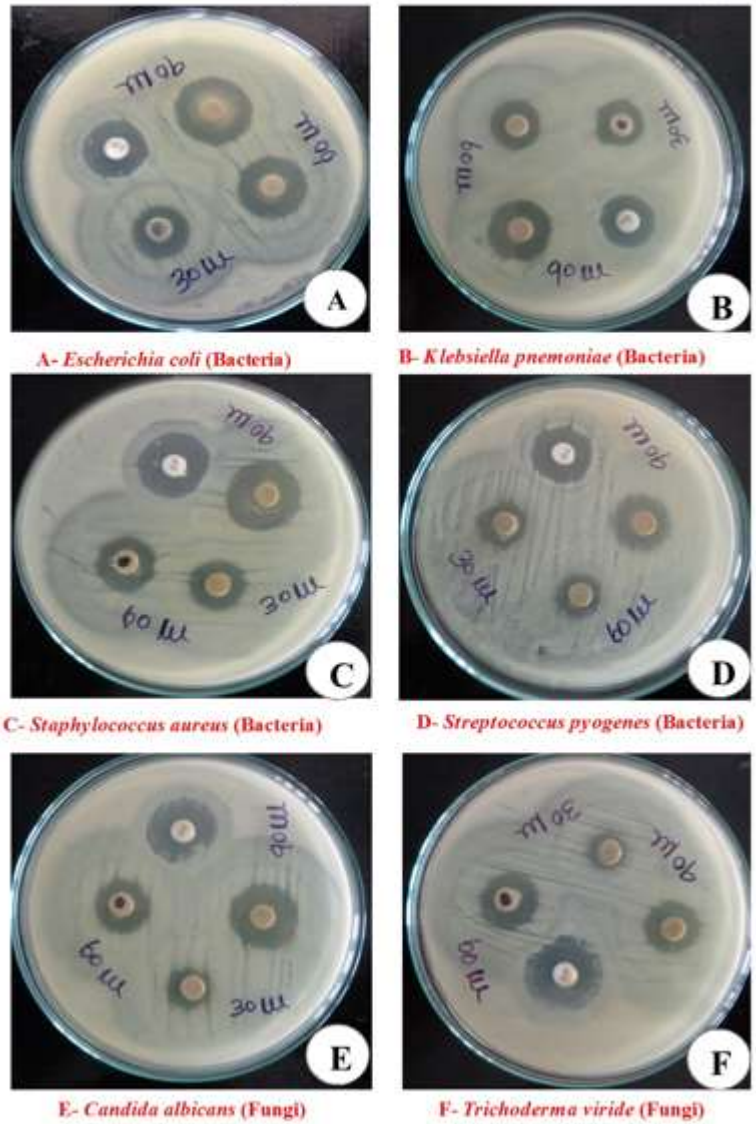
Table.1: Antimicrobial activity of ethanolic and methanolic extracts of Solanum melongena L. callus

S. No	Pathogenic Microbes	Ethanol extract Zone of inhibition (mm)			Standard (Streptocycline)	Methanol extract Zone of inhibition (mm)			Standard (Streptocycline)
		30 µl	60 µl	90 µl		30 µl	60 µl	90 µl	
1.	Escherichia coli	6.53±0.20	9.66±0.17	13.60±0.20	8.50±0.17	6.63±0.20	8.46±0.14	12.50±0.17	8.73±0.08
2.	Klebsiella pneumoniae	5.56±0.24	6.56±0.26	11.83±0.12	7.50±0.23	5.66±0.14	8.53±0.12	12.13±0.26	8.80±0.11
3.	Staphylococcus aureus	6.76±0.14	7.66±0.14	12.70±0.17	10.46±0.14	6.46±0.17	7.50±0.20	12.63±0.14	10.53±0.20
4.	Streptococcus pyogenes	5.46±0.17	6.56±0.20	9.56±0.14	10.53±0.20	6.40±0.15	8.63±0.17	11.23±0.23	10.63±0.14
5.	Candida albicans	3.60±0.26	5.66±0.24	8.63±0.21	8.63±0.12	3.73±0.21	5.56±0.26	8.73±0.21	8.43±0.23
6.	Trichoderma viride	3.63±0.21	5.56±0.08	6.80±0.15	8.70±0.15	3.53±0.23	4.53±0.21	8.56±0.24	7.76±0.18

Plate: 1 Antimicrobial activity of ethanolic extract of *Solanum melongena*



A- *Escherichia coli* (Bacteria) B- *Klebsiella pnemoniae* (Bacteria)

C- *Staphylococcus aureus* (Bacteria) D- *Streptococcus pyogenes* (Bacteria)

E- *Candida albicans* (Fungi) F- *Trichoderma viride* (Fungi)

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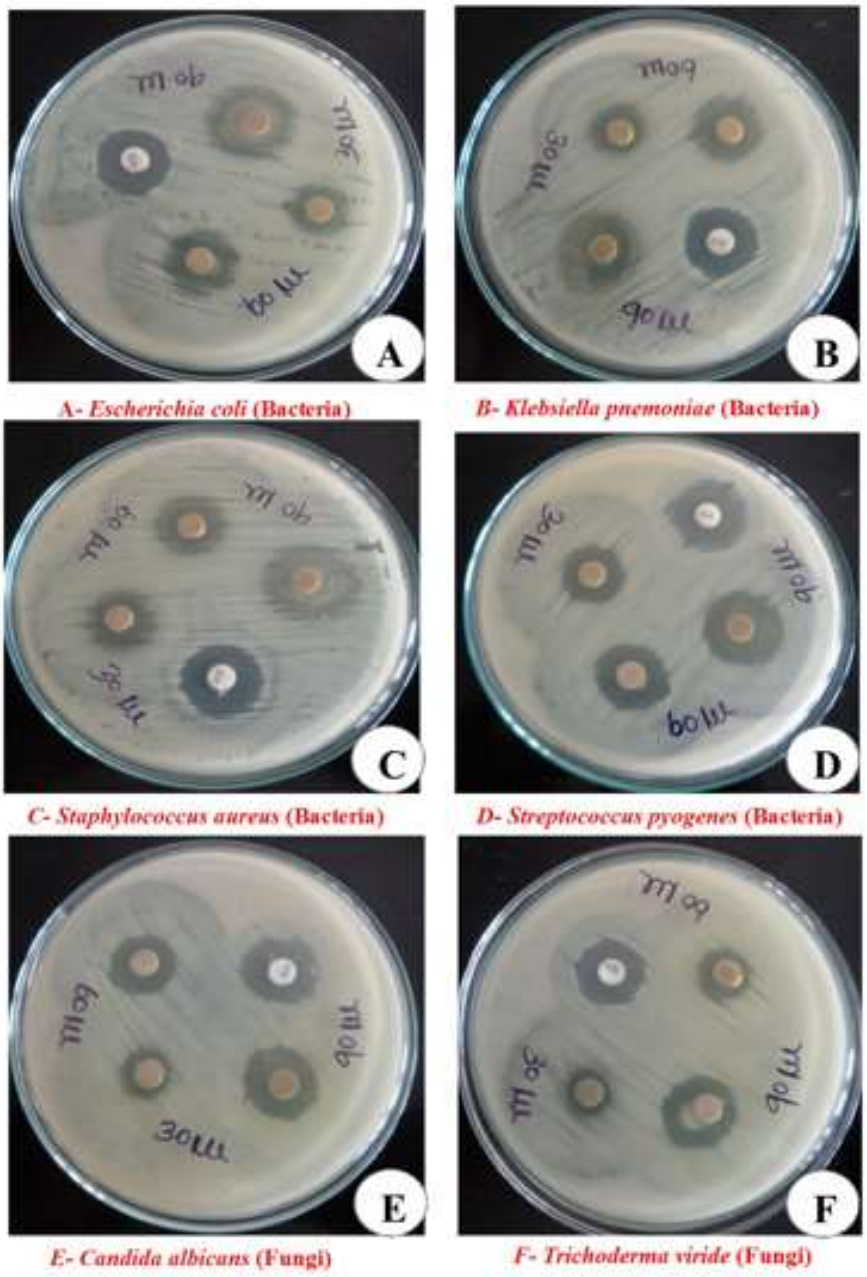
Plate 2- Antimicrobial activity of methanolic extract of *Solanum melongena*



A- Escherichia coli (Bacteria) *B- Klebsiella pneumoniae* (Bacteria)

C- Staphylococcus aureus (Bacteria) *D- Streptococcus pyogenes* (Bacteria)

E- Candida albicans (Fungi) *F- Trichoderma viride* (Fungi)

Table.2: DPPH activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena L.

S. No.		% inhibition					
		50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	IC_{50} Value(µg/ml)
1	Ethanol extract	6.34 ± 0.09	14.64 ± 0.37	22.13 ± 0.38	33.54 ± 0.61	50.30 ± 0.28	248.50
2	Methanol extract	8.12 ± 0.40	16.19 ± 0.52	28.67 ± 0.70	37.29 ± 0.27	52.25 ± 0.41	239.23
3	Ascorbic acid	16.56 ± 0.15	25.76 ± 0.92	37.30± 0.58	49.21 ± 0.20	61.53 ± 0.11	203.15

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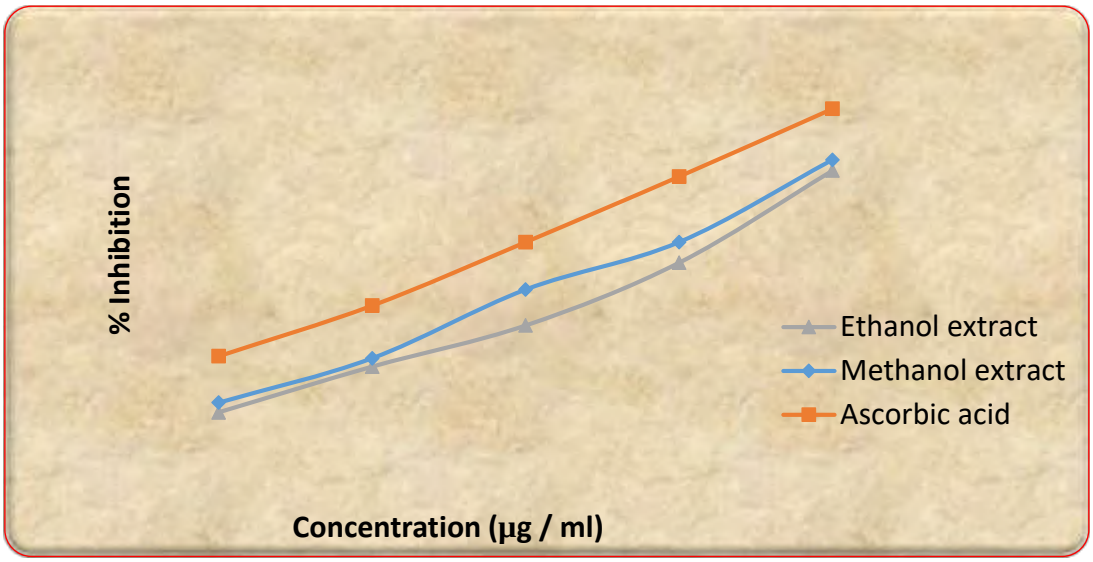

Fig.1:DPPH activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena L.

IC_{50} value of Ethanol extract : 248.50 µg/ml
IC_{50} value of Methanol extract : 239.23 µg/ml
IC_{50} value of Ascorbic acid (standard) : 203.15 µg/ml

Table.3: ABTS$^+$ activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena

S. No.		% inhibition					
		50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	IC 50 Value(µg/ml)
1	Ethanol extract	11.99 ± 0.09	20.61 ± 0.80	33.85 ± 0.19	45.32 ± 0.14	54.90 ± 0.54	227.68
2	Methanol extract	14.28 ± 0.54	26.10 ± 0. 47	39.45 ± 0.61	51.48 ± 0.69	59.25± 0.49	210.97
3	Ascorbic acid	21.45 ± 0.67	33.39 ± 0.29	47.71 ± 0.62	59.10 ± 0.20	72.10 ± 0.47	169.20

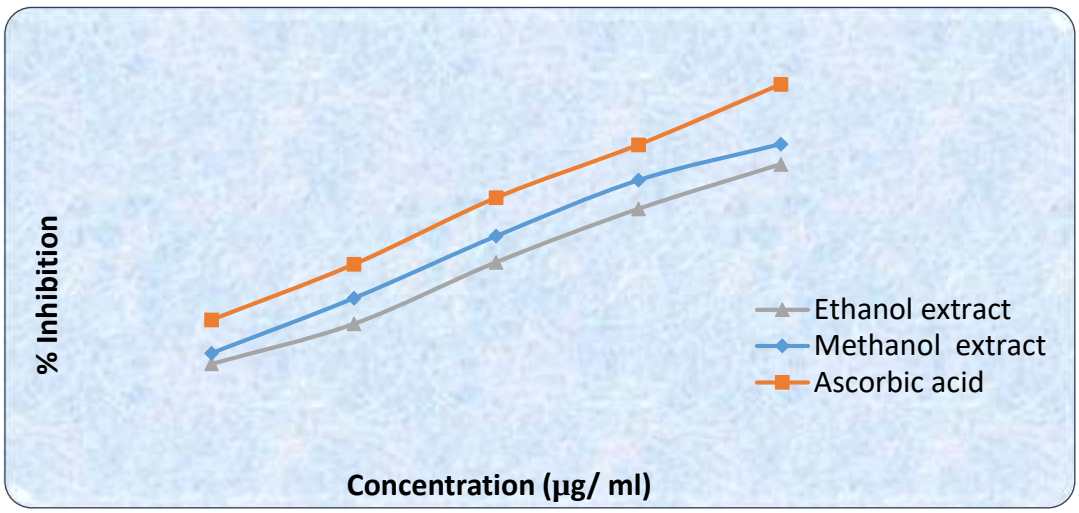

Fig.2: ABTS$^+$ activity of different concentration of ethanolic and methanolic leaf callus extracts of Solanum melongena

IC_{50} value of Ethanol extract : 227.68 µg/ml
IC_{50} value of Methanol extract : 210.97 µg/ml
IC_{50} value of Ascorbic acid (standard) : 169.20 µg/ml

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IV. DISCUSSION

Plants are employed as important source for traditional medications [16]. It is important to study scientifically, plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compounds [17]. Secondary metabolites like alkaloids, glycosides, steroids, flavonoids are potential sources of drugs present in medicinal plants.Moreover the natural antioxidants including carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols are the secondary metabolites produced by plants for their sustenance. The bioactive substances like Beta-carotene, ascorbic acid and alpha tocopherolare the free radical scavengers with enhanced potential. Natural antioxidants are the vegetables play a significant role in reducing the risk of certain types of cancer, cardiovascular diseases and other chronic diseases [18]. The question of subjecting medicinal herbs to modern scientific test has often been raised. Biosynthesis of secondary metabolites is affected strongly by salt stress resulting in considerable fluctuations in quality and quantity.

Production of secondary metabolites by callus culture have made it possible for the increased yield of a wide variety of pharmaceuticals such as alkaloids, terpenoids, steroids, saponins, phenolics, and flavonoids [19]. Environmental stresses strongly influence plant growth and development. Salinity is one of the most important of these stresses and can limit crop yield [20]. Today, 20% of the World's cultivated land and nearly half of all irrigated lands are affected by salinity [21]. Salt stress has become one of the most damaging environmental hazards to crop productivity all over the world [22].

The cultivation of medical plants using different growth regulators to enhance the production of bioactive compounds is required for commercial and research application. Bioactive compounds were found to be accumulating in culture cells at higher level than those in natural plants though optimization of culture conditions [23]. Free proline and phenol increased exponentially with the increase in NaCl level was reported in *Solanum nigrum*[24].

Antimicrobial effects of ethanolic and methanolic extracts salt callus of *S. melongena* showed good antimicrobial activities against human pathogens. Whereas another reports observed in the leaves of eggplant had antibacterial activity on gram negative only [25]. Whereas, the methanol extract of *S. melongena* seed gave maximum inhibition against *Staphylococcus aureus* and *Escherichia coli.* It is quite obvious that the inhibition level gradually increases in accordance with the level of increase in concentration. Similar observation also found in our results. However, in contrary same methanolic extract of *S. melongena* seed did not have any inhibitory activity against *Pseudomonas aeruginosa*and *Proteus vulgaris* [26].

The steroidal glycoalkaloids are the family of secondary metabolites produced by Solanaceous plants, including potato, tomato and eggplant[27]. Steroidal glycoalkaloids have antimicrobial, insecticidal and fungicidal properties which provide resistance against several insect pests and herbivores[28]. In the present study both the extracts showed good antimicrobial activity. This may be due to the presence of glycoalkaloids present in this family.

In addition to the support our results the mangrove (salt watered growing) plant *Avicennia marina* extracts showed good antimicrobial activity against *E. coli*, *S. aureus*, and *B. subtilis.* The result of present study for antibacterial activity agrees with leaf extract of mangrove plants[29,30,31]. Our reports revealed that the salt stressed *S. melongena* callus extracts shows more antimicrobial activity compared with normal brinjal plant extracts[32].

The DPPH radical were used to study the scavenging activity of some natural compounds. The results of scavenging DPPH radical ability of *S. melongena* at different concentration in comparison with standard ascorbic acid showed in the figure 1. In DPPH scavenging activity assay the IC_{50} value of ethanolic, methanolic and ascorbic acid was 248.50 µg/ml, 239.23 µg/ml and 203.15 µg/ml respectively. The extracts of *S. melongena*showed dose dependent DPPH radical scavenging activity. These findings shows that there is a strong relationship between the secondary metabolites and antioxidant activity of plant materials. Our findings are comparable with earlier reports of *S. melongenain vivo* plant extracts[33] and *S. surathense* leaf extract [34]. The DPPH scavenging activity antioxidants is due to the reaction between antioxidant molecules and radical, which occur by donating the hydrogen during the scavenging of the radical. Our*in vitro* callus extract results were comparable that of *in vivo* plant extract of *S. melongena*and *S.surathense*[33,34].

The decolorization of $ABTS^{+}$cation radical is a way to measure the antioxidant activity of extracts. Positive correlation between phenolic content and antioxidant activity was reported by Awika*et.al.* 2003[35]. Polyphenols are the major plant compounds with antioxidant activity. The activity of phenolic compounds is mainly due to their redox properties[36,37]which can do an main role in free radical absorbing and neutralizing, singlet and triplet oxygen quenching or peroxides decomposing. Result of the present study revealed that ethanolic and methanolic extracts possess superior antioxidant activities. Alcoholic extracts of *S. melongena* showed potent ABTS radical scavenging activity with IC_{50} value of 227.68 and 210.97 µg respectively. Many scientists reported the presence of steroids, terpenoids,

flavonoids, phenolic compounds, tannins in various extracts of *S. melongena*[38,39,40,41]. These reports confirmed that both ethanolic and methanolic extracts showed high level of antioxidant activity in *in vitro* system.

V. CONCLUSION

The presence of various bioactive compounds in the both ethanolic and methanolic extracts of salt stressed callus of *S. melongena* justifies that, the salt stress have been induced to produce strong bioactive compounds. However, isolation of individual phytochemical constituents and subjecting it to the biological activity from salinity tolerance callus will definitely give fruitful results. The results, shows that the salt stressed *S. melongena* callus contains various bioactive compounds. Therefore, it is concluded that *in vitro* clonal propagation with salt stress could alter the biochemical changes which could be a phytopharmaceutically and morphopotencially importance. These reports confirmed that both ethanolic and methanolic extracts showed high level of antimicrobial activity and antioxidant activity in *in vitro* system.

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Records of Arthropod Species Sampled from Avocado Plant (*Persea americana* Mill) in Small-scale Agro-ecosystems at Taita Hills and Mount Kilimanjaro

Odanga J. James[3,2,1*], Florence Olubayo[2], Richard Nyankanga[2], Sizah Mwalusepo[1, 4], Tino Johansson[1, 5]

[1]ICIPE-African Insect Science for Food and Health, P. O. Box 30772 - 00100, Nairobi, Kenya
[2]Department of Plant Science and Crop Protection, University of Nairobi, Kenya, P.O. Box 30197-00100, Nairobi, Kenya
[3]Invertebrate Zoology Section, National Museums of Kenya, P. O. Box 40658 - 00100, Nairobi, Kenya
[4]Department of General Studies, Dar es Salaam Institute of Technology, P. O. Box 2958, Dar es Salaam, Tanzania
[5]Department of Geosciences and Geography, University of Helsinki, P. O. Box 68, FI-00014, Finland

Abstract— *Avocado, Persea americana Mill, plays a central role in distribution of both beneficial and detrimental arthropods thereby influencing local species diversity in agro-ecosystems adjacent to Afromontane forests at Mount Kilimanjaro in North-eastern Tanzania and Taita Hills in South-eastern Kenya. However, little is known about arthropod species that inhabit avocado trees in the two study areas despite the fact that the crop forms the major part of agro-ecosystem in the East African highlands. A novel survey was, therefore, carried out for two years between August 2012 and July 2014 to establish arthropod species in avocado orchards along South-eastern slopes of both Mount Kilimanjaro and Taita Hills. A total of sixty one species of arthropods were recorded from the avocado crop through fruit observation and canopy sampling. The present arthropod checklist provides baseline knowledge for scientists in evaluating beneficial and pest status of each species inhabiting avocado plant in the East African agro-ecosystems.*

Keywords—Avocado, arthropods, East Africa, Mount Kilimanjaro, Taita Hills.

I. INTRODUCTION

Avocado, *Persea americana* Mill (Lauraceae), is an important crop in the world as it enhances both agro-forestry conservation concept and nutritional security (Griesbach, 2005; Bergh, 1992). The avocado trees thrives well in agro-ecosystems with relatively high altitude between 1000m a.s.l and 2600m a.s.l that receive average annual precipitation ranging from 120mm to 160mm and average temperature of 21^0C (Griesbach, 2005; Wasilwa *et al*., 2004; Whiley, 2002). Such ecosystems are located near indigenous forests with favourable agricultural conditions as exemplified by Afrotropical highlands at Mount Kilimanjaro and Taita Hills where avocado is the dominant fruit crop (Griesbach, 2005; Wasilwa *et al*., 2004). However, the potential land area available for avocado farming along slopes of Mount Kilimanjaro in North-eastern Tanzania and Taita Hills in South-eastern Kenya is shrinking as a result of ecological degradation (Conte, 2010) and human activities. The envisaged reduction of avocado orchards in these East African highlands will not only affect distribution of arthropod species but also livelihood of local farmers that depend on avocado fruits as a source of cash and nutritious food (Hemp, 2009).

Eight five percent of avocado production in Kenya and Tanzania is at small scale level with number of trees per farm varying from three to twelve where the crop is grown mainly for subsistence and local markets (Griesbach, 2005; Wasilwa *et al*., 2004). Unlike commercial plantations, the small-holder avocado cropping systems do not utilized modern-day agricultural technology leading to poor farming practices, possible increase in arthropod pests and reduction of related natural enemies (Ware *et al*., 2016; Ware *et al*., 2012; Mwatawala *et al*., 2009, Griesbach, 2005; Bale *et al*., 2002; Bergh, 1992). Moreover, there is limited information on arthropods inhabiting avocado crop in Kenya and Tanzania. This paper was, therefore, initiated to provide a checklist of arthropods sampled from different parts of avocado crop in farmlands at Mount Kilimanjaro and Taita

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Hills. Establishing checklist of arthropods inhabiting avocado plant in East Africa can furnish important baseline information on pest and beneficial status of each species

II. MATERIALS AND METHODS

2.1. Study areas

The study was carried out in avocado farmlands adjacent to East African montane forests at Mount Kilimanjaro in North-eastern Tanzania and Taita Hills in South-eastern Kenya (Fig 1a and b, respectively). Avocado crop is grown in the two study transects along altitudinal gradient from 900 to 2000m a.s.l. and form major part of agro-ecosystem in the region. Mount Kilimanjaro and Taita Hills are the first uppermost elevated montane forms inland from the Indian Ocean and these highlands are important catchment areas for surrounding lowland areas of Moshi and Voi in Tanzania and Kenya, respectively (Hemp 2006a; Hemp 2006b; Bytebier, 2001; Bennun & Njoroge, 1999). The two study areas; Taita Hills and Mount Kilimanjaro, are situated 90km apart and both are about 150km from Indian Ocean. Mount Kilimanjaro study area is located between 03^0 378`S, $37^0$ 450`E and 03^0 481`S, $37^0$ 456`E in North-eastern Tanzania (Fig 1a). Mean elevation of Mount Kilimanjaro study area is 1372.69m a.s.l, average annual rainfall was 107.83mm, an average annual temperature of 20.14ºC with a mean annual humidity of 78.97%. Taita Hills study area is located in South-eastern Kenya, 25km west of Voi town in the Taita-Taveta County between 03^0 481`S, $38^0$ 378`E and 03^0 402`S, $38^0$ 296`E (Fig 1b). Mean elevation of Taita Hills study area is 1397.02m a.s.l, average annual rainfall was 135.19mm; mean annual temperature was 19.56ºC with a mean annual humidity of 81.46%.

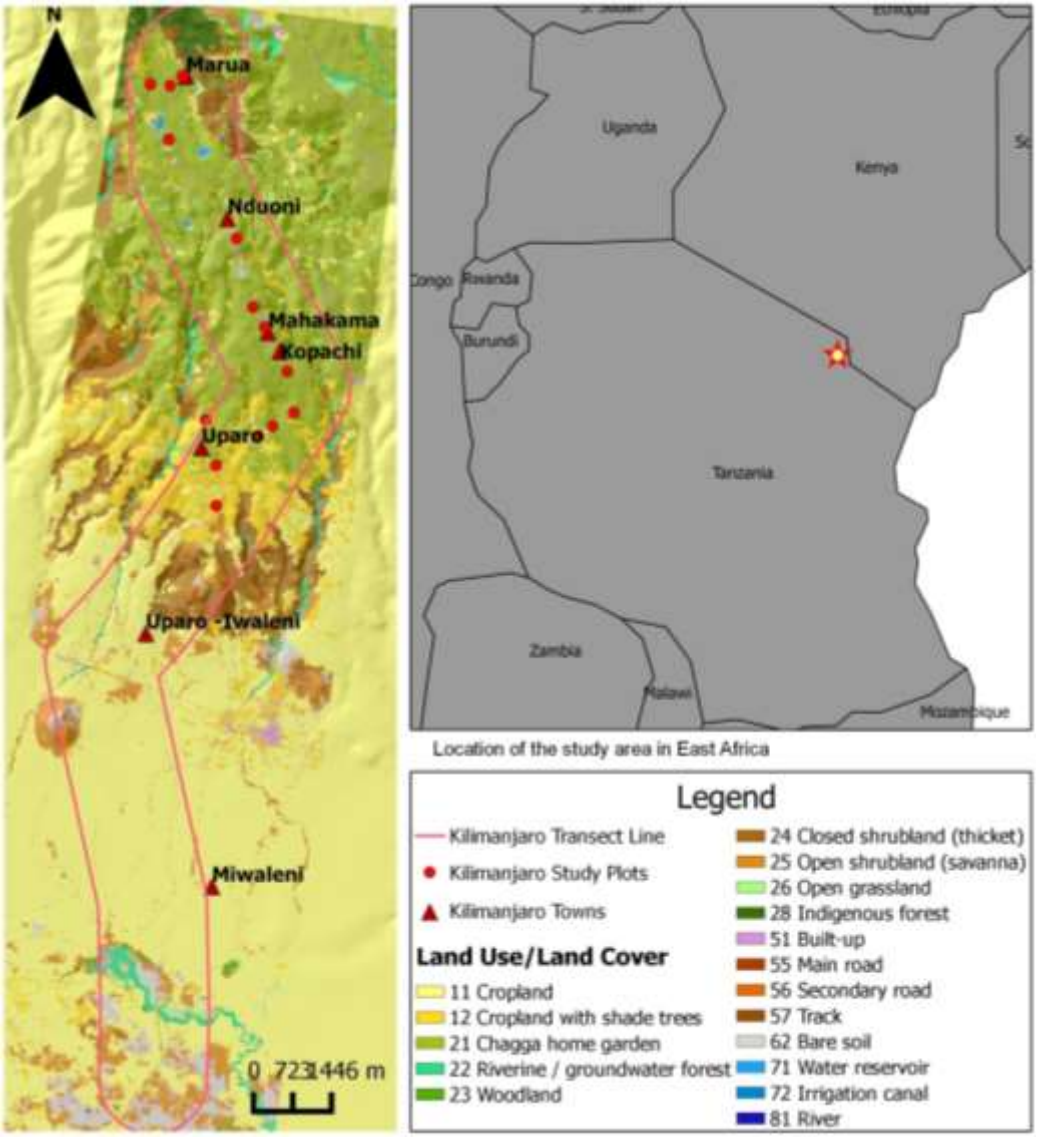

Fig.1a: Map of Mount Kilimanjaro study area in North-eastern Tanzania.

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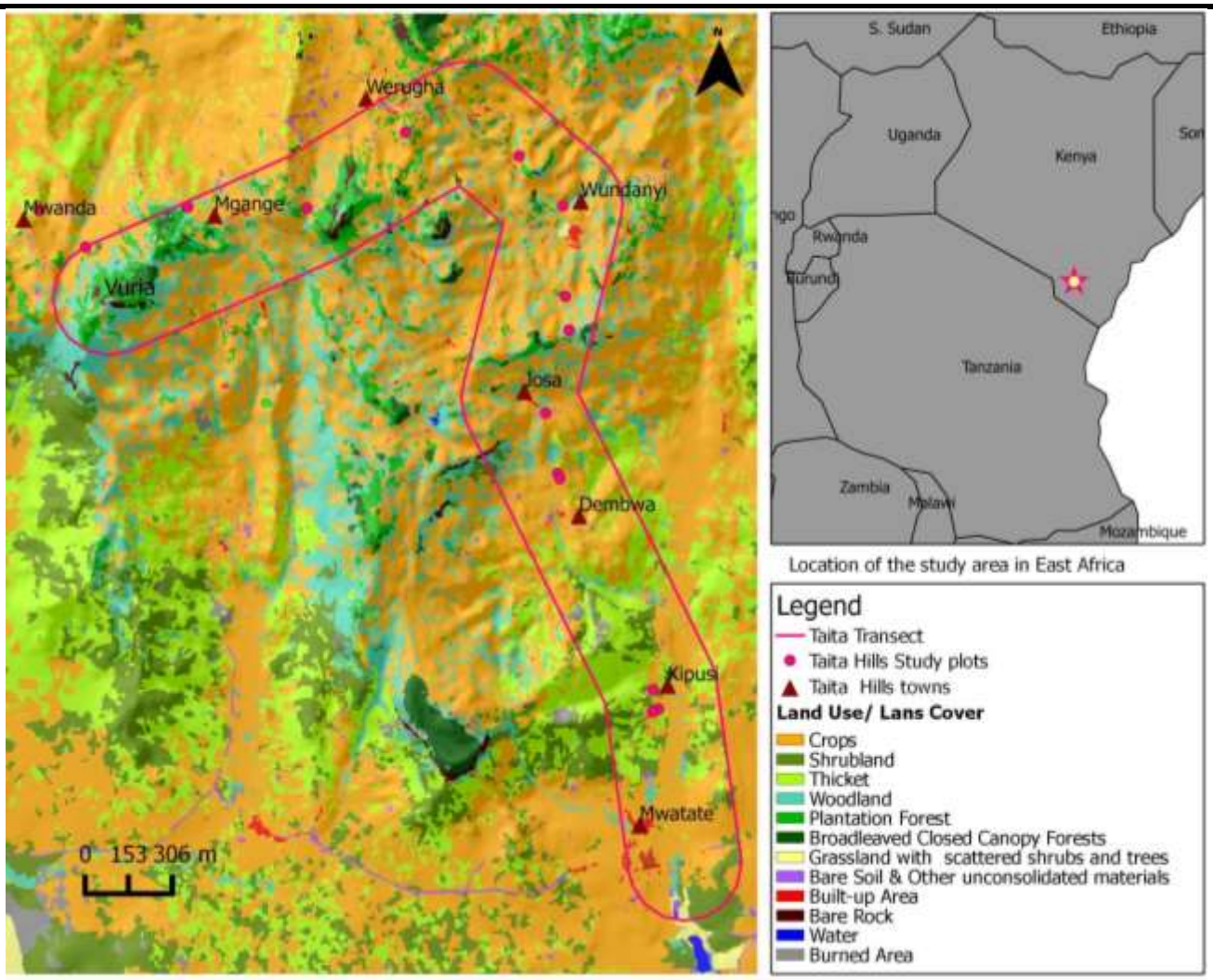

Fig.1b: Map of Taita Hills study area in South-eastern Kenya.

2.2. Sampling design

Species of arthropods were sampled randomly from avocado plants for two consecutive years between August 2012 and July 2014 along each study transect; Mount Kilimanjaro and Taita Hills. A transect comprised of fifteen blocks with each block consisting of at least a hundred avocado trees. During each survey, five avocado trees from the hundred sampling unit of the plants at each block were randomly examined for arthropods using protocol described by Ekesi *et al* (2006), Stibick (2006), Palmer (1990) and Moritz *et al* (2013). Leaves and flowers were gently shaken on a tray to sample species that inhabits plant parts as described by Palmer (1990). However, avocado fruits were observed for other arthropod species as described by Ekesi *et al* (2006). Some species were handpicked from the avocado the plant using fine forceps and aspirator following protocols described by Millar *et al* (2000). The collected specimens were preserved in vials containing 60% ethyl alcohol and later taxonomically identified at the National Museums of Kenya (NMK) entomology laboratory in Nairobi.

2.3. Statistical analysis

Rank abundance test was used to categorize arthropod species based on their population using Biodiversity-R software (R Development Core Team, 2012). Species accumulation curves were used to compare if the observed species richness at two study areas along slopes of Mount Kilimanjaro and Taita Hills reach saturation point (R Development Core Team, 2012). In order to check for the completeness of sampling, observed numbers of species were compared with projected ones (R Development Core Team, 2012). The estimated species richness was constructed for each study area using non-parametric

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estimators; Chao, Jackknife 1 and Bootstrap (R Development Core Team, 2012; Crawley, 2007; Crawley, 2005).

III. RESULTS

A total of sixty one species of arthropods were recorded inhabiting avocado plants in the farmlands adjacent to Afromontane forests within Mount Kilimanjaro in North-eastern Tanzania and Taita Hills in South-eastern Kenya. Further analysis using Rank abundance test revealed that most abundant arthropod species in avocado cropping systems at Mount Kilimanjaro and Taita Hills were; *Bactrocera* (*invadens*) *dorsalis* (Hendel) (Diptera: Tephritidae), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae), *Frankliniella schultzei* Trybom (Thysanoptera: Thripidae) and *Heliothrips haemorrhoidalis* Bouche (Thysanoptera: Thripidae) (Table 1).

*Table.1: Arthropod species recorded on avocado crop in the two study areas of Taita Hills and Mount Kilimanjaro for two years between August 2012 and July 2014. T = Taita Hills, K = Mount Kilimanjaro study area where the species were sampled. * = equals or less than 0.1 log abundance.*

Rank	Scientific name	Common name	Plant part sampled	Log abundance	Order	Habitat sampled
1	*Bactrocera* (*invadens*) *dorsalis*	Asian invasive fruit fly	Ground collected ripened fruits	5.9	Diptera	T and K
2	*Thaumatotibia leucotreta*	False codling moth	Immature fruits	4.9	Lepidoptera	T and K
3	*Frankliniella schultzei*	common blossom thrips	Flowers	3.2	Thysanoptera	T and K
4	*Heliothrips haemorrhoidalis*	Greenhouse thrips	Leaves and young fruits	2.9	Thysanoptera	T and K
5	*Megalirothrips sjostedti*	Cowpea flower thrips	Flowers and leaves	2.8	Thysanoptera	T and K
6	*Thrips austarlis*	Western flower thrips	Flowers and leaves	2.6	Thysanoptera	T and K
7	*Thrips pusillus*	Thrips	Flowers and leaves	2.5	Thysanoptera	T and K
8	*Aleyrodicus dispersus*	Spiralling whitefly	Leaves	2.4	Hemiptera	T and K
9	*Haplothrips gowdeyi*	Thrips	Flowers and leaves	2.4	Thysanoptera	T and K
10	*Cheilomenes sulphurea*	Ladybird beetle	Flowers and leaves	2.3	Coleoptera	T and K
11	*Pheidole megacephala*	Sugar ant	Flowers and leaves	2.2	Hymenoptera	T and K
12	*Rhinocoris* sp.	Assassin bug	Flowers and leaves	2.1	Hemiptera	T and K
13	*Trialeurodes vaparariorum*	Greenhouse whitefly	Leaves	2	Hemiptera	T and K
14	*Tetranychus* sp.	Red spider mite	Flowers and leaves	1.8	Trombidiformes	T and K
15	*Bactrothrips* sp.	Thrips	Flowers and leaves	1.7	Thysanoptera	T and K
16	*Nezara viridula*	southern green stink	Flowers and leaves	1.7	Hemiptera	T and K

		bug				
17	*Helopeltis schoutedeni*	Mirid (plant bugs)	Flowers and leaves	1.7	Hemiptera	T and K
18	*Cheilomenes lunata*	Ladybird beetle	Flowers and leaves	1.7	Coleoptera	T and K
19	*Thrips abyssiniae*	Thrips	Flowers and leaves	1.7	Thysanoptera	T and K
20	*Franklinothrips* sp.	Thrips	Flowers and leaves	1.6	Thysanoptera	T and K
21	*Proboscidocoris fuliginosus*	Bugs	Flowers and leaves	1.6	Hemiptera	T and K
22	*Franklinothrips megalops*	Predatory thripsand mimics ant	Flowers and leaves	1.5	Thysanoptera	T and K
23	*Dolicholepta jeanneli*	Thrips	Flowers and leaves	1.4	Thysanoptera	T and K
24	*Gynaikothrips*sp.	Thrips	Flowers and leaves	1.3	Thysanoptera	T and K
25	*Chilothrips frontalis*	Thrips	Flowers and leaves	1.3	Thysanoptera	T and K
26	*Dendrothrips* sp.	Thrips	Flowers and leaves	1.3	Thysanoptera	T and K
27	*Scirtothrips dorsalis*	Chilli thrips or yellow tea thrips	Flowers and leaves	1.3	Thysanoptera	T and K
28	*Apterygothrips sp*	Thrips	Flowers and leaves	1.2	Thysanoptera	T and K
29	*Scirtothrips* sp1	Thrips	Flowers and leaves	1.2	Thysanoptera	T and K
30	*Frankliniella occidentalis*	Thrips	Flowers and leaves	1.2	Thysanoptera	T and K
31	*Haplothrips* sp.	Thrips	Flowers and leaves	1.0	Thysanoptera	K
32	*Gigantothrips*sp.	Thrips	Flowers and leaves	1.0	Thysanoptera	T and K
33	*Microcephalothrips abdominalis*	Thrips	Flowers and leaves	1.0	Thysanoptera	T and K
34	*Vuilletia houardi*	Thrips	Flowers and leaves	0.9	Thysanoptera	T and K
35	*Ceratothripoides brunnens*	Tomato thrips	Flowers and leaves	0.8	Thysanoptera	T and K
36	*Scirtothrips* sp.2	Thrips	Flowers and leaves	0.8	Thysanoptera	T and K
37	*Ecacanthothrips tibialis*	Thrips	Flowers and leaves	0.7	Thysanoptera	T
38	*Thrips revelatus*	Thrips	Flowers and leaves	0.7	Thysanoptera	T
39	*Sericothrips* sp.	Thrips	Flowers and leaves	0.6	Thysanoptera	T

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40	*Neosmerinthothrips sp*	Thrips	Flowers and leaves	0.5	Thysanoptera	T
41	*Diarthrothrips* sp.	Thrips	Flowers and leaves	0.3	Thysanoptera	K
42	*Frankliniella williamsi*	Thrips	Flowers and leaves	0.3	Thysanoptera	T
43	*Rhipiprothrips* sp.	Thrips	Flowers and leaves	0.3	Thysanoptera	T and K
44	*Stenchaetothrips*sp.	Thrips	Flowers and leaves	0.3	Thysanoptera	T
45	*Elaphrothrips* sp.	Thrips	Flowers and leaves	*	Thysanoptera	T
46	*Pselaphothrips pomeroyi*	Thrips	Flowers and leaves	*	Thysanoptera	T
47	*Stephanothrips* sp.	Thrips	Flowers and leaves	*	Thysanoptera	T
48	*Urothripine* sp.	Thrips	Flowers and leaves	*	Thysanoptera	T
49	*Craspedothrips* sp.	Thrips	Flowers and leaves	*	Thysanoptera	T
50	*Apis mellifera*	Honey bee	Flowers	*	Hymenoptera	T and K
51	*Camponotus maculatus*	Ant	Flowers and leaves	*	Hymenoptera	T and K
52	*Oecophylla longinoda*	Weave ant	Leaves	*	Hymenoptera	T and K
53	*Crematogaster* sp.	Ant	Flowers and leaves	*	Hymenoptera	T and K
54	*Brachypeplus* sp.	Sap-feeding beetles	Flowers and leaves	*	Coleoptera	T and K
55	*Corynas*p.	Pollen beetles	Flowers and leaves	*	Coleoptera	T and K
56	*Epitrix silvacola*	Leaf beetles	Flowers and leaves	*	Coleoptera	T and K
57	*Formicomus* sp.	Ant-like flower beetles	Flowers and leaves	*	Coleoptera	T and K
58	*Nematocerus*sp.	Weevils	Flowers and leaves	*	Coleoptera	T and K
59	*Paederus sabaceus*	Short-winged beetles	Flowers and leaves	*	Coleoptera	T and K
60	*Scymnus* sp.	Ladybugs	Flowers and leaves	*	Coleoptera	T and K
61	*Camponotus rufoglaucus*	Ant	Flowers and leaves	*	Hymenoptera	T and K

Species accumulation curve revealed that 59 species of arthropods were recorded at Taita Hills and 50 arthropod species at Mount Kilimanjaro (Fig 2; Table 2).

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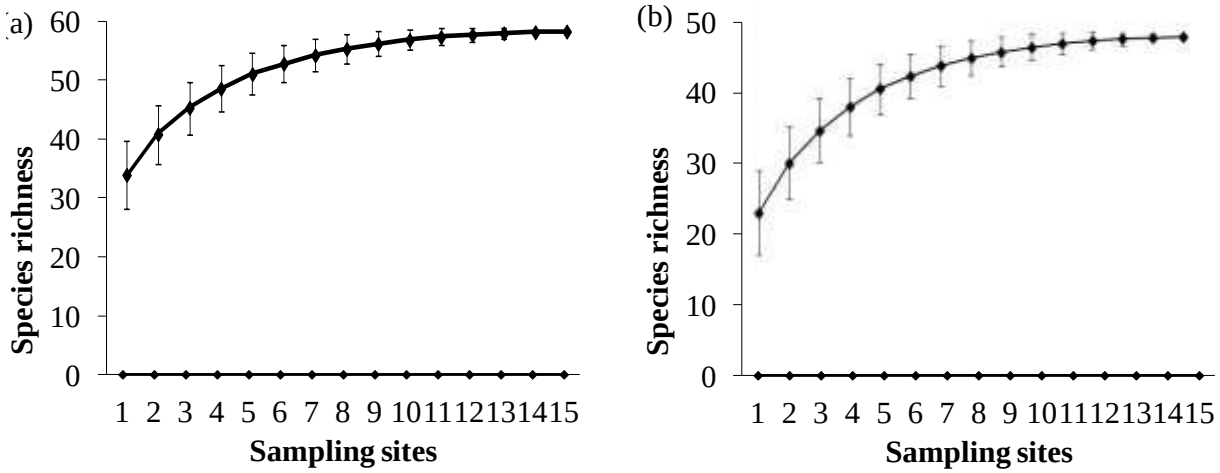

Fig.2: Species accumulation curve of arthropods recorded on avocado plants for (a) Taita Hills and (b) Mount Kilimanjaro study areas. The bars are standard errors. Sampling sites are study blocks at with each comprising of at least a hundred avocado trees.

Richness estimators (Boot, Jacknife and Chao) predicted a number of between 71 ± 3 (mean ± se) and 54 ± 2 species for Taita Hills whereas between 56 ± 2 and 43 ± 1 for Mount Kilimanjaro (Table 2).

Table.2: Observed species richness and non-parametric species richness estimators for Taita Hills and Mount Kilimanjaro transects.

Habitat	Observed species	Chao	Jacknife 1	Boot	Sampling blocks (n)
Taita Hills	59	71.51 ± 3.77	56.62 ± 2.23	54.43 ± 2.09	15
Mount Kilimanjaro	50	56.14 ± 2.81	50.20 ± 2.01	43.12 ± 1.36	15

IV. DISCUSSION

Our results revealed that the Asian invasive fruit fly (*Bactrocera dorsalis*), False codling moths (*Thaumatotibia leucotreta*), common blossom thrips (*Frankliniella schultzei*) and the Greenhouse thrips (*Heliothrips haemorrhoidalis*) are the most abundant arthropod species inhabiting avocado plant parts at Mount Kilimanjaro in Tanzania and Taita Hills in Kenya. *Bactrocera dorsalis* was recorded from ground collected ripened avocado fruits whereas *Thaumatotibia leucotreta* was sampled from immature avocado fruits confirming them as fruit a scenario that has been reported by Mwatawala *et al* (2009) and Prinsloo and Uys (2015), respectively. *Frankliniella schultzei* was recorded exclusively feeding on flower resources, however, *Heliothrips haemorrhoidalis* was recorded from leaves and young fruits proving to be pests of avocado crop as reported in Palmer (1990) and Prinsloo and Uys (2015). Beneficial or pest status of each of the species sampled was not evaluated in this novel paper since our study focused on generating the checklist of arthropods that inhabits avocado plant. However, Ware *et al* (2016), Prinsloo and Uys (2015), Ware *et al* (2012) and Mwatawala *et al* (2009) reported that *Bactrocera dorsalis* and *Thaumatotibia leucotreta* are economically important pests of avocado fruits. Moreover, beneficial insects such as honey bees (Apis melifera) was exhaustively described pollinating avocado flowers at Taita Hills in South-eastern Kenya by Luvanga (2015).

The failure of the species accumulation curves to reach saturation point indicated that more species were likely to be found if additional sampling effort continued at Taita Hills transect than at Mount Kilimanjaro study area. This hypothesizes that if surveys are conducted over multiple years, the species accumulation curves for arthropods are expected to reach saturation point probably due to a larger proportion of rare species which are often recorded in single encounters (Novotny and Basset, 2000). Most of the rare species in this study were Thysanopteran non-pest thrip species that contributed to the high projection of species richness for Taita Hills using Chao; a non-parametric estimator (R Development Core Team, 2012).

V. CONCLUSIONS

Relatively high species richness of arthropods sampled from a single crop confirmed that the avocado plant also plays vital ecosystem functioning at both Mount Kilimanjaro and

Taita Hills study areas. The present checklist provides baseline data for scientists to identify species of economic importance which can assist in designing strategies for avocado pest control and pollination services in East Africa. In order to enhance avocado farming strategies at the two study areas, spatiotemporal data is required for each species recorded. This study, therefore, recommends research on how the most abundant arthropods (*Bactrocera dorsalis*, *Thaumatotibia leucotreta*, *Frankliniella schultzei* and *Heliothrips haemorrhoidalis*) impacts on avocado farming at Mount Kilimanjaro and Taita Hills. The study, further, recommends research on possible beneficial insects such as *Apis mellifera* (Hymenoptera: Apidae), *Oecophylla longinoda* (Hymenoptera: Formicidae) and *Camponotus maculatus* (Hymenoptera: Formicidae) in the avocado orchards along slopes of Mount Kilimanjaro and Taita Hills.

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Collaborative Livelihood Strategy: A Reflection of Social Network in Economic Activity (Case Study in Small Islands, Maluku Province, Indonesian)

August E. Pattiselanno, Massie. T.F. Tuhumury, Noviar F. Wenno, Junianita F. Sopamena

Agribusiness Department, Faculty of Agriculture, Pattimura University, Ambon, Maluku, Indonesian

Abstract— *Research was aimed to analyze the existence of household livelihood strategy and to identify agreements constituting livelihood strategy adopted by households in small island community. Data were collected from questionnaire given to 200 respondents who lived in five small islands, such as Ambon Island, Saparua Island, Gorom Island, Selaru Island, and Kisar Island. Respondents were selected with simple random sampling. Depth interview was also conducted with key informant in each island to verify questionnaire data. Some findings were then obtained. It was found that 83.5% respondents have built social network based on kinship, while 38.5% created network based on friendship and 48% was based on neighborhood. Agreement in network may take few forms such as borrow-lend activity (63%), output marketing (59.5%), and using farming output as collateral (42%). Therefore, it was concluded that kinship is the most influential base underlying the economic activity of community in small islands.*

Keywords— economic behavior, social relationship, small island.

I. INTRODUCTION

The culture adopted by small island community is not emphasizing on one work only. Previous studies have suggested several ways of how to survive successfully in the environment, among others is by living with reliable resilience to cope with various conditions of changes at the surrounding. The most difficult change to be dealt with is climate change because it is mostly less predictable. Small island community still have local wisdoms they have conserved throughout times. Early local wisdom was dominated by land-based activity, whereas sea-based activity was only a supplement. When population grows, sea-based activity becomes central with land-based activity as support. The ability to set both land-based and sea-based activities into good collaboration is then becoming a local wisdom distinguishing the community of small islands in Maluku.

The community of small islands have been throughout generations conserving genetic diversity of various plant species based on rainfall pattern in the environment where they live. In general, Maluku people have been familiar with both fixed and shifted farming. Farmers till their land for perennial plants, such as coconut, clove, and pepper, and these plants are usually grown in plantation. Farmers also cultivate short-term plants such as edible tubers, nuts, and dry-land rices. The selection of plants is describing the culture professed for a long time by local community. Farmers have been since the beginning understanding the importance of plant selection because it allows them to gain sustainable harvest from the commodities. Land-based activity has benefited from the availability of natural resources and it is definitely meaningful for livelihood strategy. Other reasons are related with small island characteristics such as water scarcity, uncertain climate, and restrictive control span preventing technological inputs, supplies, or yields (outputs) from accessing or being marketed on the islands.

Small island community also utilizes sea sides to satisfy their needs. Their activity on sea sides includes catching fishes, cultivating sea grass, and collecting sea products such as sea cucumber and lola. The exploitation of resources at both sea and land sides shows a fact that the community begins to understand their environment. The ability of

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community to understand their surrounding environment is a proof that local wisdom exists and has been inherited to generations since which they conserve it until now. Natural resources at certain island can be limited due to the narrow extent of the island. Therefore, collaborative livelihood strategy is the most rational choice referring to Weber concept of Rational Choice Theory with Traditional Authority.

Rational choice values respected by small island community are absolute values and undeniable by any interventions. To stimulate the productive function of this community, a cooperation network involving many entities is then created to help "relieving" the burden of satisfying livelihood needs. Usually, the community works with local traders in several methods, such as through borrow-lend activity or having agreement on using either sea or land outputs as collateral. Some people perceive this agreement as benefiting the entities because is is a legacy of their parents. But, others consider it as harming community. Then, a simple question rises: "When they need money for important need (for example, tuition), what is the immediate source of help for them?" The answer is traders. The agreement made between community and traders is definitely mutualism because the community with urgent need could rely on traders to get immediate help.

[1] have noted that Russian global economic system has been in war with traditional economic system at North Caucasian. Slowly but sure, traditional economic system is forced to change due to the agitation of global economic. [2] indicated that cultural power still played important role in economic development, and therefore, traditional culture (Russian traditional lifestyle), if well managed, could help improving socio-economical development of the people. Moreover, [3] explained that company culture was closely related to company performance. The stronger is company culture internalized into company members, the more increasing is company performance. The reverse of this case also prevails. It begins clear that culture still plays important role in economic life. Similar finding was shown by [4] who asserted that small island community in West Southeast Maluku has applied various strategies to satisfy livelihood needs, and they choose proper commodity based on their parental legacy. They work on activity to maintain viability of the households. It must be an interesting topic if one analyzes cultural agreement made for establishing economic activity in small island community. Therefore, the objective of this research is to analyze livelihood strategy conducted by the households in small island community and to identify agreements constituting livelihood strategy adopted by households in small island community.

II. METHOD OF RESEARCH

2.1. Time and Location of Research, and Sampling Method

Research was located in five small islands in Maluku Province, and these islands were Ambon Island, Saparua Island, Gorom Island, Selaru Island, and Kisar Island. Each island was represented by one sample village selected purposively. It included Hila Village (Ambon Island), Ihamahu Village (Saparua Island), Mida Village (Gorom Island), Adaut Village (Selaru Island), and Lebelau Village (Kisar Island). The selection of this sample was made based on economic activity done by farmers and fishers in the islands. The community in five villages mostly works in agriculture as their dominant activity, and fishery or non-agriculture is only side job. Research was conducted gradually, in August 2015 (in Kisar Island), October 2015 (in Gorom Island), March 2016 (in Saparua Island), April 2016 (in Selaru Island), and June 2016 (in Ambon Island). Each location is represented by 40 respondents, and therefore, five villages give the author with 200 respondents. All respondents are selected in simple random manner and all of them work as farmer and fisher. Key informant is chosen from each village to explore further the answers of respondents. Key informant is decided based on their involvement in the agreement made by farmers and fishers at sample village.

2.2. Data Collection and Data Analysis

Data collected from questionnaire given to respondents [5] or obtained from depth interview with key informant [6] are called as primary data. Those acquired from participative observation [7], [5] are known as secondary data. Participative observation requires the author to go deep into the daily life of community in order to listen words and to distinguish actions shown by community as the subject. Data analysis uses Simple Tabulation to describe conditions and characteristics of research location. The processed data are shown on the table and diagram to facilitate the analysis.

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III. RESULT AND DISCUSSION

3.1. Collaborative Livelihood Strategy

The selected collaborative livelihood strategy would be the function of various income sources. Some income sources of the households in small island community can be used collaboratively to satisfy the needs of food, cloth, shelter, child tuition, and custom ritual. The function of each income source is shown in Table 1.

Table.1: Collaborative Livelihood Strategy, Income Source, and Function

No	Income Source	Function	Number of Respondent	Percentage (%)
1	Crop farming	Mostly are for household consumption, and few are sold for other needs.	200	100.0
2	Plantation farming	Child tuition, cloth	139	69.5
3	Livestock	Custom ritual, such as community trial, marriage, and funeral	118	59.0
4	Sea grass	Child tuition, cloth and shelter	40	20.0
5	Captured fishery	Food, child tuition, and shelter	107	53.0

Source: Result of research (2015-2016, processed)

The table above describes the variety of livelihood strategies adopoted by households in small island community. All households exploit crop farming as their main source of household food. Some commodities are planted, such as edible tubers, corn, nuts, and dry-land rice. The selection of commodity is made based on climate condition of each island or because it is the legacy of their parent. Somehow, it is always difficult to replace certain commodity with other commodity. It is not suprising then if the households in small island community still profess their ancestral habits or legacies. Referring to the table above, it is clear that community need for food is definitely fulfilled because the main focus of crop farming is indeed to satisfy food need of the households. Thus, it can be said that food scarcity is impossible in small islands of this research.

Plantation farming may support this finding. Main commodities of plantation are coconut, clove, nutmeg and orange (especially in Kisar Island). Coconut is the most favorite plant and the condition of small island is very conducive for growing coconut. Also, coconut is the biggest contributing commodity to the fulfillment of child tuition and cloth.

3.2. The Use of Income by Households in Small Island

The increase of economic activity implies on positive impact to the household income. This income is then arranged to satisfy various needs including food, child tuition, cloth, shelter, custom ritual, and daily needs. Clear description is given in the following table.

Table.2: The Impact of the Increasing Economic Activity on Income

No	Income Source	Income Average/Year (IDR)**	Use of Income (%)*						
			1	2	3	4	5	6	Total
1	Crop farming	1,684,000	50					50	100
2	Plantation farming	2,230,000		70	30				100
3	Livestock	1,500,000					100		100
4	Sea grass farming	2,530,000		60	20	20			100
5	Captured fishery	9,850,000	70	15		15			100

Source: Result of research (2015-2016, processed)

Note*):

1 = Food Consumption; 2 = Child Tuition; 3 = Cloth; 4 = Shelter; 5 = Custom Ritual; 6 = Daily Needs; ** = in monetary unit

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Above table shows that child tuition dominates the use of household income over other needs. The tuition is mostly related with the continuity of child study after graduating from Senior High School. Higher education remains only in Ambon City and it definitely requires huge costs to cover. Copra farming, sea grass farming, and captured fishery, also contribute greatly to the continuity of child study.

Custom ritual alway involves livestock (pig and cattle) as the funding source. Every ritual, such as marriage, birth, and funeral, often requires the relatives of the host to share contribution. Besides using livestock as the custom animal, the ritual also compels the host to provide local alcohol beverage called *sopi*. Therefore, custom ritual always incurs great cost to the host. Community trial for diverging society norms, such as adultery, also uses livestock for settlement.

Food is only derived from crop farming and captured fishery. Mostly, the harvest of crop farming is used for household consumption, and few, if any, are sold to satisfy other daily needs, including the supplement of main food. The haul of captured fishery is mostly consumed as household food, and the remaining is sold to fulfill needs of child tuition, snack and shelter. At certain times, shelter must be fixed and repaired, and the funding is taken from captured fishery and sea grass farming. Although most parts of the house are collected from the forest, but the construction of permanent house would need materials bought from the store, and these materials include cement, zinc plate and iron bar.

As also shown in Table 2, households prepare specific strategy to fulfill their needs. The selected work must be functional to the fulfillment of needs. It is then clear that the preferred livelihood strategy is collaborative which combines several sources of income. If one source is failed or disrupted, other source may cover the needs.

3.3. Networks in Collaborative Livelihood Strategy

Collaborative livelihood strategy is made of networks which are differentiated based on kinship, friendship, and neighborhood. The following table illustrates this position.

Table.3: Networks in Collaborative Livelihood Strategy

No	Network Models	Number of Respondents (Person)	Percentage (%)
1	Kinship Based Network	167	83.5
2	Friendship Based Network	77	38.5
3	Neighborhood Based Network	96	48.0

Source: Result of research (2015-2016, processed)

Kinship based network, according to Barnes (1969), is differentiated into two, respectively total network and partial network. Total network is when all parts of the network are owned by single individual, and this would cover various contexts or living aspects of the community. Partial network is a network owned by individual to be used for certain living aspect, such as for political affair, religion, genealogy, and others. Respondents (including farmers and fishers) create network with their customers based on kinship. All relatives are included regardless they may have same or different profession. Strong kinship network can produce a strong social unit. Indeed, strong network among them would facilitate them in selling crop harvest and fish haul. They are also given easier access to borrow-lend activity, especially when they need starting capital. Borrow-lend agreement is almost always found in all research locations. More clear description is shown in Table 4.

Frienship based network, pursuant to [8], is a network connecting someone with some others into a less official relationship. The word “friendship” means that the relationship would make friend as a requirement. As Wolf said, the households do not concern too much with the sale price of their commodities they sell because they always use their friend to market their farming commodities. In such relationship, friendship would require honesty and cooperation to produce good trading activity for the commodities.

Neighborhood based network, as explained by [9], [10], and [8], is a network relating someone with some others who live around the house, and who are perceived as neighboor. This perspective declares that the marketing of plantation commodities and sea products (sea grass and fish) often relies on neighboors who are familiar with network out of village. So far, the collaboration of these three networks is apparently presented in five

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research locations. The following table provides the detail.

Table 4. Agreements in Collaborative Livelihood Strategy

No	Forms of Agreement	Number of Respondents (Person)	Percentage (%)
1	Borrow-Lend Activity	126	63.0
2	Output Marketing	119	59.5
3	Using Farming Output as Collateral	84	42.0

Source: Result of research (2015-2016, processed)

The agreement is not unilaterally made but created by the consent of the entities. Farmers, fishers and collector-traders have built agreement for long time. Borrow-lend activity is settled after selling the harvest to the lender because the harvest is used as the collateral by farmers or fishers. Not all of them do this, but mostly their network is created based on kinship, and this kinship is often used as the collateral in borrow-lend activity. Farmers and fishers often submit some their farming output to traders as the precondition of agreement in output marketing. They trust these traders because the latter has been helping them to market their farming outputs and fishes. In other hand, outputs are sold to the lender to settle borrow-lend activity. The price is cheaper than the price received by farmers or fishers if they sell themselves to the market center in district town. However, transportation cost is quite prohibitive to them, or possibly they have tight schedule or are too busy to do the selling. Therefore, they make agreement with traders who are willing to lend them monies. Besides for capital loan, borrow-lend activity is also initiated for urgent necessities such as child tuition and custom ritual. In consistent to this finding, [11] conceded that by empowering the resilience of individual and community, then socioeconomical vulnerability would be reduced. This effort can be enforced by establishing the sophisticated community based on culture, morality and solidarity.

3.4. Economic Sociology Approach in Collaborative Livelihood Strategy

The attributes of economic sociology approach in collaborative livelihood strategy is described in the following table.

Table.5: Economic Sociology Approach in Collaborative Livelihood Strategy

No	Economic Sociology Approach	Characteristics	Actors
1	Coherence	Helping others, honest, sharing information, and trust to each other are done on collective norms.	Producers of local alcohol beverage (sopi) and traders
2	Supply intensity process	Trust, cooperation, and social exchange are built for long time, and thus, price is determined with mutual trust.	Orange farmers and traders
3	Clientelization process	Borrow-lend activity may involve harvest as the collateral.	Farmers with local traders
4	Patron-Client	Capital assistance is given in exchange for the privilege on output sale.	Farmers and the company as nutmeg buyer
5	Cultural value	The participation of women in fulfilling household needs is increasing.	Women and income

Source: Result of research (2015-2016, processed)

It seems that economic sociology approach in collaborative livelihood strategy is quite relevant to be applied to small island community. The concepts of coherence, supply intensity, clientelization, patron-client, and cultural value, were suggested by [12] and [13], and these are clearly evident in economic activity of small island community. Through these economic sociology concepts, the selected livelihood strategy must successfully keep households resilient because social agreements determine the selection of strategy. Such resilience may decline or even vanish if economic indicators are used as measurer. The relationship at household level between husband and wife, in a form of man

and woman, would then grow into relationship at community level in a form of agreement between two entities which is created based on sociocultural characteristic of each. The stronger is the sociocultural agreement, then the more certain is that livelihood strategy should fulfill household needs. [14] used Institutional Economic Theory to explain the role of knowledge in the change of European economic after the integration of European countries into European Union. Consistent to this statement, [15] has concluded that participation as the important aspect in social capital is very determining to organizational quality. The study on social network done by [16], has shown that social network is very influential to the change of social behavior perspective among individuals. The agreements made by farmers with traders were subjected to the study by [17], and they found that agreements between farmers and entrepreneurs were related closely with village isolation and personal profile.

IV. CONCLUSION

This research concludes that social relationship is often considered as the guidance in the economic activity of community. To the community of small islands, their network cannot escape from the legacy inherited by their parent. The strength of networks which are created based on kinship, frienship and neighborhood is mostly robust enough. Moreover, kinship is the strongest base that underlay the establishment of network.

There are 83.5% respondents who admit that they create kinship based network while networks based on friendship and neighborhood are developed by 38.5% and 48% of them. Agreement in network could take forms of borrow-lend activity (63%), output marketing (59.5%), and using farming output as collateral (42%). Meanwhile, kinship is the most influential base underlying the economic behavior of small island community.

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Identification of Distribution the Pineapple Mealybug Wilt Disease in the Pineapple plant in North Tapanuli

Arta Junita Hutahayan, SP, Msi

Universitas Sisingamangaraja XII Tapanuli

Abstract— *Pineapple is one of the commodities of horticultural crops of fruits that have been developed by generations of people in the North Tapanuli Regency. Pineapple is a commodity mainstay of the community, where the planting spread in several districts, such as Sipahutar, Pangaribuan, Siborongborong and Tarutung. Sipahutar District is a pineapple production center in North Tapanuli Regency. Pineapple from Sipahutar, famous since the first because it has advantages compared with other pineapple that has a sweeter taste, water content slightly and texture more dense. The problem in pineapple cultivation lately is wilting mealybug disease because the losses incurred are very large. The purpose of this study was to detect and study infections from mealybug wilt disease in the field and the spread of mealybug wilt disease in some villages of pineapple planting centers in North Tapanuli region. The results of this study are expected to be information in controlling mealybug wilt disease in pineapple plants*.

Keyword— Mealybug, North Tapanuli, Pineapple Disease, Wilt Symptom.

I. INTRODUCTION

Pineapple (*Ananas comosus*) is a commodity mainstay of society in North Tapanuli Regency. The most dominant pineapple planting is in Sipahutar, Pangaribuan, Siborongborong and Tarutung sub-districts, which are pineapple production centers in North Tapanuli Regency (TUDA, 2015). Pineapple found in North Tapanuli has advantages of pineapple contained in other areas such as those produced from Garut. Since long time, pineapple plant produced from Garut region is famous but compared with pineapple from North Tapanuli regency, Garut pineapple quality is still far below pineapple Tapanuli Utara (TUDA, 2015)

The North Tapanuli Pineapple is large and sweet, red and has plenty of water. Pineapple fruit in canned packaging from North Tapanuli Regency is also exported to several countries such as China, Taiwan, America and Sweden. The exported pineapple is the fruit of the crops of farmers in almost every sub-district in North Tapanuli namely Sipahutar, Pangaribuan, Siborong-borong and Tarutung (TUDA, 2015).Seeing pineapple as one of the much needed horticultural products, especially the North Tapanuli community, it is necessary to improve the quality and quantity of pineapple plants, and to follow up it is necessary to know the determinants of production such as soil, climate, plant species, cultivation techniques and pests and pineapple plant disease. One of the main problems in the cultivation of pineapple plants is the disease of wilting mealybug This disease has significance because of the wide spread area that is in the entire pineapple planting region of the world, but it also caused considerable losses (Pretty et al., 2012). The spread of this disease is almost in the entire pineapple planting regions of the world including Indonesia, not least the central pineapple planting area of North Tapanuli (CABI 2013). However, there is not enough information on this disease. Based on the observation, this disease is one of the important problems in pineapple plantation of PT Great Giant Pineapple Company in Lampung, the same thing happened in North Sumatra. This disease causes damage and rot to rooting. Symptoms seen in affected plants are to wither, red color on the leaves starting from the outermost, dwarf fruit and even death of the plant.

So far, control measures are still based on chemical methods. The main target is to control vector insects Dysmicoccus spp and ants as a tick symptom. But this way can be bad for people and the environment. In addition, consumer demand at this time wants the use of pesticides to a minimum, especially for consumers in developed countries. Biological control is an alternative and an important control component in Integrated Pest Management (IPM). Nevertheless, information on the existence of natural enemies of this pest in Indonesia is still very limited. For that we need a study about the detection and spread of the disease in order to support effective control efforts. This study aims to detect and study infections from Mealybug wilt disease in the field and the spread of mealybug wilt disease in some villages of pineapple planting centers in North Tapanuli region.

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II. LITERATURE REVIEW

Mealybug wilt disease was first recognized in 1910 in Hawaii. In the 1920s and 1930s almost destroyed the pineapple canning industry in Hawaii. Some of the data below mention that the decline in production caused by this wilting disease is quite high. Pretty et al. (2012) in Cuba suggests a yield loss of 40%, according to Sether et al. (2001), Hu (2001), the decrease&yield loss of 35%, even according to Sether in yields can also be experienced by asymptomatic plants that are asymptomatic. In addition to causing a decrease in yield, this wilting disease may also lead to early ripening of the fruit (Sipes et al., 2002). In plants infected with wilting mealybug disease there was a decrease of average weight of fruit by 55% when compared with plantsybug disease-free plants. If Mealybug wilt disease develops 14 months after planting, the resulting fruits weigh an average of ± 7% less than plants that are free from Mealybug wilt disease (Sether and Hu 2002).

Symptoms of the disease first appear in rooting roots that are impaired growth, collapse and decomposition, followed by wilting symptoms in the leaves. Collins (1960) divides symptoms of wilt disease on this pineapple into four stages. The first stage is a reddish leaf that starts from the outer leaves (third or fourth leaf), the edges of the leaves are rolled up, the tip of the leaf is not curved and the plant still looks normal. The second stage, the leaves are reddish, turgiditas begin to disappear, the tip of the leaves slightly brownish, sometimes leaf curling and occur nekrotis with the size of the plant is still normal. The third stage, the fourth and fifth circumference leaves bend down, the edges of the leaves are yellow or reddish, the edges curl backward and the plant is dwarfed. The fourth phase, the middle leaves look erect but have lost the turgidity, the leaf tips are bent and brown, curly leaves and dwarf plants. The wilted disease in pineapple is caused by a virus belonging to the Closteroviridae family and the Closterovirus genus. At first known, the disease was thought to be due to the presence of toxins produced by Dysmicoccus spp during feeding (Carter 1973), then further the presence of latent factor transmitted by ticks and in the 1980s was successfully isolated from plant virus ill (CABI 2013). Another commonly used name is Pineapple Mealybug Wilt associated Closterovirus. Until now it has been found a type of virus associated with this wilting disease. First discovered pineapple closterovirus (PCV) in the 1980s, then because the pineapple wilt disease is always associated with white fleas, the disease is called mealybug wilt of pineapple (MWP), which is then revised into pineapple mealybug wilt associated virus. Furthermore, the last research managed to find a rod-shaped virus or baciliform (CABI 2013). Transmission of the virus cannot occur mechanically, but must be with the help of vectors. This is the importance of the role of vector insects. Insects that can be viral vectors are *D.brevipes, D.neobrevipes* and *Pseudococcus longispinus*. Transmission is semi-persistent and non-transovarial (Brunt and Gunasinghe, 1991). Factors affecting the epidemic are complex, including multiple interactions between mealybugs, ants, predators, parasites, viruses, pineapple plants and other plants as alternative hosts such as Agavae americana and Paspalum weeds. Symptomatic expression is also influenced by environmental conditions and diversity of mealybug populations (Rohrbach *et al*., 1988). Mealybug is usually associated with ants. Ants maintain and protect mealybugs from predators by eating honey dew generated by mealybugs, also preventing the development of sooty dew caused by fungi (Beardsley *et al.*, 1982).

III. METHODOLOGY

Data and Source of Data

Study of transmission of wilt disease mealybug in the field. Identifying the role of wilt disease and mealybug in inducing wilt symptoms in pineapple plants in the field, then observations were made on pineapple plantations in Sipahutar Village Village, North Tapanuli Regency. The observed pineapple garden is a garden with an incidence of wilt disease of more than 50%. In selected gardens were observed a number of symptomatic plants withered and asymptomatic ones. In each plant sample, both symptomatic and asymptomatic, were observed for mealybug colonization for 5 months.

Data Collection Method

Mapping the geographic distribution of pineapple wilt disease. The observation of mapping of pineapple wilt disease by mealybug was conducted through surveys of pineapple in several central districts of pineapple production in North Tapanuli region, Pangaribuan, Siborongborong and Tarutung subdistricts, to observe the spread of wilt disease and variation of wilting symptoms. In each area of pineapple production centers were observed some pineapple gardens owned by local farmers. In each selected garden were observed the incidence rate of the disease and the type of wilting symptoms in the pineapple plant. This data is expected to map the spread of wilt disease in pineapple growing areas in North Tapanuli.

IV. RESULTS OF DISCUSSION

1. Study of transmission of wilt disease mealybug in the field

Observations of the studies determined to observe transmission of pineapple wilt disease in the field were in Pealinta Hamlet, Siabal-abal III, Sipahutar, North Tapanuli. In the area, pineapple planted mostly varieties of Smooth Cayenne.

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Fig.1: Location of pineapple plantation in Pealinta Hamlet Siabalabal Sipahutar Village

This variety has the following characteristics, stem height and fruit stalk about 20-50 cm. The number of leaves ranges from 60-80 strands. Leaves are shallow trough shape with straight edge, not wavy. The fruit is at the end of the fruit stalk with the lower part larger than the tip. Fruits weighing above average shapes are tapered from base to tip, while fruits weighing below average are near the cylinders. The upper leaf surface is dark green with the addition of irregular brownish red color due to antocyanin pigment in the epidermis.

At the location of this observation found widespread symptoms of widespread and most wilting symptoms are yellow wilt symptoms and curling at the tip of the plant leaves.

a.Yellow b. Curled at the tip of the plant leave

Fig.2: The pineapple plant was symptomatic withered on the research field

In the wilted pineapple plants observed in this area, the presence of white flea colonization (*D. brevipes*) was observed. Mealybug are found at the base of the leaf near the pineapple plant stems and are also found at the bottom of the base of the pine plant stems close to the roots of the pineapple plant.

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Fig.3: Mealybug colonies (Dysmicoccus brevipes)

Percentage of pineapple plants in the field showing symptoms of wilt and / or colonization of white fleas

No	Individual pineapple plant observed Percentage	Persentage
1	Showing symptoms of wilting and colonization of mealybug	71
2	Showing symptoms withered but uncolonized white fleas	-
3	Colonized white but healthy looking lice (not showing symptoms wither)	14

2. Mapping the geographic distribution of pineapple wilt disease

Mapping distribution of wilt disease mealybug by mealybug was conducted through a survey to pineapple plantation in the central area of pineapple production in North Tapanuli, namely Tarutung, Pangaribuan and Siborongborong Subdistricts.

2.1. Incidence of wilt disease in pineapple production center Tarutung Subdistrict (Desa Siarang-arang).

In this planting pineapple pineapple varieties Smooth Cayenne and in this area found quite a lot of wilting symptoms with two variations of wilting symptoms are symptoms of wilting and wilting symptoms with dead ends.

Fig.4: Location of pineapple plantation in Siarangarang Village Tarutung Sub-district

In all pineapple plants observed and showing wilting symptoms, colonization of mealybug was found at the base of the pineapple plant stem, in the lower leaf axilla or in the root of the plant

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Fig.5.a. Red wilt symptoms *5.b.Symptoms of wilting curling and dead tip*

In this area, there are three gardens observed and the three pineapple gardens are seen to be infected with wilt disease with disease severity for each garden averaging 50-60%.

2.2. Incidence of wilt disease in pineapple production center of Pangaribuan Sub-district (Lobu Gala Village)

Planting pineapple in the District Pangaribuan Village Lobu Gala commonly planted farmers are the type of Smooth Cayenne

Fig.6: Location of pineapple plantation in District Pangaribuan Village Lobu Gala

In the pineapple plantation of Lobu Gala Village Pangaribuan subdistrict wilt symptoms are also quite commonly found. In this area, there are three gardens observed and on the three pineapple gardens are seen many pineapple plants are infected with wilt disease. The most noticeable wilt symptoms are symptoms of red and curling. The severity of wilting disease in Pangaribuan area is already high, about 80% already.

Fig.7: Pineapple plant symptomatic with red in pineapple plantation Pangaribuan District

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In addition to red wilting symptoms, there are variations of wilting symptoms of yellow wilt symptoms, curl / curling and dead leaf tips. The three variations of wilting symptoms can be seen in Figure 8 below:

Fig.8: Variation of wilting symptoms: 1. Yellow; 2. Curl ; 3. Dead end

2.3. Incidence of wilt disease in pineapple production center of Siborongborong Sub-district (Hutabulu)

Pineapple is grown in this district is pineapple varieties Smooth Cayenne, with large pineapple fruit (weighing more than 2 kg), it feels a bit sour fresh and the flesh is full of fiber. Pineapple is more often canned because the flesh is not easily destroyed.

Fig.9: Location of pineapple plantation in Hutabulu village, Siborongborong sub-district

In this pineapple planting, wilt symptoms are also found quite a lot. But not as much in Tarutung and Pangaribuan area. In this Siborongborong area, there are four gardens observed and on the four pineapple gardens, two orchards of which there are almost no symptoms withered. While in two other pineapple gardens seen a lot of pineapple plants infected with wilt disease. The most noticeable wilting symptoms are yellow wilt symptoms (figure 9). The severity of wilt disease in Siborongborong is quite high, reaching 60-70% on average.

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Fig.10: The pineapple plant is symptomatic withered in Siborongborong pineapple plantation

Symptoms of wilt in this area are more commonly found in pineapple plants generative phase. In nurseries and young pineapple plants are rarely found withered symptoms. In Hutabulu Village, Siborongborong Subdistrict, there are two kinds of variation of wilting symptom that are symptom of wilting with red and withered yellow. Both variations of wilting symptoms can be seen in Figure 11 below:

Fig.11: Variation of wilting symptoms. 1. Red; 2. Yellow

In all pineapple plants observed and those with wilting symptoms, colonization of mealybug was found at the base of the pine plant stems, in the lower leaf axilla or in the root of the plant (figure 12)

Fig.12: Mealybug colonies (Dysmicoccus brevipes)

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Of the three sub-districts of pineapple production centers observed in North Tapanuli District, it was found that the highest incidence of wilt disease was in the Pangaribuan area (Lobu Gala) with the severity of the disease reaching 80%. This happens probably because the Pangaribuan area is at a higher altitude with high rainfall as well. So with the height of place and climate is optimal for the development and spread of this wilting disease. The lower incidence of wilt disease from the three central pineapple provinces observed was in Tarutung sub-district (Desa Siarang-arang) with incidence of wilt disease around 50-60%.

V. CONCLUSIONS

1. Mealybug infestation (*D. brevipes*) can aggravate and accelerate the onset of wilting symptoms, but is not a major factor in triggering symptoms of pineapple crops.
2. Mealybug wilt disease in pineapple plants has been found widespread in pineapple production centers in North Tapanuli District.
3. Almost all pineapple plants attacked by wilting disease and difficult to find pineapple plants that are free of wilting disease.

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The Quality Characteristics of Camel Sausage Formulated with Different Levels of Whey Protein Powder

Engy, F. Zaki

Department of Animal Breeding, Meat Production and Technology Unit, Desert Research Center, 11753Cairo, Egypt

Abstract*—In this study camel sausage was formulated with different levels (1, 2, 3 and 4%) of whey protein powder (WPP). Raw and cooked sausage samples were evaluated for physical properties, cooking measurements, shrinkage, color parameters, emulsion capacity (EC) and emulsion stability (ES) and sensory attributes. Using whey protein powder increased pH value, moisture retention, emulsion capacity and emulsion stability while, the cooking loss and shrinkage were decreased. Camel sausages formulated with 4% whey protein powder (WPP) had higher emulsion stability and emulsion capacity, lower cooking loss, better color and more acceptable than other sausage samples. However addition of 4% whey protein powder can be improved the quality characteristics of camel sausages.*

***Keywords*— Camel sausage; Whey protein; Quality characteristics.**

I. INTRODUCTION

Camels are used for many purposes such as meat and/or milk production, and for physical labour as well as racing. Camel meat is known to be more beneficial for health because it contains lower fat and cholesterol levels than other red meats (Gheisari and Ranjbar, 2013). The mineral and proximate composition of camel meat from young male camels (1-3 years) was generally similar to the amounts reported for these constituents in the corresponding tissues of beef (El Faer *et al.*, 1991and Mansour & Ahmed, 2000).Generally; consumers are prejudiced against fresh camel meat. If camel meat could be converted into processed products such as burger and sausage, it might be more acceptable to domestics' consumers. (Mansour& Ahmed, 2000).However, the important technological problem in manufacturing of camel meat products is the poor emulsifiability of camel fat. The high amount of connective tissue also makes camel meat a challenging raw material for producing a stable emulsion (Ulmer *et al.*, 2004).

Dairy products are widely used to improve the functional properties of meat products. Addition of whey protein improve the water holding capacity, increase juiciness of the final product, emulsion stability, provide better color properties and lowering chewiness and elasticity (Keaton, 1999). This study aims to evaluate the quality characteristics of camel sausages formulated with different levels of whey protein powder.

II. MATERIALS AND METHODS

Preparation of camel sausage

Camel meat and humped fat obtained from local slaughter house were used in this study. Left round (*Biceps femoris* muscles) of 3-4 years aged camel were pooled to form an experiment unit, with three (batches) of lean ground meat being prepared from each sausage formulation. All knives – separable fat was removed from muscles and used with humped fat as fat source. Lean meat was ground through a 3mm plate grinder. The ground meat was transferred to bowl chopper and the following additives (whey protein powder, fat, spices, salt, onion and ice) were added and mixed as given in Table (1). Each formula was transferred to sausage machine and stuffed into natural sausage casings (sheep intestines). Sausage was tiered into 10 cm length and placed in plastic foam trays, packed in polyethylene bags and frozen at -18ºC±1 until analysis.

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Table.1: Camel sausage formulation with whey protein powder

Ingredients (%)	Treatments				
	Control	WPP1	WPP2	WPP3	WPP4
Camel fat	10	10	10	10	10
Whey protein powder (WPP)	0	1	2	3	4
Onion	5	5	5	5	5
Salt	2	2	2	2	2
Spices	1.2	1.2	1.2	1.2	1.2
Ice	1	1	1	1	1

WPP 1, 2, 3, 4: Sausage formulated with whey protein powder at levels1, 2, 3 and 4%

pH and emulsion properties

pH of raw camel sausages was measured as described by Hood(1980). Five replicates were done for each treatment. Emulsifying capacity and emulsion stability of sausage were evaluated according to the method of Antipova *et al*. (2001). Three measurements were done for each treatment.

Cooking measurements and physical properties

Sausages were roasted in a preheated oven for 10 min. All cooking measurements were carried out on five replicates of each treatment as reported by Naveena et al. (2006) as follows:

Cooking loss (%):= (Uncooked sample weight) - (Cooked sample weight)/ (Uncooked sample weight) ×100

Cooking yield (%): = (Cooked sample weight) / (Uncooked sample weight) ×100

Moisture retention % was determined according to El-Magoli *et al.* (1996).Five replicates were done for each treatment. Moisture retention (%): = Cooking yield % × Moisture in cooked sample % /100

Moisture content was determined according to A.O.A.C (2000).

Water holding capacity (W.H.C) and plasticity were measured using the method of Wierbicki and Deatherage (1958). Five replicates were done for each treatment. Data were presented as cm^2 as described by Russo *et al.* (1999).

Shrinkage measurements

Raw and cooked samples were measured for width and length as described by Berry (1993) using the following equation:

Reduction in width (%) = (Uncooked sample width) - (Cooked sample width) / (Uncooked sample width) ×100

Reduction in length (%) = (Uncooked sample length) - (Cooked sample length)/(Uncooked sample length) ×100

Dimensional shrinkage % was calculated using the following equation as reported by Murphy *et al.* (1975). = [(Raw length - Cooked length) + (Raw width - Cooked width)] / (Raw length +Raw width) ×100

Color measurements

Meat color was measured by Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap supplied by the manufacturer. Color was expressed using the CIE L, a, and b color system (CIE, 1976). Five replicates were used per each treatment.

Sensory evaluation

Camel sausage was subjected to organoleptic evaluation as described by A. M. S. A. (1995). Ten panelists of staff members of Food Sciences Department, Faculty of Agriculture, Ain-Shams University were scored appearance, texture, juiciness, flavor, tenderness and overall acceptability using a 9-point hedonic scale. The mean scores of the obtained results of organoleptic evaluation were then statistically analyzed.

Statistical analysis

All data generated from each experiment were analyzed using statistical analysis system (SAS, 2000). Treatments were compared using the Duncan's multiple range test method for significant main effects at $P < 0.05$.

III. RESULTS AND DISCUSSION

pH value and emulsion properties

From data shown in Table 2. It can be found that all sausage samples formulated with whey protein powder (WPP) had higher pH value compared to control one, but the difference between formulated sausage samples was slightly significant. (Yetim *et al.*, 2006) showed slight but not significant ($P > 0.05$) increase in pH value of sausages with increasing whey substitution. Also, (Serdaroglu, 2006) reported that pH value of meatballs formulated with 2 or 4% whey protein (WP) were not significantly different at different levels of fat.

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Table.2: Emulsion properties and pH value of camel sausage

Treatment	pH	Emulsifying capacity (%)	Emulsion stability (%)
Control	5.81^{c}	60.00^{c}	32.00^{d}
WPP1	5.90^{ab}	65.75^{b}	32.50^{d}
WPP2	5.86^{bc}	67.50^{b}	38.40^{b}
WPP3	5.88^{ab}	78.00^{a}	34.37^{c}
WPP4	5.94^{a}	79.50^{a}	40.50^{a}

$^{a-d}$ means within the same column with different superscripts letters are different ($p<0.05$).

The same results were obtained by Serdaroğlu and Özsümer (2003) they reported that no significant differences in pH values of batters or finished beef sausages formulated with different levels of whey protein and fat. Whey protein powder had a significant effect on emulsion capacity. Camel sausage formulated with whey protein had higher emulsion capacity than control one. In addition, emulsion capacity increased with the increasing of whey protein level. Data of pH value are consistency with the results of emulsion capacity % of camel sausage samples, which mean that emulsion capacity increased with the increasing of pH value and whey protein level. These results are coincided with (Kurt & Zorba, 2005) they reported that addition of whey protein significantly increased the protein concentration and emulsion capacity. Also, they concluded that pH value had much higher effect than protein concentration on emulsion capacity of different type of meats (beef, turkey and chicken).Sausages formulated with whey protein powder had the higher emulsion stability (ES) than control one. Camel sausages formulated with 2 or 4% WPP had the higher emulsion stability than the other sausage samples. These results are close to that obtained by Serdaroğlu and Özsümer (2003) they found that addition of WP increased the ES of beef sausage formulated with different fat levels. In addition Kurt & Zorba (2005) reported that using WP increased significantly the emulsion stability of different type of meats (beef, turkey& chicken). These may be due to that addition of whey protein powder increased fat binding in the meat system even at lower fat levels (El-Magoli *et al.,* 1996) or the fact that whey proteins have a high capacity to bind water; i.e. high hydrophilic properties (Kocak & Aydemir, 1994).

Cooking parameters and physical properties

Data in Table 3.Showed that whey protein had a significant effect on the cooking loss of camel sausage. The lowest cooking loss was found in sausage formulated with 4% followed by sausage with 2% whey protein. No significant differences were found in sausages with 1% WPP and control. Sausage with 3% WPP had the highest cooking loss. These results are close to that obtained by Serdaroğlu (2006) which found that meatballs prepared with 2 or 4% whey protein were significantly higher for cooking yield at different fat levels. Also, Hale *et al.* (2002) found that beef patties containing textured whey protein had the lowest cooking loss than control one. In addition, Andiç *et al.* (2010) reported that addition WP improved the cooking yields of beef patties. They also found that patties formulated with 2% WP had the highest cooking yield. Sausage formulated with 1, 2 or 4% WPP had the highest moisture retention. Serdaroğlu (2006) found that addition of 2 or 4% whey protein to meatballs formulated with 5, 10, and 20 %fat significantly increased the moisture retention at each fat level. The same result was found by Andic *et al.* (2010) they noticed that beef patties formulated with 1 or 2% whey protein had higher moisture retention than the other patties.

Table.3: Cooking parameters and physical properties of camel sausage

Treatment	Cooking loss (%)	Moisture retention (%)	W.H.C (cm^2)	Plasticity (cm^2)
Control	44.45^{ab}	24.61^{b}	8.64^{a}	2.92^{c}
WPP1	43.64^{ab}	27.32^{a}	8.26^{a}	2.88^{c}
WPP2	42.34^{b}	27.35^{a}	4.74^{c}	3.60^{ab}
WPP3	44.86^{a}	24.19^{b}	6.62^{b}	3.04^{bc}
WPP4	40.13^{c}	27.48^{a}	3.00^{d}	4.04^{a}

$^{a-d}$ means within the same column with different superscripts letters are different ($p<0.05$).

Data in Table3.Represented a significantly improve in water holding capacity of camel sausage formulated with whey protein powder as compared to control one. The highest score of plasticity was found in sausage sample formulated

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with 4% WPP. These results are close to that obtained by Abdolghafour & Saghir (2014) who found a significantly increase in water holding capacity (WHC) of buffalo sausage formulated with different levels of whey protein powder as compared with control one. The same results were found by Serdaroğlu and Özsümer (2003) they reported that addition of whey protein increased WHC of beef sausage formulated with different levels of fat. Results of WHC were coincided with the results of cooking loss of camel sausage. Therefore, it can be concluded that addition of whey protein powder increased the WHC which cause a significant decrease in cooking loss%

Shrinkage measurements

Results of the reduction in width, length and shrinkage % of camel sausages were given in Table 4. Sausage formulated with 2 or 4% WPP had the lowest reduction in width, no significant differences were found in other sausage groups. Also, it can be noticed that sausage formulated with 4%WPP and control samples had the lowest reduction in sausage length. A slight difference was found between other sausage samples. All sausage samples trend to shrink during cooking process. Sausage formulated with 4% WPP recorded the lowest shrinkage %, while sausages of 3% WPP had the highest shrinkage %. A difference between the other sausage samples was not significant. Kumar and Sharma (2003) found that the higher reduction in diameter was found in control and the lowest reduction found in low-fat patties formulated with 10 % milk co- precipitates.

Table.4: Shrinkage measurements of camel sausage

Treatment	Reduction in width (%)	Reduction in length (%)	Shrinkage (%)
Control	23.71^{a}	10.99^{c}	13.40^{b}
WPP1	25.17^{a}	12.06^{bc}	13.39^{b}
WPP2	13.53^{b}	13.91^{ab}	13.79^{b}
WPP3	21.33^{a}	14.82^{a}	16.13^{a}
WPP4	15.73^{b}	10.98^{c}	11.82^{b}

$^{a-c}$ means within the same column with different superscripts letters are different ($p<0.05$).

The gain in height of patties was increased with increasing level of incorporation amongst the low-fat products. The shrinkage percent was indirectly proportional to the level of incorporation of milk co-precipitates with maximum shrinkage in the control group and minimum in the low-fat patties with 10 % milk co-precipitates. Also, El-Magoli *et al.*(1996) found that addition of increasing levels of whey protein concentrate (WPC) to low fat beef patties resulted in a linear decrease in shrinkage.

Color measurements

The effects of whey protein level on color attributes of fresh camel sausages were shown in Table 5. Sausages formulated with 4% WPP had the highest *L** value followed by sausage with2%.

Table.5: Color measurements of camel sausage

Treatment	*L**	*a**	*b**
Control	40.23^{c}	8.78^{b}	6.48^{d}
WPP1	39.90^{c}	9.26^{a}	8.66^{a}
WPP2	41.00^{b}	9.26^{a}	7.93^{b}
WPP3	40.36^{c}	9.14^{ab}	7.02^{c}
WPP4	43.70^{a}	9.01^{ab}	8.88^{a}

$^{a-d}$ means within the same column with different superscripts letters are different ($p<0.05$).

No significant differences were found in other samples. The lowest *a** value was found in control samples, slight differences were found between all sausage samples formulated with WPP at different levels. Control sample had the lowest *b** value than sausages formulated with whey protein. These results are close to that obtained by Yetim *et al.*(2006) who found that sausages formulated with different level of liquid whey protein had higher *L*,a** and *b** values compared with control one. These results go in parallel to that obtained by Abdolghafour & Saghir (2014).

Sensory evaluation

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From data in Table 6. It can found that sausage formulated with 4% WPP recorded the highest score for appearance followed by sausage formulated with 1 and 3% WPP. A slight difference was found in other sausages sample. Also, sausage with 4% WPP had the highest score for texture and no significant differences were found in the other sausage samples.

Table.6: Sensory evaluation of camel sausage

Treatment	Appearance	Texture	Juiciness	Flavor	Tenderness	Overall acceptability
Control	7.33^{b}	7.33^{b}	7.33^{b}	7.22^{b}	7.00^{b}	7.22^{b}
WPP1	7.90^{ab}	7.40^{b}	7.40^{b}	7.70^{b}	7.90^{ab}	7.40^{b}
WPP2	7.20^{b}	7.20^{b}	7.20^{b}	7.00^{b}	7.20^{b}	6.80^{b}
WPP3	8.30^{ab}	7.60^{b}	8.10^{ab}	6.60^{b}	7.10^{b}	7.50^{b}
WPP4	8.77^{a}	9.11^{a}	8.88^{a}	9.44^{a}	8.66^{a}	8.88^{a}

$^{a-b}$ means within the same column with different superscripts letters are different ($p<0.05$).

The high score for juiciness was recorded in sausage formulated with 4% WPP followed by sausage with 3% WPP and no significant differences were found in the other sausage samples. Sausage formulated with 4% WPP was more tender, more flavor and more acceptable than all sausage samples. Generally, sausage formulated with 4% WPP had the highest score for all sensory attributes and no significant differences were recorded between the other sausage samples. These results are close to that found by El-Magoli *et al.*(1996) they reported that sensory analysis showed the 4% WPC level to be preferred over lower levels with respect to juiciness and overall acceptability. Serdaroğlu (2006) reported that panels were not able to detect the addition of WP in meatball samples. Also, Andic *et al.*(2010) they found no significant differences in appearance, interior color, juiciness and flavor scores of patties formulated with 1% and 2% WP. The same results were found by Abdolghafour & Saghir (2014).

IV. CONCLUSION

Addition of whey protein powder significantly improved the quality characteristics of camel sausage formulated with 4% WPP and showed the highest emulsion capacity and emulsion stability, in addition to the highest score of flavor, tenderness and overall acceptability. Whey protein powder (WPP) can be used in camel sausage formula to improve the quality characteristics of the product.

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Isothermal and Batch Adsorption Studies of Malachite Green Oxalate Dye onto Activated Carbon from Snail Shell

Ikhazuangbe P.M.O.[1], Eruotor M.O.[2]

[1]Chemical engineering department, Federal University of Technology, Owerri, Nigeria
[2]Physics with electronics department, Western Delta University, Oghara, Nigeria

Abstract*—Adsorption efficiency, kinetic and thermodynamic studies of the adsorption of Malachite green oxalate onto activated carbon from snail shell was carried out. The cleaned Snail shell was carbonized at 400^{o}C, crushed and sieved before it was activated with 0.1m HCl at 800^{o}C in a furnace. Batch adsorption experiment was carried out at variable concentration, time and temperature while other factors are kept constant. The adsorption isotherms used show that the correlation coefficient of Freundlich isotherm is closer to unity compare to that of Langmuir isotherm. The adsorption follows the Pseudo second order kinetic with adsorption capacity of 1.7544 (mg/g) and rate constant of 0.471(g/mg.min). The thermodynamic parameters: change in enthalpy, ΔH = 15.90 KJ/mol, change in entropy ΔS = 60.16J/mol. K and the change in Gibbs free energy ΔG = -1.69, -2.98, -3.64, -3.24, -3.43 and -3.51 KJ/mol at 303, 308, 313, 318, 323 and 328K respectively. These results show that activated carbon from snail shell has the potential of a good low cost adsorbent for the removal of this hazardous dye from wastewater.*

***Keywords*— Adsorption, kinetic, Malachite green oxalate, snail shell, thermodynamic.**

I. INTRODUCTION

The textile, paper, printing and dye industries consume large quantities of water at its different steps of dyeing and finishing processes. Due to the large volume of water consumption, the production of huge volume of wastewater is inevitable. Generally, the wastewater from printing and dyeing units in these plants contain residue of dyes and chemicals [5]. The presence of these dyes in wastewater is not desirable because of their toxic nature to the life and environment into which they are discharged. Therefore, the removal of such compounds from wastewater is a vital task.

Adsorption process using activated carbons is widely used to remove pollutants from wastewaters. However, commercially available activated carbon is expensive. In the last years, special emphasis on the preparation of activated carbons from several agricultural by-products has been given, due to the growing interest in low cost activated carbons from renewable, copious, especially for application concerning treatment of wastewater. Researchers have studied the production of activated carbon from palm-tree cobs, plum kernels, cassava peel, bagasse, jute fiber, rice husks, olive stones, date pits, fruit stones and nutshells [2].

In this study, the ability of snail shell carbon to remove Malachite green oxalate by adsorption is been studied. The Langmuir and Freundlich isotherms will used to fit the equilibrium data. Pseudo-first order and pseudo-second order models will be used to fit the experimental data and the thermodynamic study will also be carried out [3].

II. THEORY

2.1 Adsorption kinetics

The pseudo first order and second order kinetic models need to be tested to determine which model is in good agreement with experiment adsorption capacity (q_e) value, thus suggesting which model the adsorption system follows.

2.1.1 Pseudo-first order equation

The Largergren model assumes a first order adsorption kinetics and can be represented by the equation.

$$\frac{dq_t}{dt} = K_1(q_e - q_t) \quad (1)$$

$$\text{Log}(q_e - q_t) = \text{Log}(q_e) - \frac{K_1}{2.303} t \quad (2)$$

The values of Log ($q_e - q_t$) were linearly correlated with time t. The plot of Log ($q_e - q_t$) versus t should give a linear relationship from which K_1 and q_e can be determined from the slope and intercept of the plot, respectively.

2.1.2 Pseudo-second order equation

The pseudo-second-order adsorption kinetic rates equation is expressed as

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$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \quad (3)$$

Where K_2 is the rate constant of the pseudo second order adsorption (g/mg.min). The plot of (t/q_t) and t of equation 3 should give a linear relationship from which q_e and K_2 can be determined [4].

2.2 Thermodynamic studies

The determination of the basic thermodynamic parameters such as enthalpy (ΔH), Gibb's free energy (ΔG) and entropy (ΔS) of the adsorption is important, as it determines if the process is favorable or not from thermodynamic point of view, to assess the spontaneity of the system and to ascertain the exothermic or endothermic nature of the process. An adsorption process is generally considered as physical if ΔH < 84 kJ mol^{-1} and as chemical when ΔH lies between 84 and 420 kJ mol^{-1} [9].

Using equations 4 to 6

$$\Delta G = -RT\ln K_d \quad (4)$$

$$K_d = \frac{qe}{Ce} \quad (5)$$

$$\ln K_d = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \quad (6)$$

The thermodynamic parameters of the adsorption process were determined from the experimental data obtained at various temperatures.

Where K_d is the distribution coefficient for the adsorption, q_e is the amount of dye (mg/l) adsorbed at equilibrium, C_e is the equilibrium concentration (mg/l) of the dye in solution, T is the absolute temperature in Kelvin, R is gas constant (8.314 $J.K^{-1}.mol^{-1}$), ΔG, ΔH, and ΔS are change in Gibbs free energy, change in enthalpy and entropy change respectively. The values of enthalpy change (ΔH) and entropy change (ΔS) are obtained from the slope and intercept of $\ln K_d$ versus 1/T plots [1].

2.3 Adsorption isotherm

2.3.1 Langmuir adsorption isotherm (model)

The Langmuir equation is probably the best known and most widely applied adsorption isotherm. It is represented as follows in equation 7

$$\frac{Ce}{qe} = \frac{1}{b\,Qo} + \frac{Ce}{Qo} \quad (7)$$

From which values of Q_o and b can be determined from the slope and intercept respectively of the plot of Ce/qe versus C_e

Where Q_o and b are Langmuir constants, q_e is amount of solute removed or adsorbed at equilibrium. C_e, is equilibrium concentration of mixture.

2.3.2 Freundlich adsorption isotherm (model)

The Freundlich isotherm is an empirical relationship which often gives a more satisfactory model of experimental data. It can be expressed as follows:

$$\text{Log} q_e = \text{Log}\,(K_f) + \frac{1}{n}\text{Log}\,C_e \quad (8)$$

Where C_e and q_e are equilibrium concentration and adsorption capacity at equilibrium stage, while K_f and n are Freundlich constants which incorporates all factors affecting the adsorption process (adsorption capacity and intensity). Values of K_f and n can be obtained from the intercept and slope of a plot of adsorption capacity, q_e against equilibrium concentration C_e[8].

III. MATERIALS AND METHODS

3.1 Preparation of adsorbent

Sample of snail shells were picked from the environment in Elele, Rivers State, Nigeria. The snail shells were washed with tap several times to remove the dust and other water-soluble materials. The process continues until the washing water was colorless, then dried in the open air. The dried snail shells were carbonized in a furnace (SX-5-12) at 400˚C for 3 hours, the charred were allowed to cool to room temperature and ground. 100 gram of the ground carbonized snail shells was added to 300 ml of 0.1M HCl solution, thoroughly mixed and heated until it formed slurry. The slurry was transferred to a crucible and heated in a furnace (SX-5-12) at 800˚C for 3 hours, allowed to cool to room temperature and washed with de-ionized water, dried in an oven at 110˚C for 2 hours [7].

3.2 Preparation of adsorbate

The malachite green oxalate used is of laboratory grade (KEM LIGHT, India). The solution was prepared in de-ionized water from Ion-exchange (Indian) Ltd, Eleme, Port Harcourt, Nigeria. 150mg of the dye was dissolved in 1000ml of de-ionized water to prepare the standard solution. Experimental solutions of the desired concentrations were obtained by successive dilutions with de-ionized water.

3.3 Adsorption experiment

1000mg of the activated carbon of snail shell was mixed with 50ml of malachite green oxalate solution of the desired concentrations (25, 50, 75, 100, 125 and 150mg/L) at 30ºC in a temperature controlled water bath with constant shaking. The samples were withdrawn after 30 minutes and dye solutions were separated from the adsorbent using Whatmann filter paper. The concentration of the filtrate was measured with a UV spectrophotometer (2OD) at 618nm. The experiment was repeated using 1000mg of the activated carbon with 50ml of 50mg/L concentration of malachite green oxalate solution at 30ºC in a temperature controlled

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water bath with constant shaking. The samples were withdrawn after 30, 60, 90, 120, 150 and 180minutes respectively and filtered using Whatmann filter paper. The concentration of the filtrate was measured with a UV spectrophotometer (2OD) at 618nm. Again 1000mg of the activated carbon mixed with 50ml of 50mg/L concentration of malachite green oxalate solution at 30, 35, 40, 45, 50 and 55ºC in a temperature controlled water bath (DK – 420) with constant shaking was also carried out. The samples were withdrawn after 30minutes respectively filtered and the concentration measured.

The adsorption amount of malachite green oxalate dye adsorbed onto the snail shell adsorbent at equilibrium was calculated with the following equation:

$$q_e = \frac{(C_o - C_e)V}{X} \quad (11)$$

Where C_0 (mg/L) and C_{eq} (mg/L) are the initial and equilibrium concentration of the dyes, V (L) is the volume of solution, X (g) is the weight of adsorbent in one container.

The percentage of snail shell adsorbed was calculated as:

$$\% \text{ adsorbed} = \frac{(C_o - C_e)}{C_o} \text{ X } 100 \quad (12)$$

IV. RESULTS

The results of the adsorption experiment are presented graphically in the figures below.

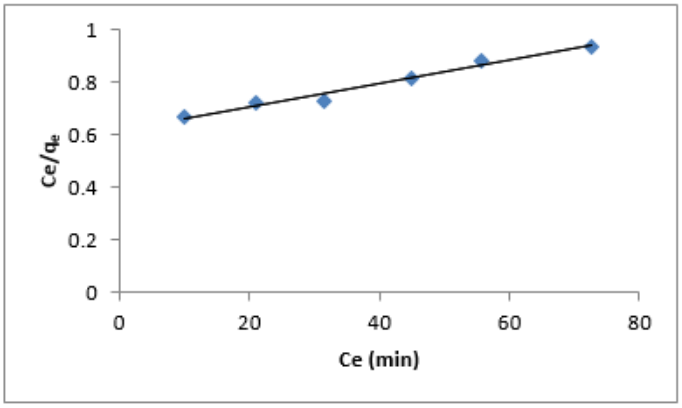

Fig.1: Langmuir model

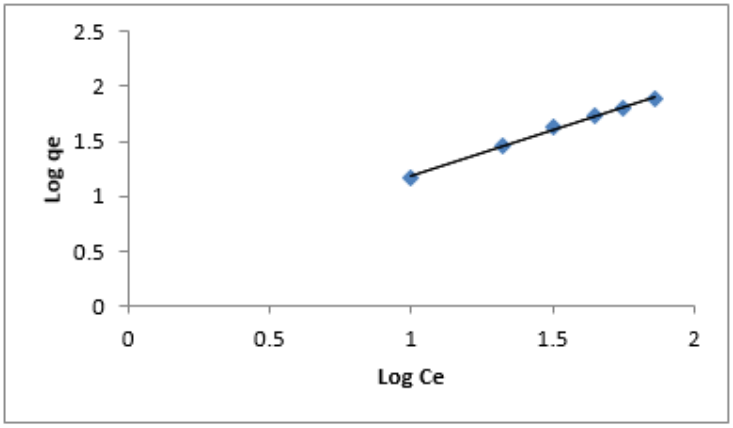

Fig.2: Freundlich model

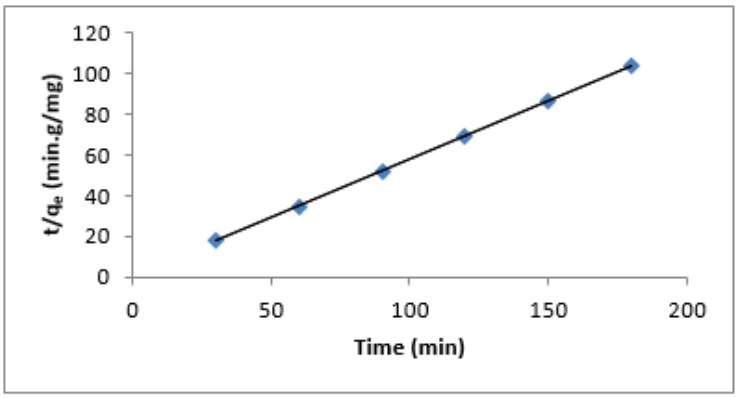

Fig. 3: Pseudo second order reaction

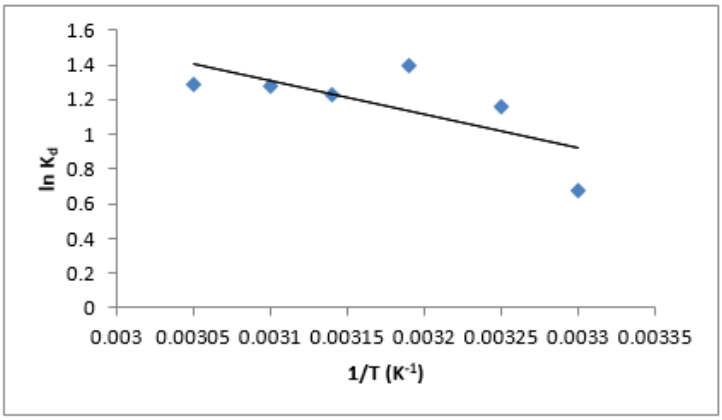

Fig. 4: Vant's Hoff model

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Table.1: Adsorption efficiency

Variable time		Variable temperature		Variable concentration	
Time (min)	% Adsorption	Temp ^oC	% Adsorption	Conc. (mg/L)	% Adsorption
30	64.73	30	64.73	25	81.09
60	75.82	35	76.55	50	79.27
90	76.13	40	76.91	75	73.33
120	76.36	45	78.18	100	72.45
150	76.36	50	78.91	125	71.71
180	76.36	55	79.09	150	70.91

Table.2: Adsorption isotherm constants for coconut fibre activated carbon

Langmuir			Freundlich		
$Q_o(\frac{g}{mg})$	$b(\frac{mg}{l})$	R^2	$K_f(\frac{mg}{g})$	$n(\frac{l}{g})$	R^2
250	0.0065	0.978	2.3067	1.206	0.995

Table.3: Thermodynamic parameters for erythrosine adsorption by activated carbon

Temperature (K)	ΔG (KJ/mol)	ΔH (KJ/mol)	ΔS (J/mol.K)
303	-1.69	15.90	60.16
308	-2.98		
313	-3.64		
318	-3.24		
323	-3.43		
328	-3.51		

V. DISCUSSION

The efficiency of the adsorption presented in table 1, shows that adsorption increases as time increases until 120min when the active sites were filled, then the adsorption efficiency becomes constant. The table also shows that adsorption increases with increase temperature, but after 35^{o}C, increasing the temperature will no longer be economical. As the concentration of the adsorbate is increased, the efficiency of adsorption decreases, though highest at 25mg/l, but it is more economical with 50mg/l.

The values of the adsorption models presented in table 2 shows that the correlation coefficient of Freundlich isotherm is closer to 1 than that of Langmuir, indicating that it a heterogeneous adsorption process.

The kinetic of adsorption of Malachite green oxalate onto snail shell was studied using pseudo first-order and second-order equations for the examined system. The pseudo second-order kinetic model provided the best correlation for the experimental data.

From the thermodynamic point of view, the positive value of ΔH indicates that the adsorption of Malachite green oxalate on snail shell is endothermic and a physical process. The positive value of ΔS shows the existence of structural changes at the solid–liquid interface and ΔS favors ion exchange and stability of adsorption.

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Table.4: Adsorption capacities of some adsorbents for MG oxalate removal

Adsorbent	T°C	Adsorption capacity(mg/g)	Reference
Rattan sawdust	30	22.4	Hameed and El-Khaiary (2008)
Prawn-Carbon	30	1.5249	Santhi (2009)
Prawn-Raw	30	5.6635	Santhi (2009)
Activated charcoal		0.180	Iqba and Ashiq (2007)
Sugar cane dust		4.88	Khattri and Singh (1999)
Bentonite		178.6	Bulut etal (2008)
Nickel ferrite		4.67	Manohar (2015)
Wood apple shell	25	34.56	Ashish etal. (2014)
Carbon prepared from Borassus bark	30	20.70	Arivoli et al. (2009)
Carbon prepared from Arundo donax root	30	8.69	Zhang et al. (2008)
Periwinkle shell	30	1.96	Ikhazuangbe etal, (2017)
Coconut fibre	30	1.88	Ikhazuangbe etal, (2017)
Snail shell	30	1.75	Present

Table.5: Enthalpy and Entropy change of some adsorbent for the dyes

Adsorbent	ΔH^o	ΔS^o	Reference
Thevetia peruviana	61.127	227.0	Baseri et al, (2012)
Pandanus leaves	60.762	231.4	Hema and Arivoli (2008)
Bentonite	13.21	62.85	Bulut et al, (2008)
Wood apple shell	1.581	6.375	Ashish et al, (2014)
Coconut fibre	20.45	74.34	Ikhazuangbe etal, (2017)
Periwinkle shell	19.74	72.21	Ikhazuangbe etal, (2017)
Snail shell	15.90	60.16	Present

VI. CONCLUSION

From the adsorption efficiency, kinetic and thermodynamic studies of the adsorption of Malachite green oxalate onto activated carbon from snail shell studied, the results obtained from the analysis show that snail shell has good potential as low cost adsorbent for the removal of this hazardous dye from wastewater.

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The nesting ecology of weaverbirds in Ekona farms, Southwest Region, Cameroon

Melle ekane Maurice*,Nkwatoh Athanasius Fuashi, Viku Bruno Agiamte-Mbom ,Tim Killian Lengha

*Department of Environmental Science, University of Buea, P.O.Box 63, Cameroon

Abstract— *Ecological factors play a key role in determining nest construction and success in weaver birds. The objective of this survey was to determine the ecological role on the nest construction in weaver birds in Ekona farms. The research data was collected from March – August 2016, by randomly laying six transects of 1km long and 100m wide each within the study area, and four different locations were visited also to observe the daily nesting-activities of the weaver birds. The ecological data of the weaver birds nesting behaviour was observed and recorded, against the day-period, weather and seasonal changes. The data was analysed using Chi-square and Pearson correlation statistical models. The result showed a positive correlation between the weaver birds' population and nest density in both seasons (R^2=0.5407 at P < 0.05). Moreover, from the analysis, the relationship between nest-building and plant-type used recorded significance (X^2= 69.1040, df= 28 at P < 0.05). In addition, it was observed that nest-building in the sunny weather was more intense than in rainy weather, 54.57% for sunny, 42.86% for rainy and 2.7% for cloudy weather conditions. Furthermore, there was a significant relationship (X^2= 830.752, df=44 at P< 0.05) between weaver birds' activities and the day-period. The study has revealed that both the seasonal and weather changes can affect the nest-building activities of the weaver birds in Ekona farming area.*

Keywords— Weaver birds, population distribution, nesting behavior, nest building, weather.

I. INTRODUCTION

Weaver birds form large foraging flocks and nesting colonies, are often involved in synchronized competitive actions such as displacing other bird species in foraging areas and mobbing intruders near and within colonies (Lahti, 2003). Weaver birds builds elaborate, enclosed nests in often dense colonies, and prefers the proximity of human habitation and agriculture (Lahti *et al*., 2002). Weaver nests represent one of the most remarkable constructions produced by any animal. In most species the male makes the major contribution to nest construction, and the female adds lining if she accepts a nest. In the 'true' weavers (subfamily Ploceinae) males construct intricately woven nests using thin strips of plant material. Typically, nest-building starts with the construction of a bridge between supports, usually thin twigs. The male then perches on this bridge while weaving the nest bowl (Collias and Collias 1964). The nest entrance is either to the side or faces vertically downwards; in some species it is extended into a tunnel from 10 cm to more than 1 m long. Nests of buffalo-weavers (*Bubalornis niger*) and sparrow-weavers (*Plocepasser mahali*) are composed of dry pieces of vegetation, inserted and interlocked into a complex structure without any weaving or knotting (Collias and Collias 1964). Several weaver species strip the leaves of the twigs around their colonies; this makes the colony more visible but it may be a displacement activity (Oschadleus 2000). In the polygynous species, males build a succession of nests to which they attract females by displaying; the females line the nest, lay, incubate, and rear the nestlings with no or little male assistance. Colonies can consist of hundreds of males or single males (Tarboton 2001). In the monogamous species, sexes share parental duties and build a single nest per breeding season (Tarboton 2001).

A diverse type of host plants preferred by *Ploceus Philippinus* was observed in various types of habitats like agricultural fields, forest areas, dams, hillslopes, open wells and irrigation channels. Selection of host plant for hanging the nest is one of the important factors for nest weaving. The plant twigs are generally thin pliable and pendant to horizontal branches (leaves in case of palms) are selected by *Ploceus philippinus* to suspend its nest. Twigs having at thickness more than a human thumb are generally not selected possibly because they can't be accommodated in the grip of claws during the knitting of fibers for the nest initiation. Strongly upward facing branches are also not preferred. Twigs which are sufficiently tough and strong as

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to support the weight of nest are selected. Their terminal or bulb terminal portions are used to anchor the report nests.

Resources that limit the size and distribution of animal populations can also determine the composition and structure of communities in ecological networks. For example, abundance of food often determines how bird species compete and coexist in communities (Mac Nally & Timewell 2005). For shelter-using species such as cavity-nesting birds and mammals, population size and community structure may be determined by the availability of shelters (Aitken & Martin 2008). The weaver nests are used both for breeding and roosting and are normally located in a compact group on one side of the nest-tree. The nest have breeding nests (with one entrance) and are easy to identify, they often do not serve a single function: roost nests are often converted into breeding nests and vice versa. Nest building is an important activity of weaverbirds in which all group members participate. Nests have a marked influence on the survival of weaver groups and the communal nest building may therefore be a form of altruism. The selective forces responsible for this altruism could be of prime importance in shaping weaver sociality. When birds build nests, more than one environmental constraint is often involved in determining the nest site. Some bird species often nest on cliffs and not in trees, presumably as an adaptation against predation. However, this nest site preference causes chicks to be exposed to a prohibitive amount of shade and cold. Mosher & White (1976) found that golden eagle (*Aquila chrysaetos*) nests were situated on cliffs where the chicks were exposed to the maximum amount of solar radiation, and that this improves survival in a cold environment. Golden eagle nest placement is thus a response to predation as well as to ambient temperatures at the nest site.

The impact of weather on the population biology of birds has been a major field of study by ornithologists over the past half century (George *et al*., 1992). Weather not only affects the metabolic rate of birds (e.g. Cold weather requiring increased energy expenditure for body maintenance), but also exerts other indirect and direct effects on bird behaviour. For example, it can influence foraging conditions and the ability to carry out other essential behaviours, such as courtship. Weather also impacts on breeding success through, for example, chilling or starvation of young (Humphrey, 2004). Extreme weather events, such as prolonged frozen spells and droughts, can have catastrophic effects on bird populations, including long-term effects on whole cohorts (Humphrey, 2004). The aim of this study was to investigate the effect of weather and seasonal changes of the nest-building activity of the weaver birds in Ekona farmlands.

II. MATERIALS AND METHOD

Study area

This study was carried out in Ekona town which is located in Muyuka Sub-Division found in Southwest Region of Cameroon. Ekona is located between latitude 4° 9' N of the equator and longitude 9° 14' E of the Greenwich Meridian. The town is located along the Atlantic Coast within the Gulf of Guinea. The area has a surface area of about 179km^2 with an estimated population of 17513 people (National institute of statistics, 2005).

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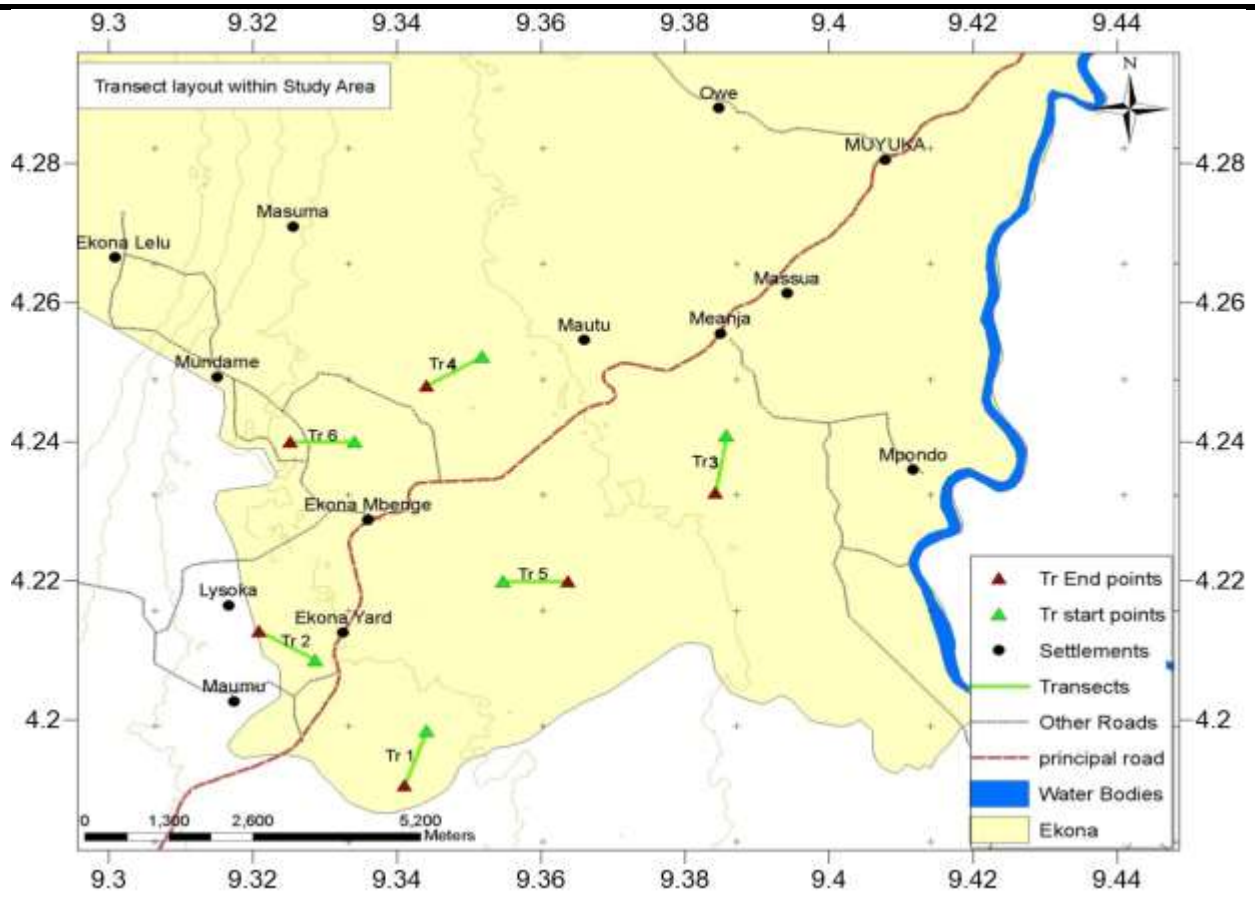

Fig.1: Map showing the location of study area, Source (Field Study, 2016)

The observation and counting of weaver birds' nest

Birds are perhaps the easiest of animals to enumerate. They are often brightly coloured, relatively easy to see, and highly vocal. They are also very popular to study, often give away their presence vocally and their calls and songs help to detect many species of birds. There are, however, some potential pit falls in birds' song as a census tool. In the present study, the field work consisted of direct observation of birds in the open and hide situations (Colinetal, 1993). During Field survey, the exact location of nesting activity of weaver birds was detected by following their calls and songs. The nest-built on tall plants and in remote places were observed from distance with binoculars. In each transect, birds' nests were counted within 10 sampling points for spot-count method. The same points were used both in dry and wet seasons. Upon reaching a point, 2-5 minutes were provided for the birds to settle in case of any disturbances (Bryan *et al*., 1984). Ten minutes were used to count and record all birds nest and activities observed or heard within 30m radius (Terborgh *et al*. (1990) and Robinson *et al*., (2000)). The study was conducted from 06:30am – 6:30pm each day (Stevenson and Fanshawe, 2002). A total number of 6 transects were laid, with a dimension of 1 kilometer in length and 100m in width within disturbed and undisturbed vegetation habitats. In laying these transects, a compass was used to get the direction and bearing readings for each of the transects. A Global Positioning System was also used to get the geographic coordinates to mark the starting and ending points of each transect. A measuring tape (100m) was used in measuring the length and width of the transect line. The direct counting method used involved the observation and counting of weaverbird nests along the transect line. This method has been used to monitor relative abundance of diurnal raptors, sparrow-weaver colonies (Douthwaite, 1992a). The nests were observed on a transect-walk for each day of the month within the entire study period. Four different zones with different groups (colony) of weaverbirds were visited for a period of 4 months. The ecological parameters like weather changes, day-period and seasonal changes were recorded at same time. The research data collected was analysed by using Chi-square and Pearson correlation statistical packages.

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III. RESULTS

Nests count

During this study, a total number of 2,710 weaverbirds nests were counted and recorded as shown in table 1. Table 1 also shows the percentage of weaverbirds' nests population found in each transect with T1 having a nest population of 777, T2= 678 nests, T3= 318 nests, T4= 323 nests, T5= 240 nests and T6= 374 nests, with percentages of 28.8%, 25.1%, 11.7%, 11.9%, 8.9% and 13.9% respectively. The total average mean nest population was 415 nests.

Table.1: Nests count

Months	T1	T2	T3	T4	T5	T6
March	66	82	33	54	9	36
April	130	131	68	56	25	60
May	98	152	46	58	40	72
June	139	113	56	75	64	95
July	199	138	57	44	50	52
August	145	62	58	36	52	59
Total	**777**	**678**	**318**	**323**	**240**	**374**
	(28.8%)	**(25.1%)**	**(11.7%)**	**(11.9%)**	**(8.9%)**	**(13.8%)**

T= transects

Relationship between weaver bird nest in the Dry and wet seasons

The results has shown (fig.2 and 3) a positive correlation on the weaverbird nest population distribution and seasonal changes (R^2= 0.5407 at P< 0.05). Also, the results showed a negative correlation of the nests distribution in the wet season,fig. 3.

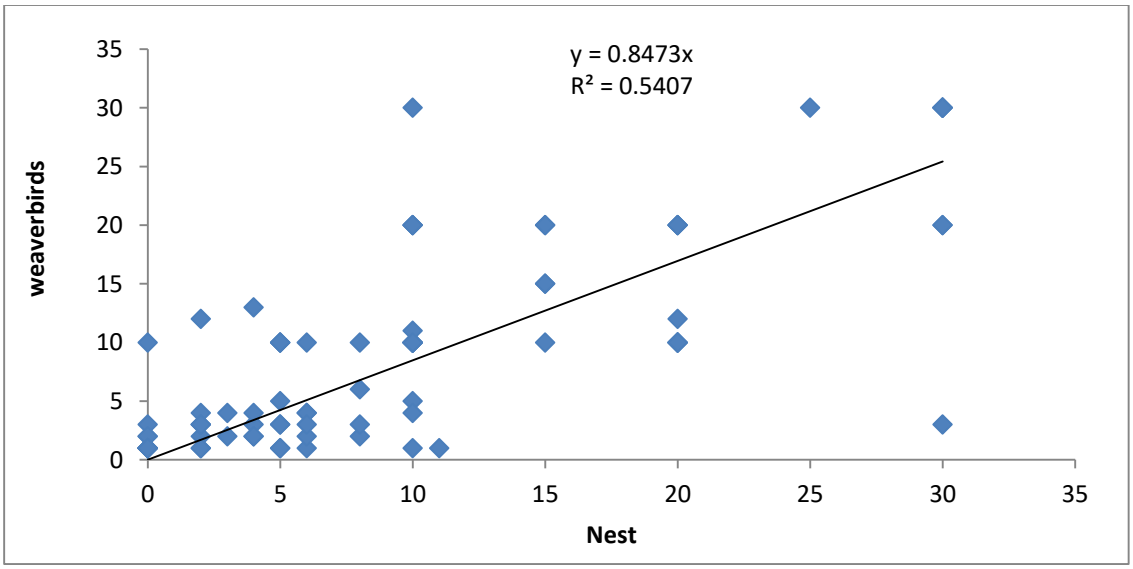

Fig.2: Relationship between weaver birds and nest population in the Dry season

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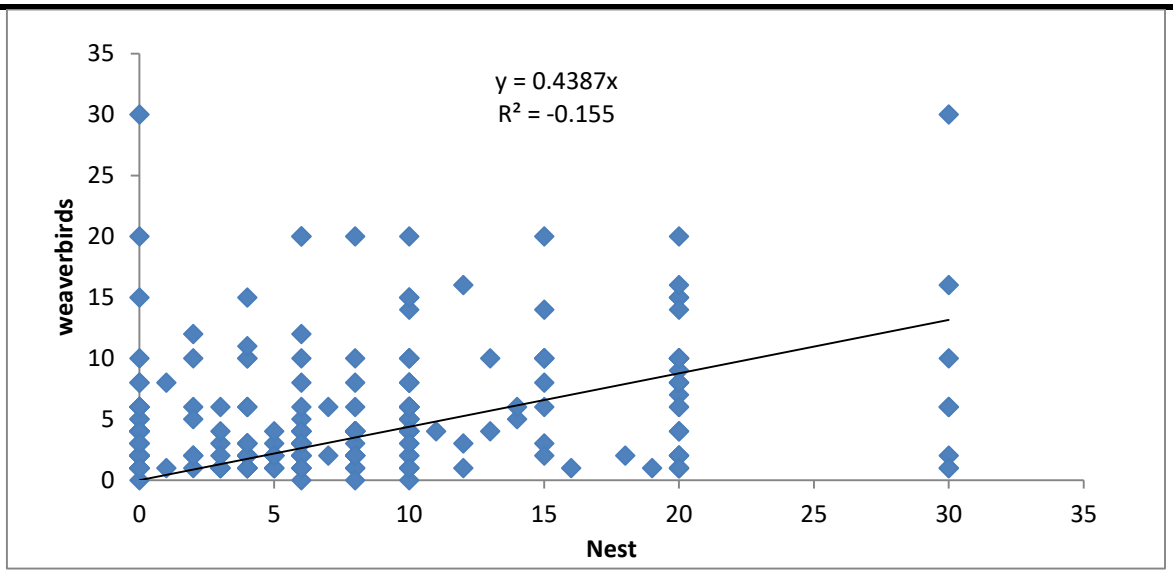

Fig.3: Relationship between weaver birds and nest population in the Wet Season

Identifying plant species used as materials used for Nest-building

Table 2 shows the plant-types on which weaverbirds were commonly observed nesting or picking nesting materials, oil-palm (22.9%), coco-nut (13.5%), maize (16.6%), Elephant-grass (10.7%), pear(8.5%), mango(9.4%) and plantain(10.0%). It was observed that nests are built on any moderately (table 2) tall trees (9-20 m), and some of these trees are of economic value (Mengesha *et al*., 2011). They include oil palm (*Elaeis guineensis*), coconut palm (*Cocos nucifera*), mango (*Mangifera indica*), plum (*Pygeum africanum*), Avocado (*Persea americana*) and sweet orange (*Citrus sp*.). Other commonly colonized plants found in the study area include bamboo (*Oxytenanthera abyssinica*) and Elephant Grass (*Pennisetum purpureum*), with plantains (*Musa paradisiaca*) and maize (*Zea mays*),as some of those economic crops in the study area (Funmilayo and Akande, 1976).

Table.2: Plant species weaverbirds perched on

Plant type and S.Name		**Transect number**						
C. Name	**Scientific ame**	**Transect 1**	**Transect 2**	**Transect 3**	**Transect 4**	**Transect 5**	**Transect 6**	**Total**
Moringa	*Moringa oleifera*	7 8.0%	2 3.9%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	**10** **3.1%**
Oil palm	*Elaeis guineensis*	20 22.7%	18 35.3%	7 16.4%	9 16.4%	9 23.7%	10 21.3%	**73** **22.9%**
Mango	*Mangifera indica*	12 13.6%	5 9.8%	3 5.5%	3 5.5%	2 5.3%	5 10.6%	**30** **9.4%**
Plantain	*Musa paradisiaca* *Musa sapientum*	7 8.0%	5 9.8%	0 10.9%	6 10.9%	3 7.9%	11 23.4%	**32** **10.0%**
Coco nut	*Cocos nucifera*	9 10.2%	2 3.9%	11 20.0%	11 20.0%	3 7.9%	7 14.9%	**43** **13.5%**
Maize	*Zea mays*	10 11.4%	5 9.8%	12 21.8%	12 21.8%	6 15.8%	8 17.0%	**53** **16.6%**
Plum tree	*Pygeum africanum*	6 6.8%	4 7.8%	1 7.3%	4 7.3%	1 2.6%	1 2.1%	**17** **5.3%**
Elephant	*Pennisetum*	12	5	0	4	9	4	**34**

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grass	*purpureum*	13.6%	9.8%	7.3%	7.3%	23.7%	8.5%	**10.7%**
Avocado	*Persea americana*	5	5	5	6	5	1	**27**
		5.7%	9.8%	10.9%	10.9%	13.2%	2.1%	**8.5%**
Total		**88**	**51**	**40**	**47**	**55**	**38**	**319**
		100.0%	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**

Nests were built on any moderately tall (9-20 m) tree, plant percentages on which weaverbird extracted materials for nest-building were, oil palm (17.2%), coco nut (2.6%), maize(26.0%), Plantains (15.3%), Elephant grass(15.8%) and pear(2.6%) table 3.

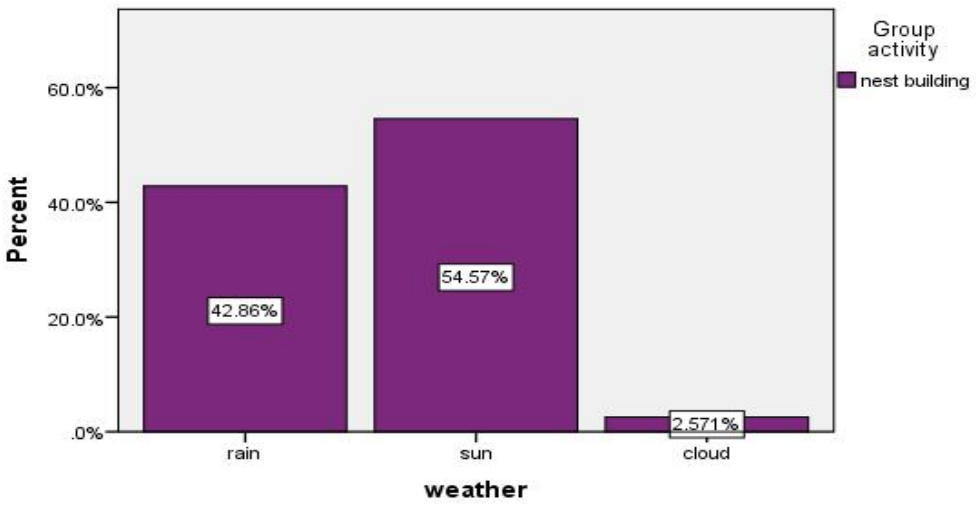
Table.3: Plant species used by weaver birds for nesting in Ekona farming area

Plant	Sc. name	Frequency	Percentage(%)	Used as Nest materials
Moringa	*Moringa oleifera*	4	0.7%	-
Oil palm	*Elaeis guineensis*	99	17.2%	√
Coco nuts	*Cocos nucifera*	15	2.6%	√
Mango	*Mangifera indica*	77	3.6%	-
Plantains	*Musa paradisiaca* *Musa sapientum*	88	15.3%	√
Plum tree	*Pygeum africanum*	8	1.4%	-
Maize	*Zea mays*	150	26.0%	√
Elephant grass	*Pennisetum purpureum*	91	15.8%	√
Sun flower	*Helianthus annuus*	23	4.0%	-
Pear	*Persea americana*	15	2.6%	√
Paw paw	*Carica papaya*	6	1.0%	-
Total		**576**	**100**	

[√]= plants commonly used as nesting materials

Nest-building and weather

It was observed that nest-building was more during the sunny weather than during the rainy weather, and very low nest-building activity was observed during a cloudy weather condition, with percentages at 54.57%, 42.86%, and 2.7% for cloudy weather conditions (fig.4).



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Fig,4: Nest-building and weather

Relationship between nest-building and plant-type

It was observed that nest-building has a strong relationship with some crops amongst other activities carried out by the weaver birds (fig.4). Nests were observed more on Maize plants (n=165), oil palms (n=114) and plantains (n=94). Moreover, the results revealed a significant relationship between nest-building and plant-type (X^2= 69.1040), df= 28, P < 0.05)), table 4.

Table.4: Relationship between nest building and plant type

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	69.104	28	.000
Likelihood Ratio	71.643	28	.000
Linear-by-Linear Association	4.604	1	.032
N of Valid Cases576			

Table.5: Relationship between weave bird activity and time of the day

Chi-Square Tests

	Value	df	Asymp. Sig. (2- sided)
Pearson Chi-Square	830.752	44	.000
Likelihood Ratio	703.484	44	. 000
Linear-by-Linear Association	.016	1	.899
N of Valid Cases	576		

From analysis, it was realized that there was significant relationship (X^2= 830.752, df =44 P= 0.000) between activity and period of the day.

IV. DISCUSSION

In many avian species, understanding the factors influencing nesting ecology can give vital insight in determining the means of handling the population. Other life cycle elements may also come into play, one of which is adult survival, however, nest-building has the greatest effect on recruitment, and is also the most easily studied and managed. Understanding the influences on nest-building is important for conservation purposes, and for predicting how a population of weaver bird in Ekona farming area may be affected by a disturbance. In many species it has been observed that nest-building declines after the breeding season. This reduction in number of nests may have a number of causes, including a decrease in food availability for nestlings, an increase in predation pressure, or a decline in habitat quality. It would seem that breeding as early as possible would be ideal, but the breeding female is constrained by low food availability (Perrins 1965).

The stripping of leaves from plant species such as oil-palm, coco-nut, maize and plantains leads to the complete damage of the plants in a long run, as they lack leaves for food production necessary for the plant growth and sustenance. Defoliation leads first to reduction in the fruit yielding capabilities of the trees and later the death of the trees (Funmilayo, 1973). The tearing down of rejected nest implies the need for materials for the construction of another in its place. These is done constantly as the female weaver will reject a nest, which therefore leads to more damages to crops in the process of acquiring more material to construct new nest. Estimates of physical losses, however, underestimate true economic losses as they do not account for costs entailed by the supply response adjustments that farmers make when faced with pests. Farms nearer to trees with extensive canopies and branches in Ekona farms were observed more attacked because of the weaverbirds colonization of these trees. In the study area, farmers identified weaverbirds as a major pest on maize, plantains and palms, hence, applied a number of traditional methods used in managing the menace from the birds. Guarding, human scaring which was used most by the

farmers, definitely consumed the time of their family, but this however was not probably included in their estimate costing of control expenditure, similar to findings from De Mey *et al*., (2013) which explains that direct economic impacts, bird damage also has substantial social consequences. On the one hand, farmers who scare birds in the field are socially separated from their family for a long time. On the other hand, traditional bird scaring is frequently undertaken by children who sometimes miss school, and in doing so, jeopardizes the key education objectives such as universal primary enrolment (De Mey *et al*., 2012).

Nest-building and the nesting materials

Weaverbirds normally use only fresh, green, flexible materials to weave their nests. The outer shell is woven by the male, into long strips torn from the leaves of elephant grass or palms. At one of the colony sites in the study area, the nest-shell was woven with strips of leaves from oil palm and maize, and lined with cassava leaves (table 3). When freshly completed the nest is approximately 18 cm long and weighs 30 g. The entrance of the nest faces downwards or sometimes sideways and is about 8 cm x 8 cm wide (*Yisau et al 2014*). Many nests are left uncompleted while many completed ones are destroyed after some time and again re-built or totally abandoned. The number of nests present in the colony tree at any one time reflects the turnover rate between nests built and nests destroyed. After a week or two a male will usually tear down a nest that has been consistently ignored or rejected by inspecting females and build a fresh model in its place (Collias and Collias, 1964).

Nest-building, weather and seasonality

Weaver bird nest-building characteristics are affected by seasonality aspects and weather conditions (fig. 4), this is in agreement with Rahayuninagsih *et al*., (2007) who found bird community structures be affected by several factors. Similarly, habitat diversity and changes, seasonal variations in climate and natural resources have affected weaver bird building structure of the study area. Thus, higher nest-building in the disturbed habitat and lower in the undisturbed habitat could be attributed to the difference in the vegetation community structure of the two habitats that determine food, water and cover availability (Mengesha *et al*., 2011).The disturbed habitat had diverse crop plant species mixed with the remnant original vegetation community. This could be the reason for high bird species richness in this habitat (Mengesha *et al*., 2011). There was increase in nest-building activities during the wet season as compared to the dry season in transect 3 and transect 5 which had undisturbed vegetation, unlike the disturbed habitat (transect 4, transect 3, transect 2 and transect 1) that were located within the agricultural farms and human settlement were remarkable with increase nesting activities in the dry season more than the rainy season. This might be due to the seasonal variation in the structure and types of plant communities that contributed to the availability of nesting material and the presence of weaverbirds in the study area. On the other hand, alteration of the shrub, tree and canopy layers might have caused a reduction in the total nesting resource availability for the weaver birds (Ukmar *et al*., 2007). The presence of more preferred trees species for nesting in this study contributed the huge number of the weaverbird population in the farms as compared to the findings of Inah, *et al.,* (1999) who noted that the presence of the birds' population could be influenced by a high agricultural activity taking place in the farming area. The observed colonization of some oil-palm, mango and coco-nut trees in transect 4 and transect 6 could be as a result of their location within and close to farms, which confirms the findings of Lahti and Lahti (2002).

V. CONCLUSION

The high ability of birds to survive in many parts of the world, even areas of extreme environmental conditions is based on their adaptability, intelligence, sight, and flight flexibility. In addition, the feeding ecology of birds is very diverse, and their feeding strategy has been survived during food scarcity by resistant long flight from one place to another and continent to continent. However, the population increase of the weaver birds in certain countries has presented challenges with severe financial implication in the agro-industry. And, the most seriously affected are the local farmers who would often not be able to afford the cost of preventing these bird-pests from visiting their farms. The study of the weaver birds' invasion and nest-building success in Ekona farming area has revealed that most of the nests were weaved and built with plantain, maize and palm leaves, materials harvested from the same farms. Moreover, the nest-building activities were high during the bright sunny weather and the dry season. The peasant farmers whose main survival force is the crop-farms are often seriously impoverished by the poor crop-harvest. Furthermore, the plantain and palm stems anchoring most of the nest and the nest-building activities would often wither, increasing more chances of unsustainable cultivation.

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Use of Remote Sensing Data to Detect Environmental Degradation in the Oil Rich Region of Southern Nigeria between 2003 and 2015

Ojiako J.C, Duru U.U*

*Department of Surveying & Geoinformatics, Nnamdi Azikiwe University Awka, Nigeria

Abstract*—The oil spill management system aims to achieve a knowledge- based system which can choose the most suitable method of response in shorter time by analyzing the various sensitivity factors of coastal environment, affecting parameters on oil spill movement, environmental concerns in oil spill response, and consequent monitoring and clean-up measurements. The major advantage of this integration is the ability to extract oil spill parameters such as location, linear size and spill areas. Spatial and temporal information, i.e. oil spill distribution at the sea, its frequency and evolution in time allow the scientists to establish the major cause and source of oil spills, and then outline the risk areas. This study has demonstrated the application of GIS and remote sensing as a decision support tool for oil spill management. Its objectives are to perform image classification and accuracy assessment, to perform post classification change detection for oil spill detection and to perform trend of change analysis for oil spill growth trend. Methodology involves planning stage, data requirements, data acquisition, data processing and results presentation. The results indicated that the annual growth rate of water bodies is decreasing at -0.16% from 2003 to 2015, settlements decreased at a rate of -1.16% from 2003 to 2015 while Mangrove and vegetation decreased significantly at the rate of -2.82% and -1.92% respectively from 2003 to 2015, this is by far the most significant decrease in the study area, as oil spill degrades farmland and plantations there by rendering it useless for economical purposes, the results also indicated that degraded environment increased at a rate of 3.39% from 2003 to 2015. It was further recommended amongst others that further studies should be on oil spill management in Gokana L.G.A as this will provide additional information on how to manage the effects of oil spill in Gokana L.G.A.*

Keywords*—*Remote Sensing, Geographic Information System, Oil spill, Environmental degradation.

I. INTRODUCTION

Petroleum products play an important role in modern society, particularly in the transportation, plastics, and fertilizer industries, there are typically ten to fifteen transfers involved in moving oil from the oil field to the final consumer (Jha et al, 2008). Oil spills are serious environmental disasters, often leading to significant, long-term impacts on the environment, ecology and socio-economic activities of area. Oil spillage refers to the discharge of petroleum onto the surface of inland or coastal waters, comprehensively; it was defined by Adelana et al, (2011) as the release of liquid hydrocarbons (crude and refined oil) and petroleum by-products into the marine, coastal areas and other environments due to human activities. Oil spills can occur during the transportation or storage of the oil, and the spillage can happen in water, ice or on land (Jha et al, 2008). This implies that oil spillage is a form of pollution that has only anthropogenic (non-natural) cause. In Nigeria, commonly reported causes of oil spillage include pipeline leaks and burst due corrosion and pressure build up, sabotage and oil theft, tankers' accidents, human error due to poor training or laxity, poor and old infrastructures, and failure to meet standards practices and infrastructures. Most of the conflicts, environmental degradation and crisis going on in the Niger Delta region in Nigeria are attributable to poor or total absence of effective environmental management (Okotoni, 2004). The poor environmental management due to business practices in this area has resulted in social, economic and political problems and this have negatively affected the well-being of dwellers in this region. In Rivers state Nigeria, the analogue methods of acquiring information necessary for mitigating oil spills

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and remediation are slow and inconsistent. There is lack of updated information and situational awareness that will enhance contingency plan in an event of spill. Many issues related to oil spill contingency planning and any emergency management has spatial dimension. There is need for a more efficient method that will speed up response time and save cost. Geographical understanding of the spillage will go a long way in devising ways to manage and control this spillage problem. It will also facilitate responds and therefore mitigate environment and economic damage caused by oil spill.

II. STUDY AREA

The study area, Gokana is a Local Government Area in Rivers State, Nigeria. Its headquarters are in the town of Kpor. It has an area of 126 km² and a population of 228,828 at the 2006 census.

The Gokana people have a rich cultural heritage. The main religions are Christianity and African traditional religions; although most of its customs, traditions and festivals have become extinct due to urbanization and rural-urban migration.

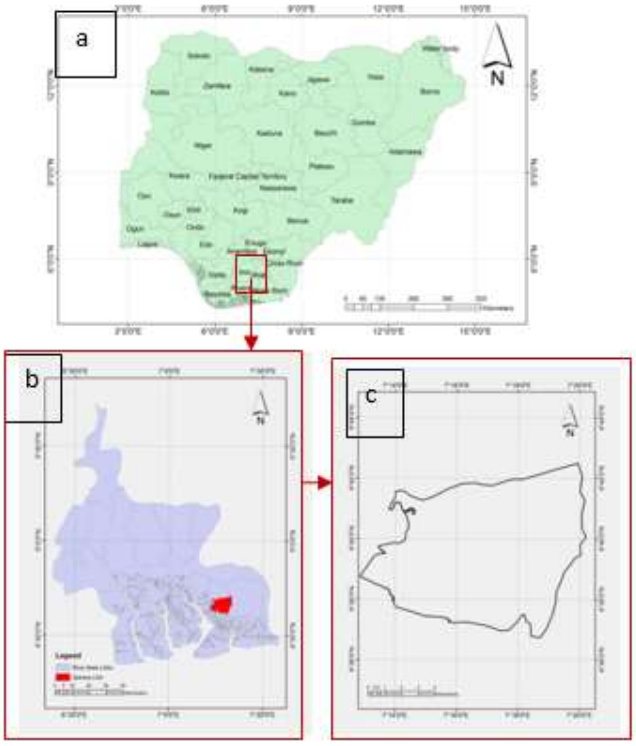

Fig.1 (a) Map of Nigeria (b) map of Rivers State showing Gokana L.G.A (c) Map of Gokana L.G.A

III. METHODOLOGY

For a proper and effective optimization, planning is very important. In this phase of the study, a user requirement analysis was done to focus on what information is presently being used, who is using it and how the source is being collected, stored and maintained. There is ideally a map of existing processes which was improved as well as being replicated by the GIS. The necessary information was obtained through interviews, documentations, reviews and workshops. This also involves the data requirement, hardware and software selections and method to be used.

3.1 Data Requirement

Data used in this research includes;

I. Landsat 7 ETM+ and Landsat 8 OLI Imagery of the study area
II. Co-ordinates of control points and other points of interest
III. Attribute Data of points of interest.
IV. Administrative map of Rivers State showing Local Government boundaries
V. Materials available in Academic journals, conference papers, relevant texts, brochures, internet and statistical files of government offices

3.2 Methods and Techniques

The method incorporated in this study involves image subset, remote sensing image classification techniques as well as spatio-temporal analysis of satellite imagery. Image subset was done on the two multi- temporal sets of images obtained (LandSat7 ETM+, LandSat8 OLI) in order to cut out the study area, after which land cover maps of the study area was produced using the supervised maximum likelihood classification algorithm in ERDAS Imagine used by (Onojeghuo and Onojeghuo, 2013). A post classification change detection technique was also employed to determine the changes that have occurred in the time period between 2003 and 2015 and trend analysis was performed to determine the trend of increase in the time period between 2003 and 2015.

IV. RESULTS

In this section, results of image analysis as obtained from the hard classification procedure of supervised (MLC) classification, change detection and trend analysis are presented. Most of the discussions are supported by maps, tables and illustrative graphs.

4.1 Land cover / Land use Distribution of Gokana L.G.A of 2003

In mapping landcover/land use, five different classes were identified to include Settlement, Degraded Environment,

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Water, Vegetation and Mangrove. The classified image of Gokana L.G.A is shown in figure 4.1

The land cover/land use distribution of Gokana L.G.A in 2003 as shown in table 4.1 and fig 4.2 indicate that Vegetation and Settlement accounted for the largest land cover/use of about 34.22% and 27.89% respectively, with areas of about 8093 hectares and 6599 hectares. Mangrove with 18.84% and an area of 4455 Hectares is 3rd, and water body has the lowest with 9.30 % with an area of 2200 Hectares. The Degraded Environment had 9.75% with a total area of 2305 hectares.

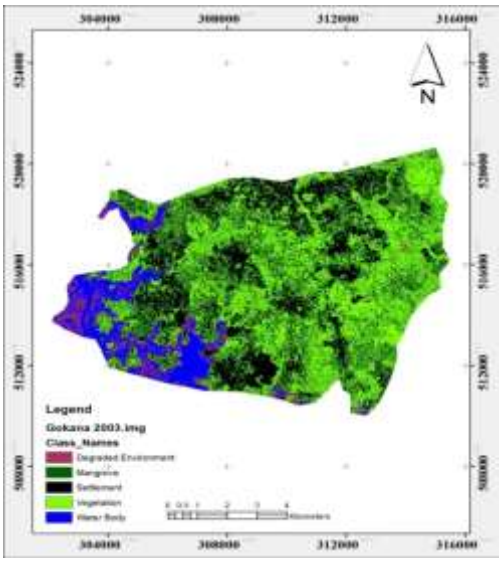

Fig.4.1: Landcover/Landuse classification of Gokana L.G.A of 2003

Table.4.1: 2 Landcover/Landuse distribution of Gokana L.G.A of 2003

Class/Region	Area (Hectares)	Percentage (%)
Water Body	2200	9.30
Mangrove	4456	18.84
Vegetation	8093	34.22
Settlement	6599	27.89
Degraded Environment	2305	9.75
Total	23,653	100

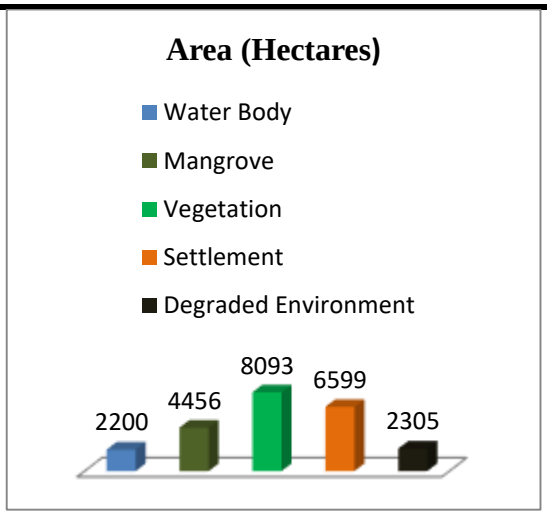

Fig.4.3: Landcover Classification Image of Gokana LGA of 2015

4.2 Land cover / Land use Distribution of Gokana L.G.A of 2015

The land cover/land use distribution of Gokana L.G.A in 2015 as shown in figure 4.3, 4.4 and table 4.2 indicate that Settlement and Vegetation accounted for the largest land cover/use of about 41.85% and 21.53% respectively, with areas of about 9899 hectares and 50.53 hectares. Degraded Environment increased to 22.43% with an area of 5505 Hectares. Then water body and Mangrove had the lowest with 8.03% and 6.16% with an area of 1900 Hectares and 1456 Hectares respectively.

Table.4.2: Landcover/Landuse distribution of Gokana L.G.A of 2015

Class/Region	Area (Hectares)	Percentage (%)
Water Body	1900	8.03
Mangrove	1456	6.16
Vegetation	5093	21.53
Settlement	9899	41.85
Degraded Environment	5305	22.43
Total	23,653	100

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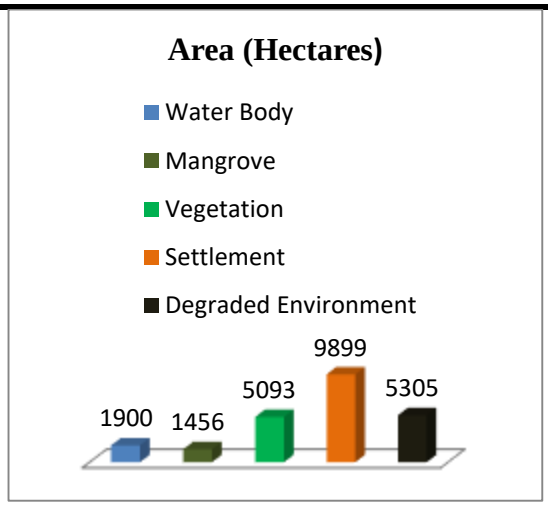

Fig.4.4: Histogram of Landcover/Landuse of Gokana L.G.A of 2015

4.3 Change Detection in Gokana L.G.A between 2003 and 2015

The classified Landsat7 ETM+ of 2003 and Landsat OLI 2015 was used to generate figure 4.5 with the attributes presented in table 4.3

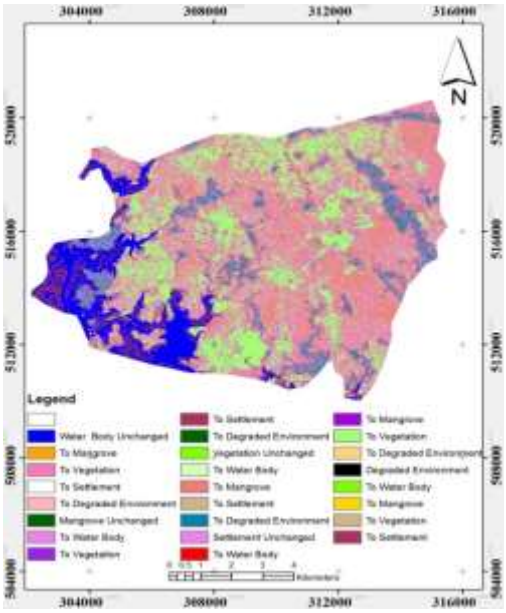

Fig.4.5: Change detection of Gokana L.G.A between 2003 and 2015

Table.5.4: Change detection distribution of Gokana L.G.A from 2003 to 2015

CHANGE DETECTION 2003 - 2015		
Class Type	Area (Hectares)	Percentage (%)
Water body Unchanged	1600	6.71
Water body to Mangrove	200	0.84
Water body to Vegetation	550	2.31
Water body to Settlement	600	2.52
Water body to Degraded Environment	1200	5.03
Mangrove Unchanged	1000	4.19
Mangrove to Water Body	150	0.63
Mangrove to Vegetation	300	1.26
Mangrove to Settlement	1400	5.87
Mangrove to Degraded Environment	1000	4.19
Vegetation Unchanged	4093	17.16
Vegetation to Water Body	110	0.46
Vegetation to Mangrove	185	0.77
Vegetation to Settlement	1200	5.03
Vegetation to Degraded Environment	500	2.09
Settlement Unchanged	6899	28.92
Settlement to Water Body	40	0.16
Settlement to Mangrove	71	0.29
Settlement to Vegetation	150	0.62
Settlement to Degraded Environment	300	1.25
Degraded Environment Unchanged	2305	9.66
Degraded Environment to Water Body	0.00	0
Degraded Environment to Mangrove	0.00	0
Degraded Environment to Vegetation	0.00	0
Degraded Environment to Settlement	0.00	0
Total	23853	100

From the data presented in table 4.3, it shows that degraded environment didn't lose any area from 2003 to 2015, but rather the other land cover features changed to degraded environment, this can be interpreted by saying that degraded environment increased over the years by gaining against other landcover features this is as a result of increased oil spillage from 2003 to 2015.

Although there was a bit of urbanization from the increase of settlements 2003 to 2015, the data shows that settlements also lost 1.25% of its area from 2003 to 2015 to oil spill events. Water body suffered the most loss with 5.03 % of its area lost to oil spillage, followed by mangrove, losing

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4.19% of its area to oil spill, then vegetation with 2.09 lost to oil spill from 2003 to 2015.

Also from the data in table 5.4, Water body had a total unchanged area of 1600 hectares, losing 0.84% to mangrove, 2.31% to vegetation and 2.52% to settlement. Mangrove had a total unchanged area of 1000 hectares, losing 0.63% to water body, 1.26% to vegetation and 5.87% to settlement. Vegetation had a total unchanged area of 4093 hectares, losing 0.46% to water body, 0.77% to mangrove and 5.03% to settlements. While settlement had a total unchanged area of 6899 hectares, losing 0.16% to water body, 0.29% to mangrove and 0.62% to vegetation from 2003 to 2015.

4.4 Trend Analysis

The results of the trend analysis from the data presented in figure 4.6 and table 4.6, indicates that the annual growth rate of water bodies is decreasing at -0.16% from 2003 to 2015, this is significant as oil spill affected water bodies are contaminated, thereby rendering it inhabitable and unusable, leading to significant decrease of clean water in the study area.

Mangrove and vegetation, is also decreasing significantly at the rate of -2.82% and -1.92% respectively from 2003 to 2015, this is by far the most significant decrease in the study area, as oil spill degrades farmland and plantain there by rendering it useless for economical purposes.

Settlements decreased at a rate of -1.16% from 2003 to 2015, oil polluted communities are forced to migrated to other unpolluted areas since oil spill pollutes the water, land and destructs daily activities. With no other way of farming and providing for themselves the inhabitants of these affected communities are forced to abandon their homes for a better environment

Degraded environment increased at a rate of 3.39% from 2003 to 2015. This signifies increased oil pollution from oil wells, oil pipelines and pipe vandalism in the study area.

The increasing trend of degraded environment in Gokana L.G.A should be monitored and clean up plans should be step up so as not to lose more landuse features in the foreseeable future.

Table.5.5: Trend of change & Annual rate of change of landcover/landuse of Gokana L.G.A from 2003 to 2015

Class Type	Difference (Hectares)	Total Area (Hectares)	Trend of Change (%)	Annual Rate (%)
	2003-2015	2003-2015	2003-2015	2003-2015
Water Body	-300	4100	-7.32	-0.61
Mangrove	-2000	5912	-33.83	-2.82
Vegetation	-3040	13186	-23.05	-1.92
Settlement	-2300	16498	-13.94	-1.16
Degraded Environment	3100	7610	40.74	3.39

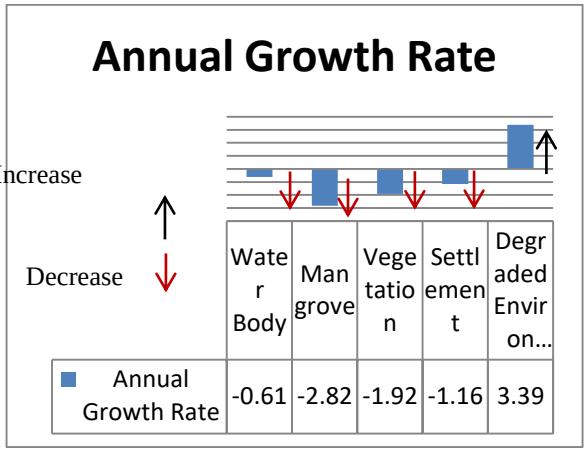

Fig.4.6: Annual growth rate of landcover/landuse of Gokana L.G.A between 2003and 2015

V. SUMMARY & CONCLUSION

This study indicated that the annual growth rate of water bodies is decreasing at -0.16% from 2003 to 2015, Mangrove and vegetation, are also decreasing significantly at the rate of -2.82% and -1.92% respectively from 2003 to 2015, this is by far the most significant decrease in the study area, as oil spill degrades farmland and plantain there by rendering it useless for economical purposes.

The study also indicated that settlements decreased at a rate of -1.16% from 2003 to 2015, oil polluted communities are forced to migrated to other unpolluted areas since oil spill pollutes the water, land and destructs daily activities. With no other way of farming and providing for themselves the inhabitants of these affected communities are forced to abandon their homes for a better environment.

Finally, the study indicated that degraded environment increased at a rate of 3.39% from 2003 to 2015. This signifies increased oil pollution from oil wells, oil pipelines and pipe vandalism in the study area from 2003 to 2015. This increasing trend of growth of degraded environment in Gokana L.G.A should be monitored and clean up plans should be step up so as not to lose more landuse features in the foreseeable future.

The oil spill management system aims to achieve a knowledge- based system which can choose the most suitable method of response in shorter time by analyzing the various sensitivity factors of coastal environment, affecting parameters on oil spill movement, environmental concerns in oil spill response, and consequent monitoring and clean-up measurements. The developed system would be able to provide a reasonable 'any- time' mechanism so that in most cases some reasonable response actions can be put forward. The system is useful in speeding of response actions especially in the regions which still suffer from the shortage of enough experts for responding the disasters.

The major advantage of this integration is the ability to extract oil spill parameters such as location, linear size and spill areas. Spatial and temporal information, i.e. oil spill distribution at the sea, its frequency and evolution in time allow the scientists to establish the major cause and source of oil spills, and then outline the risk areas. Within the GIS-approach, the tasks of analysis, modeling and forecasting of natural processes influencing the drift and spreading of oil spills can also be easily solved on the basis of standard GIS modules or linking it with other useful applications. This integration of technologies can qualitatively and quantitatively characterize not only the spatial and temporal distributions of oil spills, but also environmental conditions of the sea basins as a whole.

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The Effect on Solubility and pH of Sodium Chloride Solution by Magnetic Field

[1]Anjali Leal, [2]P. S. Tarsikka

[1,2]Department of Mathematics, Statistics and Physics, Punjab Agricultural University, Ludhiana, India
Email-anjalileal0412@gmail.com

Abstract*—On the whole world's major environmental problem is water pollution, due to pollutant water increases in microscopic harmful living organism's counts and it causes change in water odour, taste and colour, which causes the spread of dangerous epidermal diseases. A physical treatment using magnetic field it is beneficial for the medical, food industry, control and removing of the scale formation on the walls, in medical, in food industry and heating equipment's but the efficiency of this treatment is still a controversial question. In the present study, like physical parameters total dissolved salts (TDS), electrical conductivity (EC) and pH of sodium chloride solution have been evaluated under the effect of different strength of magnetic field (0.05T-0.15T) for different exposure time (3 hours, 5hours and 7 hours). The electrical conductivity and total dissolved salts rapidly increasing with the exposure of time and pH is increase with time as compared to control but for 3 hours is more and for 7 hour is less. Data collected during the experiment was analyzed statistically (SPSS-20.0). This analysis shows that the increase in TDS, EC and pH under the effect of magnetic field was significant ($p < 0.05$). The regression analysis was used to show linear relation between TDS and EC of water.*

Keywords*—Electrical conductivity, Hard water, Magnetic field, pH, Total dissolved salt.

I. INTRODUCTION

Water is essential to start and to continue the human life. It is directly related to each other. Water has spiritual values in many cultures and is associated with birth, spiritual cleansing and death. It is nutrient source and makes chemical reactions to happen. Water has unique properties it act as; solvent, an environmental, a temperature, a reactant and a molecule with cohesive properties. Use of hard water is the main problem in industry, domestic, agriculture and environmental. Scale formation is the solid deposits present in the exchanger of heat instrument and creates the heating of the hard water and makes contact with pipes and walls of the heat exchanger. Too many years used chemical method to control and remove the mineral fouling. It requires handling and disposal of hazardous chemicals, raising environmental concerns. Chlorine treatment of water is used then changes of odour, colour, hydrogen sulphide, growth of algae and germs. But due to the physical treatment it is beneficial for the control and removing of the scale formation on the walls and heating equipment's, this method is beneficial not only to the industry but also to the environment. The water treated by the magnetic field or passes through the magnetic device is called magnetized water. By the magnetic field strength change the physical and chemical properties as compared to ordinary water but not the acquired magnetic field strength. The hydrogen bond in liquid water is highly affected by magnetic field. These uncharacteristic properties of water are unique and results showed that many fluctuations of macroscopic properties Reddy *et al* (2014). Due to the magnetic field reduce the bond angle; these water clusters can break down but the increase solubility. The influence of magnetic field on liquid water has been deeply studied from last fifty years. To many people, magnets are complete mystery. Ibrahim (2006) study that the rate of flow is decreases with the increasing of magnetic field strengths, due to the application of magnetic field on water may also make alignment of water clusters and increasing of the magnetic field strength may also increases the alignment of water molecules. The alignment of water clusters may increase its electric current. According to (Gholizadeh *et al* 2008) magnetic treatment of water operates on the principle that a Lorentz force is experienced by each ion as the water is allowed to pass through a magnetic water softener. The frequency of collisions between ions increases due to redirection of the particles, positive and negative ions combine to form an insoluble compound. So, calcium carbonates dispatched from the solution as a mud which can be easily remove from the water. Musa and Hamoshi (2012) have observed that water may be levitated in very high magnetic field, which increases the tetrahedrality at the time. By the magnetic field is some disorder in the hydrogen bonding and improvement of salt mobility in hard water; the large water clusters are cut and break down to

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form smaller water clusters or twice water molecule. Hassan and Rahman (2016) observed that squeezes the bond pairs to close and deflects the bond pairs by the magnetic field then bond angle decreases from 104.5° to 103°.The magnetic charge is lost by the existence of the metallic layer inside the pipes and then purified which flows out of the tap and is no longer magnetized (Al-Khazan *et al* 2011).Such a simple technology can have many beneficial impacts on industries utilizing water, truly motivates its deeps study. Thus in view of this, the present study was planned to see the effect of magnetic field at different time intervals on the electrical conductivity, total dissolved salts and pH of the NaCl solution (hard water). The study of inherent properties of hard water such as electrical conductivity**,** TDS and pH give more insight to the concept of magnetic water treatment.

II. MATERIALS AND METHODS

The Haritron electromagnet (Model EM-20) was used for applying magnetic field to hard water. Larger magnetic field is produced when number of coil is more. The dimensions of electromagnet are diameter 9.0 cm and length 27.5 cm with total number of turns 3000 per coil. The distance between poles of electromagnet is adjustable up to 7 cm. The power supply of electromagnets has output voltage 0-100 volts and output current 0-10 ampere. To determine the nature of magnetic field, the magnetic field strength for different positions between the poles of electromagnet at different currents were measured by Digital gauss meter (DGM-102) to assure uniformity of field. Distilled water was prepared in the laboratory with of distillation of tap water. To study the effect of magnetic field on TDS, electrical conductivity and pH of hard water, at 0.05% concentrations of NaCl solution. For preparation of 0.05% NaCl solution, 0.05g of sodium chloride was dissolved in small volume of distilled water. Once the sodium chloride salt dissolved completely (after swirls the flask gently if necessary), water was added to make up the final volume as 100 ml of flask. In a similar way, other concentrations were prepared in laboratory by using distilled water. The parameters electrical conductivity and total dissolved salts of hard water were measured with the help of waterproof HANNA probe 98311 with range TDS (0-2000 ppm) and EC (0-3999 µS/cm). The HANNApH waterproof tester havingpH range from -2.0 to 16.0 was used to measurepH of hard water.Hard water solution stabilise for 1 day. After 1 day, take 40ml solution in the beaker placed in the electromagnets centre with the distance of poles. Apply the magnetic field on hard water solution for 3 hours. After 3 hour beaker out the electromagnets then HANNA EC/TDS/pH temperature meter in the solution. The EC/TDS/pH of the solution measure with variation of temperature. It is same procedure on 5 hours and 7 hours time duration. Same procedure repeated on different magnetic strength (0.05T, 0.15T, 0.25T).

III. RESULTS AND DISCUSSION

3.1 Effect of magnetic field strength on TDS and EC at different exposure time

In this experiment measurement were made on TDS/EC of NaCl solution having on 0.05% concentration at different magnetic field strength (0.05T, 0.15T and 0.25T) and different time intervals (3 hours, 5 hours and 7 hours). The plot has been shown in fig.1.1 to fig.1.6. After magnetization the solution changes the physical, chemical and microbiological properties. It has been observed that TDS/EC increases linearly with variation of temperature. The increase in electrical conductivity is more for 7 hours and less for 3 hours with exposure time for all concentrations. Which means TDS/EC depends upon time of exposure. The increase in TDS/EC with concentration is due to increase in NaCl ions concentration. The values of regression coefficients a and b, its coefficient of determination R^2 are given below in Table 1.1 to1.2.

Table 1.1 Regression coefficient for EC (µ/S) at different magnetic field

Concentration (w/v)	Magnetic field strength	Time (hrs)	a	b	R^2
0.05%	0.05T	0	17.651	481.036	0.999**
		3	17.916	493.806	0.999**
		5	17.988	508.130	0.998**
		7	17.848	532.152	0.998**
	0.15T	0	17.651	481.036	0.999**
		3	17.874	494.200	0.999**
		5	18.008	513.355	0.999**
		7	18.197	533.530	0.998**
	0.25T	0	17.651	481.036	0.999**
		3	18.372	485.785	0.999**
		5	18.842	497.355	0.998**
		7	19.186	515.427	0.997**

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Table 1.2 Regression coefficient for TDS (ppm) at different magnetic field

Concentration (w/v)	Magnetic field strength	Time (hrs)	a	b	R^2
0.05%	0.05T	0	8.832	240.170	0.999**
		3	8.963	246.506	0.999**
		5	8.995	253.927	0.998**
		7	8.932	265.730	0.998**
	0.15T	0	8.832	240.170	0.999**
		3	8.915	248.303	0.999**
		5	9.020	256.542	0.999**
		7	9.093	267.088	0.999**
	0.25T	0	8.832	240.170	0.999**
		3	9.192	242.548	0.999**
		5	9.414	248.724	0.998**
		7	9.586	257.852	0.997**

** Significant at 5% level of significance ($p < 0.05$)

The increase due to temperature is due to increase in kinetic energy of the ions. They have analyzed similar type of variation shown in Mousa *et al* (2008) and Pang (2013). According to Barron *et al* (1994) the mobility of ions in solution is increased with the increase in temperature. With the dissociation of molecules, the number of ions in solution increases on increasing the temperature. The electrical conductivity depends on these factors then an increase in the solutions temperature leads to as an increase in its electrical conductivity. Hassan *et al* (2016) investigated that magnetism decreases the bond angle between hydrogen and oxygen atoms within each water molecule from 104.5° to 103° degrees. Due to decrease in bond angle, the water molecules cluster together in groups of 6-7 rather than groups of 10-12 molecules and higher. As the cluster size decreases, consequently the absorption of water increases. Pang (2013) show that electrical conductivity of magnetized water increases with increasing the frequency of externally applied electromagnetic field and magnetized time. This is due to changes of nature of charged ions and velocity of hydrogen ions as well as the changes of polarized features under the influences of electromagnetic fields.

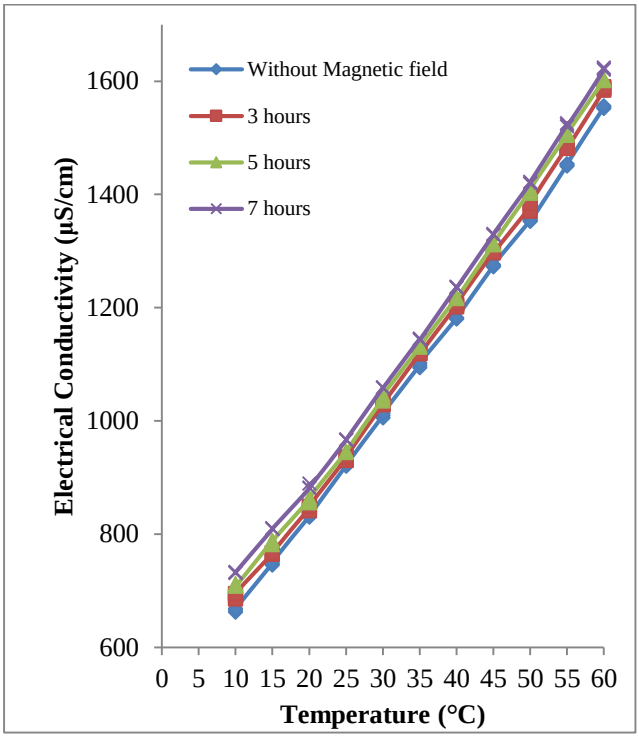

Fig.1.1Variation between the electrical conductivity and temperature for different exposure time 0.05T (0.05%)

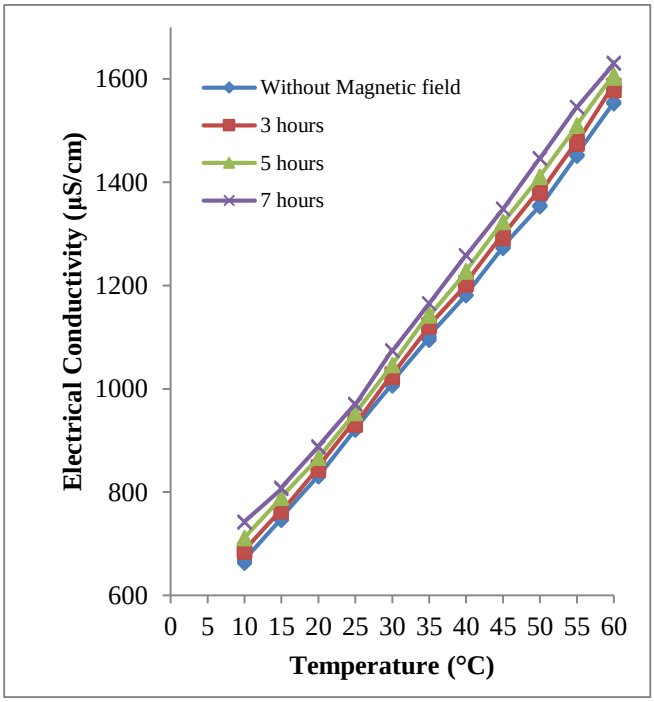

Fig. 1.2 Variation between the electrical conductivity and temperature for different exposure time 0.15T (0.05%)

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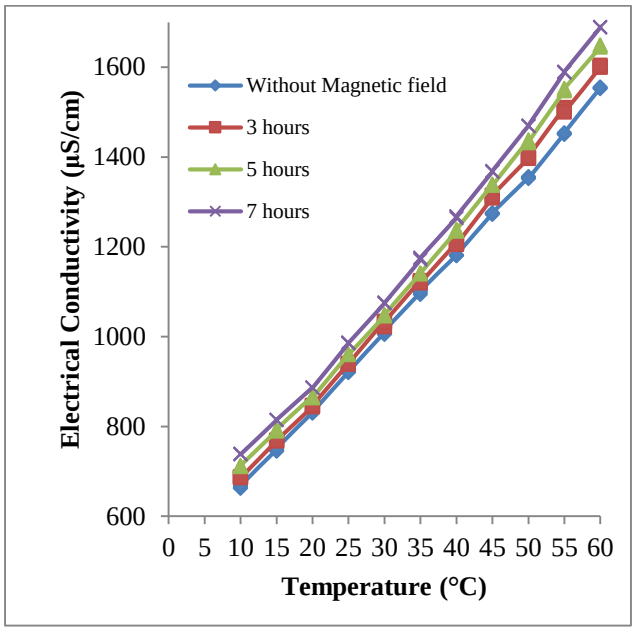

Fig.1.3 Variation between the electrical conductivity and temperature for different exposure time 0.25T (0.05%)

Moosa *et al* (2015) investigated that higher magnetic field strength increased the TDS. For exposing time less than 5 min, the rate of dissolving is slower than for exposing time greater than 5 min, where rate is much greater which mean more exposure of time greater solubility of solution. While for higher field intensity the increase in TDS is very sharp.

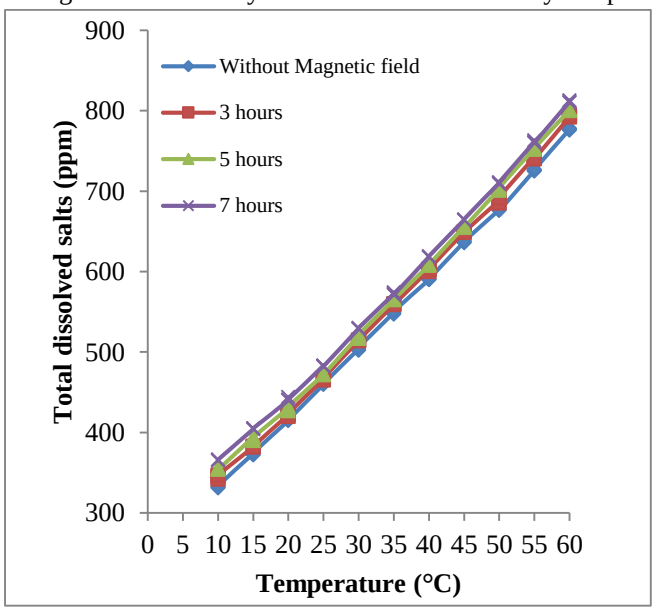

Fig.1.4 Variation between total dissolved salts and temperature for different exposure time 0.05T (0.05%)

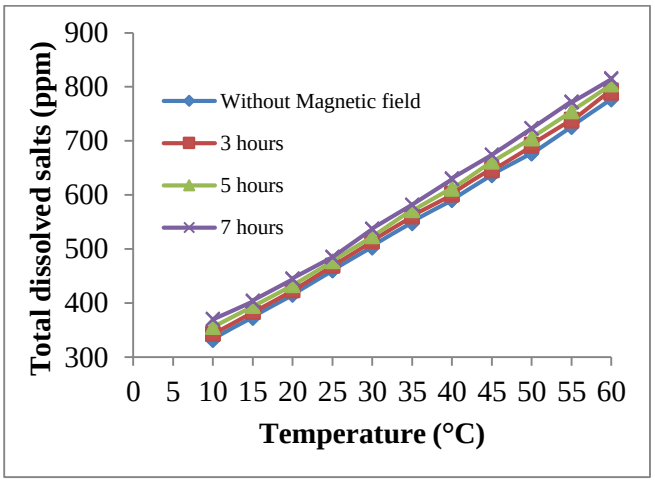

Fig. 1.5 Variation between total dissolved salts and temperature for different exposure time 0.15T (0.05%)

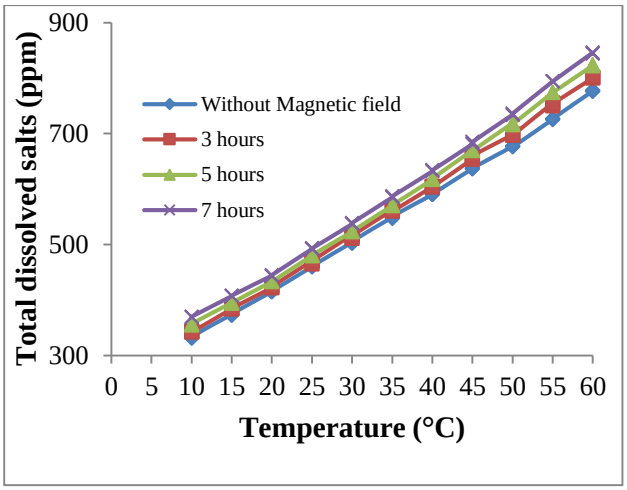

Fig. 1.6 Variation between total dissolved salts and temperature for different exposure time 0.25T (0.05%)

3.2 Relationship between the TDS and EC

The relationship between electrical conductivity and total dissolved salts shown in *fig.2.1.* It shows linear relationship between electrical conductivity and total dissolved salts for different temperature (10°C - 50°C). The conductivity is directly proportional to twice of total dissolved salts. Increase in electrical conductivity with increase of temperature and magnetic field strength. A high value of electrical conductivity indicates high total dissolved salt concentration. Iyasele *et al* (2015) found that total dissolved salts in water; an electrical conductivity value is more. Temperature effect the electrical conductivity value increases from 2 up to 3 % per 1 degree Celsius. Estimation of the electrical conductivity when number of total

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dissolved salts in solution. When conduct electrical current of water measured by electrical conductivity. Salts dissolve into positively charged ions and negatively charged ions.

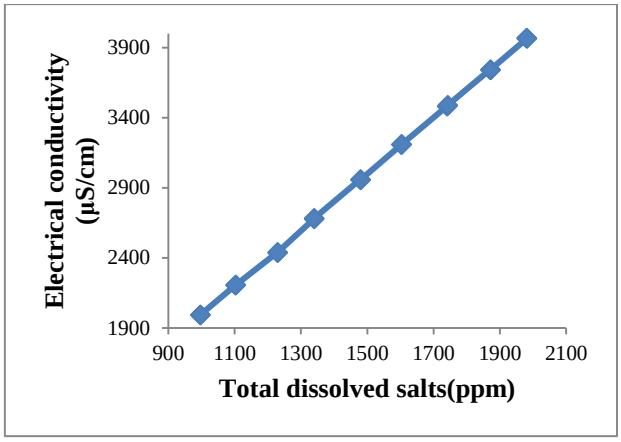

Fig.2.1 Relationship between the electrical conductivity and total dissolved salts

3.3 Effect of magnetic field on pH at different exposure of time

At 0.05% of NaCl solution at three different magnetic fields (0.05T, 0.15T and 0.25T) for different time exposure (3 hours, 5 hours and 7 hours) has been shown in fig.3.1 to fig.3.3. The variation of pH with temperature shows smooth and regular variation. The increase in pH is more for higher exposure time and higher magnetic field strength at higher concentration. The pH of 0.05% NaCl solution increases from 5.54 to 5.93 at 0.05T and from 5.64 to 6.13 for 0.15T field strength at 10°C temperature for exposure time. The pH of the solution change which means that there must be hydrolysis reaction happens in the solution because of ions polarization ability. Ionization reaction happens when inorganic salts dissolve in water, these ions forms. Then ions interact with H^+ or OH^- which are form water molecules ionization. The ions with good polarization ability can bind with H^+ or OH^- which can form weak electrolyte. Then the number of charged ion changes. This process, which is called hydrolysis reaction, promotes water hydrogen bonds breakage and breaks aqueous ionization balance.

The effect of magnetic field on NaCl solution is obtained to measurement of pH. Increasing magnetic field strength then pH of hard water is increase as compared to untreated hard water. The separation between the exposures of time is more than magnetic field strength is increase. We have more exposure of time pH is decreased for different time intervals. The reason behind pH is decreased due to large exposure of time that acidity of NaCl solution increases due to the number of hydrogen bonds increase. The total dissolved salts increased by magnetic field strength then acidity of hard water are increased.

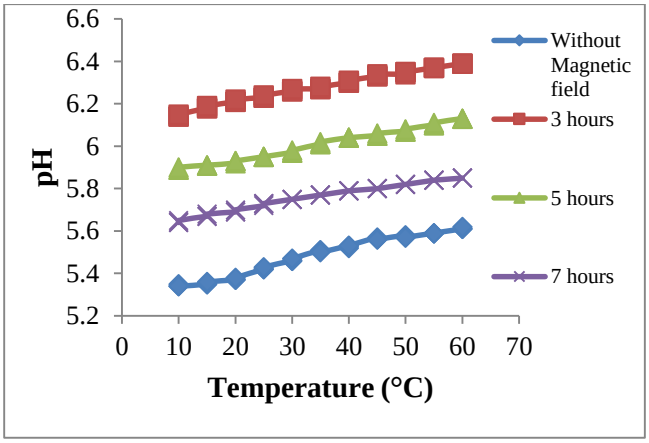

Fig. 3.1 Variation of pH with temperature for at 0.05% concentration and field strength 0.05T

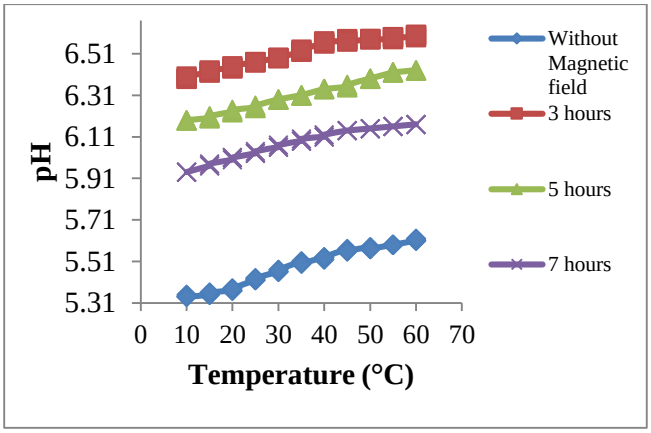

Fig. 3.2 Variation of pH with temperature for at 0.05% concentration and field strength 0.15T

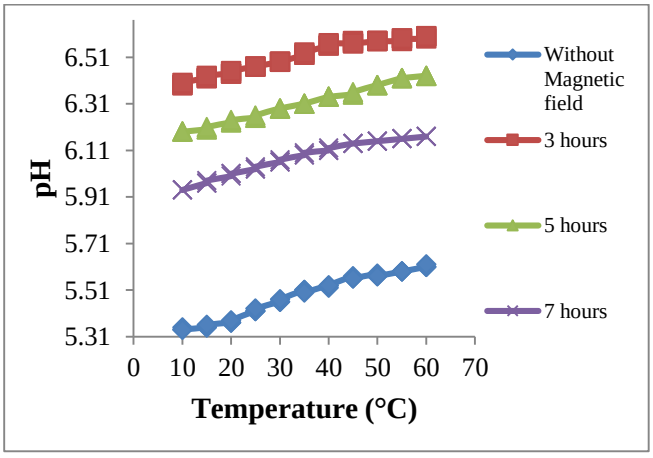

Fig.3.2: Variation of pH with temperature for at 0.05% concentration and field strength 0.25T

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Hassan and Rahman *et al* (2016) reported a higher 12% increase in water pH after magnetization. The effect of the exposure to the magnetic field was increased pH of water. The effect depends on the time of exposure to the magnetic field. Moosa *et al* (2015) studies pH value increases with exposing time this is due to the decreasing in the hydrogen ion concentration, while pH value increases with increasing magnetic field for distilled water due to the polarization of water molecules and the decreasing of hydrogen ion concentration the water molecules will arrange in one direction.

Table.3.1: Regression coefficient for pH at different magnetic field

Concentration (w/v)	Magnetic field strength	Time (hrs)	a	b	R^2
0.05%	0.05T	0	0.006	5.278	0.974**
		3	0.009	5.875	0.933**
		5	0.008	5.683	0.934**
		7	0.007	5.505	0.951**
	0.15T	0	0.006	5.278	0.974**
		3	0.005	6.111	0.988**
		5	0.005	5.836	0.991**
		7	0.004	5.615	0.982**
	0.25T	0	0.006	5.278	0.974**
		3	0.004	6.363	0.956**
		5	0.005	6.133	0.994**
		7	0.005	5.912	0.966**

** Significant at 5% level of significance (p <0.05)

3.4 Relationship between the EC and pH

There was a proportional relationship between the electrical conductivity records and pH values. It was found that the increase in pH value is more effective with the elevation in temperature degree. The reason behind pH is decreased due to large exposure of time that acidity of NaCl solution increases due to the number of hydrogen bonds increase. The electrical conductivity increased by magnetic field strength then acidity of hard water are increased.

3.5 Relationship between time and TDS/EC

The plot shown in fig 5.1 to 5.2. TDS/EC gradually increases with time at different temperature. The TDS/EC is more for 20°C and less for 60°C.

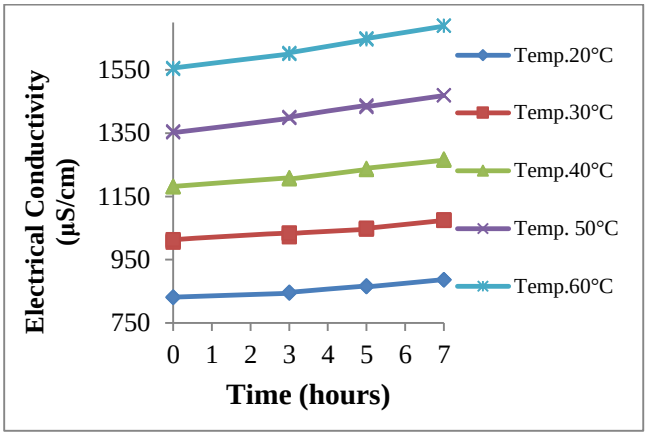

Fig. 5.1 Variation between time and EC at different temperature

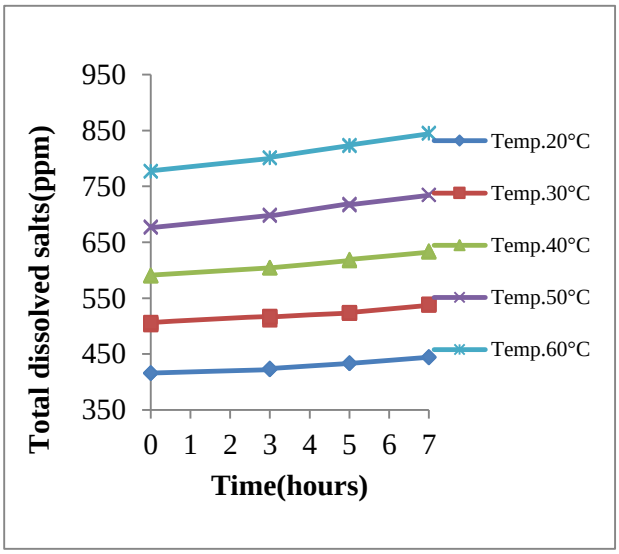

Fig.5.2 Variation between time and TDS at different temperature.

IV. CONCLUSIONS

(i) The TDS/EC of NaCl solution gradually increased with different magnetic field strength at different exposure time.

(ii) The values of pH increase with magnetic field strength; at different exposure time but for 3 hours are more and for 7 hour are less.

(iii) The changes in total dissolved salts, electrical conductivity and pH of hard water under the effect of magnetic field strengths have been observed significant at 5% level of significance.

(iv) The solubility of NaCl solution is increased because value of EC/TDS significantly increased by magnetic field strength.

(v) It results beneficial for removing of kidney stone and production of sea food.

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Bioremediation of Heavy Metals in Contaminated Soil from Abandoned Asa Dam Road Dumpsite

Abdus-Salam Nasiru[1], Ademola Olamide Sodiq[1, 2], Oyewumi-Musa Rukayat Titilayo[3]

[1]Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria
[1]Email: nasalami2002@yahoo.co.uk Telno: +2348033950752
[2]Email: Horlardiamond@yahoo.co.uk Tel. no: +2348053115015
[3]Department of Chemistry, Al-Hikmah University, P.M.B. 1016, Ilorin, Kwara State, Nigeria
[3]Email: titilayomusa@gmail.com Tel. no: +2348038083240

Abstract— *The dispersion pattern of heavy metals in soils surrounding municipal solid waste dumps was studied in a major area at Asa Dam Road, Ilorin, Kwara State. Soil samples were collected at three depths i.e.,0-15, 15-30 and 30-45 cm. The bioremediation capacity of five plants (Amaranthushybridus, Celosia agentea, Tithoniadiversifolia, Manihotescunlenta, Ipomeabatatas) grown on the dumpsite were studied for different metals. The moisture contents of the soil samples and plants were 4.55 - 18.58% and 3.75 - 13.78% respectively. The pH of soil and plants ash contents was in the range of 6.7 – 7.5 and 6.35 – 21.0 respectively. Atomic Absorption Spectroscopy method was employed in the determination of the concentration of Fe, Ca, Mg, Zn, Cd, Ca and Pb in both plant and soil samples. Amaranthushybridus and Celosia agentea were found to hyper-bioaccumulate, Tithoniadiversifoliabioaccumulate heavy metals mildly while Manihotescunlenta hyper-accumulate Iron. Cadmium and Lead were not detected in the plants. Nitrate concentration increases progressively down the soil profile and highest in Tithoniadiversifolia among the plants.*

Keywords— Bioaccumulation, bioremediation,dumpsite, heavy metal, plants, soil.

I. INTRODUCTION

It has been realized over the years that man generates waste of all sorts from his daily activities at home (domestics), farms (agricultural), industries, streets etc. These consist of combustible, non-combustible, putrescible and non-putrescible wastes (Douglas, 2013). As the wastes are being generated, they need to be collected and disposed every day. Hence, the calls for waste dump sites at various locations at the outskirts of the town to avoid pollution of the land, air and water sources and also to prevent outbreaks of diseases. However, as the towns expand (urbanization), problems continue to evolve. The dumpsites become encircled by houses and have to be abandoned, while new sites are sought for. The growing population increases the volume of waste generated (Abdus-Salam, 2009) and the existing dump sites become too small.

As regards some of the heavy metals in the waste, unfortunately dump sites rather mobilize the metals in the waste and accelerate the release of the metals to the environment. The major pathway of heavy metals release from waste to the environment is leaching of metals from wastes (Duruibe et al., 2007). Once heavy metals get into the environment, whether in small or large quantities, they cannot be completely eliminated (Ogundiran and Osibanjo, 2008). Though, several metals are essential for biological systems and must be present in a certain concentration range, too low or too high concentration lead to a decrease in metabolic activity or toxicity in plants and animals respectively. Soil is a nonrenewable dynamic resource and acts as an interface between agriculture and the environment (Ajaz et al.,2010). Excess concentrations of heavy metals such as Cd^{2+}, Cr^{4+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Fe^{2+} and Zn^{2+} in soil have caused the disruption of natural terrestrial ecosystems (Gardea-Torresdey et al.,2001).

Toxic metals when present in our body are capable of causing serious health problems, by interfering with our normal body functions, while some are useful to the body in lowconcentrations such as copper, iron and nickel but are toxic at high concentrations (Scott, 1992). They disrupt bodily functions by accumulating in vital organs and glands in the human body such as in the heart, brain, kidney, bone and liver (Salami and Adekola, 2002). They also displace vital nutritional minerals from their physiological positions. Calcium displacement from a metalloenzyme by Cd or Pb brings about disruption of enzymic reactions. Heavy metals are known to cause genotoxicity as they affect the DNA and immune-toxicity

as they are major irritants to the body (Scott, 1992). These negative consequences necessitate heavy metal remediation from pollution sites. A promising, relatively new technology for heavy metal removal from contaminated sites is bioremediation. The uptake and bioaccumulation of heavy metals in vegetables is influenced by many factors such as climate, atmospheric depositions, heavy metals concentration in soil, the nature of soil and the degree of maturity of the plants at the time of the harvest (Nwoko and Mgbeahuruike, 2011).

The objective of this study was to determine the level of heavy metals in the soil of an abandoned dump site at Asa Dam Road, Ilorin and the level of heavy metals that are hyper-accumulated in some plants, specifically planted on the polluted soil for the purpose of this research work. More so, to know which is the best plant species for bioremediation amongst sweet potato (*Ipomeabatatas*), tree marigold (*Tithoniadiversifolia*), amaranth (*Amaranthushybridus*), plumed cockscomb (*Celosia agentea*), cassava (*Manihotesculenta*) and maize (*Zea mays*).

II. MATERIALS AND METHODS

2.1 Dumpsite area:The area studied is the abandoned dump site opposite the Kwara State Metropolitan square along Asa Dam Road, Ilorin, Kwara State, Nigeria. The dump site existed for about 25 years covering 6 hectares of land space. It is a large sized dump sitecharacterized by municipal waste. As a result, surface run-offs contaminated with municipal wastes drain directly into the associated dump site soil.

2.2Sample collection:Nine soil samples were collected on the abandoned dump site and were used in this study. Three sampling points were chosen as described as site nearest to Asa Dam Road (Station 1), center (Station 2) and opposite station 1 but towards the end of the dump site (Station 3), starting from the road side. The soils were collected at (0 -15) cm, (15 – 30) cm and (30 – 45) cm depth from the three stations, using hand auger and stored in a sealed polythene bag.

Five plants samples were obtained from the dumpsite to attain the objectives of this research work. The five plants samples are: *Celosia agentea*(plummed cockscomb), *Manihotescunlenta*(cassava), *Tithoniadiversifolia*(tree marigold), *Amaranthushybridus*(pig weed) and*Ipomeabatatas*(sweet potatoes). At maturity (*Amaranthushybridus* and *Celosia agentea* 3 weeks; *Tithoniadiversifolia* 6 weeks; *Ipomeabatatas* and *Zea mays* 12 weeks; *Manihotescunlenta* 24 weeks), these plants were harvested and processed for heavy metal bioaccumulation.

2.3 Sample preparation: The plants samples were pretreated by physical removal of soil entangled to it and washed with distilled water. The plants samples were air dried in the laboratory for some days and then homogenized by grinding using a mortar and pestle.

2.4Determination of soil pH:The pH of soil samples were determined following method described by Schofield and Taylor, (1955). A 15 g of the soil sample was weighed into a 50ml beaker with a graduated scoop. A 30 ml of 0.01M $CaCl_2$ was added and stirred into suspension. It was stirred again after 15-20 minutes and allowed to stand for 30 min to allow sediment to settle. A calibrated pH meter was immersed into the partly settled suspension and the pH was recorded when the reading stabilized.

2.5 Plants moisture content determination:The moisture content of plant samples were determined following method described by UCDAVIS, (2000). A 5 g of the plant sample was weighed into the porcelain crucible and placed in the oven at temperature of 60-80 ^oC for 4 hrs. The dried sample was then weighed and the process was repeated until a constant weight was obtained. The weight lost signifies the moisture content and the percentage moisture content was calculated.

$$\% \text{ Moisture Content} = \frac{M_i - M_d}{M_i} \times 100 \quad (1)$$

M_i= Initial mass of plants sample

M_d = Dried mass of plants sample

2.6Determination of plants ash content:The ash content of plant samples were determined following method described by Prometheus wiki, (1996). The plant dried sample was weighed in a porcelain crucible and the crucible was placed in muffle furnace at the temperatures of 550 ^oC for 6 hrs. After ashing, the ashed plant sample was weighed and the percentage of ash content calculated as:

$$\% \text{ Ash content} = \frac{M_{ASH}}{M_{DRY}} \times 100 \quad (2)$$

M_{ASH} = mass of the ashed sample

M_{DRY} = mass of the dried sample

2.7 Soil moisture content determination:Soil samples moisture content were determined following method described by Hausenbuiller, (1975). The soil samples were oven dried at 105 ^oC for 24 hrs cooled in a desiccator and re-weighed. The sample was returned to the oven at 105 ^oC for 3 hrs, cooled in a desiccator and reweighed. These processes of oven-drying, cooling and re-weighing continued until the weights of the soil samples were practically constant. The soil sampleswere then gently pulverized using mortar and pestle. The crucible was removed from the oven, cooled in a desiccator and the dried soil was weighed. The percentage moisture content was calculated as follows:

$$\text{Percentage Moisture content} = \frac{M_w - M_d}{M_d} \times 100 \quad (3)$$

M_w = Mass of wet soil sample (wet weight - tare weight) (grams)
M_d = Mass of dry soil sample (dry weight - tare weight) (grams)

2.8 Perchloric acid digestion of plant materials:Perchloric acid digestion ofplant samples were determined following method described by A.O.A.C., (1970). A 1 g of ground plant sample (oven dried at 60 ^oC) was transferred into 50 ml Erlenmeyer flask which had been previously washed with acid and distilled water. A 2 ml $HClO_4$, 10 ml conc. HNO_3 and 2 ml conc. H_2SO_4 were added under a fume hood to the plant sample. The mixture was shaken and heated gently from low to medium heat on a hot plate under a fume hood. The heating was continuous until dense white fumes appeared. Finally, it was heated strongly for half a minute (i.e. medium to high). The digested sample was allowed to cool, and then 20 ml of distilled water was added. The diluted digest was boiled for 30 sec at medium heat. The digest was cooled and filtered into 50 ml Pyrex Standard flask. The filtrate was made to mark with distilled water and transferred into plastic vial for chloride determination which was done through Mohr titration method and Pb, Cd, Zn, Fe, Ca, Mg were determined using ALPHA 4 Chem Tech Analytical model of Atomic Absorption Spectrometer (AAS).

2.9 Digestion of soil samples:Digestion of soil samples were determined following method described by Nieuwenhuize et al., (1991). The soil samples were air dried and passed through 2 mm sieve. A 5 g of the air dried sieved soil was weighed and digested with 20 ml of aqua regia (HCl/HNO_3, 3:1) under fume hood, on a hot plate until a dense white fume appeared. Thedigest was cooled and 20 ml of distilled water was added into the beaker, the diluted digest was then placed on hot plate and heated strongly. The digest was filtered into 100 ml standard flask after cooling and the filtrate was made up to the mark of the standard flask. From the filtrate, Pb, Cd, Zn, Fe, Ca, and Mg were determined using ALPHA 4 Chem Tech Analytical model of Atomic Absorption Spectrometer (AAS). Chloride was also determined using Mohr titration method.

2.10Determination of nitrate in plants:Nitrate in the plant samples were determined following method described by Cataldo et al., (1975). A 0.25 ml of aliquot of extract of digestion was pipette into 50 ml Erlenmeyer flask and mixed thoroughly with 0.8 ml of 5% (w/v) salicylic acid-H_2SO_4 reagent. After 20 min of mixture, 19 ml of 2 N NaOH was added to raise the pH above 12, the sample was then cooled to room temperature. The absorbance of this mixture was measured at 410nm.

2.11Determination of nitrate in soil sample:Nitrate in the soil samples were determined following method described by Greweling and Peech, (1975). ***Extraction:*** A 5 g of the soil samples was weighed and transfer into the 100 mL conical flask. Then 0.25 g activated carbon and 20 ml of extracting solution were added to the soil sample in the conical flask. The extracting solution was prepared by adding together 100 g CH_3COONa and 30 ml of 99.58% CH_3COOH in 1000 ml standard flask. The solution was made up to mark with deionized water. The soil sample mixture was shaken for 1 min and filtered.

A 1 ml of the aliquot of the soil extract was transferred into a vial and 0.5ml of brucine reagent was added into the aliquot and 2 ml H_2SO_4 was added rapidly into the conical flask and mixed carefully for 30 sec. The sample was left to stand for 5 min before absorbance of the sample was taken at 470 nm using spectrophotometer.

A 100 ppm standard NO_3-N was prepared from KNO_3 salt as the stock solution and five different concentrations (0 – 2 ppm) were prepared from the stock solution through serial dilution. A 1 ml of each known concentration standards prepared was carried through the same procedure with the soil extracts.

2.12Phosphate determination in plant samples:Phosphate in the plant samples were determined following method described by Murphyand Riley, (1962). ***Working solution (prepared fresh daily)*** – A 12.7 g ammonium molybdate was dissolved in 250 ml of distilled water, then 0.291 g antimony potassium tartarate was dissolved in 100 ml of distilled water. Ascorbic acid was also prepared by dissolving 2.625 g in distilled water and diluting to 500 ml. The three reagents were added into 1000 ml of 5 N H_2SO_4, The solution was mixed thoroughly and make to 2000 ml with distilled water and stored in a Pyrex glass bottle in a dark compartment.

A 1ml of the digested samples that were digested via wet oxidation was quantitatively transferred into 100mlvolumetric flasks and dilute with distilled water. Using a dilutor-dispenser, the diluted samples and the standards prepared were treated in 1:100 with the working solution. Colour was allowed to develop for at least 30 min before reading. The absorbance of each sample was taken at 660 nm with a spectrophotometer.

2.13Determination of phosphate in soil samples:Phosphate in the soil samples were determined following method described by Samira et al., (2009). ***Extraction:***A 50 g of the soil samples was weighed and transferred into 250 ml conical flask and shaken with exactly 50 ml of $NaHCO_3$ at pH 8.5. The samples were shaken on an orbital mechanical shaker for 10 min.

A 10 ml aliquot of the extract was transferred into a 50 ml conical flask, 10 ml of the colour developing reagent was added, stirred and allowed to stand for 15 min. The absorbance wasmeasured using UV/ Visible Spectrophotometer and glass cells at 880 nm. Standard

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calibration curve was prepared from the standard solutions of KH_2PO_4.

III. RESULTS AND DISCUSSION

The moisture content of the soil (Table 1) decreases down the soil profile in all stations and it influenced the rate at which ionic species in the soil are been percolated down the soil. The result of plant moisture contents (Table 2) are low and it affects the availability of the ionic species (Emmanuel and Folashade, 2011) because amaranth accumulated all the ionic species studied better than others in the case of iron and nitrate. The ash content of the plant samples were also in agreement with literatures (Aletor et al., 2002;Anhwange et al., 2009).

Nitrate concentrations increase down the soil profile in all stations (Table 1), and the solubility of nitrate may have attributed to its ease of percolation (Aletor et al., 2002). Therefore, the fraction, 30 – 45 cm had the highest of nitrates ions. The nitrate level in an increasing order among the soil fractions are 0 – 15 cm < 15 – 30 cm < 30 – 45 cm.

From Table 2 the concentrations of nitrate in the five plants samples studied were 1.25, 3.25, 0.75, 0.25, 0.05 mg/l in *A. hybridus, T. diversifolia, I. batatas, C. agentea, M. escunlenta*respectively. These values are low when compared with the nitrate concentrations of some edible wild plants earlier reported (Ugur and Selima, 2011) in which plants with least nitrate concentration had 43.42 mg/l. The low result here may be due to nitrate concentration in the soil samples. This implies that the concentration of the soil at the depth of the root determines the concentration of the ionic specie in the plants. Both the plants and the soil samples did not exceed the safe limit of intake of nitrate, which is 45 mg/l according to WHO (WHO, 1987).

Phosphate concentration at the soil surface layer (Table 1) is higher than its concentration at the depth of 15-30 and 30-45 cm in station 2 and station 3. In station 1, the concentration of phosphate is higher at depth of 15-30 cm. Phosphate concentration in the dump site soil are attributed to the decaying bone ash which is slowly released into the soil and this accounted for low concentration of the phosphate in the soil (Phillip, 2004). The concentration of phosphate in the five plants samples were 0.5, 1.45, 0.4, 1.8, 0.95 mg/l for *I. batatas, T. diversifolia, M. escunlenta, A. hybridus, C. agentea*respectively. Comparing the results of a similar research on three of these plants from where the concentration of PO_4^{3-} in *C. agentea,A. hybridus* and *I. batatas* are 0.29, 0.95 and 0.62 mg/l respectively (Orhue et al., 2010; Decuypere, 2000), the concentration of phosphate in the plant samples is relatively high in *A. hybridus* and low in *I. batatas* grown on the dumpsite.

The content of chloride (mg/l) in the soil (Table 1) followed the same progression in all stations; the depth of 15-30 cm has the highest value of chloride while the chloride content in the five plants samples (Table 2) are 17.35, 12.18, 10.89, 7.94, 5.54 mg/l in *A. hybridus, I. batatas, T. diversifolia, M. escunlenta, C. agentea*respectively. It also revealed that *A. hybridus*bioaccumulated chloride most among all the plant samples studied.

From Table 1, the highest concentration of calcium was found in station 2 with (15-30 cm) and (30-45 cm) depth having 832.58 and 947.54 mg/l which are 41% and 47% of total calcium respectively. The similar results in the five plants samples studied are 577.12, 501.08, 496.07, 296.51, 173.54 mg/l in *A. hybridus, C. agentea, T. diversifolia, I. batatas, M. escunlenta*respectively. This shows that the concentration of calcium in the plants grown on the dumpsite is relatively high, when compared to calcium levels in two of these plants, *A. hybridus*(276 mg/l) and *I. batatas* (43 mg/l) from a similar research (Decuypere, 2000).

The percentage concentration of magnesium in the soil varied among the three stations and depths. Station 1 has the highest concentration (23.34 mg/l) in the depth of 15-30 cm, station2 has the concentration of magnesium increase down the soil profile and station 3 has the least concentration of magnesium (24.93 mg/l) at the depth of 15-30 cm. It was also observed that the concentrations of magnesium in the five plants samples are 324.25, 272.08, 139.25, 136.53, 61.85 mg/l in *A. hybridus, C. agentea, T. diversifolia, I. batatas, M. escunlenta*respectively. A far lower value has been reported for *A. hybridus* and *I. batatas* having values of 73 mg/l and 48 mg/l respectively (Decuypere, 2000). This shows that the concentration of magnesium in the studied plant samples is very high.

Generally, iron was the most abundant heavy metal in the soil among the heavy metals studied. The heavy metals available in the soil fell within the normal range of heavy metals in the soil except iron whose concentration in all station exceed WHO normal range (425 mg/kg) in soil.

From Table 1, the concentrations of the various metals in station 1 for 0 – 15 cm depth, Fe = 1077.5, Zn = 1.732, Cd = 0.00, Pb = 0.48 mg/l, for depth of 15-30 cm, Fe = 1072.53, Zn = 4.692, Cd = 0.00, Pb = 1.11 mg/l. The availability of some heavy metals was known to decrease with rising pH of the soil (Fargasova, 1994). The pH of the soils in station 1 are 7.5, 6.7, 6.8 in the depth of (0-15), (15-30), (30-45) cm respectively.

The concentration of heavy metals in the five plants samples studied is presented in Table 2 but Cd and Pb were not detected in all the plants samples studied. This may be due partly to below detection of AAS used, low concentration levels in the soil, or absence in the

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studied area. In comparison to an earlier research (Anhwange et al., 2009), where it was reported that the concentration of Fe, Zn, Cd and Pb in *A. hybridus*were 1.28, 0.52, 0.15 and 0.06 ppm respectively, the concentrations obtained in this study showed higher bioaccumulation (Table 2) by similar plants. This may be attributed to high metal content in the soil and/or maturity status of the plants. It was revealed that all the plant samples used are poor accumulator of Cd and Pb. The normal concentration of Fe and Zn in *A. hybridus* and *I.batatas*are 2.98, 1.16 and 1.87, 0.62 mg/l respectively, this show that the concentration of iron in the plants are generally high while the concentration of Zn relativelynormal (Decuypere, 2000). The mean concentrations of heavy metals in stations 1, 2 and 3 (Table 1) are within the normal range in the soil except iron.

IV. CONCLUSION

This study revealed that plants grown on contaminated area have the high risk of bioaccumulation of heavy metal beyond the permissible limits. The concentrations of calcium and magnesium in the plants were relatively high and are linked to high concentrations of the metals in the soil, especially in station 2. The concentrations of the anions are within the normal acceptable range except phosphate concentration in *A. hybridus.*

This research also shows that plants grown on the abandoned dumpsites of Asa Dam Road have heavy metals within the permissible limit of WHO/FAO (World Health organization/ Food and Agriculture Organization), EU (Economy Union) and EC (Economic Commission).

*Amaranthushybridus*hyper-accumulated heavy metals and anions most among other plants. This study shows that the availability of heavy metals in the soil depends on the quality and quantity of wastes heaped up at that point. None of the plants studied is a good hyper-accumulator of Cd and Pb, and the possibly low concentrations of Cd and Pb in the soil also affected the result.

Soil condition such as pH, moisture content, porosity, presence of electron acceptor all affects the soil and the type of plant to use for bioremediation also depends on the targeted pollutants. The plants grown on Asa Dam Road abandoned dump sites are not toxic to the body because of the concurrent low concentration of heavy metals available in the soil.

*Amaranthushybridus*and *Celosia agentea*are used as consumable vegetables and the consumption will raise bio-accumulation of heavy metals in the body. Since*Amaranthushybridus*and *Celosia agentea*are meant for food, they should not be planted on heavy metals contaminated soil.*Tithoniadiversifolia*is a mild hyper-accumulator and it is used as herb. So heavy metals will be release during extraction of the plant juice and long accumulation of the heavy metals in the body can cause problems that are more chronic to human. Therefore, *Tithoniadiversifolia*should be planted on the land with either no or low concentration of toxic metals. *Manihotescunlenta*and*Ipomeabatatas*are very low in bioaccumulation of heavy metals except that*Manihotescunlenta*hyper-accumulated iron most. Hence they are poor hyper-accumulator of the heavy metals studied. Most of the heavy metals in a tuber crop accumulate at the tuber and this cause its low concentration in the leaves.

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LIST OF TABLES

The comparative analytical results of the five plant samples and nine soil samples collected from the abandoned Asa dam road dump site are presented in Table 1 -3 below.

Table 1: Physicochemical analysis of soil samples collected from the abandoned Asa Dam Road dump site

	Sampling Depth (cm)	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Zn (mg/L)	Cd (mg/L)	Pb (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	Cl^- (mg)	% Moisture content	pH
Station 1	0-15	1.16	14.46	1077.5	1.732	ND	0.48	0.775	0.74	54.64	9.57	7.5
	15-30	90.64	23.34	1072.5	4.692	ND	1.11	0.875	0.745	69.23	4.69	6.7
	30-45	7.01	14.08	845.01	1.303	ND	0.35	1.575	0.58	30.27	4.55	6.8
	Average	32.93	17.29	998.3	2.576	ND	0.65	1.075	0.69	51.38	6.27	7.0
Station 2	0-15	832.58	53.35	717.52	33.451	ND	3.32	1.05	1.38	91	17.53	7.3
	15-30	947.54	82.11	1117.5	73.112	0.03	3.65	1.3	0.44	148.23	14.66	7.1
	30-45	55.66	85.49	1425.1	82.534	0.044	2.45	1.58	1.1	27.51	14.45	7.4
	Average	611.93	73.65	1086.71	63.03	0.037	3.14	1.31	0.97	88.91	15.55	7.3
Station 3	0-15	35.82	95.04	1402.5	61.491	0.065	0.64	1.18	1.1	39.87	18.58	6.9
	15-30	24.93	83.15	1437.6	53.853	0.05	1.78	1.55	0.865	44.67	12.99	7.1
	30-45	27.84	96.35	1325.2	36.156	2.416	ND	1.75	0.78	10.52	12.5	7.2
	Average	29.53	91.51	1388.43	50.5	0.84	1.21	1.49	0.915	31.69	14.69	7.1

ND: Not Detected

Station 1: site nearest to Asa Dam Road

Station 2: site at the center of Asa Dam Road dump site

Station 3: site towards the end of the dump site

Table.2: Some physicochemical analysis of the plant samples collected from the abandoned Asa Dam Road dump site

SAMPLES	Ca (mg/L)	Mg (mg/L)	Fe (mg/L)	Zn (mg/L)	Cd (mg/L)	Pb (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	Cl^- (mg/L)	% Moisture content	% Ash content
Ipomeabatatas	296.51	136.53	7.89	0.695	ND	ND	0.75	0.5	12.18	13.78	21
Tithoniadiversifolia	496.07	139.25	8.81	0.843	ND	ND	3.25	1.45	10.89	7.87	16.67
Amaranthushybridus	577.12	324.25	5.75	1.559	ND	ND	1.25	1.8	17.35	3.75	11.89
Celosia agentea	501.08	272.08	9.19	1.361	ND	ND	0.25	0.95	5.54	5.51	12.71
Manihotescunlenta	173.54	61.85	10.45	0.748	ND	ND	0.05	0.4	7.94	3.94	6.35
Average	408.86	186.79	8.42	1.0412	ND	ND	1.11	1.02	10.78	6.97	13.72

ND: Not Detected

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Table.4: WHO/FAO safe limit for heavy metals uptake in plant and soil samples (Opaluwa et al., 2012).

Metals (mg/kg)	WHO/FAO	EC/CODEX	Normal Range in Plant	Normal Range in soil
Cd	1	0.2	< 2.4	3
Pb	2	0.3	0.50 – 30	2 – 200
Zn	60	< 50	20 – 100	20 – 300
Fe	48	-	400 – 500	425

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Integrated Pest Management in Portugal: from Policies to Practices

Cristina Amaro da Costa[1], Maria do Céu Godinho[2], José Lima Santos[3], António Mexia[4], Pedro Amaro[5]

[1]Escola Superior Agrária/Instituto Politécnico de Viseu, 3500-606 Viseu, Portugal
E-mail: amarocosta@esav.ipv.pt
[2]Escola Superior Agrária/Instituto Politécnico de Santarém, Quinta do Galinheiro - S. Pedro, 2001-904 Santarém, Portugal;
E-mail: mariac.godinho@gmail.com
[3]Instituto Superior de Agronomia/Universidade de Lisboa, 1349-017 Lisboa, Portugal,
E-mail: jlsantos@isa.ulisboa.pt
[4]Instituto Superior de Agronomia/Universidade de Lisboa, 1349-017 Lisboa, Portugal,
E-mail: amexia@isa.ulisboa.pt
[5]Instituto Superior de Agronomia/Universidade de Lisboa, 1349-017 Lisboa, Portugal

***Abstract*— *Integrated pest management is an ecosystem approach to crop protection that combines different control methods to reduce pesticide use and to obtain safe food products with lower environmental impact. It has increased in Europe and since 2014, it is mandatory for farmers as a basis for their crop protection strategy.*

Using the Portuguese context as a case study, the evolution of integrated pest management adoption is analyzed. Country statistics and survey-based data are used to highlight technical differences among farmers, their motivations and attitudes that give rise to environmental benefits and food safety. A survey was applied to vineyards and apple and pear orchards in integrated pest management and in organic and conventional farming. The collected data were related with farmer profile, farm description, farmers' motivations towards sustainable farming practices, technical itinerary and practices related to pesticide use. A total of 177 questionnaires were applied.

Integrated pest management farmers are motivated to adopt biological, biotechnical and cultural solution, even if more expensive, and to give up toxic pesticides, to reduce agricultural impacts, while producing healthier and safer products. Practices that affect crop protection and soil conservation varied between agricultural systems, and can be used as lessons to improve their quality.

Keywords*— *farmers' attitudes, indicators, motivations, sustainable farming practices.

I. INTRODUCTION

Agriculture plays a key role in producing ecosystem services, such as farmland biodiversity, water and soil quality, climate stability or landscape maintenance. Simultaneously, many farming practices lead to soil depletion, water shortages, pollution and loss of wildlife habitats and biodiversity [1,2].

In the last two decades, the use of pesticides has decreased in European countries including Portugal [3,4]. However, it is still responsible for health risks and impacts on the environment. In 2011, 1.9% of total food samples analyzed in Europe exceeded the legal Maximum Residue Levels (MRL), while in Portugal that percentage rose to 3.1% of collected food samples (4.9% in fruits and nuts and 2.8% in vegetables) [4,5]. Intoxications caused by misuse or accidental exposure, and also by oral ingestion (voluntary or not) of organophosphorus compounds and herbicides were reported [6,7].

The reduction of the negative effects of intensive agriculture and pesticide use has been one of the major concerns of European policies [1,8]. Therefore, agri-environment programs were introduced in the European agricultural policy in 1985 to encourage EU farmers to adopt agricultural production methods compatible with the preservation of the environment and natural resources. The agri-environmental support aimed to promote the transformation of conventional agriculture (CA) by encouraging the adoption of IPM and organic farming (OF). These systems endorse and ensure long term adoption of practices compatible with environmental protection and food production with equivalent quality and yields [9]. This support should compensate farmers for using environmentally beneficial, but more expensive, farming techniques.

In 1992, the application of the agri-environment programs was compulsory for member states in the framework of

their rural development plans, whereas they remained optional for farmers. In fact, until 2014, agri-environment payments were implemented on a voluntary basis through contracts that established a set of commitments that the beneficiary was required to fulfill, including fertilizer reduction, pesticide reduction, extensification of livestock farming, crop rotation, maintenance of set-aside areas, prevention or reduction of soil erosion, use of local genetic resources, biodiversity conservation, upkeep of the landscape, water-related actions, buffer strips, field margins, and wetland management [8].

Since 2000, an increasing number of farmers have adopted environmental farm management practices, such as IPM or OF, in response to incentives provided through government payments and regulations, and voluntary private-led initiatives, often promoted by food processors and retailers, local markets or by individual farmers [2,10]. More than 22.2 billion euro in EU funds were allocated to encourage farmers to protect and enhance the environment on their farmland [1,11].

Thus, the evaluation of the agri-environmental policies and programs is crucial in order to determine if the cost of paying additional best practices was compensated by the environmental benefits generated [12,13]. In 2000, results from the first agri-environmental measures demonstrated that the programs have had little effect in reducing intensive practices, but in 2011, evidence proved that the conversion to friendly farming systems was effective in achieving their environmental benefits, especially in the case of IPM and OF [8,11].

Unfortunately, only a third to a half of OECD member countries is regularly monitoring these environmental benefits [2]. Primdahl et al. [13] found that half of the farming systems monitored were assessed based on general beliefs, rather than on objective evidence, of how agricultural practices are linked to environmental alterations and protection of resources and biodiversity. Only 15% were based on quantitative models that provide a statistical prediction of how changes in agricultural practices will have specific environmental impacts. Nevertheless, a survey conducted in 2005 to 62 international IPM projects covering 26 countries revealed that in over 60% of the projects pesticide use was reduced by 60%, on average, indicating a broad impact of IPM [14].

In Portugal, the agri-environmental policies have supported IPM through rural development measures; in 1999, 55 486 ha were under this production system and in 2013, the area was five times larger (248 595 ha). The total area and the number of farmers, the public investment and status given to IPM in the present European policies, especially in Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 that establishes a framework for Community action to achieve the sustainable use of pesticides, justify the analysis of the technical, environmental and economic benefits of IPM.

Establishing a framework of relationships between the agri-environmental measures and environmental pressures is required to assess the extent to which agri-environmental objectives were achieved. This can be done using test plots, case studies, quantified impact models, and surveys [8,13], as they define suitable environmental indicators (a metric or a set of metrics that helps provide insight into the linkages between agricultural activities and environmental impacts). These indicators will provide information to monitor and analyze the effects of those policies on the environment and to enhance the understanding, and study the effects, of possible policy scenarios and agricultural projections [15].

The purpose of the present paper is to understand if agri-environment support and IPM adoption truly lead to the positive environmental impact that is expected with the adoption of environmentally friendly farming practices and with the sustainable use of pesticides. More specifically, this paper seeks to determine which environmentally friendly farming practices and motivations towards the adoption of sustainable farming practices are adopted by IPM farmers, and how farmers` motivations and attitudes are related to technical options. The identification of the technical operations and tasks related to the environmentally friendly farming practices in IPM and the recognition of the technical differences among farmers that help promote environmental benefits and food safety are also pertinent aims of this study.

In the present study, the evolution of IPM is described, based on country statistics. Additionally, survey-based data are analyzed to support the definition of the farmer profile, farm description, motivations towards sustainable farming practices, technical profile and practices related to pesticide use associated with IPM strategies, by comparing it with CA and OF.

II. IPM – from the past to the present

2.1 IPM and environment policies in the period of 1994-2013

IPM began in Portugal based on the European policy supported by the Council Regulation (EEC) No 2078/92 of 30 June 1992 and evolved into 3 phases: (1) Agri-environment Program, 1994 to 1999; (2) RURIS, 2000 to 2006; (3) PRODER, 2007 to 2013.

In 1994, IPM was regulated with national laws and the Agri-environment Program started, on an annual basis, for farmers who were willing to adopt best agricultural practices during at least 5 years. The major aim was to

encourage farmers' adoption of environmentally friendly farming techniques compatible with the increasing need for natural resources protection and the upkeep of the landscape and the countryside. Within five years, the program was successful, confirmed by a large number of IPM experts (220), farmers (9 359) and IPM associations (48) and there was a significant agricultural area under IPM systems (62 831 ha) [16].

Based on the Council Regulation (EC) No 1257/1999 of 17 May, the national rural development plan was established (RURIS) and reinforced the importance of local and traditional agricultural systems. Thus, it seemed crucial to support IPM and OF for a large number of crops and to include other measures, namely pesticide risk reduction in water, minimum and zero tillage and the use of cover crops [16,17].

Finally, during the 2007-2013 period, a new rural development program – ProDer - was established, whose major goal consisted in sponsoring agriculture based on economic and sustainable principles. This program has re-introduced financial tools to encourage alternative production methods with economic and social concern for the sustainability of rural areas and the conservation of natural resources [18].

2.2 IPM in Portugal from 1994 to 2013

In 1995, the first financial protocols with farmers were established. In the first year (1996), the IPM area achieved 7 236 ha and 927 farmers. At the end of the first agri-environment program (1999), IPM was adopted by 7 450 farmers on 55 486 ha, mainly on orchards and vineyards (tables 1 and 2). The IPM area was spread over three main regions: 'Norte', 'Lisboa e Vale do Tejo' and 'Alentejo', respectively with 38%, 27% and 18% of the total area. IPM in orchards was allocated mostly in 'Lisboa e Vale do Tejo' (45%) and in vineyards in the 'Norte' region (44%) [16,19].

In 2005, the IPM area increased to 179 840 ha and 19 753 farmers, especially in vineyards (52%), olive (25%) and apple orchards (14%) (tables 1 and 2). About 11 233 ha were in the 'Norte' region with 37% of vineyards supported area in this region. In 'Alentejo', the production area was 57103 ha (32% of total area) with 2 719 farmers. In 2013, PRODER contributed to a major development of the IPM production area, and reached 248 595 ha and 6 692 farmers, with greater adoption in 'Alentejo' (62%). This increase was due to the development of IPM in new crops, in particular pastures (table 2). At this time, IPM was also adopted on 35 553 ha of vineyards and 17821 ha of orchards.

Despite the positive evolution of the IPM area from 2005 (RURIS) to 2013 (PRODER), the number of farmers did not reach the target set for this period (the PRODER target set for the 2007-2013 period was 24 000 farmers). The average area per farm was higher in 2013, compared with the previous years (table 1), mostly because farmers were required to practice IPM across the entire farm in order to get financial support [12]. This new condition has discouraged less capacitated farmers, and led to an IPM area concentration and to a reduction in the number of farmers between 2011 and 2013.

Table 1: Area, number of farmers and average farm size in IPM in Portugal [10,12,20–22]

Region	**Area (ha)**			
	1999	2005	2011	2013
Norte	21 251	66 447	41 630	41 820
Centro	6 888	18 091	22 150	21 382
Lisboa e Vale do Tejo'	15 112	34 248	28 100	27 701
Alentejo	10 149	57 103	155 387	153 820
Algarve	2 086	3 951	4 074	3 872
Total	55 486	179 840	251 341	248 595
	Farmers (number)			
Norte	3 560	11 233	3 536	3 378
Centro	1 055	2 778	1 072	1 028
Lisboa e Vale do Tejo'	1 894	2 680	858	824
Alentejo	778	2 719	1 215	1 232
Algarve	163	343	227	230
Total	7 450	19 753	6 908	6 692
	Farm size (ha)			
Norte	6,0	5,9	11,8	12,4
Centro	6,5	6,5	20,7	20,8
Lisboa e Vale do Tejo'	8,0	12,8	32,8	33,6
Alentejo	13,0	21,0	127,9	124,9
Algarve	12,8	11,5	17,9	16,8
Total	9,3	11,5	36,4	37,1

Table 2: Area of IPM crops in Portugal (ha)[20–22]

Crop	**1999**	**2011**	**2013**
Permanent crops			
Fresh fruits orchards	13 339	17 418	17281
Dry fruits and olive orchards		42 724	43329
Vineyards	42	34 543	35553

	146		
Rice		17 601	18456
Forage and non-permanent crops		25 536	27863
Vegetables	1	1 117	1168
Permanent pastures		102 962	104945
	55	251	248
Total	486	339	595

Technical support was fundamental for the adoption of IPM, particularly in the beginning of the program. In 1995, the government recognized the first IPM farmer associations. In 1998, about 28 farmer associations were recognized as IPM support structures operating mostly in vineyards and orchards, and this number continued to increase: 66 organizations in 2000, 112 organizations in 2004 and 162 organizations in 2013 [16,19,23].

The requirements for agri-environment payments in Portugal between 1995 and 2013 were mainly administrative actions undertaken by farmers: have a farm management plan, use only authorized pesticides, have a field book, keep the evidence of pesticides purchased and of the soil, water and plant material analysis, have a shelter for pesticides and fertilizers [16].

III. IPM. ANALYSIS OF FARMERS' MOTIVATIONS AND IPM TECHNICAL AND PESTICIDE USE PROFILES

3.1 Methodology

The IPM adoption in Portugal was characterized based on survey-based data collected from IPM, OF and CA farms in the most important regions and crops where pesticide use is of more concern: vineyards – Alentejo, Dão, Douro, Verdes - and apple and pear orchards - Dão, Oeste. The questionnaire was conducted between 2007 and 2009 by trained technicians working at different farmers associations, to the person responsible for the decisions at farm level. The questionnaires were applied face-to-face and a total of 177 survey questionnaires, conducted in different farms, were considered valid (13 OF farms, 91 IPM farms, 73 CA farms).

These crops were chosen based on different criteria. Firstly, vineyard was selected because it is the crop that is responsible for the largest amount of pesticides used in Portugal, mainly fungicides [3,24]. Apple and pear orchards were selected based on the fact that the key insect pests and diseases are numerous, causing serious problems to farmers, and on account of apples and pears being among the food products with the highest percentage of samples with pesticide residues above legal limits [5]. The questionnaires included data related to the farmer profile (socio-economic characterization), farm description, motivations towards sustainable farming practices, technical profile (inventory of farming practices) and crop protection practices.

3.2 Data analysis

Results from the survey were stored in a database, and categorical variables were codified as numbers (0 or 1) so that an exploratory analysis could be performed to identify which variables were related to the production system (IPM, OF or CA) or to each other. The main variables suggesting relations with IPM, OF and CA production systems were identified with a Principal Component Analysis. As this analysis was extensive [25], only the variables that explained significant levels of variance were included in the present study.

An analysis of variance (one-way ANOVA) was used to detect differences among production systems (OF, IPM and CA), in order to better understand and profile each group of variables (farmer profile, farm description, motivations towards sustainable farming practices, technical profile and practices related to pesticide use). Means were then compared using the Tukey HSD tests and Bonferroni-Holm, whether the homogeneity of variances was observed or not. Values of $P \leq 0.05$ were considered significant. All statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.).

IV. RESULTS

4.1. Farmer profile

Based on the survey results, the average farmer was 53 years old, men older (57 years average) than women (49 years average), and orchard producers older than vineyard producers. IPM and OF farmers were significantly younger than CA (table 3).

The surveyed farmers had on average a secondary level of education (ISCED 2-3) , which reveals that these farmers have an education level well above the Portuguese national average in Portugal (on average, farmers have basic education and 88% have only practical agricultural training) [26]. This education level was higher in OF and IPM farmers, as 60% of farmers had secondary or higher education. More than half of CA farmers had less than six years of education (ISCED 0-2)[1] (table 3). Most IPM

[1] The International Standard Classification of Education (ISCED) was developed by UNESCO and adopted since 1997 to facilitate comparisons of education statistics and indicators across countries on the basis of uniform and internationally agreed definitions [27]. Until 2011 ISCED had 7 levels of education, from early childhood education (ISCED 0), primary education (ISCED 1), secondary education (ISCED 2-3), post-secondary non tertiary education (ISCED 4) to tertiary education levels (ISCED 5-6).

(93%) and OF (77%) farmers had already participated in training courses related to IPM, pesticide use, and general agricultural training.

Table 3: Average values of variables related with farmer profile of IPM, OF and CA farmers

VARIABLE	OF (n=13)	IPM (n=91)	CA (n=73)
age	48 [a]	51 [a]	62 [b]
education level (ISCED)[1]	2.54 [a]	1.956 [b]	1.356 [c]
participation in training courses (%)	77 [a]	93 [a]	33 [b]
other economic activities (%)	77	53[a]	44 [b]

Note: [a,b,c] Scores in the same row with a the same superscript are significantly different at p<.05 (post hoc Bonferroni-Holm and Tukey multiple comparison tests)

Almost 50% of the inquired farmers have other economic activities meaning that a part of their income is obtained outside of the farm, and that they are not fully dedicated to farming. This figure is in line with the Portuguese reality (51% of farmers work less than 50% of a full-time equivalent and dedicate, on average, 22 hours/week of labor to their farms) [28,29]. This is especially relevant among OF farmers, wherein more than three quarters have other jobs in addition to agriculture (77%) (table 3).

4.2. Farm description

Farms included in this study have an average area of 30.9 ha, when compared to the national average farm size (12 ha of utilized agricultural area per farm) [29], and OF and IPM farms are larger than CA ones – 46.2 and 37.6 ha in average, respectively. More than 80% of the farms are held by individual farmers while only 20% work as a company (enterprises), which is higher when compared to the national figure (27% of the Utilized Agricultural Area) [1,29], probably because vineyard and orchard farms are more professionalized. The average number of workers per farm is higher in IPM (10.2workers), with twice more workers, on average, than in CA farms (4.64.0 workers) (table 4). Comparing the number of permanent hired workers on these farms, we observe that number of permanent hired workers outweigh the Portuguese national average (far less than 1 per farm) [28,30].

Table 4: Average values of variables related with IPM, OF and CA farm characteristics

VARIABLE	OF (n=13)	IPM (n=91)	CA (n=73)
farm dimension (ha)	59.75 [a]	44,50 [a,b]	10.47 [b]
number of workers	7.7 [a]	10.2 [b]	4.6 [c]

Note: [a,b,c] Scores in the same row with a the same superscript are significantly different at p<.05 (post hoc Bonferroni-Holm and Tukey multiple comparison tests)

4.3. Motivations towards sustainable farming practices

Various recent studies demonstrated that the motivations towards a particular production system go far beyond technical and economic issues [31,32]. Cultural, social and environmental beliefs underlie the choice of more sustainable production systems, such as IPM or OF. In fact, we found that some agricultural practices regarded attitudes or reasons pertaining to more sustainable practices (almost 54% of variance is explained by the two first axes) (table 5).

Forward selection results, from the principal component analysis, showed that the variables 'look for a biological, biotechnical, cultural solution' (F=18.36; p=0.002), 'look for a new pesticide' (F=21.13; p=0.002), 'not change agricultural practices in favor of species conservation' (F=4.58; p=0.008), and 'give up toxic pesticides, using them only when needed, to preserve local fishes' (F=2.79; p=0.044), were significant for p<0.05. The variable 'look for a biological, biotechnical, cultural solution' was responsible for 14.0% of variance and the variable 'look for a new pesticide' for 18% of variance. The variables 'use an expensive crop protection solution' and 'not use an expensive crop protection solution' were also significant for p<0.10.

Table 5: Principal Component analysis of the OF, IPM and CA farmers motivations towards the adoption of sustainable farming practices

Question	**Answer/variable**	**Conditional Effects**		
		λ	**p**	**F**
If it were necessary to reduce pesticide use, with economic risk to your farm, to save a very rare butterfly that was observed in the area, you would:	change agricultural practices in favour of species conservation.	0.01	0.262	1.36
	not change agricultural practices in favour of species conservation.	0.03	0.008*	4.58
If near your farm, all the fishes were found dead as a result of pesticide use, would you:	not use pesticides in the next campaign, to preserve local fishes?	0.00	0.372	1.19
	give up toxic pesticides, using them only when needed, to preserve local fishes?	0.02	0.044*	2.79
	buy pesticide equipment that	0.00	0.432	0.83

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	recovers pesticide loss, to preserve local fishes?			
	use a biological, biotechnical, cultural solution even if expensive?	0.01	0.222	1.54
	don't use pesticides, so there is no need of change to preserve local fishes?	0.01	0.122	2.24
If you had to change your agricultural practices, by government decision, you would:	reduce pesticides for a subsidy.	0.01	0.202	1.47
	reduce pesticides to have technical support.	0.01	0.280	1.18
	have already reduced the use of pesticides.	0.02	0.140	1.86
In Portugal, about 2 100 pesticide poisoning cases occur per year, and of those 360 happen with children less than 5 years old. If decreasing the use of toxic pesticides would contribute to reducing this risk, you would:	give up pesticides to reduce poisoning even 2%.	0.02	0.224	1.49
	give up pesticides to reduce poisoning 50%.	0.00	0.636	0.44
	give up pesticides to reduce poisoning 100%.	0.00	0.600	0.50
	not give up pesticides, because it would not guarantee poisoning reduction.	0.01	0.340	1.15
Facing a new or a resistant pest or disease, or even in the presence of new technical knowledge, you would:	look for a new pesticide.	0.18	0.002*	21.13
	look for a biological, biotechnical, cultural solution.	0.14	0.002*	18.36
	use the same solution.	0.00	0.878	0.13
In view of your neighbour' farming success , with the adoption of a new crop protection method that requires more work, and increases risks and costs, you would:	use a new crop protection solution.	0.01	0.662	0.39
	not use a new crop protection solution.	0.00	0.794	0.25
The adoption of new cultural practices, which increase farm costs, has led other farms to improve their results. Facing other experiences, you would:	use an expensive crop protection solution.	0.03	0.066**	2.91
	not use an expensive crop protection solution.	0.02	0.068**	2.65

* Significant at p<.05; ** Significant at p<.10

Based on the biplot analysis, it is possible to identify some relations between the adopted farming system and the attitudes or reasons for embracing sustainable practices (figure 1).

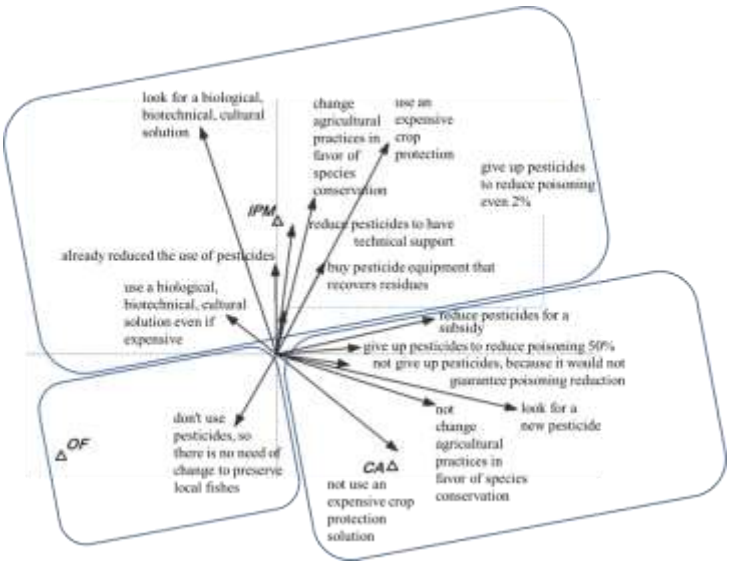

Fig.1: Biplot for motivations towards the adoption of sustainable farming practices of OF, IPM and CA farmers

IPM farmers were ready to 'look for a biological, biotechnical, cultural solution', 'give up toxic pesticides, using them only when needed, to preserve local fishes' or to 'use an expensive crop protection solution'. Meanwhile, CA farmers will 'look for a new pesticide', 'will not change agricultural practices in favor of species conservation' and 'will not use an expensive crop protection solution'. The variable 'don't use pesticides, so there is no need of change to preserve local fishes' was positively related to OF farmers, but with no significance.

4.4. Technical profile

Concerning crop management, the results from our survey indicated that, compared to CA farmers, IPM and OF farmers use less 'tillage operations', adopt 'cover crops' (especially OF farmers), prefer 'organic fertilizations'

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instead of mineral ones, perform 'soil analysis' to decide their fertilization plans (especially IPM farmers) and frequently use 'green interventions' to control canopy environment. The adoption of these practices was significantly different among IPM and CA farmers, with the exception of tillage operations (table 6).

In 2010, in Portugal, the use of cover crops was adopted by 10% of farms, with more expression in the 'Norte' and 'Centro' regions and in orchards and vineyards and only 8% of farmers decided their fertilization plans based on soil analysis (8%) [29,33]. IPM and OF farmers have exceeded these values, probably because they understand the contribution of such sustainable farming practices for the balance of the ecosystems, namely to improve functional biodiversity [34,35].

Table 6: Percentage of sustainable practices (crop management and protection) adopted by IPM, OF and CA farmers

VARIABLE (%)	OF (n=13)	IPM (n=91)	CA (n=73)
Technical profile - Practices related to crop management adopted by OF, IPM and CA farmers			
tillage	62	67	78
cover crops	77 [a]	38 [b]	11 [c]
organic fertilizations	46 [a]	79 [b]	56 [a]
soil analysis	77 [a]	82 [a]	7 [b]
green interventions	62	66	42
Pesticide use - Practices related to crop protection			
risk assessment	92	99 [a]	85 [b]
advice and national advisory services	46	78	44
reading of labels	69 [a]	85 [a]	47 [b]
evaluation of pesticide efficacy	54 a	97 b	75 c
pesticide residues analysis	39	17	7
use of protective equipment	85	95 a	85 [b]
professional applicator training	46 [a]	45 [a]	7 [b]

Note: [a,b,c] Scores in the same row with a the same superscript are significantly different at $p<.05$ (post hoc Bonferroni-Holm and Tukey multiple comparison tests)

4.5. Pesticide use

In Portugal, 71% of the total pesticides used are fungicides, with sulphur representing 90% of them (48% of total pesticides, and especially in vineyards) [3]. Herbicides represent 14% of the total sales and insecticides only 2%. From 2008 to 2011, the total pesticide sales decreased 18%, especially due to a reduction in the use of sulphur, but 3.1 % of total food samples analyzed in Portugal still exceeded the MRL [5].

According to our results, concerning the adoption of practices related to pesticide use, IPM farmers declared to use 'advice and national advisory services', practice 'risk assessment' for decision making, 'reading of labels' before pesticide treatment, 'evaluation of pesticide efficacy' regularly, request 'residues analysis' and 'use protective equipment', more often than OF and CA farmers (table 6).

The participation in 'professional applicator training' was similar between OF and IPM farmers. Differences between IPM and CA farmers were significant for 'risk assessment', 'evaluation of pesticide efficacy', 'use of protective equipment' and 'professional applicator training' (table 7).

V. CONCLUSIONS

Since 1990, the environmental performance of agriculture has improved in Europe based on agri-environment measures that were designed to protect and enhance the environment through the adoption of agricultural sustainable systems, such as IPM and OF [1,8,11]. These measures have been essential for the integration of environmental concerns by farmers and have originated a valuable contribution to the ecological balance of ecosystems.

Until 2014, IPM was adopted by farmers, on a voluntary basis, but since then, European states should promote IPM under the framework of their National Action Plans for the Sustainable Use of Pesticides.

Ultimately, farmers' actions and practices determine their environmental performance. Reacting to public concerns and policies, IPM farmers in general have become increasingly aware of the effects of their actions on the environment and have upgraded their management practices based on scientific and technical knowledge, and investments in environmentally friendly practices [2].

Based on the present case study, we can conclude that there are obvious differences between IPM and CA, related to farmer profile, farm description, motivations for IPM adoption, technical profile and practices related to pesticide use.

IPM farmers are younger, better educated, and more concerned with technical training. A significant number of IPM holdings are companies, specialized in one crop, with larger areas and employing more workers, both in orchards and vineyards, when compared to other systems.

The results from our survey proved that IPM farmers are ready to look for biological, biotechnical and cultural solutions to control pests, are willing to give up toxic pesticides and to use more expensive crop protection

solutions to preserve the environment and health. However, they usually want to be compensated with subsidies when they implement these sustainable agricultural practices.

Most IPM farmers are adopting cover crops to overcome weed problems and other technical issues (e.g. water, erosion), namely in permanent crops, such as orchards and vineyards. In fact, tillage, as a routine task, decreases from CA to IPM, and at the same time soil cover increases. Usually, IPM farmers execute green interventions to control the canopy environment (removal of side shoots, orientation of vegetation, among others). These techniques have several purposes: improve the canopy environment, reduce the number of fruits to improve size and quality, among others, and farmers don't receive any incentives to adopt them. IPM farmers also prefer organic fertilizations instead of mineral ones and organize their fertilization plans based on soil analysis.

IPM farmers are always more diligent in what pertains to pesticide use: risk assessment is the basis for decision making, advice from experts and national services is deemed indispensable, as well as information contained on labels, and they oversee the efficacy of pesticide treatments. These farmers are naturally apprehensive about the secondary effects of using pesticides and protect themselves with the appropriate equipment, more often than OF and CA farmers.

We might conclude that IPM, as a crop protection strategy, has been successful in Europe, and in particular in Portugal, in terms of area and number of farmers, due to the important technical support that was provided by farmers associations. Furthermore, the adoption of several sustainable practices, related to crop management and protection, and farmers' attitudes towards the use of pesticides are expected to have contributed to protecting, and enhancing, the environment and health safety. These variables (tables 3, 6 and 6) might be considered to establish new requirements for IPM support and used to monitor the environmental and health outcomes gained through this crop protection strategy and the sustainable use of pesticides.

The environmental and health outcomes that are obtained with these sustainable practices should be assessed, based on environmental indicators (simple metrics that disclose the linkages between agricultural activities and environmental impacts), as they will result in a balanced and sustainable management of resources and generate environmental services that have an economic value.

Some difficulties ensue when using these environmental indicators, as their monitoring (e.g. biodiversity or pesticide use) is challenging due to political, conceptual, practical (technical) and institutional factors [36], but it should be attempted. They will provide a coherent guidance for the best practices that should be defined as requirements for IPM while, at the same time, might be used in cost-benefit analysis of the most desirable policy measures and support programs, based on the identification and assessment of synergies between policy/program goals and their benefits [37].

The financial support of environmentally friendly farming systems, such as IPM, either by implementation of subsidies, by specific price policies or by ensuring a market for these specific products, should play a prominent role in supporting the sustainable development of rural areas and in responding to society's increasing demand for environmental services. It should further encourage farmers to serve society as a whole by introducing, or continuing to apply, agricultural production methods compatible with environmental and health protection, IPM being a prime example.

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Determinants of Rice Production and Marketing in low Producer Farmers: the Case of Fogera Districts, North-Western Ethiopia

Astewel Takele

Department of Agricultural Economics, College of Agriculture and Environmental Sciences. Bahir Dar University, Ethiopia.

Abstract— *Ethiopia is emerging as an important rice growing country in Eastern Africa. However, there are several constraints which drastically affect rice production and its marketing system under smallholder farmer's condition. This study aimed at examining the socioeconomic determinants of rice production and marketing in low producer farmers in the study area. A sample of 160 rice producer and 50 traders were interviewed using structured questionnaire. The result of the study showed that sex of household head is positive and statically significant in explaining rice production at 5% significant level ($p<0.05$). Oxen ownership ($p<0.07$) and land size ($P<0.067$) were directly proportional to rice production and significant at 10% level of significance respectively. Moreover, labor availability and rice seed rate was highly significant at 1% level of significance ($p<0.001$). It is recommended that farmers should use intensive farming by increasing productivity of the land using improved varities, application of other alternative traction power(oxen) , adopting labor saving technology and management of seed rate (agronomic practices)during sowing . Moreover, there is a need to consider gender differentials in rice production system. The S-C-P- model reflects that the structure of rice marketing is imperfect market (oligopsonistic), only few buyers governed the market. The Gross marketing margin indicated that assemblers harvest the highest marketing margin as compare to other market participants and farmers received below the total average share of the margin. High investment capital, and competition with unlicensed traders were the barrier in rice marketing. Hence, facilitating loan (credit services), increasing the bargaining power farmers & licensing illegal traders were the recommendation forwarded.*

Keywords— Rice, marketing, concentration ratio, S-C-P model, Ethiopia.

I. BACKGROUND

Rice *(Oryza Sativa Linu)* is the staple food of over half the world's population and at least 3.5 billion people are consuming the rice (Sreepada and Vijayalaxmi , 2013). It is one of the market oriented and strategic crop in the rice producing areas of Ethiopia. It was is a productive crop next to maize in the country (CSA, 2003) and considered as the "millennium crop" which is expected to contribute to ensuring food security in Ethiopia (Hadush, 2015,). Rice was first introduced in Ethiopia in the 1970s and has been cultivated in small pockets of the country today (Yemane, 2014). The area under rice production in Ethiopia is estimated to have increased from 5,400 ha in 1993 to about 46,832 ha in 2014 (FAOSTAT, 2017). The number of farmers engaged in rice production has also increased from about 53 thousand in 2006 to about 284 thousand in 2009 (MoARD, 2010).

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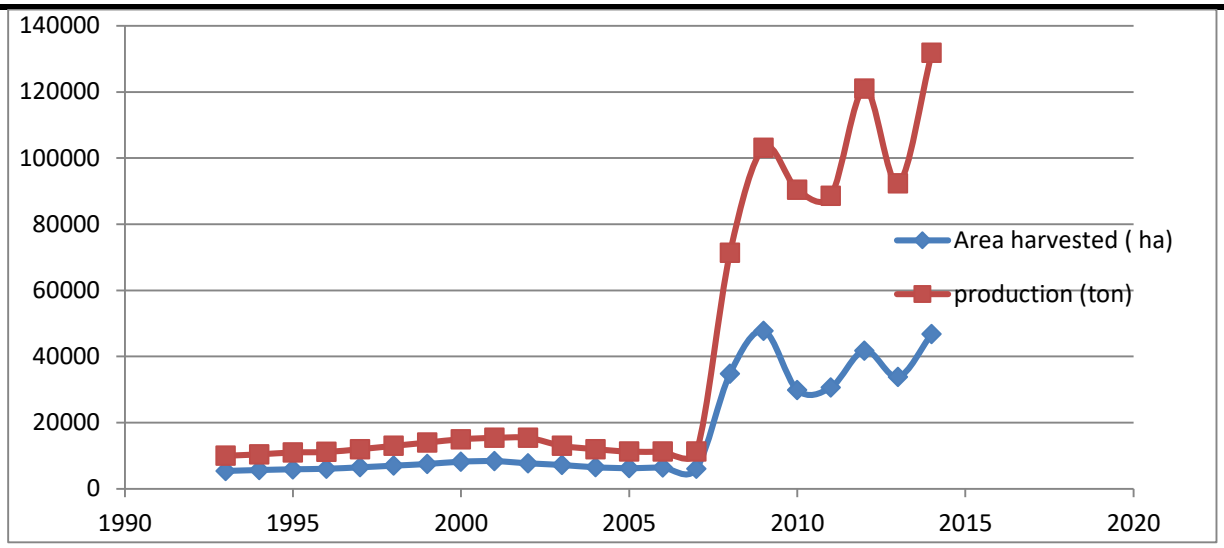

Fig.1: Area and production trend of rice in Ethiopia. Source: FAOSTAT, 2017.

The production of rice started in Amhgara Region at *Fogera* plain and at *Gambella* in Ethiopia. In the *Fogera* districts (where this research conducted) its production in hectare has been increasing year after year (CSA, 2013) and Fogera plain contributes about 32 % of rice production in the country (EIAR, 2011). Efforts have made to boost production and productivity of rice through research for the last decades, however still different production and marketing factors hindered and limited its output production. Limited input supply, farmer's inefficiency, poor adoption of technology and institutional limiting factors (credit, market, etc) and other policy issues were among the variables were considered in farming system.

Improvement in production alone was not sufficient to achieve better income unless the marketing aspect as well improved. The marketing gap enforces farmers not produce rice as much as the potential beyond fulfilling their daily consumption. It is clear that markets offer households the opportunity to specialize according to comparative advantage and thereby enjoy welfare gains from trade (Reardon *et al.*, 2005) but on the process of transaction the flow of agricultural produce from the producer to the consumer involves a long chain of intermediaries in Ethiopian grain marketing in general (Dawit, 2005, Gebremeskel *etal.*, 1998.).

In the study area, Gebremedhin and Hoekstra (2007) studied that 72% of the households and about 50% of the farmers produce and sell rice; farmers sold and marketed its output into different regions of the country (IPMS, 2005). Also Afework, (2015) indicated that rice production in *Fogera districts* is constrained by seed production and marketing. Similarly inelastic price of the product and inefficient marketing system hinders the performance of the rice market. The involvement of many intermediaries has also constrained the development of the sector and deprived the farmers of equitable returns from their produces. The factor of rice production and lack of organized marketing system have resulted in low producers' price of farmers. This causes the farmers to declines its production in amount and marketable surplus would have an impact on the farmer's income (for example for the last thirteen years from 1993 up to 2005 years) the production and area coverage was very steady but since 2005 onwards for the last 10 years (2005-2014) production has increased rapidly (FAOSTAT, 2017). Therefore, understanding its production and marketing of rice as a whole (which supports over thousand million farmers livelihood) is vital for future production plan and policy development. In view of this, the study had been conducted specifically to analyze the factors that affect rice production, to assess the structure, conduct and performance of rice marketing and finally to identify the main channels of rice marketing in the study area.

II. MATERIALS AND METHODS

The study will particular try to address the following quest ions that are very important for planning and implementation of possible intervention policies.

Research questions

- What are the factors affecting rice production in the study area?

- What are the structures of the rice marketing (who dominates the market) , what are its behavior in price setting and their strategy for sales? Who benefit from the marketing of rice?
- What are the channels of distribution (roots) of rice marketing?

Study Area: *Fogera* districts is known for rice production and located on part of the South Gonder zone. The altitude of the district ranges from 1,500 m to 2,600 m above sea level while the annual average rainfall is 1,250 mm. The district comprises of 18 rice producing peasant administrative *(Kebeles)*. Out of which majority of it is suitable for lowland production of rice and few of it used in production of upland rice cultivation.

Sampling procedure or design: The data for this study collected from randomly selected from rice farmers in South Gondar zone (Fogera district). Purposive sampling technique used in the selection of rice producing Peasant Administrative (PA) in the districts. There are classified as rice- based farming system (PAs in which rice is dominant crop) and Cereal- based farming system (PAs in which non -rice crops) are dominant (IPMS, 2005). Among ten rice producing PAs in the districts, four of them were randomly selected based on the proportion to population size. A total of 120 samples were selected and used for the study. In addition to these marketing data were also collected from assemblers, wholesalers, millers and retailers in the market.

Types and source of data: The primary data were collected from a sample of rice producers and traders in the area by preparing structured questionnaire. A total of 165 producers were randomly selected and interviewed. In addition, secondary data were obtained from various sources, woreda trade and industry office, woreda agricultural office and Agricultural Research Centers.

Method of data Analysis: Both descriptive statistics, econometrics and structure- conduct and performance model (S-C-P model) were applied. The data were analyzed using Minitab-16 and SPSS-16 software's, the specification of the model is as follows:

Econometrics models. For this analysis econometrics models were applied. Using the ordinary least square (OLS) estimator, the production function model was estimated as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 . + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 ..... + \beta_n X_{n,} \quad (1)$$

Where y = outputs
X_n = explanatory variables
β_0 = constant
β_n = the parameters to be estimated

Structure- Conduct and Performance (S-C-P) model: It originates to the work of the Harvard economist Edward Mason in the 1930s. Mason (1939)'s starting point was that market share is important in determining production and pricing policy of a firm. The SCP approach analyzed the market organization of the industrial sector and it was later applied to assess the agricultural marketing system (Scarborough and Kydd, 1992). It comprises of three major elements: these are structure, conduct and performance. According to the structure–conduct–performance paradigm, the market environment has a direct, short-term impact on the market structure (Wikipedia, 2016)

Market Structure: Refers the characteristics of the organization of a market which seem to influence strategically the nature of the competition and pricing within the market (Bain, 1959). The way in which markets fail to follow perfect competition conditions. Also, the structure of the market will always be determined by the nature of the product and the technology available (Raj kumar, 2006). The most salient characteristics of market structure according to different writers (Scarborough and kydd , 1992) Scott, 1995, Clodius and Mueller, 1961) includes:

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1. The degree of seller's and buyer's concentration which refers to the number and size distribution of firms in relation to the size of the market;
2. The degree of the product differentiation among outputs of the various sellers in the market; and
3. Barriers to entry or freedom to entry and exit from the market: this refers to the conditions for entry of new firms into the market or exit of existing firms. There are many indexes/ instruments used to analyze ones firm structure of the market, among these are Concentration indexes, Herfindahl-Hirschma Index, Gini Coefficient and Lorenz curve.

Market concentration: is defined as the number and size of sellers and buyers in the market (Scott, 1995). The greater the degree of market concentration, the greater the possibility of non-competitive behavior in the market will be. Different literatures used different types of techniques to measure d concentration. Concentration ratio is the percent of combined production of leading four or eight firms in industry. These can be explained as the four- firm concentration ratio (CR4), the four firm ratio of 51-80% implies monopolistic competition and 81-100% is high concentration –oligopoly or monopoly (Kohls and Uhl ,1985), (Karugia, 1991). In other words it can be measured by: (as expressed by Bedilu, *etal.,* 2015, Khols and Uhl , 1985).

$$CR_4 = \sum \frac{X_i}{T} \qquad (2)$$

Where, CR_4 concentration ratio of the first four dominant firms
X_i is individual firm i,
T is the total market size of firms.
.
Another popular measure of market structure of firm size is Herfindahl- Hirschman Index (HHI), means the percent of a individual sale potion in total sales and approaches zero when a market consists primarily of a large number of firms relatively equal in size (kang *etal.* , 2009). It is calculated by squaring the market share of each firm competing in the market and then summing the resulting numbers. In other words, it is equal to

$$\mathrm{HHI} = \sum_{i}^{4} (\mathrm{marketshare})^2 \qquad (3)$$

The HHI increases both as the number of firms in the market decreases and has the disparity in size between those firms' increases.. Markets in which the HHI is between 1000 and 1800 points are considered to be moderately concentrated and those in which the HHI is in excess of 1800 points are considered to be concentrated (monopoly). Another measure of market concentration is the Gini Coefficient (GC) approach named after the Italian statistician who first formulated it in 1912 (Todaro, 1998) and mathematically expressed as:

$$GC = 1 - \sum XY\,, \qquad (4)$$

Where,
X = proportion of sellers,
Y = cumulative proportion of sellers.
The value of the Gini Coefficient ranges between zero and one. The higher the coefficient, the higher the level inefficiency in the market structure (Giroh *et al.,* 2010, Nsikan E. *etal.* , 2013) and Issahaku H. *etal.,* 2012)

Additional method of computing concentration of market structure is Lorenz curves which can be used to provide a graphical overview of the distribution of market shares in an industry. The 45 degree straight line corresponds to equalized market shares. The Lorenz curve shows the quantitative relationship between the cumulative percentages of rice traders against the cumulative percentage of the volume of rice sold in the markets. To compute the cumulative percentage, the volume of rice sold will be arranged from highest to lowest. The Gini- coefficient or concentration ratio will be derived from the Lorenz curve. This measures the inequity in sales distribution among the different producer and trader groups (FAO, 2005). For this study, the concentration ratio is implemented as other methods are limited in their application for imposing additional restrictions.

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Market conduct: the set of competitive strategies that a trader or a group of traders use to run their business. It also explains that buying and selling practices are the variables which were used to determine the market conduct. Traders behavior like setting price individually or by colluding with each other and also if they jointly restricted the amount of rice for sale to raise the market price. In other words, market conduct focuses on traders' behavior with respect to various aspects of trading strategies such as buying, selling, transport, storage, information and financial strategy.

Market performance: market performance refers to the impact of structure and conduct as measured in terms of variables such as prices, costs, and volume of output (Bressler and King, 1979). It also refers to how well the market fulfils certain social and private objectives. The two major indicators of market performance are net returns and marketing margins. Estimating net returns and marketing margins provide indication of an exploitive nature when net returns of buyer are much higher than his fair amount. Net returns can be calculated by subtracting fixed and variable costs from gross returns, while marketing margin is defined as a difference between price paid by consumers and that obtained by producers or the price of collection of marketing services (Tomek and Robinson, 1990). By analyzing the level of marketing margins and their cost components, it is possible to evaluate the impact of the structure and conduct characteristics on market performance (Bain, 1968). The gross marketing margin and net marketing margin analysis is given as:

$$\text{TGMM} = \frac{\text{consumer price} - \text{producer price}}{\text{consumer price}} \text{X100} \quad (5)$$

$$\text{GMMP} = \frac{\text{consumer price} - \text{marketing gross margin}}{\text{consumer price}} \text{X100} \quad (6)$$

$$\text{NMM} = \frac{\text{Gross margin} - \text{marketing cost}}{\text{consumer price}} \text{X100} \quad (7)$$

Where:
TGMM = Total Gross Marketing Margin
GMMP = Gross Marketing Margin of the Producer
NMM = Net Marketing Margin

Many researchers applied the SCP method for conducting their study on agricultural markets, However, the SCP method has been subject to criticism; e.g Scherer (1990) stated that the SCP model is too deterministic to understand the functioning of imperfect markets. As most agricultural markets are imperfect markets, we need to develop more dynamic models showing how structure, conduct and performance interact. Others also argued that the SCP school has emphasized the private exercise of market power as a source of poor market performance, but other economists have concluded that the main source of monopoly or anticompetitive is likely to be government interference in the marketplace. Finally the dynamic behaviors of buyers and sellers have an effect on the markets, making it harder to predict and establish fixed market structures.

III. RESULT AND DISCUSSION

Descriptive results of the study: showed that The major inputs used in the production of rice in the study area identified as land, labour, oxen *(tropical livestock)* unit/ herbicides and seed (described as table 1).

Land availability : *Land* happens to be one of the main inputs used in the study area for rice production. Land ownership in Ethiopia is state ownership. From the results of the descriptive statistics, the minimum land size is 0.12 hectare and the maximum is three hectares with the mean being 1.21 ha. This means that on the average people cultivate more than one hectare of land. It is clear that land ownership is found to be the main determinant factor that affects rice production in the study area.

Labour amount: Labour as an input is very important; The results of the survey showed that the maximum labour was 45 man-days with 2 man-days being the minimum and the mean labour in the study area was 12 man-days per year . The source of labour for the production was 44% family labour and 36% both family and hired labour. The Family size of the households has a mean of 6 in each household. It is also observed that labour amount of households significantly affect rice production.

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Seeds: Seeds are the paddy rice used for the production of rice. In the study area different varieties used for production, theses are *X-Jigina* varity (local), *Gumara* varity (improved) and other Nerica varieties were available on the area. Some farmers in the study area prepared their own seed, from the previous harvest whiles others buy from the market. On the average, farm households in study area uses a minimum rate of 15kg per hectare and a maximum of 400 kg/ha with average of 98 kg /ha of seed respectively. The agronomic recommendation rate used in the area was 120 up to 140kg/ha of seed. The result from the econometric model also revealed that appropriate seed rate is the limiting factor for rice production.

Oxen ownership: oxen are one of the inputs used in rice production**.** it is the only traction power used for 300 years using local plough called *Maresha*[1] , the result of the survey shows that farmers has on average of two and a maximum of six pair of oxen respectively. There are also households without oxen and can be used as either rent in or hired oxen labor (specially female HH) the area is known with its type of livestock breeds called *Fogera* breeds which is able to adopt for ploughing purpose in area where there is wet (marsh) area. It is also found that traction power using oxen is one of the inhibiting factors for rice production.

Fertilizer: The chemical fertilizer (DAP and UREA) are very important inputs used in the production of rice. Farmers use different rate of fertilizer application at different growing stage. In the study area only few farmers (highland area) farmers used fertilizer for rice production. Due to the area is near to the great Lake *Tana*, farmers less to apply fertilizer for growing rice production. Application of chemicals was not common in the study area and farmer used weeding by hand weeding than use of chemical control.

Table.1: Descriptive statistics of farm inputs in rice production.

Description		yield/ha	Seed kg/ha	Family labor /AE/	Labor amount /year/	Oxen No/HH	UREA kg/ha	DAP kg/ha	Insecticide lt/ha	Herbicide lt/ha
N	Statistic	165	155	165	164	165	164	163	164	164
Minimum	Statistic	5.6	15	1	2	0	0	0	0	0
Maximum	Statistic	112	400	6.15	45	6	25	10	9	3
Mean	Statistic	31.17	98.03	2.68	12.21	1.91	0.43	0.06	0.08	0.16
Std. Deviation	Statistic	17.74	128.37	0.88	11.17	1.04	2.62	0.78	0.72	0.44
Skewness	Statistic	1.75	1.48	1.12	1.19	1.10	7.08	12.76	12.07	3.02
	Std. Error	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Kurtosis	Statistic	3.96	2.83	1.57	0.44	2.66	55.56	163.00	150.72	11.73

Source: Own Survey

Econometric result of the study

According to the econometric result (ordinary least square analysis) four variables were found to be significant, namely sex of households, oxen number, land size, amount of labour availability and seed rate. The result implied that gender (sex of HH farmers) is positively related to rice production and it was significant at 5% percent *(P<0.002)* and land size is also positively related with the rice production and it is also at 10% level of significance *(P<0.067)* and number of oxen ownership is more significant positively with rice production at 5% significant level *(P<0.07).* In the production of rice, labour is highly significant at 1% level *(P<0.002),* and it is the most binding factor among all variables, likewise amount of seed used in the production is also highly significant at 1% level *(P<0.002).*

[1] A wooden and a metal made material used for oxen ploughing

The overall analysis (ANOVAs) F-statistic shows that it is highly significant at 1% level *(P<0.000),* showing that there is significant variation of rice production among predicted variables in the study area. The adjusted R-square is 21%, it is showed that 21 % of the variation is explained by the independent variables of the model under ceteris paribus assumption (other things held constant) and other variation is coming from external factors (exogenously).

Table.2: Factors (determinants) of rice production.

Model	Unstandardized Coefficients		Standardized Coefficients	t-statistics	Sig.
	B	Std. Error	Beta		
(Constant)	-8.891	8.545		-1.04	0.300
Age of HH	-0.081	0.084	-0.085	-0.968	0.335
Sex of HH	**15.321****	7.471	0.144	2.051	0.042
Education level of HH	1.127	0.996	0.086	1.132	0.259
Family size of HH	0.401	0.526	0.065	0.761	0.448
Amount of labour used	**0.243*****	0.076	0.232	3.209	0.002
Oxen number	**0.931***	0.51	0.133	1.825	0.07
Land size(ha)	**3.098***	1.679	0.156	1.845	0.067
Amount of seed (qt.)	**0.037*****	0.012	0.231	3.147	0.002
a. Dependent Variable: Quantity of paddy rice produced R-square =0.257 Adjusted R-square=0.218 F statistics =6.687*** N=163					

Source: own computation

Problems Encountered by the Farmers.

There are many problems encountered farmers during production in the study area one of it the problem of rice thresher (unavailability of appropriate machinery). For instance there was no rice thresher or miller in the nearby area (Peasant Administrative) and opt to travel along way near cities. Through direct observation it was realized that farmers lose a lot during harvesting when they used threshing by beating with stick. Majority of the interviewed (56%) cited this problem. Farmers however, also claim that the varity of seed was few, they usually used local varity which is available in the area, commonly called *X-Jigna* varity.. **A**ccessibility of roads that link the fields to the main roads is a problem cited by respondents especially during rainy season. finally capital shortage and price fluctuation were considered to be the problems in the study area.

Market analysis of the study (S-C-P –mode):

Market structure: Even though different types of traders were available in the study sites market concentration ratio has been calculated for one trader to analyze the type of markets prevailed (Table 3). The results indicated that the structure of the market was measured by using concentration ratio (CR4), it was found that the four-firm Concentration ratio was about 0.77 for wholesale buyers. The concentration ratio of 0.77 indicates that 77% of the market volume was occupied by few buyers. Therefore; the market was governed by few wholesale buyers (i.e. strongly oligopsonistic). As Bain (1959), said the market structure has influence on the market conduct and this can be influence on the nature of competition and pricing within the market.

Table.3: Market concentration of rice wholesalers.(CR4-ratio)

	Amount of rice purchase				
wholesalers'	qt/month	% share	Rank	4 -firms	Main Destinations
1	2200	24.58	1st	*	*Addis Abeba, Wollo*
2	1350	15.08	4th	*	*Addis Abeba, Wollo*

3	1750	19.553	2nd	*	*Addis Abeba,*
4	1650	18.44	3rd	*	*Addis Abeba,*
5	1250	13.97			*Addis Abeba*
6	750	8.38			*Addis Abeba*
	8950	100			
Concentration ratio (CR4 in %)=77.65					

Source: own computation

The Gini coefficient was also used to determine the market structure of the rice market . The market structure analysis for wholesalers reveals a Gini coefficient of 0.58 as table-4 below . Since the coefficients are closer to one, the concentration of the market is relatively high indicating the existence of inefficiency in the market structure.

Table.4: Market Structure Analysis of Wholesalers.(GC)

Monthly Sales ETB (000)@ price of 708/q	Number	Proportion(X)	Cumulative proportion	Monthly sales ETB[2] *(000)*	Prop. of cum. Total sales (Y)	XY
<800	1	0.17	0.17	531	0.08	0.01
800 -1,000	2	0.33	0.50	1840.8	0.29	0.10
>1,000	3	0.50	1.00	3964.8	0.63	0.31
Total	6	-	-	6336.6	-	0.42
GC=1–Σxy, 1– 0.42=0.58						

Source: own computation

Barriers to entry in rice market are:

Investment capital: Another factor affecting the market structure is entry and exit barriers. To enter in the rice market initial capital investment is one of the barriers observed in the area that means more capital (demands more money) is an entry barrier to enter to the rice market . It is needed to handle more quantity of rice (during peak period) as its unit price is very high when compared to the other commodities.

Trader license: The barriers associated with assemblers on top of the regulatory framework require a trader's license from the government office. The cost of the license depends on the type of license and the total sales volume of trader. Many traders (as par time business) also indicated that high taxation rate forced traders working without licensing. Hence competition with unlicensed traders became a barrier to enter into rice marketing in general. .

Experience and price information: it was observed that about 69% of the sampled households had price information access (knowledge) of the nearby market price before they sold their output. Hence unless it's spontaneous fluctuation, price information of the commodity was not barrier for them. The survey result indicate that about 47% of the respondents had experience in rice trading between 2-5 years, 40% of them had experience of six up to ten years and the rest 6.7% had above 21 years of experience respectively.

Market conduct: in the study area there were no traders who specialized in rice trading but they were grain traders in general. During the study many occasional traders purchase d target during peak harvest times. Most grain traders were licensed and some other were trading rice without license (assemblers and brokers). Market traders can be characterized from the point of rice trading into producers, millers, retailers, urban distributors, urban and rural assemblers. The survey result indicated that most of the time wholesalers and millers buy 80% of rice from *Woreta* market (on their ware house) and 20% from village market. The reason to stay more in that area was due to high supply. The purchasing strategy for wholesalers was based on the long term

[2] Ethiopian birr

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client establishment**,** infra- family link and spontaneous purchasing. About 53% of sample traders indicated that price was set by the market but 27% of them were setting prices by themselves (price makers), 13% set by negotiation of buyer and traders and few (7%) of them set market price by getting information from the District Agriculture office.

Performance of rice marketing: The two major indicators of market performance are net returns and marketing margins. Estimating net returns and marketing margins provide indication of an exploitive nature when net returns of buyer are much higher than his fair amount. (Table-4) below gives an overview of distribution of marketing margin among different actors in the channel. The total gross marketing margin (complete distribution channel) was about 54% and the farmer's market participation was found to be 46% which is below the average percent share. Rural assemblers get the highest gross marketing margin compare to the other market participants.

Table.4: Summary of Gross marketing margins of traders in rice marketing.

	Market participants	Buying price (average)	selling price (average)	Gross marketing margin (GMM) (% share)
i	Farmers	-	387.63	46%
ii.	Rural Assemblers (collectors)	393	592.37	24%
iii.	Millers	619.54	656.63	8%
iv.	Wholesalers	670	708	6%
v.	Urban distributors	696	782	9%
vi.	Retailers	724.5	844.08	7%
vii	Consumer	844.08	-	100%

Note: [1] $TGMM = \text{End buyer price} - \textit{first seller price} \,/\, \text{End buyer price (the terminal market)}$

Rice marketing channels: The rice marketing, from producer to consumer, is a complex process involving handling from multiple intermediaries. There are three separate levels of marketing as shown in Figure 2: These are four main types of channels in consumers' markets, zero-level, is the simple one in which goods flow directly to the end-users., One level in which one intermediary in between, and multilevel in which two and more than two intermediaries in between (Amarchand and Varadharajan, 1979). The major receivers of rice from farmers were, wholesalers (45%), Millers (27%), rural assemblers (14%) and urban collectors (12%) in order of volume of purchase respectively.

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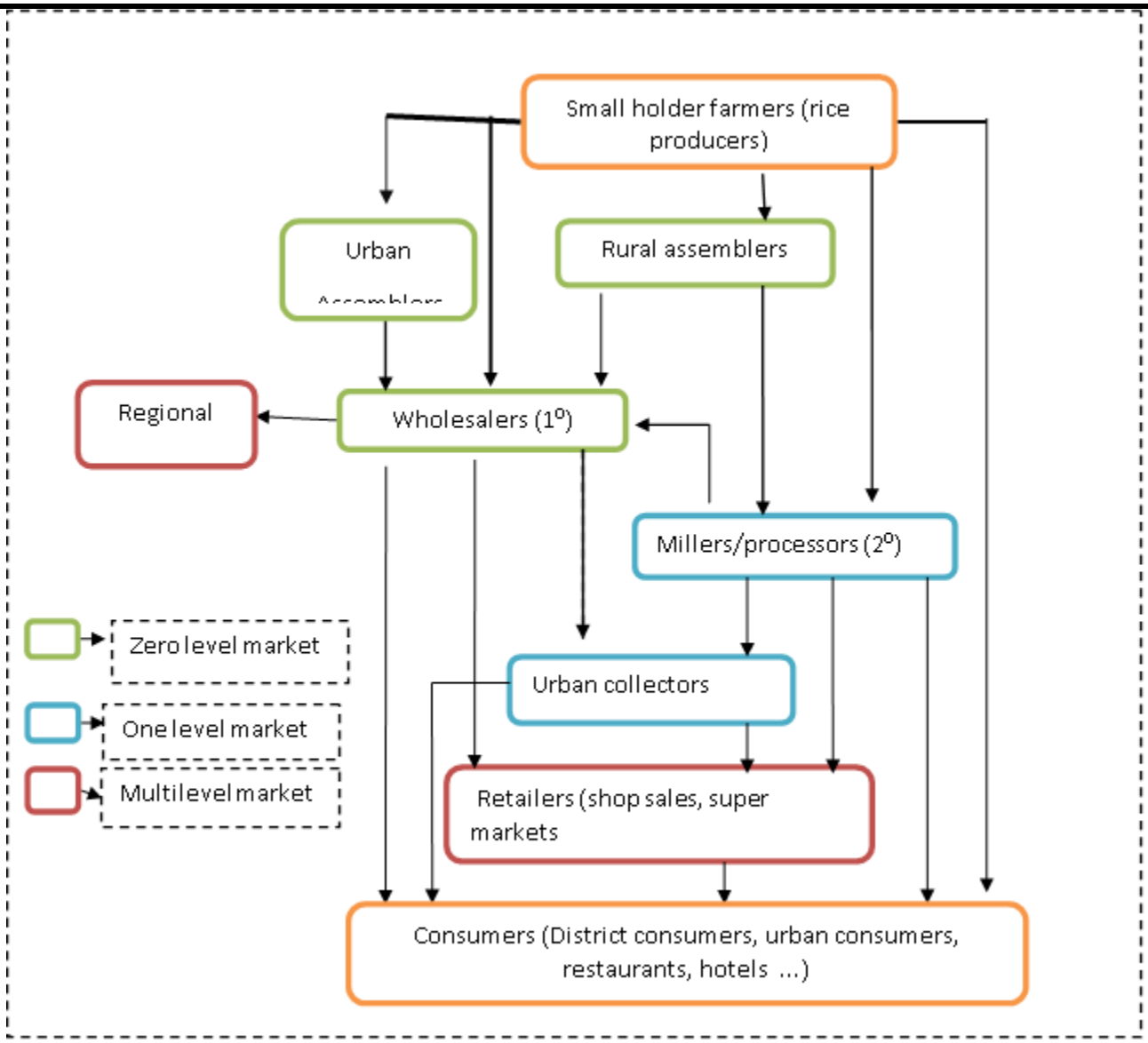

Fig.2: Rice marketing channels in Fogera Districts.

IV. CONCLUSIONS

In general, the following conclusions were derived from this study:-

- Sex of households, labour amount, oxen number ,Land size and seed rate were the main socio economic factors that determines rice production in the study area .
- The rice market structure revealed that there is imperfect (uncompetitive) market environment among rice traders. The structure was strongly oligopsonistic i.e the market was governed by few wholesalers' (buyers).
- The barriers to enter to the market were investment capital, prior control of farmers or clients and competition with unlicensed traders. Marketing margin analysis indicated that rural collectors (assemblers) get higher gross market margin as compare to other market participants in the rice trade farmers obtain below the average.
- In the rice marketing chain the main rice receivers in the channel were wholesalers (1st), Millers (2nd), rural assemblers (3rd), and urban assemblers respectively.
- Rural assemblers were found to harvest the higher marketing margin from all the marketing participants and farmers portion were obtain below the average percentage share of the gross marketing margin.

- The common problems identified in rice production and marketing were to be lack of miller machine, shortages of seed varity and its rate of application by the farmers, transportation facilities, investment capital and price fluctuations were found to be the main one.

Recommendations

- ❖ Market intervention to make the market competitive (increase the bargaining power of farmers) e,g linkage formation to Ethiopian commodity marketing system (ECX)
- ❖ Use of intensive farming, developing varities with agricultural researchers specially lowland and high land varity
- ❖ Adoption of alternative traction power (like two wheel tractor) and introducing labour saving technology.
- ❖ Demonstration of appropriate seed rate application to farmers
- ❖ Considering gender differences during rice cultivation system.
- ❖ Credit facilities for capital investment and awareness creation on importance and use of licensing trade

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Assessment of the Relative Suitability of Three Different Soils for Dry Season Lettuce Production in Ghana

Benette Yaw Osei[1], Martha Agyiri [2], Emmanuel Kwasi Aseidu [3], Kofi Agyarko[4]

[1,3,4] Department of Crop and Soil Sciences Education, College of Agriculture Education, UEW, Ghana
[2]Presbyterian College of Education, Akropong Akwapim, Ghana

***Abstract*—** *The research was conducted at the project site of the University of Educatoion, Winneba - Mampong campus between mid - November 2007 and Mid-April 2008. The main objective of the study was to compare the relative suitability of three soils in supporting lettuce production in the dry season. The treatments were Calcic Vertisol (Akuse scries), Rodic Nitisol (Ejura sesries) and Chromic Luvisol (Bediest series). The randomized complete block design was used in a pot experience with the three treatments and each replicated three times. Plant height, fresh leaf mass, leaf dry matter yield, fresh root mass, gravimetric moisture content, total porosity, drainability and bulk density were the parameters considered. From the result Bediesi Series recorded the highest growth rate as measured by plant height (266.5mm), fresh leaf weight (30.6g), leaf dry matter weight (4.9g) at 7 weeks after transplanting as well as been the most succulent with 84% succulent. Fresh root weight however, was highest with Akuse Series followed by Bediesi Series and Ejura Series in that decreasing order. Ejura Series recorded the least value for all growth and yield parameters measured. For soil parameters, Akuse Series recorded the highest value for porosity (43.0%) and gravimetric moisture of 6.43 throughout the period of field drying for 8weeks. Ejura Series Bediesi Series also recorded the highest value for drainability after 25 minutes of drainage, followed by Bediesi Series and then Akuse Series. The result of this work indicated that the Bediesi Series is the best soil type among the soils evaluated for lettuce production in the dry season in Ghana.*

Keywords*— *Calcic Vertisol (Akuse series), Chromic Luvisol (Bediest series), Lettuce, Rodic Nitisol (Ejura sesries).

I. INTRODUCTION

The increasing population in the world makes a great demand on food production and consequently calls for an increase in food supply by various countries. Food supplies are insufficient not only in quantity but also in quality thus the essential nutrients required by man for proper growth and development are lacking. Therefore during the World Food Summit of 1996 (FAO, 1996), some targets were set and among them were to; Give a strong food and nutrition orientation to programming and projects of development co-operation; support the implementation of the right to food for all people; support national and international alliance, network and partnership in the field of nutrition security and the introduction and reinforcement of food security and nutrition aspects information agreements.

Ghana, like many other countries in the sub-Sahara - Africa has poverty as one of the main reasons for malnutrition. However, poverty alone does not account for this precarious situation in the country but other factors such as socio - economic conditions, stability of population growth and government polices also contribute to this state of affairs (FAO. 1998). In order to alleviate poverty among farmers to reduce the rate of malnutrition, there is the need to encourage all year round production, including the use of improved varieties, suitable soils and improved agronomic practices. Vegetable as sources of food forms a major contributing factor for the protection and defense of human body against diseases. According to Addo - Quaye et al (1993), vegetables are rich in minerals; among the elements, iron, sodium, iodine and calcium are the most important for vital activity. Apart from providing income and employment for those involved in its production, vegetables provide nutritional supplements for people and in a way check the high rate of malnutrition in the sub-Sahara Africa.

There is increasing demand for vegetables in the country. This could be attributed to the increasing change in the diet of many Ghanaian in preference for western diets as well as increase in the tourism and the hospitality industry in Ghana. The short shelf life of lettuce, coupled with the fact that the

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crop is eaten fresh makes it necessary for all year round production of lettuce. However, most farmers in Ghana depend on rainfall for production which becomes a problem in the dry season. Yields are often very low on poor and unsuitable soils. To ensure continuous production of the crop, there is the need to find out which type of soil will be most suitable for all-year-round lettuce production, especially in peri - urban agriculture in Ghana.

The objective of the study was to find out the relative performance of the three selected soils in supporting lettuce production in Ghana.

II. MATERIALS AND METHOD

2.1 Study Area

The project was conducted at the experimental site of University of Education, Winneba, Mampong campus between December 2007 and April 2008. Mampong Ashanti lies between latitude 0.7°, 0.4° N of the equator and longitude 1°, 0.24° W of the equator. It is also situated at an elevation of 457.5m above sea level. The experimental area, falls within the transitional zone of Ghana's agroclimatology. It experiences two main seasonal rainfalls annually, with the major season rains falling between late April and late June and the minor season rains between September and mid-November (CR1, 2001). The mean monthly rainfall is about 109.4mm and the monthly temperature is about 25-32°c.

Soil types: Three different types of soils were used. And they are the Ejura Series, Akuse Series and Bediesi Series. The soil samples were collected from Ejura, Kpong and Mampong respectively and used in post experiments.

Ejura series is classified as Rhodic Nitisol (FAO UNESCO legend). It is dark brown to brown and has a fine loamy overlying. It has high water permeability, high pH and higher in nutrients, (Adu and Mensah-Ansah, 1995). Alhassan et al. (2004) recorded that Ejura Series promotes the cultivation of maize, groundnut, cowpea, garden eggs and okro. According to Morgan (1972), the Rhodic Nitisol is prone to erosion due to its low structural stability, slacking and caking of surface, prone to leaching and also has low rate moisture content in soil due to the presence **of** predominant sand particles.

Bediesi series (Chronic Luvisol as classified in the FAO/UNESCO legends) is derived from the voltaian sandstone. It occurs on upper and middle slopes of the catena. It is moderately shallow to moderately deep. Its colour is orange red to reddish brown. The soil is free from stones and concretions, is well drained and friable with satisfactory moisture holding capacity. The soil has an average pH value of 6-6.5 (Opoku, 1993). It is easy to cultivate by hand and machines but care must be taken when machines are used in tilling the land to avoid the isolated boulders of sandstones (Adu, 1992).

Akuse series is a coluvial material derived from the weathering of garnetiferous hornblende gneiss (Brammer, 1967). It is classified as Calcic Vertisol in the FAO/UNESCO legends. Locally, it is the tropical black clay and belongs to the Akuse series (Adu, 1995). The Akuse Series is very heavy clayey (30-95% clay) soils. It develops deep and wide cracks during dry season. It is sticky and plastic when wet and traffic capability is poor as moisture status is high. It supports crops like millet, sorghum, cotton, rice, wheat, barley, flax and sugarcane. Tree crops are seldom recommended because trees' roots find it difficult to establish themselves in the subsoil without being damaged by shrinking and swelling phenomena.

2.2. Preparation of Site

The experiment was carried out in plastic pots. Ninety plastic pots were assembled and perforated at the base to drain excess water from the soil samples. Each of the soil samples: Akuse series, Bediesi series and Ejura Series were loosened and all unwanted materials found in it removed. Equal quantity of each soil sample was filled into thirty (30) plastic pots for a treatment. The pots filled with the soil samples were saturated with water and allowed to settle for 3 weeks to attain the natural state

2.3. Experimental design and treatments

The Randomized Complete Block Design (RCBD) was used. The experimental field was divided into nine plots with 0.6m paths between each pot for easy movement. There were three- (3) treatments and each replicated three (3) times. Each replication had ten (10) pots.

2.4. Planting materials and method of planting

The lettuce seeds (crispa variety) were used. The seed were planted 2-4cm deep in a prepared seed box at 5-10cm between rows using the trench-drilling method. The nursed seeds were heavily watered and a shed was raised over it with palm fronds to provide shade. Sprouting and germination of seeds was observed on the 4th and 5th day after sowing. The seedlings were pricked out two weeks after planting.

2.5. Post planting activities

Equal volume of water was applied to the lettuce at regular intervals to prevent the plants form wilting since the experiment was carried out in the dry seasons. The soils in the pots were stirred intermittently to improve aeration and seepage of water. Weeds in between the pots were controlled by using a hoe and hard picking respectively, to reduce competition and provision of hide-out for pests. A fungicide

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(Top cost) was applied at a rate of 20ml per 21 to control fungi infections like dumping off and root rot every two weeks after transplanting; until two weeks to harvesting. An insecticide (Pyrical 480 EC) was also administered at rate of 20ml per 15 L (to control insects like grasshoppers, leaf miners, crickets and aphids every week after transplanting until two weeks to harvesting as there was heavy infestation of these insects on the field. Both the fungicide and the insecticide were applied by using a hand pump.

2.6 Determination of parameters

The dry bulk density was determined from soil cores collected with core sampler (Klute, 1986). Moisture content was determined on gravimetric basis using the formula $\theta_g = \left(\frac{M_w}{M_s}\right) \times 100$ (Hillel, 1982) where, M_s is the mass of the solid components of the soil and M_w is the mass of water contained in the soil. Total porosity was calculated by the formula; f = 1- ρb/ρs where f is total porosity, ρb is bulk density and ρs is particle density (2.65 g cm^{-1}) (Hillel, 1982). With drainability, soil sample of each soil treatment were collected and sifted through a sieve. Soil samples were air dried for 48 hours before they were sifted. 100g of soil from each treatment (three replication each) was weighed and poured on the funnels with cotton stuck in their necks. 75ml of water was poured onto soil in the funnels which had been placed on 100ml measuring cylinders. The volume of water drained in each measuring cylinder was recorded every minute for the first 10minutes, and every 5 minutes for the next 20 minutes. The mean of the volume of water drained for each treatment was determined and the data presented graphically.

Plant height measurement began three weeks after transplanting. Ten plants from selected pots of each of the treatments were chosen for the measurement of this parameter. A meter rule as used to measure the height of the plant (from the soil level to the tip of the leaf of the plant).The fresh roots weight were measured with an electronic balance after they had been allowed to dry off the water on the roots when the soil particles was been washed off it.

The initial summery of mechanical and chemical analysis of the various treatments are shown in Table1 and Table 2.

Table.1. Soil mechanical analysis of treatments

Treatments	% Sand	% Silt	% Clay	Texture
Akuse Series	39.6	13.2	47.0	Clay
Ejura Series	75.52	20.05	4.11	Loamy sand
Bediesi Series	66.92	27.05	6.03	Sandy loam

Table.2: Results of soil chemical analysis or the treatments

Treatment	pH H_2o	Org.C %	Total N	OrgM	Exchangeable cations me/100g				E.C.E.C Me/100g
					Ca	Mg	K	Na	
Akuse Series	7.81	0.65	0.07	1.12	17.62	10.15	0.68	1.31	29.8
Ejura Series	4.66	0.12	0.02	0.21	0.80	0.27	0.07	0.05	1.54
Bediesi Series	6.42	0.46	0.04	0.79	7.48	6.14	0.53	0.22	14.4

III. RESULTS AND DISCUSSION

3.1 Plant height

From Fig.1, Bediesi Series recorded the highest mean height of lettuce plant at 7 weeks after transplanting (7WAT) followed by Akuse Series and Ejura Series in that decreasing order. When the result was subjected to statistical analysis it was observed that the mean value for Bediesi Series was significantly (p=0.005) higher than that of Ejura Series but not significantly different from that of Akuse Series. .It was also observed that of Akuse Series was not significantly different from that of Ejura Series (p=0.005).

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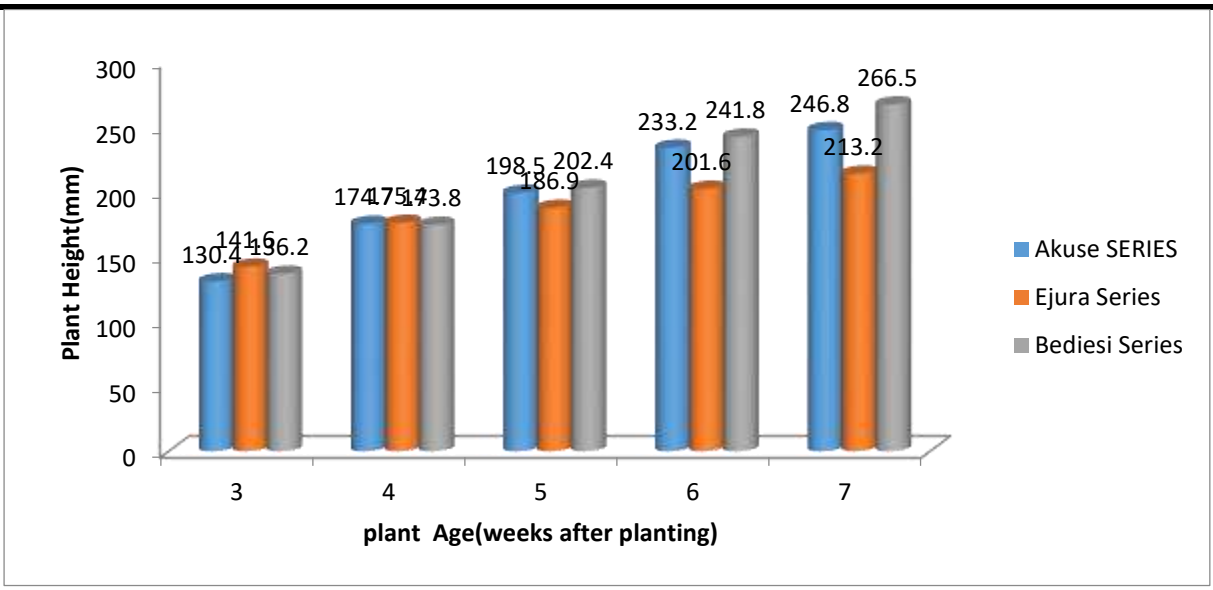

Fig.1: Weekly plant height of lettuce on treatments

Bediesi Series recording the highest value for plant height could be attributed to its contained adequate quantity of nitrogen when the three treatments were chemically analysed (Table 2). This assertion is in conformity with Akinyosaye (1986) findings that, moderate to high nitrogen supply to plants promotes vegetative growth where in this case can be equated to plant height. The result also confirms the finding of Tweneboah (2000) that adequate quantity of nitrogen leads to leaf growth which is reflected increase in height.

Although, Akuse Series had the highest nitrogen value (Table 2) it did not support the highest plant height. This could be attributed to nitrogen not being the only factor for the growth of a plant but there are other domineering factors. E.g. Bediesi Series had the higher amount of potassium than Akuse Series which promotes the efficient use of water, nitrogen uptake and protein synthesis (Tisdale et al, 1985). Bediesi Series recording the highest plant height value might also be due to suitable drainage and pH conditions it recorded. Tindall (1983) stated that well drained sandy loams with pH of 6.0-6.8 are generally considered preferable for the lettuce crop. Ejura Series however, had a pH of 4.66 which is acidic. Acidic soils promote phosphorus fixation and unavailability of some basic cations like Ca, Mg, and K which are needed for healthy and proper growth of the plants.

3.2 Fresh Leaf Weight and Dry Leaf Mater Yield of lettuce

From the statistical analysis of the data on the mean fresh leaf weight, it was observed that mean value for Bediesi Series was significantly higher than that of Ejura Series and Akuse Series at 0.05 probability level. This could be attributed to suitable nitrogen content of Bediesi Series which conforms to research work by Kwakye et al (1995) that, different crops including lettuce respond to moderate to high content of nitrogen in the soil.

Table.3: Fresh leaf weight and Dry leaf mater yield of lettuce.

Treatment	Fresh Leaf Weight (g)	Leaf Dry Matter Yield (g)
Akuse Series	19.50	4.1
Ejura Series	12.40	3.7
Bediesi Series	30.60	4.9
LSD (0.05)	5.77	0.555
C.V.	12.34%	10.02%

The difference could also be attributed to the fact that the pH of Bediesi Series falls within the require pH range for lettuce production as stated by Tindall (1983) that, a pH of 6.0-6.8 is considered preferably for lettuce production while Akuse Series and Ejura Series recorded pH of 7.81 and 4.66 respectively. From Table 2, it can observed that the pH of Ejura Series was too acidic for the lettuce plant. This can explain why Ejura Series T_2 recorded the lowest mean leaf

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weight. Although, Akuse series retained the highest amount of water in the soil, it did not record the highest mean fresh leaf weight. This might be due to the peculiar physical and hydrological characteristic of the Vertisol. It was observed that when water was applied to the treatments, Akuse Series always retained much water at the base of the crop for longer time which could have been detrimental to be the proper growth of the lettuce crop. Baffour (1998) confirms this observation, that suitable aeration and adequate supply of water, among others, affects seedling emergency, growth and yield.

Leaf dry matter yield also follows the same trend as fresh leaf weight. Just that, for dry matter yield, mean values for Akuse Series and Ejura Series were not significantly from each other at 0.05 probability level. The difference in values may be explained with the same reasons as fresh leaf weight since their pattern of growth is not different from each other.

Comparing the difference between mean fresh leaf weight and mean leaf dry matter yield of treatment, it was observed that lettuce leaves from Bediesi Series had the highest moisture content, which means Bediesi Series produced the most succulent leaves among the treatments followed by Akuse Series and Ejura Series with 84%, 79% and 71.8% moisture respectively. In the marketing of lettuce, the more succulent the leaves are the more appealing they become to the eyes of the buyer. Therefore, if succulence correlates positively with high market value, then lettuce from Bediesi Series having the highest level of succulence will have higher market value than produce from the rest of the treatments.

3.3 Fresh Root Mass

The results (Table 4) indicates that, fresh root mass from Akuse Series and Bediesi Series were significantly different from that of Ejura Series. However, there was no significant difference between the root mass value for Akuse Series and Bediesi Series at 0.05 probability level. Akuse Series recording the highest root mass could probably be as a results it having the highest nitrogen and organic matter levels from the chemical analysis (Table 2). This is in conformity with Akinyosaye (1986), that an increase in nitrogen supply to plants promotes vegetative growth which includes plant roots.

Table.4: Fresh Root Mass

Treatment	Mean Fresh Root Mass (g)
Akuse Series	3.4
Ejura Series	2.4
Bediesi Series	3.2
LSD (0.05)	0.453
C.V.	6.667%

Akuse Series had the least bulk density (Table 5) which is a measure of how soil particles are compacted together. Therefore, it could be deducted that the relatively less compaction of Akuse Series might have resulted in best root growth. Ejura Series recording the least value in root weight could that Ejura Series recorded the lowest value for porosity which means that there were not enough spaces between soil particles for air and water to occupy which could affect the growth of roots growth. It confirms the assertion by Russell (1997) that, the rate of root growth depends on water and air supply in the soil. It could also be explained that since porosity is inversely proportional to bulk density and Ejura Series had the least porosity, therefore, had the highest bulk density and high bulk density which means more compaction of soil might have hindered the growth in the roots of the soil.

3.4 Gravimetric Moisture Content

The moisture contents retained by the soils during the eight weeks of filed drying were found to be significantly different from each other at 5% probability level at the terminal reading. Akuse Series retained the highest moisture of 6.433% followed by Bediesi Series with 4.1% and then Ejura Series with 0.8%.

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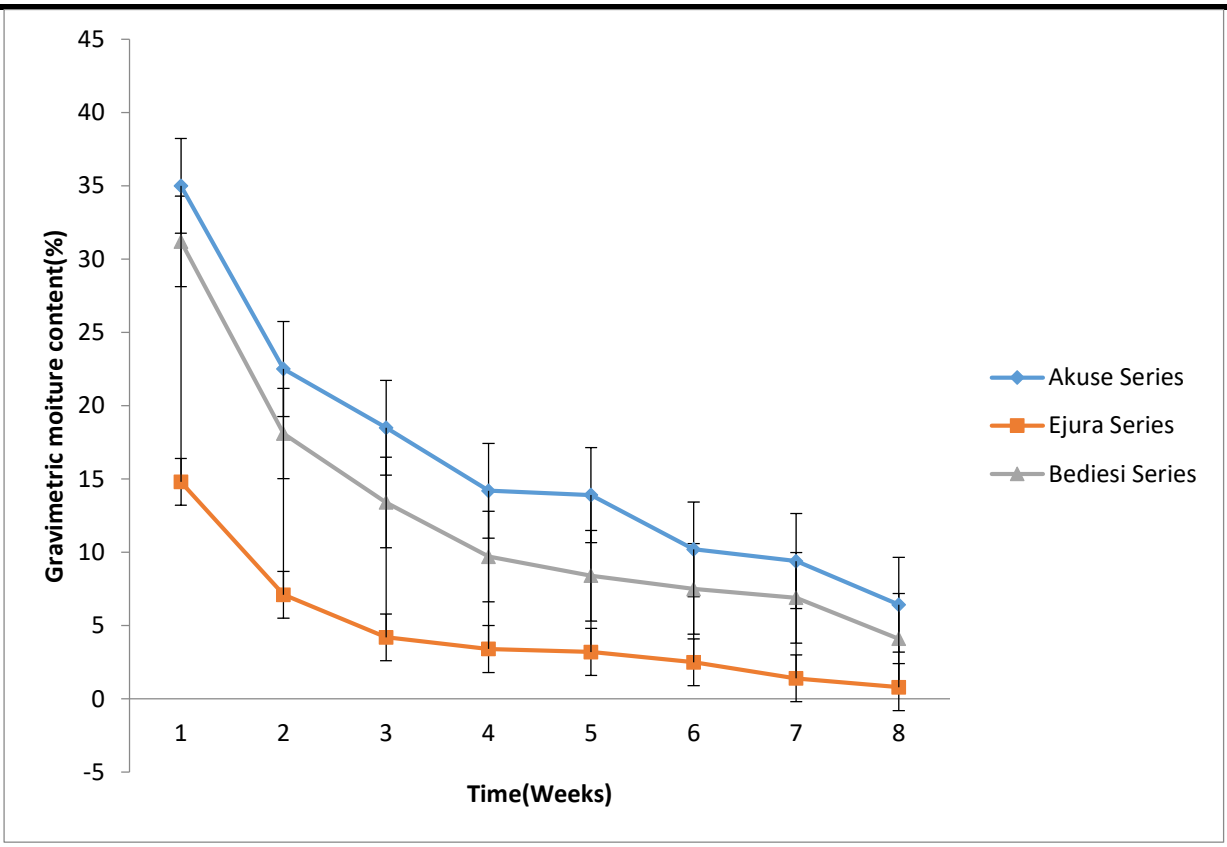

Fig.2: Soil drying of the treatments

The significant difference in gravimetric moisture of the treatments may be attributed to the fact Akuse Series recorded the highest organic matter which helps it to retain moisture better than the other treatment. This observation confirms with what Elliot and Wilding (1992) reported that, soil organic matter has a marked influence on soil structural development, it reduces seals, and crust information, improves soil micro-climate, infiltration and moisture retention of soils. This explains why the treatment which had the least organic matter recorded the least gravimetric moisture. Also, Akuse Series recording the highest value for total porosity followed by Bediesi Series and Ejura Series could be a contributing factor to its highest gravimetric content. This is because Akuse Series which has high clay content has many micropores than macropores which turn to have strong adhesive forces to hold water for longer period for plant use than Ejura Series whose large soil particle size allows more macropores and drains moisture fastest after irrigating or rainfall.

3.5 Total porosity and Bulk density

From Table 5, Akuse Series is the most porous followed by Bediesi Series and Ejura Series with mean total porosity values of 43.0%, 40.67% and 38.5% respectively. There was no significant difference between mean value of Bediesi Series and Akuse Series, and Bediesi Series and Ejura Series at 0.05 probability level but there was a significant difference between mean values of total porosity for Akuse Series and Bediesi Series.

Table.5: Total Porosity of the Soils

Treatment	Mean Total Porosity (%)	Mean Bulk Density(g/cm^3)
Akuse Series	43.00	1.50
Ejura Series	38.50	1.60
Bediesi Series	40.67	1.53
LSD (0.05)	3.81	
C.V.	4.12%	4.32

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This may be attributed to their relatively lower bulk density value recorded by the Akuse series, thus the less compacted the soil is, the higher the pores. Also the organic matter content and the particle sizes of the Akuse Series could have resulted in the observed difference. This is confirmed by Akinsanmi (1987) that organic matter opens the soil particles, increases aeration and hence makes the soil porous and the higher the pore space the better for crops production.

The mean bulk densities determined showed no significant different at 0.05 probability level.

3.6 Soil Drainability

The study showed that water drains fastest through Ejura Series followed by Bediesi Series and then Akuse Series. When the amount of water drained at the 25th minute was analyzed statistically, it was realized that the values for Ejura Series was significantly (p=0.005) higher than the mean value for Bediesi Series and Akuse Series.

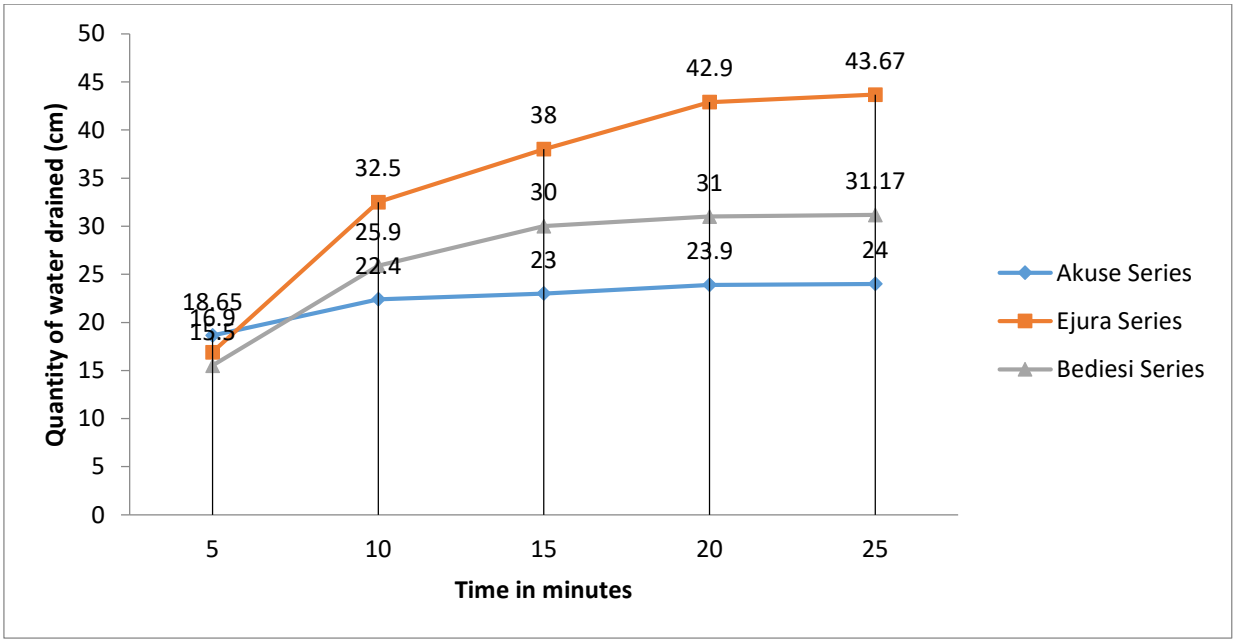

Fig.3: Soil drainability of treatments

This may be as a result of the particle sizes of Ejura Series being relatively larger than Bediesi Series and Akuse Series and hence has more macro-pores which drains water easily (faster) than the many micro-pores that may be present in Akuse Series and Bediesi Series which have stronger adhesive force to hold much water.

On the other hand, the least volume of water drained through Akuse Series after 25 minutes is an indication that much water was retained which was equally not very good for lettuce production as asserted by Thompson and Kelly (1957) that, soils for lettuce production should be well drained but retentive of moisture.

IV. CONCLUSION

Bediesi Series which drained water moderately had the capacity to retain adequate moisture for plant use without suppressing the air requirement of the roots which explore the soil for moisture and nutrients and hence recorded the highest value in most of the plant parameters measured in the experiment. Mordi et al. (1992) confirm this assertion by reporting that drainage improves soil aeration and enables crops to develop deeper root system for good plant growth and yield.

Finally, for dry season lettuce production, Bediesi Series is found most suitable since it retained moderate moisture with time hence produced the highest yield in terms of leaf height, fresh weight and leaf dry matter yield. It also produced the most succulent leaves and will therefore give a higher economic return to farmers. The Akuse Series (Calcic Vertisol (Akuse Series)) can provide relatively high yield of lettuce if some interventions are made to reduce water saturation at the base and root zone of lettuce since the plant required a well-drained soil for proper growth. Perhaps, a moderation in the quantity of water applied during irrigation may be necessary to realize the full potential of the vertisol.

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Chemical and Functional Characterization of Baobab (*Adansonia Digitata L.*) Seed Protein Concentrate using Alcohol Extraction Method

Adenekan M.K.[1], Fadimu G.J.[2,3*], Odunmbaku L.A.[1], Nupo S.S.[1], Oguntoyinbo S.I.[1], Oke E.K.[2]

[1]Department of Food Technology, Moshood Abiola Polytechnic, Nigeria
[2]Department of Food Science and Technology, Federal University of Agriculture, Nigeria
[3]Department of Food Science and Human Nutrition, King Saud University, Saudi Arabia

Abstract**—** *This study investigated the characteristics of baobab protein concentrate prepared using alcohol extraction method. Baobab fruit and the seeds were washed out, cleaned to remove dirt, sundried for three days and finally ground in an electric mill, sieved and stored. The flour was defatted with hexane under constant magnetic stirring for 3hrs. The slurry was vacuum filtered through filter paper and the residue was used for subsequent extraction. The result obtained showed that alcohol extraction method significantly ($p<0.05$) affected the chemical composition and functional properties of the baobab protein isolate. Result of functional properties shows that the alcohol extracted baobab protein concentrate displayed higher solubility index and emulsifying capacity. Baobab protein concentrate can be considered as potential functional food ingredient.*

Keywords**— Baobab, protein concentrate, alcohol, chemical composition, functional properties.**

I. INTRODUCTION

Intense efforts are currently made in the search of cheap protein sources with good nutritional and functional properties, to attenuate the problem of protein malnutrition widely spread in developing countries [1]. Plant proteins play significant roles in human nutrition particularly in developing countries where average protein intake is less than that required. Plant protein products are gaining increased interest as ingredients in food systems throughout many parts of the world and the final success of utilizing plant proteins as additives depends greatly upon the characteristics they impart to foods.

Baobab (*Adansonia digitata L.*), locally called kuka (hausa) and luru (yoruba) is a very long lived tree with multipurpose uses [2]. Baobab is a high yielding, draught resistant and all season plant belonging to the Malvaceae family, it is widely spread throughout the hot, drier regions of tropical Africa [3]. The vernacular name for baobab means "fruit with many seeds" [4]. The seeds are eaten raw or roasted and have a pleasant nutty flavor [5].

Murray *et al*. [6] reported that baobab seed flour is an important source of energy and protein. The nutritious seed have high values for proteins (33.7%), fats (30.6%), fibre (16.9%) and most minerals. Protein extracts have superior functional properties and are more effectively used in the formulation of foods as compared to seed flours [7].

The acceptability and optimal utilization of baobab seed as a protein source may be limited by the presence of antinutritional factors such as tannins, oxalate and phytate [8]. Nevertheless, techniques employed for extracting protein there from are known to be effective in the elimination of the above antinutrients [9].

Since oilseeds are valuable sources of lipids as well as proteins, numerous studies on protein functionality of major and minor oilseeds such as soybean [10], peanut [11], rapeseed [12], sunflower [13], almond [14], winged bean [I5], groundnut [16], have been reported. The chemical and functional characterization of baobab protein concentrate is scarce. The main objective of this present study is to investigate the properties of protein concentrate from baobab which is produced using alcohol precipitation method in order to establish the potentials application.

II. MATERIALS AND METHODS

Materials

Baobab fruits were collected from Anigbado village, Ayepe, Abeokuta, Ogun State, Nigeria.

Methods

Preparation of Baobab Seed Flour

Baobab seed flour was prepared according to the method described by Nkafamiya *et al.* [17].The dried pulp was scraped from the baobab fruit and the seeds were washed out, cleaned to remove dirt, sundried for three days and

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finally ground in an electric mill. It was then passed through a 40 mesh sieve and stored for use

Preparation of Defatted Baobab Flour

Defatted baobab flour was prepared according to the method described by Xiaoying and Yufei, [18]. The flour was defatted with hexane (flour/hexane ratio of 1:10 w/v) under constant magnetic stirring for 3hrs. The slurry was vacuum filtered through filter paper and the residue was used for subsequent extraction. Hexane extractions were repeated until the filtrate was clear. Residue from the last extraction and filtration step was air-dried. Defatted baobab flour (DBF) was ground to 150 meshes for further use.

Preparation of Baobab Protein Concentrate (Alcohol extraction method)

Baobab protein concentrate was prepared according to the method described by Wolf [19] with minor modifications. Defatted baobab flour was mixed with 95% aqueous alcohol (1:10 w/v) and stirred for 1hr at ambient temperature (about 25ºC). The suspension was filtered and the residues were dispersed in de-ionized water (1:10 w/v) at room temperature and stirred for 1hr. The suspension was finely filtered and the pH of the filtrate was adjusted to 4.5 by addition of 1NHCl (to precipitate out the protein). The slurry was then centrifuged (3500rpm, 15min, 36ºC). The supernatants were discarded and the precipitates (protein concentrate) dried in a conventional oven. The protein concentrate was pulverized into a powdered form and stored for subsequent analysis.

Protein Recovery and Chemical Composition Analysis

The chemical composition of baobab meal protain concentrate were determined according to AOAC standard method [20]. The carbohydrate content was estimated by substracting the sum of percentage of moisture, crude fat,crude protein and ash content from 100% the protein recovery was as follows

$$\text{Protein Recovery (\%)} = \frac{weight\ (g) of\ BPC \times protein\ content\ (\%) of\ BPC \times 100}{weight\ (g) of\ DPC \times protein\ content\ (\%) of\ DPC}$$

Determination of Funtional Properties

Determination of Protein Solubility (PS)

Baobab protein concentrate solution (20 % w/v) were prepared with dispersing powdered protein into distilled water, adjusted to pH 3 to 9. The protein solutions were stirred with a magnetic stirrer at 4°C overnight, centrifuged at 2,500rpm for 30min. The protein sample was directly solubilized by 0.5m NaOH for determination of total protein. The protein content of the content of the supernatants was determined by the (Biuret method 1940) using bovine serum albumin (BSA) as a protein standard. Protein solubility was calculated as :

$$Ps\ (\%) = 100 \times Ps/\ Pt$$

where Ps is the protein content in the supernatant after centrifugation and filtration and PT is the total protein content present in the protein sample.

Determination of Water Holding Capacity (WHC) and Fat Absoption Capacity (FAC)

WHC and FAC of Baobab protein concentrate (BPC) were determined by the method of Gandhi and Srivastara [21].

Determination of Emulsifying activity and Emulsifying stability Index

Emulsifying activity index (EAI) and Emulsion stability index (ESI) of baobab protein flour were measured by the method described by Lopez *et al*. [22].

Determination of Foaming Capacity and Foaming Stability

Foaming capacity and foaming stability were determined by the method described by khalid *et al*. [23].

Determination of Anti nutritional Factors

The tannin content of the sample was determined by the method described by Swain [24], while Phytate and oxalate in the sample was determined by the method describe by Onwuka [25].

Statistical Analysis

Data obtained were subjected to statistical analysis. Means, Analysis of variance (ANOVA) were determined using SPSS Version 21.0 and the differences between the mean values were evaluated at $p \leq 0.05$ using Duncan's multiple range test.

III. RESULT AND DISCUSSION

Result of chemical composition of alcohol extracted baobab protein concentrate is shown in Table 1. The percentage yield of baobab protein concentrate was 51.74%, this agrees with the findings of Arnold *et al.* [5] that the nutritious seeds have high values for proteins, fats (oils), fibre and most minerals. The result indicates that the protein concentrate contains 8.16% moisture, 90.36% protein, 1% crude-fibre and very low/insignificant levels of fat, ash and carbohydrate of 0.13%, 0.20% and 0.13% respectively. The protein content which is the major concern and important constituent (90.36%) was high and compared favourably with 90.05% reported for walnut protein isolate by Xiaoying and Yufei [18] and this value

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is higher than 85%, 83% and 70% reported for roasted peanut protein concentrate [26], sesame protein concentrate [27] and bambara bean protein concentrate [28] respectively. Low percentages recorded for other constituents of the cellular matrix as presented in Table 1 is an indication of purity of the protein concentrate obtained and the efficiency of the alcohol extraction method adopted.

Table.1: Chemical properties of baobab protein concentrate

Chemical properties (%)	Composition
Protein yield	51.74±0.12
Moisture	8.16±0.15
Protein	90.36±0.00
Fat	0.13±0.05
Ash	0.20±0.10
Crude Fibre	1.00±0.10
Carbohydrate	0.13±0.05

Mean values with different superscripts within the same column are significantly different ($p < 0.05$)

Result of functional properties of alcohol extracted baobab protein concentrate is presented in Table 2. The foaming capacity of baobab protein concentrate obtained in the present study was 67.36% and it compared favourably with 59% in sesame protein concentrate [27] and higher than 38% in walnut protein concentrate [18]. The foaming capacity was enhanced by high protein concentration, as high protein concentration increases the formation of a multilayer, cohesive protein film at the interface [29]. The high foaming stability in this study (44.86%) shows that baobab has enough flexible protein structure in aqueous solutions and interacted strongly with the air-water interface to form more stable foam. The water absorption capacity of the alcohol extracted baobab protein concentrate recorded was 130%. However, 220% and 610% were reported for the water absorption capacity of cashew nut protein concentrate [30] and sesame protein concentrate [27] respectively. Aletor *et al.* [31] reported that water absorption capacity of the range of values from 149% to 472% is considered critical in viscous food. The oil absorption capacity was 150% and this compared better than 102% reported for bambara groundnut protein isolate [32], but lower than 294% and 306% in sesame protein concentrate [33] and lupin protein isolate [34] respectively. Elnasri and Eltmay [35] reported that high protein content shows high fat absorption capacity. Kinsella [36] also reported that the ability of protein to bind fat is very important for such applications as meat replacement and extenders principally because it enhances flavor retention and improve mouthfeel. Therefore baobab protein concentrate may be used as thickener and binder in food system. The solubility of the alcohol extracted baobab protein concentrate at the isoelectric point of the protein was 97.26%.The emulsifying capacity of the alcohol extracted baobab protein concentrate was 14.66%. This value compared favourably with 14% reported for sesame protein concentrate [27] but higher than 5% reported for soy protein isolate [27] but lower than 27% reported for walnut protein concentrate [18].

Table.2: Functional properties of baobab protein concentrate obtained through alcohol extraction method

Functional properties (%)	Composition
Foaming capacity	67.36±0.25
Foam stability	44.86±0.05
Water absorption capacity	130.0±5.00
Oil absorption capacity	150.0±5.00
Solubility	97.26±0.15
Emulsifying capacity	14.66±0.15

Mean values with different superscripts within the same column are significantly different ($p < 0.05$)

Table 3 shows the protein solubility and emulsifying capacity of the alcohol extracted baobab protein concentrate as affected by different pH as shown in Table 3. The mean values for the solubility index at pH_5, pH_6, pH_7 and pH_8 are 90.46%, 93%, 97.73% and 98.63% respectively. These solubility index compared significantly ($p \leq 0.05$) higher than 83% and 47% reported for sesame protein concentrate [27] and walnut protein concentrate [18]. These solubility indexes ensure the usefulness of the baobab protein concentrate in applications such as in beverages. It was observed that as the pH increases, the solubility index and emulsifying capacity were also increasing. This indicates that both solubility and emulsification are pH dependent. Similar observation was reported for Bambara groundnut protein concentrate [32].

Table.3: Solubility and emulsifying capacity of baobab protein concentrate obtain at different pH

Sample pH	Solubility	Emulsifying capacity
pH_5	90.46±0.15	10.80±0.30
pH_6	93.00±0.26	12.33±0.15
pH_7	97.73±0.11	14.76±0.15
pH_8	98.63±0.15	17.53±0.15

Mean values with different superscripts within the same column are significantly different ($p < 0.05$)

The result of the antinutritional properties is presented in Table 4. This shows that the oxalate, phytate and tannin were undetectable in the baobab protein concentrate. This may be due to the earlier report of Mwasaru *et al.* [9] that

the techniques employed for extracting protein from baobab seeds are known to be effective in the elimination of the above antinutrients.

Table.4: Antinutritional properties of baobab protein concentrate

Antinutritional factors	Baobab concentrate
Oxalate	ND
Phytate	ND
Tannins	ND

ND= Not detected.

IV. CONCLUSION

The result obtained from this present study indicates the efficiency of the alcohol extraction method adopted as high purity of the protein concentrate was obtained and the oxalate, phytate and tannin content of the protein concentrate were at undetectable level. The functional properties of the protein concentrate compared favourably with other legumes especially with significantly higher solubility index and emulsifying capacity at different pH. This suggested that the baobab protein concentrate can find useful application in food systems.

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Response of hydro-physical properties of a Chromic Luvisol in Ghana to different methods of application of Mucuna pruriens as a soil amendments

Benette Yaw Osei, Kofi Agyarko, Kwabena Kyere, Emmanuel Kwasi Asiedu

College of Agriculture Education, University of Education, Winneba, Mampong-Campus, Ghana.

Abstract**—** *The study assessed the response of hydro-physical properties of Chromic Luvisol to different methods of application of Mucuna pruriens as a soil amendments in two separate experiments. A Randomized Complete Block Design (RCBD) with three replications was used with the following treatments: 7.04t/ha Mucuna pruriens as green manure (**GM**), 7.04t/ha Mucuna pruriens as live mulch (**LM**), 7.04t/ha Mucuna pruriens as in-situ mulch (**IM**) and a control plot which had no Mucuna pruriens as soil amendment. Data were collected on gravimetric (θ_g) and volumetric moisture content (θ_v**)**, residual moisture storage(R), sorptivity(s), cumulative infiltration (I), bulk density (ρ_b), total porosity (f), aeration porosity (ξ_a), aggregate stability (ASt) and soil temperature, for assessment of hydro-physical properties of the soil. The results from the experiments indicated that Mucuna pruriens as live mulch used as amendment significantly reduce bulk density (ρ_b), increased total porosity (f) and aeration porosity (ξ_a) thus it gave significant improvement on those soil physical properties measured while Mucuna pruriens as in-situ mulch improved aggregate stability (ASt) and gave optimal soil temperature. In the assessment of soil volumetric moisture content (θ_v**)**, residual moisture storage(R), sorptivity(s), cumulative infiltration(I), the study shows that Mucuna pruriens as in-situ mulch recorded the optimal values and was closely followed by Mucuna pruriens as live mulch.*

Keywords**— Mucuna pruriens, live mulch, in-situ mulch, green manure, Chromic Luvisol.**

I. INTRODUCTION

Most tropical soils are inherently low in nutrients and are prone to erosion, especially after deforestation and subsequent cultivation with conventional mechanical tillage (Hartmans *et al.*, 1982). Vargas- Ayala′ *et al.* (2002), noted that farmers in their response to combat hunger rather deplete the soil resources through continuous cropping, bush burning and environmentally unfriendly systems of farming. According to Nyakande *et al.* (1981), ecosystems need to be conserved so as to support plant and animal lives. To reverse this trend of loss of soil fertility and to increase food production, practices such as green manuring, mulching, composting, agro-forestry as well as the use of short-duration improved fallow leguminous plants have evolved for adoption (Whitehead, 1995).

Mucuna pruriens, commonly known as velvet bean is one of the herbaceous leguminous crop which has been used for mulching purposes is botanically known as *Mucuna pruriens* var utilis (Buckles *et al.*, 1998).The genus, *Mucuna*, is used to describe various species of annual and perennial legumes belonging to the Fabaceae family including the velvet bean (Buckles, 1995). According to Duke (1981) *Mucuna pruriens* is self-pollinating, hence natural out-crossing is rare. Bailey (1993) gives the gestation period (from flowering to dry seed harvest) of *Mucuna pruriens*as between 100-290 days. Gardener (1965) wrote that *Mucuna pruriens* grows well on a wide range of soil types including heavy clays provided they are well drained. Velvet bean thrives best with warm, moist conditions with plenty of rainfall (Chavez, 1993). Most *Mucuna* spp. exhibit reasonable tolerance to a number of abiotic stresses, including drought, low soil fertility, and high soil acidity, although they are sensitive to frost and grow poorly in cold, soils (Duke,1981; Hairiath, *et al.*1993). Gallagher *et al.* (1999) asserted that *Mucuna pruriens*, due to its vegetative cover, reduces the impact of the rain drop splashes which create soil crust and therefore enhances percolation. As a cover crop, Reijntes (1997) noted that, *Mucuna pruriens* helps to reduce the rate of run-off

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especially on hilly and sloppy areas and also reduces the rate of leaching and losses of mineral nutrients.

Although mulch farming has proven to be effective in controlling soil erosion and in improving soil properties, resource-poor farmers have always been reluctant to adopt conventional mulching, because of the large amount of labour needed to gather, transport and apply the mulch. The search for inexpensive and more attractive mulching methods has led to the development of *in-situ* and live mulch. Therefore, the objective of this work was to determine the influence of different methods of application of *Mucuna pruriens* as soil amendments on the hydro-physical properties of a Chromic Luvisol in Ghana.

II. MATERIALS AND METHOD

2.1 Study area

The experiment was carried out at the Multi-purpose nursery of the University of Education, Winneba, Mampong – Campus. Mampong lies at 457.5meters above sea level and falls within the transitional zone that is between the southern rain forest and Guinea Savannah belt of the North. Rainfall distribution in the area is bimodal and classified into major and minor rainy seasons. The major season commences from April to July and the minor season from early September to late November (Meteorological Service Department, Mampong, 2012). The soil is of the savannah Ochrosol type which belongs to the Bediesi series known as Chromic Luvisol in F.A.O/UNESCO classification, 1990 and derived from the voltaian sandstone (Soil Research Institute, 1999).

2.2 Treatments and design

There were four treatments and arranged in a Randomised Complete Block Design (RCBD) with 3 replications. These treatments were (i) *Mucuna pruriens* as green manure (ii) *Mucuna pruriens* as live mulch (iii) *Mucuna pruriens* as *in-situ* mulch and (iv) Control (no soil amendment)**.** Each plot measured 6m by 4m giving a total area of 24m^2 per plot. A path of 1m was left between each plot for easy movement. The demarcated field of 6m by 4m per plot was left for five months to be stable as it had recently been farmed on. The plots were all sprayed with an herbicide (Sunphosate with glyphosate as active gradient at a rate of 2.5L ha^{-1}) to control the weeds on them. Lining and pegging was done for planting of *Mucuna pruriens* at a planting distance of 0.8m by 0.8m. The plots with treatment (i), (ii) and (iii) were planted (mid-May, 2012) with *Mucuna pruriens* for the first experiment while for the second experiment the *Mucuna pruriens* was planted in mid-January, 2013.

***Mucuna pruriens* as green manure.** On this particular plot, the *Mucuna pruriens* which was at the flowering stage (a growth period of 120 days for maximum foliage) was incorporated into the soil using a hoe. The plot was left for three weeks to ensure that at least the decomposition of simple sugars had taken place and the heat produced during the initial stage of decomposition had reduced.

***Mucuna pruriens* as live mulch.** The *Mucuna pruriens* which was at the flowering stage (a growth period of 120 days for maximum foliage) was allowed to grow without any disturbance till the end of the experiment.

***Mucuna pruriens* as *in-situ* mulch.** The term *in-situ mulch* refers to the residues of dead or chemically killed cover crops which are used on the same land on which they were grown as mulch (Wilson *et al*,. 1982). On this plot *Mucuna pruriens* at the flowering stage (a growth period of 120 days for maximum foliage) was cut at the base with a knife. The *Mucuna pruriens* was then left on the plot as *in-situ* mulch.

Control (No *Mucuna pruriens*). This plot did not have any *Mucuna pruriens* planted on it from the inception of the experiments.

2.3 Determination of parameters

The dry bulk density was determined from soil cores collected at 0-15cm depth on the field with core sampler (Klute, 1986). Moisture content was determined on gravimetric and volume basis (Hillel, 1982) while residual moisture storage was obtained from the measurement of the gravimetric moisture content of the soil at the end of the experiment, using the method by Gardener (1965). Total porosity was calculated by the formula; $f = 1- \rho b/\rho s$ where f is total porosity, ρb is bulk density and ρs is particle density (2.65 g cm^{-1}) (Hillel, 1982). Air filled porosity was calculated by the formula, $af = f - \theta v$ where *af* is air filled porosity (Klute, 1986), *f* is the total porosity and θv is volumetric water content.

A modified wet sieving method was used to measure the aggregate stability (ASt) (Kemper and Rosenau, 1986). Twenty grams (20 g) of the aggregates were weighed unto a 0.25 mm sieve. The sieve was immersed in water contained in a basin and manually rotated gently for five minutes. The wet sieved aggregates were dried to a constant mass. Another 20 g sub sample was weighed and oven dried to a constant mass. After oven drying, the wet sieved aggregates were divided by the sub sample to give the aggregate stability, which was expressed as a percentage

Sorptivity was measured by dividing the first 5-minute cumulative infiltration by the square root of the time (Philip, 1957).The single ring infiltrometer method (Klute, 1986) was used to determine the cumulative infiltration in the flied.

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Soil temperature was determined by inserting a soil thermometer into the soil to a depth of about 5cm for 8 days. The readings were taken every 6:00 am, 12:00 noon and 6:00 pm for each day and recorded.

2.4 Data analysis.

The data collected on various parameters were subjected to analysis of variance using Genstat software programme 2013. The means were separated using Least Significant Difference (LSD) at 5% probability level.

III. RESULTS AND DISCUSSIONS

Results of initial analysis of hydro-physical soil properties of the area are presented in Table. 1

Table 1 Summary of initial hydro-physical soil properties

Soil property	value
Bulk density (ρ_b) (g/cm3)	1.6
Total porosity (f) (%)	40
Aeration porosity (ξ_a) (%)	29.26
Gravimetric moisture content (θ_g) (%)	6.71
Volumetric moisture content(θ_v) (%)	10.736
Degree of saturation (θ_s) (%)	26.84
Sand (%)	73.18
Silt (%)	16.54
Clay (%)	10.28
Texture	Sandy loam

Table 2 shows that *Mucuna* as live mulch recorded the least bulk density in both experiments while the control recorded the highest. It was significantly ($p<0.05$) different from the in-situ mulch and the control but not from the green manure. This could have been as result of the biological activity provided by the roots of the live *Mucuna pruriens* and the burrowing activity of earth worms since live *Mucuna pruriens* promotes earthworm activity. This confirms the assertion made by Houngnanadan *et al.* (2000), that the presence of live *Mucuna pruriens* in the soils as well as its biomass application to the soil provides favourable conditions for growth, survival and breeding of earthworms. The control recorded the highest bulk density and can be explained by the fact that it had the least volume of loose soil as it had no *Mucuna pruriens* on it and thereby less interference by biological or mechanical agents. Lower bulk densities are important productivity index in agriculture. According to Devis and Freitas (1970), the lower the bulk density the more productive the soil is, as it allows for easy root penetration.

Table.2: Influence of Treatments on Dry Bulk Density, Total Porosity and Air-Filled Porosity

	Dry Bulk Density(g/cm^3)		Total Porosity (%)		Air-Filled Porosity (%)	
	2012	2013	2012	2013	2012	2013
Control	1.567	1.550	40.88	44.51	28.00	26.21
Green manure	1.330	1.370	49.81	48.30	39.05	34.58
Live mulch	1.303	1.330	50.82	49.81	39.82	35.61
In-situ mulch	1.470	1.460	44.53	44.90	30.39	27.88
L S D (0.05)	0.0935	0.02825	3.523	1.067	3.667	2.008
C V (%)	2.0	0.4	2.3	0.4	4.6	2.2

The data (Table 2) revealed that *Mucuna* as live mulch recorded the highest total porosity in both experiments. This trend could have been as results of the burrowing activities of the roots of live *Mucuna pruriens* and earthworm. The result affirms Akinsanmi (1994), assertion that high total porosity of soil is a result of high organic matter obtained from *Mucuna pruriens* fallow. And also according to Devis and Freitas (1970) *Mucuna pruriens* fallows produce crumb structure which facilities soil cultivation and growth of roots of crop plants, as it permeates the soil with roots and pores, and are able to loosen, break up and process the soil**.** The control recorded the least total porosity in both experiments (Table 2). This might be explained with the fact that the control recorded the highest bulk density therefore had more compaction hence less pores. It could also be that, it had fewer disturbances as it had no *Mucuna pruriens* on it.

From the results of the experiments (2012 and 2013), it was observed that the *Mucuna* as live mulch had the highest air-filled porosity ,this could have been as result of it recording the least bulk density (Table 2) therefore least compaction, highest total porosity and coupled with one of the lowest volumetric moisture content. This trend in air-filled porosity

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is common knowledge, in that the moisture content of the soil is a principal determinant of the content of soil air. This affirms Hodgson and Macleod (1989) that air-filled porosity of a soil increases with decreasing moisture content. It also conforms to Brady (1990) that the moisture content of the soil principally determines the content of soil air, since the soil pores not filled with water are occupied by air. The control also recorded the least air-filled porosity in both experiments. This might be attributed to it recording the least aggregate stability (Table 3), highest bulk density and therefore the least porosity. This affirms (Brady, 1990) that Soil texture, bulk density and aggregate stability are among the soil properties that influence soil aeration.

Table.3: Influence of Treatments on Aggregate Stability

	Aggregate Stability (%)	
	2012	2013
Control	41.82	41.20
Green manure	41.16	41.64
Live mulch	43.74	44.71
In-situ mulch	51.68	52.77
L S D (0.05)	6.50	5.932
C V (%)	4.0	3.8

Stability of soil aggregates is defined as the ability of soil aggregate to resist disruption when outside forces (usually associated with water) are applied (USDA-NRCS, 2008). The results of the experiment (Table 3) shows that in both experiments the *Mucuna* as *in-situ* mulch recorded aggregate stability values that were significantly ($p<0.05$) higher than all the other treatments. This trend could be attributed to the fact that *Mucuna* as *in-situ* mulch had its surface cover with *Mucuna pruriens* throughout the experiment and therefore it provided protection against the full impact of raindrop. Gallagher *et al.* (1999) asserted that *Mucuna pruriens*, due to its vegetative cover, reduces the impact of the rain drop splashes which create soil crust and therefore enhances percolation. The result could also be due to the decomposition of the *Mucuna* which helped to stabilize the soil aggregate. This confirms Van-Camp *et al*. (2004) that organic matter from organic materials stabilizes soil structure by at least two different mechanisms: by increasing the inter-particle cohesion within aggregates and by enhancing their hydrophobicity, thus decreasing their breakdown. In experiment two (2013) the control recorded the lowest aggregate stability. It might have been as results of the control being bare throughout the experiment and had no protection from the full impact of the raindrop. However *Mucuna* as green manure recorded the lowest aggregate stability in experiment one, might be due to the mechanical forces operating on the soil surface during the incorporation of the *Mucuna pruriens* and tillage could cause significant soil loosening which causes the destruction of soil aggregates (Aksakal and Oztas, 2010)

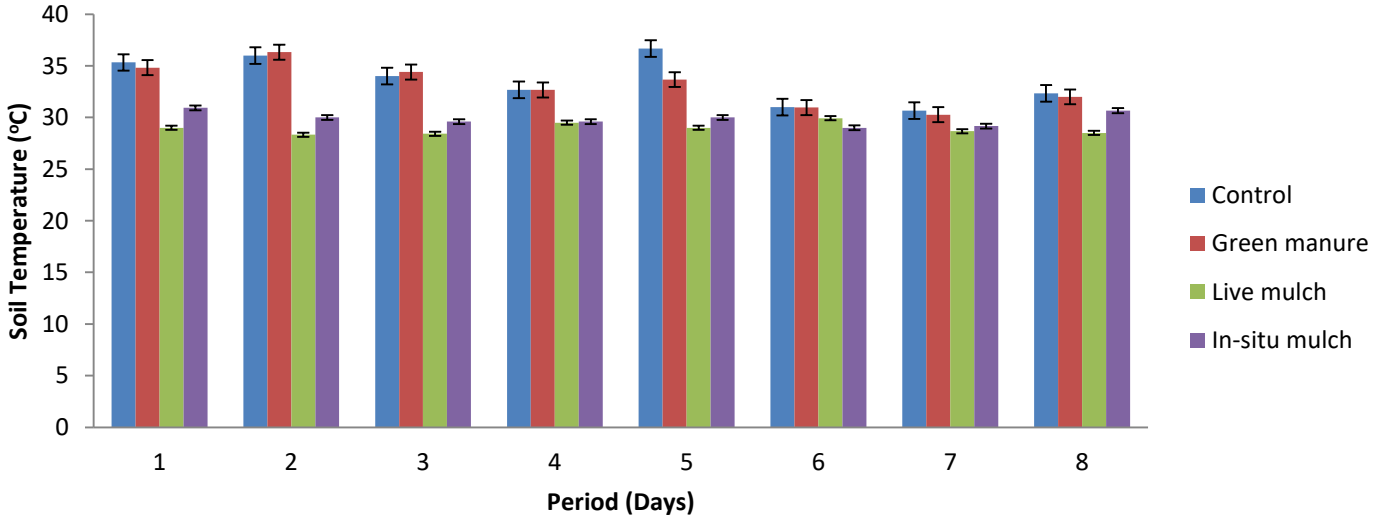

Fig.1: Daily Soil temperatures reading of treatments (2012)

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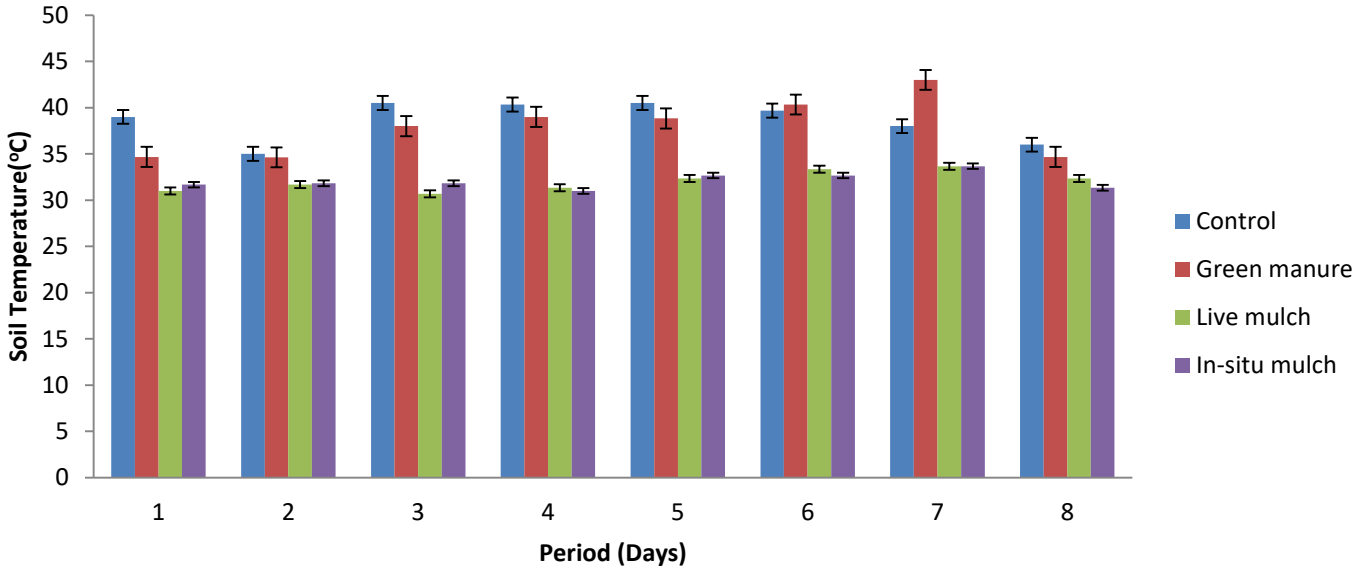

Fig.2: Daily Soil temperatures reading of treatments (2013)

By the fifth day of measuring daily temperature(Figure 1) *Mucuna* as live mulch recorded the lowest temperature (29^0 C).However, it was not significantly ($p<0.05$) different from *Mucuna* as *in-situ* mulch(30^0C) but they were both significantly($p<0.05$) different from *Mucuna* as green manure (33.67^0C)and the control(36.67^0C) in experiment one. A similar trend was observed in the second experiment (2013), where by the seventh day of measuring daily temperature, *Mucuna* as live mulch (33.67°C) and *Mucuna* as *in-situ* mulch (33.67°C) recorded the lowest temperature which was significantly ($p<0.05$) different from *Mucuna* as green manure (43^0C) and the control (38^0C) in experiment two. This trend (Figure 1 and 2) could be attributed to the fact that, both *Mucuna* as live mulch and *Mucuna* as *in-situ* mulch had *Mucuna* giving a protective cover and thus reducing the effect of direct sunshine and hence leading to lower temperatures. This affirms the assertion of Parson (1996), that *Mucuna pruriens* is a successful cover crop because it exerts certain influence on the soil, protecting the soil surface from direct heat of the sun, keeping the soil cool and warm in the extremes of the weather. Organic mulch maintains more uniform soil temperature by acting as an insulator that keeps the soil warm during cool spells and cooler during the warm month of the year (Ameroso, 2010).Generally the low temperature recorded by *Mucuna* as live mulch and *Mucuna* as *in-situ* mulch could be that they recorded the highest gravimetric moisture content (Table 4). This conforms to Raison (1986) who after series of trials in Australia, reported that soil temperatures reduced as the soil water content increased.

Table.4: Influence of Treatments on Soil Moisture

	Gravimetric Moisture Content (g/g)		Volumetric Moisture Content (cm^3/cm^3)		Residual Moisture Content (cm)	
	2012	2013	2012	2013	2012	2013
Control	8.13	9.87	12.74	15.29	1.93	2.26
Green manure	8.22	10.03	10.93	13.74	1.61	2.06
Live mulch	8.44	10.93	11.00	14.53	1.65	2.18
In-situ mulch	9.61	11.66	14.14	17.03	2.12	2.55
L S D (0.05)	NS	1.468	2.272	1.983	0.3449	0.3102
C V (%)	3.3	2.0	5.0	2.4	4.9	1.9

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Table 4 indicates that the *Mucuna* as *in-situ* mulch recorded the highest volumetric moisture content in both experiments with the control recording the lowest. This result could be attributed to the fact that *Mucuna* as *in-situ* mulch and the live mulch had a ground cover which reduced water loss through evaporation. A similar trend was observed by Akubundu *et al*, (2000) that *Mucuna pruriens* plant serves as a ground cover to prevent loss of water through evaporation and decreases soil run off and thus keeping the soil moist.

Erenstein (2002) also observed that there was a reduction in runoff, evaporation and increased infiltration for effective soil water storage for crop's water requirement after mulching.

The high residual moisture content (Table 4) recorded by *Mucuna pruriens* as *in-situ* mulch in both years could be attributed to the fact that the *Mucuna pruriens* on that plot reduced the contact between the soil and dry air and this reduced the water loss into the atmosphere through evaporation from sunshine and desiccating winds (Olabode *et al.*, 2006). It also conforms to Agyenim-Boateng and Safo (1999) in an experiment conducted on *Mucuna pruriens* as a cover crop that there was improved moisture content of the soil; from 9.4% to between 10.30% and 11.40%. Hartfield *et al*.(2001) in farming matters found 34-50% reduction in evaporation and a considerable decrease of soil temperature as results of the ground cover protection by mulch (*Mucuna pruriens* as *in-situ* mulch).The trend observed might have been as a result of the low temperatures recorded by *Mucuna pruriens* as *in-situ* mulch (Figure1 and Figure 2).This conforms to Srivastava (1992) who observed that low temperature leads to a low evaporative demand and increases the scope for excess soil water conditions. The lower the temperature, the lower the evaporation losses and the more moisture retention for cultivated crop. The highest residual moisture content recorded by *Mucuna pruriens* as *in-situ* mulch could be attributed to the highest gravimetric and volumetric moisture content recorded (Table 4) as antecedent moisture is key to residual moisture content.

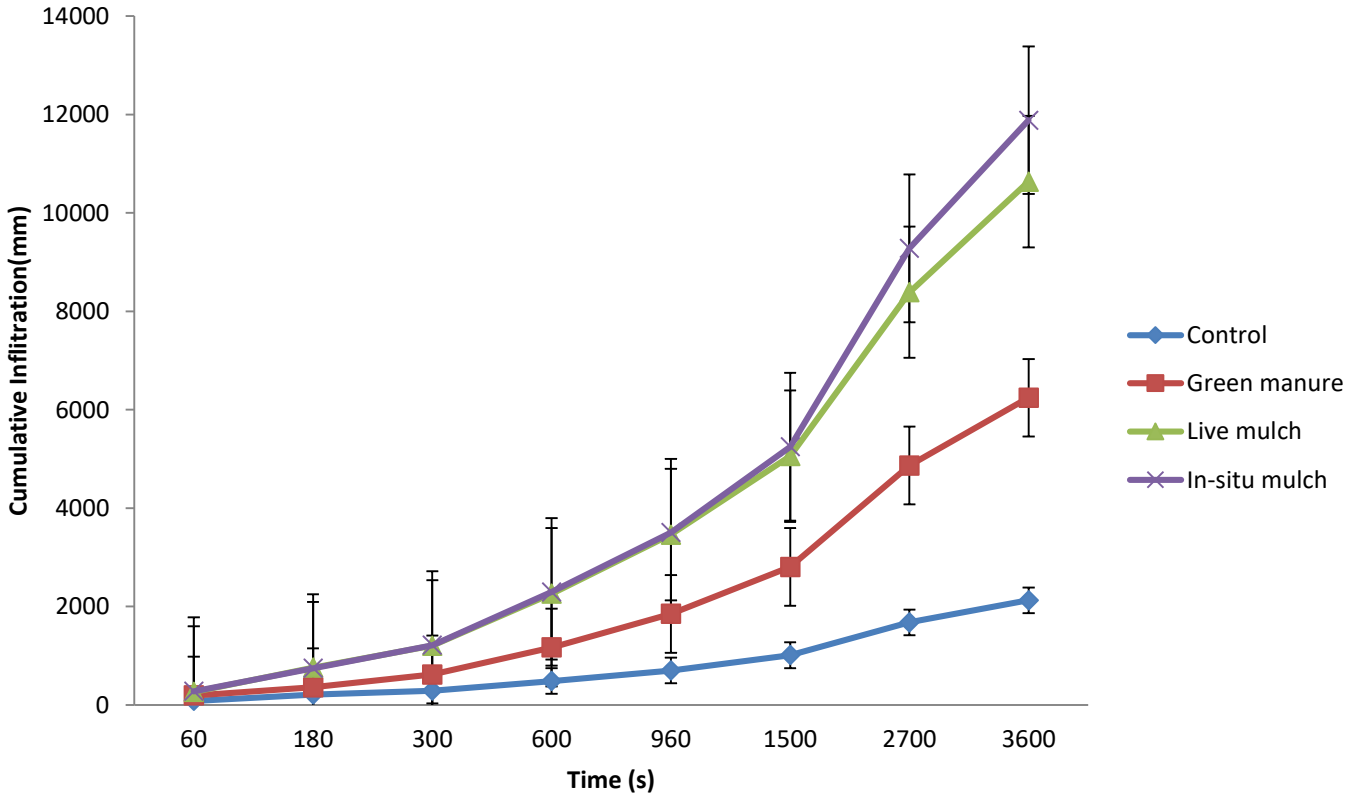

Fig.3: Cumulative infiltration curves for treatments (2012)

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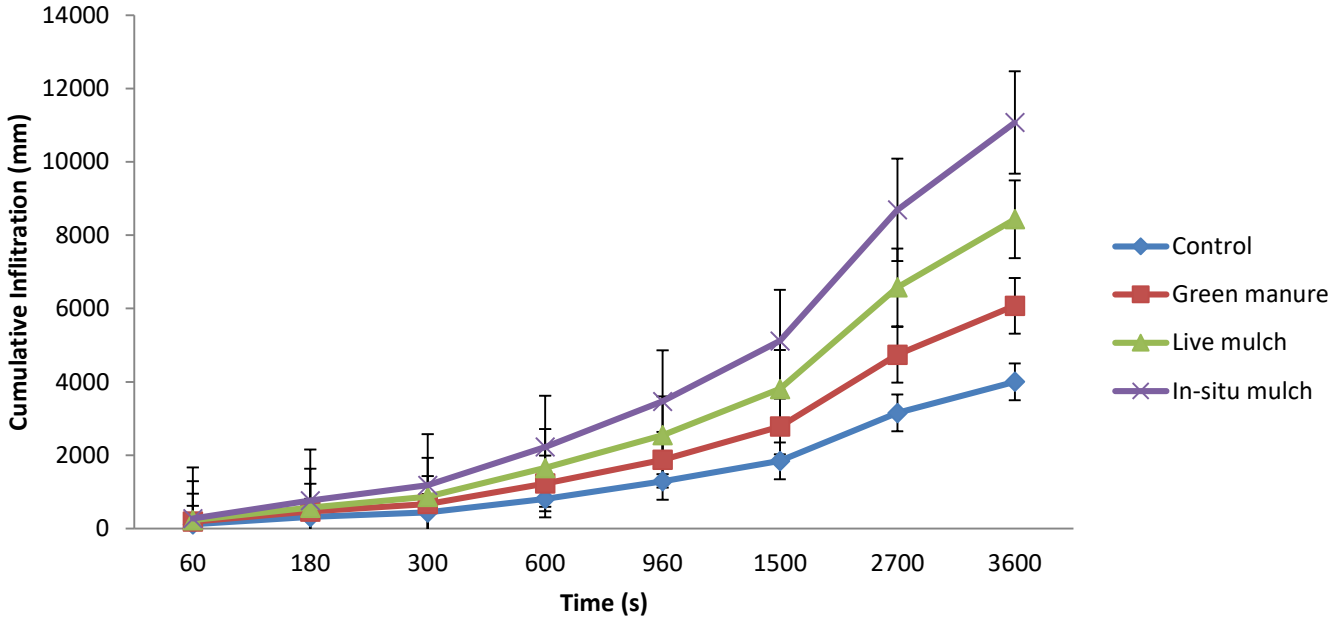

Fig.4: Cumulative infiltration curves for treatments (2013)

The results (Figure 3 and 4) of the experiment showed that, by the hour mark, *Mucuna pruriens* as *in-situ* mulch recorded the highest cumulative infiltration amount in both experiments with the control recording the least value. *Mucuna pruriens* as *in-situ* mulch was significantly different from *Mucuna pruriens* as green manure and the control but not significantly different from *Mucuna pruriens* as live mulch in the first experiment. This findings could also be attributed to the fact that *Mucuna pruriens* as live mulch plot recorded the highest Total porosity and air-filled porosity (Table 2) and likely to have more macro pores for good water infiltration. This confirms that it is not only antecedent water that influences infiltration but other factors like structure, texture porosity and tillage Jury *et al*. (1991). These findings are very important as infiltration influence runoff and time of ponding. The temporal distribution of soil moisture controls numerous catchment processes including runoff generation, groundwater recharge, ET, soil respiration, and biological productivity (Williams *et al*., 2009).

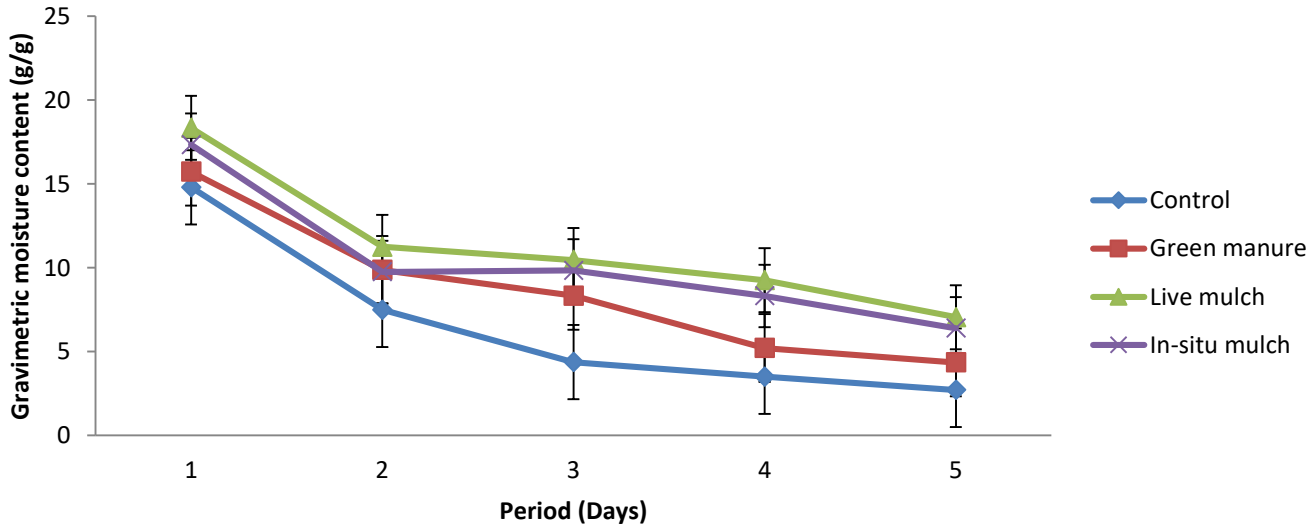

Fig.5: Influence of mucuna on soil drying (2012)

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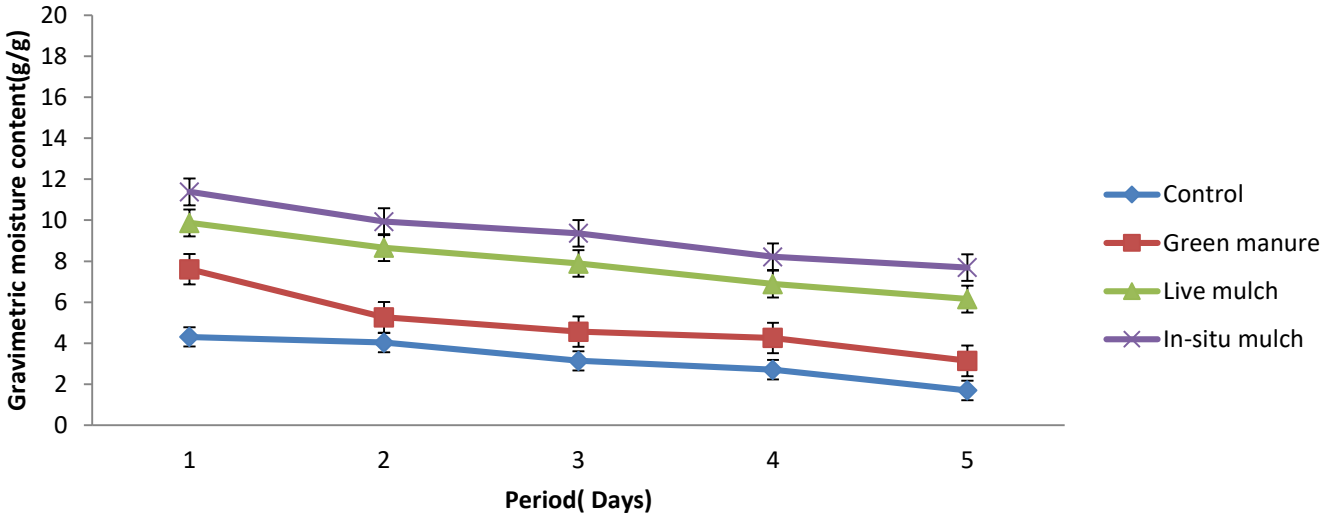

Fig.6: Influence of mucuna on soil drying (2013)

It was observed that, *Mucuna pruriens* as live mulch scored the highest gravimetric moisture content value (7.05) after the five days of studying soil drying in experiment one (2012) with the control scoring the lowest (2.71). In 2013 the in-situ mulch recorded the highest value (7.69) while the control again recorded the least value (1.70). The observation above could be attributed to the fact that the treatment *Mucuna pruriens* as *in-situ* mulch and *Mucuna pruriens* as live mulch had mulch which reduced water loss by evaporation and reduced direct impact of sunshine on the surface of the soil. It could also be attributed to the low temperature recorded by *Mucuna pruriens* as *in-situ* mulch as low temperatures also reduces soil moisture loss. This affirms Parson (1996) assertion that *Mucuna pruriens* is a successful cover crop because it exerts certain influence on the soil, protecting the soil surface from direct heat of the sun, keeping the soil cool and warm in the extremes of the weather.

Table.5: Influence of Treatments on Sorptivity

	SORPTIVITY(mm/s) 2012			SORPTIVITY (mm/s) 2013		
	S60	S180	S300	S60	S180	S300
Control	10.3	15.6	16.7	14.84	25.72	25.40
Green manure	24.7	27.9	35.7	24.73	37.82	42.02
Live mulch	34.2	56.5	69.4	29.46	42.60	50.95
In-situ mulch	35.7	55.5	70.1	35.27	56.76	67.80
LSD	7.77	16.02	20.35	8.8	11.12	12.18
CV	7.3	6.8	4.8	9.2	6.5	4.1

Sorptivity is a measure of the soils ability to absorb water. Generally *Mucuna pruriens* as *in-situ* mulch recorded the highest sorptivity value followed by the live mulch in both experiments with the control recording the lowest sorptivity value (Table 5). This could be attributed to *Mucuna pruriens* as live mulch and *Mucuna pruriens* as *in-situ* mulch were better drained with higher porosity and therefore, likely to have more macro pores and the greater ability to absorb and conduct initial water during infiltration. Also the Mucuna treatment had a protective ground cover by the *Mucuna pruriens* and therefore reduced the impact of raindrop and splash which in turn reduced soil compaction, reducing the surface sealing and increasing porosity, reducing surface runoff and increasing infiltration, and readily making soil water available to the plant. This assertion is supported by Chancellor (1977) and Olabode *et al*. (2006). The reverse is true for the control plot which recorded the lowest sorptivity value. This was supported by Jury *et al*. (1991), that besides,

antecedent moisture, sorptivity is influenced by porosity and pore size distribution, aggregate stability, bulk density, permeability.

IV. CONCLUSION AND RECOMMENDATION

Mucuna pruriens as *in-situ* mulch, *Mucuna pruriens* as live and *Mucuna pruriens* as green manure as soil amendments improve gravimetric (θ_g) and volumetric moisture content (θ_v), residual moisture storage(R), sorptivity (s), cumulative infiltration(I) with *Mucuna pruriens* as *in-situ* mulch recording significantly ($p \leq 0.05$) higher values while the control recorded the least values in the above mentioned parameters. The soil bulk density (ρ_b), total porosity (f) and aeration porosity (ξ_a), were significantly improved by *Mucuna pruriens* as live and *Mucuna pruriens* as green manure as they were significantly ($p \leq 0.05$) higher than *Mucuna pruriens* as *in-situ* mulch and the control in both years. *Mucuna pruriens* as *in-situ* mulch in both years was significantly higher in aggregate stability (ASt) than all the other treatments while there was no significant difference amongst them. Soil temperature was steadily reduced by *Mucuna pruriens* as live and *Mucuna pruriens* as *in-situ* mulch with each been significantly different from the control in the first and second year respectively. From this results, the application of *Mucuna pruriens* as *in-situ* mulch, *Mucuna pruriens* as live as soil amendments is recommend as the most effective soil conservational technology for improvement soil hydro-physical properties.

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Bioadsorption of Pb^{2+} and Cu^{2+} on Eucalyptus Camaldulensis Leaves

Zeinab Ezzeddine[1], Effat Al Sayed[2], Hassan Rammal[1], Akram Hijazi[1], Hussein Hamad[*1,3], Hanane Akhdar[4]

[1]Platform for research and analysis in environmental sciences (PRASE), Lebanese University, Beirut, Lebanon
[2] Lebanese University, Faculty of Sciences (I), Hadath, Beirut, Lebanon
[3,4]Lebanese University, Faculty of Sciences (V), Nabatieh, Lebanon

***Abstract*—** *Herein, the efficiency of Eucalyptus camaldulensis leaves as biosorbent for lead and copper was investigated. The particle size distribution was determined by Granulometric analysis and the functional groups were identified by FT-IR spectroscopy. The effects of contact time, pH and initial metal ions concentration were investigated. The experimental kinetic data were well fitted by the pseudo-second order kinetic model and Langmuir isotherm with a maximum adsorption capacity up to 71 mg g^{-1} and 37 mg g^{-1} for Cu^{2+} and Pb^{2+} respectively. The selectivity was examined in a binary ions solution where the adsorbent showed preference for lead over copper.*

***Keywords*— Biosorption, Eucalyptus leaves, copper, lead.**

I. INTRODUCTION

Access to clean water is essential for the development of any country. Currently, many water resources are contaminated due to the direct discharge of domestic and industrial wastewater [1]. Contrary to organic pollutants, heavy metals are more dangerous since they are not biodegradable and thus persist in nature. The latter cause heavy metals transformation to the food chain through their accumulation by plants and animals which leads to several toxic health effects [2]. The ingestion of copper, zinc, cadmium, lead, mercury, iron and nickel in amounts exceeding guidelines damages severely human physiology [3]. Several treatment techniques for heavy metals removal from wastewater have been reported in literature [4]. Chemical precipitation, ion exchange and adsorption are among these methods are. For low metal ions concentrations in wastewater, adsorption is recommended for their removal. It is advisable that the adsorbent is available in large quantities, easily regenerable, and cheap [5]. Biosorption of heavy metals from aqueous solutions has proven very promising. Materials derived from low-cost agricultural wastes can be used for the effective removal and recovery of heavy metal ions [6]. The major advantages of biosorption over conventional treatment methods include: low-cost; high efficiency; minimization of chemical sludge; and the possibility of metal recovery [7]. The cost advantage of this technology would guarantee a strong penetration of the large market of heavy metal polluting industries. Several studies have shown that non-living plant biomass materials are effective for the removal of trace metals from the environment [8].

Eucalyptus tree is one of the most widely distributed trees in most of arid and semiarid areas. This kind of tree exists in Lebanon. Accordingly, the objective of this study is to investigate the adsorption potential of Eucalyptus leaves for the removal of Cu^{2+} and Pb^{2+} from wastewater within various experimental conditions.

II. MATERIALS AND METHODS

2.1. Adsorbent preparation

Eucalyptus camaldulensis leaves were collected from Bekaa-Lebanon. The leaves were thoroughly rinsed with water to remove impurities like soluble materials and dust then dried in the oven at 50 ºC for 48 hours. The dried Eucalyptus leaves were then grinded to fine powder.

2.2. Characterization of the Eucalyptus leaves

The granulometric analysis was done to the grinded leaves in order to determine size distribution before and after modification using Partica LA-950V2 Horiba. The functional groups were identified by Fourier Transform Infrared (FTIR) Spectroscopy in the range of 4000–400 cm-1. The samples were first mixed with KBr and then pressed into pellets and analyzed with FT-IR – 6300 JASCO.

2.3. Batch adsorption tests

Lead and copper solutions were prepared from the corresponding nitrate salts in distilled water to obtain solutions of different concentrations. The pH of the solution was adjusted using 0.1M HNO_3 and 0.1M NaOH solution. In typical batch studies, 0.5 g of eucalyptus leaves powder was placed in a flask containing 50 mL of a metal solution with the desired concentration. The flask was continuously stirred at room temperature (25 ± 2ºC),

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except where the effect of temperature was being investigated, at 250 rpm for 180 min. At the end of each step the solution was filtered and the metal ion concentration was determined for each metal using Atomic Adsorption Spectrophotometer (RAYLEIGH WFX-210) equipped with Automatic hollow cathode lamp changeover and air–acetylene burner. The equilibrium time was determined as the contact time required for the metals in the solution to reach equilibrium. The lead adsorption percentage was calculated by equation (1):

$$R = \frac{C_0 - C_t}{C_0} \times 100$$

where R is the adsorption rate (%), C_0 is the initial concentration and C_t is the concentration at time t. The adsorption capacity of the adsorbent at equilibrium was calculated by equation (2):

$$q_e = \frac{(C_0 - C_e)V}{m}$$

Where qe is the equilibrium adsorption capacity in mg g^{-1}, C_0 is the initial concentration and C_e is the concentration at equilibrium, V is the volume in L of metal solution and m is the mass in g of the adsorbent. For obtaining the isotherms, the batch experiments the initial metal ions concentrations were varied between 50 mg L^{-1} and 500 mg L^{-1}. The solutions were then filtered by a 0.45 µm nylon syringe filter and the remaining metal ions were measured by AAS in order to calculate Ce and qe.

III. RESULTS AND DISCUSSION

3.1. Granulometric analysis

Fig. 1.a shows the size distribution of the grinded powder of Eucalyptus leaves. The obtained results showed two particle distribution populations with average diameter 14.5 and 115 µm.

3.2. FT-IR spectroscopy

The obtained spectra for Eucalyptus leaves are shown in Fig. 1.b. The peak between 3400–3200 cm^{-1} is due to OH stretch. At 2925 cm^{-1}, the peak could be assigned to C-H stretching vibration. The absorption peak at 1750 cm^{-1} is characteristic to -COOH group and that at 1647 cm^{-1} can be attributed to HN_2 bending vibration. The C-N stretching vibration is at 1318 cm^{-1}, while C-O stretch is at 1032 cm^{-1}. The peak at 886 cm-1 and 646 cm^{-1} may be caused by C-H $–CH_2$ bending. The peak at 500-473 cm^{-1} is attributed to polysulfide (S-S stretch).

3.3. Effect of pH on Pb^{2+} and Cu^{2+} adsorption

Fig. 2 shows the adsorption capacity of Eucalyptus leaves as a function of pH. The obtained adsorption capacities were high over the studied pH range (between 2 and 8);

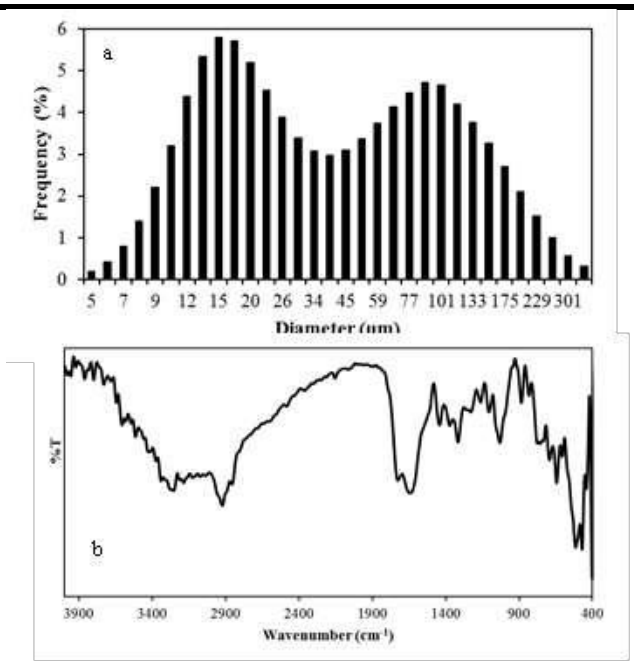

Fig. 1: Particle size distribution (a) and FT-IR spectrum (b) of Eucalyptus leaves.

However, the maximum adsorption capacity for Pb^{2+} was at pH = 4 and at 6 for Cu^{2+}.

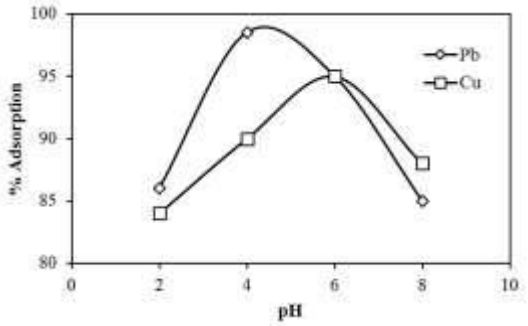

Fig. 2: Effect of pH on metal ions biosorption ((m)/(V) = 0.5/50 mg mL^{-1}, at 25°C, 300 rpm and [M^{2+}] = 50 ppm).

3.4. Adsorption Kinetic models

3.4.1. Pseudo-first order

The pseudo first-order kinetic model is expressed as follows:

$$\ln (q_e - q_t) = \ln q_e - k_1 t$$

Where q_t and q_e are the quantity of metal ions adsorbed (mg g^{-1}) at time t (min) and at equilibrium respectively, and k_1 is the rate constant of adsorption (min^{-1}). The plot of ln (q_e - q_t) versus t should give a linear relationship from which k_1 and q_e can be determined from the slope and intercept of the plot, respectively [9].

3.4.2. Pseudo-second order

The pseudo-second-order sorption rate is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

where k_2 is the pseudo-second order rate constant (g mg^{-1} min^{-1}), q_t and q_e are the quantity of metal ions adsorbed at

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t time and at equilibrium (mg g^{-1}) respectively. A plot of t/q_t versus t should yield a straight line from which q_e and k_2 can be determined from the slope and intercept of the plot, respectively [10].

Lead and copper adsorption as a function of contact time is illustrated in Fig. 3.a. Equilibrium was reached within the first 30 min for both metal ions which indicates the high affinity of the adsorbents towards them. The kinetic parameters as well as the correlation coefficients (R^2) and the experimental equilibrium capacities are reported in Table 1. The obtained results show that Pb^{2+} and Cu^{2+} adsorption on Eucalyptus leaves followed the pseudo-second order kinetic model (Fig. 3.b). This suggests that the adsorption rate depends mainly on the interaction between different functional groups present on the leaves and the metal ions.

Table.2: Comparison of Langmuir and Freundlich models for Me^{2+} adsorption on Eucalyptus leaves

Me^{2+}	Langmuir model			Freundlich model		
	q^{exp}_{max}	K_L	R^2	n	K_f	R^2
Cu^{2+}	71	0.02	0.875	1.46	2.15	0.996
Pb^{2+}	37	0.06	0.988	2.3	4.5	0.983

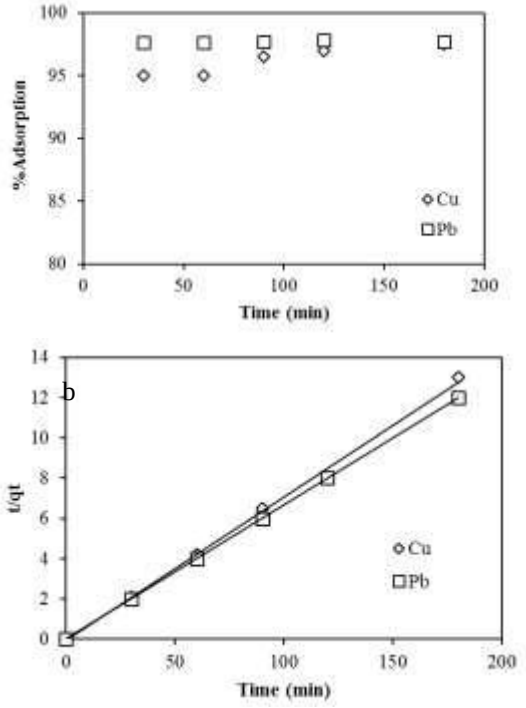

Fig.3: biosorption of M^{2+} as a function of time (a) and pseudo-second-order model (b) ((m)/(V) = 0.5/50 mg mL^{-1}, at 25°C, 300 rpm and [M^{2+}] = 50 ppm)

3.5. Adsorption Isotherms

Langmuir Isotherm:

Table.1: Comparison of the first and the second order kinetic models

Eucalyptus leaves							
	qe exp	First order kinetic model			Second order kinetic model		
		k_1	qe^{cal}	R^2	k_2	qe^{cal}	R^2
Cu^{2+}	14.3	0.023	13	0.949	0.087	14.7	0.999
Pb^{2+}	15	0.009	15.6	0.948	0.514	14.7	0.999

Langmuir Isotherm is a model that assumes monolayer coverage of a finite number of identical sites present on the surface such that no further adsorption takes place.

Based on these assumptions, Langmuir represented the following equation [11]:

$$q_e = \frac{K_L \times q_{max}\ C_e}{1 + K_L C_e}$$

where q_{max} is the maximum adsorption capacity (monolayer coverage), i.e. mmol of the adsorbate per (g) of adsorbent and K_L is Langmuir isotherm constant. The adsorption parameters of Langmuir model can be determined from its linear form by sketching C_e/q_e versus C_e so that the values of q_{max} and K_L can be calculated from the slope and intercept of the linear plot respectively:

$$\frac{C_e}{q_e} = \frac{1}{K_L q_{max}} + \frac{C_e}{q_{max}}$$

Freundlich Isotherm:

This model describes the non-ideal and reversible adsorption, not limited to monolayer formation. It can be applied to multilayer adsorption, with non-uniform distribution of adsorption heat and affinities over a heterogeneous surface [12]. The equation is expressed as follows:

$$q_e = K_f C_e^{1/n}$$

Where K_f is Freundlich isotherm constant (mmol g^{-1}) and n is the adsorption intensity. The linear form:

$$\log q_e = \log K_f + \frac{1}{n}\log C_e$$

The theoretical fittings for the experimental data are shown in Fig. 4 and the calculated parameters are shown in Table 2. The linear regression correlation coefficients R^2 show that the equilibrium data could be well interpreted by the Langmuir isotherm with maximum adsorption capacity q_{max} equals to 71 mg g^{-1} and 37 mg g^{-1} for Cu^{2+} and Pb^{2+} respectively.

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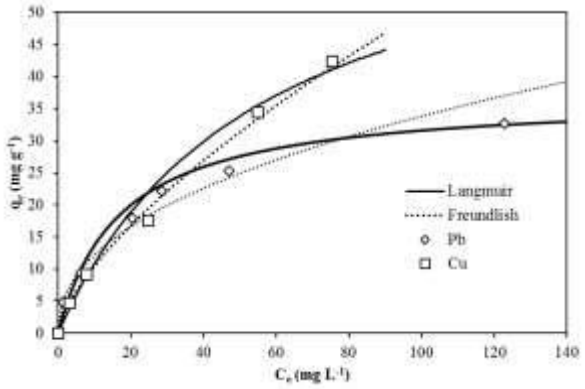

Fig. 4: Langmuir and Freundlish fit for the experimental points((m)/(V) = 0.5/50 mg mL^{-1}, at 25ºC, 300 rpm and [M^{2+}] = 50-500 ppm)

3.6 Selectivity for Pb^{2+}/Cu^{2+} in binary ions solution

The competition in binary solutes was obtained by testing the adsorption capacity of the adsorbent in a mixed solution containing both lead and copper ions (both at 50 ppm). Figure 5 shows the adsorption percentages of these two metals. Eucalyptus leaves showed preference for lead over copper and this could be explained by the smaller size of hydrated lead ions.

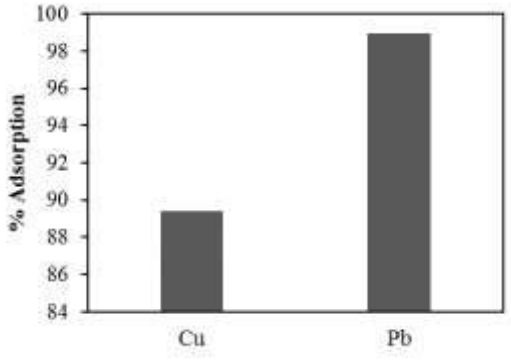

Fig. 5: Selectivity of Eucalyptus leaves in a binary metal ions solution ((m)/(V) = 0.5/50 mg mL^{-1}, at 25 ºC, 300 rpm and [M^{2+}] = 50-500 ppm).

IV. CONCLUSION

In this study, the biosorption of lead and copper on Eucalyptus leaves was studied. The adsorption capacity was high at different pH values. The adsorption process followed the pseudo second order kinetic model. The maximum adsorption capacity was determined from Langmuir isotherm. The adsorbent was selective for lead in a binary metal solution containing both lead and copper.

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Genotype by environment interaction and stability of extra-early maize hybrids (Zea Mays L.) for yield evaluated under irrigation.

M. S. Koroma[1], M. Swaray[1], R. Akromah[2], K. Obeng-Antwi[3]

[1]Sierra Leone Agricultural Research Institute, Tower Hill, P.M.B. 1313, Freetown
[2]Department of crops and soil sciences, Faculty of agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[3]CSIR-Crops Research Institute, Kumasi, Ghana

Abstract— Maize *(Zea mays L.) is the most important cereal crop produced in Ghana.*
However the change in environmental conditions, the expansion of maize to new agro-ecologies coupled with inadequate maize varieties available for the different environments affects yield improvement programmes in Ghana. Hence, the study is to investigate the influence of genotype by environment interaction on the maize hybrids and to identify stable and high yielding hybrids with superior agronomic for famers use in the country.
The objectives of the study was to investigate the influence of genotype by environment interaction on the maize hybrids and to identify stable and high yielding hybrids with superior agronomic performance for famers use in Ghana. Thus, fifteen extra-early maize hybrids and three locally released checks were evaluated in a randomized complete block design with three replications in two locations in Ghana. The experiment was carried out at KNUST and Akomadan which represent the forest and forest transition zones of Ghana. Nine of the hybrids out of the fifteen hybrids evaluated produce above the average yield and the effect of genotype, location and genotype by location interaction was significant for grain yield. The GGE biplot used in this study revealed that TZEEI-1 x TZEEI-21, TZEEI-6 x TZEEI-21, TZEEI-15 x TZEEI-1 and TZEEI-29 x TZEEI-21 were high yielding and stable hybrids because they were closer to the ideal. The GGE biplot also identified Akomadan as the most ideal testing environment for these hybrids under irrigation.

Keywords— Genotype, Interaction, Genotype by Environment.

I. INTRODUCTION

Maize (*Zea mays* L.) is cultivated globally and being one of the most important cereal crops produced world-wide after wheat and rice (Golbashy *et al*., 2010). More maize is produced annually than any other grain, and about 50 species exist and they consist of different colours, textures and grain shapes and sizes. It has become Africa's dominant food crop since its introduction in 1500.

Like many other regions, it is consumed as a vegetable and contains excellent quality edible oil, carbohydrate, starch, protein, minerals and vitamins A (Amaregouda, 2007). The grains actually contain 72 % starch, 10 % protein, 4.8 % oil, 8.5 % fibre, 3.0 % sugar and 1.7 % ash (Chaudhary, 1983). In developed countries, maize is mainly utilized as feed for domestic animals and at the same time as raw material for manufacturing products, although in developing countries, it is really utilized as food for human consumption (Badu-Apraku *et al*., 2012; IITA, 2009). Maize is produced mostly by small holder resource poor farmers under rain- fed conditions (SARI, 1996). In spite of this, the production rate of maize in farmer's fields in the country is low. The average grain yields of maize nationwide are around 1.89 metric tons ha^{-1}(MOFA-SRID, 2011). However, yields as much as 5.0-5.5 metric tons per hectare have been achieved by farmers using improved seeds, fertilizer, mechanization and irrigation (MiDA, 2010). Low yields of maize have been as a result to traditional farming practices, the use of low-yielding varieties, poor soil fertility and limited use of fertilizers, low plant population density, and inappropriate weed control. These biotic and a biotic factor have lead to the tremendous limit in productivity of maize across countries in the region (Fajemisin *et al*., 1985). High yields could be achieved through the use of hybrid maize varieties (Agribusiness Trade Project, 2008).

Stability of desirable genetic characters is important for development of improved varieties and useful for the commercial exploitation over a wide range of agro-climatic conditions. According to (Esechie, H.A, Rodriguez, V. and Al-Asmi, H.) (2004), the consistency in performance for both high and low yields across different environment is referred to yield stability. It is

more practical to develop and release varieties which are adapted to more than a single environment and can be successfully grown over a range of environments. Thus the use of extra-early hybrid maize is required because of the short raining seasons, resulting from climate change. A need to fit crops to the seasons and hence will be very important in improving maize productivity and enhancing food security in Ghana.

Plant breeders have been trying to develop genotypes with better qualities and other worthy characteristics over a wide range of environmental conditions. Genotype by environment interaction in multi-environment trials refers to differential responses of genotypes across a range of environments (Kang and Gorman, 1989). In addition, (Casper Nyaradzai Kamutando *et al.,* 2013) started the important cross-over genotype by environment that raised the need to indentify hybrids that performed superior in particular environments. The most important agronomical and economical traits such as grain yield are quantitative in nature and usually exhibit genotype by environment interaction (Fan *et al*., 2007). Genotype by environment interactions determined in multi-location trials reduced the correlation between phenotypic and genotypic values and have been found to reduce gain from selection (Comstock and Moll, 1963). Also the knowledge of correlation between yield and its component characters is essential for yield improvement programmes (Ofori *et al.,* 2015; Baudh Bharti *et al.,* 2017). Genotype by environment interaction (GEI) according to (Yau, 1995) defined it as the degree of different reactions of a genotype for an exact trait across environments and (Nzuve *et al.,* 2013) mentioned the importance of genotype by environment interaction for various traits. The development of maize hybrids which are high-yielding and relatively stable when grown in different environments is of fundamental importance to commercial maize production (Gama and Hallauer, 1980). Hence, the study is to investigate the influence of genotype by environment interaction on the maize hybrids and to identify stable and high yielding hybrids with superior agronomic performance for famers use in the country. The objectives of this research therefore were to evaluate the presence of genotype by environment interactions in the 15 maize hybrids and their agronomic performance and identify stable and high yielding hybrids and the pattern of response of the hybrids at different agro-ecologies.

II. MATERIALS AND METHODS

Fifteen extra-early maize hybrids, including three locally released checks, were use. The study was carried out in two experimental locations in order to estimate G x E interaction. The first site is the drip irrigation site at the Department of Animal Science which lies in the Forest ecology and the second site is at Akomadan which lies in the Forest transition ecology zones. A dialled cross was made among the inbred lines involving reciprocals at the drip irrigation site. The experimental fields were ploughed with a disc-ploughed and harrowed before planting to achieve a minimum tillage.

Genotypes were planted in one row plots and the plots were 5 m long, spaced 0.75 m apart, with 0.40 m spacing between plants within a row. The experiment was conducted in randomized complete block design (RCBD) with three replications at each location. Three maize seeds per hill were initially planted in each trial but were later thinned to two per hill at two weeks after planting (WAP). Pre-emergence and post-emergence chemical weed control was done with an application of Gramoxone and Atrazine respectively. Hand weeding was also done when necessary to control weeds during the growing period. NPK 15-15-15 fertilizer was applied at the rate of 30 kg N ha-1 and 60 kg P2O5 ha-1 as basal fertilizer at two weeks after planting and top-dressed with additional N at 60 kg N ha-1 at four weeks after planting. At the same time urea was also applied as top dressing after six weeks of planting for optimum plant growth at each location and all management practices were based on recommendations for each location.

III. DATA COLLECTION AND ANALYSIS

The agronomic parameters recorded were days to anthesis (were recorded as number of days from planting to the time 50% of plants had shown complete tassels emergence in each plot), days to silking (were recorded as number of days from planting to the time 50% of plants had shown complete silk emergence in each plot), plant height (the height of five randomly selected plant were measured with a graduated measuring stick from soil surface to the last node in each plot and average), ear height (the height of five plant in centimetres from to the soil surface to the node on which the uppermost ear sits were measured from the same plant from which plant heights were recorded and averaged), anthesis-silking interval (were calculated as the differences between days to 50% silking and days to 50% anthesis),

Cob length (five randomly selected cobs from each plot were selected and measured using a vainer calliper from the base of the ear to the tip and the average was determined), Cob width (five randomly selected cobs from each plot were selected and measured using a vainer calliper at the middle of the cob and the averaged was also determined), fresh weight (the weight of cobs per plot was measured in kilograms using a measuring scale and the values were recorded) and grain yield at 15%

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moisture content based on 80% shelling percentage was also recorded.

IV. DATA ANALYSIS

Data were analysed using the Genstat Statistical package version 12.1. Data from each location were subjected to Analysis of Variance (ANOVA) individually to explore differences among entries for all traits and pooled across locations to determine G x E
Interactions. Means separation was carried out using least significant differences (lsd). Correlations among grain yield and yield contributing characters were examined. GGE biplot analysis (Yan, 2002) was used to asses yield stability among the maize hybrids.

Table.1: Description of the maize hybrids tested across the two locations in 2014/ 2015

Entry number	Entry name (single-cross)
CR1	TZEEI-1 X TZEEI-21
CR2	TZEEI-1 X TZEEI-4
CR3	TZEEI-15 X TZEEI-1
CR4	TZEEI-6 X TZEEI-15
CR5	TZEEI-6 X TZEEI-29
CR6	TZEEI-21 X TZEEI-4
CR7	TZEEI-29 X TZEEI-4
CR8	TZEEI-29 X AZEEI-21
CR9	TZEEI-6 X TZEEI-4
CR10	TZEEI-6 X TZEEI-21
CR11	TZEEI-6 X TZEEI-1
CR12	TZEEI-15 X TZEEI-4
CR13	TZEEI-29 X TZEEI-15
CR14	TZEEI-29 X TZEEI-1
CR15	TZEEI-15 X TZEEI-21
CR16	AKPOSOE
CR17	MAMABA
CR18	ETUBI

V. Results and Discussion

The combined mean square analysis for grain yield indicated significance differences among the hybrids across the two trial locations (Table 2). Therefore, the significant mean square analysis for location revealed that genetic potentials of the genotypes were predisposed by the surroundings owing to the consequence of diversity in the surroundings. Similar findings were reported by Butron *et al*. (2002) where they mentioned that genotype by environment effect for grain yield in maize in particular were mostly owed to environment yield limiting factor such as minimum temperature, relative humidity, moisture stress and pest and diseases.

Table.2: Combined mean squares analysis of variance of grain yield (t/ha) across the two locations.

Source	df	Mean squares
Replication	4	1.18
GENOTYPE(G)	17	1.38*
Location(L)	1	229.97**
G x L	17	1.63**
Error	68	0.67

** = Significant at 1% level of probability * = Significant at 5% level of probability

The percentage sum of squares for genotype, location and genotype by location interaction (Table 3) revealed that the location contributed the highest proportion 69.25 % of the total variance for grain yield while genotype contributed 7.08 % and the interaction between the genotype and environment contributed 8.37 % .The result is in similar findings of Badu-Apraku *et al*. (1995, 2003) who reported that the largest proportion of total variation in multi-environmental trials is attributed to locations, whereas G and G × L sources of variation are relatively smaller.

Table.3: Percentage sum of squares attributed to genotype (G), location (L) and genotype by location interaction and error as the percentage of the total sum of squares.

Source	df	grain yield
Replication	4	1.42
Genotype (G)	17	7.08
Location (L)	1	69.25
G x L	17	8.37

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Error	68	13.85

Genotype + Genotype × Location Interaction Biplot Analyses

Best Hybrid in each Location

The GGE biplot can be used to identify superior maize genotypes for target locations (Dehghani *et al*., 2009). The biplot (Fig 1) represent a polygon which indicates some of the hybrids located on the vertexes and the others within the polygon. The perpendicular lines split the biplot into different parts and the winning entry for each part is located on the individual vertex (Yan and Tinker, 2006). Therefore, entry CR10 (TZEEI-6 x TZEEI-21) obtained the highest yield at Akomadan and CR7 (TZEEI-29 x TZEEI-4) obtained the highest yield at KNUST.

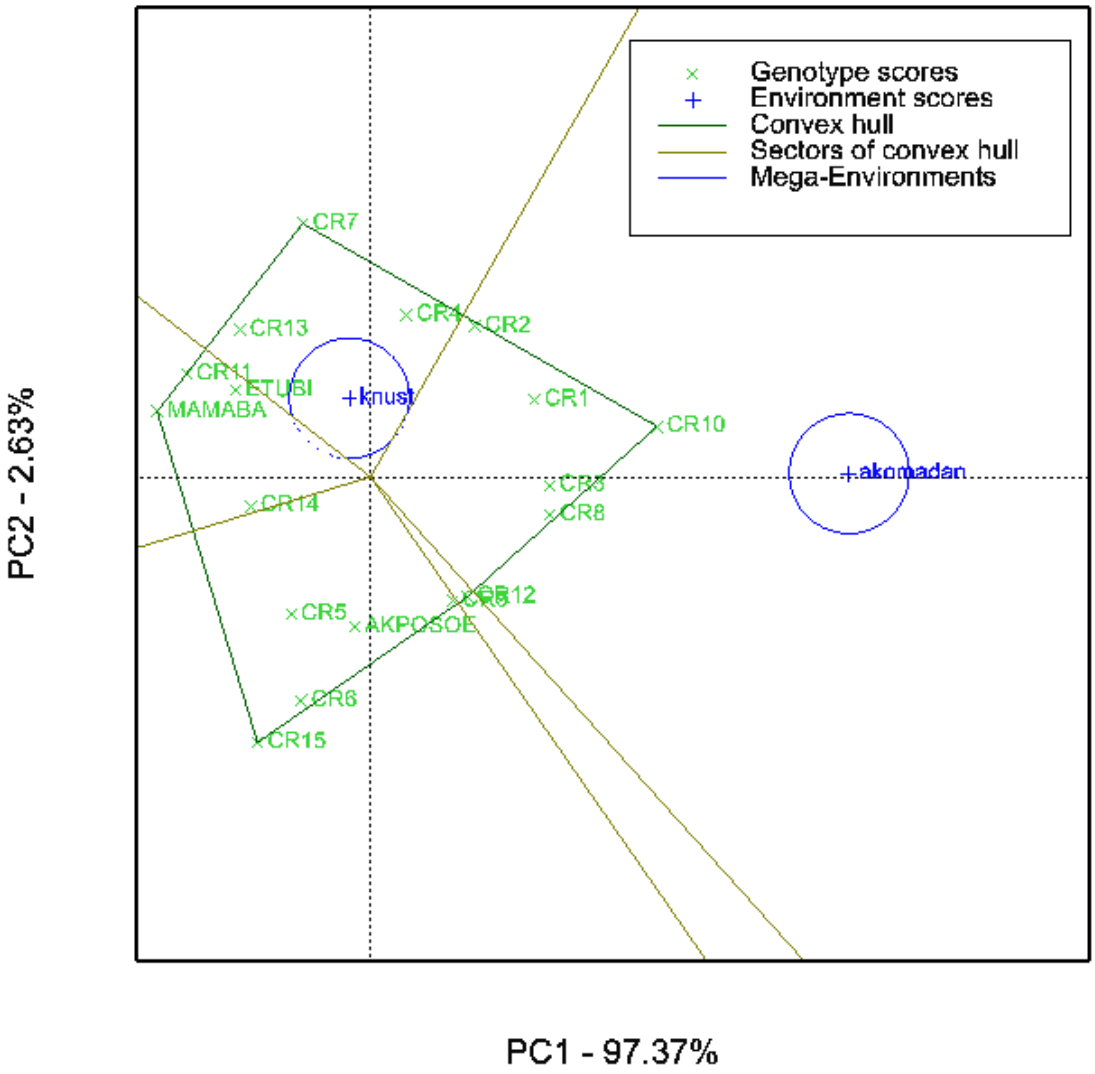

Fig.1: A which-won-where or which was best for what view of the GGE biplot of grain yield for 18 hybrids evaluated across the two locations

Average Yield and Stability of Hybrids

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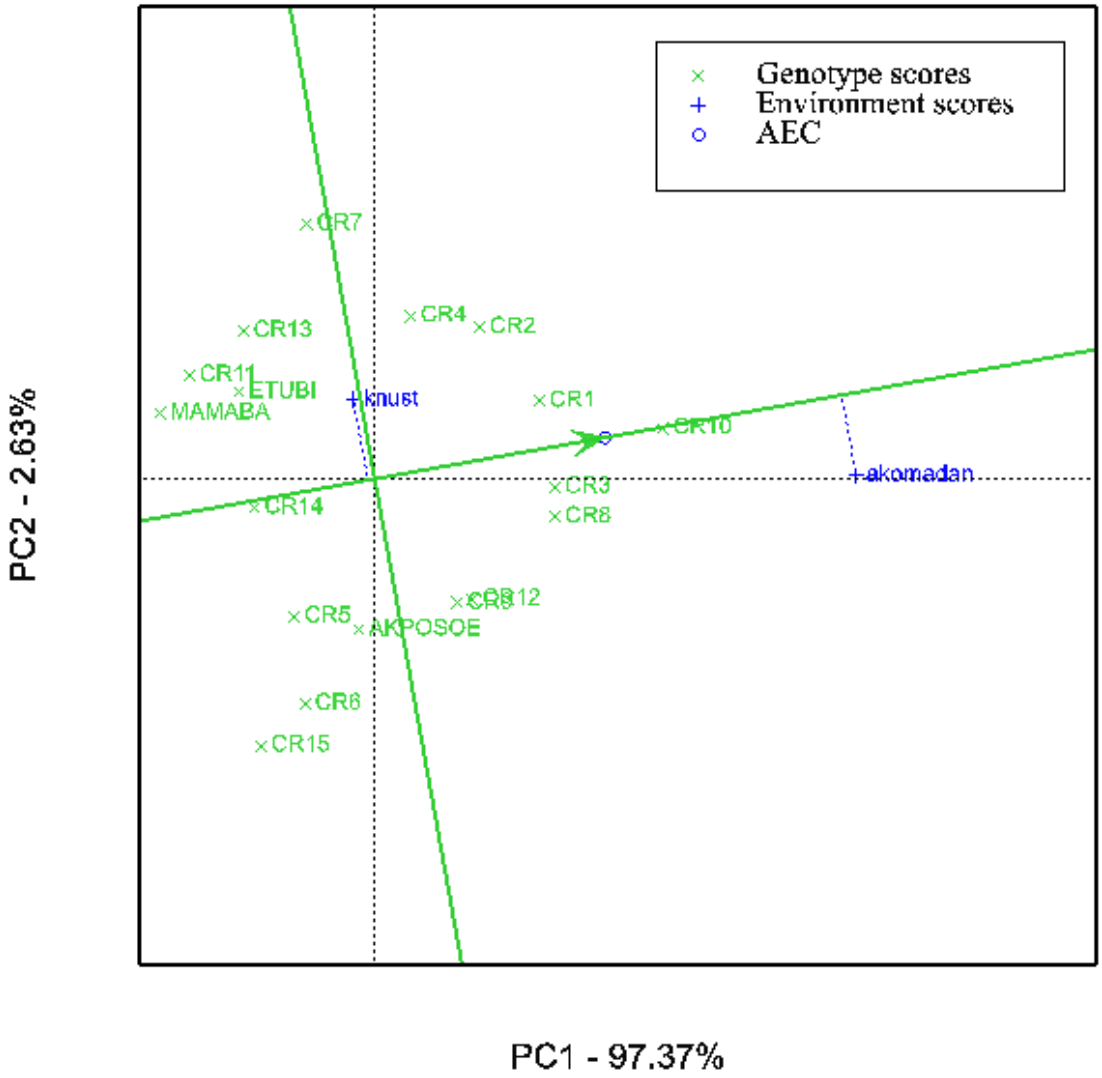

Fig.2: The "mean vs. stability" view of the GGE biplot of grain of grain yield for 18 hybrids evaluated across the two locations

In (Fig 2), the biplot is divided into four parts with an arrowed line (AEC abscissa or x-axies) and a vertical line without an arrow (AEC ordinate or y-axis). The vertical line separates the entries with below average yield from those with above average yield. This simply indicate that entries on the left side of the vertical line obtained lower yield than the average yield while those on the right obtained higher yield than the average yield. The blue circle on the x-axis in the biplot is referred to as the average tester yield. Therefore entry CR10 (TZEEI-6 x TZEEI-21) acquired the highest yield followed by entries CR1 (TZEEI-1 x TZEEI-21), CR3 (TZEEI-15 x TZEEI-1), CR8 (TZEEI-29 x TZEEI-21), CR2 (TZEEI-1 x TZEEI-4), CR4 (TZEEI-6 x TZEEI-15), CR12 (TZEEI-15 x TZEEI-4), CR9 (TZEEI-6 x TZEEI-4), AKPOSOE and CR7(TZEEI-29 x TZEEI-4) in that order. According to Yan *et al*. (2007), the stability of a genotype is determined by their protrusion against the y-axis, hence the nearer the protrusion of the genotype the more stable it is. Therefore, the biplot revealed that the entries CR1 (TZEEI-1 x TZEEI-21), CR10 (TZEEI-6 x TZEEI-21), CR3 (TZEEI-15 x TZEEI-1), CR8 (TZEEI-29 x TZEEI-21) were the most stable hybrids among the highest yielding hybrids because they were closer to the ideal. Similar result of genotypes for their stability under varying conditions was reported by Tiwari *et al.*, (2014). In contrast, entry CR7 (TZEEI-29 x TZEEI-4) was the least stable among the highest yielding hybrids. However, CR14 (TZEEI-29 x TZEEI-1) which is among the lowest yielding is more stable. Entries CR6 (TZEEI-21 x TZEEI-4) and CR15 (TZEEI-15 x TZEEI-21) were not only low yielding but also amongst the least stable hybrids. Among the checks themselves, Akposoe was highly unstable.

Location Ranking Based on both Discriminating Ability and Representativeness

Discriminating ability and representativeness of the trial environment is presented in (Fig 3). An ideal trial

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environment may be defined as one that is most discriminating for genotypes and representative of all other environment (Yan, 2001; Yan and Kang, 2003). Although in real life situation an ideal environment might not exist, it can be used as a reference for genotype selection in multi-location yield experiment. It is represented in the biplot by a tiny blue circle with an arrow it (Yan *et al*., 2007), and the longer the projection, the more discriminative the environment. On the bases of this requirement, KNUST was highly discriminating but least representative of the test environments whiles Akomadan was most representative and discriminating of the test environments. Hence Akomadan was identified as the most ideal trial environment under irrigation. A similar finding was obtained by Abdulai *et al*. (2007).

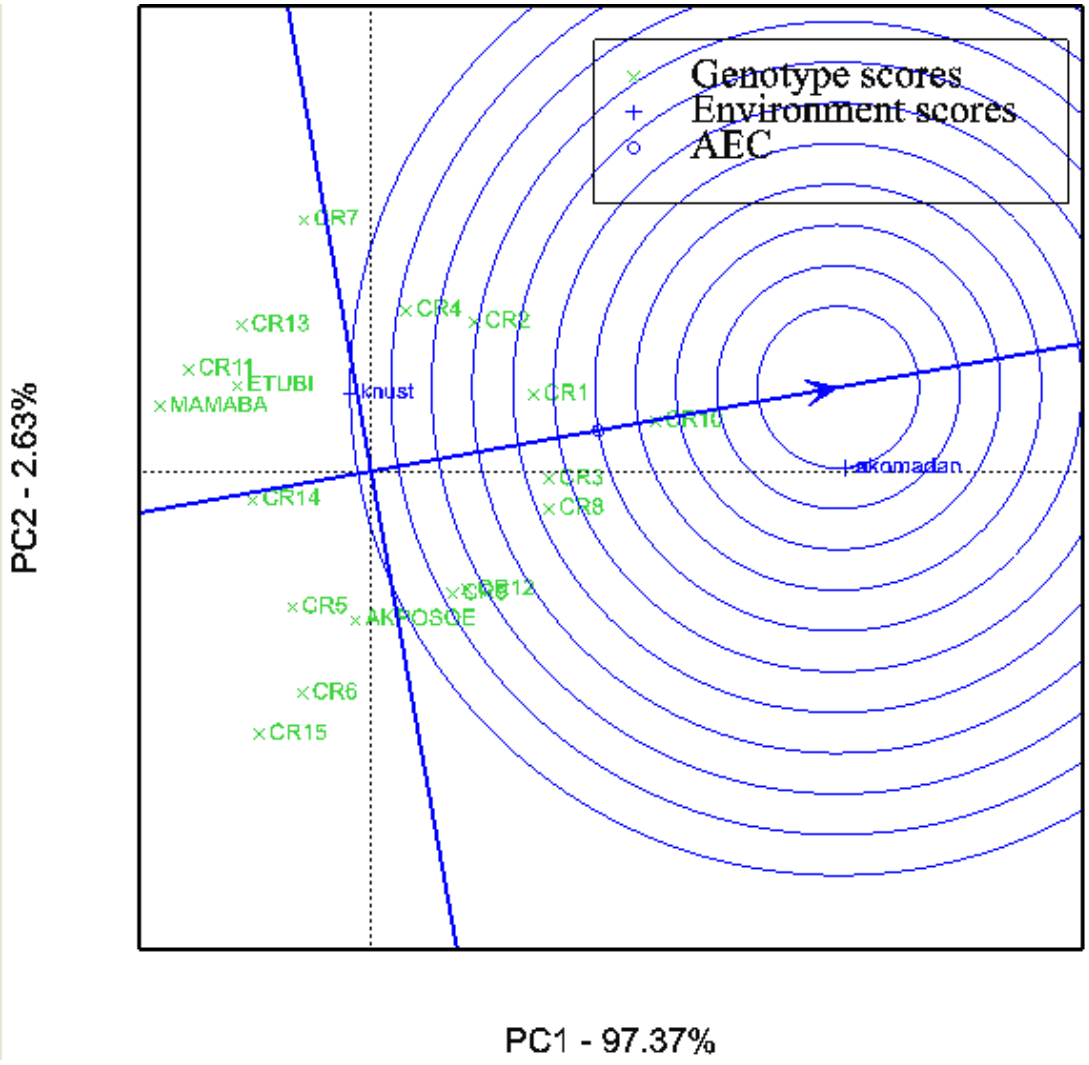

Fig.3: The ranking of locations based on discriminating ability and representativeness GGE biplot of grain yield for the 18 hybrids evaluated across the two locations

VI. CONCLUSION

Out of the 18 genotypes evaluated across the two locations, the result from the combined analysis revealed that the location contributed the highest proportion 69.25 % of the total variance for grain yield while genotype contributed 7.08 % and the interaction between the genotype and environment and error contributed 8.37 % and 13.85 %, respectively. This indicates that the environment plays a vital role in selecting hybrids for higher grain yield and adaptation. The use of GGE biplot analyses provided clear bases for determining stability and performance of the eighteen extra-early maize hybrids. Akomadan was the best environment test location for selecting genotypes with wide adaptability. TZEEI-6 x TZEEI-21, TZEEI-1 x TZEEI-21, TZEEI-15 x TZEEI-1 and TZEEI-29 x TZEEI-21 obtained higher yield potential. Hence these hybrids were considered high yielding and stable.

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Forecasting Biomass Loss and Carbon Released to the Atmosphere as a Result of Habitat Conversion of Eastern Selous-Niassa TFCA

Adili Y. Zella[1], Josephat Saria[2], Yohana Lawi[3]

[1]Department of Economic Studies, The Mwalimu Nyerere Memorial Academy (MNMA)-Zanzibar, P.O Box 307, Zanzibar, Tanzania.

[2,3]Department of Environmental Studies, Faculty of Science, Technology and Environmental Studies, Open University of Tanzania, P.O Box 23409, Dar es Salaam, Tanzania.

***Abstract*—** *Terrestrial climate change predictions use various models that are based on atmospheric parameters combined with projected carbon emission scenarios. Increased levels of carbon emissions into the atmosphere are accelerated by human activities and are the main reason of climate change (CC). CC threatens networks of protected areas (PAs) and forced many species out of PAs. Unfenced PAs gives species opportunity to migrate from one PA to another or other unprotected areas to sustain their climatic niche. Many PAs in SADC countries including transfrontier conservation areas (TFCA) are unfenced; hence, connectivity of PAs uses corridors. However, many of these corridors are unprotected and advocacies adaptation of reserved fauna and flora under CC. This paper explains the less known amount of biomass loss and carbon released to the atmosphere as result of habitat conversion of eastern corridor of Selous – Niassa TFCA which connecting the two PAs of Tanzania and Mozambique. Specifically, the study predicts amount of biomass loss, amount of carbon released to the atmosphere and amount of conservation profit disposed as a result of habitat conversion from 2015 to 2035. Existing data on spatial and temporal changes in land use and land cover (LULC) of eastern corridor of Selous – Niassa TFCA from 1986 – 2016 was analysed and used to forecast LULC from 2015 to 2035 by using CA-Markov model. The forecasted LULC from 2015 to 2035 was analysed to get intended results. The results revealed that, an average amount of 29559.8 tons of biomass (above ground + below ground + deadwood) loss annually from 2015 to 2035. Consequently, average amount of 40217.2 tons of carbon (above ground + below ground + deadwood) released to the atmosphere annually from 2015 to 2035 equivalent to US$ 160868.6 per annum if REDD+ implemented. The study concludes that, there is a need to include virgin corridors into core PAs network or formulation of sustainable conservation strategies that will consider climatic niche of both flora and fauna without compromising livelihoods of corridor dwellers.*

Keywords*— *Habitat conversion, Climate change mitigation and adaptation, Biomass, Carbon

I. INTRODUCTION

1.1 Background information

Terrestrial climate change predictions use various models that are based on atmospheric parameters combined with projected carbon emission scenarios. However, uncertainty is associated with these predictions, and at least 16 different models are in use (Nature Conservancy, 2009; Malimbwi *et al*., 2016). These global models have high uncertainties as emission scenarios vary depending on future energy use choice of different communities; and those models are suitable in predicting changes in temperature rather than precipitation. These global models have advantage of being, in principle, applicable anywhere. However, due to great variation in climatic and edaphic factors, such models can yield large error locally. Thus, a model developed on data from a smaller region, will within that region give more accurate estimates (Malimbwi *et al*., 2016; Kulindwa *et al.*, 2016; Lobora *et al*., 2017).

Increased levels of carbon emissions into the atmosphere accelerated by human activities are the main reason of climate change. Terrestrial carbon sinks include soils, trees and other vegetation soaks up at least half of annual greenhouse gases emissions from fossil fuels resulting to slow down of climate –warming gases in our atmosphere (Kulindwa *et al.*, 2016). Carbon dioxide (CO_2) sequestration is one of great role of forests and woodlands ecosystem, other roles include protection of watersheds, soil conservation, conservation of biodiversity, sustaining

cultural values, climatic amelioration and eco-tourism. Despite all the valuable and invaluable goods and services provided by forests, there are high rates of deforestation and forest degradation in developing countries (Malimbwi*et al*., 2016). For instance, Tanzania is among the developing country in East Africa where the aforementioned scenario accelerating deterioration of over 10,000 plant species, hundreds of which are nationally endemic. Among plant and animal species in Tanzania, 724 are identified as "Threatened" in the World Conservation Union (IUCN) Red List with 276 species classified as "Endangered" (IUCN, 2013). Some of these forests and woodlands are within protected areas (PAs) rich of biodiversity (fauna and flora) of different categories and others unprotected areas termed as public good (general land). These scenarios necessitate development of allometric models for measuring biomass and volume of different forest and woodland species of Tanzania (URT, 2015).

All PAs in Tanzania are unfenced. Wildlife uses that opportunity to migrate from one PA to another or other areas for climate change adaptation. One of the unprotected areas for adaptation are wildlife/biodiversity corridors which connect two or more PAs within the country or transfrontier conservation areas (TFCA). Connectivity of PAs through corridors advocacy adaptation of reserved fauna and flora under climate change. The protection of corridor biodiversity relies on the ability to assess hot spots, quantify and predict spatial and temporal trends of key species, maintain a natural disturbance regime, and limit harmful human activities (Stohlgren *et al.*, 1999).

1.2 Problem statement

Classification and management of networks of protected areas (PA) is pre-requisite for sustainable biodiversity conservation. However, these networks are commonly considered static because areas that have been classified as PAs almost never declassified (Mascia and Pailler, 2011; Malimbwi *et al*., 2016). Habitat loss, fragmentation, over-hunting and resource depletion are among drivers of populations decline which PAs safeguarding species from those shocks(UNEP 1992); consequently, climate change forced many species out of PAs (Kulindwa *et al*, 2016; Lobora *et al*., 2017). Certainly, models project that several PAs will lose suitable habitats for species of high conservation concern (Hole *et al*., 2009; Kharouba and Kerr, 2010; Ara_ujo *et al*., 2011; Virkkala *et al*., 2013; Malimbwi *et al*., 2016; Kulindwa *et al*, 2016; Lobora *et al*., 2017). To address this challenge, researchers and conservation bodies recognize that new conservation areas will need to be designated in future (Hannah & Salm 2003; Ara_ujo 2009b; Lobora *et al*., 2017). This scenario faces many transfrontier conservation areas (TFCAs) in Africa including Selous – Niassa TFCA.

Selous - Niassa TFCA is connected by the corridor between Selous Game Reserve (Tanzania) and Niassa National Reserve (Mozambique) making an area of 154000 km^2 of natural miombo woodlands ecosystem. The TFCA consist a network of PAs of various categories of protection; an area of 110,000 km^2 of this ecosystem is presently under conservation (Baldus and Hahn, 2009). The corridor connecting these two PAs to form TFCA is unprotected ecosystem. However, areas adjacent to TFCA PAs formulated WMAs (wildlife management areas) so as to involve community in conservation of wildlife outside PAs. Though, the effects of climate change and variability suggests that, WMAs is not enough strategy as species use the corridor for migration and others adapted in the corridor due to its suitability for their climatic niche.

Therefore, the increasing concerns over the impacts of climate change necessitate the inclusion of new PAs categories to increase connectivity between PAs through wildlife/biodiversity/habitat corridors. This study intended to forecast amount of biomass loss, carbon released to the atmosphere, and conservation profit to be disposed as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

1.3 Objectives

1.3.1 Main objective

The main objective of this study was to predict amount of biomass and carbon released to the atmosphere as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA

1.3.2 Specific objectives

Specifically the study intends to:

(i) predict amount of biomass loss of eastern corridor of Selous-Niassa TFCA from 2015 to 2035
(ii) predict amount of carbon released to the atmosphere as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035
(iii) predict amount of conservation profit disposed as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Description of the Study Area

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The study was carried out in eastern Selous-Niassa TFCA with an area of 1, 462, 560 hectares called Selous-Niassa wildlife corridor (SNWC) which extends across southern Tanzania into northern Mozambique (Figure 1). Administratively passes in Liwale, Nachingwea,Masasi, and Nanyumbu Districts. Migration of elephants, buffalos and zebras has been observed (Pesambili, 2003; Ntongani *et al*., 2007). Two migratory routes have been identified as follows:

(i) From Selous through Nahimba, Nakalonji, Mbondo, Kilimarondo, Matekwe and Kipindimbi proposed game reserve (GR) in Nachingwea District and then via Msanjesi, Mkumbalu, Sengenya, Nangomba and Nanyumbu in Nanyumbu District to Lukwika-Lumesule GR and then crosses Ruvuma River to the Niassa GR.

(ii) From Selous to Kiegei, Namatumu, Kilimarondo in Nachingwea then along Mbangala and Lumesule rivers to Mchenjeuka and Mitanga in the Lukwika-Lumesule GR, from where they cross the Ruvuma River to the Niassa Reserve.

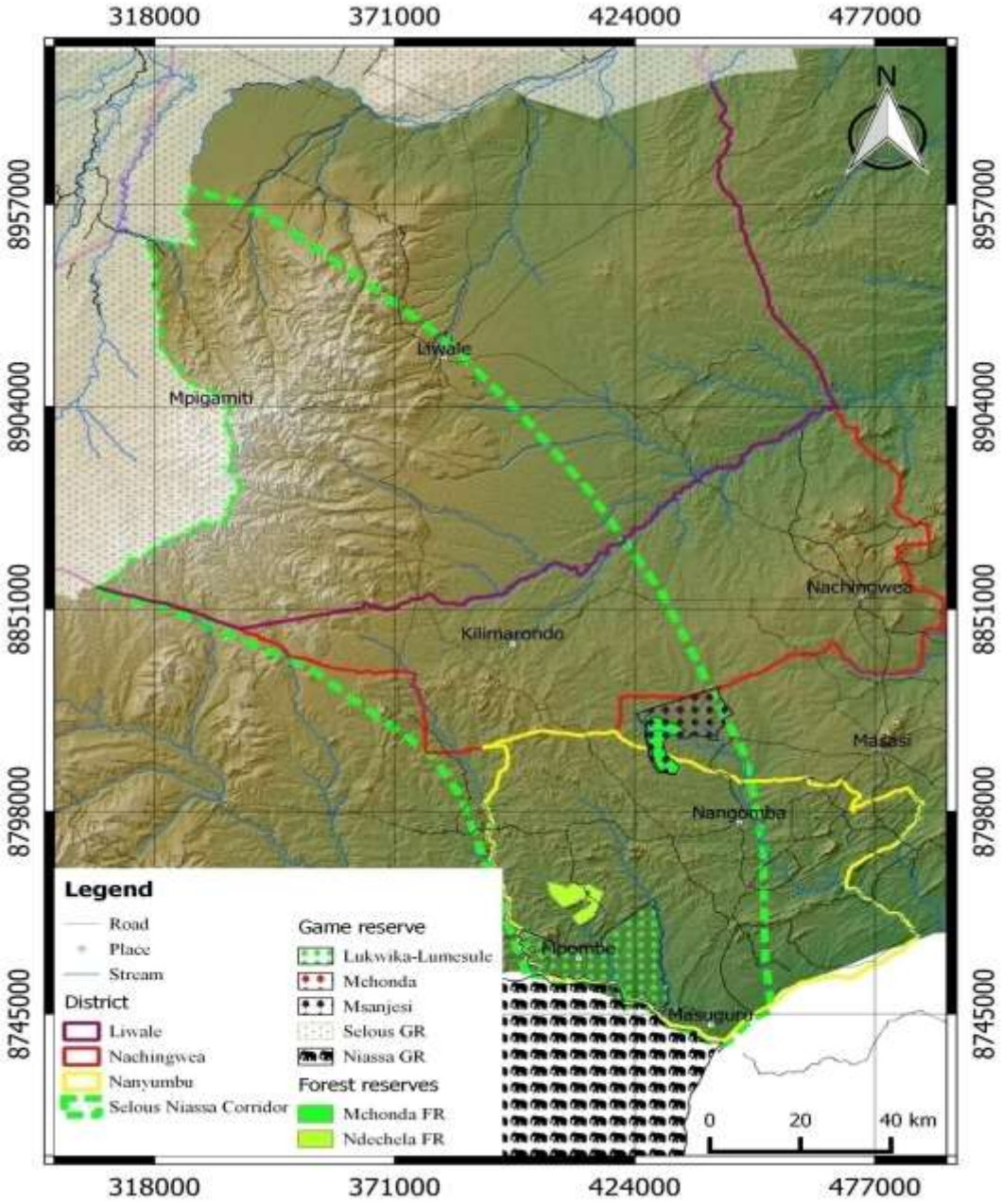

Fig.1: The Map of the study area

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These routes forms SNWC called Selous-Masasi corridor includes the Msanjesi (2,125 ha) and the Lukwika-Lumesule (44,420 ha) GRs in Masasi and Nanyumbu Districts respectively; wildlife management areas (WMAs) bordering Selous, Msanjesi and Lukwika-Lumesule game reserves (MAGINGO WMA, NDONDA and MCHIMALU proposed WMAs respectively) which are within Liwale, Nachingwea/Masasi and Nanyumbu Districts respectively.

2.2 Methods

Prediction of land use and land cover change of eastern corridor of Selous - Niassa TFCA from 2015 to 2035 using Markov Chain Analysis and Cellular Automata Analysis, jointly called CA–Markov was employed as shown in Figure 2 and Table 1.

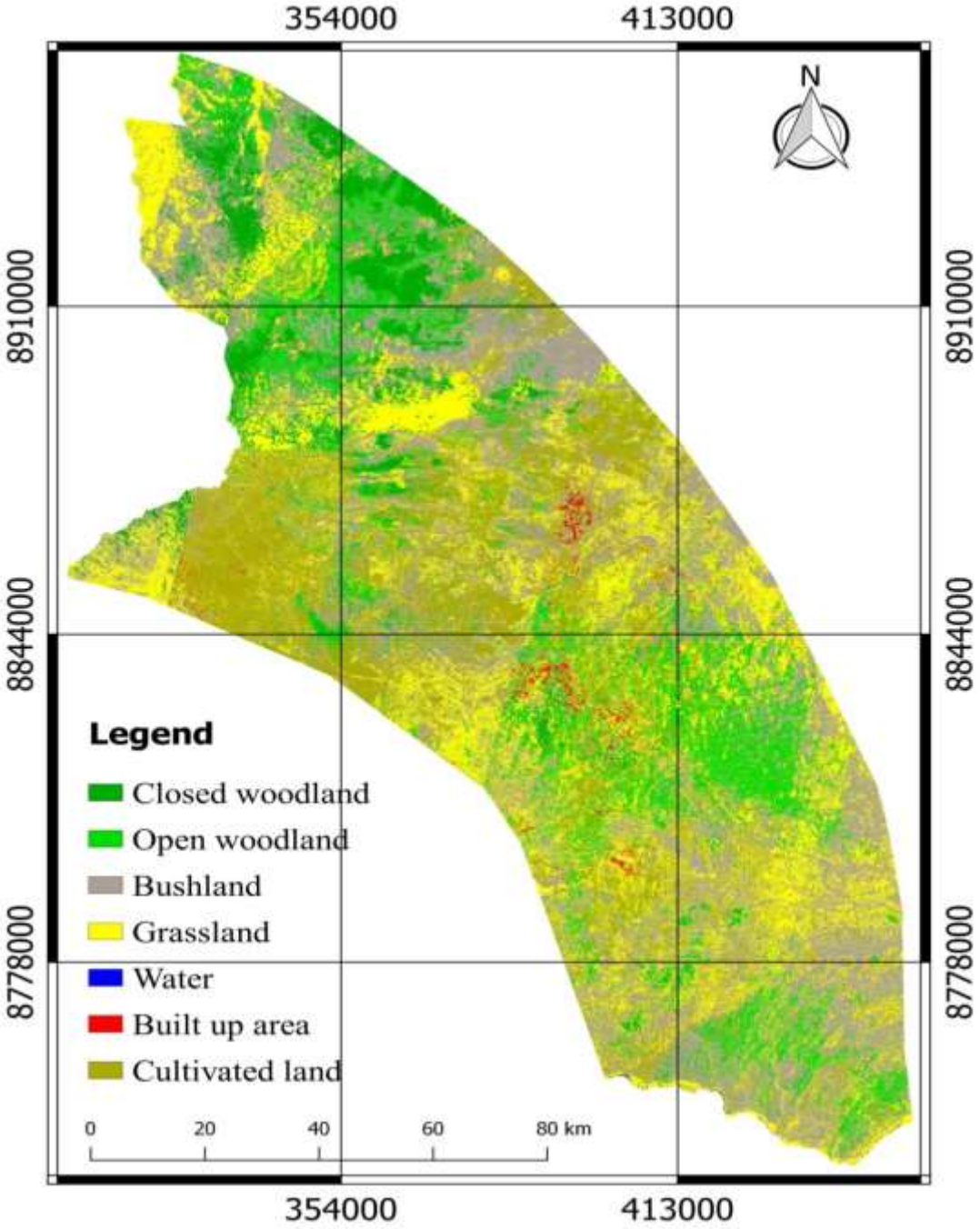

Fig.3: Projected Land use/cover map for eastern corridor of Selous-Niassa TFCA for 2035

Table.2: Land use/cover area distribution in 2035

LULC	2015		2035	
	(Ha)	(%)	(Ha)	(%)
Closed woodland	89923	6.15	81981	5.61
Open woodland	220217	15.06	211690	14.47
Bushland	480269	32.84	411950	28.17
Grassland	394461	26.97	411272	28.12
Water	646	0.04	242	0.02
Built up area	8851	0.61	12749	0.87
Cultivated land	268193	18.34	332676	22.75
TOTAL	**1462560**	**100**	**1462560**	**100**

2.2.1 Data analysis

To predict amount of biomass loss of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

2.2.1.1 Biomass Stocks

2.2.1.1.1 Living Biomass Stocks

Tanzania forest Carbon can be estimated in three pools namely AGB (above ground biomass), BGB (below ground biomass) and DW (dead wood) (URT, 2015). BGB was estimated as a fraction of AGB. AGB and BGB were estimated as follows:

(i) AGB (tonnes/ha) = Tree stem volume (m^3/ha) * wood density/1000; and

(ii) BGB (tonnes/ha) = AGB * 0.25 (as default), or root to shoot ratios.

URT (2015) uses conversion factors into programmed NAFORMA analysis system by tree species or species groups to provide standards in each terrestrial ecosystem of Tanzania as shown in Table 3.

Table.3: Living tree stemwood biomass by primary vegetation type

Primary Vegetation Type	CWD	OWD	BS	GL	WTR	CL	BLT
Aboveground Biomass (t/ha)	59.5	27.7	11.0	2.9	4.6	5.9	2.9
Belowground Biomass (t/ha)	18.2	9.5	4.4	1.1	1.7	2.1	1.1

CWD = Closed woodland, **OWD** = Open woodland, **BS** = Bushland, **GL** = Grassland, **WTR** = Water, **CL** = Cultivated land and **BLT** = Built Up area

2.2.1.1.2 Deadwood Biomass Stocks

Dead wood (DW) biomass is estimated from the volume computed using Smalian formula multiplied by wood density of 619 kg/m^3 (Chidumayo, 2012 cited by URT, 2015). URT (2015) through NAFORMA reveals the dead wood Biomass of Tanzania (Table 4) is relatively low since most dead wood in accessible areas is collected as fuelwood. As woodlands are generally more accessible than forests, collection of deadwood for fuelwood from these areas is easier. The relatively high volume of dead wood in water is assumed to be because dead trees lying in areas with water / wetlands are difficult to access and decay slowly and because they are wet and therefore unattractive for fuelwood.

Table.4: Dead wood biomass by primary vegetation type

Primary Vegetation Type	CWD	OWD	BS	GL	WTR	CL	BLT
Biomass (t/ha)	4.87	1.82	0.73	0.35	1.31	0.91	0.22

CWD = Closed woodland, **OWD** = Open woodland, **BS** = Bushland, **GL** = Grassland, **WTR** = Water, **CL** = Cultivated land and **BLT** = Built Up area

To predict amount of carbon released to the atmosphere as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

2.2.1.2 Carbon Stocks

According to URT (2015), carbon in terrestrial ecosystems of Tanzania can be computed as follows:
Carbon (tonnes/ha) = Biomass * 0.47
Living tree stemwood and dead wood carbon (t/ha) by primary vegetation type are illustrated in Table 5& 6.

Table.5: Living tree stemwood Carbon (Aboveground + Belowground) by primary vegetation type

Primary Vegetation Type	CWD	OWD	BS	GL	WTR	CL	BLT
Carbon (t/ha)	36.5	17.5	7.2	1.8	3.0	3.8	1.9

CWD = Closed woodland, **OWD** = Open woodland, **BS** = Bushland, **GL** = Grassland, **WTR** = Water, **CL** = Cultivated land and **BLT** = Built Up area

Table.6: Dead wood Carbon by primary vegetation type

Primary Vegetation Type	CWD	OWD	BS	GL	WTR	CL	BLT
Carbon (t/ha)	2.39	0.89	0.36	0.17	0.64	0.45	0.11

CWD = Closed woodland, **OWD** = Open woodland, **BS** = Bushland, **GL** = Grassland, **WTR** = Water, **CL** = Cultivated land and **BLT** = Built Up area

To predict amount of conservation profit disposed as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

The study adopted from Jenkins (2014), Malimbwi *et al.* (2016), Kulindwa *et al.* (2016) and Lobora *et al.* (2017) emphasized different carbon market per ton depending on different countries and continents, but the standard carbon market suggested is US$ 4 per ton if REDD+ is implemented. This was used to predict amount of conservation profit that will be disposed from 2015 to 2035 as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA.

III. RESULTS AND DISCUSSION

3.1 Amount of biomass that will be loss in eastern corridor of Selous-Niassa TFCA from 2015 to 2035

The results in Table 7 and Table 8 revealed that, 124.5% of biomass will be loss in woodland, bushland and water; while, 24.5% of biomass will be stored by other vegetation type as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA . Bushland alone will loss 67.7% of biomass, followed by woodlands (56.7%). This implies that, average amount of 36801.95 tons of biomass (above ground + below ground + deadwood) from woodlands; bushland; and water will be loss annually from 2015 to 2035. Moreover, average amount of 7242.19 tons of biomass (above ground + below ground + deadwood) from other vegetation type will be stored annually from 2015 to 2035. The results shows that, the natural vegetation will be degraded and new tree species will take (under succession) place. The degradation will impacts negatively ecosystem services offered to wildlife residing or using the area for migration or adapting to climatic change. The degraded area will be converted to bushland, cultivated land and built up area due to increase of human population, livestock, and dependence of corridor dwellers on existing natural resources in the ecosystem for their livelihoods. Thereof, the average total annual loss will be 29559.76 tons of biomass (above ground + below ground + deadwood) in all vegetation type from 2015 to 2035. These results necessitated the emergence of new management strategies of the area which will assure the survival of wildlife without compromising livelihoods of corridor dwellers. The existing formulation of wildlife management areas (WMAs) of Liwale (MAGINGO), Nachingwea (NDONDA) and Nanyumbu (MCHIMALU) districts relies only adjacently to core PAs of Selous, Msanjesi and Lukwika-Lumesule game reserves, and forgetting other areas which are crucial to wildlife, using their living habitat and migration trails.

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Table.7: Amount of living tree stemwood biomass (Aboveground + Belowground) loss of eastern corridor Selous-Niassa TFCA from 2015 to 2035

Primary Vegetation Type	Total area converted (ha)	Above ground biomass loss (t/ha)	Below ground biomass loss (t/ha)	Total Biomass loss (t)	Biomass loss (%)
Closed woodland	7942	4.87	18.2	183221.9	35.91
Open woodland	8527	1.82	9.5	96525.64	18.92
Bushland	68319	0.73	4.4	350476.5	68.69
Grassland	-16811	0.35	1.1	-24376	-4.78
Water	404	1.31	1.7	1216.04	0.24
Cultivated land	-3898	0.91	2.1	-11733	-2.3
Built up area	-64483	0.22	1.1	-85117.6	-16.68
Total				**510213.6**	**100.00**

Table.8: Amount of dead wood biomass loss of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

Primary Vegetation Type	Total area converted (ha)	Biomass loss (t/ha)	Total Biomass loss (t)	Biomass loss (%)
Closed woodland	7942	4.87	38677.54	47.76
Open woodland	8527	1.82	15519.14	19.16
Bushland	68319	0.73	49872.87	61.59
Grassland	-16811	0.35	-5883.85	-7.27
Water	404	1.31	529.24	0.65
Cultivated land	-3898	0.91	-3547.18	-4.38
Built up area	-64483	0.22	-14186.3	-17.52
Total			**80981.5**	**100.00**

3.2 Amount of Carbon released to the atmosphere as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

The results in Table 9 and Table 10 revealed that, 122.29% of carbon will released to the atmosphere from in woodland, bushland and water; while, 22.9% of carbon will be stored by other vegetation type as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA. Bushland alone will loss 64.1% (516491.6 tons) of carbon, followed by woodlands 57.89% (465675.9 tons). This implies that, average amount of 49181.91tons of carbon (above ground + below ground + deadwood) from woodlands; bushland; and water will be loss annually from 2015 to 2035. Moreover, average amount of 8964.75 tons of carbons (above ground + below ground + deadwood) from other vegetation type will be stored annually from 2015 to 2035. This is something that we can never stay quiet; and the need to act urgently is unquestionable. Thus, the need for sustainable utilization and management of natural resources in the area is vital. Nevertheless, the average total annual loss will be 40217.16 tons of Carbon (above ground + below ground + deadwood) from 2015 to 2035. Since, climate change is a result of increasing greenhouse gases in the atmosphere, there are must be strategies to reverse the situation. If, we decide to include the area into core PA network, we must revise the current participatory management strategies which insist on formulation of Wildlife Management Areas (WMAs) but forgetting that those WMAs are only adjacent to core PAs which in other scenarios doesn't fit. Thus, the need to formulate other management strategies that will include all areas in the corridor which has wildlife climatic niche; economical and ecological importance for corridor dwellers is unavoidable.

Table.9: Amount of living tree stemwood Carbon (Aboveground + Belowground) that will be released to the atmosphere as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

Primary Vegetation Type	Total area converted (ha)	Carbon loss (t/ha)	Total Carbon loss (t)	Share (%)
Closed woodland	7942	36.5	289883	37.91
Open woodland	8527	17.5	149222.5	19.52
Bushland	68319	7.2	491896.8	64.33
Grassland	-16811	1.8	-30259.8	-3.96
Water	404	3.0	1212	0.16
Cultivated land	-3898	3.8	-14812.4	-1.94
Built up area	-64483	1.9	-122518	-16.02
Total			**764624.4**	**100**

Table.10: Amount of dead wood Carbon that will be loss in eastern corridor of Selous-Niassa TFCA from 2015 to 2035

Primary Vegetation Type	Total area converted (ha)	Carbon loss (t/ha)	Total Carbon loss (t)	Share (%)
Closed woodland	7942	2.39	18981.38	47.79
Open woodland	8527	0.89	7589.03	19.11
Bushland	68319	0.36	24594.84	61.92
Grassland	-16811	0.17	-2857.87	-7.19
Water	404	0.64	258.56	0.65
Cultivated land	-3898	0.45	-1754.1	-4.42
Built up area	-64483	0.11	-7093.13	-17.86
Total			**39718.71**	**100**

3.3 Amount of conservation profit that will be disposed as a result of habitat conversion of eastern corridor of Selous-Niassa TFCA from 2015 to 2035

Results in Table 11 revealed that, eastern corridor of Selous – Niassa TFCA will loss an average amount of US$ 160868.6 of carbon trade annually from 2015 to 2035 due to habitat conversion of the area. Woodlands, bushland and water pioneered degradation on which they will loss an annual average of US$ 196727.6 from 2015 to 2035. It seems that the area have potential hard wood species which are regarded as commercial rewarding but environmental destructive by corridor dwellers. Sustainable utilization of natural resources in the area is of important priority. Thus, we need to integrate community in management of the area by combined PFM (Participatory Forest Management), JFM (Joint Forest Management) and WMA (Wildlife Management Areas) and having one entity which will be integral and community-centered in decision making on corridor management.

Table.12: Amount of conservation profit disposed as a result of habitat conversion of eastern Selous-Niassa TFCA from 2015 to 2035

Primary Vegetation Type	Total Carbon loss (t)	Amount of money loss (US$)	Share (%)
Closed woodland	308864.4	1235458	38.4
Open woodland	156811.5	627246.1	19.5
Bushland	516491.6	2065967	64.21
Grassland	-33117.7	-132471	-4.12
Water	1470.56	5882.24	0.18
Cultivated land	-16566.5	-66266	-2.06
Built up area	-129611	-518443	-16.11
Total	**804343.1**	**3217372**	**100**

IV. CONCLUSION AND RECOMMENDATIONS

This study predicted amount of biomass loss and carbon released to the atmosphere as a result of habitat conversion of eastern corridor of Selous – Niassa TFCA from 2015 to 2035**.** The findings have revealed that, the study area will undergo notable biomass loss of 591195.1 tons due to socio-economic activities performed by corridor dwellers. Also amount of carbon released to the atmosphere of 804343.1 tons can contribute much to climate change and climate variability. The amount of conservation profit disposed of annual average of USD 160868.6 seems to offset amount of benefit received by corridor dwellers from their destructive activities if adopted REDD+ strategies. The scenario necessitates formulation of sustainable management strategies that will emphasis on species adaptability in the corridor ecosystem in regard to their climatic niche without compromising livelihoods of corridor dwellers. Furthermore, adequately adapting conservation policies to climate change requires a paradigm shift. Specifically, planners need to adopt a long-term view and accept that under budgetary constraints the release of PAs areas that become redundant at some point in time might be required if new conservation areas (wildlife corridors) are to be designated to meet conservation targets.

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Functional plasticity and tolerance to drought conditions of 11 apple tree varieties grown in Morocco.

Ouassat S. [1*], Allam L.[2]

[1]Department of Biology, Laboratory of Health and Environment, team of plant protection; Faculty of sciences, Meknes-Morocco

[2]Supervisor, Tel +212 674 904 640

***Abstract*—** *In this study, 11 varieties were grown in the experimental chamber in order to determine the morphological, anatomical and physiological characteristics of each. The experimental protocol was carried out under controlled conditions of irrigation, temperature and hygrometry. The values obtained of the thickness, the cuticle, length of the ostiole, density and size of the stomata and then of stomatal and cuticular sweat have made it possible to calculate the stomatic resistance of each variety and to evaluate its potential to adapt to drought conditions. We have highlighted significant differences related to variety through the Duncan test. 4 groups are identified and the results are discussed in this article.*

Keywords —Malus, Apple varieties, transpiration, stomata, stomatal resistance.

I. INTRODUCTION

The apple tree belongs to the Rosacea family, such as pear, peach, and requires for its development the deep, moist soils with a generally neutral PH, the apple tree shows an important requirement for water and cold[1].The apple tree is irrigated. Nearly 99% of the planted area is irrigated, compared with only 1% in the countryside. The most used irrigation method is gravity irrigation with 61% of the area compared with 39% for the localized irrigation system [2]

Water, a precious element, is notalways available in quantitysufficient for plants, whichgrow in arid and semi-arid regions. Among the factors influencing the decision-making process in irrigation, the threshold level of available soil water content is critical for irrigation timing [3]According to water requirements, plants are divided into three distinct groups: hygrophytes adapted to wet bioclimate and grow in aqueous media and tolerate high humidity levels, from mesophytes to sub humid to semi-arid bioclimates and xerophytes to bioclimate arid and high mountains.

Within the xerophytes, we distinguish between two different types, the ephemerophytes, which show nodrought resistance mechanism and whose adaptation is manifested by the development of a short vegetative cycle; on the other hand, physiologically active xerophytes which resist drought by physiological, biochemical, morphological and anatomical mechanisms and structures [4]. They avoid the dehydration of their tissue; by maintaining a high water potential [5], the reduction of transpiration, increased pubescence, development of a strong root system, leaf rolling, sinkingstomata in the epidermis, transformation of the leaves into needles andor scales or spines and the decrease of the conductancestomatic. The varieties examined in this study resist senescence by the acquisition of certain characteristics of adaptations. We then seek to get them out by comparing the morphological and anatomical characteristics of adaptation to drought conditions

II. MATERIEL AND METHOD:

In this study, 11 varieties of apple trees grown in the Arbor orchard were subjected to examination to raise their morphological, anatomical and physiological characteristics of seed tolerance, Jeromine ,It ,Red shift, skarlet, Royal-galla ,Obro-galla,Buckey ,Brookfield ,Golden reinders ,Golden Delicious and Anna. For each variety, 5trees are used. ***0.0)***

opted for the black peat ,because its organic matter content is very high (about 90%) and its water retention rate is important (60-70%), no factor limiting the growth of plants is recorded during our test.

Table.1 shows the general characteristics of each cultivar

Culture conditions:

The protocol was carried out in the experimental greenhouse. The seedlings of these 7 varieties are grown in plastic of 30 cm diameter and 50 cm of height, we

Varietal density of stomata:

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In the apple tree (rosacea family), the stomata are located on the underside of the leaves. The density of stomata was determined from five measurements. Five fully developed leaves were selected from five plants of the same variety. Five Repetition for each variety to produce a representative mean value. Practically, the density of stomata (number of stomata per square millimeter) is obtained from the foliar epidermal implications of a colorless adhesive tape; the prints obtained are then glued to a slide. Stomata are counted microscopically on slides graduated in millimeters.

Length of the stomata ostiole:

The measurements were carried out under a microscope(Nikon E200 LED) with a graduated eyepiece micrometer (µm). Thin sections leaves were prepared and their contents was destroyed by the hypochlorite of sodium. The cuts were doubled coloration with alumina carmine and green of iodine to differentiate the different cell compartments. For each variety, five plants been used and, for each plant, the length of stomatal diffusion or length of the ostiole was measured in average over five cuts.

Varietal resistance of stomata:

The stomatal resistance of the pores as described by[6]is computed by the following general equation:

$R_s = [(L / ncb) + (\log (4c/b))/nc]*(1/D_v n)$

It is first necessary to determine the fundamental parameters of this equation such as:

Rs: Stomatal resistance (s / m)
c: half-length of the stomatal pore (m)
b: half width of the stomatal pore (m)
L: length of the ostiole or diffusion of the pore (m)
n: density of stomata (number of stomata per m2)
Dv: diffusion of water vapor into air (m2 / s)

At each luminous intensity applied to the plants, epidermal fragments of 5 leaves of each variety are taken to determine the dimensions of the stomatal pore at the opening.

Thickness of the cuticle and Vessel-cuticle length:

For this purpose, we have applied the same preceding preparations for the determination of the length of the ostiole or stomatal diffusion in order to determine the thickness of the cuticle and the length separating the vessels of the cuticle from the seven varieties. Five plants are retained for each variety and 10 leaves for each plant, 10 sections are observed under the microscope (NikonE200 LED).

Cuticular sweat:

The experimental protocol related to the determination of cuticular sweat consists in placing the plants in a chamber for an hour in a dark tight chamber to prevent any stomatal sweat. To avoid the evaporation of water from the substrate-culture system, pots are wrapped perfectly in plastic. We then proceed to measure the difference in weight related to the time interval used and to the leaf area of the plant, hence the loss of water dissipated cuticularly.

The sweat is due essentially to the opening of the stomata during the phase of light. We have projected light fuses at varying light intensities. The light source (100 W thermal projector) can be fixed at different positions. For each position (height), five plants of the same vegetable variety, receive an intensity of luminous intensity for an hour. Thus, by the difference in weight projected at the leaf surface, one can determine the stomatal sweat of each cultivar and for each luminous intensity.It should be noted that the luminous intensity was measured using a photopile (LI 1600) and expressed in micromoles of photons per square meter per second (µmoles / m2 / s).

III. RESULTS

Thickness of the cuticle and cuticular sweat

The average values of the cuticular thicknesses are given in ***0.0)***

lowest values are observed in the Golden (0.33± 0.02), while the most are recorded in Jeromine (0.58± 0.0)

Table.1. In general, the cuticular thickness varies according to the varieties and age of the leaves, the

Table.1: Mean height of the scions, mean thickness of the cuticle (µm).
Mean values with the same letter do not differ significantly according to the Duncan test at the 1% threshold. The pear did not show a variation in sound cuticular thickness

variety	Average height of plants (cm)	tree1	tree2	tree3	tree4	tree5	average	st dev
Jeromine	95,4	0,58	0,59	0,56	0,58	0,58	0,58a	0,01
Red shift	96,3	0,55	0,55	0,54	0,58	0,53	0,55a	0,02
skarlet	92,7	0,58	0,56	0,56	0,54	0,59	0,57a	0,02
It	95	0,55	0,54	0,54	0,54	0,56	0,54a	0,01

Golden Deliciuos	96,8	0,32	0,35	0,31	0,36	0,33	0,33c	0,02
Golden reinders	99,5	0,35	0,36	0,35	0,36	0,36	0,35c	0,00
royal Gala	98,4	0,46	0,47	0,46	0,46	0,47	0,46b	0,01
Obro-galla	98,3	0,45	0,46	0,45	0,45	0,45	0,45b	0,00
Brookfield	99,4	0,43	0,42	0,43	0,44	0,44	0,43b	0,01
Buckey	97	0,46	0,46	0,46	0,47	0,47	0,46b	0,01
Anna	101,2	0,50	0,51	0,51	0,50	0,52	0,51a	0,01

The measurements taken at the end of each hour of weight of the 10 plants of each variety gave the mean values of cuticular losses of water.***Fig****.1* represents the results.

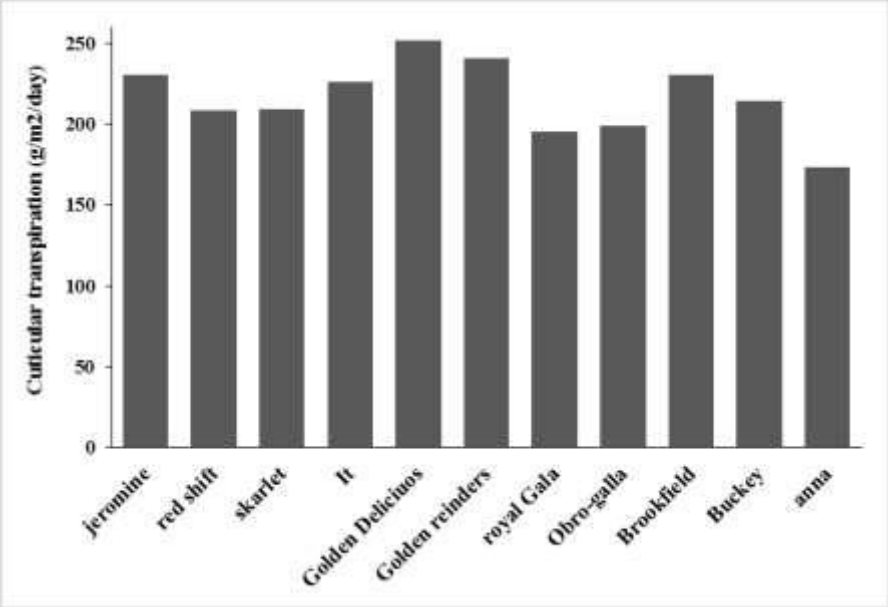

Fig.1: The cuticular transpiration of the 11 varieties studied (g/m2/day).

Vessel-cuticle length

The length from the vessels to the cuticle represents the path traveled by the water to pass to the outside air through the path of stomatal sweat or through the path of cuticular sweat. When the distance traveled by the water *Table.2*. The difference between these two limit values is 1.7 µm between the vessels and the cuticle is great, the resistance to the outflow is great. The lowest length is 7.33 ± 0.17 µm (in Golden reinders) and the highest is in the Anna variety of 9.03 ± 0.37 µm

Table.2: Mean lenght from vessels to cuticle (µm), mean stomatal density (number of stomata per mm^2),mean lenght of the stomata ostiole(µm).

The means followed by the same letter do not differ significantly according to the Duncan test at the 1% threshold.

variety	Vessel-cuticle length(µm)	number of stomata/mm^2	Length of the stomata ostiole(µm)
Jeromine	8,05c±0,29	345,7b±3,4	1,58b± 0,17
Red shift	8,4b±0,21	335b±3,1	1,56b ± 0,17
Skarlet	8,02c±0,18	334,6b±1,6	1,49b ±0,20
It	8,08c±0,32	343b±4,1	1,48b± 0,22
Golden Deliciuos	7,35d±0,22	361,7a±3,7	0,95c ±0,11
Golden reinders	7,33d±0,17	360a±2,6	0,97c± 0,16
royal Gala	8,97a±0,16	329,4c±2,8	1,08c± 0,23
Obro-galla	8,89a±0,14	330bc±1,8	1,09c ±0,15
Brookfield	7,42d±0,32	344,2b±2,1	1,47b± 0,35
Buckey	8,33b±0,29	334,7b±2,1	1,53b± 0,14

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Anna	9,03a±0,37	312,9d±4,9	2,11a± 0,22

Stomatal resistance and stomatal sweat

The average stomatal density is 339.3 in the apple tree, the minimum density is observed in the Anna variety (312.9d ± 4.9)and the high-priced Golden Delicious variety (361.7a ± 3.7).

During stomatal sweat, water vapor passes from the sub-stomatic chambers to the outside air, the length of the ostiole (the length of stomatal diffusion). This depends on the variety. It is 0.95± 0.11 and 0.97± 0.16 µm respectively for Golden delicious and Golden reinders whereas for Anna it is larger (2.11±0.22 µm)

Table.2

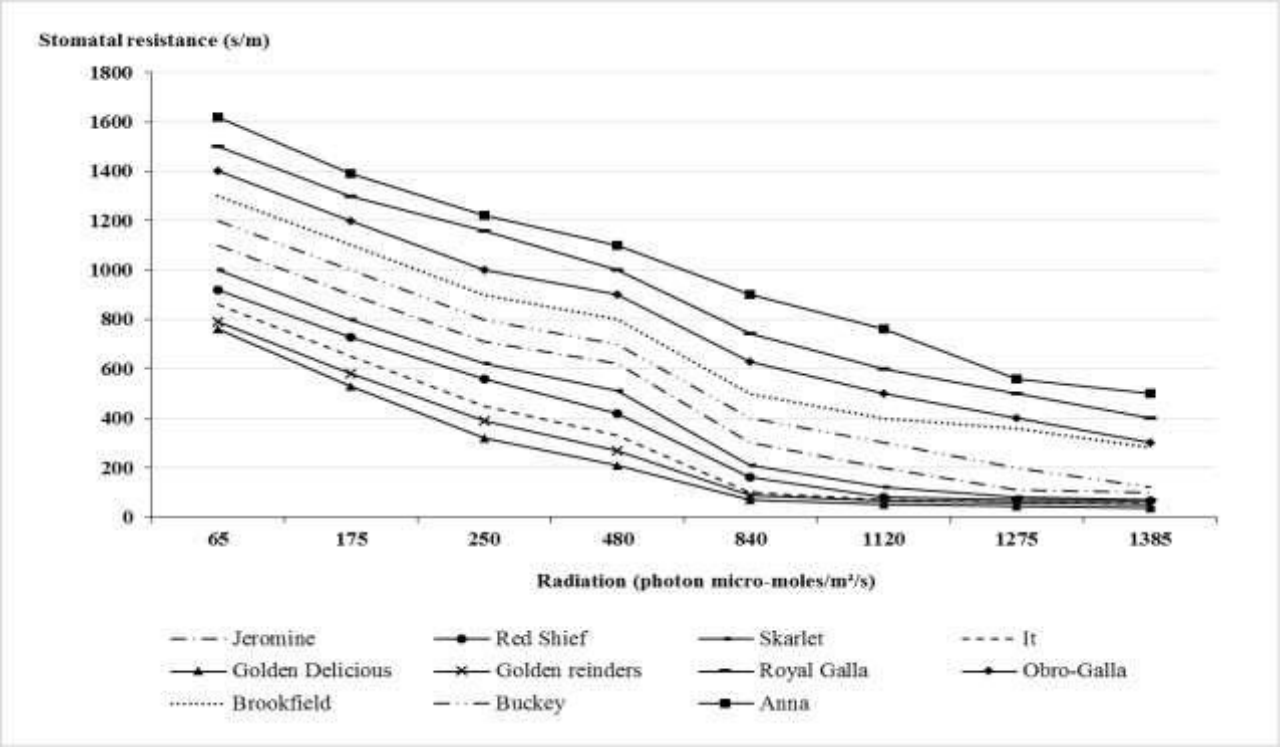

Fig.2: variation of stomatal resistance (s/m) with shortwave radiation.

The measurements of the stomatal sweat in the 11 varieties are shown in ***Fig.3***. The first reading reports the higher values of the golden varieties than the intensity of the light.

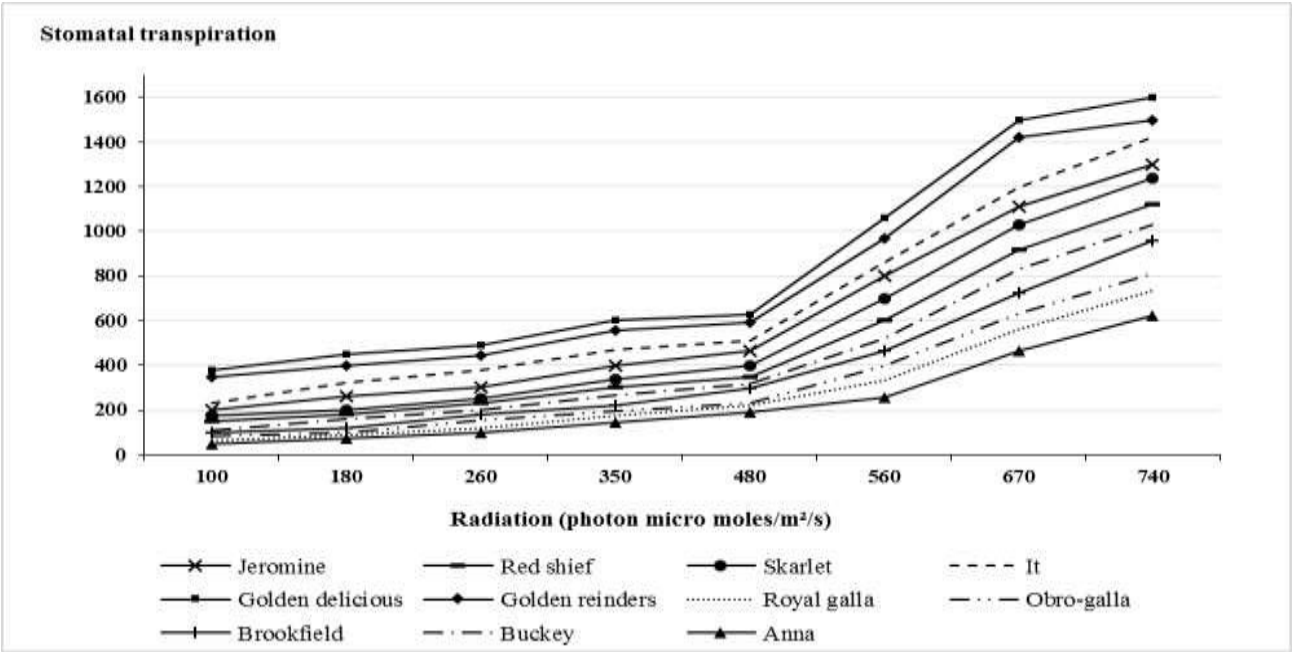

Fig.3: variation of stomatal transpiration (g/m2/h) with shortwave radiation

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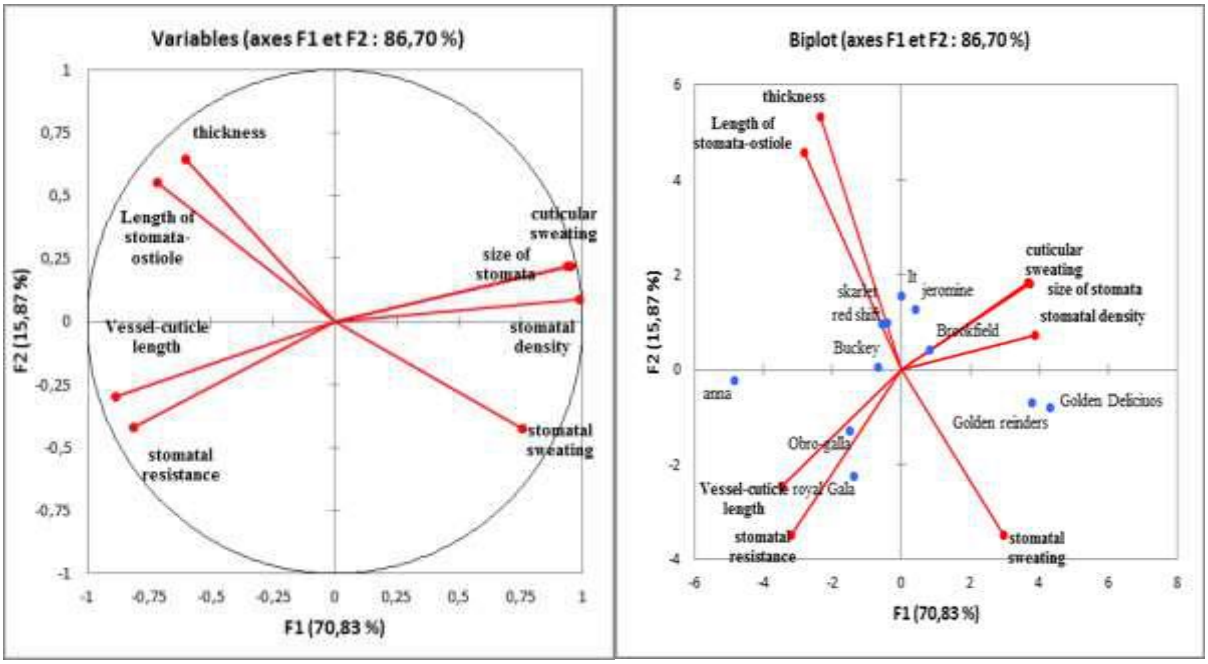
The analysis of the main components of the morphological and anatomical parameters in the cited varieties makes it possible to describe their physiological behavior in order to adapt to drought conditions, the results are shown in ***Fig.4***



(a) (b)

Fig.4: Principal Component Analysis (PCA). (a): circle of correlations. (b): projection of the varieties in the plane (Axis F1 and F2 86.70%).

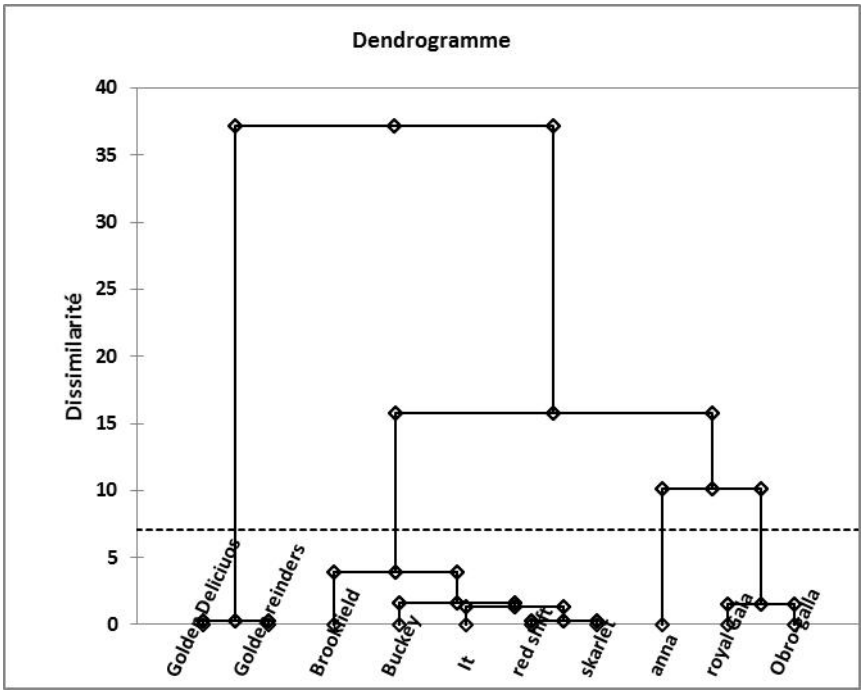


Fig.5: Dendrogram of the grouping of the varieties studied according to the method of Ascending Hierarchical Classification (AHC)

IV. DISCUSSION

The *vascular* plant is always placed in a compromise between photosynthesis and sweat. In developing the dry matter, the plant loses in aconcomitant water through perspiration.In water-deficient areas ofrainfall, to adjust the level of production agriculture to water requirements, it is imperative to practice irrigation. However, water resources are limited and the demands are constantly increasing parallel to the areas growing crops. So, one aspect of the research is attached to the determination of performing varieties adapted or tolerant to drought, able to improve efficiency of water use in irrigated cropsor

dry. Physiologically, the decrease of water in the soil is manifested bystomatal regulation in the leaves[7]. This phenomenon is often married to a fall in stomatal conductance [8]which is of course at the expense of other physiological processes such as photosynthesis [9]. Faced with this problem of drought, plants that tolerate water deficit, resistsenescence and adjust their need for production to the environmental conditions. Putting this form of adaptation, it is not necessary to bring all the water irrigation required by crops.

According to the results, the Golden varieties appear to be water demanding, their anatomical characteristics such as cuticle thickness, vessel-cuticle length, stomatal density and the diffusion length favoring high perspiration. On the other hand, the red varieties of Jeromine, Red shield and It varieties have anatomical characteristics that reduce transpiratory water losses relatively more than Anna, which is a variety adapted to dry climatic conditions.

The Galla, Obro-galla, Brookfield, and Buchey varieties show no significant differences in anatomy and morphology as well, cuticle thickness, vessel-cuticle distance, theirstomatic densities, and diffusion length decrease stomatal transpiration (**Table.1**). The thickness of the cuticle has no effect on cuticular transpiration, whereas the diffusion path between the vessels and the cuticle acts on stomatal sweat[10].The it is true for the Jeromine, Red shield and Skarlet varieties and the clon of the golden, the Anna variety is significantly different from the other varieties.Sweat depends mainly on stomatal conductance, density and size of stomata. This dependence is verified for the varieties studied and our result is similar to that obtained by [11] under three water regimes.The varieties Galla and Obro-galla show similar stomatal resistance due mainly to the long length of their ostiole. Similarly, for the Golden varieties, which resemble each other anatomically, and physiologically by a high density of stomata and in one particular case the Anna constitutes a variety apart but the journey traversed by water approaching the varieties Galla and Obro-galla in the areas of transition to the mountain

Fig.*2*.The regulation of cuticular permeability depends essentially on the composition and spatial conformation and structural structuring of the cuticle as well as on its thickness[12]Among the 11 apple varieties studied, the varieties Golden delicious, golden reinders, Red chief, Skarlet and Jeromine are classified as demanding varieties of water. Under conditions where irrigation water is limited, it is necessary to cultivate the Anna and Royal Galla varieties or, if necessary, the Obro-galla or Buckey varieties. The size of the stomata is estimated by measuring the length L of the guard cells. For each clone. The number of stomata per leaf can be expressed either by the stomatal density (number of stomata per mm^2 or by cm^2) or by the stomatal index (percentage of stomata relative to the total number of epidermal cells). This index is not usablein the apple tree because cells with very irregular contours are difficult to identify; we retained the stomatal density expressed in the number of stomata per mm^2[13].The multiple comparison of the mean stomatal size showed a significant difference between the golden and Anna varieties on the one hand and the galla varieties on the other hand, whereas it is not significant between Jeromine and red shield as well as skarlet which seem to occupy the same geographies

With respect to stomatal density, the "multiple" comparison of the means shows a significant difference at the threshold $\alpha = 0.001$ between the 4 clones. The stomatal densities of the Golden varieties are the highest and the lowest value is recorded in the Anna. The Duncan comparison test revealed a significant difference between the stomatal densities of the golden and red varieties as well as with the royal Galla Obro-galla, Brookfield and Buckey. On the other hand, the difference between the Jeromine, skarlet and red chief varieties and between the Galla, Brookfield and Buckey varieties is not significant.

The anatomical parameters studied in apple varieties are regulators of adaptation to environmental and production conditions. In mountain areas, the Golden delicious, golden reinders, Jeromine, red shield and skarlet varieties form more spurs and twigs, indicative of a good adaptation to the environment. Its flowering is relatively clustered and production is better. During the maturity of the fruits, the rapid disappearance of chlorophyll gives way to a more intense coloration. Moderate temperatures and high thermal amplitudes favor the synthesis of the pigments responsible for good coloring, especially red[14]. The fruit is firm, crunchy and more fragrant when fully mature. Under these conditions, the usual range of Golden Delicious and Golden reinders is to be extended with the Red Chief, Jeromine and other non-studied cultivars. There is a strong demand for red fruits, indicating that future plantings should be based on colored varieties.

The varieties show an increasing stomatal resistance by applying increasingly higher light radiations. In fact, plants open their stomata to light in order to ensure their gaseous exchange and to perspire, but mechanisms of resistance to dehydration are developed and expressed in stomatal resistance, which is also explained by the set of anatomical, morphological and physiological parameters mentioned above. In Fig.3, the Anna variety appears to be more resistant to dry conditions, the others less, whereas the Golden varieties are less resistant and this is due to their particularities discussed.

The projection of the values confirms this result Fig.4 , the thickness of the cuticle is negatively correlated with

cuticular (-0.45) and stomatal sweat (-0.6). The transpiration through the stomata ;depends mainly to the density ,and the length of the ostiole that when it increases, the stomatic losses of the water become minimal, joining to it the geometry of the leaves[6] The Jeromine, It, Skarlet and Red chief varieties, constitute a clon ;whose ,morphological, anatomical and physiological characteristics are homogeneous Fig.4. To which group Brookfield varieties can be affiliated by the similar size of the stomata and by values close to cuticular sweat and Buckey variety on the basis of stomata length ostiole.

The average thickness, cuticular thickness, stomatal density and average ostiole length give the Royal Galla, Obro-galla, Brookfield and Buckey varieties a physical adaptation to the conditions of the transitional media towards altitude, these semi-early varieties are perfectly adapted and their maturity comes to fill a vacuum in the production schedule. They give respectively light yellow and light carmine red fruits. Their texture is crisp and the flow on the market is easy

The Golden delicious and golden reinders varieties have high stomatal densities and short water vapor diffusion lengths, their stomatal resistancebeing lower than those of the other varieties

Fig.*2* showsat low light, the Anna varietyhas the most stomatalhigh while the highlights, the Brookfield varietyshows a stomatal resistance similar to that of Obro-galla. The Golden varieties show the valuesthe lowest for all intensitiesillumination. For the 11 varieties studied, thestomatic resistance is dependent onthe light.The Anna variety has a stomatal regulation that makes it possible to reduce the lossestranspiratory moisture while these regulations are very low in golden delicious and reinders varieties.The application of the Hierarchical Ascending Classification (HAC) method makes it possible to group all the varieties in question, the result confirms the morphological, anatomical and physiological convergences and divergences that contribute to the classification of the varieties. Class 1 of Red shield, Skarlet, It, Brookfield and Buckey. Class 2 of Golden delicious and golden reinders. Class 3 of Royal Galla and Obro-Galla and a class with unique variety of Anna (***Fig.5***).Note that this classification brings together the Brookfield and Buckey varieties of Skarlet and Red chief, even though the anatomical and physiological characteristics measured resemble those of the Galla varieties.

V. CONCLUSION

Thanks to their anatomical and physiological characteristics, the Anna varieties and the Galla, Obro-Galla and Buckey varieties can tolerate the conditions of lack of water; these conditions characterize the plains and the zones of transition towards the mountain in Morocco and in the southern outline of the mediteranea.The long path of diffusion, the large thickness of the cuticle and the lower stomata size and density give them a resistance to dryness. On the contrary, the varieties of red apple trees and varieties Golden are much more demanding in terms of water and cold, their anatomical and physiological characteristics classify them in the range of altitude variability, in some cases they may be present in the transition zones but the quality and volume of production not meet the wishes of producers.

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The Effects of Climate Change Phenomena on Cocoa Production in Malaysia

Ali Chizari[1], Zainalabidin Mohamed[2], Mad Nasir Shamsudin[3], Kelly Wong Kai Seng[4]

[1,2,3,4] Department of Agribusiness and Bioresource Economics, Faculty of Agriculture, UPM
Corresponding Author: Ali Chizari

***Abstract*—** *Climate change is arguably one of the most important factors influencing agricultural production in developing countries such as Malaysia. Therefore, it becomes important to explore the impacts of climate change on agricultural yield and production. Cocoa was brought to Malaysia for commercial planting in the 1950s. The cocoa industry grew to become the third major commodity crop in Malaysia after oil palm and rubber. In 2013, Malaysia became 28th among the Cocoa-producing countries in the world. The way forward requires increased understanding and awareness to cope with the interdependencies and interactions of natural resources and climate change, the vulnerabilities and interdisciplinary efforts. This study applied the autoregressive distributed lag (ARDL) co-integration approach over the periods (1980 – 2014). There are two main methods including the Regional Climate Model (RCM) which can reasonably produce appropriate projections that can be used for climate scenario generation in a country-scale. Based on this information, this study considered three scenarios: 1) First Scenario, Rainfall changes 2) Second Scenario, Temperature changes 3) Third Scenario, Scenario 1 and 2 simultaneously. Preliminary results from the Autoregressive Distributed Lag (ARDL) model applied indicated that despite the projected changes in the climate variables (temperature and rainfall), in scenario 1 (the projected changes (5% increase) in rainfall), cocoa yield is expected to decline from 0.148 tonne per hectare (t/ha) in 2015 to 0.143 t/ha in 2020. The average trend compared to the baseline is positive and expected to develop by +3.83% annually. In scenario 2 (the projected changes (2% increase) in temperature), cocoa yield is expected increase from 0.149 t/ha in 2015 to 0.155 t/ha in 2020. The average trend compared to the baseline is positive and expected to increase by +1.76% annually. Similarly, in scenario 3 (the projected simultaneous changes (+5%) and (+2%) in rainfall and temperature respectively), cocoa yield is expected to increase from 0.154 t/ha in 2014 to 0.189 t/ha in 2020.*

***Keywords*— Cocoa, Climate Change, ARDL, RCM, MCB.**

I. INTRODUCTION

Cocoa was brought to Malaysia for commercial planting in the 1950s (Malaysia Cocoa Board (MCB), 1991). The cocoa industry grew to become the third major commodity crop in Malaysia after oil palm and rubber. In 2013, Malaysia became 28th among the Cocoa-producing countries in the world (World Cocoa Foundation, 2015). Currently, Cote d'Ivoire, Ghana, and Indonesia are the three largest producers of cocoa bean (Department of Statistics Malaysia, 2016).

The Malaysian cocoa beans and cocoa products export continued to increase as it rose from about 600,000 tonne in 2000 to 5 million tonne on 2015 (Department of Statistics Malaysia and Malaysian Cocoa Board, 2016). In 2015, cocoa beans and cocoa products was the second food exports with RM4.1 million and currently Malaysia is the largest cocoa processors in Asia (Department of Statistics Malaysia, 2016).

Presently, cocoa is cultivated in Sabah and Sarawak with about 6,260 ha (38.9%) and 6,020 ha (37.4%) respectively. However, as the price of cocoa went down, numerous plantations moved to palm oil production. The smallholder producers also declined, though at a slower rate compared to the estate cocoa producers (MCB, 2014).

In 2014, 95% of cocoa is grown mainly by smallholding plantations on an area estimated around 15 thousand hectares. The areas under cocoa declined by almost half from around 30 thousand hectares to 15 thousand hectares in just a decade. Unfortunately, the plantation under the state also dropped dramatically from about 11,000 hectares to less than 900 hectares during the same period (2004–2014) (MCB, 2015). However, a fall in the global price of cocoa from RM14,323.01per Metric tonne to RM10,770.30 in 2015 to 2016 had significant negative impact on the expansion of cocoa plantations, thus making many smallholders to either destroy or abandon their cocoa plantations for other crops such as pepper and oil palm (World Bank, 2016).

The production of cocoa beans follows almost the same pattern with the planted area. Sabah is the largest producer with about 59.7%, followed by Peninsular Malaysia (33.4%) and Sarawak (6.9%) (MCB, 2015). During the period (1985-1996), Malaysia average annual

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production of cocoa beans peaked at around 180,000 tonne. However, it declined in subsequent years by almost 98.6% to 2,665 tonne in 2014.

Yield measures profitability and also represent one of the most vital monetary elements influencing the cost of production per ton of cocoa beans. At higher efficiency, the cost of production per ton of cocoa will be lower and vice versa. In this regard, efforts need to be made to improve efficiency so as to ensure profit maximization.

The Malaysian national yield in cocoa increased from 0.79 t/ha in 2004 to 1.3 t/ha in 2008. However, in 2014 the yield was just 0.166 t/ha. At these levels of national production efficiency, Malaysia can be considered the most profitable cocoa producer on the planet. Nonetheless, this level of national profitability is far lower than the hypothetical potential yield of 11 t/ha (Corley, 1967) and the feasible yields of somewhere around 2.0 and 6.8 t/ha (MohdYusof et. al, 1998). Interestingly, the smallholder cocoa producers under the recovery program organized by the Malaysian Cocoa Board accomplished a substantial increase in yield from less than 0.5 t/ha to 2.07 t/ha (Ministry of plantation industries and commodities (MOPICO), 2014).

Climate changes have been affected on cocoa production like other commodities in the world. These changes are wide and depends on the place are different. Kenneth and Baba Insah in 2014 found that increasing temperature and decreasing rainfall have negative impact on the cocoa production. Martin Noponenin 2015 figure out that drought in Indonesia has led to higher seed mortality and higher mortality for younger trees that are vulnerable to diseases. Also, Justina O. Lawal and Leo A. Emaku found out that there is weak negative correlation for both rainfall and relative humidity on cocoa yield over the years (-0.257 and -0.196) respectively while positive correlation (0.595) was established for temperature on yield. Furthermore,NwaJesus Anthony Onyekuru and Rob MarchantYork (2016) demonstrated that the results show positive impact of precipitation during the spring and adverse impact in the summer and autumn are also in agreement with works on plantation agriculture in Nigeria (Fonta et al., 2011), on cocoa production in Nigeria (Lawal and Emaku, 2007), in African cropland (Kurukulasuriyer and Mendelsohn, 2008) and on Ethiopian Agriculture (Deressa, 2007). Therefore, the impact of the climate changes are too various and need to find in each specific place and as I already mention because of the great exercise in Malaysia in cocoa industry it is really important to investigate of this impacts on coca production and yield.

Finally, the impact on agriculture due to the threats and effects of climate change while large and serious, is therefore compelling and urgent. Not addressing the challenges and the urgency of collective actions is going to be catastrophic. The way forward requires increased understanding and awareness to cope with the interdependencies and interactions of natural resources and climate change, the vulnerabilities and interdisciplinary efforts.

Econometric Model is applied in this study simply because it has competencies to set the climate change and economic variables as a climate-economic model (CEM) (Auffhammer et al., 2013; Pindyck, 2013; Nelson et al., 2014; Dell et al., 2014). The calculated F-statistics value is compared with two sets of critical values estimated by Pesaran et al. (2001). One set assumes that all variables are *I(0)* and other assumes they are *I(1)*. If the calculated F-statistics exceeds the upper critical value, the null hypothesis of no co-integration is rejected irrespective of whether the variable are *I(0)* or *I(1)*. If it is below the lower critical value, the null hypothesis of no co-integration cannot be rejected. If it falls inside the critical value bands, the test is inconclusive.

II. OBJECTIVES

The general objective of this study is to find impacts of climate change on cocoa production and yield.

The specific objectives are:

1) To develop cocoa market model
2) To investigate the relationship between climate change and yield of cocoa
3) To estimate, forecast and simulate the level of cocoa production based on climate changes until 2020
4) To suggest policy alternative to mitigate impact of climate changes in sustaining cocoa production.

III. METHODOLOGY

The structure of the Malaysian cocoa model is presented in Figure 1. As it has been displayed, productions of dry cocoa beans hang on the harvested area and the yield in the corresponding sector. Besides, the yield of cocoa is predictable to be influenced by climate factors such as temperature and rainfall, technology and fertilizer price such as rubber and oil palm. Another more component of the cocoa beans supply is import which depends on the world prices of cocoa beans as well as the industrial production index in addition to the Malaysian exchange rate.

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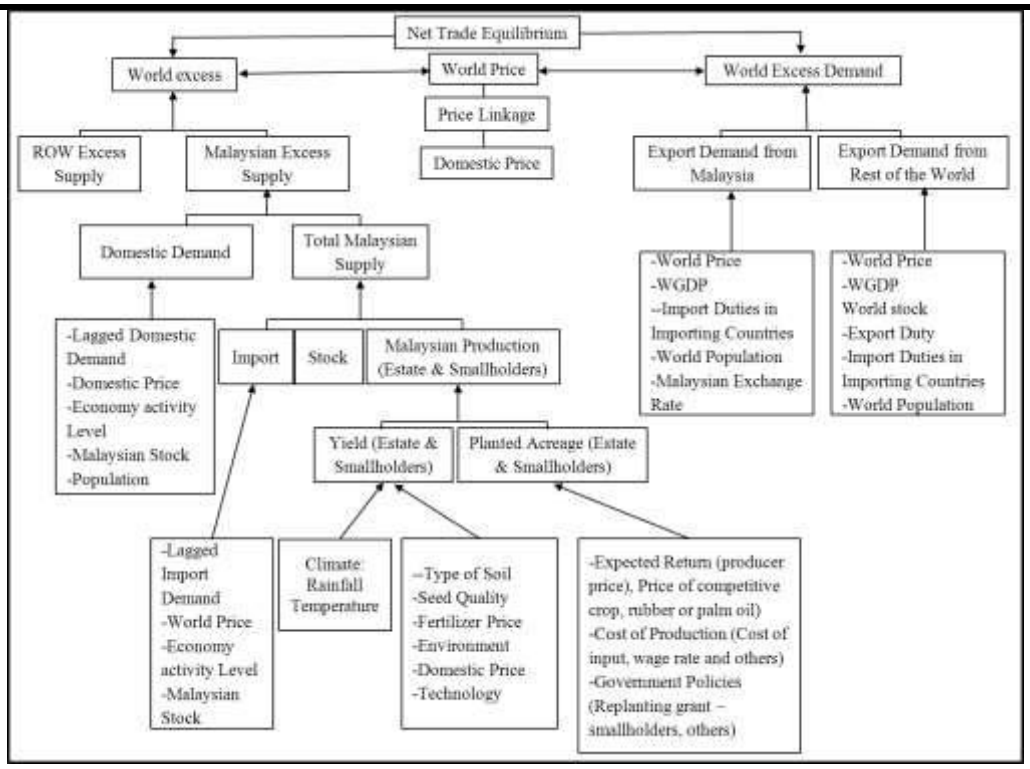

Fig.1: Conceptual Framework of Cocoa Market Model

Model specification of Yield

The yield of Cocoa Beans is dependent on its previous year's level, Climate (rainfall, temperature), the type of soil, seed quality, fertilizer and insecticide prices, and the environmental condition in addition to advancement in technology represented by a time trend.

The yield of cocoa beans Malaysia in can be presented as follows:

$CBYDMY_t = f(FTP_t, RAIN_t, TEMP_t, trend)$

Where;

CBYDMY = Cocoa Bean yield in Malaysia (tonne/hectare)
FTP = Fertilizer price (RM/tonne)
RAIN = Average annual Rainfall (mm)
TEMP = Average annual Temperature (°C)
Trend = Trend dummy proxy for technology
t = Time period

Diagnostic Tests

This study applied ARDL model and it has to adopt the Unit test (Table 1), ARDL bounds test (Table 2), and a series of diagnostic tests and stability test. Cocoa model should be validated through historical simulation. The model is selected on the basis of the Schwartz-Bayesian Criteria (SBC) and Akaike's Information Criteria (AIC) (Table 3).All the results of simulation will compare and contrast with the actual data. The closeness and deviation of the estimation results and actual values are scaled by Root Mean Square Error (RMSE), Root Mean Square Percentage Error (RMSPE), and U-Theil inequality coefficient (Table 4).The results indicate the absence of any instability of the coefficients because the plot of the CUSUM and CUSUMSQ statistic fall inside the critical bands of the 5% confidence interval of parameter stability (Figure 3, 4).For the out-of-sample validation purposes, the endogenous variables are projected based on the actual values of exogenous. The comparison results in out-of-sample are shown in Table 5.

IV. SIMULATION MODEL

In order to forecast and simulation of the commodity model, we determined 2014 as a base year. According to the Kwan Kok Foo (2010) it has two main methods which call Regional Climate Model (RCM) can produce reasonably appropriate projections to be used for climate-scenario generation in country-scale.Based on this information this study has been considered three scenarios:

1. First Scenario, Rainfall changes: Based on rainfall changes in Malaysia in 2020 which will increase +6% more than normal trend
2. Second Scenario, Temperature changes: Based on temperature changes in Malaysia in 2020 which will increase +1.15°C more than normal trend
3. Third Scenario, Scenario 1 and 2 combined together

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V. RESULTS AND DISCUSSION

In Table 3, the Climate Cocoa Yield equation was determined by the technology trend (T), lagged one and two year annual yield adjusted ($CBYDMY_{t-1}$,$CBYDMY_{t-2}$) and cocoa farm price (RCBFP), Fertilizer price in current and previous year (FTP, FTP_{t-1}), rainfall (Rainfall) and temperature (TEMPER). The empirical results show that all climates the determinant variables (rainfall and temperature) have been estimated positive sign and rainfall statistically is significant at 10% significance level, but temperature is statistically insignificant. The results are supported by Just in a Oluyemisi Lawal and Omonona, (2014), Omolaja et al. (2009) Oyekale et al. (2009). In addition, Farm price and trend are estimated positive sign however, statistically is not significant. Furthermore, fertilizer price has negative impact and the yield especially in lagged one is statistically significant at 5% level. The values of climate coefficients (rainfall and temperature) convey that they have a solid impact on cocoa yield and it displays that, if the temperature increase by 1% then coca yield would have increase 1.96387% and if the rainfall increase by 1% the yield will enhance 0.578657%.

Table.1: Augmented Dickey Fuller (Unit Root) Test Results

Variable	Augmented Dickey Fuller				Stationary
	Level		First Difference		
	Constant Without Trend	Constant With Trend	Constant Without Trend	Constant With Trend	I(0)/I(1)
FTP	-2.767618*	-3.730227**	-7.702798***	-3.191559**	I(0)
RAINFALL	-4.021853***	-4.135785**	-4.287250***	-4.168478**	I(0)
TEMPRATURE	-0.33788	-6.269207***	-8.263086***	-7.951693***	I(1)
CBYDMY	-1.942392	-2.270397	-4.521666***	-4.478893***	I(1)
RCBFP	-2.740792*	-2.574133	-6.206393***	-6.322402***	I(0)

Table.2: ARDL Bound Test of Long-Run Cointegration

Equation	Lag	F-statistic	Wald test (Fs)
Cocoa: CBYDMY= f(FTP, RAIN, TEMP, RCBFP)	3	14.4560***	16.18206 ***

Table.3: The ARDL Results of Climate Cocoa YieldModel (CBYDMY)

C	CBYDMY(-1)	CBYDMY(-2)	FTP	FTP(-1)	RCBFP	RAINFALL	TEMPR	T
-9.21	1.330	-0.406	-0.037	-0.329	0.0202	0.578657	1.963869	0.011
-1.006	8.3725***	-1.8948*	-0.3123	-2.7615**	0.222	1.993*	0.8057	1.432

Diagnostic Tests

Test Statistics		Test Statistics	F [prob]
R-Squared	0.947001	Serial Correlation	0.0084[.977]
R-Bar-Squared	0.929334	Functional Form	4.2400[.051]
F Test	53.60468 [0.000]	Normality	5.7821[.056]
DW-statistic	1.898858	Heteroscedasticity	0.2490[.621]

Table.4: The Summary Results of the Validation Tests

Endogenous	RMSE	MAE	U^T	U^B	U^V	U^C
Cocoa LCBYDMY	0.183943	0.141889	0.173712	0.008493	0.027477	0.964030

*Note: ***, **, and * denote significant at 1%, 5%, and 10% significance levels, respectively.*

Note: RMSE = Root Mean Squared Error; MAE = Mean Absolute Error; U^T = Theil Inequality Coefficient; U^B = Fraction of error due to bias; U^V = Fraction of error due to variance; U^C = Fraction of error due to covariance.

Table.5: The Summary Results of the Validation Out of the Sample Test

Endogenous	RMSPE (%)	U^T
Cocoa LCBYDMY	18.93484	0.107331

Note: RMSPE = Root Mean Squared Percentage Error; U^T = Theil Inequality Coefficient

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Figure 2 shows the simulation results for cocoa yield under the three scenarios (scenario 1, 2 and 3). All projections are between the periods (2015 – 2020). In scenario 1 (the projected changes in rainfall), cocoa yield is expected to decline from 0.148 tonne per hectare (t/ha) in 2015 to 0.143 t/ha in 2020. The average trend compared to the baseline is positive and expected to develop by +3.83% annually. In scenario 2 (the projected changes in temperature), cocoa yield is expected increase from 0.149 t/ha in 2015 to 0.155 t/ha in 2020. The average trend compared to the baseline is positive and expected to increase by +1.76% annually. Similarly, in scenario 3 (the projected simultaneous changes (+5%) and (+2%) in rainfall and temperature respectively), cocoa yield is expected to increase from 0.154 t/ha in 2014 to 0.189 t/ha in 2020. The average trend compared to the baseline is also positive and expected to develop by +6.06% annually. Finally, the results revealed that the overall trend is positive and climate change will also have positive impacts on the industry.

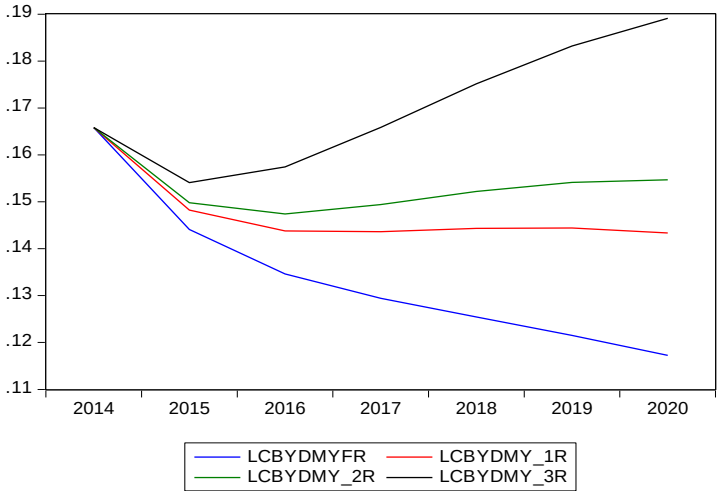

Fig.2: Simulation Results of Cocoa Yield Scenario1, 2, 3 and Base line

VI. CONCLUSION

Cocoa represents one of the commodities that will take central role in the per capita gross national production in the coming years. Based on the result of the study, cocoa is expected to be an important commodity in the economic development of the agricultural sector. The findings from the research indicate that changes in temperature and precipitation will have no negative impact on cocoa yield in the coming years. The production trend is positive and the projected increase in temperature and rainfall will lead to about 6.06% rise in yield annually. Thus, investment in this sub-sector can be very effective in increasing the commodity's GDP share of the agricultural sector. With regards to the operational experience of the farmers in this sub-sector, it can become one of the most important commodities in Malaysia.

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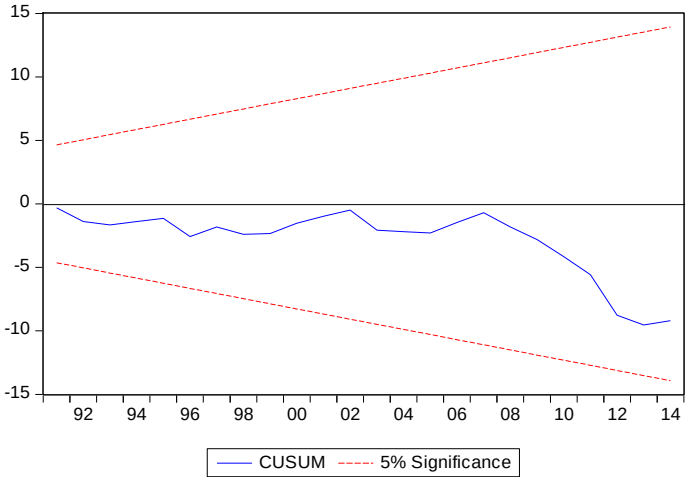

Fig.3: Cusum Test of Cocoa Yield Model

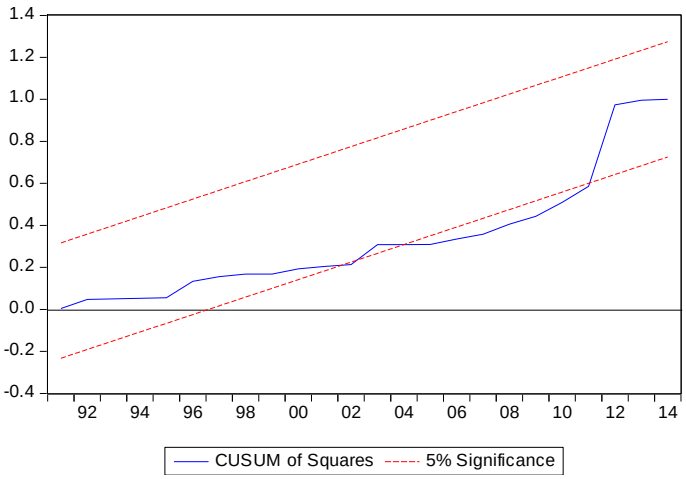

Fig.4: Cusum of Square Test of Cocoa Yield Model

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Comparative Economic Analysis of Cassava Mosaic Disease-Resistant Varieties and Non-Resistant Varieties Production in Akwa Ibom State of Nigeria

Rachel G. Isonguyo, Raphael A. Omolehin

Department of Agricultural Economics and Rural Sociology, Faculty of Agriculture, Ahmadu Bello University, Zaria, 800001, Nigeria

Abstract— *Comparative Economic Analysis of Cassava Mosaic Disease (CMD)-resistant Varieties and Non-Resistant Varieties (NRV) Production in Akwa Ibom State of Nigeria is the research. The CMD, which causes reduction in yield to about 20-30%, or 90-100% is a problem to farmers. Multi-stage sampling procedure was used to select 80 CMD-resistant varieties and 80 NRV farmers, while descriptive statistics, net farm income and production function analysis were used in analyzing the data. The study was to provide useful information to students, policy makers, investors and researchers to aid them in their various fields. The study revealed the socio-economic characteristics, such as farming experience, educational level, number of extension contact and farm size to positively influence the CMD-resistant varieties farmers' income. The R^2 of 0.83454 variability in the income of the CMD-resistant farmers was explained by the socio-economic variables in the model. The R^2 of 0.6696 variability for the non-CMD-resistant farmers' income was also explained by the socio-economic variables in the model. The CMD varieties production at ₦91,270 Net Farm Income against ₦41,170 of NRV productions, indicated both productions' profitability. Average rate of return indicated every Naira invested by CMD-resistant farmers, earned ₦2.49 profit, while NRV farmers earned ₦1.67 profit. CMD-resistant farming was thus, more profitable than the NRV farming. The Z-test of the mean income (3.5271) at 1% level of significance against tabulated Z-value (1.96) causes the hypothesis' rejection. Production of CMD-resistant varieties was more profitable and farmers are, advised to produce it and form cooperatives for .wider dissemination of research information.*

Keywords— Comparative economic analysis: Cassava Mosaic Disease-resistant, non-resistant-varieties production.

I. INTRODUCTION

Cassava is one of the major food crops of Nigeria. It has high starch content with useful extracts for food, both for humans and animals, or industrial use as starch, gum and dye. Cassava was first introduced into Africa during the slave trade era of 1558, and cultivated as a source of food for the slave ships [10]. The crop was not popular until late 1890s when famine forced the people that live around the coastal regions to accept and cultivate the crop [13]. Cassava production has been on the increase in Nigeria since 1960s, and today, the country ranks as the world's largest producer of cassava [6]. It plays a particularly important role in developing countries, especially in sub-Saharan Africa as it does well on poor soils with low rainfall, and is a perennial crop that can be harvested as required [22]. Like most other root-crops, it has long growth cycles, high perish-ability, and slow multiplication rates of propagation and is subject to several stresses like insects, mites, nematodes, weeds and diseases, including Cassava Mosaic Disease (CMD) [15]. Cassava production has been on increase in Nigeria since the mid-1960s when estimated to rise to about 8 million tonnes produced from 0.83 million hectares. Food and Agriculture Organization (FAO) in 2001 pronounced Nigeria as the world's largest producer, estimated to be about 34 million tonnes per year from about 3.1 million hectares [14]. [16] estimated that about 42% of harvested cassava roots in West and East Africa are processed into dried chips and flour for easier storage. In Nigeria, however, cassava production has helped in increasing food availability, reducing rural

poverty and unemployment and enhancing agro-industrial and socio-economic growth.

Cassava and its products are used for food, feed, and industrial use [14]. Industrially, its product is used in the production of ethanol [18]. Cassava tubers can be peeled, dried and blended into flour and used by confectionery industries. It has an average yield of about 11 tonnes per hectare, mainly from the numerous small-scale, subsistence farmers from the southern and central regions of Nigeria, [8]. It is a very important crop for food security and income in the tropics and Africa, which translate into 300 calories per day for more than 200 million Nigerian people. It is a prolific crop and can survive on wide range of soils which are acidic with low fertility. In recent times, despite its versatility, its production has been on the decline due to the presence of CMD virus in the country [18]. [17] in a diagnostic survey in Nigeria revealed that CMD symptoms were mild in most farms in Akwa-Ibom, Lagos, Delta and Edo States (South-South) geopolitical zone; Anambra and Enugu State (South-East zone); Kwara, Nassarawa and Niger (middle belt); Jigawa (North-East) and Kaduna (North-West). CMD symptoms were either moderately severe or severe in most farms in Cross River and Bayelsa (South-South); Abia, Ebonyi and Imo (South-East); Ekiti and Ondo (South-West); Plateau, Federal Capital Territory, Benue and Kogi (Middle belt); Bauchi, Gombe, Adamawa, Borno and Yobe (North-East); while Kano, Katsina and Sokoto (North-West) showed CMD symptoms of either mild, moderately severe or severe in various proportions. In the entire country, the farms, although randomly distributed, showed that 48% of the farms had cassava with moderately severe or severe symptoms [11]. These were about the same proportion of farms with mild symptoms which were about 52% [5]. [11] diagnostic survey in Nigeria also revealed that about 74% of the 1397 cassava leaves samples tested positive for African Cassava Mosaic Virus (ACMV). Whiteflies (the disease vectors) were not found in a lot of the farms in Northeast and Northwest [21]; [17]. This is because the geographical climatic condition of Northern Nigeria (Semi-arid/arid) does not favour the spread of CMD as whitefly population is very low unlike in the South humid region. The potentials of the crop has made the government of Nigeria in 2004 to suggest it to be treated as one of the major source of foreign exchange and a food security crop.

However, Akwa Ibom States was chosen for this study due to the intensity of cassava production and, possibly, exchange of the cassava stems among farmers across neighbouring country, such as Cameroun. The cultivation of CMD-resistant genotype by farmers have led to increase in cassava production in the State (Dixon *et al.*, 2005). Improved cassava varieties planted provided about 590 farmers with the planting stem, which were replanted in 2004 to form another source of planting materials for 2005. The improved cassava varieties were planted by the Cassava Development Committee (CDC) in 2005 for stem multiplication at Ube/Obufi and Ebigbi in Okobo Local Government Areas of the State. Nine hundred and twenty five hectares have been cultivated through farmer to farmer transfer within the State and the overall hectares of improved cassava varieties cultivated within the State are about 785 hectares [1]. Presidential initiative of Cassava Enterprise Development Project (CEDP) was being made to enhance its processing, encourage its trade, market its products, as well as encourage the adoption of the CMD-resistant varieties. Since these varieties were introduced in 2002, and adopted by farmers in Akwa Ibom State, few studies have evaluated the cassava farmers' performances economically, in terms of cassava varieties produced. Thus, this study of comparative economic analysis of CMD-resistant varieties and NRV production in Akwa Ibom State becomes very necessary.

Problem Statement

Introduction of CMD-resistant varieties in 2002 has boosted the crop yield substantially, although still inadequate in supply relative to demand [2]. Some farmers have adopted the new varieties, while many have not due to lack of information on the economic advantage and profitability of the new varieties. The presence of CMD is a problem to the farmers and manifests in chlorosis of the cassava leaf blade, reduction, twisted and yellowish leaves with bright areas separated by normally green areas [11]. The disease causes reduction in yield to about 20 to 30% and the cultivation of the susceptible cassava genotypes can lead to greater losses of about 90 to 100% [9]. Perfect control of CMD is said to be rare, but its economic control may be possible if the increase in yield is greater than the cost of production through planting of healthy cassava stock, using disease-resistant varieties, adopting protective measures, immunizing, eradicating diseased plants, and avoiding infested stock. However, most farmers in Akwa Ibom State are yet to become fully aware of the potentials of the CMD-resistant varieties and adoption benefits. Although, certain improved production techniques and CMD-resistant varieties have been adopted by farmers, the desired level of the crop's productivity is yet to be achieved. This may be due to high cost of production,

insufficient planting materials, or none evaluation of the outcome of the production. This study is to provide the needed statistical information on cassava production, processing and distribution, based on such research questions as; What are the socio-economic characteristics of farmers growing CMD-resistant varieties and NRV in the study area?; What is the relationship these characteristics and their income?; What are the costs and returns of CMD-resistant varieties and NRV production?; What is the input-output relationship for the crops, the resource use efficiency and constraints faced by both farmers in their production?

Objectives and Justification of the Study

The aim of this study was to carry out a comparative economic analysis of CMD-resistant varieties and NRV production in Akwa Ibom State of Nigeria. The objectives were to: describe the socio-economic characteristics of farmers growing CMD-resistant varieties and NRV in the study area, determine the relationship between farmers' socio-economic characteristics and their income, estimate the costs and returns of production of CMD-resistant varieties and non-resistant varieties, determine the input-output relationship for the CMD-resistant varieties and NRV, evaluate the resource use efficiency in CMD-resistant varieties and non-resistant varieties production, and identify constraints faced by both farmers in their production. Cassava is the most important singular staple food crop in every home in Akwa Ibom State of Nigeria, supplying about 70% of the daily calorie intake, and recently, the second most important cash crop after palm oil [3]. It provides most of the dietary intake of carbohydrate of the average population of southern Nigerians. It is one of the major staple food crops produced at a range of 0.5 to 1.0 tonnes per hectare from the local varieties [2]. This quantity is yet to meet the high demand for the crop within the State and is thus substituted with other food crops like yam, cocoyam, and plantain.

The nature and harvesting duration of cassava enables it to act as famine reserve crop and is invaluable in managing labour schedules. It is flexible for resource-poor farmers as it serves as both subsistence and cash crop as well as gives the highest yield of food energy per cultivated farmland area per day among crop plants. Research studies have not shown clearly, the comparative economic analysis of CMD-resistant varieties and NRV production in the State. However, it is expected that the findings of this study will be found useful to agricultural students in providing useful academic information for their studies. Researchers will find the information to be a relevant feedback for further studies. Policy-makers will be guided in agricultural policy formulation that will contribute to the sector's development, while investors will be able to backup their decisions on cassava production with reliable data provided by this study. The information from this study will also help stimulate more production of either CMD-resistant varieties or NRV by the resource-poor, small-scale farmers in the agricultural sector.

Hypotheses:- There is no significant relationship between farmers' socio-economic characteristics and the incomes of CMD-resistant and NRV farmers. Also, there is no significant difference between the mean incomes of CMD-resistant varieties' farmers and NRV farmers.

II. MATERIALS AND METHODS

2.1. Study Area

The study was carried out in Akwa Ibom State of Nigeria in the South-South geo-political zone as it is involved in massive production of cassava. The State is about 7,245,935 square metres in land area [7], and is divided into 31 Local Government Areas (LGAs) and 3 Senatorial Zones, with a population of about 5,304,318 people as at 2009, based on the 2006 population estimate of 4.8 million people at 2.5% growth rate. It has a temperature that varies between 28^0C and 30^0 C, and a relative humidity that varies between 63% in December to February and 79% from June to September [7]. It is located between longitudes $7^0$35' and $8^0$25' East and latitudes $4^0$33' and $5^0$33' North of the Equator. The State lies within the humid rainfall zones of Nigeria, has a relief of gently undulating plains with sandy, loamy, deep and well drained soil derived from alluvium and coastal deposits. It has rain forest mangrove vegetation, and shares boundaries with Abia State in the North-East and West; Cross River State in the South-East; Rivers State in the South-West; and the Atlantic Ocean in the South-South. The Ibibio, Annang and Oron people make up the major ethnic groups of the State. These people are mostly Christians of various denominations. Eighty percent of the rural people are farmers and cassava is the major agricultural crop of the people in all the 31 LGAs of the State. The remaining twenty percent are made up of white and blue-collar workers, fishermen, traders, artisans and transporters. There are about 0.8 million registered cassava farmers in Akwa Ibom State, according to [3]. Some of these farmers also cultivate other crops such as maize, plantain, yam, cocoyam, vegetables, and swamp-rice, but in a smaller quantity. Mixed cropping, both in compound and farmland environments are practised in the State. Every

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household processes the cassava for consumption or for sale as *garri*, chips, pellets and *fufu*. The people also keep some domestic animals such as goat, sheep, pig, chicken and turkey. Head carriage, and use of bicycles, motorcycles, pick-up vans, cars, truck and wheel-barrows are the major means of transportation for the people and their produce.

2.2. Method of Data Collection

Only Primary data was used for the study. Primary data was collected with the help of interview method using structured questionnaire with the assistance of the extension staff of Akwa Ibom State Agricultural Development Project, on the socio-economic characteristics of the cassava farmers and their production variables. These socio-economic variables included age, educational status, years of cassava farming experience, household size, farm-size, number of contact with extension agents and membership of cooperative societies. The production variables included quantity and cost of planting materials, quantity and cost of fertilizers, cost of labour, quantity and value of the cassava output, and problems that both CMD-resistant and non-CMD-resistant varieties' farmers face in the course of their production.

2.3. Analytical Techniques

2.3.1. Descriptive statistics

These include means, ratios, percentages and frequency distributions and were used to achieve objectives i, ii, and v.

2.3.2. Gross margin analysis

This was used to partially achieve objectives iii. It is the evaluation of the efficiency and profitability of an individual farm enterprise or farm plan that enables one to compare different farm enterprises or farm plans. [19] refer to Gross Margin (GM) as a very useful tool in a situation where fixed capital is a negligible portion of the farm enterprise. The formula is:

$$GM = GI - TVC \quad \text{-(1)}$$

Where: GM refers to the gross margin (₦/ha); GI refers to gross farm income (₦/ha) and TVC refers to total variable cost (₦/ha).

2.3.3. Net farm income analysis

This was used to also achieve objective (iii) of the study. According to [19]. It is expressed as :

$$NFI = GFI - TVC - TFC \quad (2)$$

Where: NFI refers to net farm income (₦/ha); GFI refers to gross farm income (₦/ha); TVC refers to total variable cost (₦/ha) and TFC refers to the cost of fixed input (₦/ha for cutlasses, hoes, axes and rakes). Straight-lie depreciation method was used to estimate depreciation value for the fixed assets used for the farming activities and the assets are hoe, cutlass, axe and rake.

2.3.4. Regression analysis

Ordinary Least Square technique (OLS) was used to treat objective (ii), the model in the simplified form is thus expressed as;

$$Y = f(X_1, X_2, X_3, X_4, U) \quad (3)$$

Where: $Y_1 = a + \beta X_1$

Y_1 refers to Income (Naira); X_i refers to the socio-economic characteristics of i^{th} individual; β refers to the regression coefficient; and a refers to the constant term. For this study Y_i is explicitly expressed as:

$$Y_1 = a + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + U \text{ -----(4)}$$

Where: -

Y refers to the income (Naira), X_1 is the age of the farmer (expressed in the number of years); X_2 refers to farming experience of farmers (expressed in years); X_3 refers to the educational level (number of years spent in a formal school); X_4 refers to household size (number of persons in the household); X_5 refers to membership status in an association. (Years) ; X_6 refers to extension contact (number of visits to, and received from an extension officer); X_7 refers to the farm size (hectares cultivated per season); X_8 refers to the amount of credit obtained (Naira) and U is the disturbance term. The socio-economic data collected were fitted into the linear functional form expressed thus;

$$Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4 + U$$

Linear

2.3..5. Production function analysis

This was used to achieve objective (iv) of the study. The production function establishes the physical or technical relationship between inputs and output in any production process [19]. Researchers have estimated production function with such equations as linear, quadratic, Cobb-Douglas, Spillman, semi-log, square-root and exponential. Production Function for this study is expressed in implicit form as:

$$Y = f(X_1, X_2, X_3, X_4, U) \quad (5)$$

Where; Y refers to the output (Kg/ha); X_1 refers to the farm size (ha); X_2 refers to the quantity of cassava stem(Kg/ha) ; X_3 refers to the quantity of fertilizer used (Kg/ha); X_4 refers to units of labour used (manday/ha); and U is the error term. Data collected were fitted into these three functional forms and the best fitted equation selected for further analysis, based on the magnitude of co-efficient

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of multiple determination (R^2), signs of regression co-efficient, significance of *t-values* and *F-values.* The three functional forms are expression as;

$Y = a + b_1 X_1 + b_2 X_2 + b_n X_n + e$ Linear

$Y = a + b_1 \ln X_1 + b_2 \ln X_2 + b_n \ln X_n + e$ Semi-log

$\ln Y = a + b_1 \ln X_1 + b_2 \ln X_2 + b_n \ln X_n + e$ Double Log

Where; - b_1 - b_n refers to the regression co-efficient of inputs X_1 - X_n; a refers to the constant; ln refers to the log, and e is the error term

2.3.6. Estimation of resource use efficiency

This was used to achieve objective (v) of the study, and is computed thus;

$$r = \frac{Marginal\ Value\ Product}{Marginal\ Factor\ Cost} = \frac{MVP}{MFC} \text{ --} \quad (6)$$

Where: r refers to the efficiency ratio; MVP refers to the marginal value product; and MFC refers to the marginal factor cost. However, when: r = 1, it implies efficiency in resource use; and when $\Gamma > 1$, it implies under-utilization in resource use; $\Gamma < 1$, it implies Over-utilization in resource use, Where: =, >, < , refer to: equal to, greater than, and less than, respectively

2.3.7 Specification of hypothesis testing, using mean incomes and z-test

This was also used to achieve objective (iii) of the study. It involved carrying out a Z-test of the mean incomes of the CMD-resistant varieties' producers and non-CMD-resistant varieties' producers. The mean incomes were tested for significance at 1%. 5% and 10% levels of probability. If the calculated Z-value was greater than tabulated Z-value, it means that there is a significant difference between the mean output, income and profit of CMD-resistant varieties' farmers and non-CMD-resistant varieties' farmers. The formula is:-

$$Z - test = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \text{ -} \quad (7)$$

Where: Z refers to the Z-test value; $\bar{X}_1$ refers to the mean incomes of those who produced CMD-resistant varieties; $\bar{X}_2$ refers to the mean incomes of those who did not produce CMD-resistant varieties; $S_1^2 \text{ and } S_2^2$ refer to standard deviations for the two groups (CMD-resistant varieties and non-producers); and $n_1 \text{ and } n_2$ refer to the sample sizes for the two different groups

III. RESULTS

3.1. Socio-economic Characteristics of Respondents

The socio-economic characteristics of the sampled cassava farmers analyzed included among others, gender, age, marital status, educational level, household size, cassava farming experience, number of extension contact, sources of farm labour, farm size, method of farm-land acquisition, reason for preference of particular cassava varieties, sources of non-farm income and other crops grown by the sampled farmers. Majority (63%) of the CMD-resistant and non-resistant varieties (60%) farmers were found to be male, while 38% of the CMD-resistant and 40% non-resistant varieties farmers were found to be female as shown in Table 1.

Table.1: Gender and age distribution of respondents

Variable	MD-resistant varieties Farmers		Non-CMD-resistant varieties armers	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Male	50	62.5	48	60
Female	30	37.5	32	40
Total	**80**	**100**	**80**	**100**
Age	16	20.00	0	0
Less than30	33	41.25	8	10.0
31 – 40	18	22.50	34	42.5
41 – 50	13	16.25	38	47.5
51 and above				
Total	**80**	**100**	**80**	**100**

The Table also revealed that the highest number (41%) of the CMD-resistant varieties farmers was found to be within the active age bracket of 31-40 years old. This indicates that most of the farmers were young and were likely to be more receptive to innovation and energetic for increased production, [20]. However, the highest population of the

non-CMD-resistant varieties farmers (48%) was found to fall within the age bracket of 51 years old and above, which indicates that they were much older, less active and are likely to be less receptive to innovation, and more conservative.

However, according to Table 2, over 80% of the CMD-resistant varieties and 78% non-resistant varieties farmers respectively were found to be married. Eleven percent, 1% and 8% of the CMD-resistant varieties farmers were single, divorced and widowed, respectively. However, none of the non-resistant varieties farmers were single, but 6% and 16% were divorced and widowed respectively. These indicate that both farmers were able to rely on family support in their farm work, since family is known to play critical role in provision of labour for farm work in the State, . [20]..

The results also showed the CMD-resistant varieties farmers, which is 3%, to have formal education, while on the other hand, among the non-resistant varieties' farmers, the highest percentage (48%) of them also had no formal education.. This is in line with the findings of [23]. who agreed that education significantly enhance farmer's ability to make accurate and meaningful management decision as he is able to read and interpret the recommended practices.

Table.2: Distribution of Farmers According to Marital Status and Educational Level

Variable	CMD-resistant varieties Farmers		Non-resistant varieties Farmers	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Marital Status:				
Married	64	80	62	77.5
Single	9	11.25	0	0
Divorced	1	1.25	5	6.25
Widowed	6	7.5	13	16.25
Total	**80**	**100**	**80**	**100**
Educational Level:				
No Formal Education	2	2.5	38	47.5
Primary Education	33	41.25	32	40
Secondary Education	39	48.75	10	12.5
Diploma and above	6	7.5	0	0
Total	**80**	**100**	**80**	**100**

Household Size of the farmers, was measured by adding up the number of wives, children, relatives and dependents actually living with the respondents as at the time of the survey. This information is important since agriculture in the study area is traditional and the primary source of cheap labour for farm work is the farmer's household. Table 3 indicates majority (51%) of the CMD-resistant varieties farmers to have household sizes of less than 5 members. Non-resistant varieties farmers, rather, had most of their members (41%) having between 6 and 10 household members,. Large households adopt fewer innovations, due to insufficient financial resources to acquire modern inputs after the other commitments of the family have been taken care of. Thus, innovative farmers tend to have smaller families.

Table.3: Distribution of Respondents According to Household Size and Years of Cassava Farming Experience

Variable	CMD-resistant varieties Farmers		Non-resistant varieties Farmers	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Household Size:				
Less than 5	41	51.25	24	30.00
6-10	25	31.25	33	41.25
11 and above	14	17.5	23	28.75
Total	**80**	**100**	**80**	**100**
Farming Experience				

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Less than 10	25	31.25	12	15.00
11-20	37	46.25	15	18.75
21-30	15	18.75	23	28.75
More than 31 years	3	3.75	30	37.5
Total	**80**	**100**	**80**	**100**

The Table also indicates majority (46%) of the CMD-resistant varieties farmers to have between 11 and 20 years of cassava farming experience, while non-resistant varieties farmers, on the other hand, had the largest percentage (38%) that had more than 31 years of cassava farming experiences. Most (38%) of the CMD-resistant varieties farmers had up to 3 times visits from extension officers or contacts during their cassava production period, while the highest population (50%) of the non-resistant varieties farmers reported to have had only a single contact with the extension officers, and the least population (15%) indicated to have had up to 3 times contacts, as shown in Table 4. This may have contributed to their non-production of the improved cassava varieties due to insufficient information from less extension contact. The highest proportion of the farmers, 64% and 58% of CMD-resistant varieties and non-resistant varieties farmers according to Table 4 employed both family and hired labour respectively. It has been argued that availability of family labour influences the adoption of new practices positively as it reduces the labour constraints faced by the farmers. However, farm size determines the scale of production in agriculture.

Table.4: Distribution of respondents according to number of extension contact and sources of farm- labour

Variable	**CMD-resistant varieties Farmers**		**Non-CMD-resistant varieties Farmers**	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Extension Contact:				
1	0	0	40	50.00
2	23	28.75	28	35.00
3	30	37.50	12	15.00
4	27	33.75	0	00.00
Total	**80**	**100**	**80**	**100**
Source of Labour				
Family	17	21.0	30	37.5
Hired Labour	12	15.0	4	5.0
Both family and hired labour	51	64.0	46	57.5
Total	**80**	**100**	**80**	**100**

The study also showed that 43% of the CMD-resistant varieties farmers cultivated cassava on less than 1 hectare of land, as shown in the Table 5. Similarly, 53% of the non-resistant varieties producers cultivated less than 1.0 hectare of cassava farm lands, while, 31%, 11% and 5% of them cultivated their non-improved cassava varieties on 1.1 – 2.0, 2.1 – 3.0 and 3.1 hectares and above, respectively. This implies small-scale farming on less than 2.0 hectares of farm land.

Table.5: Distribution of the farmers according to farm sizes and method of land acquisition

Farm size (ha)	**CMD-resistant varieties Farmers**		**Non-resistant varieties Farmers**	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Less than 1.0	34	42.5	42	52.5
1.1 – 2.0	31	38.75	25	31.25
2.1 – 3.0	11	13.75	9	11.25
3.1 and above	4	5.0	4	5.0

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TOTAL	**80**	**100**	**80**	**100**
Method of Land Acquisition:				
Inheritance	60	75.0	62	77.5
Purchase	14	17.5	16	20.0
Rent	6	7.5	2	2.5
TOTAL	**80**	**100**	**80**	**100**

Method of land acquisition revealed in Table 5, that about 75% and 78% of the CMD-resistant and non-CMD-resistant varieties farmers respectively, acquired their lands through inheritance. The least form of land acquisition was by rent, by 8% CMD-resistant and 3% non-CMD-resistant varieties farmers. This implied that farmers were restricted in terms of farm size due to land fragmentation, and those who have large farms were in bits and scattered in different locations. These impede easy access to more farm land for expansion and mechanization. Most of the CMD-resistant varieties farmers (71%) gave high yield of the improved varieties as the main reason for their adoption of the varieties. However, twenty percent of CMD-resistant and 31% of non-resistant varieties farmers gave high starch content, as their reasons for cultivating the improved varieties (Table 6). Forty-five percent of the non-resistant varieties farmers indicated that they preferred the local varieties because these tend to last longer on the farm. None of the non-resistant indicated high yield as a reason for their choice of their varieties. Experienced farmers have learnt over the years not to rely solely on any one agricultural activity for economic survival. Thus, they tend to generate additional incomes from other sources.

Table.6: Distribution of the respondents according to reasons for their choices of cassava varieties and sources of non-farm income

Reasons for Preference	**CMD-resistant varieties Farmers**		**Non-resistant Varieties Farmers**	
	Frequency	**Percentage**	**Frequency**	**Percentage**
High Yield	57	71.25	0	0
High Starch Content	16	20.0	25	31.25
Better Taste	3	3.75	19	23.75
Matures Early	4	5.0	0	0
Last Longer in farm	0	0	36	45.0
Total	**80**	**100**	**80**	**100**
Sources of Non-Farm Income				
Artisan	13	16.25	26	32.5
Manual-Labour	10	12.5	8	10.0
Transportation	10	12.5	5	6.25
Salaried work	18	22.5	6	7.5
Tailoring	16	20.0	11	13.75
Petty trade	13	16.25	16	20.0
Total	**80**	**100**	**80**	**100**

The results in Table .6 also shows that the highest percentage (23%) of the CMD-resistant varieties farmers earned non-farm income through salaried works, while, the greatest population (33%) of the non-resistant varieties farmers earned their non-farm incomes through artisan work. Meanwhile, Table 7 indicated majority (40%) of the CMD-resistant varieties farmers to have earned between ₦21,000 and ₦40,000 average annual income from their non-farm activities. The highest percentage (43%) of the non-CMD-resistant varieties farmers, too, earned average

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annual non-farm income of less than ₦20,000. These non-farm incomes are important since they act as financial security against risk.

Table.7: Distribution of the respondents according to average amount of non-farm income per annum

Average Amount of Non-Farm Income (Naira)	CMD-resistant varieties Farmers		Non-CMD-resistant varieties Farmers	
	Frequency	Percentage	Frequency	Percentage
Less than 20,000	21	26.25	34	42.5
21,000 – 40,000	32	40.00	23	28.75
41,000 – 60,000	15	18.75	12	15.00
61,000 and above	12	15.0	11	13.75
Total	**80**	**100**	**80**	**100**

Farmers in the study area practiced mixed cropping. A proportion (33%) of both CMD-resistant and non-resistant varieties farmers (33%) were sole cassava producers. Twenty-five percent, 9%, 5%, 11% and 18% of the improved varieties farmers produced maize, yam, plantain, vegetables and palm fruit respectively alongside the cassava as shown in Table 8. Similarly, 13%, 23%, 8%, 15% and 10% of the non-CMD-resistant varieties farmers combined their local cassava varieties production with maize, yam, plantain, vegetables and palm fruit, respectively. The farmers practiced mixed cropping as a way of diversification so as to increase their revenue, food supply and insurance bases, in case of poor yield from cassava production.

Table.8: Distribution of farmers according to cropping system

Cassava/Other Crop Produced	CMD-resistant varieties Farmers		Non-CMD-resistant varieties Farmers	
	Frequency	Percentage	Frequency	Percentage
Sole Cassava	26	32.5	26	32.5
Cassava / Maize	20	25.0	10	12.5
Cassava/ Yam	7	8.75	18	22.5
Cassava/ Plantain	4	5.0	6	7.5
Cassava/ Vegetables	9	11.25	12	15.0
Cassava/ Palm Fruit	14	17.5	8	10.0
Total	**80**	**100**	**80**	**100**

Table 9 contains the result of the relationship between CMD and non-CMD-resistant varieties farmers' socio-economic characteristics and their income. The linear form of the regression analysis was found to be the best in explaining the relationship for both groups of farmers. The R^2 was about 0.8354 for CMD and 0.6696 for non-CMD-resistant varieties farmers, which indicate that 84% and 67% of the variability in the incomes of CMD and non-resistant varieties farmers respectively was explained by the socio-economic characteristics. The F-values of 45.03 and 17.73 were significant at one percent level of probability, and indicated the overall statistical significances of the regression equations of both varieties as all the variables jointly determined the incomes of both farmers. Five, out of the eight socio-economic variables in the regression equation were found to be statistically significant at 10%, 5% and 1% levels of probability for the CMD-resistant varieties farmers, while four of the socio-economic variables were found to be statistically significant at 5% and 1% levels of probability for the non-resistant varieties farmers.

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Table.9: Socio-economic Determinants of Income of CMD-resistant and Non-resistant Varieties Farmers in the Study Area.

Variables		CMD-resistant Varieties Farmers		Non-resistant varieties Farmers	
		Linear	t-value	Linear	t-value
Age	(X_1)	-0.0388663	-0.57	0.0439739^{NS}	0.50
		(0.0679821)		(0.0871843)	
Farming Experience	(X_2)	0.1428501*	2.16	0.0023414^{NS}	0.04
		(0.0661088)		(0.0631227)	
Educational Level	(X_3)	0.1833944**	1.66	-0.3611233***	3.61
		(0.110422)		(0.1001117)	
Household Size	(X_4)	0.0906855**	1.64	0.4219649***	2.93
		(0.055296)		(0.1443919)	
Yrs of membership	(X_5)	-0.0925512	-0.57	0.9842953**	2.50
Of Association		(0.1635484)		(0.3937395)	
No. Extension Contact	(X_6)	0.2733607***	4.35	-0.3065236^{NS}	-0.54
		(0.0628415)		(0.5649307)	
Farm Size	(X_7)	6.563861***	13.13	2.701899***	5.40
		(0.4999238)		(0.5004699)	
Amount of Credit	(X_8)	-0.000031^{NS}	1.30	0.0000147^{NS}	0.63
		(0.0000239)		(0.0000233)	
Constant	(a)	7.0255***	2.85	-4.976575^{NS}	-1.17
		(2.467453)		(4.262384)	
R^2		0.8354		0.6696	
R^2 - adjusted		0.8168		0.6318	
F value		45.03***		17.73***	

***, ** , * = Significant at 1%, 5%, 1% levels of probability ; ^{NS} = Not Significance.

Values of Standard Error are in parenthesis

Farming experience had positive co-efficient and was significant at 10% level of probability for the CMD farmers but was not significant for the non-CMD farmers. This implied that farming experience had a direct effect on the income of CMD-resistant varieties farmers, as the positive sign suggests that increase in farming experience led to increase in the production and thus, income of CMD-resistant varieties farmers. This is consistent with the findings of [20], who found farming experience to be the main determinants of production efficiency and better income.

Educational level had positive coefficient that is significant at 5% level of probability for CMD but negative coefficient that is significant at 1% level of probability for non-CMD farmers. These indicate a positive relationship with the incomes of CMD-resistant varieties farmers and negative relationship with the incomes of non-CMD farmers. The implication is that the more educated the CMD farmer is, the more his income, since he is more knowledgeable in better production techniques. On the other hand, the more educated the non-CMD farmer is, the less he is likely going to continue to produce the non-resistant varieties thus, the less his income. This is consistent with the findings of [23]., who confirmed from their various studies that education was a predetermining factor in information assimilation and technological adoption among farmers of diverse socio-economic environment. The coefficient of household size was found to be positive as expected and significant at 5% and 1% levels of probability for CMD and non-CMD farmers respectively. This positive sign indicates that the higher the size, the higher the incomes of both farmers, since the assumption is that the more the number of members of the household in a subsistence set-up, the more the availability of cheap and ready family labour and thus, the more the output and income [20]. Family labour availability stimulates increase in production activities as labour constraint is reduced

Farm size also had positive coefficients and was significant at 1% level of probability for both groups of farmers. These indicate positive relationships with the incomes of the

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farmers and the implication is that the larger the farm size, the more the farm area cultivated and thus, the more the incomes of both farmers. However, the coefficient of number of extension contact was also found to be positive and significant at 1% level of probability for only CMD farmers. This implied that more contact with extension agencies enhanced information acquisition which encouraged investment in the CMD-resistant varieties production for a more rewarding income. However, based on this, therefore, the hypothesis that there is no significant relationship between farmers' socio-economic characteristics and their income from the production of CMD-resistant varieties and non-resistant varieties was rejected since all the variables' coefficients were statistically different from zero.

3.3. Costs and Returns of Cassava Production

The average costs and returns of cassava production for both the CMD-resistant and non-resistant varieties are as presented in Table 10. The CMD-resistant varieties farmers utilized about 21,000Kg of cassava stem cuttings, 225Kg of fertilizer and 205 man-days per 1hectare of farmland, and thus incurred about ₦66,750 Total Variable Cost (TVC). The non-resistant varieties farmers, on the other hand, utilized about 21,000Kg of cassava stem cuttings, 123Kg of fertilizer and 230 man-days per 1hectare of farmland, and thus incurred a Total Variable Cost of about ₦74,450. The farm land of CMD farmers was valued at ₦8,000 per hectare according to the prevailing rent value, while that of the non-CMD farmers was valued at ₦8,500 per hectare. Farm tools/implements were depreciated using the straight line method, and valued at market price of ₦10,400 for CMD farmers and ₦8,500 for non-CMD farmers. The CMD farmers were able to produce about 12,440Kg of cassava tubers valued at ₦99,520 at the rate of ₦8/Kg and 665 bundles of cassava stem valued at ₦66,500, at the rate of ₦100/bundle and thus, generated about ₦166,020 Total Revenue, a Gross Margin of ₦99,270, and Net Farm Income of ₦91,270. The non-CMD farmers on the other hand were able to produce about 8,165Kg of cassava tubers valued at ₦65,320 (at the rate of ₦8.00/Kg) and 588 bundles of cassava stem valued at ₦58,800 (at the rate of ₦100/bundle), to generate a Total Revenue of about ₦124,120, a Gross Margin of ₦49,670, and a Net Farm Income of ₦41,170. The CMD farmers were able to get an Average Rate of Return of 2.49 against 1.67 ARR of the non-CMD farmers. This 2.49 ARR meant that to every ₦1 spent by the CMD farmers a return of ₦2.49 was made, whereas, to every ₦1 spent by the non-CMD farmers a return of ₦1.67 which is less was made. In comparison therefore, the production of CMD-resistant varieties is more profitable than that of the non-resistant varieties since there was a better response of output to input in CMD-resistant varieties production than that of the non-resistant varieties.

Table.10: Costs and Returns of Cassava Production of CMD-resistant and Non-resistant Varieties.

Categories		**CMD-resistant Varieties Production**			**Non-resistant Varieties Production**		
A.	**Inputs/Costs:**	**Quantity**	**Cost/ Value**	**%**	**Quantity**	**Cost/ Value**	**%**
I.	Variable Inputs:		(₦)			(₦)	
	Cassava-stem cuttings(Kg)	21,000	7,000	8.00	2,100	4,800	4.80
	Fertilizer (Kg)	225	6,750	7.71	123	7,500	7.51
	Labour (man-day):	(205)			(230)		
	Family labour	100	30,000	34.29	121	36,300	45.65
	Hired labour	105	36,750	50.00	109	38,150	42.04
	Farm Size (ha)	1	0	0	1	0	0
a	***Total Variable Cost (TVC)***		***66,750***	***100***		***74,450***	***100***
2	Fixed Inputs:						
	Farm land (Rent)	1ha	8,000		1ha	8,500	
b	***Total Fixed Cost (TFC)***		***8,000***			***8,500***	
	Total Cost (TC=a+b)		***74,750***			***82,950***	
B	Outputs/Revenue:						

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	Tubers (Kg)	12,440	99,520	8,165	65,320
	Stem (Bundles)	665	66,500	588	58,800
C	***Total Revenue (Naira)***		***166,020***		***124,120***
	Gross Margin *(C – a)*		***99,270***		***49,670***
	Net Farm Income(C-b)		***91,270***		***41,170***
	Average Rate of Return	***(TR/TVC)***	***2.49***		***1.67***

3.3.1. Statistical difference between the mean incomes of both cassava farmers

The mean incomes of the CMD-resistant varieties farmers and that of non-resistant varieties' farmers were tested using the Z-test as shown in Table 11. This was necessary to achieve the second hypothesis, which states that 'there is no significant difference between the mean incomes of CMD-resistant varieties' farmers and non-resistant varieties farmers'. The CMD-resistant varieties farmers mean income was estimated to be ₦166,020 and that of the non-CMD-resistant varieties farmers was ₦124,120. The Z - calculated was found to be 3.5271 at 1% level of significance. This is greater than the tabulated (1.96), meaning that, there is a significant difference between the mean incomes of the CMD-resistant varieties and non-CMD-resistant varieties' production. The coefficient of variation of the mean incomes of the two groups of farmers was found to be 34%. The hypothesis that there is no significant difference between the mean incomes of CMD-resistant varieties' producers and non-CMD producers is thus rejected.

Table.11: Test of Statistical Difference in Income of the Cassava Farmers Using the Z-Test.

Group	Mean Income	Standard Deviation	Standard Error	Z-Calculated	Z-table	Sign.
CMD-resistant varieties Farmers	166,020	65134.60	7,636.2	3.5271	1.96	0.006***
Non-CMD-resistant varieties Farmers	124,120	32067.30				
Difference	41,900					
Co-efficient of Variation (%)	**33.76**					

*** = Significant at 1% level of probability

3.4. Input–output Relationships for the Production of CMD-resistant and Non- resistant Varieties

According to Table 12, the input–output relationships for the production of both the CMD-resistant and non-resistant varieties were best explained by the double-log forms of the production model. The R^2 for the CMD and non-CMD farmers were 0.831and 0.634 respectively, which meant that about 83% and 63% of the variability in the incomes of CMD and non-resistant varieties respectively was explained by the input variables. The F-value of 92.02 and 32.43 for CMD and non-CMD respectively were significant at 1% level of probability, which indicate that the independent input variables included in the models were important in explaining the variations in the incomes of the farmers. All the variables, such as stem-cutting, fertilizer, labour, and farm size had positive coefficients but only fertilizer and farm size were statistically significant at 1% and 5% levels of probability respectively for the CMD farmers, whereas, for the non-CMD farmers fertilizer, labour and farm size had positive coefficients and were significant at 1% level of probability. The quantity of cassava stem cuttings indicated a positive relationship for CMD farmers and negative for non-CMD farmers but was not significant. The possible explanation here is that increases in the quantity of fertilizer, labour and farm size increase the farmers' incomes.

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Table.12: Production Function Result for Cassava Production.

	CMD-resistant Varieties	Non-resistant Varieties
Variables	**Double-log**	**Double-log**
Constant	2.270***	3.926***
	(0.423)	(0.644)
Stem X_1	0.008	-0.100
	(0.067)	(0.114)
Fert. X_2	0.689***	0.537***
	(0.057)	(0.058)
Labour X_3	0.172	0.248***
	(0.144)	(0.217)
Farm S. X_4	0.095**	0.726***
	(0.129)	(0.219)
R^2	0.831	0.634
R^2–Adjusted	0.822	0.614
F – Value	92.02***	32.43***

F – Value = Significant at 1% level of probability

3.5. Resource Use Efficiency

The results of the calculations of resource use efficiency (Table 13) revealed that the CMD-resistant varieties farmers were efficient in the use of cassava stem cuttings, since the efficiency ratio is equal to 1.00. They, however, over-utilized fertilizer (0.69) and farm size (0.50), and under-utilized labour (1.15). The non-CMD-resistant varieties farmers, on the other hand, under-utilized cassava stem cuttings (2.5) and farm size (1.60), and over-utilized fertilizer (0.14) and labour (0.84).

Table.13: Estimated Marginal Value Product and Marginal Factor Cost

Production Resources	**CMD-resistant Varieties Efficiency**			**Non-CMD-resistant Varieties Efficiency**		
	MVP	**MFC**	**r=$\frac{MVP}{MFC}$**	**MVP**	**MFC**	**r=$\frac{MVP}{MFC}$**
Stem (Kg)	29,830	29,830	1.00	29,547	92,400	2.5
Fertilizer (Kg)	52,450	75,900	0.69	4,050	67,500	0.14
Labour (manday)	74,130	64,320	1.15	72,640	86,750	0.84
Farm Size(ha)	500	1,000	0.50	800	500	1.60

3.6. Constraints Faced by Farmers in the Production of CMD-resistant and Non-resistant Varieties in the Study Area

Majority (15%) of the CMD-resistant varieties and non-resistant varieties (16%) farmers reported high cost of production as the most important and ranked it as the first constraint in their cassava production (Table 14). This may be due to the low-income, poor and rural background of the cassava farmers. Eleven percent CMD and 15% non-CMD farmers ranked scarcity of the cassava stem cuttings at the peak of planting season as their second constraint.

Ten percent of the CMD farmers ranked difficulties in maintaining the cassava farm in terms of weeding and fertilizer application, and low farm gate price for the cassava outputs as third constraints, while 10% of the non-CMD farmers on the other hand ranked short storage duration and also low farm gate price as their third complaints in their cassava production.. However, nine percent of the CMD farmers ranked the need for the cassava stem to be planted on time for high yield and short storage duration as their fourth constraint, while 9% of the non-CMD farmers too ranked difficulty in maintaining the cassava farm in terms of weeding and insufficient fertilizer

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supply as at when needed as their fourth constraints. Eight percent of the CMD farmers ranked difficulty in getting enough CMD cassava stem during planting season and insufficient fertilizer supply as at when needed as the fifth constraints, while 8% of the non-CMD farmers on the other hand only ranked need for the cassava stem to be planted on time for maximum yield as their fifth constraint.

Table.14: Distribution of CMD-resistant varieties farmers according to the constraints of production of CMD-resistant varieties

S/N.	Constraints	CMD-resistant Varieties			Non-resistant Varieties		
		Freq	%	Rank	Freg.	%	Rank
1	High cost of production	12	15.00	1st	13	16.25	1st
2	The Cassava stem is scarce at the peak of planting season when needed most	9	11.25	2nd	12	15.00	2nd
3	Seen other farmers who plant CMD-resistant varieties fail	3	3.75	8th	5	6.25	6th
4	Needs to be planted on time for maximum yield.	7	8.75	4th	6	7.50	5th
5	It does not stay long in the farm but decays fast after maturity.	7	8.75	4th	8	10.00	3rd
6	Difficult to maintain in terms of weeding and fertilizer application.	8	10.00	3rd	7	8.75	4th
7	Difficult to get enough CMD cassava stem during planting season.	6	7.50	5th	5	6.25	6th
8	Low farm-gate price for output.	8	10.00	3rd	8	10.00	3rd
9	Poor means of transportation of output to the nearest market.	5	6.25	6th	3	3.75	8th
10	Insufficient fertilizer supply as at when needed.	6	7.50	5th	7	8.75	4th
11	Difficult to store/preserve produce after a certain period	5	6.25	6th	4	5.00	7th
12	Lack of financial assistant from the Government.	4	5.00	7th	2	2.50	9th
	Total	**80**	**100**		**80**	**100**	

Six percent of the CMD farmers ranked poor means of transportation of output to the nearest market and difficulty in storing or preserving produce after a certain period as the sixth constraints, whereas their counterpart ranked seeing other farmers who plant CMD-resistant varieties fail, and difficulty in getting enough CMD cassava stem during planting season as their sixth constraints. Five percent of the CMD farmers ranked lack of financial assistant from the Government as the seventh while 5% of the non-CMD farmers ranked difficulty in storing or preserving produce after a certain period as their seventh constraints too. The eight constraints ranked by the farmers were that about 4% of the CMD and non-CMD farmers complained to have seen other farmers who plant CMD-resistant varieties fail, and poor means of transportation of output to the nearest market, respectively. Only 3% of the CMD farmers rated lack of financial assistant from the Government as their ninth constraints

IV. CONCLUSION

Comparative Economic Analysis of Cassava Mosaic Disease-resistant varieties (CMD) and Non-Cassava Mosaic Disease-resistant varieties production in Akwa Ibom State is the main purpose of this study. Respondents were selected with the use of multi-stage sampling procedure and data collected with interview method and well structured questionnaires from 160 respondents.. Descriptive statistical analysis described the socio-

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economic characteristics of respondents, while, the Gross Margin Analysis enabled the evaluation of returns to investment of the CMD-resistant and non-CMD–resistant varieties farmers to enable their performances to be compared. Linear form of the regression analysis was found to be the best in explaining the socio-economic determinants of both farmers. Mean incomes and Z-test established the effects of production of the varieties on their incomes.

Majority (63% and 60%), of the respondents were male among the CMD and non-CMD-resistant varieties farmers respectively. But the CMD-resistant varieties farmers were found to be younger (35 years old) than the non-CMD-resistant varieties farmers (45 years old) on the average. Fifty-one percent of the CMD-resistant varieties farmers had house hold size of less than 5, against the majority (70%) of the non-CMD-resistant varieties farmers, who had more than 5 members in their house hold. Both groups of farmers were fully aware of the CMD-resistant varieties. The highest populations (43% CMD and 53% non-CMD) of both farmers produced their cassava on less than 1 hectare of farm land and the mean difference in farm size between the CMD-resistant and non-CMD-resistant varieties were found to be 0.913 and significant at 5% level of probability. Also, majority (75% and 78%) of the CMD-resistant and non-CMD-resistant varieties farmers respectively acquired their farm lands through inheritance. They both earned non-farm incomes within the range of slightly less than N20,000 and N61,000. Majority (76%) of the CMD-resistant varieties farmers had been in cassava production for less than 20 years, while 66% of the non-resistant varieties farmers had been in the cassava production for more than 20 years. The socio-economic determinants of the income of the CMD-resistant-varieties farmers were determined by a regression analysis. The linear form was found to be the best in explaining the relationship, since the magnitude of co-efficient of multiple determinations (R^2) was 0.8354 and the F-value was 45.03. These indicate that 84% of the variability in the income of CMD-resistant varieties was determined by the socio-economic characteristics and the statistical significance of the regression of the variables were important in explaining the variations in the income. The coefficients of farming experience, educational level, household size, and farm size were positive and statistically significant at various levels. However, for the non-CMD-resistant varieties the R^2 was about 0.6696, thus indicating that 67% of the variability in the income of the non-CMD-resistant varieties farmers was also determined by their socio-economic characteristics. The coefficients of household size, years of membership of association and farm size except educational level, positive and statistically significant at various levels too.

Cost and return analysis of the cassava production activities revealed that the CMD-resistant varieties farmers used ₦66,750 Total Variable Cost to generate an Average Revenue of about ₦166,020, a Gross Margin of ₦99,270 and a Net Farm Income of ₦91,270 and Average Rate of Returns of 2.49. The non-CMD-resistant varieties on the other hand, used a Total Variable Cost of about ₦74,450 to generate an estimated income of about ₦124,120, a Gross Margin of ₦49,670, and a Net Farm Income of ₦41,170 at an Average Rate of Returns of 1.67. The Farm Income of the improved varieties farmers increased by ₦50,100 or 121% over that of the non-improved varieties and was significant at 1% level of probability. Also, to every ₦1 spent by the CMD-resistant varieties farmers, a return of ₦2.49 was made, while for their counter-part, only ₦1.67 return was made. The input-output relationship for the CMD and non-CMD-resistant varieties, determined with the production function analyses revealed that the double log forms of the production function were found to give the best fit as the R^2 were 0.831 and 0.634 for CMD and non-CMD-resistant varieties respectively. These meant that 83% and 63% of the farmers' variations in the income of the improved or non-improved cassava varieties were explained by the input and cost variables. Positive signs and significant coefficients of the variables indicated that the production of the CMD-resistant varieties relate with the producers' incomes, while the reverse was the case if the sign or coefficient was negative as is seen in labour for the non-CMD-resistant varieties farmers in the study area. The resource use efficiency analysis revealed that the CMD-resistant varieties farmers were more efficient (1.00) in their cassava stem cutting usage and less efficient in the use of other inputs than the non-CMD-resistant varieties farmers who were inefficient in all their inputs allocation, since the efficiency scores were either less or more than 1.00. The Mean Incomes and Z-test revealed that the value of the Z-calculated (3.6128) was greater than the table Z (1.96) at 1% level of significance. Thus, the null hypotheses which state that 'there is no significant relationship between farmers' socio-economic characteristics and the income of CMD-resistant varieties and non-CMD-resistant varieties farmers and their mean incomes' were all rejected. However, both the CMD-resistant varieties farmers (16%) and non-CMD-resistant varieties farmers (19%) reported high cost of production, scarcity of planting material and low farm gate price for

output as the three major constraints in their cassava production. This may be due to the low-income, poor and rural background of the cassava farmers.

Socio-economic variables such as farming experience, education level, household size and farm size were the major determinants of CMD-resistant varieties' income. The non-CMD-resistant varieties farmers on the other hand, were influenced by their house hold size, membership of an association and farm size. The CMD-resistant varieties farmers earned more net farm income (₦91,270) than the non-CMD-resistant varieties farmers (₦41,170). The mean incomes of both farmers varied at 34%, Z-test indicated the Z-calculated (3.5271) was greater than the Z-tabulated (1.96) and the hypothesis which stated that there is no significant difference between the mean incomes of CMD-resistant and non-resistant varieties farmers was rejected. Fertilizer and farm size were the major inputs that determined the incomes of both farmers positively, while 83% and 63% of the variability in incomes of CMD-resistant and non-resistant varieties farmers respectively were explained by the input variables. The F-values of 92.02 and 32.43 for CMD-resistant and non-resistant varieties farmers respectively indicate that the independent input variables contained in the model were important in explaining the variation in the farmers' incomes. Only the CMD-resistant farmers were efficient in their stem cutting allocation and inefficient in the allocations of other resources, while the non-resistant varieties farmers were inefficient in all their inputs allocations. The most common constraints faced by both farmers in their cassava production were high cost of production, scarcity of planting materials during planting season, short storage duration and low farm-gate price for the cassava output. Based on the findings of the study these recommendations were made:

- Farmers were recommended to produce the CMD-resistant varieties since it is more profitable.
- They should be encouraged to form cooperatives through which extension workers can easily pass down research information to the practicing farmers, distribute the planting materials on time and help them market their produce.
- Cassava farmers should be encouraged to device means of earning more non-farm incomes, and to increase their farm holdings to enable them generate more income.
- Improved cassava stem should be made readily available as at when needed through farmers' co-operatives and associations.
- Occasional trainings should be organized for the farmers on the benefits of innovations, and they should be taught and encouraged to add value to their produce before sale by processing them first.

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Growth Pattern of *Pseudomonas aeruginosa* in different wastewater media

Aderiye Babatunde Idowu; Oluwole Olusola Adeoye*, Sulaimon Adebisi M, Bello Mustapha Oladapo

Department of Microbiology, Ekiti State University, Ado-Ekiti

***Abstract*—** *Restaurant wastewater are a major cause of environmental pollution with the indiscriminate release of the effluent to the environment resulting in blocking of drainages and eutrophication thereby causing serious threat to aquatic life. The growth pattern of a dietary rich oily wastewater degrading bacteria strain, Pseudomonas aeruginosa in different wastewater media composed to mimic the possible constituents of restaurant wastewater was investigated in this study. There was noticeable microbial growth in the synthetic and domestic wastewaters after 48h while the detergent wastewater and heated oil-detergent wastewater did not support the strain's growth. The decrease in the fat content with a corresponding increase in the ash content after 120h was due to the test strain's metabolic activity, which is slightly higher in domestic wastewater than the heated oil-detergent solution. Also, the potassium (K^+), Mg^{2+}, Fe^{2+} and Ca^{2+} contents increased within the same period in both media except in the domestic wastewater where the Ca^{2+} content reduced. Protease enzyme activity (46.440mM/min) was considerably higher in the domestic wastewater than lipase (3.322mM/min) and amylase activity (14.244mM/min) after 72 hours of incubation. The pristine genetic properties of Pseudomonas aeruginosa altered when cultured in various wastewaters probably due to variation in the composition of the substrates.*

***Keywords*— restaurant, domestic wastewater, Pseudomonas aeruginosa, synthetic wastewater, lipase, 16s rRNA sequencing.**

I. INTRODUCTION

Restaurant wastewater usually results from water that has been used for cleaning food products such as meats and vegetables, washing dishes and cooking utensils, or cleaning the floor of restaurants (Zulaikha et al., 2014). The effluent comprises leftovers of food, soup, detergents, fats, oil and grease which generates unpleasant odor when released into drains without proper pre-treatment processes (Xue et al., 2016).

The domestic wastewater is composed of proteins and carbohydrates, smaller amounts of lipids (Odeyemi et al., 2011), anthropogenic organic chemicals and some microbial pathogens (Tchobanoglous et al., 1991). The lipids (oils) that are released into the environment have been reported to be responsible for the clogging of sewer networks and unsettling the balance of water in the treatment plants and implicated as environmental pollutants (Saifudin et al., 2006; Xue et al., 2016).

Several authors have reported different wastewater contaminants in soil and aquatic environments in different parts of Nigeria (Nwachukwu et al., 2001; Adeyemo, 2003; Akpan, 2004; Efe, 2005; Zulaikha et al., 2014). In Nigeria, domestic wastewater especially from bukateria undergoes little treatment (Adeyemo, 2003). It is usually disposed on open lands or drainages where it empties into water bodies causing eutrophication (Akpan, 2004). Studies have shown that the chemical oxygen demand (COD) concentration, animal and vegetable oils and suspended solids are 16 times higher in restaurants wastewaters than domestic wastewater (Fadile et al., 2011; Kshirsagar, 2013; Xue et al., 2016) Leaching into groundwater is also a major part of environmental concern, especially due to the recalcitrant nature of some contaminants (Lapygina et al., 2002).

Biodegradation of fats and oils in wastewater has a potential role in pollution control employing the metabolic capacity and diversity of microorganisms to breakdown these complex substrates and reduce its toxicity (Nelson, 2009). Bioremediation offers a clean and cheaper alternative to conventional clean-up methods (Zhu et al., 2001; Xia et al. 2006; Calvo et al., 2008).

Lipase producing bacteria have been isolated from oil contaminated sites and exploited for their ability to remediate domestic wastewater polluted environments (Odeyemi and Aderiye, 2011). The bioremediation process is typically enzymatic, where amylases, oxygenases and lipases are secreted into the medium which facilitate the breakdown. Predominant among degraders of complex organic substrates including lipids and oils is the genus Pseudomonas which has been investigated for its ability for bioremediation (O'Mahony, 2006; Odeyemi and Aderiye, 2011). With domestic oil waste constituting a big threat to clean and hygienic environment in Nigeria, there is need to further exploit the potential of Pseudomonas aeruginosa previously

associated with the degradation of oil rich waste water (Odeyemi et al., 2014).

Therefore, this study was aimed at observing the growth pattern of P. aeruginosa in different wastewater media and monitoring the effect of some growth conditions on the genetic makeup of the microbe.

II. HEADINGS

Collection of domestic oil wastewater

Fresh wastewater samples containing a mixture of waste food debris, detergents and domestic oils were collected into sterile 2.5L sampling bottles from Falegan restaurant situated along Ekiti State Secretariat road, Ado-Ekiti, Ekiti State of Nigeria. The restaurant opens between 8am and 4pm daily and samples were collected at 2½h intervals (10:30am and 1pm and 3:30pm). The samples were transported in cold storage to the Microbiology laboratory of the Ekiti State University, Ado-Ekiti for further analyses.

Source of *Pseudomonas aeruginosa*

P. aeruginosa was obtained from the work of Odeyemi *et al*. (2013), previously isolated from domestic oil-rich wastewater sample collected from the same source and purified by sub-culturing on *Pseudomonas* agar (HiMedia, India).

Preparation and analyses of wastewater media

The growth culture media for *P. aeruginosa* include restaurant wastewater, palm oil/detergent solution, detergent solution and synthetic wastewater. Two hundred millilitre (200mL) of fresh restaurant wastewater was homogenized and sieved before autoclaving to prepare sterile wastewater media. For preparation of palm oil/detergent solution, 20mL of palm oil was heated for 15minutes in a ventilated oven at 100ºC, allowed to cool and 20g of detergent (OMO Unilever PLC.) was mixed with the heated palm oil and then dissolved in 200mL of distilled water in 1000mL Erlenmeyer flask prior to autoclaving. Detergent solution was prepared by dissolving 20g of the detergent in 100mL of distilled water, made up to 200mL in 250mL Erlenmeyer flask before autoclaving. Synthetic wastewater was formulated using a modification of the method of Foglar (2004) by dissolving 1g of sodium acetate, 0.1g $MgSO_4.7H_2O$, 0.1 g KNO_3, 0.1g KH_2PO_4, 0.2g meat extract and 0.1g NaCl in 200ml of distilled water prior to autoclaving. The media were then inoculated with the test strain.

Twenty millilitre (20mL) of standard inoculum was transferred into each of the four media, incubated at 30ºC and monitored spectrophotometrically for 5days at 24h intervals. Also, the microbial load, proximate components (moisture, ash, crude protein, crude fibre, fat and total carbohydrate contents) (AOAC, 2005) and mineral analyses (Fe^{2+}, K^+, Ca^{2+}, Mg^{2+}) (AOAC, 2005) were carried out during the same period.

Analysis and Production of Enzymes:

The wastewater media was prepared for enzyme production, with 100mL of each wastewater inoculated into five different 250mL flasks, incubated in an orbital shaker (Stuart shaker) at 150r.p.m and 30ºC. Samples were drawn from each of the flasks at 6h intervals for a period of 48h and each sample was centrifuged at 5000 rpm for 30 min at 4ºC. Cell free supernatant corresponding to each growth phase was used for the assay of crude enzyme (An *et al*., 1994). The activity of protease and amylase was determined according to the methods of An *et al.* (1994) and Berfield *et al*. (1995) respectively while lipolytic activity was determined by colorimetric method of Lotrakul and Dharmsthiti (1997).

Molecular characterization of *P. aeruginosa* (CP004061.1) grown in different wastewater media

Genomic DNA was isolated according to the method of Sambrook *et al*. (1989). The appropriate primer used for the work was designed by Inqaba Biotechnical Industries (South Africa). DNA isolation, PCR and sequencing were carried out at the International Institute for Tropical Agriculture (IITA, Ibadan, Nigeria). The sequences were analyzed using the BLAST (Basic Local Alignment Search Tool) bioinformatics program on the NCBI (National Center for Biotechnology Information) website.

III. RESULTS AND DISCUSSION

The growth and the degrading potential of Pseudomonas aeruginosa (CP004061.1) was monitored in different wastewater media for about 120h. This bacterial strain recovered from restaurant wastewater, after dish washing (with soap solution, 5g/100ml detergent in water), first and second rinsing of the dishes, and run-off into open sewers along the drainage was capable of growing well in dietary oil (Odeyemi et al., 2014).

The synthetic wastewater and detergent solution were used as experimental controls. It was observed that the synthetic media was best suited for the growth of the organism after 144h when compared to other media. There was about 553% increase in microbial growth than when cultured in the domestic wastewater. Meanwhile, there was no visible growth in either the detergent wastewater or the heated detergent wastewater (Figure 1). However, Odeyemi et al. (2013) reported that this Pseudomonas strain was capable of growing in detergent concentration as high as 5g/100ml which is lower compared to the 20g/100ml used in this study. Ambilly et al. (2014) reported the growth of Pseudomonas

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aeruginosa (MTCC 10311) in 96% of Sodium dodecyl sulfate after 48h incubation in detergent contaminated soil.

The various mineral salts present in the synthetic wastewater might have influenced the high count of the Pseudomonas sp. (Usharani et al., 2011). Odeyemi et al. (2013) also reported an appreciable increase in the weight of Pseudomonas aeruginosa with 66.7% weight gain after 5days incubation in domestic oil wastewater. The increase in the weight may be attributed to the ability of the strain to produce lipase which is responsible for dietary oil degradation.

The nutritive quality of any medium is better evaluated by assessing its proximate composition which provides information on the basic chemical components of the medium and the type of growth that takes place within such medium (Adeolu and Enesi, 2013). The nutritive qualities of the fresh heated oil/ detergent wastewater and domestic wastewater were examined. Similarly, these qualities were investigated after 144h incubation.

The proximate components of the culture media revealed that the ash content increased in both the synthetic and domestic waste water after 120h (0.16% to 0.24% and 0.21% to 0.26% respectively). However, the crude protein was found to decrease in the synthetic wastewater (0.45-0.37%) but increased in the domestic wastewater (0.38-0.40%). Also, there was reduction in the fat content of both media (0.11 to 0.07% and 0.10 to 0.06% respectively). There was however no significant difference in the moisture content in the synthetic wastewater and the domestic wastewater (0.02%) (**Table 1**). This report is similar to that of Odeyemi *et al*. (2014) where a low value of 3.2% crude protein, 3.1% carbohydrate and 1.2% fat was reported in the domestic waste water. The low organic matter observed might be due to variation in the organic components in the wastewater.

Ash content provides an estimate of the inorganic quality of a substrate (Adebowale and Bayer, 2002; Adeolu and Enesi, 2013). There was notable increase in the ash content of the two wastewater media after 120h incubation as shown in Table 1. The various activities of *Pseudomonas aeruginosa* on the organic component of the wastewater are likely responsible for the level of ash detected in the wastewater medium. Tchobanoglous *et al*. (1991) indicated that the organic matter in wastewater is mostly composed of proteins and carbohydrates and smaller amounts of lipids. The rate of microbial activity (fermentation) on the fibre content of the wastewater sample may have contributed to the slight increase in the carbohydrate and ash contents. This study also revealed the low crude protein content of the domestic wastewater and the heated oil/detergent wastewater during incubation. This observation is contrary to the findings of Effiong *et al*. (2009), who reported high values (between 26.2% and 36.8%) for ash in wastewater after treatment. According to Hanif *et al*. (2006), these amounts may be attributed to high nitrogen contents in those wastewaters with vegetables.

The mineral concentrations of both the heated oil/domestic and domestic oil wastes also increased after 120h. There was increase in the potassium (K^+) 7.20mg/L - 7.50mg/L (4.2%), Mg^{2+} (25.00mg/L - 28.10mg/L) (12.4%), Ca^{2+} (11.30mg/L - 11.35mg/L) (0.4%) and Fe^{2+} (0.01mg/L - 0.03mg/L) (200%) in the heated oil/detergent wastewater after 120h. Similarly, there was slight increase in the potassium content (6.50mg/L-7.00mg/L) (7.7%), Mg^{2+} (27.01mg/L-29.30mg/L) (8.5%), Fe^{2+} (0.01mg/L-0.03mg/L) (200%) in the domestic oil wastewater. Meanwhile, calcium reduced significantly (99.75%) from (12.00mg/L-0.03mg/L). Phosphorus was not detected in both media. The increase in the mineral contents of the waste media may be responsible for the high ash content observed after 120h in the waste media (**Table 2**).

Similar report was obtained by Odeyemi *et al.* (2013) where the mineral content of the domestic wastewater rose on the ninth day of degradation of the samples. Despite the low mineral contents, the media were still rich enough to support microbial growth due to low nutrient demand of microorganisms.

The pH and temperature of the wastewater culture media were also monitored. There was no significant change in the temperature of the media, which averaged 27.25ºC. However, there was a significant drop in the pH of the media except the synthetic media. Synthetic wastewater showed a pH range of 8.80-10.24, while the pH values in the domestic wastewater varied between 9.06 and 10.5; detergent wastewater varied between 9.85 and 11.31 and the heated oil wastewater between 8.75 and 11.15 (**Fig. 2)**.

The various biochemical reactions that took place within the medium depend on the enzymes which facilitate the processes (Kirk *et al*., 2005). The activity of the enzymes (amylase, lipase and protease) secreted by *Pseudomonas aeruginosa* into the growth media during incubation was examined at different time intervals. The activities of these enzymes increased during the incubation of domestic oil waste water. It was however observed that lipase activity (0.333, 2.778 and 3.322 mM/min) in the domestic wastewater was low when compared to amylase and protease activities. The results obtained for lipase activity were similar to those of Orapin *et al*. (2002) who reported higher fat (73%) and oil (88%) degradation in the wastewater treated with a pure culture of *Pseudomonas aeruginosa* after 7days.

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Amylase activity in the domestic oil waste water was 378.4% greater (14.24mM/min) after 120h storage than its initial value with protease activity exhibiting a tremendous influence on biodegradation of the waste water (46.44mM/min after 120h). Meanwhile, Laura *et al*. (2013) reported some toxicity impacts of sewage effluent on the amylase activity of pigeon pea (*Cajanus cajan* L.) in 50% and 100% sewage effluent for 8days. Interestingly, a corresponding increase in the protein concentration in the waste water was recorded after 120h (41.82mg/ml). The protease activity in the domestic oil wastewater was similar to the report of Irina and Yana (2010) who observed an accelerated increase in protein hydrolysis and the rate of protein removal from 10% to an average of 74% within 72h in kitchen wastewater treated with proteolytic bacteria which without the organism would have taken 135h. The high production of protease can be attributed to the meat, fish and other proteinaceous foods present in the domestic wastewater (**Table 3**).

The recovered genomic DNA extracted from the strain grown at optimal conditions in the different wastewater samples was studied. The entire 1.5 kb 16S rRNA gene was amplified (Figure 2) and sequenced. A search of the Genbank using the BLAST tool showed that the pristine strain cultured in the heated oil wastewater and in the domestic wastewater, at 98% identity value and 0.0 E-value was similar to *Pseudomonas* sp. (R3. 1B) and *Pseudomonas* sp. strain (PO150) respectively. Also, it showed 98% identity value and 0.0 E-value similarity and 97% identity value and 0.0 E-value similarity with *Pseudomonas* sp. strain (YR20) and *Pseudomonas* sp. strain (PO150) respectively, after BLAST.

Sequences reported here are available at the Genbank Nucleotide Sequence Database under the accession numbers KM058081.1, HG93439.1, HM224401.1 and KC433649.1 respectively.

The 16s rRNA sequencing analysis revealed that the *Pseudomonas* sp. strain (PO150) was present in both the domestic wastewater and synthetic wastewater and these can be named *Pseudomonas* sp. strain (PO150a) and *Pseudomonas* sp. strain (PO150b) respectively to distinguish them. *Pseudomonas* sp. (PO150b) had the highest degrading activity and this might be attributed to the composition of the synthetic wastewater (Figure 3). This is in agreement with high cell concentration observed in the synthetic medium after 120hours incubation.

The PCR reveals that there has been genetic modification in the pristine *Pseudomonas* strain when grown in domestic wastewaster and heated oil/ detergent media (Fig. 3). The molecular weight of the pristine isolate and synthetic media DNA sequence was around 0.75kbp compared to that from the same isolate grown in domestic wastewater and heated oil/ detergent media which was about 1.5kbp thus suggesting that the conditions present in the culture media of the domestic wastewater and heated oil/ detergent media may have affected the genome sequence. The nucleotide sequence tree relates the similarity of all the isolates; *Pseudomonas* sp. strain (Heated), *Pseudomonas* sp. strain (Domestic), *Pseudomonas* sp. strain (Pristine) *Pseudomonas* sp. strain (Synthetic) grown under the same condition in wastewater media.

IV. FIGURES AND TABLES

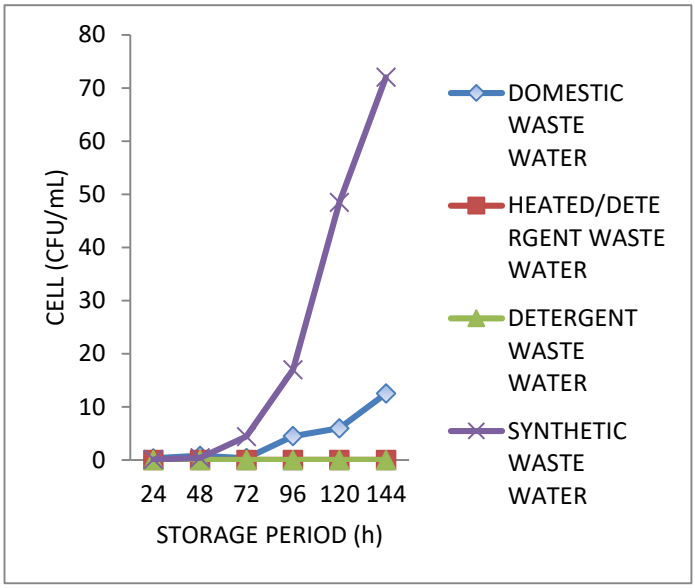
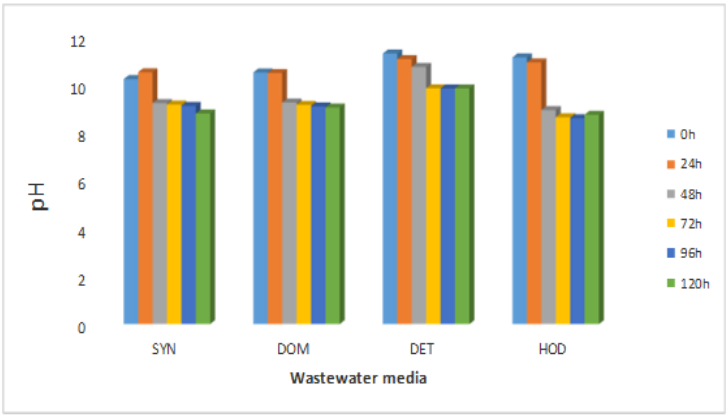


Figure 1: Growth of *Pseudomonas aeruginosa* in the different media



Legend: SYN (synthetic media), DOM (domestic oil wastewater media), DET (detergent wastewater media), HOD (heated oil detergent wastewater media)

Fig.2: The pH values of the wastewater media

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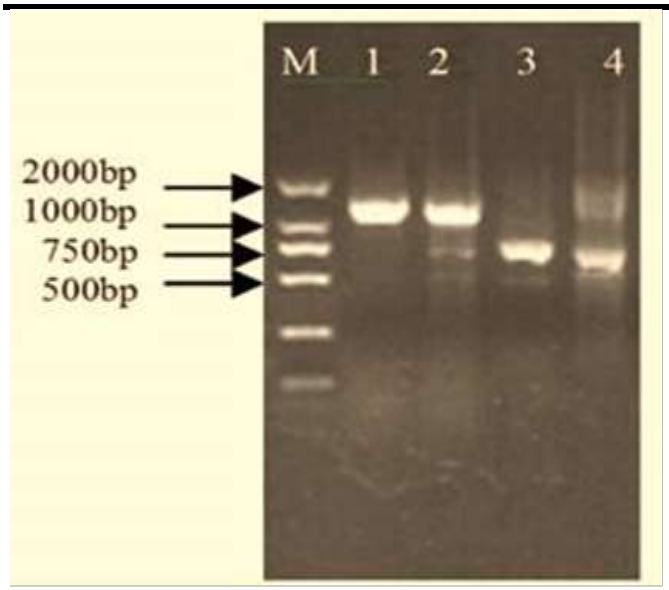

Fig.3: PCR results of the amplified genomic DNA of Pseudomonas aeruginosa.

Keys: M-DNA ladder: 2000bp, 1- *Pseudomonas* sp. (Domestic), 2- *Pseudomonas* sp. (Heated), 3- *Pseudomonas* sp. (Pristine) and 4- *Pseudomonas* sp. (Synthetic).

Fig.4: Nucleotide sequence tree of the Pseudomonas aeruginosa

Table 1: Proximate component of wastewater

Wastewater Sample	Proximate component (%)				
	Ash	Crude Protein	Carbohydrate	Fat	Moisture
Fresh Wastewater					
Heated oil and detergent wastewater	0.16±006^{c}	0.45±010^{a}	1.05±010^{a}	0.11±010^{a}	98.24±010^{c}
Domestic oil-rich wastewater	0.21±010^{b}	0.38±010^{bc}	0.12±010^{d}	0.10±010^{a}	99.21±010^{a}
After 120h Incubation					
Heated oil and detergent wastewater	0.24±010^{a}	0.37±010^{c}	0.60±10^{b}	0.07±010^{b}	98.71±010^{b}
Domestic oil-rich wastewater	0.26±010^{a}	0.40±010^{b}	0.40±10^{c}	0.06±010^{b}	98.89±015^{b}

All data were mean ± standard deviation of triplicate determinations

V. CONCLUSION

Microbial communities are prone to adapt to a substrate when it is a regular contaminant, such is the situation for the Pseudomonas sp. inoculated into the wastewater media. The genetic sequence of the original inoculum changed when grown in the different wastewater media which might be due to the variations in the media composition since the organism was exposed to the same environmental conditions. However, the nature and mechanism of the genetic changes of this organism in the different growth media is still open to investigation.

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Table 2: Mineral component of fresh growth media and after 120h of incubation

Sample	Mineral content (mg/L)			
	Potassium	Magnesium	Calcium	Iron
Fresh medium				
Heated oil and detergent wastewater	7.20±0.06^{b}	25.00±0.00^{d}	11.30±0.00^{a}	0.01±0.00^{a}
Domestic oil-rich wastewater	6.50±0.00^{d}	27.01±0.06^{c}	2.00±0.00^{b}	0.01±0.00^{a}
After 120hours Incubation				
Heated oil and detergent wastewater	7.50±0.00^{a}	28.10±0.00^{b}	11.55±0.10^{a}	0.03±0.10^{a}
Domestic oil-rich wastewater	7.00±0.00^{c}	29.30±0.00^{a}	0.03±0.10^{c}	0.03±0.10^{a}

All data were mean ± standard deviation of triplicate determinations

Table 3: Activity of enzymes secreted by *Pseudomonas aeruginosa* in domestic oil rich wastewater

Incubation period	Enzyme activities (mM/min)			Protein concentration (mg/mL)
	Amylase	Lipase	Protease	
0h	3.764	0.333	0.485	4.318
72h	10.258	2.778	24.515	17.500
120h	14.244	3.322	46.440	41.818

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Allelopathic Effects of Sweet Basil (*Ocimumbasilicum* L.) on Seed Germination and Seedling Growth of some Poaceous Crops

Awadallah B. Dafaallah*, Sara Al. Ahmed.

Crop Protection Department, Faculty of Agricultural Sciences, University of Gezira, Sudan.

Abstract— *Laboratory and greenhouse experiments werecarried out at the Faculty of Agricultural Sciences, University of Gezira, Sudan in season 2014/15 to study the allelopathic effectsof aboveground parts ofsweet basil (Ocimumbasilicum L.) on seed germination and seedling growth of some poaceous crops.Laboratory experiments were conducted to study the allelopathic effects of aqueous extract of aboveground parts ofsweet basilon seed germination of sorghum (Sorghum bicolor [L.] Moench), millet (Pennisetumglaucum [L.]R. Br.), maize (Zea mays L.) and wheat (TriticumvulgareL.).Six concentrations (0, 20, 40, 60, 80 and 100%) of the aqueous extract of the aboveground parts of sweet basilwere prepared from the stock solution(50 g / l). Treatments, for each crop, were arranged in completely randomized design with four replicates. The seeds were examined for germination at three days after initial germination.Greenhouse experimentswere conducted to study the allelopathic effects of powder of aboveground parts ofsweet basil on seedling growth of the same poaceous crops. The powder of aboveground parts was incorporated into the soil at rate of 0, 1, 2, 3, 4 and 5% on w/w bases in pots. Treatments, for each crop, were arranged in completely randomized design with four replicates. The experiments were terminated at 30 days after sowing and the plant height, number of leaves and root length of crop seedlings were measured as well as plant fresh and dry weight.Data were collected and subjected to analysis of variance procedure. Means were separated for significance using Duncan`s Multiple Range Test at $p \leq 0.5$. The results showed that the aqueous extract of aboveground parts of sweet basil significantly reduced seed germination of the tested poaceous crops and there was direct negative relationship between concentration seed germination. Also, the results showed that incorporating powder of aboveground parts into the soil significantly decreased plant height and root length of crop seedlings as well as seedling fresh and dry weight. In addition, the reduction in seedling growth was increased as the powder increased in the soil. Based on results supported by different studies, it was concluded that sweet basil has allelopathic affects on seed germination and seedling growth of the poaceous crops.*

Keywords— Allelopathic; Allelochemicals; Sweet Basil;Ocimum; Poeaeae; sorghum; millet; maize; wheat.

I. INTRODUCTION

The genus *Ocimum*, Lamiaceae, collectively called basil, is comprises more than 30 species of herbs and shrubs from the tropical and subtropical regions of Asia, Africa, and Central and South America, but the main center of diversity appears to be Africa (Paton, 1992). It is a source of essential oils and aroma compounds (Simon *et al.*, 1984, 1990), a culinary herb, and an attractive, fragrant ornamental (Morales *et al.*, 1993; Morales and Simon, 1996). The seeds contain edible oils and a drying oil similar to linseed (Angers *etal.*, 1996). Extracts of the plant are used in traditional medicines, and have been shown to contain biologically active constituents that are insecticidal, nematicidal, fungistatic, or antimicrobial (Simon*et al.*, 1990; Albuquerque, 1996).

The *Ocimum* plants have been investigated as potential allelopathic plants (Baličević*et al.*, 2015). Allelopathy refers to direct or indirect negative effects of one plant on another through the release of chemical compounds into the environment (Delabays*et. al.*, 2004). These biochemicals are known as allelochemicals (Singh and Chaundhary, 2011). Allelochemicals are released from plant parts by means of leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Chou, 1990). The allelochemicals can reduce cell division or auxin that induces the growth of shoot and roots (Gholami*et al.*, 2011). Allelochemicals such as phenolic compounds inhibit root and shoot length (Hussain and Reigosa, 2011). Growth inhibition caused by

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these allelochemicals may probably be due to its interference with the plant growth processes (Gholami*et al.*, 2011). Allelochemicals released to the environment can either inhibit shoot and/or root growth, nutrient uptake, or may attack a naturally occurring symbiotic relationship thereby destroying the plant's source of a nutrient.

Baličević*et al.*(2015) demonstrated that aromatic plants show allelopathic effect toward germination, root and shoot length and fresh weight of weeds, both inhibitory and stimulatory. The allelopathic effect depended on donor and target species.Đikić (2005) reported inhibitory effect of caraway, dill, basil and coriander on germination of hoary cress(*Lepidiumdraba*L.). Dhima *et al.* (2009) found that water extracts of aboveground mass of basil, coriander and oregano reduced germination and growth of barnyardgrass(*Echinochloa crus-galli*[L.] PB.), while in field experiments reduced plant number of different weed species when incorporated as green manure.

Understanding well the mechanism of allelopathic interactions between aromatic plants andcrops will enable to come up with proper and effective management of the agricultural ecosystem. Considering the economic importance of poaceouscrops, these studies were carried out to investigate the allelopathic effects of sweet basil (*Ocimumbasilicum* L.), on seed germination and seedling growth of some poaceouscrops, particularly sorghum (*Sorghum bicolor* [L.] Moench), millet (*Pennisetumglaucum* [L.]R. Br.), maize (*Zea mays* L.) and wheat (*Triticumvulgare*L.).

II. MATERIALS AND METHODS

2.1. Experimental site

A series of experiment wascarriedout at Faculty of Agricultural Sciences (FAS), University of Gezira (UofG), Sudan, comprised germination test and pot experiments. The germination test was conducted in the biology laboratory having an average temperature range of 25 - 30°C and the relative humidity ranging from 60 to 70 %. The pot experiment was conducted in a greenhouse of horticulture nursery under field conditions. The experimental site was located at Latitude 14° 24′ N, Longitude 33° 29′ E and 407m asl. The climate of the region is semi-desert with a mean annual precipitation of 100-250 mm/year, with the rainy season extended from June to October and the dry season from March to June. The mean annual evapotranspiration is 2400 mm/year. The mean annual minimum and maximum temperatures are 12 °C in January and 42°C in May, respectively. The soil of the area is characterized by heavy clay soil (clay 60%), with pH 8-8.5, low organic matter and nitrogen, adequate potassium and low available phosphorous(Elbasher, 2016).

2.2. Materials collection

Mature plants of sweet basil plants were collected from Experimental Farm of the FAS in season 2014/15. The plants were transferred to the biology laboratory of theFAS. The aboveground parts of plants were collected and then washed with sterilized distill water, air dried on bench for 15 days at room temperature in a dark room to avoid the direct sun light that might cause undesired reactions. The dried aboveground parts were then crushed into powder and kept in brown bottles till used. Certified commercial seeds of sorghum (cv. *Tabat*), millet (cv. *Baladi*), maize (cv. *Hudeiba* I) and wheat (cv. *Imam*), that have a germination percentage of 95-100% and purity of 100%, were obtained from the central market of Wed Medani city, Gezira state, Sudan. The seeds were surface sterilized by sodium hypochlorite, (NaOCl) 1% (v/v), solution, for 3 min continuously agitated to reduce fungal infection. Subsequently the seeds were washed with sterilized distill water for several times and stored at room temperature till used.

2.3. Laboratory experiments

These experimentswere conducted in the biology laboratory to study the allelopathic effects of aqueous extract of aboveground parts of sweet basilon seed germination of sorghum, millet, maize and wheat. Fifty grams of the powder of aboveground parts of sweet basilwere placed in a conical flask,sterilized distill water was added to give a volume of 1000 ml and then the flasks wereshaken for 24 hours at room temperature (27±3°C) by an orbital shaker (160 rpm). The extracts were drained through double layers of cheese cloth and then through 2 layers of WhatmanNo-2 filter paper to remove solid material. The filtrate was centrifuged at 3000 rpm for 20 min. The supernatant was collected and filtered through a 0.22 µm membrane filter paper. The stock solution was stored at 4°C until further use. Six concentrations (0, 20, 40, 60, 80 and 100%) of the aqueous extract were prepared from the stock solution.Seeds of sorghum, millet, maize and wheat (100 seeds each) were put on Glass Fiber Filter Paper (GFFP) (Whatman GF/C) placed in a glass Petri-dish (GPD), 9 cm internal diameter (i.d). Each GPD moistened with 20 ml of aqueous extract of aboveground parts of sweet basil, sealed with Parafilm, covered with black polyethylene bag and incubated at 30^0C in the dark.The treatments, of each crop, were arranged in completely randomized design with four replicates. The seeds were examined for germination at three days after initial germination for three days.

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2.4. Greenhouse experiments

These experiments were conducted at the greenhouse of horticulture nursery to study the allelopathic effects of powder of aboveground parts ofsweet basil on seedlings growth of sorghum, millet, maize and wheat.Plastic pots, 10 cm i.d. and 18 cm high with drainage holes at the bottom, were filled with Gezira soil and river silt that at the ratio 1:1, oven dried at 120 C for 48 h and screened to pass a 2-mm sieve. The powder of aboveground parts ofsweet basilwas incorporated into the soil at rate of 0, 1, 2, 3, 4 and 5% on w/w bases.Five seedsof each cropwere sownin pots. The pots were kept weed free, irrigated and then seedlings were thinned to 3 plants per pot, 7 days after emergence. Treatments, for each crop, were arranged in completely randomized design with four replicates. At 30 days after sowing the experiments were terminated and plant height (cm), number of leaves and root length (cm) of crop seedlings were measuredas well as seedlings fresh and dry weight (g).

2.5. Statistical analysis

Data were collected and subjected to analysis of variance procedure. Means were separated for significance using Duncan`s Multiple Range Test at $p \leq 0.05$.The statistical analysis was done using the Statistical Analysis System software v.9.0 (SAS, 2004).

III. RESULTS

3.1. Laboratory experiments

The results of laboratory experimentsshowed that the aqueous extract of aboveground parts of sweet basilsignificantly ($P \leq 0.05$) reduced seed germination of the tested poaceouscrops compared to the controls(Table 1).The reduction in seed germination increased with concentration of aqueous extract of aboveground parts. The highest seed germination was observed in the corresponding controls.However, the highest concentration (100%) displayed lowest seed germination which was71.5, 74.3, 58.3 and 67.3 % in sorghum, millet, maize and wheat, respectively. Maize seeds were highly affected by the aqueous extract of aboveground parts of sweet basil in comparison to other tested crops.

3.2. Greenhouse experiments

The results of the greenhouse experiments showed that incorporatedpowder of aboveground parts of sweet basil into the soil significantly ($P \leq 0.05$) decreased seedling growth attributes of tested poaceouscrops in comparison to the controls(Table 2, 4, 5 and 6).

3.2.1. Effects onplant height

At 30 days after sowing, the highest plant crop seedlings were observed in the control treatments (Table 2). The plant height of sorghum, millet, maize and wheatin the control treatmentswere37.5, 30.3, 43.5 and 24.3cm, respectively.However, increasing the concentration of powder of aboveground parts of sweet basilinto the soil exhibited lowest plant height in all tested crops.The powder of aboveground parts of sweet basilwhen incorporated into the soil at rate of 1 to 5% decreased the plant height of poaceouscrops in comparison to control treatments(Table 2). Moreover, the reduction in the plant height was increased as powder of aboveground parts increased in the soil. The greatest reduction in plant height was observed when powder of aboveground parts was incorporated intothe soil at the rate of 5%. At high concentration of powder of aboveground parts, the plant heights were significantly($P \leq 0.05$) decreased to 31.3 cm in sorghum, 15.0 cm in millet, 35.8 cm maize and 10.8 cm in wheat seedlings.

3.2.2. Effects on number of leaves

At 30 days after sowing, the results showed that incorporated powder of aboveground parts of sweet basilinto the soil at rate of 1, 2, 3, 4 and 5% negatively affected the leaves number of seedlings of all testedcrops compared to the control treatments (Table 3). The highest leaves numbers of crop seedlings were obtained in the control treatments.The leaves number of sorghum, millet, maize and wheat in the control treatments was 6.8, 7.5, 7.0 and6.0, respectively(Table 3). Incorporating powder of aboveground parts of sweet basil into soil at the rate of3% or more significantly ($P \leq 0.05$) reduced leaves number of seedlings of milletin comparison to the control treatments. While, significant reduction in leaves number of seedlings of maize and wheatwere obtained as powder of aboveground parts incorporated into soil at the rate of 5%. However, sorghum seeds were not significantly affected by incorporated powder of aboveground parts of sweet basilinto the soil at rate of 1 to 5% in comparison to other tested crops.

3.2.3. Effects on root length

Incorporation of powder of aboveground parts of sweet basilinto the soil significantly ($P \leq 0.05$) reduced root length of seedlings of all testedpoaceous crops (Table 4). The reduction in root lengths was increased with concentration of powder of aboveground parts in the soil.At 30 days after sowing, the longest root lengths of crop seedlings were observed in the control treatmentsand amounted to 21.0, 25.8, 20.3 and 15.8 cm in sorghum, millet, maize and wheat, respectively.The root length was decreased to

10.3cm in sorghum, 15.5cm in millet, 15.3 cm maizeand 7.8 cm in wheatseedlingswhen powderof aboveground parts of sweet basil was incorporated into the soil at concentration of 5%.

3.2.4. Effects on fresh weight

The greatest fresh weights of crop seedlings, at 30 days after sowing, were recorded in control treatments (Table 5). The incorporationpowder of aboveground parts of sweet basil into soil at the rate of 2% or more significantly ($P \leq 0.05$) reduced fresh weight of sorghum, millet, maize and wheatin comparison to control treatments.Moreover, the reduction in the fresh weight was increased as the powderincreased in the soil.The incorporation of powder of aboveground parts of sweet basil into the soil at rate of 5% resulted in seedling fresh weights amounted to 6.2, 5.2, 9.3 and 4.3 g in sorghum, millet, maize and wheat, respectively.

3.2.5. Effects on dry weight

The results of incorporatedpowder of aboveground parts of sweet basil into the soil at rate of 1, 2, 3, 4 and 5% on seedling dry weight had same trend as seedlings fresh weight (Table 6).Incorporating powder of aboveground parts of sweet basil intothe soil at rate of2% or more significantly reduced fresh weight of sorghum, millet and wheatin comparison to the control treatments. While, significant reduction in dry weight of maizeseedlings were obtained when the powder incorporated intothe soil at rate of 3% or more compared to the control treatments. The incorporation of powder of aboveground parts of sweet basil into to the soil at concentration of 5% (w/w) decreased the seedlingdry weight to 1.1 g in sorghum, 1.1 g in millet, 2.2 gmaizeand 0.8 g in wheat seedlings.

IV. DISCUSSION

The results of these studies revealed that the aqueous extract of aboveground parts of sweet basil significantly reduced seed germination of the tested poaceouscrops and there was a direct relationship between concentration and reduction in germination. These findings were in agreement with observation made by Sharmal and Singh (2003) that evaluated the allelopathic effects of basil (Tulsi) (*Ocymumsanctum*) on the germination of someweedspecies. They found that the germination of radish, redroot pigweed, hairy beggarticksandguineagrass was completely inhibited with addition of 7.5 g basil leaf powder to 100 gofsand as compared to plants grown in sand alone or in a mixture of sand and sphagnum. Also, the germination of seeds was significantly inhibited in redroot pigweed and hairybeggarticks when grown in 10% (w/v) basil leaf extract as compared to distilledwater. Baličević*et al.*, (2015) studied the allelopathic effect of basil (*Ocimumbasilicum*] on germination and early growth of weeds under laboratory conditions and found that basil reduced germination of hoary cress from 13.8 to 27%. These effects of basil powder were possibly due to the release of allelochemicals after decaying (Chou and Patrick, 1976).

This study indicated that incorporating powder of aboveground parts of sweet basil into the soil at rate of 1, 2, 3, 4 and 5% (w/w) significantly decreased plant height, number of leaves per seedling, root length of crop seedlings as well as plant fresh and dry weight. In addition, the reduction in seedling growth was increased as seed powder increased in the soil. These results are in lined with the findings reported by Đikić (2005) and Dhima *et al.* (2009). The study pertains to the exploration of the phytotoxic (allelopathic) potential of aqueous extracts derived from leaf, root and seeds of *Ocimum* on some commercially important agricultural crops like wheat, gram lentil, mustard, barley, okra and pea, in terms of seed germination, root and shoot elongation. The inhibitory effect was exhibited by all the extracts with maximum in leaf followed by root and seed extract (Verma*et al*., 2012). Baličević*et al*. (2015) reported that the extracts from dry plant biomass of basil in higher concentration completely (10%) inhibited germination and weed seedling growth of scentless mayweed (*Tripleurospermuminodorum*[L.] C.H. Schultz). On average, the extracts from dry plant biomass had higher inhibitory effect. Reduction in weed seed emergence and growth was recorded when dry plant residues in rates of 10 and 20 g/kg were incorporated in the soil.

Moreover,the aqueous leaf extract of Basil (*Ocimum sanctum* L.) plants was prepared in different concentrations and was tested on some legumes like Green gram (*Phaseolusradiata*[L.]Wilczek), Cow pea (*Phaseolusunguiculata*(L.)Walp), Pigeon pea (*Cajanuscajan*L.), Chickpea (*Cicerarietinum L.*), Black gram (*Phaseolusmungo*(L.) Heeper) and Moth bean (*PhaseolusaconitifoliusJacq.).* Some concentrations were also used to see the effect on *Dichanthiumannulatum*L., *Chlorisbarbata*L., *Acalyphaindica*L .and *Amaranthusspinosus*L. The study was conducted at laboratory condition to see the effect of extracts on seed germination and seedling growth. The objective of the study was to find out suitable concentration which inhibits weed germination but not of legume crops. The study showed that Basil had differential effects on each legume at different concentration.Based on results supported by different studies, it was concluded that sweet basil has allelopathic

affects on seed germination and seedling growth of the tested poaceouscrops(Purohit and Pandya, 2013).

V. CONCLUTION

- The aqueous extract of the aboveground parts of sweet basil, significantly, reduced seed germination of the poaceouscrops;sorghum, millet, maize and wheat.
- Incorporating powder of the aboveground parts of sweet basil into the soil, significantly, decreased plant height, number of leaves and root length of crop seedlings as well as seedlings fresh and dry weight of all tested crops.
- The reduction in seedling growth was increased as seed powder increased in the soil.
- Sweet basil has allelopathic effects on seed germination and seedling growth of the testedpoaceouscrops.
- More studies related to the effects of sweet basil allelochemicals over cultivated plants and other weed plants are required.

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Table.1: Allelopathic effects of aqueous extract of aboveground parts of sweet basil on seed germination of some poaceouscrops

Concentration extracts (w/v)	Seed germination (%)			
	Sorghum	Millet	Maize	Wheat
0%	97.3 a	98.8 a	96.5 a	94.5 a
20%	92.5 b	97.8 a	92.5 a	91.3 a
40%	89.3bc	94.0ab	81.3 b	81.3 b
60%	86.3 c	89.8bc	77.5bc	72.0 c
80%	81.5 d	85.0 c	76.3 c	63.0 d
100%	71.5 e	74.3 d	58.3 d	67.3 e
$SE_{\pm}$	1.45	1.90	1.51	2.05
$CV_{\%}$	3.4	4.2	3.8	3.7

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table.2: Allelopathic effects of incorporated powder of aboveground parts of sweet basilinto soil on plant height of some poaceouscrops

Concentration the powder (w/w)	Plant height (cm)			
	Sorghum	Millet	Maize	Wheat
0 %	37.5 a	30.3 a	43.5 a	24.3 a
1 %	37.0 a	25.0 b	39.0 b	23.8 a
2 %	36.5 a	22.0 c	37.3 b	22.5 a
3 %	35.8ab	21.3 c	36.8 b	16.8 b
4 %	33.8 b	17.3 d	36.0 b	11.8 c
5 %	31.3 c	15.0 e	35.8 b	10.8 c
$SE_{\pm}$	0.81	0.67	1.00	1.17
$CV_{\%}$	4.5	6.1	5.2	12.8

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table.3: Allelopathic effects of incorporated powder of aboveground parts of sweet basilinto soil on number of leaves of some poaceouscrops

Concentration the powder (w/w)	Number of leaves			
	Sorghum	Millet	Maize	Wheat
0 %	6.8 a	7.5 a	7.0 a	6.0 a
1 %	6.0 a	7.5 a	7.0 a	5.8ab
2 %	6.0 a	7.0ab	6.3ab	5.5ab
3 %	5.8 a	6.0bc	6.3ab	5.3ab
4 %	5.5 a	5.0 c	5.5ab	4.8ab
5 %	5.3 a	5.0 c	5.0 b	4.3 b
$SE_{\pm}$	0.46	0.41	0.48	0.50
$CV_{\%}$	15.7	12.9	15.5	19.1

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

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Table.4: Allelopathic effects of incorporated powder of aboveground parts of sweet basilinto soil on seedlings root length of some poaceouscrops

Concentration the powder (w/w)	Seedlingsroot length (cm)			
	Sorghum	Millet	Maize	Wheat
0 %	21.0 a	25.8 a	20.3 a	15.8 a
1 %	17.8 b	22.5 b	19.8ab	15.5 a
2 %	15.8 c	18.8 c	17.8bc	13.8ab
3 %	14.0 cd	17.8 cd	16.8 cd	12.0bc
4 %	12.8 d	17.3 cd	16.5 cd	10.5 c
5 %	10.3 e	15.5 d	15.3 d	7.8 d
$SE_{\pm}$	0.64	0.87	0.67	0.73
$CV_{\%}$	8.3	8.9	7.6	11.6

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table.5: Allelopathic effects of incorporated powder of aboveground parts of sweet basilinto soil on seedlings fresh weight of some poaceouscrops

Concentration the powder (w/w)	Seedlingsfresh weight (g)			
	Sorghum	Millet	Maize	Wheat
0 %	12.7 a	10.4 a	14.4 a	8.3 a
1 %	12.1 a	10.2 a	14.3 a	8.3 a
2 %	10.6 b	8.1 b	12.3 b	6.2 b
3 %	8.2 c	8.1 b	12.3 b	6.1 b
4 %	7.1 d	6.2 c	11.3 c	5.2 c
5 %	6.2 e	5.2 d	9.3 d	4.3 d
$SE_{\pm}$	0.20	0.13	0.21	0.13
$CV_{\%}$	4.1	3.2	3.4	4.0

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table.6: Allelopathic effects of incorporated powder of aboveground parts of sweet basilinto soil on seedlings dry weight of some poaceouscrops

Concentration the powder (w/w)	Seedlingsdry weight (g)			
	Sorghum	Millet	Maize	Wheat
0 %	3.9 a	2.4 a	3.2 a	1.9 a
1 %	3.7 a	2.3 a	3.2 a	1.7ab
2 %	3.0 b	2.0 b	3.1 a	1.6bc
3 %	1.8 c	1.5 c	2.7 b	1.3 cd
4 %	1.4 d	1.3 cd	2.4 c	1.2 d
5 %	1.1 e	1.1 d	2.2 c	0.8 e
SE±	0.11	0.08	0.08	0.08
CV%	8.7	9.3	5.4	11.1

* Means in the same column followed by the same letter(s) are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

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Effects of Electromagnetic fields on the Physicochemical Properties of Waste Water Samples from Selected Industries in Akure Metropolis.

Boboye B.[1], Adetuyi F. C.[1]., Balogun O. B.[2]

Department of Microbiology Federal University of Technology, Akure Ondo State, Nigeria.
Department of Microbiology Joseph Ayo babalola University, Ikeji-arakeji Osun State, Nigeria.

***Abstract*—** *Wastewater is one of the most critical problems of both middle and low income countries is improper management of vast amount of wastes.The research is to determine the physicochemical characteristics of the wastewaters and also to asses the effect of the Electromagnetic field (EMF) on the physicochemical factors of the waste watersWaste water samples were collected from two industries in Akure Metropolis. The waste water samples were subjected to physicochemical analyses before and after exposure to Electromagnetic field (EMF) at 1150nT, 1310nT, 3000nT, 5000nT. The presence of some bacteria in the waste water collected from different companies showed their occurrence at different hours during the treatment of the wastewater sample with different EMF strength. a. From the two industries, before EMF treatment industry A had the highest pH value (7.74), Temperature (27.00^{o}C), Total Solid (277.00mg/l.), Total Disolved Solids (256.00mg/l.) Industry B had Total hardness (994mg/l), Chemical Oxygen Demand (13.20mg/l), Potassium (13.23g/l), Biochemical Oxygen Demand (9.60mg/l) Zinc (1.24ppm) and Copper (0.07ppm). From the two industries, after EMF treatment pH (6.47), Turbidity (0.29NTU), Biochemical Oxygen Demand (4.60), Chemical Oxygen Demand (5.40). Industry B had Chloride (10.47mg/l), (600 mg/l). Sulphate (8.70mg/l), after exposure to EMF, the values above listed shows physicochemical factors reduced significantly. Therefore from the study, it was observed that EMF treatment has a significant effect on the bacteria load and physicochemical condition of the waste water samples.*

Keywords*— *Wastewater, Electromagnetic field, Microorganisms, bacteriological analysis.

I. INTRODUCTION

Wastewater, is any water that has been adversely affected in quality by anthropogenic influence. Is one of the most critical problems of developing countries is improper management of vast amount of wastes Wastewater can originate from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water, and from sewer inflow or infiltration (2). Municipal wastewater (also called sewage) is usually conveyed in a combined sewer or sanitary sewer, and treated at a wastewater treatment plant. Industrial development and uncontrolled increase of rural-urban migration that lead to growth of the urban population have resulted in an increase in the unavailability of good quality water resulting to drinking any water available whether is wastewater discharge from industry, which have adverse effects on human populace (4) . Management problems such as poor wastewater collection, an indiscriminate disposal of wastewater.

Wastewater can originate from human waste (such as faeces, used toilet paper or wipes, urine, or other bodily fluids), also known as blackwater, usually from lavatories; Cesspit leakage; septic tank discharge, sewage treatment plant discharge, washing water (personal, clothes, floors, dishes, etc.), also known as greywater or sullage, rainfall collected on roofs, yards, hard standings, etc. (generally clean with traces of oils and fuel); groundwater infiltrated into sewage; surplus manufactured liquids from domestic sources (drinks, cooking oil, pesticides, lubricating oil, paint, cleaning liquids, etc) (7).

These effluent from industries have a great deal of influence on the pollution of the water body, these effluent can alter the physical, chemical and biological nature of the receiving water body. Increased industrial activities have led to

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pollution stress on surface waters both from industrial, agricultural and domestic sources (16).

Water of good drinking quality is of basic importance to human physiology as well as indispensable to man's continued existence (1). Its role as a medium of water borne disease which constitutes a significant percentage of the diseases that affect human and animals cannot be underestimated. This is the most important concern about the quality of water. Guideline for physicochemical composition of water differs from country to country but they all conform to WHO recommendation (8) The standards for drinking water are more stringent than those for recreational waters. Investigations of how magnetic and electric fields affect living organisms at the molecular level have revealed impacts on the biological functions of organisms via changes in the concentration of hormones, activity of enzymes, transport of ions by the cell membrane or changes in the synthesis or transcription of DNA (19).

Natural water is never absolutely pure, as it carries traces of other substances which bestow on it physical, chemical and bacteriological characteristics. The nature and amount of these substances called impurities vary with sources of the water. Although, most of the water on earth is not accessible, the surface water, which is the most accessible, represents only about 0.02% of the total water resources (6).

The industrial, domestic and agricultural wastes that are discharged into this river contains harmful chemicals such as heavy metals, oil, settle able solids, nutrients and ammonia which may affect the resident species in receiving water body. In addition, plants and animals inhabiting the water body are not spared as their normal functioning and population dynamics is affected by pollution. All these effects will go back to man as its insatiable consumption of fresh water resources remains unending. Thus, man may be facing the physiological threat. Many people in developing countries like Nigeria do not have easy access to it.In 2004, the World Health Organization reported that about 1.1 billion people representing 17% of the global population were without safe drinking water. Substantial number of these people lives in China, India, Africa and Middle East. The report also had it that 42% of Sub-SaharaAfrica lacks drinking water. By the end of 2008, an estimated 884 million people in the world lacked access to improved sources of drinking water and 2.6 billion people lack access to improved sanitation facilities (18). Forecast has shown that more than 47% of the global population will face severe water hardship by the year 2030 (21). Despite increasing public sensitization, water pollution continues to generate unpleasant implication for health and community development. The protection of water quality and aquatic ecosystem as a vulnerable resource essential sustainable development is of utmost important to prevent water pollution and degradation of fresh water resources in this region. It is important to continually to develop means of having water resources management policies to prevent discharging of wastewater into the environments.Chemicals that have been used to inhibit the microorganisms can cause deteriorating effects on aquatic microbiota and humans (Aiyesanmi, 2012). better alternative that does not have adverse effect is by the use of Electromagnetic Field. This study therefore is aimed at investigating the effect on water physicochemical properties.

II. MATERIALS AND METHODS

2.1 Study Area

Akure is situated at 7.25^0 North latitude, 5.19^0 East longitude and 396 meters elevation above the sea level. Akure is a big town in Nigeria, having about 420,594 inhabitants. Owena which is located in the suburb of Owena town in Ifedore Local Government Area of Ondo-State, between latitude 7.15^0 N, longitude 5.05^0 E

2.2 COLLECTION OF WASTEWATER SAMPLE

Wastewater sample were collected at different companies from septic tank using polyethylene bottles which was washed, rinsed with dilute nitric acid solution, and rinsed two to three times with some of the water been sampled and transported to the laboratory for experiment

2.3 PHYSCOCHEMICAL PARAMETERS

2.3.1 DETERMINATION OF pH

The pH values of the wastewater samples were monitored using an electronic pH meter, (Jenway, 2015). The electrode of the pH meter was dipped into a beaker containing 100ml of buffer solution pH 4 and pH 9 in order to calibrate the instrument. The standardized electrode was removed from the buffer solution and rinsed with sterilized distilled water. The sample was placed into 50ml clean glass beaker into which an electrode of a standardized pH meter was inserted. The values were immediately read on the meter record and the values were recorded in triplicate (Ademoroti, 1996)

2.2.2 DETERMINATION OF ELECTRICAL CONDUCTIVITY (EC)

The conductivimeter used in conductivity measuring bridge type MC3 instrument. The samples were thoroughly mixed together before an aliquot was poured into the meter sample holder. Immediately the reading knob was depressed, the reading was taken and recorded for each sample. (20).

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2.3.3 DETERMINATION OF TOTAL SOLIDS, DISSOLVED SOLIDS AND SUSPENDED SOLIDS

(a) Total solids

The sample was thoroughly shaken together and 50ml of unfiltered sample was measured and transferred into a previously weighed evaporating dish. The dish was then placed on an electric hot plate for evaporation. After evaporation, it was dried in an oven at 105^{o}C, cooled in the desiccators and weighed. The drying, cooling and weighing on the balance continued until a constant weight was obtained (8).

Total solid is expressed as: total solid (mg/l) = $\frac{\text{total solid (mg)} \times 100}{\text{Sample (ml)}}$

(b) Total dissolved solids

The sample was first filtered using a Whatman filter paper. 50ml of the filtrate was then transferred into a previously weighed evaporating dish. This was evaporated to dryness on an electric hot plate before drying to a constant weight in the oven at 105^{0}C. The weight of the dish was subtracted from the final weight to obtain the weight (mg) of the total dissolved solids (9). Total dissolved solid (mg/l =$\frac{\text{Total dissolved solid (mg)} \times 10)}{\text{filtrate taken (ml)}}$

(c) Suspended solids

Apparatus: Gooch funnel, filtering flask, oven, dessicator, vaccum pump, 100ml pipette.

Procedure: Dry glass filter papers, 5.5cm in diameter to constant weight at 103^{0}C-105^{0}C in oven, cool to room temperature in a dessicator. Note the weight. Then prepare Gooch funnel and rubber adapter and fix to a filtering flask. Place the filter paper into the Gooch funnel carefully with the aid of a pair of tongues. Mix the water sample thoroughly and withdrawn 100-250ml with a pipette. Filter quickly using the filtering apparatus. Using a pair of tongues, remove the filter paper carefully from the Gooch and then dry to constant weight at 103-105^{0}C. Weigh it, subtract the weight of the filter paper to obtain the weight of the suspended solids (9).

Suspended Solid (SS) = $\frac{\text{Suspended Solid (mg)} \times 100}{\text{Sample (ml)}}$

2.3.4 Determination of sulphate

A 10ml of the sample was introduced into 25ml volumetric flask and 10ml of distilled water was added. This was followed by addition of 1ml of gelatin-$BaCl_2$ reagent. The mixture was made up to the mark with distilled water. The mixture was allowed to stand for 30mins before the optical density was determined at 420nm (19).

Calculation

SO_4^{2-} (mg/l) = $\frac{\text{mass of } SO_4^{2-} \text{ from cruve} \times 1000 \times D}{\text{Sample (ml)}}$

Where D is the dilution factor

D = $\frac{\text{total volume of mixture}}{\text{Sample volume}}$

2.3.5 Determination of biochemical oxygen demand

Determination of the initial dissolved oxygen of the water samples, the water samples was properly shaken and 250 ml of each sample was taken aseptically into 250 ml black bottle. The bottle was kept in the incubator at 20^{o}C for 5 days. After 5 days of incubation, the dissolved oxygen analyzer; Model JPB-607 was used to determine the final dissolved oxygen. The analyzer was calibrated in distilled water before and after use for each sample. (Ademoroti, 1996)

The biochemical oxygen demand (BOD) was calculated as follows;

$$BOD = (Do_i - Do_f) \times \frac{\text{volume of bottle}}{\text{volume of sample used}}$$

Where:

BOD = Biochemical Oxygen Demand

DO_i= Initial Dissolved Oxygen

DO_f= Final Dissolved Oxygen

2.3.6 Determination of potassium

It's also measured with the help of flame photometer. The instrument is standardized with known concentration of potassium solution in the range of 1mg to 5mg/l. The sample having higher conc is suitably diluted with distilled water and dilution factor is applied to the observed values (8).

2.3.7 Determination of chloride

It is measured by titrating a known volume of sample with standardized silver nitrate solution using potassium chromate solution in water or eosin/fluorescein solution in alcohol as indicator. The latter indicator is an adsorption indicator while the former makes a red colored compound with silver as soon as the chlorides are precipitated from solution. (9)

2.3.8 Determination of silicates and phosphates

Apparatus: Spectrometer (Gallenkamp)

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Analytical balance, pipette, burette, standard flask and funnel.

These are also measured spectroscopically. 50 ml of water samples were pipette into 100 ml standard flask followed by 8 ml of Murphey and Riley reagent and made up to mark with distilled water. The solutions were allowed to stand for 30 minutes. The absorbance of the standard and samples were read from spectrophotometer at 660 nm. The graph of absorbance against concentration of standards were plotted and sample concentration evaluated from the graph(9).

Calculation:

$$PO_4 \text{ mg/l} = \text{Reading from graph} \times \frac{100}{50} \times \frac{1000}{50}$$

2.3.9.Determination of nitrate

Apparatus: Spectrometer (Gallenkamp)

Analytical balance, pipette, burette, standard flask and funnel.

0.5ml of samples and working standard were pipette into test tubes. 1ml of 5% salicyclic acid solution was added to each test tube and mixed. This was allowed to stand for 30 minutes, after which 10 ml of 4M NaOH solution were added. It was allowed to stand for one hour for colour development, colour stable for 12 hours. The absorbance were read from spectrophotometer at 410nm.

Calculation:

$$NO_3\text{-N mg/l} = \text{Reading from graph} \times \frac{11.5}{50} \times \frac{1000}{50}$$

2.3.10 DETERMINATION OF METALS

The sample for metal analysis was prepared prior to determinadtion. 5ml of concentrated HNO_3 was added to 200ml of water sample in a 250cm^3 beaker. The solution was evaporated to dryness (less than 25ml). After cooling, the solution was made up to 25ml with conc. HNO_3 and transferred into sample bottle prior to analysis (11). The heavy metals were determined with Atomic Absorption Spectrometer (AAS) by using appropriate wavelength for each. The alkali metals were determined by using flame photometer. The absorbance and the concentration of the metals in the sample were thereby obtained. (8).

III. RESULTS AND DISCUSSION

3.1 Physicochemical composition of wastewater

Tables 1and 2 shows physicochemical composition (i.e both physical and chemical composition) of wastewater from two different industries in Akure Metropolis. The physical parameter for raw and treatedsample or industry A has various triplicate results but the mean values are temperature (26.8^{0}C), treated (25.0^{0}C),colour (15.00pt/co unit),turbidity (4.65FTU),treated (0.27FTU),electrical conductivity (43.00µS/cm)treated (0.01µS/cm). The physical parameter for raw sample or industry B hasvarious mean values temperature (27^{0}C), treated (26^{0}C), colour (19.00pt/co unit), turbidity (8.50NTU),treated (0.01 NTU) ,electrical conductivity (39.00µS/cm)treated (0.01µS/cm). The chemical parameter for rawand treated sample A hasvarious mean values pH(7.74) treated (6.44), chloride (18.00mg/l), treated (8.30mg/l), Total Hardness (382 mg/l), treated (68.20mg/l), sulphate (15.60mg/l), treated (6.30 mg/l), nitrate (7.20 mg/l), treated (4.20 mg/l), Phosphate (10.60 mg/l) treated (3.50 mg/l), total solid (276 mg/l) treated (66 mg/l), Total dissolved solid (276 mg/l), treated (66mg/l), Total soluble solid (64.70 mg/l) treated (32.20 mg/l), total alkalinity (74 mg/l) treated (37 mg/l) total acidity (6.40mg/l) while treated (2.90mg/l), sodium (14 mg/l), treated (5.40 mg/l), potassium (12.10 mg/l) while treated (3.25 mg/l) DO (5.20 mg/l), treated (1.70 mg/l) BOD(9.20 mg/l), treated (4.20 mg/l),COD (13.02 mg/l), treated (5.10 mg/l).The physical parameter for raw and treated sample/industry A hasvarious mean values temperature (26.8^{0}C), treated (25.0^{0}C), colour (15.00pt/co unit), turbidity (4.65FTU),treated (0.27 NTU),electrical conductivity (43.00µS/cm) treated (0.01µS/cm). The physical parameter for raw and treated sample/industry B has various mean values temperature (27^{0}C), treated (26^{0}C), colour (19.00pt/co unit), turbidity (8.50NTU), treated (0.01 NTU),electrical conductivity (39.00µS/cm)treated (0.01µS/cm). The chemical parameter for raw and treated sample Bvarious mean values pH (7.65) treated (6.40), chloride (26.08mg/l), treated (10.46 mg/l), total hardness (990mg/l), treated (600mg/l), sulphate (22.20mg/l), treated (8.50 mg/l), nitrate (6.80 mg/l), treated (6.00 mg/l), Phosphate (14.40 mg/l) treated (4 mg/l), total solid (154.50 mg/l) treated (51.50 mg/l), total dissolved solid (150.30 mg/l), treated (50.30 mg/l), total soluble solid (72.50 mg/l) treated (20.40 mg/l), total alkalinity (90mg/l) treated (65 mg/l) total acidity (6.00mg/l) while treated (2.50mg/l), sodium (13.20 mg/l), treated (4.24 mg/l), potassium (16.30 mg/l) while treated (3.25 mg/l) DO (3.40 mg/l), treated (1.30 mg/l) BOD (7.50 mg/l), treated (2.50 mg/l), COD (10.95mg/l), treated (2.50 mg/l).

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TABLE.1: Statistical Analysis Physicochemical Parameters of Raw and Treated Waste Water From Industry A.

Physical parameter	**Range**	**Grand mean**	**Standard Deviation**	**CV%**	**T cal**
Temperature (°C)	25.00 – 27.00	7.07	0.69	9.77	1.04
Colour(Pt/Co unit)	15.45-15.55	15.50	0.05	0.31	Colorless
Taste	Objectionable	Objectionable	Objectionable	Unobjectionable	Unobjectionable
Turbidity (NTU)	0.27-4.75	4.70	0.05	1.11	1.07
Conductivity(μS/cm)	0.01-43.00	43.00	0	0	1.81
Chemical parameters					
pH	6.41-7.74	7.07	0.69	9.77	66.27*
Chloride (mg/l)	8.28-18.50	13.16	5.33	40.5	-89.35*
Total hardness (mg/l)	68.20-384.0	225.13	171.85	7.63	271.63*
Sulphate (mg/l)	6.00-16.20	10.95	5.11		
Nitrate (mg/l)	4.24-7.50	5.75	1.60	46.68	24.01*
Phosphate (mg/l)	4.00-10.64	7.05	3.90	27.83	16.42*
				0.55	24.52*
Total Solid (mg/l)	64.00-277.00	171.00	115.03	67.27	
Total Dissolved solids (mg/l)	55.00-256.00	152.7	106.65	69.85	162.67*
					79.09*
Total Suspended solids (mg/l)	32.00-71.10	49.45	19.05	38.52	1.75
Partial Alkalinity	ND	ND	ND	ND	ND
Total Alkalinity (mg/l)	30.00-75.00	55.50	20.75	37.39	1.83
Total Acidity (mg/l)	2.85-6.43	4.65	1.92	41.23	1.06
Sodium (mg/l)	5.00-14.50	9.7	4.73	48.74	1.15
Potassium (mg/l)	3.25-12.30	7.69	4.84	62.95	1.15
DO (mg/l)	17.0-5.40	3.60	1.87	51.88	1.06
BOD (mg/l)	3.80-7.53	6.70	2.76	41.22	1.08
COD (mg/l)	4.90-10.98	9.03	4.29	47.53	1.13

LEGEND:ND = Not detected,CV = Coefficient of variation;t cal = t values calculated for test of significant difference between raw and treated waste wate, BOD=Biological oxygen demand, COD=chemical oxygen demand.

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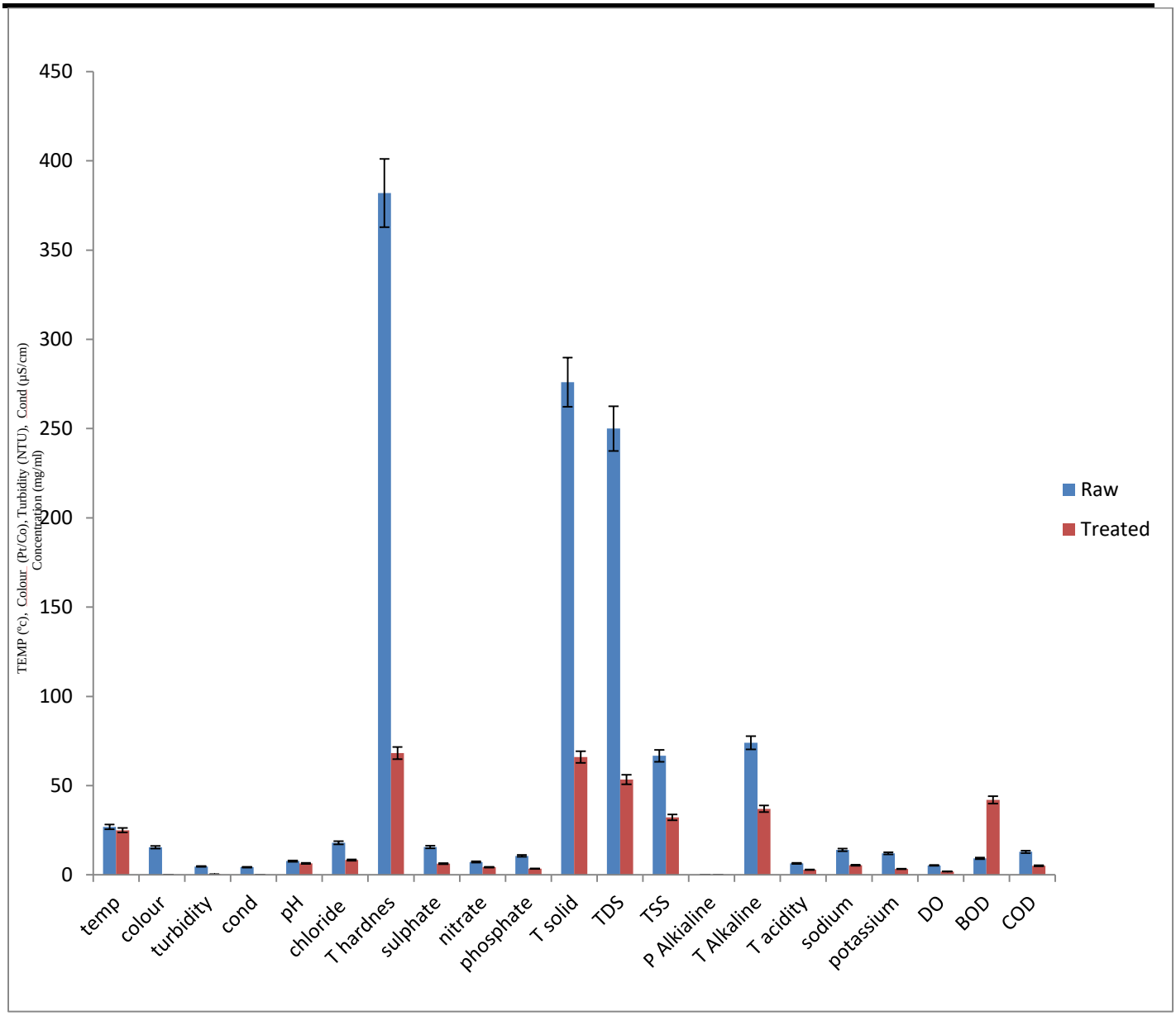

Fig.1: Physicochemical Factors Of Raw and EMF Treated Waste Water From Industry A
LEGEND : Temp= Temperature, Cond= Conductivity, T = Total, DO= Dissolve oxygen BOD=Biological oxygen demand, COD=chemical oxygen demand.

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TABLE 2: Statistical Analysis Physicochemical Parameters of Raw and Treated Waste Water From Industry B.

Physical parameter	**Range**	**Grand mean**	**Standard Deviation**	**CV%**	**T cal**
Temperature (°C)	26.00-27.00	26.57	0.53	2.01	1.04
Colour(Pt/Co unit)	18.50-15.55	8.43	2.20	26.87	Colorless
Turbidity (NTU)	0.01-8.50	4.86	4.54	93.35	1.07
Conductivity(µS/cm)	39.00	39.00	20.85	53.45	1.81
Chemical parameters					
pH	6.39-7.66	7.04	0.671	9.53	
Chloride (mg/l)	10.41-27.13	8.43	2.20	26.12	66.27*
Total hardness (mg/l)	598.0-994.0	795.00	213.63	26.87	89.35*
Sulphate (mg/l)	8.30-23.20	15.35	7.53	49.07	271.63*
Nitrate (mg/l)	5.50-6.85	6.40	0.54	8.46	
Phosphate (mg/l)	4.00-14.80	9.20	5.70	61.98	24.01*
					16.42*
Total Solid (mg/l)	51.45-154.8	103.00	56.42	54.78	24.52*
Total Dissolved solids (mg/l)	50.0-150.4	100.30	2882.69	227.52	162.67*
Total Suspended solids (mg/l)	20.00-74.50	46.45	28.57	6150	79.09*
Partial Alkalinity	ND	ND	ND	ND	1.75
Total Alkalinity (mg/l)	65.00-91.00	77.5	13.71	1.35	
Total Acidity (mg/l)	2.00-6.43	4.26	1.98	46.38	ND
Sodium (mg/l)	4.21-14.50	8.55	4.74	55.42	17.69
Potassium (mg/l)	3.22-12.30	9.79	7.14	72.94	
DO (mg/l)	1.26-5.40	2.35	1.15	48.98	1.06
BOD (mg/l)	2.43-7.53	5.00	2.74	54.78	1.15
COD (mg/l)	2.46-10.98	6.73	4.63	68.82	1.15
					1.06
					1.08
					1.13

KEY ND = Not detected, CV = Coefficient of variation;t cal = t values calculated for test of significant difference between raw and treated waste water.

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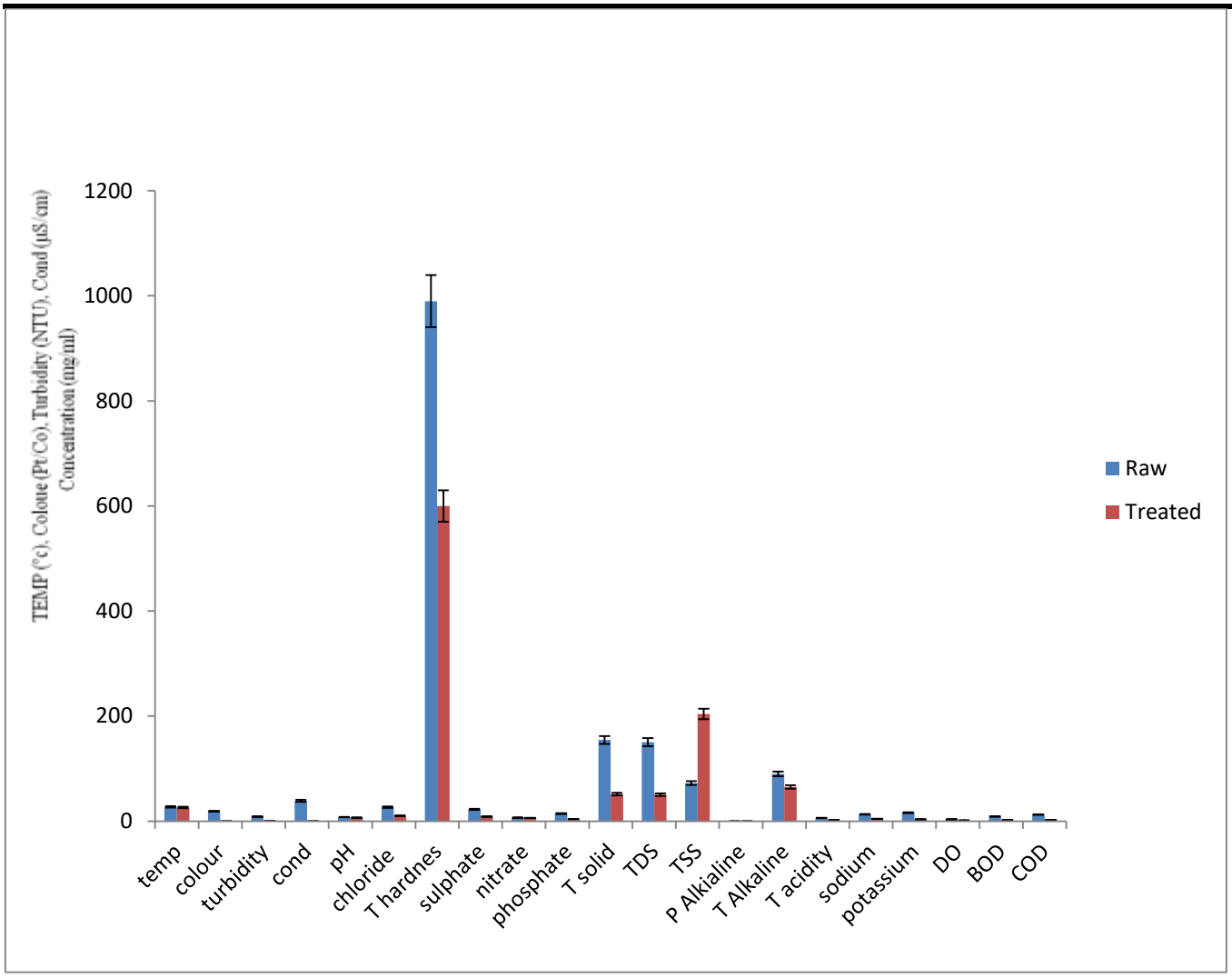

Fig.2: Physicochemical Factors Of Raw and EMF Treated Waste Water From Industry B
LEGEND : Temp= Temperature, Cond= Conductivity, T = Total BOD=Biological oxygen demand, COD=Chemical oxygen demand, DO= Dissolve Oxygen

3.2 Mineral composition of wastewater

Tables 3 and 4shows mineral composition of wastewater from different food companies. The mineral composition includes the iron, zinc, lead, chromium, cadmium, copper, manganeseand Nickel. For industry A the raw and treated sample with EMF, the highest mean values are iron(1.66 ppm), treated (0.84 ppm), zinc (0.42 ppm), treated (0.24ppm), copper (0.12 ppm), treated (0.06 ppm), nickel (0.08ppm), treated (0.04 ppm), manganese (0.06 ppm), treated (0.04ppm), lead, chromium and cadmium werenot detected. For industry B the raw and treated sample with EMF, the highest mean values are iron (0.53mg/l), treated (0.22 ppm), zinc (0.24ppm), while treated has a higher value (1.20ppm), manganese (0.04mg/l), while treated has a higher value (0.05mg/l), copper (0.04mg/l),while treated has a higher value (0.06 mg/l), nickel has lowest mean value (0.01mg/l), treated (not detected) lead, chromium and cadmium were not detected.The concentrations of heavy metals analysedin the waste water for both raw and treated samples. Statistical analysis of the data showed significant difference ($p < 0.05$) in the mean values between the raw and treated sample forPb, Cu, Cr,, Mn, Zn, whereas Cd and Ni Whereas Statistical analysis of the data showed no significant difference ($p < 0.05$) in the mean values between the raw and treated sample for Zn and Fe whereas Cu and Mn there was no significant difference for industry B.

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TABLE. 3 :Mineral Composition of Waste Water From Industry A

SAMPLE	Cu	Cr	Zn	Fe	Cd	Pb	Mn	Ni
RAW	0.12±0.02[a]	0±0	0.47±0.02[a]	1.63±0.02[a]	0.14±0.01[a]	0±0	0.07±0.01[a]	0.14±0.02[a]
1150nT	0.10±0.01[ab]	0±0	0.44±0.04[ab]	1.60±0.02[a]	0.12±0[a]	0±0	0.06±0.025[a]	0.12±0.02[b]
1310nT	0.07±0.01[bc]	0±0	0.42±0.02[ab]	1.58±0.02[ab]	0.10±0[ab]	0±0	0.04±0.02[a]	0.06±0.01[b]
3000nT	0.05±0.01[c]	0±0	0.40±0.04[b]	1.54±02[bc]	0.08±0[b]	0±0	0.02±0.02[a]	0.05±0.01[b]
5000nT	0.02±0.01[c]	0±0	0.36±0.02[b]	1.50±0.02[c]	0±0[c]	0±0	0.024±0.02[a]	0.04±0.01[b]

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TABLE.4: Mineral Composition of Waste Water From Industry B

SAMPLE	Cu	Cr	Zn	Fe	Cd	Pb	Mn	Ni
RAW	0.08±0.04[a]	0±0	1.40±0.02[a]	0.53±0.02[a]	0±0	0.±0	0.45±0.01[a]	0.42±0.01[a]
1150nT	0.06±0.01[b]	0±0	1.2±0.04[b]	0.22±0.02[b]	0±0	0±0	0.053±0.03[b]	0.40±0[ab]
1310nT	0.05±0.01[b]	0±0	1.08±0.02[b]	0.18±0.02[bc]	0±0	0±0	0.034±0.02[b]	0.38±0.01[ab]
3000nT	0.04±0.01[b]	0±0	1.06±0.04[b]	0.14±0.02[c]	0±0	0±0	0.030±0.02[b]	0.36±0.01[b]
5000nT	0.02±0.01[b]	0±0	1.02±0.02[b]	0.13±0.02[c]	0±0	0±0	0.023±0.02[b]	0.03±0.01[c]

Legend: Data are presented as Mean ± SD (n=2) from triplicate determinations, different superscripts in the same row are significantly different (P< 0.05)

3.3 Physicochemical Factors Of Raw and EMF Treated Waste Water From Industry A and B

In the chemical composition the chemical parameters of wastewaters collected from the different companies, the raw wastewater without treatment collected from industry A has the highest pH level range, after the treatment the pH decreases. For industry B the pH was dwindling and after the treatment the pH also elided further in values . The pH of the water samples ranged from very slightly acidic value to slightly basic value which is identical to the findings of (21).

The pH of all waste water (i.e the raw and the treated) falls under the internationally recommended standard, for both surface and groundwater system. Although pH usually has no direct impact on consumers, it is one of the most important operational water quality parameters. Extremes of pH can affect the palatability of a water but the corrosive effect on distribution systems is a more urgent problem (18).The pH is of the utmost importance in determining the corrosivity of water (16). In general, the lower the value of pH, the higher the level of corrosion. It has been observed that in some cases decrease in pH is accompanied by the increase in bicarbonate, carbonate and hydroxyl ions. Decrease in pH can be caused by the increase in the amount of organic carbon, total carbonate by the use of sewage.

The wastewater collected from industry A has the highest dissolved oxygen demand value from which also reduces to after application with EMF, the industry B has lower dissolved oxygen demand than industry A which the value after application with EMF also reduces which is in congruent to findings (2).

Organic wastes and other nutrient inputs from sewage and industrial discharges, agricultural and urban runoff can result in decreased oxygen levels. Nutrient input often leads

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to excessive algal growth; when the algae die, the organic matter is decomposed by bacteria, a process which consumes a great deal of oxygen that could lead to oxygen sag (2). A high DO level in a community water supply is good because it makes drinking water taste better. However, high DO levels speed up corrosion in water pipes.

3.4 Physicochemical factors of raw and emf treated waste water from industry A and B

In the chemical composition the chemical parameters of wastewaters collected from the different companies, the raw wastewater without treatment collected from industry A has the highest pH level range, after the treatment the pH decreases. For industry B the pH was dwindling and after the treatment the pH also elided further in values . The pH of the water samples ranged from very slightly acidic value to slightly basic value which is identical to the findings of (21).

The pH of all waste water (i.e the raw and the treated) falls under the internationally recommended standard, for both surface and groundwater system. Although pH usually has no direct impact on consumers, it is one of the most important operational water quality parameters. Extremes of pH can affect the palatability of a water but the corrosive effect on distribution systems is a more urgent problem (9).The pH is of the utmost importance in determining the corrositivity of water (6). In general, the lower the value of pH, the higher the level of corrosion. It has been observed that in some cases decrease in pH is accompanied by the increase in bicarbonate, carbonate and hydroxyl ions. Decrease in pH can be caused by the increase in the amount of organic carbon, total carbonate by the use of sewage.

The wastewater collected from industry A has the highest dissolved oxygen demand value from which also reduces to after application with EMF, the industry B has lower dissolved oxygen demand than industry A which the value after application with EMF also reduces which is in congruent to findings of (4).

Organic wastes and other nutrient inputs from sewage and industrial discharges, agricultural and urban runoff can result in decreased oxygen levels. Nutrient input often leads to excessive algal growth; when the algae die, the organic matter is decomposed by bacteria, a process which consumes a great deal of oxygen that could lead to oxygen sag (16). A high DO level in a community water supply is good because it makes drinking water taste better. However, high DO levels speed up corrosion in water pipes.

Dissolved oxygen is an important environmental parameter for the survival of aquatic life. The wastewater collected from industry A has lower biological oxygen demand value range and industry B had the higher biological oxygen demand from which devaluate after application with EMF, the industry. Unpolluted, natural waters should have a BOD of (5 mg/l or less), and there are no direct health implications for BOD, but an important indicator of overall water quality according to United State Environmental Protection Agency, "Current Drinking Water Standards. So before application the waste water was polluted but after application with EMF it reduces the pollution level to unpolluted because the values fall beyond 5 mg/l.

The wastewater collected the industry B has higher chemical oxygen demand value than Similar observation was reported on the study of chemical oxygen demand in some industries in Ado-Ekiti by (2). No direct health implications for COD, but also an important indicator of overall water quality according to United State Environmental Protection Agency, "Current Drinking Water Standards. The wastewater collected from industry A has the lower total solid value, while industry B has the higher soluble solid presence in the wastewater sample then after treatment it decreases in the value after application but also an important indicator of overall water quality which fall into 5000 mg/ l similar to findings of (19) which research was done on some waste water from some industries.

The wastewater collected from industry A has the lower total dissolved solid value while industry B has the higher value of soluble solid, then after treatment both industry A and B values decreases consubtantial observation was reported by (17) on the study. The wastewater collected from industry A has the higher total soluble solid value between presence then after treatment it decreases while industry B has the lower soluble solid presence then after treatment it decreases in which the values was higher than industry A after treatment. Higher chloride levels were measured in the raw waste water sample A, while after. Lower chloride levels were measured in the raw waste water sample B. The consistently higher values recorded in the sample A could be as a result of concentration of this anion from excessive water evaporation from the waste water. Similar to what was recorded in this study which the values reduces after exposure to EMF, and much below the permissible drinking water standard of 250 mg/l similar to findings of (8).

Nitrate level in the raw waste water from industry A was lower in values than nitrate level in the raw wastewater from industry B the results was in congruent to findings of (11). Nitrate level in the raw waste water from the two industries compared to what is normally found in an unpolluted natural fresh waters, relatively little of the nitrate found in natural waters is of mineral origin, while most

coming from organic and inorganic sources, including waste discharges and artificial fertilisers. Also, bacterial oxidation and fixing of nitrogen by plants can both produce nitrate (11). Interest is centred on nitrate concentrations for various reasons. Most importantly, high nitrate levels in waters to be used for drinking will render them hazardous to infants as they induce methaemoglobinaemia ("blue baby" syndrome).

The nitrate itself is not a direct toxicant but is a health hazard because of its conversion to nitrite, which reacts with blood haemoglobin to cause methaemoglobinaemia. Hence, 100 mg/l nitrate is set as Guideline value for nitrate in drinking water (20). The values recorded in this study were well below the guideline value suggesting that water from the dam is considered safe for drinking. In aquaculture, nitrate is considered a less serious environmental problem, it can be found in relatively high concentrations where it is relatively nontoxic to aquatic organisms, but stimulates the growth of plankton and water weeds that provide food for fish. This may increase the fish population, but when concentrations become excessive, and other essential nutrient factors are present, eutrophication and associated algal blooms can become a problem.

The significance of nitrite (at the low levels often found in surface waters) is an indicator of possible sewage pollution and as earlier mentioned, it is of concern for its toxicity. Concentrations of phosphate in the raw waste water sample A was higher values and it was lower after subjecting it to EMF which has a value of (4.00 mg/l), concentrations of phosphate in the raw waste water sample B was lower than sample A which is in range to values from (3) findings. Phosphorus from where phosphate is derived occurs widely in nature in plants, in microorganisms, in animal wastes; and large quantities of phosphate are applied as fertilizers in agriculture for which runoff from this area will often contains elevated concentrations of phosphate (9). Hence, (250 mg/l) phosphate is set as Guideline value for phosphate in drinking water (20).

Partial Alkalinity was not detected in both industries may be due to less carbonates. The total alkalinity level in the raw waste water from industry A was higher range between while the treated sample reduces in value. The total alkalinity level in the raw waste water from industry B was lower in values, while the treated sample reduces in value. Total alkalinity is a measure of the ability of the water to neutralize acids.

The constituents of alkalinity in neutral system include mainly carbonate, bicarbonate, hydroxide and other components (17). These compounds result from dissolution mineral substances in the soil and atmosphere. The carbonates was more and later becomes lesser after the application of EMF because of the values of raw and treated samples water (19). Partial Alkalinity was not detected in both industries may be due to less carbonates. The total acidity level in the raw waste water from industry A was lower ranged between while the treated sample reduces in values similar to the findings of (17). The total acidity level in the raw waste water from industry B was higher ranged between while the treated sample reduces in value, it ranged from.

The sodium level in the raw waste water from industry A was lower than treated sample reduces in value. The sodium level in the raw waste water from industry B was higher, while the treated sample reduces in value, it ranged from, which is in correlation to the finding of (12). Abnormally large concentrations may indicate natural brines, industrial brines, or sewage, so because of lower values of sodium it shows lesser concentrations of natural brines, industrial brines, or sewage even the lesser concentrations was reduced to minimal level after exposure to EMF.

The potassium level in the raw waste water from industry A was higher while the treated sample reduces in value . The sodium level in the raw waste water from industry B was lower between while the treated sample reduces in value, it ranged from, Similar results were reported by (4).

In the physical parameters, the raw waste water collected from site A has the higher temperature mean value 27 ^{0}C, which reduces after the treatment with EMF to 26 ^{0}C and raw sample collected from industry B has the lower temperature value range, which reduces after the treatment with EMF to the mean temperature value (25 ^{0}C) Similar (9). The temperature values of the industrial waste water fell within the optimal water temperatures (Target Guidelines) of 28 °C – 30 °C, within which maximal growth rate, efficient food conversion, best condition of fish, resistance to disease and tolerance of toxins (metabolites and pollutants) are enhanced (3).

The raw waste water without treatment collected from industry A the taste is objectionable while after the treatment with EMF the taste was unobjectional which fell under WHO standard, so exposure to EMF changes the taste to unobjectionable which is good for drinking (20).

The raw waste water collected from site A has the lower colour value ranged between, which reduces after the treatment with EMF to colourless, and raw sample collected from industry B has the lower temperature value range, Similar results were reported by (4) which reduces after the treatment with EMF to colourless. High colour units measured during before exposure to EMF can be attributed to runoff into water bodies with high entrained suspended

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suspended particles and coloured substances predominantly of organic origin. Because of its origins mostly in vegetable matter the degree of colour in a water may vary widely in space and in time. Limits for colour in potable water have traditionally been based on aesthetic considerations rather than on the basis of a health hazard, and this has been set at 15.00 Pt/Co units (20). This calls for attention because the presence of colour on a persistent basis in a water to be disinfected by chlorination is highly undesirable.

There is high tendency for the colour-causing substances to react with the added chlorine giving rise to the presence of trihalomethanes (THMs), which are potential hazards to public health (2). So it's better for industrial waste water should be disinfected with EMF instead of chlorine because of these disadvantage mention earlier (15).

The turbidity level in the raw waste water from industry A was higher while the treated sample reduces in value the turbidity level in the raw waste water from industry B was lower was while the treated sample reduces to minimal value, In addition, high turbidity can lead to an increase in the amount of disinfection byproducts (THMs) that form in treated water and could interfere with sunlight penetration, thus reducing photosynthesis. The low values after subjecting to EMF indicate that it has disinfect byproducts (THMs)(3).

The electrical conductivity value level in the raw waste water from industry A was higher than industry B. The electrical conductivity level in the raw waste water from industry B was lower was while the treated sample reduces to minimal value, it has the highest while site A of the treated sample has the lowest electrical conductivity value, similar to the findings of (14). The wastewater collected from industry B of the raw sample has the highest chemical oxygen demand value while industry A of the treated sample has the lowest chemical oxygen demand.

In the mineral and elemental composition, There is variation in the mineral composition among the raw sample and the ones treated with EMF strength. For wastewater collected from industry A, the treated sample has the higher value of iron while the raw sample has the lower iron value. For wastewater collected from industry B, The raw sample has the highest value of iron while the treated sample has the lowest iron value Similar results were reported by (6)

Wastewater collected from industry A, the raw sample has the higher value of zinc than while the treated sample, for wastewater collected from industry B, the treated sample has the higher value of zinc ranged while the raw sample has the lower iron value which is similar to the findings of (13).Chromium was not detected in both industries in the raw and treated samples.

For wastewater collected from industry A, the raw sample has the higher value of copper while the treated sample has the lower copper value, for wastewater collected from industry B, the treated sample has the higher value of copperSimilar observation was reported by (17). While the raw sample has the lower copper value. For wastewater collected from industry A, the raw sample has the higher value of cadmium range while the treated sample has the lower cadmium value ranged cadmium was not detected in industry B in the raw and treated samples, (9) for wastewater collected from industry A.

Lead was not detected in both industries in the raw and treated samples. For wastewater collected from industry A, the raw sample has the higher value of manganese while the treated sample has the lower cadmium value. For wastewater collected from industry B, the raw sample has the higher value of manganese while the treated sample has the lower value of manganese, this is in agreement to the findings to (15).

For wastewater collected from industry A, the raw sample has the higher value of nickel while the treated sample has the lower cadmium value ranged while the treated sample was not detected this in agreement to the results of (5). While zinc and iron recorded higher concentrations than their guideline values but after the treatment with EMF for industry it reduces below guildlines values but in industry A it did not reduces below guildlines values but still reduce in value this in agreement to the results of (1). Toxic effects have resulted from the ingestion of large quantities of iron, but there is no evidence to indicate that concentrations of iron. The presence of some microorganisms in the waste water, hence, a maximum acceptable concentration has not been set. At concentrations above 0.3 mg/l (19). Statistical analysis of the data showed significant difference ($p < 0.05$) in the mean values between the raw and treated sample for Pb, Cu, Cr, Mn, Zn, where as Cd and Ni showed no significant difference for industry A. Whereas Statistical analysis of the data showed significant difference ($p < 0.05$) in the mean values between the raw and treated sample for Zn and Fe where as Cu and Mn showed no significant difference for industry A.

IV. CONCLUSION

From the research, it was observed that EMF treatment has a significant effect on the physicochemical parameters of the industrial wastewater.significant effect was observed on the physicochemical properties as for the the exposed wastewater to electromagnetic field but no significant effect was observed on the elemental composition of the waste water sample. The EMF treatments reduced the microbial

population as well as the rate of contamination in the wastewater samples as the exposure time increased. It is therefore recommended that wastewater from industries should be treated with EMF before discharging them to the other water bodies so as to avoid contamination. This will help reduce microbial population that constitute a serious hazard to public health. The electromagnetic field treatments could also help protect other life forms inhabiting the water body and thus guard against ecological imbalance of the microbiota.

COMPETING INTERESTS

Authors have declared that no competinginterests exist.

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Yellow Cassava Attributes Influencing its Utilization among Cassava Processors in Oyo State, Nigeria.

R. G. Adeola[1], K. Y. Ogunleye[1*], I. F. Bolarinwa[2]

[1]Department of Agricultural Extension and Rural Development, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso, Oyo State, Nigeria.

[*2]Department of Food Science and Engineering, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo State, Nigeria.

Abstract— *The research focused on attributes determining utilization of Yellow cassava (YC) varieties. Two of the four agricultural zones in the state namely; Ogbomoso and Oyo zones were covered in the study. Structured interview schedules were used to elicit information from 302 cassava processors who were selected through multi-stage sampling procedure. Data was presented using descriptive statistics and analysed with inferential statistical tools. Findings showed age of respondents was 46 years, about 92% were females with more than two-thirds (75.5%) having formal education. Awareness of YC was substantial among sampled processors. Virtually all the respondents (99.3%) claimed to be aware of TMS 01/1368 variety of YC and majority were using this particular variety. Extension agents from Oyo State Agricultural Development Programme (OYSADEP), Harvest plus and International Institute of Tropical Agriculture (IITA) formed leading sources of information on YC varieties among the respondents. Gari and Fufu were the common products people in the study area made from YC. The processors are favourably disposed to utilization of YC. Critical constraints faced in the utilization of YC were non availability of market for YC products and inadequate information on the potentials of yellow root cassava. Pearson Product Moment Correlation revealed that taste of YC products (r=0.813), consumer's acceptability of the products (r=0.758) and multiple usage of the YC (r=0.818) are important attributes that influences the utilization of YC. More awareness campaign on potential of YC should be made so as to create market for its products thereby increasing the income of the processors.*

Keywords— Yellow cassava, Processors, Utilization, Vitamin A, Attributes.

I. INTRODUCTION

Globally, Vitamin A deficiency (VAD) is the world's commonest cause of blindness among children. Approximately 228 million children are affected sub-clinically and 500,000 children become partially or totally blind every year due to VAD {World Health Organization(WHO)/Food and Agricultural Organization(FAO), 2003}. Therefore, VAD has been a major public health problem in many developing countries. In Nigeria, about 30 percent of children under age five and almost 20% of pregnant women are deficient in micronutrients like Vitamin A. Vitamin A deficiency in children leads to stunted growth, diarrhoea, measles and premature death. According to Maziya-Dixon et al., (2007) and World Health Organizations (2017), Vitamin A deficiency can cause severe night blindness and high mortality rate in pregnant women.

Many Nigerians irrespective of age, gender and geographical location get less Vitamin A than the required amount. The major determinants of Vitamin A deficiency are low availability and inadequate consumption of Vitamin A diets. Animal foods that are good sources of Vitamin A are not affordable by the poor communities and thus leaving foods of plant origin as an important source of pro-Vitamin A in developing countries (Tumuhimbise *et al*,2013). Recognizing the severity of the problem, Nigerian government had embarked on supplementation programs with Vitamin A for children within the age range of 6 months to 5 years during immunization days and has mandated the fortification of certain food items like sugar, wheat flour and vegetable oil with Vitamin A since the year 2000. In order to combat the prevalence of VAD, various strategies including fortification and bio-fortification methods have been developed by scientists across the world (www.harvestplus.org). Cassava (*Manihot esulenta Crantz.*), is the chief source of dietary food energy for the majority of the people living in the low land tropics and much of the sub-humid tropics of west and central Africa (Echebiri and Edaba, 2008). Cassava is a hardy crop that is extremely adaptable to severe weather conditions and drought tolerant. It can grow well on soils of limited fertility. Cassava is an important food and subsistence

crop in Nigeria and one of the staple foods generally consumed by the majority of the populace that is vulnerable to VAD. It has been estimated that 600–700 million people obtain more than 500 calories/day from cassava (Maziya-Dixon et al., 2007; Nuwamanya et. al., 2010) but the commonly available white cassava lacks micro nutrients like Vitamin A (www.harvestplus, 2014). Economic problems, political unrest, reduced soil fertility, drought, and the population explosion all have increased the need for cassava as a cheap, common, versatile crop that is resistant to adverse environmental factors, such as poor soil fertility, drought (Osiru et. al., 1992) and disease (Asonye, 2001). Considering the important role of cassava in the diets of Nigerians, National Root Crops Research Institute (NCRI) Umudike and International Institute of Tropical Agriculture (IITA), Ibadan jointly developed cassava varieties bio-fortified with Vitamin A in order to complement government efforts to check Vitamin A deficiency and malnutrition in the country. These varieties are yellow in colour owing to their high beta-carotene (pro-Vitamin A) content; hence they are called Yellow Cassava. The new yellow varieties are also high yielding and resistant to major diseases and pests. It is strongly believed that the YC varieties being introduced to farmers would be an effective tool in reducing VAD among poor people.

This study;

1. described the socio-economic and enterprise characteristics of the processors
2 determined extent of awareness and utilization of YC varieties
3. investigated the processors' perception of yellow cassava,
4. determined level of yellow cassava utilisation
5. identified YC attributes that determines its use by the processors
6. know constraints to the utilization of YC
7. identified factors influencing the utilization of YC

II. METHODOLOGY

Four (4) Local Government Areas (LGAs) were purposively selected for the survey because they were noted for production and consumption of cassava in large quantity and formed part of the targeted areas by the Oyo State Agricultural Development Programme (OYSADEP) for YC introduction and delivery. The LGAs included in the survey were; Afijio and Ojongbodu in Oyo Agricultural zone while Orire and Surulere LGAs were selected in Ogbomoso Agricultural zones of the state. All the villages with a high concentration of cassava processors in the selected LGAs were listed through the assistance of OYSADEP Women In Agriculture (WIA) extension agents and two villages were randomly selected from each LGA; thus a total of eight (8) villages for the four (4) LGAs selected for the survey. The WIA agents were tasked to make the list of all the processors within the selected villages which resulted in sampling frame of 604 from which fifty (50) percent (302) of the processors were randomly selected to form the sample for the study. Interview schedule was developed and used to collect data for the study. The processors were asked to give their ratings of cassava attributes that will encourage the utilisation of cassava using a Likert type scale ranging from 3 (very important) to 1 (somewhat inmportant). Processors' perception of YC and its products were operationalized as follows: Strongly Agreed (SA) = 5, Agree (A) =4, Undecided (U) =3, Disagree (D) =2 and Strongly Disagreed (SD) = 1 for positive statements and these values were reversed for the negative statements. Data collected was subjected to descriptive (frequency counts, percentage distribution, mean standard deviation) and inferential statistics (Chi-square and Pearson Product Moment Correlation) at p 0.05

III. RESULTS AND DISCUSSION

3.1 Socio-economic characteristics of the respondents

Results in Table 1 revealed that few (4.3%) of the processors were within the age group of 20 to 29 years, majority were between 30 and 49 years while 33.8% were within the age group of 50 to 69 years. The mean age of the processors was 46 years and as such, an average processor in the study area is still economically active and could result in a positive effect on adoption as he or she would be willing to take risks in expectation of more profit.

Majority (92.4%) of the respondents were females. The implication of this is that females are most active and involved when it comes to garri processing in the study area. This corroborates the findings of Nweke et al.,(2002) and Ogunleye, Olaniyi and Adedeji (2012) that women specializes in cassava processing. Also, 94.4% of the respondents were married while others were either single or divorced. This could help in the dissemination of information, because according to Ojo and Jibowu (2008), married people being responsible, their views are likely to be respected within rural communities as they take decisions on the use of agricultural inputs.

Education is very important for farmers to understand and interpret any agricultural information coming to them from any direction. It enables one to access information needed to use and practice an innovation. About 76% of the respondents had formal education while others had no formal education. Given this level of literacy, the implication is that information could be disseminated with ease among the processors. Majority (66.6%) of the respondents spent between 1 and 9 years in obtaining basic education, while only a few (0.7%) spent between

20 and 29 years in obtaining basic education. The average number of years spent in obtaining basic education by respondents was approximately 7 years. This implies that the level of education of the processors was low although they had one form of education or the other.

Household size is considered to be the number of individuals who reside in a family. Large household size is assumed as an indicator of labour availability in the family. Table 1 further shows majority (94.0%) of the respondents interviewed had between 1 – 10 household members, while only 0.7% had between 21 – 30 members within their household. The average number of members within a household is 6. Very few (9.6%) of the respondents used family labour, 44.7% used hired labour for processing activities while 45.7% used both family and hired labour. This might be due to the small household size. Findings also showed that majority (74.5%) received information about YC through Oyo State Agricultural Development Programme (OYSADEP), 43.0% obtained information through relations while 4.0% and 6.0% of the respondents obtained information through Harvest Plus and International Institute for Tropical Agriculture respectively.

Table.1: Distribution of respondents' socio-economic characteristics

Socio-Economic Characteristics	Category	Frequency	Percentage
Age (Years)	20 – 29	13	4.3
	30 – 39	74	24.5
Mean=45.78	40 – 49	113	37.4
SD=(9.847)	50 – 59	66	21.9
	60 – 69	36	11.9
Sex	Male	23	7.6
	Female	279	92.4
Marital status	Single	4	1.3
	Married	285	94.4
	Divorced	13	4.3
Educational status	No Formal Education	74	24.5
	Primary Education	132	43.7
	Secondary Education	79	26.2
	Tertiary Education	17	5.6
Years spent in school	**0**	74	24.5
	1 – 9	127	42.0
Mean=6.85	10 – 19	99	32.8
SD=(5.132)	≥20	2	0.7
Household size (People)	1 – 10	284	94.0
Mean=**6.39**	11 – 20	16	5.3
SD=**(3.036)**	21 – 30	2	0.7
Religion	Islam	142	47.0
	Christianity	160	53.0
Source of labour	Family	29	9.6
	Hired	135	44.7
	Both	138	45.7
Source of information*	Extension Agents	225	74.5
	Family Relations	130	43.0
	Harvest Plus	12	4.0
	IITA	18	6.0

Note: * multiple responses

3.2 Enterprise characteristics of the processors

Table 2 shows majority (92.7%) of the processors used local method for processing while very few (7.3%) used

improved method. This might be because of sharp reduction in carotenoid content due to losses during processing (Aniedu and Omodamiro, 2012).

The result further revealed that 61.0% had spent between 10 and 29 years in cassava processing, with the average number of years being 21 years. It shows the processors are veterans in cassava processing. About 92.0% of the respondents are into farming and had farm size of less than 10 hectares, 7.3% had 10 to 29 hectares while very few (0.7%) had above 30 hectares. Nonetheless, their average farm size was approximately 3 hectares. However, 93.4% used 5hectares or less to cultivate cassava while 4.6% used 6 to 10 hectares and 0.7% cultivated 16 to 20 hectares. The average land cultivated to cassava was 2 hectares meaning they are not large scale farmers.

The result further revealed that only about half (53.3%) of the respondents used 1 hectare to cultivate YC while very few (6.0%) used 2 ha but 40.7% did not cultivate YC. This implies that majority of the processors operate on a small scale production in the study area. This is because they had farm size of less or equal to 5 hectares which is considered as small scale based on classification of Federal Office of Statistics (1999). This supports Erhabor and Emokwo (2007) who stated that most cassava farmers' are small-holder farmers.

Furthermore, majority (74.5%) of the respondents obtained their planting materials from other farmers while the remaining (25.5%) obtained their planting materials from either IITA or relatives. Extension visits afford farmers easy exposure to new technologies, how to go about them and the benefits. The greater the visits by extension agents, the better the farmers are informed about new technology (Manyong and Houndekon, 1997; Wejnert, 2002; Berisso, 2008). Furthermore, majority (72.5%) of the respondents had contact with extension agent, though the frequency of contacts was not the same. The reason for the respondents' access to extension agents may be due to membership of social organization. It could therefore be seen that extension visits is a determinant of investment decision in new technologies. Therefore, Oseni *et al*. (2015) posited that farmers, through extension visits become better informed about farm management planning and new technologies, hence improving their efficiency in production. Result also revealed that all the respondents were aware of YC as 33.8% had been aware for 1 to 2 years, 64.2% had been aware for 3 to 4 years while very few (2.0%) had been aware for 5 or more years. Majority (74.2%) of the respondents in the study area preferred the yellow variety of cassava to the white variety. The reason for their preference was adduced to the fact that products from YC sells faster, the YC gives more product after processing, and that there are more buyers for the products. This finding corroborates Oparinde *et al*. (2014) that yellow cassava products is most preffered in Oyo state.

Table.2: Enterprise characteristics of the respondents

Enterprise characteristics	**Category**	**Frequency**	**Percentage**
Methods of processing	Local method	280	92.7
	Improved method	22	7.3
Years spent in Cassava processing	1 – 9	36	11.9
	10 – 19	89	29.5
Mean=21.04	20 – 29	95	31.5
SD=(9.823)	30 – 39	66	21.9
	40 – 49	16	5.3
Farm size (Ha)	< 10	278	92.1
Mean=3.25	10 – 19	14	4.6
SD=(4.688)	20 – 29	8	2.7
	> 30	2	0.7
Farm size planted to cassava (ha)'	≤ 5	282	93.4
Mean=2.00	6 – 10	14	4.6
SD=(2.385)	>10	6	2.0
Farm size planted to YC (ha)	0	123	40.73
Mean=0.69	1	161	53.31
SD=(0.439)	2	18	5.96
Source of planting materials	IITA	60	19.9
	Self/Relative	17	5.6
	Other farmers	117	38.7
Contact with extension agent	Contact	219	72.5
	Non-contact	83	27.5

Enterprise characteristics	Category	Frequency	Percentage
Years of awareness of YC varieties (years)	1-2	102	33.8
	3-4	194	64.2
	≥5	6	2.0
Preferred Cassava variety	Yellow	224	74.2
	White	78	25.8

*** Multiple responses recorded**

3.3 Awareness of white and yellow varieties of cassava

According to Agricultural Development Office in Ogbomoso agricultural zone, three varieties were introduced namely; TMS 01/1368, TMS01/1412 and TMS01/1371. From the findings of the study 99.3% of the processors were aware of TMS 01/1368 while 95.7% used the variety by processing into products. This implies TMS 01/1368 is the most adopted YC variety and the least adopted was TMS01/1412. Lack of awareness and poor use of other varieties might be because the varieties did not trive well in the area. This is similar to the findings of Umunakwe *et al* (2015) that low planting of some cassava varieties by farmers could be due to farmers unfamiliarity of the varieties and lack of desirable characteristics that may encourage their cultivation.

Table.3: Extent of awareness and utilization of YC varieties

Varieties	Aware	Unaware	Using	Not using
TMS 01/1368	300 (99.3)	2 (0.7)	287 (95.67)	15 (5.0)
TMS01/1412	25 (8.3)	277 (91.7)	14 (4.6)	288(95.4)
TMS01/S371	62 (20.5)	240(79.5)	60 (19.9)	242 (80.1)

3.4 Frequency of YC processing, quality and attractiveness of product

Majority (85.1%) of the respondents always make Garri from YC while 13.6% occasionally make Garri from YC. About 42% occasionally make Lafun from YC. This might be due to the colour that the YC will bring out in the making of Lafun which will nonetheless affect its sale. Findings also showed that most (35.4% and 35.1%) of the respondents either rarely or always make fufu from YC respectively while only 27.5% occasionally make fufu from YC. Results also revealed that majority (68.5%) of the respondents never make starch from YC while only 22.2% of the respondents rarely make starch from this variety of cassava. Also, majority (69.5% and 93.4%) of the respondents never make chips and cassava cake from YC. This could be due to their acceptability in the market. Almost all (98.7%) the respondents claimed that Garri made from YC had an excellent taste; 43.7% and 46.0% claimed that Lafun and Fufu made from this variety of cassava tasted good respectively. Majority (93.7%) of the respondents believed that Garri made from YC had a very attractive colour, while about half (50.7%) of the respondents believed that Lafun made from YC was attractive. Also, 62.9% of the respondents were convinced that the colour of the Fufu made from YC was attractive while 45.4% claimed that the colour of cassava chips made from YC was attractive (Table 4).

Table.4: Respondents' frequency of YC processing, quality and attractiveness of product

Frequency of YC processing	Gari	Lafun	Fufu	Cassava Chips
Always	257 (85.1)	14 (4.6)	106 (35.1)	2 (0.7)
Occasionally	41 (13.6)	126 (41.7)	83 (27.5)	0 (0)
Rarely	4 (1.3)	28 (9.3)	107 (35.4)	18 (6.0)
Never	0 (0)	134 (44.4)	6 (2.0)	282 (93.4)
Quality of product				
Excellent	298 (98.7)	6 (2.0)	74 (24.5)	33 (10.9)
Good	2 (0.7)	132 (43.7)	139 (46.0)	37 (12.3)
Fair	2 (0.7)	79 (26.2)	85 (28.1)	120 (39.7)
Poor	0 (0)	85 (28.1)	4 (1.3)	112 (37.1)
Attractiveness of product				
Very attractive	283 (93.7)	28 (9.3)	103 (34.1)	39 (12.9)
Attractive	5 (1.7)	153 (50.7)	190 (62.9)	137 (45.4)
Not Attractive	14 (4.6)	121 (40.1)	9 (2.98)	126 (41.7)

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3.5 Perception of processors on Yellow cassava utilization

As shown on Table 5, majority (95.0%) of the processors strongly agreed that yellow root cassava is very rich in Vitamin A, 99.3% agreed that consumption of yellow root cassava products can help prevent blindness in children and disease infection in reproductive women and 98.1% agreed that improvement in children growth and development can be achieved by feeding them with yellow root cassava products. The findings also revealed that most of the respondents did not agree with the statements that were not in favour of the YC and its products which was indicative of their favourable disposition to such statements. These perceptions of the respondents with respect to YC and its products could have been mostly shaped by the information they received from OYSADEP extension agents during the introduction of YC varieties to them. This implies that the processors have a good perception of YC and its products for consumption.

Table.5: Respondents' perception of Yellow cassava utilization

S/N	Perception Statements	SA	A	U	D	SD	Mean
1	Utilizing yellow root cassava is very rich in Pro-Vitamin A	287 (95.0)	15 (5.0)	0 (0)	0 (0)	0 (0)	4.95
2	The yellow root cassava does not produce good quality cassava products	6 (2.0)	4 (1.3)	8 (2.6)	92 (30.5)	192 (63.6)	1.48
3	Consumption of products from yellow root cassava can help improve health conditions of my family	141 (46.7)	157 (52.0)	0 (0)	2 (0.7)	2 (0.7)	4.43
4	Consumption of yellow root cassava products can help prevent blindness in children and disease infection in reproductive women	165 (54.6)	135 (44.7)	2 (0.7)	0 (0)	0 (0)	4.54
5	Improvement in children growth and development can be achieved through feeding them with yellow root cassava products	143 (47.4)	153 (50.7)	4 (1.3)	0 (0)	2 (0.7)	4.44
6	The appearance and colour of products made from yellow root cassava are not attractive to encourage its consumption	8 (2.6)	4 (1.3)	6 (2.0)	156 (51.7)	128 (42.4)	1.70
7	Many products cannot be made from yellow root cassava compared to other varieties	31 (10.3)	24 (7.9)	6 (2.0)	172 (57.0)	69 (22.8)	2.26
8	Consumers do not patronize products made from yellow root cassava	7 (2.3)	58 (19.2)	29 (9.6)	167 (55.3)	41 (13.6)	2.41
9	Taste of yellow root cassava products is not as palatable as products from other cassava varieties	22 (7.3)	8 (2.6)	10 (3.3)	177 (58.6)	85 (28.1)	2.02
10	Consumption of yellow root cassava products will help reduce the money that I usually spend on my family members as hospital	182 (60.3)	106 (35.1)	8 (2.6)	4 (1.3)	2 (0.7)	4.52
11	Quick discoloration of yellow root cassava during processing makes it unattractive to buyers and consumers	14 (4.6)	20 (6.6)	88 (29.1)	91 (30.1)	89 (29.5)	2.27

Figures in brackes are percentages

3.6 Yellow Cassava attributes that determines its use by the processors

Table 6 shows the importance of Yellow cassava attributes in its use by the processors. Majority (71.2%) of the processors considered consumer acceptability of products from yellow cassava as important. However, other attributes such as ease of processing (67.5%), taste of the products (67.2%) and multiple usage of the tuber (65.9%) were also considered important for its processing by the processors. This is similar to the findings of Agwu

and Anyaeche (2007) who opined that high product quality, ease of processing and ability of the processed cassava to taste well to the consumer are reasons for continued use of some cassava cultivar.

Table.6: Attributes of Yellow Cassava that determines its use by the processors

Attributes of Yellow Cassava	Very important	Important	Somewhat important
Taste of the products	40(13.2)	203(67.2)	59(19.5)
Ease of Processing	44(14.6)	204(67.5)	54(17.9)
Consumer acceptability of the product	33(10.9)	215(71.2)	54(17.9)
Multiple usage of the tuber	42(13.9)	199(65.9)	61(20.2)

3.7 Constraints faced by processors in the utilization of Yellow cassava

As shown in Table 7, majority non-availability of market for yellow root cassava products (93.0%) of the respondents claimed that the constraints to the utilization of yellow root cassava were non-availability of market for yellow root cassava products, inadequate information on the potentials of yellow root cassava (90.4%), non-acceptance of yellow root cassava products by the consumers (54.6%) and inadequate knowledge about other products that can be made from YC (51.3%). However, 42.1% and 23.2% of the respondents indicated that low or poor quality products as well as the appearance and colour of yellow root cassava products makes it unattractive to some consumers respectively.

Table.7: Constraints to the utilization of Yellow cassava

Constraints	Yes	No
Low or poor quality products	127 (42.1)	175 (57.9)
Inadequate information on the potentials of yellow root cassava	273 (90.4)	29 (9.6)
Non acceptance of yellow root cassava products by the consumers	165 (54.6)	137 (45.4)
Appearance and colour of yellow root cassava products that makes it unattractive to the consumers	70 (23.2)	232 (76.8)
Non availability of market for yellow root cassava products	281 (93.0)	21 (7.0)
Inadequate knowledge about other products that can be made from yellow root cassava	155 (51.3)	147 (48.7)

3.8 Result of correlation analysis between attributes of yellow cassava and it's utilization by the processors.

The result of correlation analysis in Table 8 revealed there were positive and significant relationship between utilization of yellow cassava and the taste of its products (r=0.813), consumer's acceptability of the product (r=0.758) and multiple usage of the tuber (r=0.818). These results implied that the processors are likely to use Yellow cassava more as these attributes; taste of the products improves and the processing becomes easier. Also, acceptability of the products and its multiple use might increase the utilization of yellow cassava.

Table.8: Correlation analysis between attributes of yellow cassava and it's utilization by the processors

Variables	r-value	*p-value*	Decision
Taste of the product	0.813	0.000	**Significant**
Ease of Processing	0.109	0.059	Not Significant
Consumer acceptability of the product	0.758	0.000	**Significant**
Multiple usage of YC	0.818	0.000	**Significant**

***p*≤0.005**

3.9 Chi-square result of relationship between selected socio-economic characteristics and utilization of Yellow cassava

The result of the chi-square test on table 9 shows sex (x^2=30.141), marital status (x^2=84.919), educational level has significant relationship with utilization of YC at p≤0.05. The significant relationship between sex and utilization implies that gender affects the utilization of YC. This still emphasises the age long practice that women are more into the processing of cassava (Nweke *et. al.*,(2002) and Ogunleye, *et.al*. (2012). That of marital status implies that the married use YC as a major part of their meal. Also, the higher their education the more they utilize YC. This is because education influences ability to

adopt innovation like various ways to process the variety. As the extension visits to the respondent increases their utilization of YC increases. This might be because respondents were enlighten more on the benefits of the consumption of the varieties and also because of the trainings received in the processing of the varieties to products which are good sources of additional income as well as increase the shelf life.

Table.9: Chi-square result showing the relationship between selected socio-economic characteristics and utilization of Yellow cassava

Socio economic Characteristics	x^2 value	df	p-value	Remark
Sex	30.141	10	0.001	**Significant**
Marital Status	84.919	30	0.002	**Significant**
Educational level	112.925	40	0.001	**Significant**
Extension visit	48.656	10	0.001	**Significant**
Religion	17.707	10	0.060	**Not Significant**

IV. CONCLUSION AND RECOMMENDATIONS

The study concludes that the processors who were mostly women were in there active age and majority had formal education. Awareness of YC (pro-Vitamin A cassava varieties) was substantial among the processors. Virtually all the respondents were aware of TMS 01/1368 variety of YC and majority of them were using this particular variety to produce *Gari*, and *Fufu*. Extension agents from the Oyo State Agricultural Development Programme (OYSADEP), Harvest plus and International Institute of Tropical Agriculture (IITA) formed the leading sources of information on YC varieties among the respondents. Most severe constraint experienced by the processors was non availability of market for yellow root cassava products. It is recommended that effort should be intensified on awareness campaigns about the benefit of the varieties to increase their use and invariably provide market for the products. Also, training should be organised for the processors from time to time to upgrade their knowledge to process more products that will have better taste that is acceptable to consumers.

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Life Cycle Analysis of the *panela* agroindustry: Intensification for its development

Walter Francisco Quezada Moreno[1]; Walter David Quezada Torres[2]; Erenio González Suárez[2]; Marcia Judith Torres Tambo[2]; Franklin Antonio Molina Borja[1]; Nancy Fabiola Moreano Terán[1]; Amaury Pérez Martinez[3]

[1]Department of research, Universidad Técnica de Cotopaxi. Latacunga, Ecuador.
[2]Department of Chemical Engineering. Universidad "Marta Abreu" de Las Villas. Santa Clara, Cuba.
[3]Department of research, Universidad Estatal Amazonica. Puyo, Ecuador.

Abstract— *The research made it possible to identify sensitive environmental factors generated in all the operations carried out in an intensified panela agroindustry for the purposes of diversification, productivity, quality and safety. Results of the Environmental Impact Assessment (EIA), according to the Life Cycle Analysis (LCA) methodology show that in all impact categories, the industrial stage of the production of honey, panela and sugar, cause greater environmental impact, being the two latter the most representative. However, according to the Ecuadorian environmental legislation, the impacts of the panela agroindustry are considered to be moderate and cataloged type II, therefore it does not require intensive corrective practices. However, it requires preventive actions aimed to mitigate impacts, considering that it is a subsector of the sugar cane industry that is present as production units throughout the country, where there are favorable conditions for the cultivation of sugar cane.*

Keywords— Agroindustry panelera, Sweeteners, Intensification, Environmental assessment.

I. INTRODUCTION

The traditional, artisanal and inefficient way used in the processes of producing sweeteners from sugar cane in the *panela* agroindustry affects the productivity, quality and safety of the final product. However, globalization and increasingly demanding quality product markets promote a common approach based on an intensification of the processes [1], an approach that has emerged as an intelligent engineering discipline in the field of scientific research and advancement in industrial processes. Cleaner and more efficient productions that allow improvements in the general processes can be included in the *panela* agroindustry, as an action for business development. These productions are safe, economical and necessary for the sustainability of a technological and organizational process. The sugar cane industry is aimed at developing productive activities that comply with the technical obligations of the environmental legislation.

In that sense, environmental assessments are valid in the business and governmental world since they have turned into actions to understand and manage the risks and opportunities for the introduction of scientific knowledge through the Life Cycle Analysis of a product or process, in order to diminish their impact on the environment during the production process. The new applications of this knowledge, are effective to solve problems of sustainability and, necessary for the quality of life and sustainability of a company.

1.1 *Panela* agroindustry

The purpose of the *panela* agroindustry in Ecuador is to develop productive activities from the cultivation, processing of sugar cane and commercialization of its by-products such as honey, *panela* and natural sugar, as well as biomass. The *panela* industry transforms sugar cane juice into a solid product, known as *panela*, which is considered an important sweetening agent in the diet of Ecuadorians because of its characteristic taste and nutritional value [1][2]. If this industry is so important in terms of high employment multiplication and product value addition [3] [4] then it is necessary to optimize resources for its modernization and development, while increasing job opportunities. It is estimated that there are 610 units of *panela* production in the country that use as bagasse, firewood, tires and chemicals as fuels in the technological process for juice clarification [1][5].

In this industry, artisanal activities still prevail rather than industrial activities, due to the use of rudimentary practices in the manufacturing process, which have limited its development. On the other hand, the lack of control and

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application of the regulations contained in the environmental legislation, have made the activity be considered hardly eco-friendly. Thus, the lack of management, technology and improvements in its processes have led to low productivity, competitiveness, quality and safety of its products. All these problems have an impact on the environment and led to the search of feasible alternatives for intensifying the industrial processes and diversifying the *panela* industry production for productivity and sustainability.

The quality and safety of its products, the competitiveness and the development of the industry must be fully justified by actions of continuous improvement and innovative efforts for using clean and safe production [6]. Despite the importance of the sugar cane industry and the existence of an environmental legislation, the compliance with technical and environmental obligations is not always fulfilled in the *panela* industry, affecting its development and impacting the environment. Hence, innovative efforts are required from the technical-economic and environmental point of view to achieve improvements with fewer resources, which must be environmentally assessed to favor their investment and secure the market.

1.2 Intensification of industrial processes

The quality, safety, profitability, productivity and competitiveness, support the stability of the products in the global market; therefore, it is necessary to introduce scientific and technological development to move from the traditional and artisanal practices to the industrial ones with a focus on sustainability.

Intensification of Processes consists of the development of innovative equipment and techniques that offer substantial improvements in the production process, by reducing the volume of equipment, energy consumption and waste generation, resulting in cheaper, safer and sustainable technologies [11] [12] [13]; it is a revolutionary approach of development to process and design a plant [14], with industrial creativity including Research and Development techniques [15].

Intensification seeks to improve traditional processes, but the environmental impact is imminent and for doing this some actions are required to mitigate it. A balance between production and consumption is needed and it can be largely achieved with a responsible attitude of all the actors involved in the market [7]. Intensification of Processes (IP) develops safe actions, with highly efficient equipment, reduced in size that allows to obtain greater productivity with less amount of raw material and waste generation. It is important that the intensification of processes continues to be carried out and organized, so that the new generations could collaborate to develop technologies by promoting innovations and contributions to industry [8] [9] [10].

Leading scientists in the industry and in the US academic settings outline seven key issues to strengthen the development of these changes: reducing capital investment, reducing energy use and raw material costs, increasing flexibility in the process and reduction of the investments, increasing the safety of the process, increasing the attention to the quality and improvement of the environmental performance [16] [17]. Most entrepreneurs argue that increasing global competition will require significant changes in the way plants are designed. So, knowing how to understand and define the IP to achieve efficiency and productivity is imperative for the engineer and entrepreneur. The IP is necessary and consists of research actions and innovations development to optimize the processes in the different stages to obtain a quality product or service, through cleaner and safer productions that are reverted in satisfaction and social welfare. People committed to the development of scientific research and technological progress should prioritize improvement and evaluation processes, which guide the application of intensification processes and environmental assessment alternatives with a comprehensive approach, through the Life Cycle Analysis (LCA).methodology.

1.3 Environmental assessment of the *Panela* Agroindustry

There is widespread concern of reducing the environmental effects caused by economic activities at the global level. Research led by Rememen Arne and colleagues point out that the aim is to minimize the environmental impacts of a product or service from their portfolio throughout their entire life cycle [18]. LCA is a tool that can be applied in the optimization of production systems, improving market competitiveness through policies and practices that improve or increase sustainability [19]. LCA is the collection and evaluation of inputs and outputs and the potential environmental impacts of a product system throughout its life cycle [20].

It is a new way of applying knowledge, which allows to solve existing environmental problems generated throughout the production chain to achieve significant improvements in intensification processes. Assessing the sustainability of policies, processes, products and actions requires addressing this concept in depth, avoiding a fragmented vision by applying the Life Cycle Approach, a form of analysis that goes beyond the traditional approach to include

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environmental, social and economic impacts throughout the life cycle. The life cycle of a product begins with the extraction of the raw material from its natural sources and the generation of energy necessary for all manufacturing processes (transportation, production, packaging, distribution, use, maintenance, etc.) to eventual recycling, reuse, recovery or final disposal.

This process requires an infrastructure that reduces environmental impact. Adequate planning in the protection of the environment is necessary as the main factor to guarantee the quality and safety of the product, the health of the community and good life standards.

The increasing recognition of environmental protection and the potential impacts associated with the manufacture and consumption of products has increased the interest in developing methods for the evaluation and reduction. The LCA studies the environmental aspects and the potential impacts throughout the life cycle of a product or process [21]. Defining and categorizing the industry as well as assessing the environmental effects generated throughout the production chain, supports the evaluation of the LCA. ISO 14040 defines the "life cycle" as the consecutive and interrelated stages of a product system, from the acquisition of raw material or its generation from natural resources to the final disposal [22].

The Mexican Center for Cleaner Production also defines LCA as a tool that establishes market strategies and helps to plan prevention activities to achieve cleaner production in the industry [23]. In fact, LCA is an internationally accepted and recognized methodology for the evaluation of environmental loads and impacts associated with the elaboration of a product or process, taking into account all the stages of their life [24]. It means to reduce the consumption of resources necessary for the elaboration of a product and also emissions to the environment; it is the management of sustainability of the supply chains. Eventually, LCA, is fundamental in daily decision making [25], to make better use of resources since every productive activity is prone to generate negative environmental impacts.

Thus, it is significant for the agroindustrial sector, to pass a law that promotes its development, contributing to food security and sovereignty for good living standards, through a safe and permanent access to healthy and nutritious food [26] [27] [28].

According to Annex I of the National Environmental Categorization Catalog (NECC), for the construction and and/or operation of factories for the production of *panela*, this industry is categorized in group II, considered of low impact, therefore it requires an environmental record [29]. Despite the non-compliance and lack of environmental control in this industry, the use of bagasse as fuel material, less costly for industries as well as wood and tires, cause harmful environmental effects.

II. MATERIALS AND METHODS

A bibliographical review of the sector was carried out and data were collected to evaluate the environmental impact of an intensified *panela* industry with improvements in each stage of the process and optimization of by-products such as *cachaza* and bagasse in the production of alcohol and as nutritional supplement for animals. The optimization in each of the stages of the process, allowed the intensification in the industry, increasing the production capacity from 20 to 30 t cane / day with a 65% extraction. Meanwhile, diversification paved the way for the development of new products with and without aromas according to different variants: Variant 1: hydrolyzed honey (50%) - *panela* (50%); Variant 2. hydrolyzed honey (50%) - natural sugarcane (50%); Variant 3. *panela* (50%) - natural sugarcane (50%); Variant 4. honey (30%) - *panela* (35%) - natural sugarcane (35%).

The environmental assessment was performed using the LCA methodology and the software Simapro7. The intensification was initiated by applying mass and energy balances and the input and output inventory of materials that triggers the production process. The combustion of gases was measured using the E-instrument 4400. The limits of the system in the agricultural and industrial stages were assessed and are shown in figure 1.

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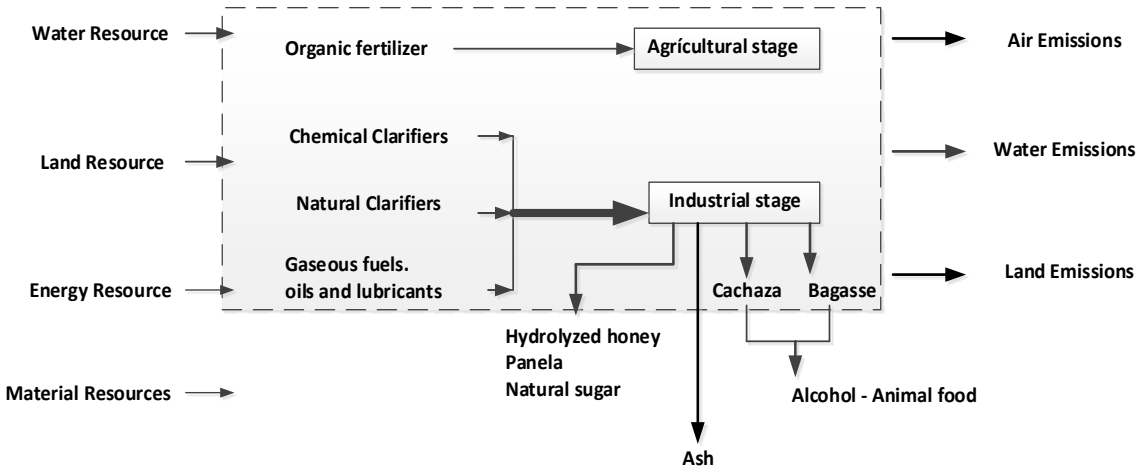

Fig.1: Limits for the production of panela, natural sugarcane and hydrolyzed honey

III. RESULTS

As a result of the intensification and diversification, the efficiency of the production of the sweeteners with and without aromas in the different variants got scores between 13.24 and 14.92%, higher than those calculated in the current *panela* industry, which are encouraging with respect to those reported in Colombia and India [30] [31]. It is noted that the scores are better for the variant where the hydrolyzed honey appears in the process. Figure 2 shows the diagram for obtaining three sweeteners.

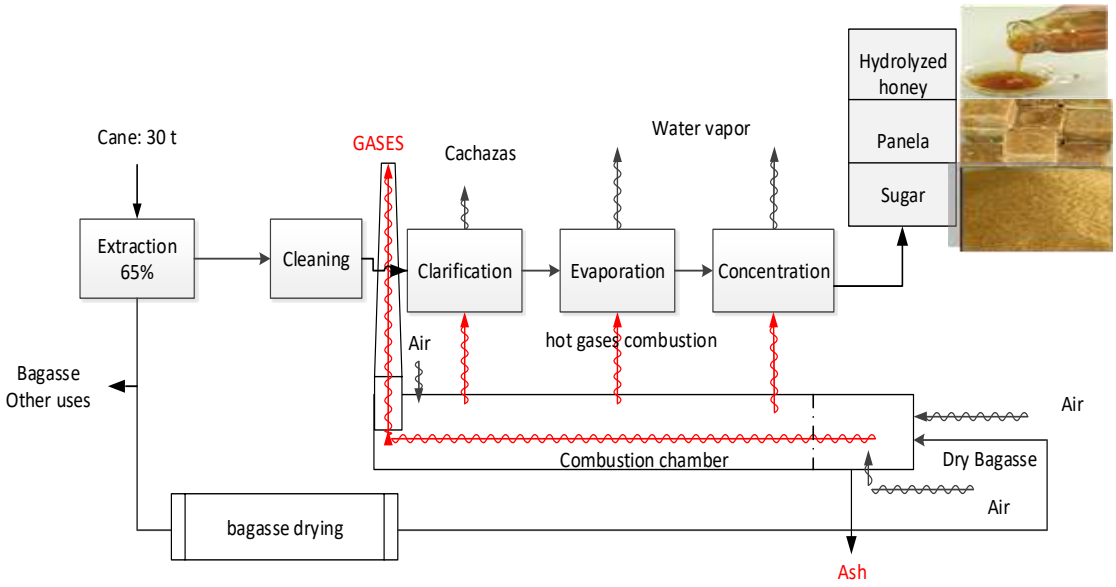

Fig.2: Diagram of the production process of hydrolyzed honey, panela and natural sugarcane.

The results of Figure 3 show that the major contribution to pollution occurs in the industrial stage, specifically in the production of honey, *panela* and natural sugar, being the two latter the most significant.

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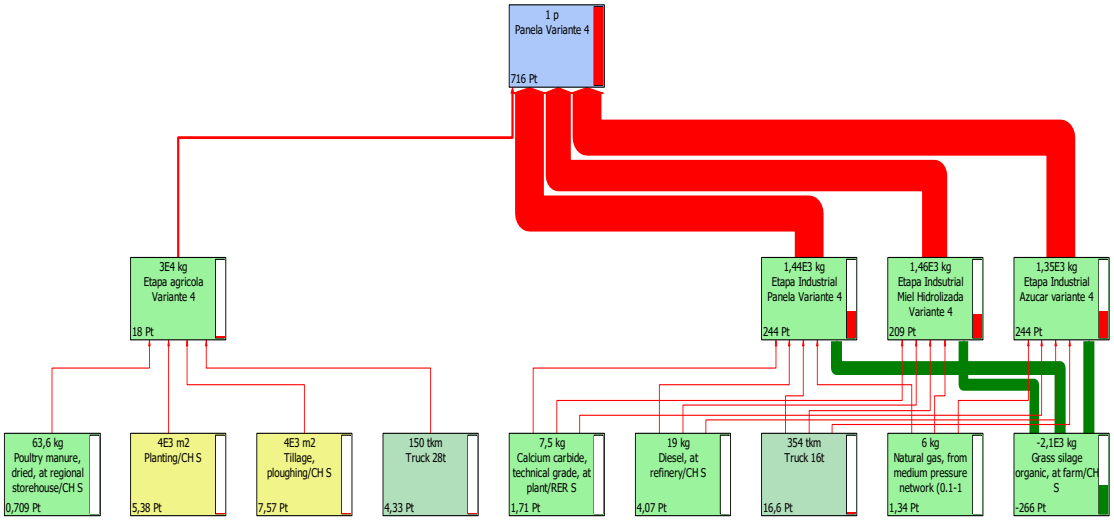

Fig.3: Impacts in the production variants

According to figure 4 of the industrial stage, the impact of inorganic respiration compounds is higher due to the use of diesel and bagasse as fuel in the combustion process. Unlike the industrial stage under current conditions, this variant significantly reduces the impact on the categories acidification - eutrophication, soil use, minerals, radiation, fossil fuels, ecotoxicity and climate change, which is attributed to the use of by-products as animal food. At the agricultural stage the impact is significant in the categories of fossil fuels, respiration of organic compounds, climate change, radiation, minerals and ecotoxicity.

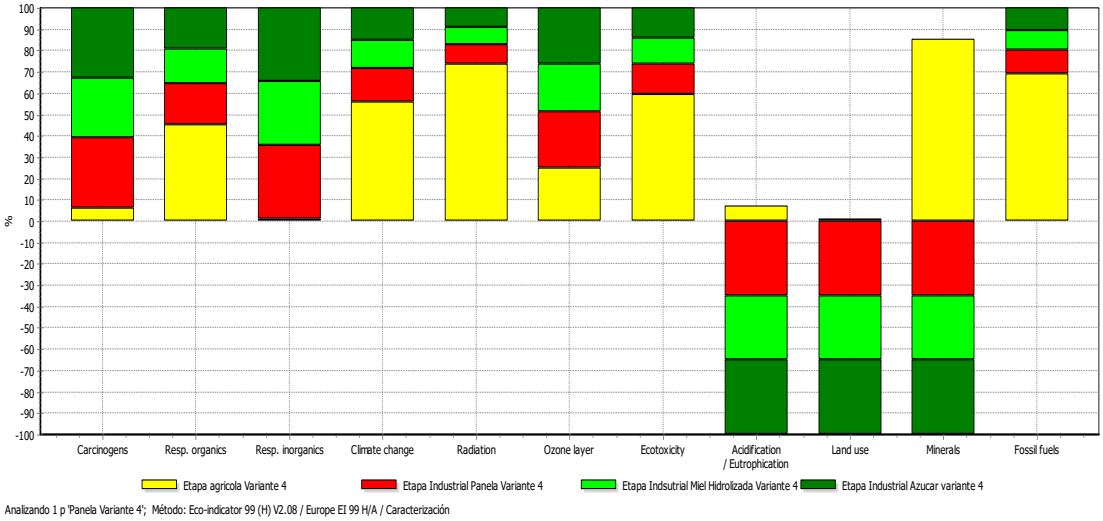

Fig.4: Contribution of impact categories according to Eco-indicador 99

Figure 5 shows the comparison of the environmental profiles of the current variant with Variant 4, where the contribution is lower in the following impact categories: climate change, radiation, acidification / eutrophication, land use, minerals and fossil fuels. However, in the categories carcinogenesis, respiration of inorganic compounds and ozone layer, the results in variant 4 are higher. This is due to the fact that in this alternative more sugar cane is used, because three products have to be produced, therefore the amount of fuel increases, causing more air emissions.

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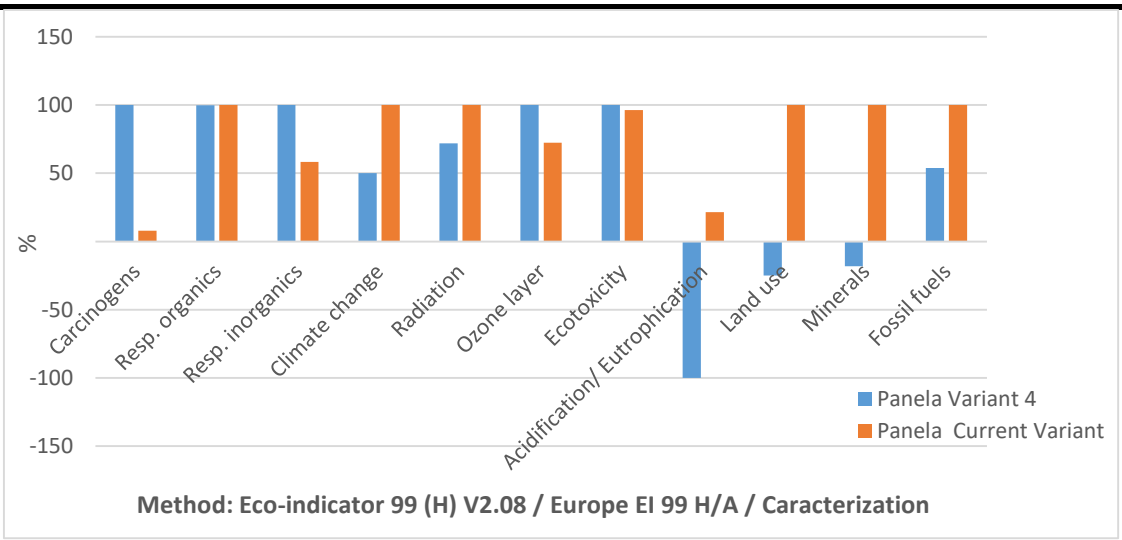

Fig.5: Comparison of the environmental profiles of the Current Variant and Variant 4

A comparison of the total score of the current variant, where only *panela* is produced, and variant 4, where three products are produced, (Figure 6) shows that the total impact in Variant 4 is greater, because of the increase in the amount of sugar cane processed (10 t / day), consequently the air emissions associated to the combustion processes are increased. However, this impact is conditioned to the production of three products, unlike the current variant where only *panela* is produced.

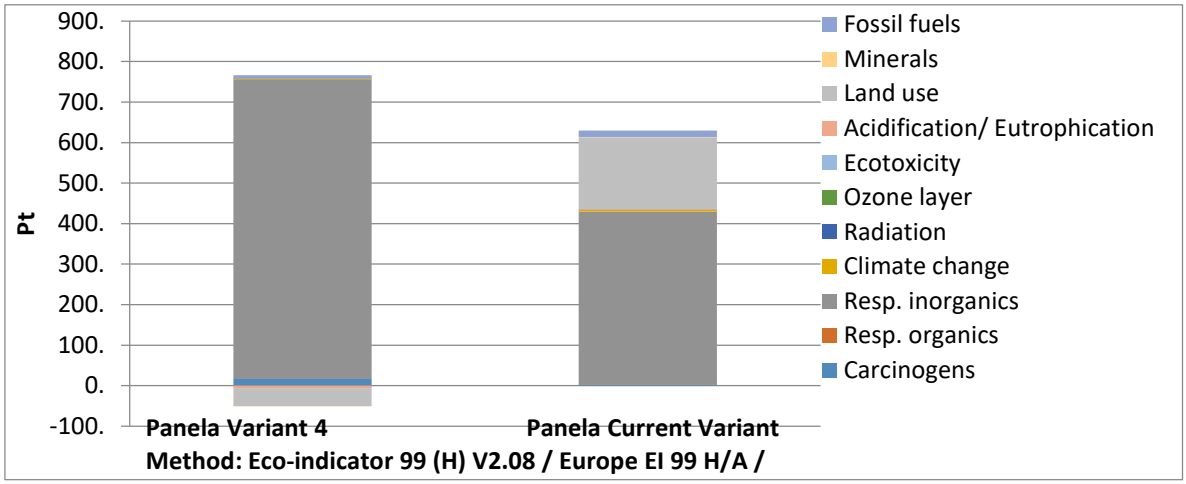

Fig.6: Comparison of the total impact of the Variant 4 with the Current variant

In figure 7, results from the comparison of the production of 1 kg of *panela* under current conditions with the production of 1 kg of a product in Variant 4, that considers in its percentages the production of honey, *panela* and natural sugar, evidence that the proposed variant is more feasible from the environmental point of view.

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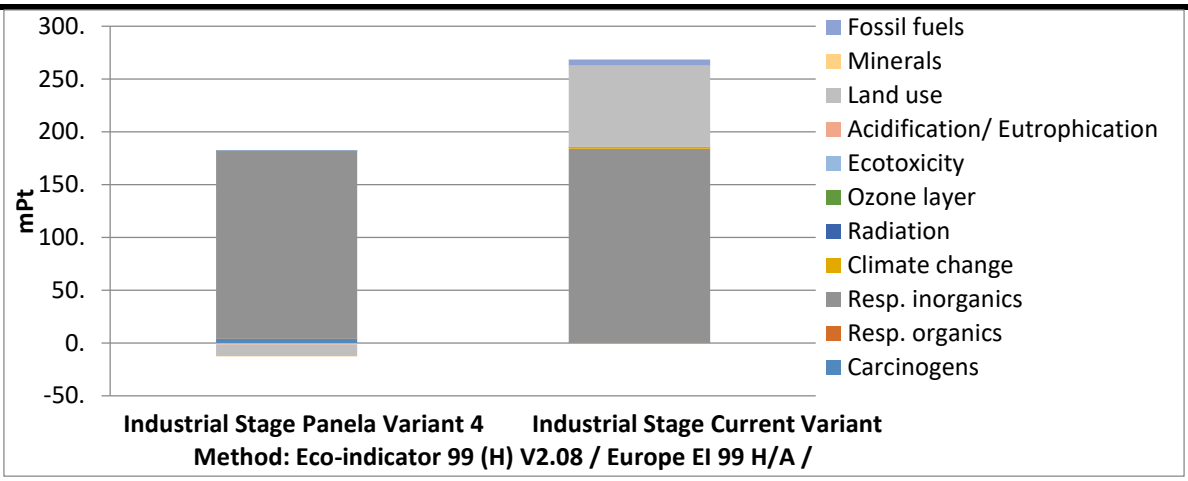

Fig.7: Comparison of the total impact of the Variant 4 with the Current variant per kilograms of the product

This shows that IP applied to the *panela* industry favors the development of new products as proposed in Variant 4, compared to the current Variant dedicated to the exclusive production of *panela*. It also optimizes processes and increases production capacity, taking advantage of by-products and the alternative use of natural clarifiers. This ensures the use of the LCA methodology as a viable environmental assessment tool in this sector and allows this industry be categorized as low-impact industry [32].

IV. CONCLUSIONS

The Life Cycle Analysis (LCA) is a viable concept useful as a design and planning tool in decision making, project execution and is increasingly used for the evaluation of environmental loads for the purpose of improvement and consequently with the environmental and economic benefits.

It is feasible to intensify the *panela* industry, based on theoretical principles with technical results, through the incorporation of new production lines and combinations, depending on internal and external factors considered by the industry.

The environmental assessment of the *panela* industry carried out through the LCA methodology confirms that the industrial stage is the one with greater contribution in all the impact categories. The impact of inorganic respiration compounds is higher, due to the use of diesel and bagasse in the combustion processes.

Of all the alternatives of production, the variant for the elaboration of hydrolyzed honey is feasible and technically and economically favorable. Considering the aspects of diversification, Variant 4 is the one that responds to the interests of the producers, since it considers optimization criteria for making the intensification of a dynamic and competitive agroindustry more feasible. Therefore, it reveals the need for management with immediate application to provide solutions and ensure the progress and productivity of the *panela* industry for sustainability.

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Assessment of the Effects of Growth Enhancement Support Scheme (GESS) on the Output of Dry Season Rice Farmers before and after Scheme Participation in Sokoto State, Nigeria

Sidi S.H., Abubakar B. Z, Ango A. K.

Department of Agricultural Extension and Rural Development, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto.

Abstract— *The study assessed the effects of Growth Enhancement Support Scheme (GESS) on the output of dry season rice farmers before and after participation. A multistage sampling technique was used to select farmers for the study. Data for the study were collected from 250 farmers using structured questionnaire. The data obtained was analyzed using descriptive and inferential statistics. The results of the showed that the age of the majority of the farmers fall between the ages of 30-39 years, married and had one form of education or the other. Based on the findings, the main source of information (46.8%) regarding the awareness of GESS programme was the district heads and majority (94.4%) of the farmers were registered with the scheme. About 40% of the farmers registered with the scheme because inputs provided by the scheme are supplied to only register farmers at a subsidized rate. The result of t-test analysis showed a significant difference (P<0.001) between farmers' output before and after GESS participation. The major challenges facing registered GESS farmers was that of untimely and inadequate supply of production inputs and manipulation of GESS register by agro dealers. It is therefore, recommended that effort should be geared towards ameliorating the aforementioned shortcomings.*

Keyword— Assessment; Effects; GESS; Output; Dry season farmers; before and after; Participation.

I. INTRODUCTION

The Nigerian agricultural sector over the years has witnessed efforts of its transformation. Many agricultural extension programmes were launched by various governments with the aim of improving the sector and make Nigeria self-sufficient in food production. The last administration headed by President GoodluckEbele Jonathan launched agricultural Transformation Agenda (ATA) and which was done through a set of complementary programme interventions aimed at solving, in a holistic and integrated manner, the constraints and weaknesses that held down agricultural development of Nigeria for a long time. The ATA seek to grow and develop agriculture as a business and thereby create jobs, assure food security, promote private sector investments for wealth creation and maximize the sector's contribution to the country's economic growth (APNET,2013). The specific objectives of the agricultural sector as envisioned in ATA blueprint document are to:

i. Secure food and feed for the needs of the nation;
ii. Enhance generation of national and social wealth through greater exports and import substitution;
iii. Enhance capacity for value addition; efficiently exploit and utilize available agricultural resources,
iv. Enhance the development and dissemination of appropriate and efficient technologies.

These objectives are to be achieved by focusing attention on five priority areas:

a. Commercial agriculture development aimed at developing major crops, livestock, and fisheries along their entire value chains;
b. Construction, completion, and rehabilitation of silos and warehousing for agricultural commodities;
c. Research and development, including equipping existing institutes for research in agricultural biotechnology;
d. Completion and rehabilitation of existing irrigation schemes and dams.
e. Restructuring of agricultural commodity marketing companies as enunciated in the firstimplementation plan (Olomola, 2015).

Based on Okafor and Malizu, (2013) the major implementation strands for the ATA includes:

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i. Growth Enhancement Support Scheme (GESS) – designed to enhance agricultural productivity through timely, efficient and effective delivery of yield-increasing farm inputs;
ii. Staple Crops Processing Zones (SCPZs) – to promote private sector investments for agribusiness development and establish public-private partnership framework for the sustained development of commodity value chains;
iii. Nigeria Incentive-based Risk Sharing for Agricultural Lending (NIRSAL) – designed to derisk agricultural financing by banks and enhance the flow of credit to agricultural sector value chain actors;
iv. Commodity Marketing Corporations (CMCs) – aimed at improving the marketing environment for agricultural commodities and assuring sustainable pricing and market development.

Among the above four ATA components, the GESSprovides a unique connecting link as it targets the farmers directly with critically needed modern farm inputs on real-time basis. Understandably, the implementation of GESS seems to be ahead of other components because of the primacy and urgency of boosting farm-level outputs and productivity.

In July, 2012, the Federal Government of Nigeria introduced the Growth Enhancement Support Scheme (GESS) which was designed to deliver government subsidized farm inputs directly to farmers via Global System for Mobile Communication (GSM). The GESS scheme was powered by e-wallet, an electronic distribution channel which provides an efficient and transparent system for the purchase and distribution of agricultural inputs based on a voucher with which thefarmers can redeem assorted fertilizers, seeds and other agricultural inputs from agro dealers at less than 50% of the total cost of the inputs , the other half of the cost being shouldered by the Federal and State Governments in equal proportion (Okafor and Malizu, 2013).

Under the Scheme, an accredited farmer will receive agro-chemicals and other inputs allocation through an e-wallet that hosts unique voucher numbers sent to his/her phone, and the farmer will then go to an accredited agro dealer to redeem his/her inputs. It is expected that this effort by the Federal Government should lead to improvements in agro - inputs distribution and marketing by private sector; as well as consequent improvement in crop and agricultural productivity; and profitability for both the input supplier/dealer and farmer. Adedapo(2013) reported that the programme had so far registered about 14 million farmers throughout the federation for direct redemption of farm inputs through the e - wallet system. Federal Ministry of Agriculture and Rural Development (FMA&RD)(2013) disclosed that 4 million were registered in 2012, while over 10 million were registered by the year 2013.

A recent stock-taking by the FMA&RD shows that the process of targeting farmers to benefit from the input subsidy programme under the GESS scheme started with the registration of 3.9 million farmers in 2012. The number increased to 9.5 million in 2013 and 10.5 million in 2014. The number of farmers targeted to benefit from the subsidy also continued to increase from 1.1 million in 2012 to 7.2 million in 2013 and 8.3 million in 2014. Redemption of inputs by the farmers was also on the increase yearly (Adesina, 2013).

In the past,fertilizer procurement and distribution in particular has been fraught with fraud, discrepancies and inefficiencies. Governments at the Federal and State levels spent a lot of money on procurement and distribution of farm inputs which unfortunately does reach the real farmers (small holder farmers) and thus, does notsignificantly having impact on the national food output. The involvement of Federal Government in the direct procurement and distribution ofagro-chemicals has succeeded in weakening the ability of private companies to actively participate in the development of the agricultural sector and their ability to compete efficiently for market share among their business partners. In order to address this problem of direct involvement of the Federal Government in procurement and disbursement of agro-chemicals and other agricultural inputs, the government decided from the year 2012 farming season to opt out of direct procurement and distribution of inputs by instituting the Growth Enhancement Support Scheme (GESS)which aimed at delivering subsidized farm inputs to farmers through an electronic wallet. It is against this background that this study addressed the following objectives:

i. Describe the socio-economic characteristics of participating farmers in the study area
ii. Describe the participating farmers sources of information regarding GESS
iii. Identify the participating farmers reasons for registration with GESS
iv. Examine the difference between the output of farmers before and after participation in the GESS.

II. RESEARCH METHODOLOGY

The study was conducted in Sokoto State, Nigeria. The state located in the extreme end of the north western Nigerian,

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close to the confluence of the Sokoto Rima River. The study area is located between latitude 11º 00` and 14º 00`N and longitude 3º 50` to 8º 00`E.Rainfall in the area is highly seasonal. In terms of vegetation, the State falls within the Savannah zone.Daily maximum temperature is about 36ºC. During the Harmatan season, daily minimum temperature of the area falls below 17ºC, and sometimes it reaches up to 44ºC. Rainfall starts late and ends early, the dry seasons start from October and lasts up to April in some parts and may extend to May or June in other parts. The wet season on the other hand begins in most parts of the State in May and lasts up to September or October. The average rainfall is about 550mm per annum. Relative humidity of the studyarea is between 15-20% during the dry season and up to 70-75% during the rainy season (Audu and Zubairu, 2013).

The State has a projected population of 4,850,374 in ten years at 3% population growth rate (NPC, 2015). The State shares common boundary with Kebbi State to the south-east, Zamfara State to the east and Niger Republic to the north. The study area is basically an agrarian society with over 90% of the population involved in one form of agricultural activity or the other.

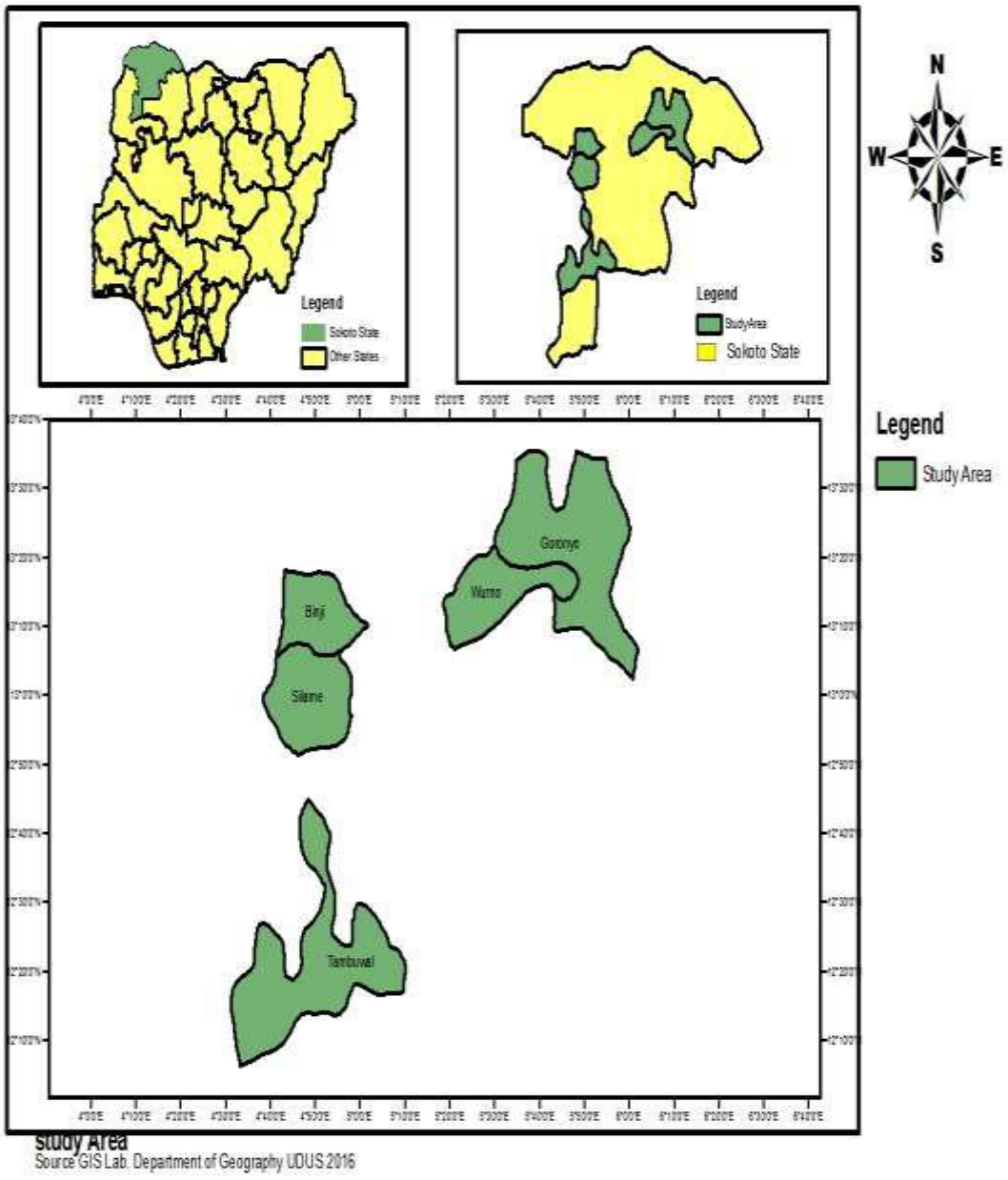

Fig.1: Map of the study area

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Sampling Procedure and Sample Size

The population of the study includes all the dry season rice farmers participating in the GESS intervention programme in the 23 Local Government Area of Sokoto State. The study adopted a multi-stage sampling technique. In the first stage Five Local Government areas were purposively due to high number of GESS farmers. The second stage involved the random selection of five (5) villages from each of the selected Local Government areas.The third stage included the selection of ten (10) GESS farmers from each of the villages. Giving a total of fifty (50) farmers from each of the selected Local Government Areas, making the sample size of the study to 250 farmers

Structured questionnaire was used to collect the primary data for the study while the secondary information was sourced from text books, journals, GESS office record and internet sources.The data collected were analyzed using descriptive (frequency counts and percentages) and inferential statistics (paired t-test analysis).

Table.1: Sampling procedure and sample size

Number of LGAs in Sokoto state	Selected LGAS	Number of GES registered farmers	Sampled villages	Number of respondents	Sample size
23 LGAs	Goronyo	12621	Goronyo	10	
			Taloka	10	
			Birjingo	10	
			Gorau	10	
			Keta	10	
	Silame	22250	Jekanadu	10	
			Silame	10	
			Maje	10	
			Gittarana	10	
			Kubodu	10	
	Wurno	14000	Lugu	10	
			Wurno	10	
			GidanBango	10	
			Dimbiso	10	
			Kwargaba	10	
	Tambawal	11100	Tambawal	10	
			Kaya	10	
			RomonSarki	10	
			RomonLiman	10	
			Jabo	10	
	Binji	8500	Gawazzai	10	
			Inname	10	
			Binji	10	
			SoroYamma	10	
			SoroGabbas	10	250

III. RESULTS AND DISCUSSION

Socio-economic Characteristic of the Farmers.

Table 2 presents the socio-economic characteristics of the sampled farmers. Majority of GESS farmers (30.8%) were within the ages of 30-39. Only 6.8 percent were above 60 years old. The mean age was established as 40.7 years. This is fairly youthful age which can spur inquisitiveness to participate in agricultural extension programmes. Low number of farmers for age group above 60 is likely caused by retirement from agricultural activities or delegation of production activities to young family members. The result is in agreement with Nwaru, 2004 who reported the most productive age to be in the range of 20-50 years. Main farming activities were known to be practiced by the male farmers, while female farmers in most cases participate in processing and other value addition activities. The result indicated that majorities (98.4%) of the farmers were males and only few (1.6%) were females. This imbalance

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according Ango*et al.* (2013) could be attributed to either the stress involved with farming activities, gender division of labour or access of women to land due to their cultural background as well as prevailing norms and values of the people of the study area. Similarly, majority of the farmers (93.6%) were married. This offers the challenge to strive to improve agricultural productivity to adequately feed family members. On the educational attainment, the result evidently indicated that larger percentage (54.8%) of the farmers had formal education. By implication, it would be easier for farmers in the study area to accept and adopt new innovations and technologies that are vital to enhancing farm production. With regards to monthly income of the farmers, it is shown that majority of the farmers (43.6%) had monthly income of N20, 000 and below, 30.4 percent had monthly income of between N21, 000 to 40,000 while only 0.8 percent had N100, 000 and above. The implication of this is that the farmers in the study area may not be opportune to take credit facility. This is because; credit use is associated with higher income than average economic performance. They may not also be able to invest in capital projects like modern technology as this normally attract huge financial obligation considering their low financial status.

Table.2: Distribution of farmers according to socio-economic characteristics

Variable	Frequency	Percentage	Mean	SD
Age (Years)				
20-29	41	16.4		
30-39	77	30.8		
40-49	65	26		
50-59	50	20		
60 and above	17	6.8	40.7	11.2
Total	**250**	**100**		
Level of education				
Primary education	36	14.4		
Secondary education	54	21.6		
Tertiary education	35	14		
Adult literacy	12	4.8		
Qur'anic education	113	45.2	8.85	4.53
Total	**250**	**100**		
Marital Status				
Single	12	4.8		
Married	234	93.6		
Widow/ divorcee	4	1.6		
Total	250	100		
Income				
<20,000	109	43.6		
21,000-40,000	76	30.4		
41,000-60,000	43	17.2		
61,000-80,000	12	4.8		
81,000-100,000	8	3.2		
N100,000 and above	2	0.8	32132.4	21858.2
Total	**250**	**100**		

Source: Field study, 2016

Sources of GESS Information to Farmers

The highest percentage of farmers 46.8 percent sourced information regarding GESS programme from their district heads, 38.8 percent sourced information regarding GESS from Media sources while Only 28 percent sourced information on GESS programme from Rice Farmers Association.

The result of the study indicated that District Heads were the most popular source of information regarding GESS programme, followed by neighbors and friends. This is in

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agreement with the finding of Ajeigbe and Dashiell (2010) who reported that the first step for an extension agent or researcher to build trust among community members is to arrange a meeting with community leaders to explain, discuss, and gain their support for the process of participatory research and extension approach. This could be the approach used by the Federal Ministry of Agriculture and Rural Development to enlighten public on the significance of the GESS programme.

Table.3: Distribution of Farmers According to their Sources of GES Information

Sources of Information	Frequency	Percentage
ATA office	51	20.4
Neighbors and friends	85	34
District head	117	46.8
Media	97	38.8
Rice Farmers Association	70	28
Total	420*	

*Multiple responses.

Registration with GES Programme.

Agriculture progresses technologically as farmers adopt new innovations. The extent to which farmers adopt available innovations and the speed by which they do so determines the impact of innovations in terms of productivity. It is a common phenomenon that farmers like any other kind of entrepreneurs; do not adopt innovations simultaneously as they appear in the market. Apparently some farmers choose to be innovators (first users) while others prefer to be early adopters, late adopters or non-adopters (Paul*et al.*, 2003). The process of targeting farmers to benefit from the input subsidy programme under GESS scheme started with registering 3.9 million farmers in 2012. The number increased to 9.5 million in 2013 and 10.5 million in 2014 (Olomola, 2015). The result of the study indicated that majority of the farmers 94.4% registered with GES programme immediately they heard about the programme. Only 5.6 % registered later.

Reasons for Registration with GESS scheme

The level of awareness about the scheme was the major reason why farmers register. Majority(62.3%)of the farmers registered with the scheme because the inputs provided were subsidized, 49.6 percent registered because the programme support both rainy and dry season farming while only22.4 percent registered because the existing input supply was not reliable. Furthermore, the finding also indicated that majority of the farmers 62.8 percent participated in the programme for three years, 32.8 percent participated for two years and only 4.4 percent participated for only one year.

Table.4: Distribution of Farmers According to Reason for Registering to GESS

Scheme

Variables	Frequency	Percentage
Reasons for registration		
Because the programme is new	61	24.4
Because it is federal government programme	72	28.8
Because existing input supply is not reliable	56	22.4
Because the programme support both rainy and Dry season farming	124	49.6
Because inputs are subsidized	156	62.3
Total	469*	

*Multiple responses

Analysis of the Difference between Outputs Obtained Before and After GESS Programme

t- test was conducted to determine the difference between output of farmers before and after participation to GESS programme. The result of the analysis is presented in table 3.

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Table.5: Analysis of the Difference in the Output of farmers before and after GES programme.

Variable	No. of Farmers	Mean output (kg)	Std dev.	t- value
Output Before	250	4402.1413	3928.99060	**10.67**
Output After	250	6756.3920	5571.96426	

Analysis in table 5 shows that the mean difference between the output of farmers before GESS programme was 4402.1413, while the mean output of farmers after GESS programme was 6756.3920 and the mean difference was 2354.25 The results showed that there was significant difference in the output of farmers before and after GESS participation, meaning that dry season rice farmers in the study area recorded significant improvement in the output obtained after the intervention of GESS programme. Thus, the null hypothesis is rejected that there is no significant difference in the output obtained by farmers before and after participation in the GESS programme. The GESS programme in the study area has been able to achieve its cardinal objective of increasing rice production among participants.

Constraint Facing Farmers Regarding GESS

There were appreciable numbers of GESS farmers in the study area. However, there were problems affecting them regarding GESS programme that could have effect on their output.

Sangoi*et al*. (2007) reported that farm input subsidy programme have once again become a popular policy tool that many African governments use to improve agricultural productivity and address rural poverty. Nigeria is one of the countries in Africa that has revived input subsidy programme through GESS. One of the stated goals of GESS is to ensure timely, effective and adequate supply of agricultural inputs to GESS target farmers in the form of fertilizer, chemicals and hybrid seed. However, timely delivery of GESS inputs has been a longstanding constraint, despite persistent calls by farmers to correct this problem. From the study, result shows that 35.6 percent of the farmers reporteduntimely supply of inputs as the major constraint regarding GESS. It is possible that late delivery of GESS inputs may significantly affect farmer's production.

In 2012, when GESS was introduced, the beneficiaries were entitled to 2 bags of 50kg fertilizer and 25kg bag of hybrid seed; quantity which most farmers considered inadequate, considering their farm size. This might be the reason why 32% of the farmers reported inadequate supply of inputs as a constraint.

Olukayode (2014) reported that, when GESS was introduced, a major criticism was that many beneficiaries were unable to redeem their inputs due to GSM network failure or an absence of it in many remote areas. To solve the problems of poor mobile phone network, multiple registration, corruption and easy inputs redemption process, the FMA&RD, in collaboration with International Fertilizer Development Centre (IFDC), introduced a new technology known as "GES TAP" for farmer's registration. The GES Touch and Pay (TAP) is an offline technology that captures the data of farmers along with their photographs, and at the end of the registration exercise, a green card is issued to the registered farmers which can be used in redeeming subsidized inputs (FEPSAN, 2014). But, findings from this study show that 21.2 percent of farmers' alleged manipulation of register by agro dealers byconniving with some farmers to collect their TAP card, redeem the inputs and give a token to farmers, and later sell the inputs at market price.

IV. CONCLUSION AND RECOMMENDATIONS

The study was carried out to assess the effect of GESS programme. The t-test analysis shows significance difference in the output after GESS participation. Null hypothesis was tested and rejected. From the study, it could be concluded that GESS programme is promising, and if sustain properly, the goal of the programme can be achieved and agricultural production can be enhanced in terms of the output of dry season rice farmers in the study area. As a result of the impressive improvement in the output of GESS farmers after participation, it is recommended that growth enhancement support scheme be retained and encouraged by the federal ministry of agriculture and rural development.

Based on the findings of the study, the following recommendations are hereby made.

i. Inputs should be delivered to farmers before the planting season commences.
ii. Increase GESS input allocation to farmers.

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iii. Farmers should be enlightened not to sell their TAP cards for a token.

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SHORT COMMUNICATION

Spatial characterization of common blossom thrips (*Frankliniella schultzei*) in smallholder avocado orchards along slopes of Taita Hills and Mount Kilimanjaro

Odanga J. James[1, 2a*, 6], Samira Mohamed[1], Florence Olubayo[6], Richard Nyankanga[6], Irine A. Otieno[3], Sizah Mwalusepo[1, 4], Geoffrey Mwachala[2b], Tino Johansson[1, 5]

[1]ICIPE-African Insect Science for Food and Health, P. O. Box 30772 - 00100, Nairobi, Kenya
[2a]Invertebrate Zoology Section, National Museums of Kenya, P. O. Box 40658 - 00100, Nairobi, Kenya
[2b]East African Herbarium, National Museums of Kenya, P.O. Box 40658 - 00100, Nairobi, Kenya
[3]Faculty of Geosciences, Utrecht University, Netherlands
[4]Department of General Studies, Dar es Salaam Institute of Technology, P. O. Box 2958, Dar es Salaam, Tanzania
[5]Department of Geosciences and Geography, University of Helsinki, P. O. Box 68, FI-00014, Finland
[6]Department of Plant Science and Crop Protection, University of Nairobi, Kenya, P.O. Box 30197-00100, Nairobi, Kenya
*Corresponding author: jkodss@yahoo.com

Abstract *– Frankliniella schultzei Trybom (Thysanoptera: Thripidae) is an important flower pest of avocado crop (Persea americana Mill) at Taita Hills in South-eastern Kenya and Mount Kilimanjaro in North-eastern Tanzania. However, its geographical distribution is not known in the East African avocado cropping systems. In order to generate the spatial data of the common blossom thrips (Frankliniella schultzei), a survey was carried out in small-holder avocado orchards along altitudinal gradient (900 - 1800m.a.s.l.) of Taita Hills and Mount Kilimanjaro using a white coloured beating tray and camel brush. Once the specimens of thrips were taxonomically verified, the abundance data was tabulated into three altitudinal zones, namely; lowland (900-1199m.a.s.l.), sub-montane (1200-1499m.a.s.l.) and montane (1500-1799m.a.s.l.). Frankliniella schultzei was recorded in all altitudinal zones of both transects with mean abundance being highest at Taita Hills (5.4) compared to Mount Kilimanjaro (0.9). However, abundance of the pest was greater in cooler highlands (>1200m.a.s.l.) than warmer lowland areas (<1200m.a.s.l.) of both transects. The findings of this study contributes significantly towards spatial mapping of Frankliniella schultzei in East Africa and this information is important in developing strategies aimed at controling infestation of avocado flowers by the insect pest at the two study transects.*

Keywords— Avocado, East Africa, Frankliniella schultzei, Mount Kilimanjaro, Taita Hills.

I. INTRODUCTION

Common blossom thrips (*Frankliniella schultzei* Trybom) is anthophilous pest species (Milne *et al*., 1996; Odanga *et al*., 2017b) whose diet is predominantly pollen and floral tissues (Kakkar *et al*., 2012). Being a polyphagous insect pest, it feeds on flowers of various ornamental, vegetable and fruit crop hosts in different parts of the world (Milne *et al*., 1996; Kakkar *et al*., 2012). Palmer (1990), Palmer (1992) and Milne & Walter (2000) reported *Frankliniella schultzei* on 83 species of plants from 35 families with important hosts being cotton (*Gossypium* spp.), groundnut (*Arachis hypogaea*), beans (*Phaseolus vulgaris*) and pigeon pea (*Cajanus cajan*) and avocado (*Persea americana*). In Afrotropical highlands of Taita Hills and Mount Kilimanjaro, *Frankliniella schultzei* is a pest of avocado crop that feeds on floral resources (Odanga *et al*., 2017a; Odanga *et al*., 2017b) thereby contributing to flower abortion and subsequent low fruit-set. This pest, therefore, impacts negatively on livelihood of small-scale farmers as it contributes to low yield of avocado fruits which the local growers depend on as a source of cash and nutritious food. Although, Odanga *et al* (2017b) described in detail temporal fluctuations of the pest abundance at Taita Hills and Mount

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Kilimanjaro, limited information is available on geographical distribution of *Frankliniella schultzei* in the East African highlands. This study was, therefore, initiated to provide first-ever spatial distribution data of *Frankliniella schultzei* in avocado orchards along altitudinal gradient of Taita Hills in South-eastern Kenya and Mount Kilimanjaro in North-eastern Tanzania.

II. MATERIALS AND METHODS

2.1. Study areas

This study was carried out in farmlands at Taita Hills in South-eastern Kenya and Mount Kilimanjaro in Northern-eastern Tanzania as described by Mwalusepo *et al* (2015) and Odanga *et al* (2017b). The study regions were selected because the avocado plant (*Persea americana* Mill) is the major fruit crop cultivated by the small-holder farmers at South-eastern slopes of Taita Hills and Mount Kilimanjaro. Furthermore, farming along the two study transects is rainfed and the small-holder growers do not use chemicals to control insect pests or diseases of avocado crop.

2.2. Study design

The survey was carried out along altitudinal gradient from 900 to 1800 m.a.s.l. in small-scale avocado orchards at the two study areas between August 2012 and July 2014. The common blossom thrips, *Frankliniella schultzei* Trybom, was sampled from avocado trees using a white coloured beating tray and camel brush as described by Palmer (1990). Six hundred avocado trees were sampled at each altitudinal zone, namely; lowland region (900-1199m a.s.l.), sub-montane (1200-1499m a.s.l.) and montane (1500-1799m a.s.l). Geographical coordinates and elevation of every study site was verified using a hand-held Garmin GPS model eTrex 30. Collected specimens of the thrips were mounted and identified at the National Museums of Kenya entomology laboratory in Nairobi using taxonomic manuals; Palmer (1990), Palmer *et al* (1992), Moritz *et al* (2001) and Mound (2010). The fully identified and confirmed thrip species were deposited in the entomology collection at the National Museums of Kenya.

2.3. Data analysis

Wilcoxon signed rank test was employed to test differences between paired datasets sampled at Taita Hills and Mount Kilimanjaro (R Development Core Team, 2012; Crawley, 2007). Sets of variables were normalized for further analysis using Tukey's HSD (Honestly Significant Difference) post hoc test to pinpoint what exact sub-sets within a data that had significant differences from each other (R Development Core Team, 2012). Spatial mapping was generated using kriging method by interpolating mean abundance of *Frankliniella schultzei* along altitudinal gradient of the two transects using QGIS version 1.8.0.

III. RESULTS

Mean abundance of *Frankliniella schultzei* differed significantly between Taita Hills (5.4±0.8) and Mount Kilimanjaro (0.9±0.1) ($V=1726.4$, $P<0.0001$). For both transects, mean abundance of *Frankliniella schultzei* was smaller at lowland zone (900-1199m a.s.l) than the highlands. However, the abundance of *Frankliniella schultzei* at Taita Hills study area was highest at mid-altitudinal range (1200-1499m a.s.l) (Figure 1a). For Mount Kilimanjaro, the abundance was highest at montane zone (1500-1799m a.s.l) (Figure 1b). Tukey's HSD pair wise comparison of mean abundance of *Frankliniella schultzei* between agro-ecological zones at Taita Hills revealed a significant difference in two pairs (lowland and sub-montane, $P<0.0001$; sub-montane and montane, $P<0.0001$) except between montane and lowland ($P=0.344$) (Figure 1a). For Mount Kilimanjaro transect, the mean abundance of *Frankliniella schultzei* between agro-ecological zones revealed a significant difference in only one pair; lowland and montane ($P=0.011$), however, the rest did not show a significant difference (lowland and sub-montane, $P=0.312$; sub-montane and montane, $P=0.317$) (Figure 1b).

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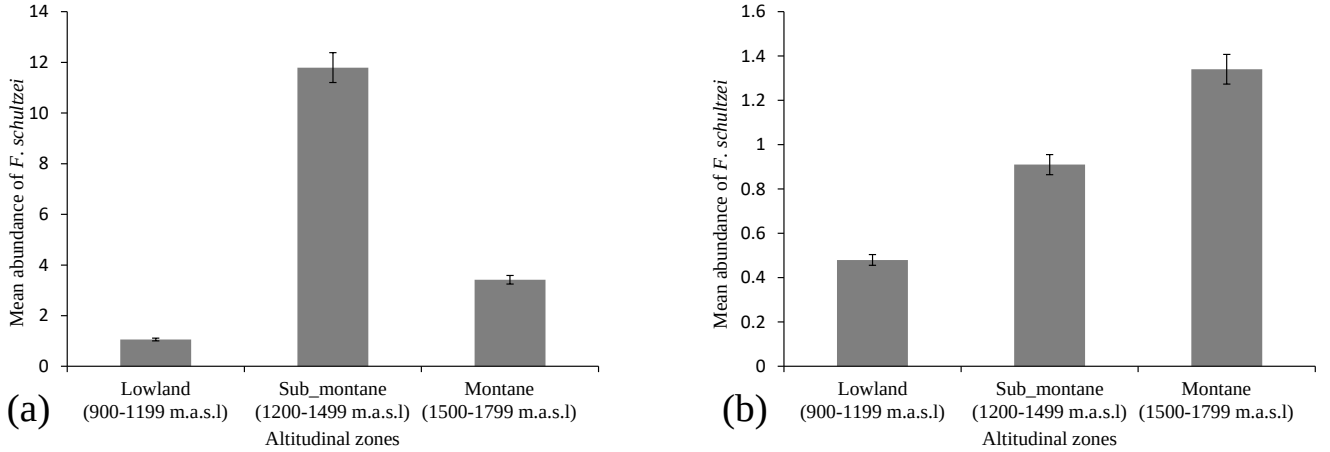

Fig.1: Distribution of Frankliniella schultzei within altitudinal zones of (a) Taita Hills and (b) Mount Kilimanjaro transects. Similarly, spatial distribution pattern for Frankliniella schultzei using kriging method revealed that the pest is highly abundant in highlands above 1200m a.s.l than lowlands of both Taita Hills and Mount Kilimanjaro study areas (Figure 2a & b).

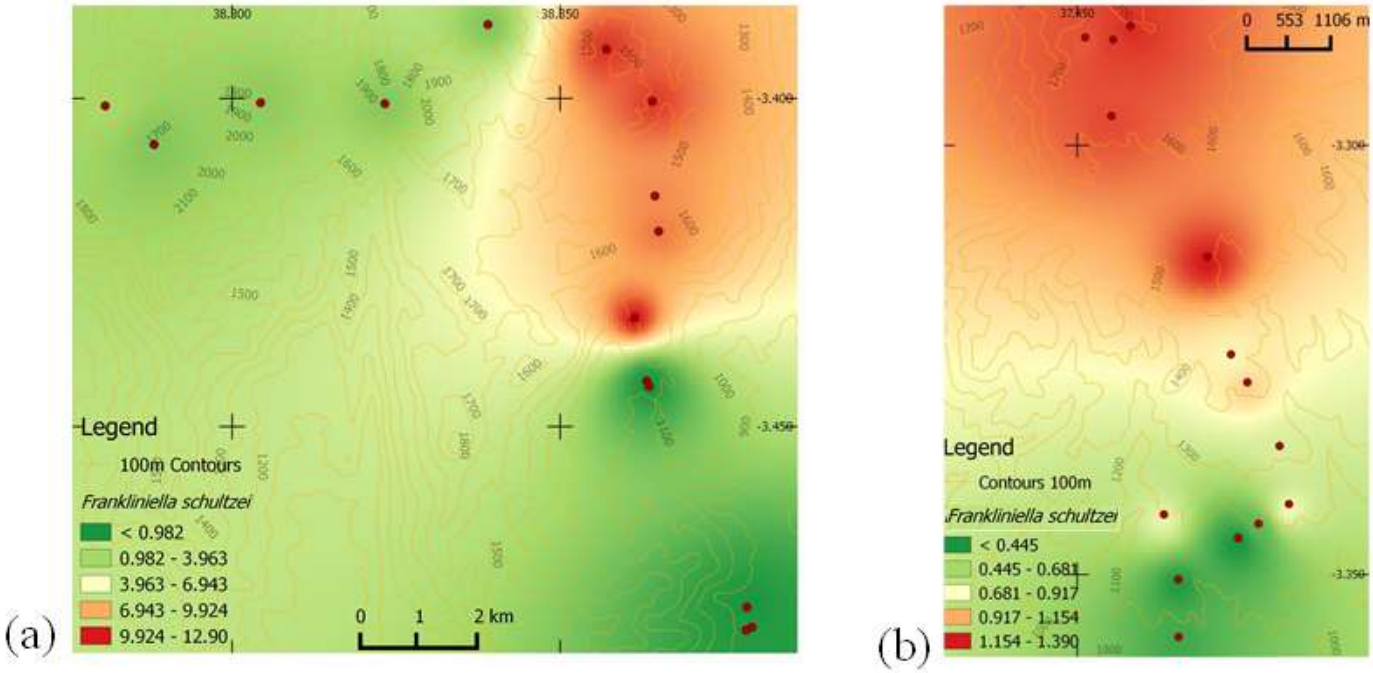

Fig.2: Geographical distribution of Frankliniella schultzei based on its mean abundance along altitudinal gradient of (a) Taita Hills in South-eastern Kenya and (b) Mount Kilimanjaro in North-eastern Tanzania. Red pattern shows highest whereas green reveals least mean abundance of Frankliniella schultzei. Dotted red points are the sampling sites along each study transect.

IV. DISCUSSION

Mean abundance of common blossom thrips (*Frankliniella schultzei*) was greater in highlands above 1200m.a.s.l because these zones had plentiful host avocado trees with abundant floral resources at both study transects. Availability of productive host trees at highlands provided the pest with enough food and habitat (Palmer *et al*., 1992). Consequently, the abundance of *Frankliniella schultzei* was very low at the lowland zone (1200m.a.s.l.) because the region is warmer compared to ever cold highlands. Generally, the common blossom thrips thrives well in areas with mild temperatures (Milne *et al*. 1996; Kakkar *et al*. 2012; Palmer *et al*., 1992) which were available in sub-montane (1200m – 1600m a.s.l) and montane (1500-1799m a.s.l) zones at both Taita Hills and Mount Kilimanjaro agro-ecosystems. However, distribution patterns of *Frankliniella schultzei* along altitudinal gradient of Taita Hills and Mount Kilimanjaro may shift drastically in future with changing climate and associated agricultural activities.

V. CONCLUSION

The common blossom thrips (*Frankliniella schultzei*) is major flower pest of avocado crop and the insect species is well established along altitudinal gradient of Taita Hills and Mount Kilimanjaro. In order to enhance productivity of the avocado trees, control measures for the pest should be focused in all altitudinal zones at both transects. However, applied research is required to develop a universal protocol for indexing floral infestation levels by *Frankliniella schultzei* in East African avocado orchards. Regional studies that integrate biogeographical approaches to predict shifts in distribution and exact pest status of *Frankliniella schultzei* as a function of future climate change are highly recommended.

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Stem Cells from a Biological Perspective in Animals: A Review

M. A. Khan, Archana Jain, J. Shakkarpude*

Department of Veterinary Physiology, College of Veterinary Science & A.H., Mhow, NDVSU (MP) 453446

Abstract**—** *Cells are the smallest living units of living system. Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide to produce more stem cells. Embryonic stem (ES) cells are the inner cell mass of a blastocyst, an early-stage embryo. (*Thomson et al., 1998). *ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. Adult stem cells, also called somatic stem cells, they are stem cells which maintain and repair the tissue in which they are found (*Behrens et al., *2014). Pluripotent adult stem cells are rare and generally small in number, but they can be found in umbilical cord blood and other tissues. Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues. Cell potency is a cell's ability to differentiate into other cell types. Stem cells resembling totipotent blastomeres from 2-cell stage embryos can arise spontaneously in the embryonic stem cell cultures. (Macfarlan et al., 2012). New research related to multipotent cells suggests that multipotent cells may be capable of conversion into unrelated cell types. In one case, fibroblasts were converted into functional neurons. Stem-cell therapy is the use of stem cells to treat or prevent a disease or condition.*

Keywords— Stem cell, Types, Identification, Properties, Cell potency, Stem cell therapy, Medical use.

I. INTRODUCTION

Stem cells are a pervasive component of embryonic and foetal development, tissue maintenance and of regeneration and repair. Stem cells are potential source of new cells for the therapeutic regeneration of diseased or damaged tissue. Embryonic Stem cells are pluripotent and have retained their embryonic capacity to give rise to most, probably all, cell types. The potential advantage of ES cells is their ability to be isolated and grown in large numbers coupled with their ability to differentiate into any other cell of the body. Tissue transplantation carries with it the risk of immune reaction and rejection unless the donor cells are closely immunologically matched or (ideally) identical to those of the patient. One seductively enticing scenario would be to provide a source of ES cells, genetically identical to the patient from which that required specialised pre- cursor cells might be differentiated. This would involve "de-differentiation" of a cell from the patient to an ES state. The only method so far known to work in animal model experiments is nuclear transplantation into an oocyte and establishment of the ES cell line from the early embryo formed. Such somatic cell nuclear transfer (SCNT) technique could be used to generate a patient specific ES cell line. This scenario of generating an embryo by nuclear transfer of an adult cell nucleus into an enucleated oocyte, making an ES cell line and using this to generate cells which will repopulate tissues of an adult, has been fully demonstrated in a mouse model.

Stem Cell

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to produce more stem cells. They are found in multicellular organisms. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells ectoderm, endoderm and mesoderm but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

Stem cells are distinguished from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue or organ-specific cells with special functions.

History of stem cell

In the mid 1800s it was discovered that cells were basically the building blocks of life and that some cells had the ability to produce other cells. In 1968, the first bone marrow transplant was performed to successfully treat two siblings with severe combined

immunodeficiency. Other key events in stem cell research include:

1978: Stem cells were discovered in human cord blood
1981: First in vitro stem cell line developed from mice
1988: Embryonic stem cell lines created from a hamster
1995: First embryonic stem cell line derived from a primate
1997: Cloned lamb from stem cells
1997: Leukaemia origin found as haematopoietic stem cell, indicating possible proof of cancer stem cells.

Properties of all stem cells

Stem have three general properties:

They are capable of dividing and renewing themselves for long periods, they are unspecialized and they can give rise to specialized cell types.

Embryonic stem cells

Embryonic stem (ES) cells are the cells of the inner cell mass of a blastocyst, an early-stage embryo. Thomson *et al.,* (1998). ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. During embryonic development these inner cell mass cells continuously divide and become more specialized.

Embryonic stem cells grown in the laboratory

Growing cells in the laboratory is known as cell culture. Embryonic stem cells are generated by transferring cells from a pre-implantation stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as culture medium. The cells divide and spread over the surface of the dish. The inner surface of the culture dish is typically coated with mouse embryonic skin cells that have been treated so they will not divide. This coating layer of cells is called a feeder layer. The mouse cells in the bottom of the culture dish provide the cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium. Researchers have devised ways to grow embryonic stem cells without mouse feeder cells. This is a significant scientific advance because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.

The process of generating an embryonic stem cell line is somewhat inefficient, so lines are not produced each time cells from the pre-implantation-stage embryo are placed into a culture dish. However, if the plated cells survive, divide, and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of re-plating or sub-culturing the cells is repeated many times and for many months. Each cycle of sub-culturing the cells is referred to as a passage. Once the cell line is established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

Laboratory tests are used to identify embryonic stem cells

However, laboratories that grow human embryonic stem cell lines use several kinds of tests, including:

1. Growing and sub-culturing the stem cells for many months. This ensures that the cells are capable of long-term growth and self- renewal.
2. Using specific techniques to determine the presence of transcription factors that are typically produced by undifferentiated cells. Two of the most important transcription factors are Nanog and Oct 4.
3. Using specific techniques to determine the presence of particular cell surface markers that are typically produced by undifferentiated cells.
4. Examining the chromosomes under a microscope. This is a method to assess whether the chromosomes are damaged or if the number of chromosomes has changed. It does not detect genetic mutations in the cells.
5. Determining whether the cells can be re-grown, or sub-cultured, after freezing, thawing, and re-plating.
6. Testing whether the human embryonic stem cells are pluripotent by allowing the cells to differentiate spontaneously in cell culture, manipulating the cells so they will differentiate to form cells characteristic of the three germ layers and injecting the cells into a mouse with a suppressed immune system to test for the formation of a benign tumor called a teratoma.

Adult stem cells

Adult stem cells, also called somatic stem cells, they are stem cells which maintain and repair the tissue in which they are found. Pluripotent adult stem cells are rare and generally small in number, but they can be found in umbilical cord blood and other tissues. (Ratajczak *et al.,* 2007). Bone marrow is a rich source of adult stem cells, which have been used in treating several conditions including liver cirrhosis, chronic limb ischemia and end stage heart failure.

Adult stem cells are also used in veterinary medicine to treat tendon and ligament injuries in horses. The history

of research on adult stem cells began about 50 years ago. In the 1950s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the types of blood cells in the body. A second population, called bone marrow stromal stem cells (also called mesenchymal stem cells, or skeletal stem cells by some) were discovered a few years later.

Identification and function of stem cells

Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (called a "stem cell niche"). In many tissues, current evidence suggests that some types of stem cells are pericytes, cells that compose the outermost layer of small blood vessels. Stem cells may remain quiescent (non-dividing) for long periods of time until they are activated by a normal need for more cells to maintain tissues, or by disease or tissue injury.

Tests are used to identify adult stem cells

Scientists often use one or more of the following methods to identify adult stem cells:

(1) Label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate.

(2) Remove the cells from a living animal, label them in cell culture, and transplant them back into another animal to determine whether the cells replace (or "repopulate") their tissue of origin.

Adult stem cell differentiation

Normal differentiation pathways of adult stem cells. In a living animal, adult stem cells are available to divide for a long period, when needed, and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue.

1. Hematopoietic stem cells:-Hematopoietic stem cell give rise to all the types of blood cells.
2. Mesenchymal stem cells:-Mesenchymal stem cell have been reported to be present in many tissues.
3. Neural stem cells:-Neural stem cells in the brain give rise to its three major cell types: nerve cells (neurons).
4. Epithelial stem cells:-Epithelial stem cell in the lining of the digestive tract occur in deep crypts and give rise to several cell types: absorptive cells, goblet cells, Paneth cells, and enteroendocrine cells.
5. Skin stem cells:-Skin stem cells occur in the basal layer of the epidermis and at the base of hair follicles.

Differences between embryonic and adult stem cells

One major difference between adult and embryonic stem cells is their different abilities in the number and type of differentiated cell types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging.

Cell Potency

Cell potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency. Potency is taken from the Latin term "potens" which means "having power."

Totipotency

Totipotency is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and zygotesare examples of totipotent cells. *Toti* comes from the Latin *totus* which means "entirely". Stem cells resembling totipotent blastomeres from 2-cell stage embryos can arise spontaneously in the embryonic stem cell cultures. (Macfarlan *et al*., 2012). and also can be induced to arise more frequently in vitro through down-regulation of the chromatin assembly activity of CAF-1.

Pluripotency

Pluripotency (from the Latin plurimus, meaning *very many*, and potens, meaning *having power*) refers to a stem cell that has the potential to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm and nervous system.

Induced pluripotency

Induced pluripotent stem cells, commonly abbreviated as iPS cells or iPSCs are a type of pluripotent stem cell artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a "forced" expression of certain genes and transcription factors. These transcription factors play a key role in determining the state of these cells and also highlight the fact that these somatic cells do preserve the same genetic information as early embryonic cells. (Stadtfeld and Hochedlinger 2010). The ability to induce cells into a pluripotent state was initially pioneered in 2006 using

mouse fibroblasts and four transcription factors, Oct4, Sox2, Klf4 and c-Myc; this technique, called reprogramming, earned Shinya Yamanaka and John Gurdon the Nobel Prize in Physiology or Medicine 2012.

Multipotency

Multipotency describes progenitor cells which have the gene activation potential to differentiate into multiple, but limited cell types. New research related to multipotent cells suggests that multipotent cells may be capable of conversion into unrelated cell types. In one case, fibroblasts were converted into functional neurons. (Vierbuchen *et al*., 2010). Multipotent cells are found in many, but not all human cell types. Multipotent cells have been found in cord blood, (Yong and Theodore 2010). Adipose tissue, cardiac cells, bone marrow, and mesenchymal stem cells (MSCs) which are found in the third molar. Hematopoietic stem cells are an example of multipotency.

Oligopotency

Oligopotency is the ability of progenitor cells to differentiate into a few cell types. It is a degree of potency. Examples of oligopotent stem cells are the lymphoid or myeloid stem cells.

Unipotency

A unipotent cell is the concept that one stem cell has the capacity to differentiate into only one cell type.

Stem cell therapy

Stem-cell therapy is the use of stem cells to treat or prevent a disease or condition: Bone marrow transplant is the most widely used stem-cell therapy, but some therapies derived from umbilical cord blood are also in use. Research is underway to develop various sources for stem cells, and to apply stem-cell treatments for neurodegenerative diseases and conditions such as diabetes, heart disease, and other conditions.

Medical uses

For over 30 years, bone marrow has been used to treat cancer patients with conditions such as leukaemia and lymphoma; this is the only form of stem-cell therapy that is widely practiced. (Karanes *et al*., 2008). Another stem-cell therapy called Prochymal, was conditionally approved in Canada in 2012 for the management of acute graft vs host disease in children who are unresponsive to steroids. It is an allogenic stem therapy based on mesenchymal stem cells (MSCs) derived from the bone marrow of adult donors.

Neuro-degeneration

Research has been conducted on the effects of stem cells on animal models of brain degeneration, such as in Parkinson's, Amyotrophic lateral sclerosis, and Alzheimer's disease. There have been preliminary studies related to multiple sclerosis.

Heart

Possible mechanisms of recovery include:
Generation of heart muscle cells, stimulation of growth of new blood vessels to repopulate damaged heart tissue, secretion of growth factors and assistance via some other mechanism.

Wound healing

Stem cells can also be used to stimulate the growth of human tissues. In an adult, wounded tissue is most often replaced by scar tissue, which is characterized in the skin by disorganized collagen structure, loss of hair follicles and irregular vascular structure.

Infertility

Culture of human embryonic stem cells in mitotically inactivated porcine ovarian fibroblasts (POF) causes differentiation into germ cells (precursor cells of oocytes and spermatozoa), as evidenced by gene expression analysis. (Richards *et al*., 2008). The stem cell, therapy may also be used in the treatment of the following: Brain and spinal cord injury, Blood cell formation, Missing teeth, Cochlear hair cell re-growth, Blindness and vision impairment, Pancreatic beta cell, Orthopaedics etc.

Veterinary medicine

Research currently conducted on horses, dogs, and cats can benefit the development of stem cell treatments in veterinary medicine and can target a wide range of injuries and diseases such as myocardial infarction, stroke, tendon and ligament damage, osteoarthritis, osteoc hondrosis and muscular dystrophy both in large animals, as well as humans. (Murphy *et al.*, 2003).

Sources of stem cells

Veterinary applications of stem cell therapy as a means of tissue regeneration have been largely shaped by research that began with the use of adult-derived mesenchymal stem cells to treat animals with injuries or defects affecting bone, cartilage, ligaments and/or tendons. There are two main categories of stem cells used for treatments: allogeneic stem cells derived from a genetically different donor within the same species and autologous mesenchymal stem cells, derived from the patient prior to use in various treatments. A third category, xenogenic stem cells, or stem cells derived from

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different species, are used primarily for research purposes, especially for human treatments.

Stem cells and hard-tissue repair

Because of the general positive healing capabilities of stem cells, they have gained interest for the treatment of cutaneous wounds. In one trial, stem cells were isolated from the Wharton's jelly of the umbilical cord. These cells were injected directly into the wounds. Within a week, full re-epithelialization of the wounds had occurred, compared to minor re-epithelialization in the control wounds. This showed the capabilities of mesenchymal stem cells in the repair of epidermal tissues.

Stem cells and orthopaedic repairs

Logous stem cell-based treatments for ligament injury, tendon injury, osteoarthritis, osteocho ndrosis, and sub-Auto-chondral bone cysts have been commercially available to practicing veterinarians to treat horses since 2003 in the United States and since 2006 in the United Kingdom. Autologous stem cell based treatments for tendon injury, ligament injury, and osteoarthritis in dogs have been available to veterinarians in the United States since 2005. Over 3000 privately owned horses and dogs have been treated with autologous adipose-derived stem cells. The efficacy of these treatments has been shown in double-blind clinical trials for dogs with osteoarthritis of the hip and elbow and horses with tendon damage. (Nixon *et al.*, 2008).

Tendon repair

The embryonic stem cells were shown to have a better survival rate in the tendon as well as better migrating capabilities to reach all areas of damaged tendon. The overall repair quality was also higher, with better tendon architecture and collagen formed.

Joint repair

Horses and dogs are most frequently affected arthritis. Adipose-derived mesenchymal cells are currently the most often used because of the non-invasive harvesting. There has been a lot of success recently injecting mesenchymal stem cells directly into the joint. This is a recently developed, non-invasive technique developed for easier clinical use. Dogs receiving this treatment showed greater flexibility in their joints and less pain. (Guercio *et al.*, 2012).

Bone defect repair

Stem cells have been used to treat degenerative bone diseases. The normally recommended treatment for dogs that have Legg Calve Perthes disease. Recently, mesenchymal stem cells have been injected directly in to the head of the femur, with success not only in bone regeneration, but also in pain reduction.

Stem cells and muscle repairs

Stem cells have successfully been used to ameliorate healing in the heart after myocardial infarction in dogs. Adipose and bone marrow derived stem cells were removed and induced to a cardiac cell fate before being injected into the heart. The heart was found to have improved contractility and a reduction in the damaged area four weeks after the stem cells were applied.

Stem cells and nervous system repairs

Spinal cord injuries are one of the most common traumas brought into veterinary hospitals. Mesenchymal stem cells that are induced to a neural cell fate are loaded on to a porous scaffold and are then implanted at the site of injury. The cells and scaffold secrete factors that counteract those secreted by scar forming cells and promote neural regeneration. Eight weeks later, dogs treated with stem cells showed immense improvement over those treated with conventional therapies. Dogs treated with stem cells were able to occasionally support their own weight, which has not been seen in dogs undergoing conventional therapies. (Sung Su Park *et al.*, 2012).

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Effects of Electromagnetic Fields on the Bacterial Load of Waste Water Samples from Selected Industries in Akure Metropolis

Adetuyi F. C.[1],Boboye B.[1], Balogun O. B.[2]

Department of Microbiology Federal University of Technology, AkureOndo State, Nigeria.
Department of Microbiology Joseph Ayo babalola University, Ikeji-arakejiOsun State, Nigeria.

***Abstract*—** *Wastewater is any water that has been adversely affected in quality by anthropogenic influence which can serve as habitat for pathogenic microbes and can constitute to health hazard of the populace. The present study was designed to enumerate and identify microorganisms in wastewaters and to investigate the effect of Electromagnetic Field (EMF) on the populations and identities of bacteria in the wastewaters from selected industries in Akure Metropolis. Wastewater samples were collected from two different industries in Akure Metropolis. The waste water samples were subjected to microbiological analyses before and after exposure to Electromagnetic field (EMF) at 1150nT, 1310nT, 3000nT, 5000nT. The presence of some bacteria in the waste water collected from different companies showed their occurrence at different hours during the treatment of the wastewater sample with different EMF strength. It was observed that at the early part (hours) of the experiment the heavy presence of microbes were seen but as the experiment progresses the microbial population were observed been reduced. It is therefore recommended that wastewater from industries should be treated with EMF before discharging them to the other water bodies so as to avoid contamination. This will help reduce microbial population that constitute a serious hazard to public health. And could also help protect other life forms inhabiting the water body and thus guard against ecological imbalance of the microbiota.*

Keywords*— *Wastewater, Electromagnetic field, Microorganisms, bacteriological analysis.

I. INTRODUCTION

Wastewater: Is any water that has been adversely affected in quality by anthropogenic influence. Is one of the most critical problems of developing countries is improper management of vast amount of wastes Wastewater can originate from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water, and from sewer inflow or infiltration (7). Municipal wastewater (also called sewage) is usually conveyed in a combined sewer or sanitary sewer, and treated at a wastewater treatment plant. Treated wastewater is discharged into receiving water via an effluent pipe (10). Wastewaters generated from industries and there should be an on-site treatment unit. The management of wastewater belongs to the overarching term sanitation, just like the management of human excreta, solid waste and stormwater (drainage)(9).

Water of good drinking quality is of basic importance to human physiology as well as indispensable to man's continued existence. It serve as a medium of water borne disease which constitutes a significant percentage of the diseases that affect human and animals cannot be underestimated. This is the most important concern about the quality of water. Guideline for bacteriological water differs from country to country but they all conform to WHO recommendation (22). The standards for drinking water are more stringent than those for recreational waters. Investigations of how magnetic and electric fields affect living organisms at the molecular level have revealed impacts on the biological functions of organisms via changes in the concentration of hormones, activity of enzymes, transport of ions by the cell membrane or changes in the synthesis or transcription of DNA (13)

Natural water is never absolutely pure, as it carries traces of other substances which bestow on it physical, chemical and bacteriological characteristics. The nature and amount of these substances called impurities vary with sources of the water. Although, most of the water on earth is not accessible, the surface water, which is the most accessible, represents only about 0.02% of the total water resources (2). In Nigeria Akure metropolis precisely there is limited knowledge on wastewater treatment technology for small-

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scale wastewater such as cassava power plant and some water industry. Moreover, a greater population of Nigerians lack knowledge concerning the reusability potentials of treated wastewater. The waste generated from both small and large scale industry therefore does not go through any segregation and treatment Polluted water has been the cause of all such cases, in which the major sources of pollution are domestic and municipal wastes from urban and industrial activities, runoff from farmland, etc. (14). Most countries of the world now have water resources management policies aimed at achieving sustainable use Preliminary studies have suggested that the application of electric and electromagnetic fields are potentially useful methods of non thermal decontamination (9).The menace of water-borne diseases and epidemics still looms large on the horizon of developing countries as a result of lack of accessibility to good quality water (13). Chemicals that have been used to inhibit the microorganisms can cause deteriorating effects on aquatic microbiota and humans (22). Better alternative that does not have adverse effect is by the use of Electromagnetic Field.The aim of this study was to assess and to isolate and enumerate microorganisms associate with waste water, characterize microorganisms associated with wastewater from some industries in Akure. Metropolis, investigate the effects of electromagnetic field on wastewater microorganism.

II. MATERIALS AND METHODS

2.1 Study Area

Akure is situated at 7.25° North latitude, 5.19° East longitude and 396 meters elevation above the sea level. Akure is a big town in Nigeria, having about 420,594 inhabitants.Owena which is located in the suburb of Owena town in Ifedore Local Government Area of Ondo-State, between latitude 7.15° N, longitude 5.0°E.

2.2 Collection of wastewater samples

Wastewater sample were collected at septic tank of different companies using sterile container and transported to the laboratory for experiment.

2.3 Characterization and Identification of Bacterial Isolates

The isolates were characterized using colonial and cellular morphology as well as biochemical reactions as described in Benson's microbiological application (5). They were subsequently identified using cowan and steels manual for identification of bacteria (17).

2.4 Serial dilution

The wastewater sample was prepared using serial dilution. 1.0ml of waste water sample was taken into 9ml sterilized distilledwater in a test tube to form a stock solution. The solution was mixed vigorously and 1ml was taken with the aid of sterile syringe into 9ml of sterilized water in another test tube under aseptic condition. These were repeated until 8th dilution factor from which 0.5ml was taken into a sterile plate and then pour plate. The inoculated nutrient agar was incubated at at 37°C for 24hours.

2.5Determination of the effects of EMF on bacterial load of waste waters.

An electric circuit that generated the electromagnetic field wave used for this research work was designed and constructed at the Department of Physics, Federal University of Technology, Akure, Nigeria. The electromagnetic field pulse was generated from solenoid coil of hundreds of turns of copper wire. The coil was connected across a voltage source of which induced magnetic field around the coil. 1ml inoculums was taken and introduced into 9 ml freshly prepared test tubes which was serially dilutedtubes. Then, the conical system were treated with electromagnetic field generated at different EMF strength respectively. Treatment of waste water with EMF: (A= 1150nT, B= 1310nT, C=3000nT, D=5000nT) and sampling was done before exposure and at four (4) hours interval until 24 hours and 24 hours interval until 168 hours.

III. RESULTS

3.1 Occurrence of the bacteria isolate

Table 1 to 8 below shows the occurrence of the bacteria isolated from wastewater from different food companies in South West, Nigeria. The different companies where the wastewater samples were collected were named industry A and B. In the table below, the suspected organisms, the various time in which the wastewater was exposed to different EMF strengths were shown. The time of exposure and the EMF strength affects the population of the isolates, different isolates had different reaction to these paramount and ponderous factor. The lowest EMF strength (1150nT) had the least effect on all the isolates while the highest EMF strength

Table 1 shows the effects ofemf (1150nT) on the occurrence of the bacteria on waste water from industry A from 0 hours to 144 hours i.e (7days) whichhad the lowest emf strength (nT).

Table 2 shows the effects ofemf (1310nT) on the occurrence of the bacteria in waste water from industry A from 0 hours

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to 144 hours which had higher effect on microbial load than the emf strength with (1150nT).

Table 3 shows the effects of EMF (3000nT) on the occurrence of the bacteria in waste water from industry A from 0 hours to 144 hours whichhad higher effect on microbial load than the emf strength with (1310nT).

Table 4 shows the effects of EMF (5000nT) on the occurrence of the bacteria in waste water from industry A from 0 hours to 144 hours which had higher effect on microbial load than the previous emf strength.

Table 5 shows the effects of EMF (1150nT) on the occurrence of the bacteria in waste water from industry B from 0 hours to 144 hours whichhad the lowest emf strength (nT).

Table 6 shows the effects of EMF (1310nT) on the occurrence of the bacteria in waste water from industry A from 0 hours to 144 hours whichhad higher effect on microbial load than the emf strength with (1150nT).

Table 7 shows the effects of EMF (3000nT) on the occurrence of the bacteria in waste water from industry A from 0 hours to 144 hours whichhad higher effect on microbial load than the emf strength with (1310nT).

Table 8 shows the effects of emf (5000nT) on the occurrence of the bacteria in waste water from industry A from 0 hours to 144 hours whichhad higher effect on microbial load than the previous EMF strength.

3.2 Bacteria load of the wastewaters

Figure 1 and 2 shows the microbial loads of wastewater samples from different industrial site that was subjected to different EMF strength (A= 1150nT, B= 1310nT, C=3000nT, D=5000nT) at various time. The values of the bacteria colony count was counted and recorded. Sample collected at industry A had the highest mean values with EMF strength 1150nT, after exposure to EMF at lowest hour at hours were 230cfu/ml, 225cfu/ml and 220cfu/ml respectively, while the lowest value with EMF strength at 5000nT were 0cfu/ml, 2cfu/ml, 4cfu/ml, respectively. For sample collected at industry B had the highest values with EMF strength 1150nT, after exposure to EMF at lowest hour at 4 hours were 179cfu/ml, 176cfu/ml and 173cfu/ml respectively, while the lowest value with EMF strength at 5000nT were 1cfu/ml, 3cfu/ml, 5cfu/ml, respectively.

T*ABLE 1: Microganisms isolated from industry A wastewater*

ISOLATE CODE	PROBABLE MICROORGANISM
IAI	*Enterobacter aerogenes*
IA2	*Klebsiella oxytoca*
IA3	*Bacillus subtilis*
IA4	*Proteus vulgaris*
IA5	*Staphylococcus aureus*
IA6	*Bacillus cereus*
IA7	*Staphylococcus saprophyticus*
IA8	*Lactococcus lactis*
IA9	*Salmonella typhi*
IA10	*Enterococcus faecalis*
IA11	*Micrococcus luteus*
IA12	*Pseudomonas aeruginosa*
IA13	*Escherichia coli*

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Table.2: Effects of EMF (1510nT) on the occurrence of the bacteria on waste water from industry A.

	TIME (hr)												
Isolate code	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IA1	+	+	+	+	+	+	+	-	-	-	-	-	-
IA2	+	+	+	+	+	+	+	+	+	-	-	-	-
IA3	+	+	+	+	+	+	+	+	-	-	-	-	-
IA4	+	+	+	-	-	-	-	-	-	-	-	-	-
IA5	+	+	+	+	+	+	+	+	+	-	-	-	-
IA6	+	+	+	+	+	+	+	+	+	+	-	-	-
IA7	+	+	+	+	+	+	+	+	+	-	-	-	-
IA8	+	+	+	+	+	+	+	+	+	+	-	-	-
IA9	+	+	+	+	+	+	+	+	-	-	-	-	-
IA10	+	+	+	-	-	-	-	-	-	-	-	-	-
1A11	+	+	-	-	-	-	-	-	-	-	-	-	-
IA12	+	+	+	+	+	+	+	+	+	+	+	-	-
IA13	+	+	+	+	+	+	+	+	+	+	+	-	-

Table.3: Effects of emf (1310nT) on the occurrence of the bacteria on waste water from industry A.

	TIME (hr)												
Isolate code	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IA1	+	+	+	+	+	+	+	-	-	-	-	-	-
IA2	+	+	+	+	+	+	+	+	+	-	-	-	-
IA3	+	+	+	+	+	+	+	-	-	-	-	-	-

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IA4	+	+	+	-	-	-	-	-	-	-	-	-	-
IA5	+	+	+	+	+	+	+	+	+	-	-	-	-
IA6	+	+	+	+	+	+	+	+	+	-	-	-	-
IA7	+	+	+	+	+	+	+	-	+	-	-	-	-
IA8	+	+	+	+	+	+	+	+	+	-	-	-	-
IA9	+	+	+	+	+	+	+	+	-	-	-	-	-
IA10	+	+	+	-	-	-	-	-	-	-	-	-	-
1A11	+	+	-	-	-	-	-	-	-	-	-	-	-
IA12	+	+	+	+	+	+	+	+	+	+	-	-	-
IA13	+	+	+	+	+	+	+	+	+	+	-	-	-

Legend: (+) Present, (-) Absent

IA1-IA13=First Isolate –Thirteen Isolate A13

Table.4: Effects of EMF (3000nT) on the occurrence of the bacteria on waste water from industry A.

	TIME (hr)												
Isolate code	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IA1	+	+	+	+	+	+	+	-	-	-	-	-	-
IA2	+	+	+	+	+	+	+	+	+	-	-	-	-
IA3	+	+	+	+	+	+	+	-	-	-	-	-	-
IA4	+	+	+	-	-	-	-	-	-	-	-	-	-
IA5	+	+	+	+	+	+	+	+	+	-	-	-	-
IA6	+	+	+	+	+	+	+	+	+	-	-	-	-
IA7	+	+	+	+	+	+	+	+	+	-	-	-	-
IA8	+	+	+	+	+	+	+	+	+	-	-	-	-
IA9	+	+	+	+	+	+	+	+	-	-	-	-	-
IA10	+	+	+	-	-	-	-	-	-	-	-	-	-
1A11	+	+	-	-	-	-	-	-	-	-	-	-	-

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IA12	+	+	+	+	+	+	+	+	+	-	-	-	-
IA13	+	+	+	+	+	+	+	+	+	-	-	-	-

Legend: (+) Present, (-) Absent

IA1-IA13=First Isolate –Thirteen Isolate A1

Table.4: Effects of EMF (5000nT) on the occurrence of the bacteria on waste water from industry A.

Isolate code	**TIME (hr) 0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IA1	+	+	+	+	+	+	+	-	-	-	-	-	-
IA2	+	+	+	+	+	+	+	+	-	-	-	-	-
IA3	+	+	+	+	+	+	-	-	-	-	-	-	-
IA4	+	+	+	-	-	-	-	-	-	-	-	-	-
IA5	+	+	+	+	+	+	+	-	-	-	-	-	-
IA6	+	+	+	+	+	+	+	-	-	-	-	-	-
IA7	+	+	+	+	+	+	+	-	-	-	-	-	-
IA8	+	+	+	+	+	+	+	-	-	-	-	-	-
IA9	+	+	+	+	+	+	+	-	-	-	-	-	-
IA1	+	+	+	-	-	-	-	-	-	-	-	-	-
1A11	+	+	-	-	-	-	-	-	-	-	-	-	-
IA12	+	+	+	+	+	+	+	+	-	-	-	-	-
IA13	+	+	+	+	+	+	+	+	-	-	-	-	-

Legend: (+) Present, (-) Absent
IA1-IA13=First Isolate –Thirteen Isolate A13

TABLE 6 :Microganisms isolated from industry B wastewater

ISOLATE CODE	PROBABLE MICROORGANISM
IAI	*Micrococcus luteus*
IA2	*Lactococcus lactis*
IA3	*Listeria* spp
IA4	*Escherichia coli*
IA5	*Erwinia caratova*
IA6	*Proteus vulgaris*
IA7	*Staphylococcus saprophyticus*
IA8	*Enterobacter aerogenes*
IA9	*Bacillus subtilis*
IA10	*Pseudomonas aeruginosa*
IA11	*Salmonella typhi*

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Table.7: Effects Of EMF(1150nT) On The Occurrence Of The Bacteria On Waste Water From Industry B.

	TIME (Hr)												
Isolate code	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IB1	+	+	-	-	-	-	-	-	-	-	-	-	-
IB2	+	+	+	+	+	+	+	+	+	+	-	-	-
IB3	+	+	+	+	+	+	+	+	+	+	-	-	-
IB4	+	+	+	+	+	+	+	+	+	+	-	-	-
IB5	+	+	+	+	+	+	-	-	-	-	-	-	-
IB6	+	+	+	-	-	-	-	-	-	-	-	-	-
IB7	+	+	+	+	+	+	+	-	-	-	-	-	-
IB8	+	+	+	+	+	+	+	-	-	-	-	-	-
IB9	+	+	+	+	+	+	+	+	+	+	-	-	-
IB10	+	+	+	+	+	+	+	+	+	+	+	-	-
IB11	+	+	+	+	+	+	+	+	-	-	-	-	-

Legend: (+) Present, (-) Absent

IA1-IA13=First Isolate –Thirteen Isolate A13

Table.8:effects of EMF (1310nT) on the occurrence of the bacteria on waste water from industry B.

	TIME (Hr)												
Isolate code	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IB1	+	+	-	-	-	-	-	-	-	-	-	-	-
IB2	+	+	+	+	+	+	+	+	+	-	-	-	-
IB3	+	+	+	+	+	+	+	+	+	-	-	-	-
IB4	+	+	+	+	+	+	+	+	+	+	-	-	-

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IB5	+	+	+	+	+	+	-	-	-	-	-	-	-
IB6	+	+	+	-	-	-	-	-	-	-	-	-	-
IB7	+	+	+	+	+	+	+	-	-	-	-	-	-
IB8	+	+	+	+	+	+	+	+	-	-	-	-	-
IB9	+	+	+	+	+	+	+	+	+	-	-	-	-
IB10	+	+	+	+	+	+	+	+	+	+	-	-	-
IB11	+	+	+	+	+	+	+	+	-	-	-	-	-

Legend: (+) Present, (-) Absent

IA1-IA13=First Isolate –Thirteen Isolate A13

Table. 9: Effects of EMF (3000nT) on the occurrence of the bacteria on waste water from industry A

	TIME (Hr)												
Isolate code	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IB1	+	+	-	-	-	-	-	-	-	-	-	-	-
IB2	+	+	+	+	+	+	+	+	-	-	-	-	-
IB3	+	+	+	+	+	+	+	+	-	-	-	-	-
IB4	+	+	+	+	+	+	+	+	+	-	-	-	-
IB5	+	+	+	+	+	+	-	-	-	-	-	-	-
IB6	+	+	+	-	-	-	-	-	-	-	-	-	-
IB7	+	+	+	+	+	+	+	-	-	-	-	-	-
IB8	+	+	+	+	+	+	-	-	-	-	-	-	-
IB9	+	+	+	+	+	+	+	+	-	-	-	-	-
IB10	+	+	+	+	+	+	+	+	+	-	-	-	-
IB11	+	+	+	+	+	+	+	+	-	-	-	-	-

Legend: (+) Present, (-) Absent

IA1-IA13=First Isolate –Thirteen Isolate A13

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Table.10: Effects of EMF (5000nT) on the occurrence of the bacteria on waste water from industry B.

	TIME (Hr)												
Isolate code	**0**	**4**	**8**	**12**	**16**	**20**	**24**	**48**	**72**	**96**	**120**	**144**	**168**
IB1	+	+	-	-	-	-	-	-	-	-	-	-	-
IB2	+	+	+	+	+	+	+	-	-	-	-	-	-
IB3	+	+	+	+	+	+	+	-	-	-	-	-	-
IB4	+	+	+	+	+	+	+	+	-	-	-	-	-
IB5	+	+	+	+	+	+	-	-	-	-	-	-	-
IB6	+	+	+	-	-	-	-	-	-	-	-	-	-
IB7	+	+	+	+	+	+	+	-	-	-	-	-	-
IB8	+	+	+	+	+	+	-	-	-	-	-	-	-
IB9	+	+	+	+	+	+	+	-	-	-	-	-	-
IB10	+	+	+	+	+	+	+	+	-	-	-	-	-
IB11	+	+	+	+	+	+	+	-	-	-	-	-	-

Legend: (+) Present, (-) Absent

IA1-IA13=First Isolate –Thirteen Isolate A13.

IV. DISCUSSION

The presence of some microorganisms in the wastewater collected from different companies is demonstrating their occurrence at different hours during the treatment of the wastewater sample with different EMF strength. It was observed that at the early part (hours) of the experiment the heavy presence of microbes were seen but as the experiment progresses the microbial population were reduced.The EMF had most dwinding effect on *Micrococcus luteus* because it was not visible after four hours of exposure for each EMF strength. Even some microbes (*Bacillus subtillis, Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella oxytoca*) were not visible at 96 hours of exposure for three EMF strength(1150nT, 1310nT and 3000nT) and it was not visible after seventy two hours of exposure for the last EMF strength (5000nT). (*Enterococcus faecalis, Proteus vulgaris*)were no longer visible after twelve hours for all EMF strength (1150nT, 1310nT, 3000nT and 5000nT) similar to the findings of (12). *Enterobacter aerogenes* after fourty eight hours for all EMF strength (1150nT, 1310nT, 3000nT and 5000nT).*Bacillus cereus* and *Lactococcus latis* was no longer visible after visible after One hundred and twenty hours of exposure, while at (1310nT and 3000nT) EMF strength it was no longer visible after ninty six hours of exposure, then at (5000nT).EMF strength it was no longer visible after twenty four hours of exposure. *Salmonella typhi* was no longer visible after seventy two hours of exposure for all the EMF strength (1150nT, 1310nT, 3000nT and 5000nT). (*Esherichia coli* and *Pseudomonas*

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aeruginosa) was not visible after one hundred and forty four (144) hours of exposure at 1150nT, it was no longer visible, while at 1310nT it was no longer apparent after. Industry B had lesser number of microbes than industry A is consistent to the finding of (13). The EMF had most dwinding and receeding effect on *Micrococcus luteus* because it was not visible after Eight hours of exposure for each EMF strength(1150nT, 1310nT, 3000nT and 5000nT). *Esherichia coli* and *Pseudomonas aeruginosa* was observed after 120 hours of the wastewater sample with EMF treatment and. For industry B *Proteus vulgaris* is no longer visible after twelve hours at all the EMF strength (1150nT, 1310nT, 3000nT and 5,000nT) which is in contrast to what of (6) found where certain bacteria responds positively well in terms of increased growth rate and activities to electromagnetic field treatment. .*Enterobacter aerogenes* and *Salmonella typhi* is no longer visible after forty eight hours from the wastewater collected from industry B after the treatment of the wastewater sample, *Lactococcus lactis*, *Listeria spp*, *Bacillus subtilis* is no longer visible after one hundred and twenty hours with EMF treatment at 1150nT, while at 1310nT it was not visible after exposure to ninty six hours, then after exposure to seventy two hours it was not visible at 3000nT and after exposure to seventy two hours it was not visible at 5000nT and other microbes were observed but in reduced population which is in contrast to what of (12) found where certain bacteria responds positively well in terms of increased growth rate and activities to electromagnetic field treatment. .*Klebsiella oxytoca*, *Staphylococcus saprophyticus*, *Staphylococcus aureus* was no longer visible after seventy two hours of exposure at 5000nT which also correlate to work done by (22) he allot that exposure of some bacterial cells to electromagnetic fields causes inhibition of the growth, reproduction and activities of such microorganisms. .*Erwinia caratova* was inhibited during the course of the experiment. It was observed that the EMF treatment has a significant impact on the microbes present in the wastewater collected from different industries because it was observed that has the wastewater is been exposed to the different EMF for a longer period of time the microbial population were seen been reduced to the lowest minimum even some were not longer present in the sample before the completion of the experiment it is in agreement with the findings of (10), One of the probable explanations of the effect of electromagnetic field on the bacterial isolates might be due to the denaturing effect of EMF on the metabolites resulting from microbial activity. It could also be as a result of the rotating electric field formed by the variable magnetic field. The pulsed-electric field treatment, enhanced the bactericidal action (11), who found that electromagnetic field treatments significantly reduced the growth and proliferation of bacteria.

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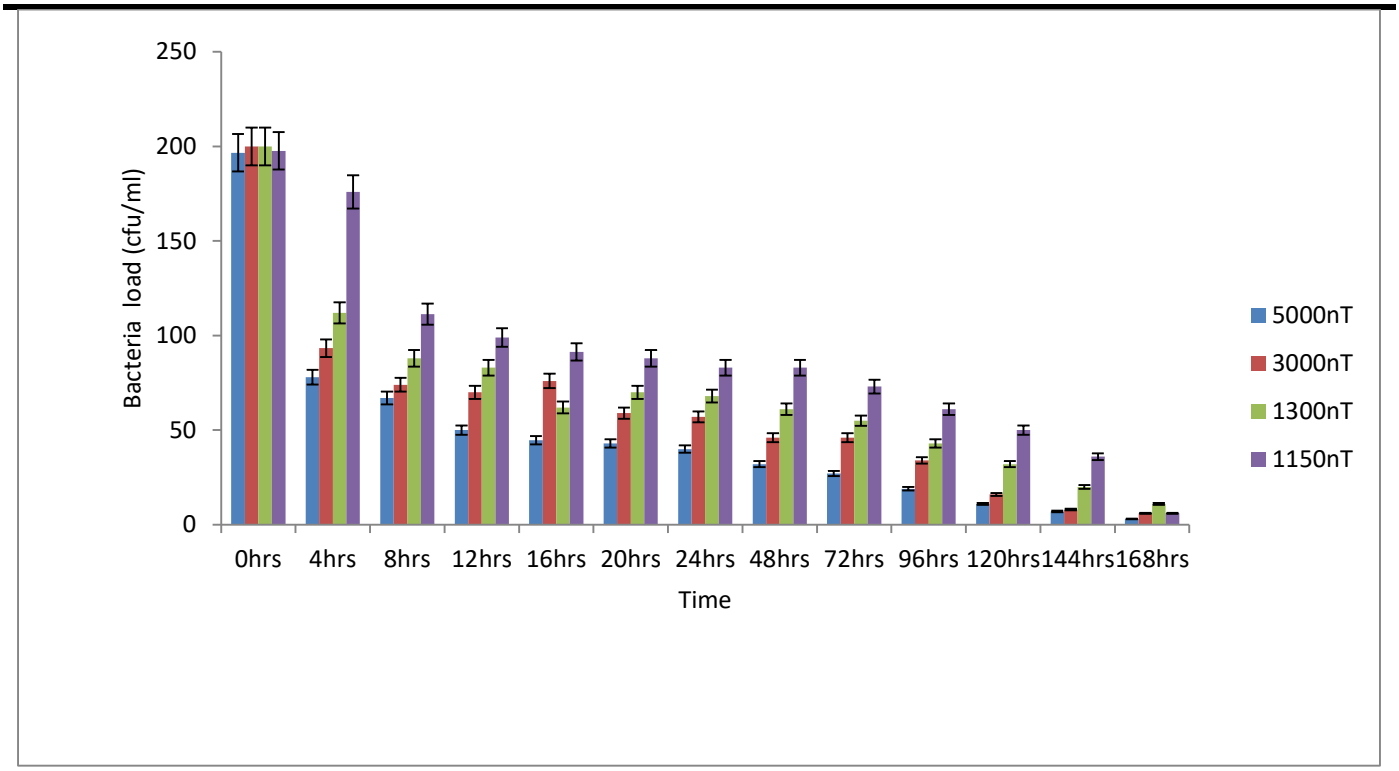
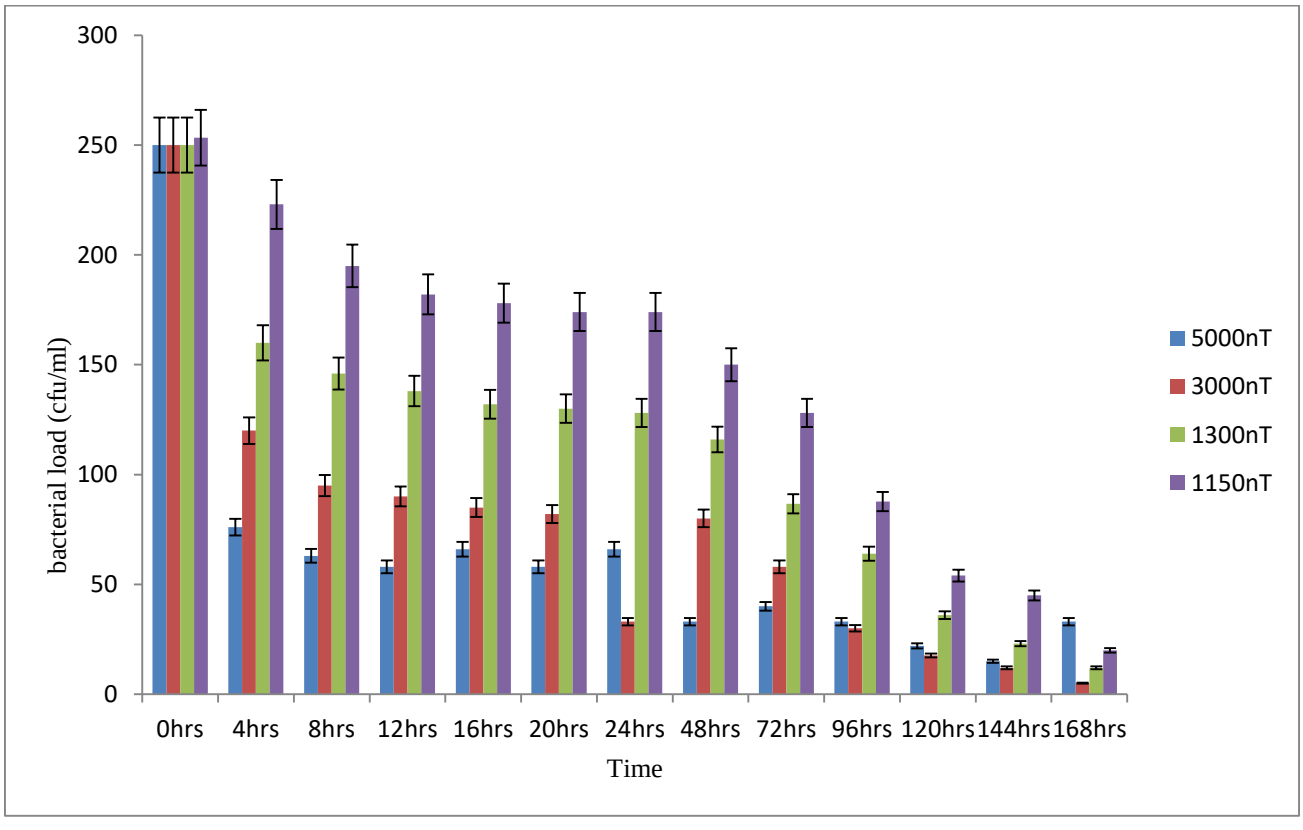


Fig.1:Effect of EMF on Bacteria Loads in Waste Water fromIndustry A



Error bars ± 1 SE

Fig.2: Effect of EMF on Bacteria Loads In Waste Water From Industry B.

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The results of this investigation also revealed that increased duration of exposure of bacteria to electromagnetic field resulted in the reduction in the population of the bacterial isolates as there was a considerable decrease in the colony count. The period of exposure of microbial cells to electromagnetic field plays an important role in the production of the biological effect and this can cause desirable changes in them. Human activities such as pollution also affect the activity of microbes in water and this can lead to Eutrophication, consequently have a negative effect on the relationship that exist between the microbial groups.

V CONCLUSION

The EMF treatments reduced the microbial population as well as the rate of contamination in the wastewater samples as the exposure time increased and it also inhibit growth and proliferation of microorganisms thus making it to have both bacteriostatic and bacteriocidal effect.

VI. RECOMMENDATION

It is therefore recommended that wastewater from industries should be treated with a better alternative mean by the use of electromagnetic field before discharging them to the other water bodies so as to avoid contamination. This will help reduce microbial population that constitute a serious hazard to public health. EMF treatments could also help protect other life forms inhabiting the water body and thus guard against ecological imbalance of the microbiota.

Plate 1: Electromagnetic field (EMF) apparatus set up

Plate 2: Setup of EMF with wastewater inside the conical flasks during the experiment

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Response of Irrigated Groundnut to Polythene Mulching on Broad Bed and Furrows during the Low Temperature Months in Nigeria

Hakeem Ayinde Ajeigbe[1], Babu Nagabushan Motagi[1,2], Shiyanbola Abiodun Abdulsalam[3]

[1]International Crops Research Institute for the Semi-Arid Tropics, Kano, Nigeria
[2]Present address: Department of Genetics and Plant Breeding, UAS, Dharwad, Inda
[3]Department of Agronomy, Bayero University Kano

***Abstract*—** *Experiments were conductedduring 2014-2015 and 2015-2016 dry seasons to evaluate the response of selected groundnut varieties to Polythene Mulching (PM) onBroad Bed and Furrows (BBF)in the Sudan Savanna of Nigeria.The treatments consisted of Polythene mulch vs without mulch (control) and four groundnut varieties,laid outin Split plot design with four replications. The result showed that polythene mulch positively and significantly influenced the phenological and physiological variables as well as the yield and yield component of groundnut.Plot withPM emergence at mean of 8 days earlier and attained days to 50% flowering and maturity 11 and 10 days earlier than the control.Polythene mulch had positive and significant effects on all of the phenological, growth and yield parameters (100 seed weight,Spad Chlorophyll Meter Reading, LAI and shelling percentage) of groundnut. These effects ranged from 5% advantage in Spad Chlorophyll Meter Reading at 40DAS to 29% at LAI 60DAS.Mean pod yield of the mulch treatments (3401 kgha^{-1}) was 39% higher than the control (2102 kgha^{-1}). Samnut-24 had highest pod yield of 4009 kgha^{-1}under the polythene mulch treatments. Polythene mulch also increased the haulm production by 26% over the control treatment (4775 vs 3505 kgha^{-1}). The experiment showed that it is possible to produce high groundnut pod and haulm yields using PM onBBFin the Sudan Savanna of Nigeria..*

***Keywords*—Broad Bed and Furrow, Groundnut varieties, Polythene Mulch, Temperature and Yield**

I. INTRODUCTION

Groundnut (*Arachis hypogaea.* L.) is leguminous crop that is grown in the moist and dry savanna zones of Nigeria.Groundnut is grows for its nut, oil and haulms, though the yield is low because of the several environmental factors especially moisture and temperature (Ravindra *et al*., 1990; Karim, 1990 and Ntare *et al*., 2001), weed management and diseases. The average yields of groundnut in Nigeria and most parts of West Africa are lower (903 kg ha^{-1}) than those in South Africa (2000 kg ha^{-1}), Asia (1798 kg ha^{-1}), or the rest of the world (1447 kg ha^{-1}) (FAOSTAT, 2015).Mulching is the practices of covering the soil to make more favorable conditions for plant growth, development and efficient production. It plays a paramount role for conserving the moisture in the soil profile for the success of groundnut production, which totally depends on the precipitation received before and during crop growth period. The practice of mulching has been widely used as a management tool in many parts of the world. It dampens the influence of environmental factors on soil by increasing soil temperature controlling diurnal/seasonal fluctuations in soil temperature (Yang *et al*., 2006; Lalitha *et al*., 2001). However, the effect varies with soils, climate and kind of mulch material used and the rate of application. Singh(1994) and Lalitha *et al*., (2001) stated that variation of the soil microclimate by mulching favors seedling emergence and root proliferations and suppress weed population. The surface mulch favorably influences the soil moisture regime by controlling evaporation from the soil surface, improves infiltration, soil water retention, decreases bulk density and facilitates condensation of soil water at night due to temperature reversals (Yang *et al*., 2006; Pawar *et al*, 2004).

Broad Bed Furrow (BBF) is a method of cultivation by which farmers used to increase the density of groundnut population. Purcell *et al*. (2002) and Ball *et al*., (2000) reported that increasing plant density increased LAI and light inception of soybean which significantly increased soybean production, this is in agreement with findings of Ajeigbe *et al*, (2016) whoreported that increasing density in groundnut led to increase in high leaf area index (LAI) and the fraction of intercepted photosynthetic active radiation and high pod yield. Broad Bed and Furrow have been practiced in some parts of China and India for many

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decades to increase their groundnut productivity particularly during post-rainy season.

Many researchers (Sun *et al*., 2015 and Malekar *et al*., 2011) conducted researches to determine the effect of polythene mulch on groundnut production. These studies showed that the application of mulch increased pod yield of groundnut in comparison withcontrol groundnut. The major reason for mulch raising groundnut yield are soil and water conservation, improved soil physical and chemical properties and enhanced soil biological activity (Yang et al., 2006). ICRISAT and it National partners in Nigeria are encouraging the cultivation of groundnut for seed, grain and fodder under irrigation in the Sudan savanna zone during the dry season. During this period temperature is generally low compared during the beginning of the dry season in Nigeria With the advent of this technology, the core objective of the present studies was to investigate the response of irrigated groundnut varieties to polythene mulch during the low temperature months in the Sudan savanna of Nigeria.

II. MATERIALS AND METHODS

2.1 Description of the Experimental Site

Theirrigated experiments were conducted at ICRISAT experimental station in Wasai situated at (Latitude 12.14^{0}N and Longitude 08.67^{0}E with an elevation of 441 above sea level), Minjibir local government area of Kano State, Nigeria during 2014-2015 and 2015-2016 dry seasons (Late December when the temperature was cold). The study area has a Semi-arid climate with mean annual rainfall of 862.8mm in 2014 and 564.8mm in 2015 with peak rain around August for the both years. The minimum and maximum mean temperature during the experiment in 2014–2015 and 2015–2016 are given in figure (1). The soil texture is loamy sandy with pH of 6.54 and organic carbon of 0.219.

2.2 Field management and Experimental Design

The soil was ploughed two times to ensured removal of some noxious weeds and stumps that might cause damages to polythene mulch and planked to get a fine seedbed. After the seedbed preparation, pre-emergence herbicide (Pendimenthaline@3Lltrs/ha) was sprayed followed by application of basal fertilizer Single Super Phosphate (SSP) @100kg/ha and Gypsum at@400kg/ha prior to sowing, the seeds of groundnut cultivars were treated with Apron star @10g/4kg of seed and sown immediately.

The experiment was a Split plot designed with four replications. The mulching (white polythene mulch or control) was the main plot while the groundnut varieties (Samnut 23, Samnut 24, Samnut 26 and Ex-Dakar) were the sub-plot. Each plot consisted of 4 beds, each single bed measured in 4m length by 1m wide alternated with 0.25m furrow (20m^2).To effect the mulching treatment, 7 microns white polythene mulch having holes of 20 × 20cmhexagonal spacing was spread on the broad bed prior sowing. While the control (without) plots were conducted on the bare BBF. Sowing was done on 24th of December 2014 repeated same date in 2015 by dibbling 2 seeds per hole at 5cm depth. Care was taken to ensure uniform depth of planting.Irrigation was administeredtwice a week for the first two weeks and there after it was observed once a week. Plots were regularly observed for good agronomic control (weeding where necessary) during the life period of the crop.Harvesting was done when thegroundnut varieties attained their physiological maturity.

2.3 Sampling and Measurement

Observations on plant growth were measured at 40, 60 and 80 DAS. Parameters such as SPAD chlorophyll meter reading (SCMR) and Leaf area index (LAI) leaf area index was also measured with the Ceptometer (AccuPAR PAR/LAI Ceptometer Model LP-80), at 12:00h noon prior irrigation.Fully expanded third tetrafoliate leaf from the apex of the main axis on all the five sampled plants was used to record SCMR. Care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and the interference from veins and midribs was avoided.

Germination percentage was recorded at 8, 15 and 30 DAS while the destructive sampling was carried out at 15days interval from 30 DASuntilharvest. For biomass fresh weight, 5 plants were randomly selected in 2 border rows of each plot, the root system having soil particles were thoroughly cleaned, weighed on electronic digital balance with precision of 0.1g and kept in the paper bags. The Samples were oven-dried for 48 hours at 70^{0}C and the final weight was taken. Data was recorded on plot basis for days to first flowering, 50% flowering(number of days from sowing to when at least 50% of the plants had begun flowering), pod yield and haulm yield and converted to kg ha^{-1}using conversion factor. Groundnut was harvested from the net plot avoiding the border rows when at least 80% of the plant has attained their physiological maturity (Days to maturity), pods were stripped and air-dried for the determination of the yield and its components. Weight of dry haulms after 1 week of air-drying was recorded. Pods were shelled and 100-g matured pods were used to estimate 100-seed weight (g) and shelling percentage. GENSTAT 17th Edition was used to analyze for Split plot design and means were compared by using Least Significant Difference (LSD) test. Data recorded on different parameters were subjected to Analysis of variance (ANOVA) techniques to find out the different between the treatments and their interactions.

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III. RESULTS

Figure1 shows theaverage monthly minimum and maximum temperature during the experimental period. The average monthly minimum temperature ranged from 12.3ºC at planting to 25ºC at harvest while average monthly maximum temperatures ranged from 27ºC at planting to 41ºC at harvest. Planting was done during the cold months and harvest done during the hot months.

The means square from the analysis of variance (ANOVA) for germination percentage at 8, 15 and 30 DAS, days tofirst, 50% flowering and physiological maturity are presented in Table (1a).Significant differences were observed between the years for germination percentage at 30 DAS, days to first and 50% flowering. Polythene mulchand varieties (V) have significant effect on germination percentage at 8, 15 and 30 DAS, days to first and 50% flowering and days to physiological maturity, though the varieties did not differ for germination percentage at 8 DAS. The Year (Y) × Mulch (M)interaction was significant for germination percentage at8 and 15 DAS, days first and 50% flowering. The Y×V interaction was significant for germination percentage at15 and 30 DAS, days to first and 50% flowering and days to physiological maturity.While no significant M×V interaction were foundat all the germination stages, significant M×V interaction were found for days to first flowering, 50% flowering and days to physiological maturity. The Y×M×Vinteraction was significantonly for days to flowering.

Table 1b, shows the analysis of variance of combined mean squaresfor the yields and yield attributes. Polythene mulch and groundnut varieties had significant effect on pod and haulm yields as well as on 100 seed weight and shelling percentage. Y×M interaction was significant for haulm yield(kgha^{-1}) and 100 seed weight, while M×Vand Y×M×V interactions were significant for pod yield.

Table 1c, shows the combined mean squares for analysis of variance of physiological parameters. Year and groundnut varieties have significant effect only on LAI at 40 DAS. Polythene mulch had significant effect on chlorophyll content at 40, 60 and 80 DAS, and LAI at 40 and 60 DAS. Significant Y×M interactions were observed for chlorophyll content at 40, 60 and 80 DAS, and LAI at 40, while significant M×V and Y×M×V interactions were observed forchlorophyll content.

Table 1d shows the combined mean squares for analysis of variance of dry matter weight from 30 DAS to harvest. Significant differences were observed in dry matter weight at 30, 60, and 75 DAS and non-significant at 45, 90, 105, 120 DAS and harvest between the year of experiment conducted. Polythene mulch significantly influenced the dry matter weight from 30 DAS to harvest. Variety showed significant differences in all intervals except for 120 DAS and at harvest. All interactions were not significant except for dry matter weight at 60 DAS in Y×V and 30 DAS in M×V.

The effect of mulch on selected phenology, growth and yield characters of irrigated groundnut in the cool dry season in the Sudan savanna of Nigeria is given in table 2. Mulched plots recorded higher mean % germination at 8, 15 and 30 DAS (27, 51, 70% respectively) than control plots (0, 25 and 63% respectively). Mulched plots recorded higher mean days to first and 50% flowering and physiological maturity (31, 35, 134% respectively) than control plots (38, 46 and 144% days respectively). Mean pod (3401 kg/ha) and haulm (4775 kg/ha) yields, 100 seed weight (36 g) as well as shelling percentage (72%) of the mulched plots were significantly higher than the control plots (2102 kg/ha, 3505 kg/ha, 34gand 67% respectively). Also mean SCMR at 40 (37.9), 60 (44.35) 80DAS (50.03), mean LAI at 40 (1.32), and 60 (3.3) were significantly higher than control plots. However mean LAI at 80 DAS (4.43) on mulched plot was not significantly higher than mean LAI at 80 DAS (4.08) of control plots.The SPAD Chlorophyl Meter Reading (SCMR) was higher in polythene mulch treatment than controlat all the observation stages (Table. 2).

Table 3. Shows the interaction between polythene mulch and variety treatments on pod yield (kgha^{-1}). A significant interaction was observed for pod yields. Though all varieties positively responded to polythene mulch, the extent of response varies significantly. Samnut 24 produced the highest pod yields (4009 and 2261 kgha^{-1} mulched and control respectively), while Ex-Dakar produced the lowest pod yields (2906 kgha^{-1}) under mulched condition though Samnut 23 produced the lowest pod yields (1986.8kgha^{-1}) in the control. The polythene mulch increases pod yield increase by 30% in Ex-Dakar to 44%.

Fig. 2. Illustrate the effect of polythene mulch on dry matter weight of groundnut. The comparative percentage values between the mulched area and control for the dry matter weight were 67%, 50%, 45% 59%, 60%, 63%, 67% and 69% at 30 DAS to harvest respectively. The maximum dry matter weight was still achieved at the harvest.

IV. DISCUSSIONS

The first germination was observed at 8 DAS in mulch treatment as compared to control (without mulch) at 15 DAS indicating 7 daysdifference. The earlier germination under mulched condition (8 days) could be attributed to prevailing higher soil temperature as a result of heat entrapment by the polythene mulch, and moisture conservationby the polythene filmcompared to the cold

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stress by lower diurnal range of soil temperature experienced by the control causing the seeds to emerge in 15 days. At 30DAS, germination percentage was also discovered to show some disparity in phenological development (onset flowering R1 and pegging R2)between the mulched and control. Temperature is one of the key benefits of mulching in groundnut production. It was reported that soil temperature lower than 18^0C reduces germination and crop growth and temperature higher than 37^0C during pod development restricts pod and kernel growth resulting in lower pod yield (Reddy *et al*., 2003). After 30 days of planting, the plants under mulched treatment flowered earlier before control due to initial temperature stress experienced by plants in controlplot. Hence, given the groundnuts under mulched better chance for yield increment.

The results revealed in the current study indicated that days to first and 50% flowering and maturity were significantly lower (7, 11 and 10 days respectively) under polythene mulching indicating that the groundnut did not recover from late germination and flowering. The response of varieties under mulched treatment matured earlier due to higher photosynthetic rate on account of high mean soil temperature, sufficient moisture, less competition with weeds, functional microbial activities, and undisturbed soil structure coupled with nutrients availability beneath the mulch thereby shortens the crop duration. Polythene mulch had positive and significant effects on all of the phenological, growth and yield parameters (100 seed weight,Spad Clorophyl Meter Reading, LAI and shelling percentage) of groundnut. These effects ranged from 5% advantage in Spad Clorophyl Meter Reading at 40DAS to 29% at LAI 60DAS.Disparity in dry matter accumulation could be due to differences in the germination percentage, leaf area production and leaf area index. Bolaji *et al*., (2015) reported that LAI showed positive correlation with the dry matter accumulation. Likewise decrease in weed competition for limited resources in treatments that had higher dry matter weight and sufficient moisture under mulched which continued to act as substrate for other biochemical reaction which might have stimulated stronger carbohydrates sinks via photosynthesis. Zagade *et al*., (2006) also observed higher dry matter weight in treatments, where effects of polythene mulch, moisture regimes and plant densities were practiced on groundnut in Ratnagiri.Greater SCMR in treatments showed that the effect of mulching could include higher photosynthetic rate on account of sufficient moisture, light, and adequate nutrients uptake. Singh (2004) observed that photosynthetic rate of leaves in groundnut reduces as relative water content and water potential decreases.

Mean pod yield of the mulch treatments (3401 $kgha^{-1}$)was 39% higher than the control (2102 $kgha^{-1}$). Similar findings were reported by Hu, *et al*., (1995) and also in agreement with Zagade *et al*., (2006). The mean pod yields(2752 $kgha^{-1}$) obtained in this trial is much higher than mean yield (2067 $kgha^{-1}$) obtained in same location by Ajeigbe *et al*., (2016)under a population of 133,333 hill ha^{-1}. However the mean yields of the polythene mulched plots (3401 $kgha^{-1}$) was 40% higher the mean yield obtained by Ajeigbe *et al*., (2016). These differences can be attributed to the higher population in the polythene mulch trial (250,000hill ha^{-1}). It is also in agreement with Ajeigbe *et al*., (2016) who recommended higher plant population for groundnut production in the Sudan savanna zone of Nigeria. Groundnut haulm is a very important commercial product of dry season groundnut cultivations. The haulm comes at the peak of dry season when fodder cost is also at its peak. The groundnut haulms is therefore as important as pod to the farmers overall productivity. Polythene mulch increased the haulm production by 26% over the control treatment (4775 vs 3505 $kgha^{-1}$). This is a significant increase in the income of farmers as well as important in the crop-livestock integration continuum. Among the tremendous challenges facing Sub Saharan Africa agriculture is the need to generate a sustainable food and feed supply to match the expected high demand without destroying the natural resource base. Technologies like the polythene mulch and broad bed cultivation of groundnut in the dry season is a good option not only to increase production of quality legume fodder for livestock but to also break the cereal-cereal (rice-wheat) cycles normally foundin the irrigated schemes and Fadama in Sudan Savanna zone of Nigeria.

V. CONCLUSION

Polythene Mulching on Broad Bed and Furrows is recommended cultivation technology for dry season production in the Sudan Savanna of Nigeria,since it increase both the pod and haulm yields. The system also have additional advantage of increased water use efficiency because of conservation of moisture by the mulch as well as reduced cost on weeding since the polythene mulch reduced weed germination and emergence. It was also noticed that even when polythene mulch is not used, the Broad Bed and Furrows offer advantage of yields over the traditional cropping pattern in the area. Samnut 24 is the highest yielding of the tested varieties and is recommended for cultivation, though Samnut 26 and Samnut 23 also produced appreciable pod and haulm yields and can be used when Samnut 24 is not available.

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Table.1a:Combined mean squares from the analysis of variance for Phenology on groundnut in 2014 and 2015 dry season at Minjibir LGA of Kano state, Nigeria.

Source of variation	d.f	Germination (%) at 8 DAS	Germination (%) at 15 DAS	Germination (%) at 30 DAS	Days to first flowering	Days to 50% flowering	Days to maturity
Replication	3	54.69	411.1	237.70	0.1875	0.7292	1.792
Year (Y)	1	1255.26^{ns}	273.0^{ns}	3595.31*	33.063**	42.250**	1.000^{ns}
Mulch (M)	1	11564.65**	10626.7**	754.10*	715.563**	1660.563**	1521.0**
Variety (V)	3	242.70^{ns}	2162.7**	2361.46**	19.271**	32.687**	24.667**
Y.M	1	1255.26*	7608.5**	153.33^{ns}	45.563**	30.250**	4.000^{ns}
Y.V	3	50.69^{ns}	1770.2**	2173.89**	1.6042*	8.458**	7.000*
M.V	3	80.90^{ns}	13.1^{ns}	40.94^{ns}	10.188**	3.020**	11.000*
Y.M.V	3	50.69^{ns}	31.7^{ns}	135.78^{ns}	2.521**	2.792**	2.000^{ns}
Residual	36	32.89	112.2	92.57	0.2847	0.3229	1.792
Total	63						

*Significant at 5% probability level, **Significant at 1% probability level, ^{ns}: Non significant

Table.1b: Combined mean squares from the analysis of variance for yield and yield attributes of groundnut in 2014 and 2015 dry season at Minjibir LGA of Kano state, Nigeria

Source of variation	d.f	Pod yield (kgha^{-1})	Haulm yield (kgha^{-1})	100 Seed weight (g)	Shelling percentage (%)
Replication	3	454445.0	310282.0	1.658	13.422
Year (Y)	1	138337.0^{ns}	470510.0^{ns}	27.040*	26.904^{ns}
Mulch (M)	1	26997045.0**	25803225.0**	60.062*	435.227*
Variety (V)	3	1234611.0**	4629208**	199.720**	32.176*
Y.M	1	121974.0^{ns}	3451467*	31.360*	1.327^{ns}
Y.V	3	2300.16^{ns}	421982^{ns}	0.971^{ns}	13.239^{ns}
M.V	3	534924.0*	224358^{ns}	2.500^{ns}	12.612^{ns}
Y.M.V	3	323886.0*	52288^{ns}	1.445^{ns}	7.281^{ns}
Residual	36	104142	203847	4.474	5.429
Total	63				

*Significant at 5% probability level, **Significant at 1% probability level, ^{ns}: Non significant

*Table.1c: Combined mean squares from the analysis of variance for SCMR and LAI at 40, 60, and 80 DAS of groundnut varieties in 2014 and 2015 dry season at Wasai, Minjibir LGA of Kano state, Nigeri***a.**

Source of variation	d.f	SCMR at 40 DAS	SCMR at 60 DAS	SCMR at 80 DAS	LAI at 40 DAS	LAI at 60 DAS	LAI at 80 DAS
Replication	3	13.617	11.860	30.00	0.01683	0.2802	1.1170
Year (Y)	1	0.375^{ns}	40.641^{ns}	14.92^{ns}	1.57816*	1.0379^{ns}	0.1089^{ns}
Mulch (M)	1	61.819*	165.122*	293.69*	1.12625*	15.0059*	1.9252^{ns}
Variety (V)	3	3.645^{ns}	10.787^{ns}	10.66^{ns}	0.24548*	0.0730^{ns}	1.7495*
Y.M	1	76.781*	329.423**	243.75*	1.08941*	1.2572^{ns}	0.7788^{ns}
Y.V	3	5.152^{ns}	7.837^{ns}	5.46^{ns}	0.22684*	0.3480^{ns}	0.0979^{ns}

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M.V	3	24.357*	7.596^{ns}	4.98^{ns}	0.01783^{ns}	1.0174^{ns}	0.6195^{ns}
Y.M.V	3	20.213*	13.964^{ns}	6.47^{ns}	0.11732^{ns}	0.2480^{ns}	0.0547^{ns}
Residual	36	5.445	9.269	13.06	0.07340	0.3769	0.3086
Total	63						

*Significant at 5% probability level, **Significant at 1% probability level, ^{ns}: Non significant, SCMR= SPAD Chlorophyll meter reading, LAI= leaf Area Index

TABLE.1d: Combined mean squares from the analysis of variance for Dry matter weight (g) from 30 DAS to harvest of groundnut varieties in 2014 and 2015 dry seasons at Wasai, Minjibir LGA of Kano state, Nigeria.

Source of variation	**d.f**	Dry matter weight (g) at 30 DAS	Dry matter weight (g) at 45 DAS	Dry matter weight (g) at 60 DAS	Dry matter weight (g) at 75 DAS	Dry matter weight (g) at 90 DAS	Dry matter weight (g) at 105 DAS	Dry matter weight (g) at 120 DAS	Dry matter weight (g) at harvest
Replication	3	0.68266	22.401	35.22	989.7	380.9	1467.0	6960.0	6522.0
Year (Y)	1	0.87891*	110.513^{ns}	5187.60*	9530.6*	10251.6^{ns}	885.0^{ns}	47579.0^{ns}	36577.0^{ns}
Mulch (M)	1	17.32641**	558.731**	4928.04*	9433.3*	40804.0*	70623.0*	150253.0*	136161.0*
Variety (V)	3	1.16516**	41.353*	329.70*	1884.1*	5622.2**	4970.0*	6226.0^{ns}	6968.0^{ns}
Y.M	1	0.15016^{ns}	0.238^{ns}	691.69^{ns}	1.3^{ns}	441.0^{ns}	856.0^{ns}	24219.0^{ns}	11556.0^{ns}
Y.V	3	0.24641^{ns}	13.636^{ns}	264.23*	330.7^{ns}	770.4^{ns}	2293.0^{ns}	3808.0^{ns}	2828.0^{ns}
M.V	3	0.58057*	8.701^{ns}	4.70^{ns}	201.9^{ns}	376.8^{ns}	239.0^{ns}	5129.0^{ns}	5503.0^{ns}
Y.M.V	3	0.06432^{ns}	8.698^{ns}	14.88^{ns}	107.8^{ns}	87.8^{ns}	807.0^{ns}	2757.0^{ns}	1001.0^{ns}
Residual	36	0.09953	7.889	50.99	287.8	526.1	1582.0	3725.0	3509.0
Total	63								

*Significant at 5% probability level, **Significant at 1% probability level, ^{ns}: Non significant

Table.2: Effect of Mulch on selected Phenology, Growth and Yield Characters of Groundnut

Treatments	**Mulched**	**Control**	**F-Probability**	**LSD**
Percentage germ (%) at 8 DAS	27	0	**<.001**	6.01
Percentage germ (%) at 15 DAS	51	25	**<.001**	5.55
Percentage germ (%) at 30 DAS	70	63	**0.005**	3.86
Days to first flower	31	38	**<.001**	0.197
Days to 50% flower	35	46	**<.001**	0.3
Days to maturity	134	144	**<.001**	0.819
100 Seed weight (g)	36	34	**0.003**	0.977
Shelling percentage (%)	72	67	**0.001**	2.26
SCMR at 40 DAS	37.9	35.9	**0.042**	1.87
SCMR at 60 DAS	44.35	41.13	**0.005**	1.845
SCMR at 80 DAS	50.03	45.74	**0.021**	3.389
LAI at 40 DAS	1.32	1.05	**0.037**	0.243
LAI at 60 DAS	3.3	2.33	**0.003**	0.4766
LAI at 80 DAS	4.43	4.08	**0.084**	0.411
Pod yield (kg/ha)	3401	2102	**<.001**	125.88
Haulm yield (kg/ha)	4775	3505	**<.001**	400.6

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Table.3: Interaction effect of PM and variety on maturity and pod yield (kg/ha)

Variety	Pod yield (kg/ha)		
	Mulched	Control	%Reduction
Ex-Dakar	2906	2043	30
Samnut 23	3346	1987	41
Samnut 24	4009	2261	44
Samnut 26	3343	2116	36
Mean	3401	2102	37
F-Probability	**0.005**		
LSD	**300.55**		

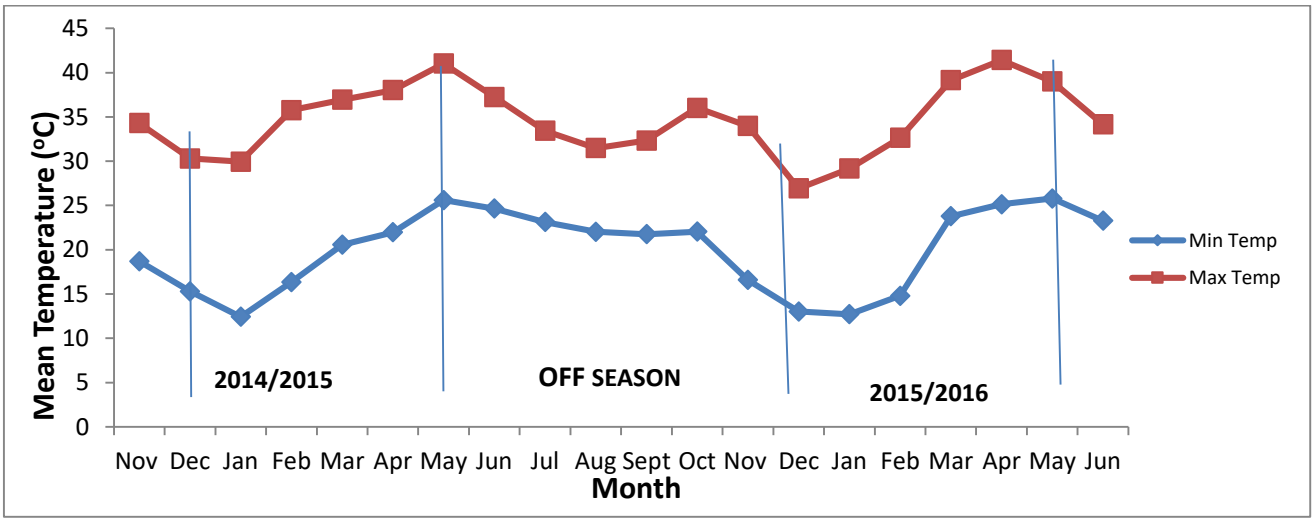

Fig.1: Monthly Minimum and Maximum Mean temperature during the experiment 2014–2015 and 2015–2016

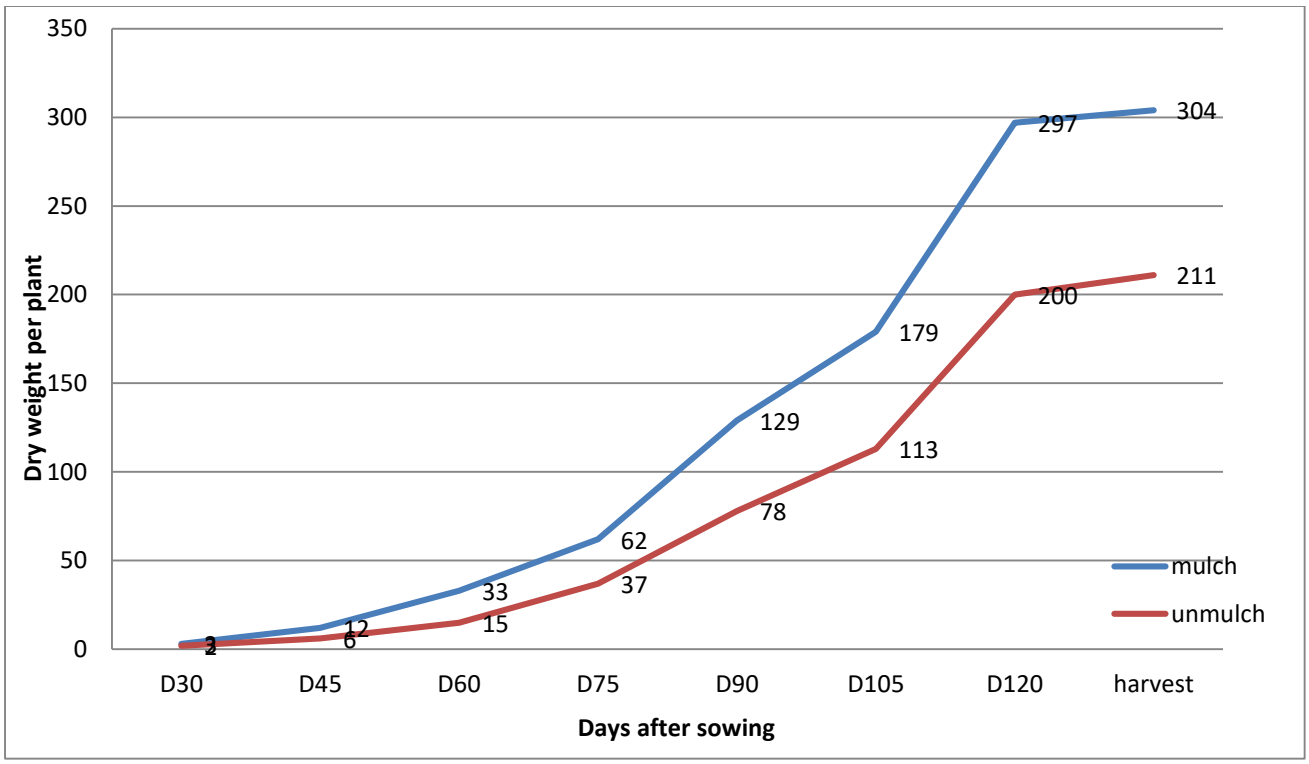

Fig.2: Effect of Polythene mulch on Dry matter weight of groundnut

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Assessment of Yield and Yield Attributing Characters of Hybrid Maize using Nutrient Expert® Maize Model in Eastern Terai of Nepal

Samjhana Khanal[1*], Bishal Dhakal[2], Keshav Bhusal[3], Lal Prasad Amgain[4]

[1] Institute of Agriculture and Animal Science (IAAS), Sundarbazar, Lamjung, Nepal
[2] Regional Agricultural Research Station (RARS), Bhagetada, Dipayal, Doti, Nepal
[3] Agriculture and Forestry University, Rampur, Chitwan, Nepal
[4] Institute of Agriculture and Animal Science (IAAS), Sundarbazzar, Lamjung, Nepal
*Corresponding author: samjhanakhanal04@gmail.com

Abstract— *Indiscriminate use of fertilizer and lack of site specific nutrient management technology is the main cause of low maize productivity in Nepal. Thus, field experiments on farmer's field were conducted on maize to assess the productivity at two sites of Jhapa district viz. Damak and Gauradaha using Nutrient Expert® Maize model from November 2015 to May 2016. The experiment was laid out in Randomized Completely Block Design consisting two treatments viz. NE (Nutrient Expert recommendation) and FFP (Farmer's Fertilizer Practice) with twenty replications. The result revealed significant differences in terms of grain yield, stover yield, biological yield, and yield attributing characters. NE based practices produced higher grain yield (9.22 t ha^{-1}), which was 86.6 percent higher than FFP (4.94 t ha^{-1}). Similarly, higher average cob number m^{-2} (8.2), average kernel rows cob^{-1} (14.2), average kernels number row^{-1} (589.9) and test weight (361.4 g) were recorded in NE based practice. Thus, NE based practice can be adopted for obtaining higher productivity in eastern terai region of Nepal.*

Keywords— grain yield, maize, nutrient expert.

I. INTRODUCTION

Maize (*Zea mays* L.) is the second most important cereal crop after rice in Nepal. It is used as food, feed, fodder and raw materials for industries. It is cultivated in 891,583 hectares of land with production and productivity of 2,231,517 tons and 2.5 t ha^{-1}, respectively (MoAD, 2017). It is the major food crop in the hills of Nepal and accounts about 71% of maize production of the country (MoAD, 2017). The demand of maize grain has increased, but the productivity in farm level is almost stagnant around 2-2.5 t ha^{-1} in last decade (MoAD, 2017). The farm level yield of maize (2.5 t ha^{-1}) is not satisfactory as compared to attainable yield (5.7 t ha^{-1}) in Nepal (MoAD, 2017; KC *et al.*, 2015). Indiscriminate use of fertilizer and lack of site specific nutrient management technology is the main cause of low maize productivity in Nepal. Therefore, nutrient management is always the major concern in maize for increasing production in Nepal.

Site specific nutrient management (SSNM) is a plant based approach for supplying crops with nutrients in right amount and time. It strives to enable farmers to adjust fertilizer use dynamically to make up the deficit in nutrients needs between that required by a high-yielding crop and nutrient supply from naturally occurring indigenous sources (i.e. soil, crop residues, manures and irrigation water) (Ghimire *et al.*, 2015). Based on SSNM principles, a dynamic nutrient management tool, Nutrient Expert® (NE), was developed that can generate farm-specific fertilizer recommendation for maize (Majumdar *et al.*, 2014).

Many researches concerning about SSNM has been carried out around the globe. Similarly, Nutrient Expert has been tested earlier in India (Majumdar *et al.*, 2014), Indonesia and Philippines (Pampolino *et al.*, 2014) and found valid. But, In Nepal, limited research has been carried out concerning about SSNM and Nutrient Expert. Therefore, the present investigation is planned, executed and accomplished with the objective of assessing yield and yield attributing characters of maize using Nutrient Expert®-Maize.

II. MATERIALS AND METHODS

The study was carried out at two sites of Jhapa district viz. Damak and Gauradaha from November 2015 to May 2016. The experiment was laid out in single factorial Randomized Completely Block Design consisting two treatments *viz.* NE (Nutrient Expert recommendation) and FFP (Farmer's Fertilizer Practice) in twenty farmer's field, considering one farmer as one replication. The gross plot and net plot size for each treatment was maintained 100 m^2 and 10 m^2, respectively. The NE plot consist the cultivation of maize under Nutrient Expert- Maize

recommended spacing, seed rate, fertilizer dose and other factors of production. FFP plot consist of maize cultivation under farmer's own practice of spacing, seed rate, fertilizer dose and other factors of production. Data of observations on yield attributing characters, grain yield and stover yield were recorded from net plot. These recorded data were tabulated in MS-Excel which was subjected to ANOVA (Gomez and Gomez, 1984), after analysis through GENSTAT-C, computer based program at 5% significance level. The grain yield was adjusted at 14% moisture level.

III. RESULTS AND DISCUSSION

3.1 Grain yield

The grain yield of maize was highly influenced by nutrient management practices (Table 1). The grain yield of maize under Nutrient Expert (NE) (9.22 t ha^{-1}) was highly significant than grain yield of maize under farmer's fertilizer practice (FFP) (4.94 t ha^{-1}). The significant increase in yield attributing characters under NE (Table 2) might be mainly responsible for obtaining the higher grain yield of maize under NE. The increase in grain yield of maize under SSNM based practices and NE was also reported in previous experiments (Kumar *et al.*, 2014; Majumdhar *et al.*, 2014; Pampolino *et al.*, 2014; Chauhan, 2015; Kumar *et al.*, 2015a; Vikram *et al.*, 2015; Sinha, 2016). Further, it was revealed that NE produced 86.6% more grain yield than farmer's fertilizer practice. Similar results were also reported by previous researchers in their studies (Kumar *et al.*, 2015b; Pooniya *et al.*, 2015; Sinha, 2016).

Table.1: Grain yield and stover yield of maize as affected by nutrient management practices at Damak and Gauradaha, Jhapa, Nepal, 2015/16

Treatment	Grain Yield (t ha^{-1})	Stover Yield (t ha^{-1})	Biological yield (t ha^{-1})
NE	9.22	12.70	21.92
FFP	4.94	8.62	13.55
SEm (±)	0.14	0.24	0.28
LSD $_{(0.05)}$	0.413	0.699	0.827
P-value	<.001	<.001	<.001
CV (%)	8.8	9.9	7.0
Grand Mean	7.08	10.66	17.74

The higher yields in NE may be ascribed to efficient adjustments in applying nutrients to accommodate field specific needs of the crops for supplementing plant nutrients (Pooniya *et al.*, 2015). The increased availability of nutrients at critical physiological phases results in better translocation of photosynthates from source to sink, resulting better growth and yield attributing characters, and finally increasing the grain yield (Vikram *et al.*, 2015). Similarly, broadcasting of seed in FFP had caused patchy growth of crop, characterized by improper spacing. This led to increased incidence of insect, pest and diseases in FFP, which also led to reduced grain yield.

3.2 Stover and biological yields

The stover yield was highly influenced by nutrient management practices (Table 1). The stover yield under NE was found to be 12.7 t ha^{-1}, which was highly significant than stover yield under farmer's practice (8.62 t ha^{-1}). Inadequate supply of nutrients in farmer's practice might have led to reduced plant height, leaf area, etc. due to improper growth and development, which in turn results the lower stover yield of maize. Higher stover yield of maize under SSNM based practice was also agreed by earlier experiments (Kumar et al., 2015a; Kumar et al., 2015b; Vikram et al., 2015).

Similarly, the biological yield of maize under NE practice (21.92 t ha^{-1}) was significantly higher than farmer's practice (13.55 t ha^{-1}). The higher biological yield under NE practice was due to dynamic adjustment of fertilizer application rates based on crop requirement. Further, the judicious nutrient management under NE based nutrient management practice has led to the higher grain, stover and biological yield over farmer's practice of nutrient management and has clearly indicated its benefit. Higher biological yield under SSNM based practice was also reported by Kumar et al. (2015b).

3.3 Yield attributing characters

The result showed that yield attributing characters viz. average plant number per m^2, average cob number per m^2, average kernel row per cob, average kernel number per row, average kernel number per cob and test weight were highly influenced by nutrient management practices (Table 2). The average plant number per m^2 (7.6), average cob number per m^2 (8.2), average kernel row per cob (14.2), average kernel number per row (42.4), average kernel number per cob (589.9) and test weight (361.4 g) under NE practice was found to be highly significant than the farmer's fertilizer practice. Optimum plant population was found under NE due to recommendation from nutrient expert with proper spacing, whereas lower plant population in FFP was due to improper spacing and seed rate. The higher cob number per m^2 in NE practice was due to higher number of plants per m^2. The difference in kernel number in row and cob under NE and FFP, although there is no difference in cob length (Table 2), suggest us that there was better translocation and assimilation of photosynthates from source to sink in NE practice. Further, it suggests us that lower kernel number in row and cob in FFP might be due to incomplete grain filling in the rows and cob under farmer's fertilizer

practice. Similar results were also obtained by various researchers in their experiments (Kumar *et al.*, 2014; Chauhan, 2015; Kumar *et al.*, 2015a; Vikram *et al.*, 2015 and Sinha, 2016).

Table.2: Yield attributes of maize as affected by nutrient management practices at Damak and Gauradaha, Jhapa, Nepal, 2015/16

Treatment	Avg. Plant no. m^{-2}	Avg. Cob no. m^{-2}	Avg. Kernel row cob^{-1}	Avg. Kernels no. row^{-1}	Avg. Kernels no. cob^{-1}	Test Weight (g)	Avg. Cob length (cm)
NE	7.6	8.2	14.2	42.4	589.9	361.4	18.1
FFP	5.5	5.8	13.4	38.6	502.4	310.4	17.3
SEm (±)	0.15	0.15	0.13	0.54	10.58	4.15	0.71
LSD $_{(0.05)}$	0.431	0.446	0.378	1.601	31.310	12.270	ns
P-value	<.001	<.001	<.001	<.001	<.001	<.001	0.433
CV (%)	10	9.6	4.1	6	8.7	5.5	18
Grand Mean	6.51	7.01	13.82	40.47	546.1	335.9	17.67

IV. CONCLUSION

Indiscriminate use of fertilizer and lack of site specific nutrient management technology is the main cause of low maize productivity in Nepal. Therefore, nutrient management is always the major concern in maize for increasing production in Nepal. The productivity of maize was increased under NE based nutrient management practice. Thus, NE based practice can be adopted for obtaining higher productivity in eastern terai region and similar agro-climatic condition of Nepal.

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Aproveitamento Do Nitrogênio Da Adubação Verde Na Produção De Hortaliças

Adielle Rodrigues da Silva

Doutoranda em Genética e Biologia Molecular pela Universidade Estadual de Santa Cruz; Mestra em Ciências Agrárias pela Universidade Federal do Recôncavo da Bahia; Engenheira Agrônoma pela Universidade Estadual do Sudoeste da Bahia; Especialização em Meio Ambiente e Sustentabilidade pela Universidade Cândido Mendes.

Resumo— *A produção de hortaliças é uma atividade agrícola de grande importância econômica para a geração de renda para os produtores, e uma parceira indispensável em propostas de políticas públicas que visem o desenvolvimento sustentável e humano. Dessa forma, busca-se por sistemas de produção orgânica que utilizam técnicas agroecológicas que contribuem com a fertilidade do solo e proporcionem a produção de alimentos saudáveis, a exemplo da adubação verde que utiliza espécies fixadoras de nitrogênio. Sendo assim, o presente artigo pretende analisar trabalhos sobre o aproveitamento de nitrogênio da adubação verde, por meio de uma revisão de dados coletados na literatura cientifica especializada. Para isso, o estudo partiu de uma revisão bibliográfica exploratória para o levantamento do conceito de aproveitamento de nitrogênio, para possibilitar o embasamento necessário para a compreensão do uso da adubação verde no cultivo de hortaliças, visando à segurança alimentar e a sustentabilidade ambiental. Dessa forma, os principais dados levantados mostram as contribuições da adubação verde no cultivo de hortaliças, apontando que há possibilidades da utilização de sistemas sustentáveis, evidenciando os efeitos da incorporação de biomassa provenientes de espécies fixadoras de nitrogênio na fertilidade do solo e na produtividade de hortaliças cultivadas de forma orgânica. Assim, o uso de adubos verdes pode ser uma alternativa para reduzir a aplicação da quantidade de fertilizantes minerais sintéticos e devolver nutrientes retirados do solo. Apesar, de ainda ser um desafio implantar e manter essa prática, uma vez que o sistema convencional ainda vem sendo bastante utilizado por muitos produtores.*

Palavras-chave—Produção orgânica, Sustentabilidade, Fertilidade do solo.

Nitrogen Adequacy of Green Fertilizer in the Production of Vegetables

Adielle Rodrigues da Silva

PhD student in Genetics and Molecular Biology from the State University of Santa Cruz; Master in Agricultural Sciences from the Federal University of the Recôncavo of Bahia; Agronomist from the State University of Southwest of Bahia; Specialization in Environment and Sustainability at Cândido Mendes University.

Abstract— *The production of vegetables is an agricultural activity of great economic importance for the generation of income for the producers, and an indispensable partner in proposals of public policies that aim at the sustainable and human development. In this way, organic production systems are used that use agroecological techniques that contribute to soil fertility and provide healthy food production, such as green manuring using nitrogen-fixing species. Therefore, the present article intends to analyze works on the nitrogen utilization of the green manure, through a review of data collected in the specialized scientific literature. For this, the study was based on an exploratory bibliographical review to survey the concept of nitrogen utilization, to provide the basis for understanding the use of green manuring in the cultivation of vegetables, aiming at food safety and environmental sustainability. Thus, the main data collected show the contributions of green manure in the cultivation of vegetables, pointing out that there are possibilities for the use of sustainable systems, evidencing the effects of incorporation of biomass from nitrogen fixing species on soil fertility and vegetable yield grown organically. Thus, the use of green manures can be an alternative to reduce the application of the amount of synthetic mineral fertilizers and to return nutrients withdrawn from the soil. Despite this, it is still a challenge to implement and maintain this practice, since the conventional system is still widely used by many producers.*

Keywords— Organicproduction, Sustainability, Soilfertility.

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I. INTRODUÇÃO

O cultivo de hortaliças é considerado como uma importante atividade econômica de geração de renda para os produtores, ou seja, uma parceira indispensável em propostas de políticas públicas que visem o desenvolvimento sustentável e humano, pois o desenvolvimento rural não se restringe somente ao desenvolvimento econômico, vai muito além, necessita ser construído socialmente.

Segundo dados da Food and Agriculture Organization of the United Nations a produção de vegetais frescos no Brasil no ano de 2013 foi de 2 milhões e 900 mil toneladas (FAOSTAT, 2016). Dentre os alimentos mais consumidos podem ser citados as hortaliças, espécies de ciclo curto que geralmente são produzidas em sistemas intensivos.

O cultivo intensivo de hortaliças é uma prática que reduz a fertilidade do solo, já que promove a perda de matéria orgânica e nutrientes, devido à aplicação de elevada quantidade de fertilizantes minerais sintéticos ou adubos químicos. Dessa forma, o uso elevado desses fertilizantes pode tornar o solo inadequado para o plantio e causar risco para o consumidor, pelo o acúmulo de nitrato e nitrito no solo e nos tecidos vegetais da planta.

Por outro lado, em sistemas de produção orgânica não se aplica fertilizantes minerais sintéticos de alta solubilidade, como por exemplo, os nitrogenados e os estercos obtidos sob manejo não orgânico. Daí a busca por sistemas de produção orgânica que visam o uso de técnicas agroecológicas que contribuem com a fertilidade do solo e proporcionem a produção de alimentos saudáveis.

Assim, temas atuais como segurança alimentar e alimentos orgânicos tem incentivado o desenvolvimento de tecnologias limpa para a produção de hortaliças. Nesse sentido, o uso de adubos verdes pode ser uma alternativa para reduzir a aplicação da quantidade de fertilizantes minerais sintéticos e devolver nutrientes retirados do solo.

As principais espécies utilizadas na adubação verde são as leguminosas com considerável capacidade de fixação biológica do nitrogênio (N). Conforme Barros Júnior et al. (2009), no bioma caatinga as espécies que se destacam com potencial para uso como adubo verde na produção de hortaliças são a jitirana (*Merremia aegyptia* L.), mata pasto (*Senna uniflora* L.) e flor-de-seda (*Calotropis procera* L.).

Segundo Garcia e Cardoso (2013), grande parte do N dos alimentos retorna ao ambiente como produtos obtidos pela a degradação biológica em estação de tratamento de esgoto ou por processos biogeoquímicos nos corpos de água, que transformam os compostos nitrogenados em amônio ou nitrato. Além dessas fixações de N sem intenção, atividades antrópicas que empregam processos de combustão fixam grandes quantidades de N de forma intencional.

Sendo assim, o presente artigo por meio de uma revisão de dados coletados na literatura científica especializada, busca investigar a viabilidade do uso do adubo verde para o aproveitamento do N, como um insumo alternativo capaz de elevar a fertilidade do solo sem agredir o meio ambiente, contribuindo com a produção orgânica e reduzindo gastos com o uso de fertilizantes minerais na produção de hortaliças.

II. METODOLOGIA

O método de pesquisa adotado nesse estudo partiu de uma revisão bibliográfica exploratória para o levantamento do conceito de aproveitamento do N, de forma a possibilitar o embasamento indispensável para a compreensão do uso da adubação verde no cultivo de hortaliças, visando à segurança alimentar e a sustentabilidade ambiental.

Assim, a pesquisa utilizada é exploratória por permitir maior caraterização do problema, buscando explicá-lo e construindo hipóteses a respeito dele. É bibliográfica que segundo Prodanov e Freitas (2013) refere-se a uma revisão realizada a partir de materiais já publicados.

Dessa forma, a pesquisa partiu de dados encontrados pela consulta ao Portal de Periódicos da Capes. A busca foi refinada as palavras chave "aproveitamento de nitrogênio e adubação verde". Diante dos resultados encontrados, realizou-se a leitura seletiva, na qual permitiu selecionar os materiais considerados de maior contribuição para o estudo. Esses trabalhos científicos foram analisados da seguinte forma: pré-análise, exploração do material e interpretação dos resultados.

Para analisar a viabilidade do uso da adubação verde foi realizado um levantamento de dados sobre trabalhos que avaliaram o uso de diferentes espécies como adubo verde, a exemplo de Alves et al. (2004) que avaliaram a produção de cenoura, beterraba e feijão-de-vagem em consócio com guandu (*Cajanus cajan*), Castro et al. (2005) que estudaram o plantio direto da berinjela na palhada de crotalária, milheto e vegetação espontânea, Oliveira et al. (2013) que utilizaram flor-de-seda no cultivo do rabanete no consórcio de beterraba e rúcula, Viola et al. (2013) que avaliaram o uso de nabo forrageiro, ervilha forrageira, ervilhaca comum e tremoço, no cultivo do trigo e Corrêa at al. (2014) que avaliaram o uso de milho e crotalária, com plantio posterior de couve-folha.

III. APROVEITAMENTO DO NITROGÊNIO DA ADUBAÇÃO VERDE

Para o crescimento e desenvolvimento das plantas é indispensável à presença de nutrientes que irão participar dos processos fisiológico e bioquímico. Sobre isso,

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Garcia e Cardoso (2013) explicam que o N é um macronutriente essencial, que deve estar sempre disponível no solo para o crescimento dos vegetais. Ainda, em ambientes naturais a ciclagem do N entre as várias espécies reativas garante a disponibilidade necessária para manter o equilíbrio do ecossistema. Isso, devido o ciclo do N possuir várias espécies gasosas e íons altamente solúveis em água que facilita a dispersão dos compostos de N no ambiente.

Dessa forma, a utilização de insumos alternativos, como os adubos verdes, pode contribuir para reposição de nutrientes ao solo, a exemplo do N, como também auxiliar na ciclagem dos nutrientes ao trazer para a superfície do solo nutrientes que estão em maior profundidade. Além de, favorecem a manutenção da matéria orgânica do solo e o "sequestro" de carbono da atmosfera, recuperando solos degradados e controlando plantas daninhas (OLIVEIRA et al., 2015).

Segundo Garcia e Cardoso (2013) o aporte de N nos cultivos agrícolas até o século XIX era oriundo de excrementos de animais e de salitre retirado de minas no deserto do Chile. Outro fertilizante utilizado e que estava quase esgotado no final do século XIX foi o guano (fezes de pássaros) que se localizavam em ilhas ao largo da costa oeste da América do Sul. Já no século XX, surgiu a síntese e produção comercial da amônia que promoveu a produção de alimentos com a aplicação de fertilizantes nitrogenados.

Entretanto, a aplicação inadequada dos fertilizantes nitrogenados causa poluição ao meio ambiente e reduz a eficiência da adubação. Isto ocorre, quando são aplicados no solo e perdidos por lixiviação ou desnitrificação (SILVA et al., 2011). Assim, a segurança alimentar tem estimulado o crescimento da demanda por alimentos orgânicos, já que estes são produzidos sem o uso de fertilizantes químicos que podem causar danos à saúde humana e comprometer o equilíbrio ambiental.

Dessa forma, Castro et al. (2005) comentam que o sistema de produção orgânica passou a ser normatizado pela legislação, com credenciamento e certificação de estabelecimentos rurais. Sendo que o desenvolvimento de sistemas de produção orgânica depende do manejo conservacionista do solo e do acúmulo de nutrientes oriundos de fontes renováveis, provenientes de resíduos orgânicos facilmente disponíveis, que sejam de origem animal e vegetal. Sobre isso, Alves et al. (2004) comentam que no sistema de produção orgânica de hortaliças, geralmente emprega-se esterco animal, composto ou outros fertilizantes orgânicos.

No entanto, Castro et al. (2005); Oliveira et al. (2013) comentam que umas das dificuldades de produzir em sistemas orgânicos é o aporte de nutrientes, principalmente o N, que em condições tropicais ocorre rápida mineralização da matéria orgânica, em função de elevadas temperaturas e umidade. Dessa forma, a adubação verde com leguminosas pode ser uma alternativa para fornecer N no período de maior exigência da espécie cultivada, auxiliar no controle de ervas espontâneas e no transporte de nutrientes de camadas mais profundas do solo, melhorando o aproveitamento de nutrientes.

Nesse sentido, Alves et al. (2004) comentam que há possibilidade de cultivar hortaliças em consórcio com outras espécies de plantas, já que algumas espécies possuem a capacidade de fixar N do ar e absorver esse nutriente de locais em que as hortaliças não conseguem acessar, acumulando o na sua biomassa. Além disto, o consórcio pode proporcionar sombra, proteger dos ventos e favorecer um microclima capaz de reduzir pragas e patógenos.

Sobre isso, Garcia e Cardoso (2013) explicam que o processo de fixação do N baseia-se na transformação do gás N_2 em uma forma que pode ser manipulável, como por exemplo, a amônia na forma líquida ou o sal NH_4NO_3. Grande parte do N fixado naturalmente é oriunda de bactérias adaptadas como as do gênero *Rhizobium* que vivem em nódulos de raízes de plantas leguminosas.

Diante disso, a adubação verde vem sendo apontada como alternativa capaz de se enquadrar no conceito de produção orgânica. Entretanto, produzir sem o uso de herbicidas e fertilizantes minerais sintéticos ainda é um desafio e, por isso, estudos têm sido realizados para avaliar a viabilidade do uso de diferentes espécies como adubo verde no cultivo de hortaliças.

IV. ADUBAÇÃO VERDE NA PRODUÇÃO ORGÂNICA DE HORTALIÇAS

Experiências sobre o uso de adubos verdes foram relatados por Oliveira et al. (2013) que constataram que a incorporação residual de 55 t de flor-de-seda ao solo, no cultivo do rabanete no consórcio de beterraba e rúcula proporcionou maior produtividade de raízes, confirmando que a adubação verde com flor-de-seda promoveu uma redução nos custos de produção, não sendo necessário uma nova adubação. Dessa forma, esse estudo mostra que há possibilidade da substituição de adubos sintéticos pelo adubo verde, uma vez que essa ação não afetou a produtividade da cultura.

Já Corrêa et al. (2014) avaliaram três cultivos para adubação verde sendo, compostos por milho, consórcio de milho com crotalária e crotalária, sob duas formas de preparo do solo, plantio direto e preparo convencional, com plantio posterior de couve-folha, observaram que a emissão de folhas da couve-folha foi superior no plantio direto, sendo 1.967.083 unidades ha^{-1}. Esses autores

explicam que provavelmente isso se deve a manutenção da palhada no solo, que permite uma mineralização mais lenta da matéria orgânica, quando comparada ao plantio convencional em que há a incorporação ao solo, conferindo a liberação de N de forma mais acentuada com a demanda das plantas.

Nesse sentido, observa-se que são necessários cuidados na escolha da espécie que será utilizada como adubo verde e também na forma como esse adubo será aplicado. Segundo Tavares Júnior et al. (2015) o feijão de porco solteiro, assim como a crotalária juncea solteira e/ou consorciada com as demais fabaceas, Mucuna preta (*Styzolobium aterrimum*), Feijão de porco (*Canavalia ensiformis*) e (*Crotalária ochroleuca*), apresentam boa adaptação para produção de adubos verdes nas condições do agreste paraibano.

Em relação ao tipo de espécie usada como adubo verde, Castro et al. (2005) verificaram que a palhada da crotalária é mais eficiente que a do milheto e da vegetação espontânea no controle da população de ervas espontâneas no plantio direto da berinjela. Além disso, observaram que os cultivos simultâneos da berinjela e crotalária ou caupi não diminuem a produtividade quando comparados ao monocultivo da hortaliça e, a berinjela responde a doses de N até 391 kg ha^{-1}, na forma de adubação suplementar de cobertura com cama de aviário, alcançando produtividade de 50,6 t ha^{-1}.

Já Alves et al. (2004) constataram efeitos benéficos do cultivo em aléias, em que é a cultura principal é cultivada entre faixas de leguminosas. Na produção de cenoura, beterraba e feijão-de-vagem em consócio com guandu (*Cajanus cajan*), a produção orgânica foi comparável ao sistema de produção convencional. Esses autores comentaram também, que nesse sistema de cultivo em aléias, podas periódicas devem ser realizadas nas leguminosas, sincronizadas com o ciclo da cultura principal.

Diante disso, a utilização de adubos verdes como adubos alternativos, permite a manutenção da matéria orgânica do solo e o "sequestro" de carbono da atmosfera, recuperando solos degradados e controlando ervas espontâneas. Uma vez que, as plantas espontâneas conhecidas como "plantas daninhas", podem causar prejuízos à cultura principal, devido à competição por nutrientes, água e luz. No entanto, estas espécies podem ser usadas para adubação verde, como cobertura do solo, produção de biomassa e ciclagem de nutrientes (OLIVEIRA et al. 2013).

Estudando a relação C/N dos adubos verdes, nabo forrageiro, ervilha forrageira, ervilhaca comum e tremoço, no cultivo do trigo em sucessão, Viola et al. (2013) verificaram que a mineralização foi superior à imobilização, elevando a disponibilização de N durante a decomposição dos resíduos vegetais. Sobre isso, Alves et al. (2004) explicam que o aproveitamento do N irá depender da sincronização entre o período de ciclagem dos nutrientes e da fase de maior absorção pela cultura, da capacidade de fixação biológica do N e dos nutrientes disponibilizados nos resíduos, e da poda da leguminosa consorciada. Além disso, a leguminosa possui menor relação C/N que favorece a mineralização e menor imobilização de N mineral (SILVA et al., 2006; SILVA et al., 2009).

Sendo assim, a adubação verde apresenta-se como um insumo alternativo. Visto que, os fertilizantes nitrogenados quando usados de forma inadequada mostram-se como possíveis poluidores do meio ambiente, porque podem ser perdidos quando aplicados no solo por lixiviação ou desnitrificação, reduzindo a eficiência da adubação (SILVA et al., 2011).

V. CONSIDERAÇÕES FINAIS

A produção orgânica e sustentável de hortaliças pode ser realizada com o uso de adubos verdes, de forma a aproveitar o N fixado por determinadas espécies, de forma a contribuir com a conservação do ambiente. A utilização dessa prática, motiva os produtores uma vez que produz alimentos mais saudáveis para a população e reduz os custos com fertilizantes minerais.

Apesar de se observar os benefícios obtidos com o uso da adubação verde no cultivo de hortaliças na literatura consultada, relacionados à reposição da fertilidade do solo e ao aumento da produtividade, o que se evidenciou foi à importância dessa atividade agrícola para o desenvolvimento sustentável.

Dessa forma, o desenvolvimento de novos estudos poderá vir a contribuir com pesquisas que buscam aprimorar o manejo e a conservação do solo, incentivando à produção de hortaliças saudáveis para uma melhor qualidade de vida e beneficiando os produtores que utilizam essa prática como geradora de renda.

Pesquisas para avaliar o aproveitamento do N por meio da adubação verde estão sendo desenvolvidas, evidenciando os efeitos da incorporação de biomassa provenientes de espécies fixadoras de N na fertilidade do solo e na produtividade de hortaliças cultivadas de forma orgânica. Entretanto, ainda é um desafio implantar e manter essa prática, uma vez que o sistema convencional ainda vem sendo bastante utilizado por muitos produtores.

Com isso, observa-se que é preciso mudanças no sistema convencional de cultivo, a fim de promover a sustentabilidade ambiental e fornecer a população alimentos com qualidade nutricional. Para isso, tornam-se necessárias políticas públicas que auxiliem os produtores a produzirem hortaliças orgânicas, em que a relação custo benefício é viável, pois um bom aproveitamento requer

produção limpa e maximização de lucro, com possibilidades de empregar novos sistemas para executar atividades agrícolas sustentáveis.

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Bioremediation of Nitro-aromatics: An Overview

N.S. Kasture

Assistant Professor, Department of Microbiology, Siddharth College, Jafrabad, Dist. Jalna (M.S.), India

Abstract**—** *Since last two three decades due to industrialization, globalization there is tremendous change in human life that means to fulfil the need various industries are flourishing. We are facing the problem of environmental pollution and also facing hazards to biodiversity. So it becomes our duties to remediate the environment by using scientific tool like bioremediation. This is emerging as an effective innovative technology for treatment of a wide variety of contaminants. Bioremediation involves various approaches like phytoremediation (plants) and rhizoremediation (plant and microbe interaction). Bioremediation is most effective technology for treatment of soil and water which are mostly contaminated by human activities. It is an economical process that means operation cost is less. In current review contamination of water and soil by nitro-aromatic compounds and the role of bacteria and fungi and their enzyme activity to enhance bioremediation process is studied by literature review. Nitro-aromatic compounds are used worldwide as explosives, pesticides and as a feedstock for the manufacture of many products, including dyes, pharmaceuticals, fungicides and plastics. On the contrary, nitro-aromatic compounds are released into the biosphere exclusively from the anthropogenic sources. Nitro-aromatic compounds do not only come from manmade sources; they also are formed by some natural processes, such as photochemical reactions in the atmosphere. Extensive production and indiscriminate application of nitro-aromatic has led to environmental pollution. Hence, nitro-aromatic compounds are recognized as Hazardous Rating-3.*

Keywords**— *Bioremediation, enzyme, nitro-aromatic compounds, phytoremediation, rhizoremediation.***

I. INTRODUCTION

One of the major environmental problems examined by the world today is the contamination of soil, water and air by toxic chemicals. Eighty billion pounds of hazardous organopollutants are produced annually and only 10% of these are disposed of safely. The estimated for their decontamination using traditional approaches such as incineration and landfilling is approximately one trillion [1]. Nitro-aromatic compounds are toxic to plant, animal and human health and poses significant health and environmental risk due to its mutagenic and carcinogen activity. Nitro-aromatic compounds are generally considered to be highly resistant to microbial degradation. The purification of wastewater contaminated with these pollutants is very difficult since they are resistant to the conventional treatment techniques. Although several investigators have used physical and chemical methods such as volatilization, photodegradation, photo-catalysis and advanced oxidation to treat the wastewater containing nitro-aromatic compounds.

1.1 Bioremediation: a brief introduction

The term bioremediation was firstly introduced by scientists in early 1980s. Bioremediation is the use of the living organism to reduce or eliminate environmental pollution by various hazardous chemicals. Bioremediation involves transformation of complex or simple chemical compounds into nonhazardous forms by microbes [2]. The past two decades have seen a tremendous upsurge in the search for cost-effective and environmentally benign alternatives to the conventional methods for remediation of hazardous wastes. The use of microbes to clean up polluted environments is a rapidly changing and escalating area of environmental biotechnology. The explanation for their remarkable range of degradative abilities is that, by the time human beings came on the scene, microbes had already coexisted for billions of years with an immense variety of pollutants. The vast diversity of potential substrates for growth led to the evolution of enzyme capable of transforming many unrelated natural pollutants by many different catalytic mechanisms. The resulting giant 'library' of microbial enzymes serves as raw material for further revolution whenever a new chemical becomes available [3].Being eco-friendly, this mode is sustainable too. The general approaches to bioremediation are basically (i) intrinsic bioremediation, (ii) biostimulation and (iii) bioaugmentation.

II. NITRO-PHENOLS: A BRIEF ACCOUNT

Nitro-phenols (NPs) are among the most important and versatile industrial organic compounds with applications as ingredients in pesticides, pharmaceuticals, pigments, dyes, and rubber chemicals [4]. Among the mono-nitrophenols, *p*-nitrophenol (PNP, also known as 4-

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nitrophnol or 4-NP), is the most common and important in terms of quantities manufactured and extent of environmental contamination. PNP is (i) used for synthesis of medicines, dyes, explosives, leather colouring, wood preservatives, and rubber chemicals [5] and (ii) generated during formulation, distribution, and field application of pesticides or photodegradation of pesticides that contain the nitro-phenol moiety [6]. Consequently, PNP has often been detected in wastewater, rivers, soils, and ground water. Most nitro-phenols, including PNP and 3-nitrophenol, enter the environment through manufacturing and processing. Nitro-phenols are toxic to plants, microorganisms, animals and humans [7, 8]. The US EPA (1980) lists *p*-nitrophenol and 2-nitrophenol as priority pollutants, and restricts their concentrations in natural waters to 10 ngL^{-1}[9]. The presence of substituted groups, i.e., nitro- and chloro-, on phenols increases the toxic effects on ecosystem and human health due to their persistence in the environment. Most of these compounds are resistant to microbial degradation, especially at high concentrations.

2.1 *p*-nitrophenol(PNP)

p-nitrophenol (PNP), a priority environmental pollutant, occurs in industrial effluents posing esthetic and health problems [10] .*p*-nitrophenol (PNP) was first registered in the United States in 1963 for use as a fungicide to control fungal mold on leather. In 1980, its application was registered for the protection of leather and military products at a concentration of 0.7% of dry leather weight. Its use was further extended for treatment of cork used in missile silo construction [11, 12]. It is primarily used as (i) solvent, (ii) commodity chemical for the synthesis of azo and sulphur dyes, (iii) explosives, (iv) number of intermediates, (v) pesticides (parathion, methyl parathion),(vi) insecticides (carbofuran, phosphalon, flurodifer), (vii) herbicides (nitrofen, bifernose), (viii) tubercutostatic 4-aminosalycilic acid, (ix) analgesic (4-acetoaminophenol/ paracetamol). PNP is manufactured on large scale (20 million Kg per annum) by many companies from Europe, USA and Japan. In 1996, environmental release of PNP as reported by USA manufacture was 45-450 MT per year. Its hazardous consequences are summarized in Table 1.

2.1.2 Toxicity

p-nitrophenol is a corrosive eye irritant (Toxicity Category I, indicating the greatest degree of acute toxicity) and a potential dermal irritant.*p*-nitrophenol is acutely toxic (Toxicity Category II) via the oral route and moderately toxic (Toxicity Category III) via the dermal route.

A subchronic oral toxicity study in rats showed an increased incidence of acute mortality, while a dermal study in mice resulted in dermal irritation and mortality. Chronic toxicity has not been conclusively evaluated. *p*-nitrophenol has been classified as Group D for carcinogenicity, indicating that there is inadequate information to determine its cancer potential. *p*-nitrophenol is not believed to cause reproductive or developmental toxicity, but additional studies are needed to confirm these tentative findings [12].

Since *p*-nitrophenol is classified as Toxicity Category I for eye irritation potential and since data on skin irritation potential are not available, the Agency is imposing risk reduction measures including use of personal protective equipment (chemical-resistant gloves and apron, and protective eyewear) as well as a long sleeved shirt, long pants, shoes and socks [12].

III. BIOLOGICAL SOLUTION TO THE POLLUTION

Nitro-aromatic compounds, now a days are recognized as high risk contaminants. Hence, their removal from environment is the first priority. Chemical approach may offer a temporary solution and may pose environment threat. On the contrary, microbial system is ubiquitous and versatile. It is well known that microbial entity plays central role in nutrient cycling on this planet [13]. Current routes for amelioration therefore, employ microorganisms which either immobilize or transform contaminants to innocuous end products. The incredible metabolic versality of microbes permits them to (i) inhabit in hostile ecological niches and (ii) exploit compounds as a source of carbon, nitrogen and energy. Such microbial metabolic potential must be harnessed to develop process (es) for detoxification of recalcitrant(s). The strategy [14] offers many advantages like

Table.1: Hazardous consequences of nitro-aromatic compounds [15]

F-hazard/Exposure	Acute hazard/Symptoms	Prevention	First Aid/Fire Fighting
1. Fire	Combustible	No open flames, no contact with oxidant.	Powder, water spray, foam, carbon dioxide.
2. Explosion	Risk of Fire and explosion on contact with acid(s), oxidant.	Deposition of dust, close system, dust	In case of fire: keep drums etc. cool by

		explosion. Proof electrical equipment and lightening.	spraying with water.
3. Exposure		Prevent dispersion of dust! Strict hygiene	
4. Inhalation	Blue lips/fingers, nails, blue skin, cough. Burning sensation, confusion, convulsion, dizziness, headache, nausea, sore throat, unconsciousness, weakness.	Local exhaust or breathing protection.	Fresh air, rest, refer for medical attention, artificial respiration may be needed.
5.Skin	May be absorbed, redness(further sea inhalation)	Protective gloves, protective clothing.	Remove contaminated clothes. Rinse and then wash skin with water and soap. Refer for medical attention.
6. Eyes	Redness, Pain.	Safety spectacles face shield or eye protection in combination with breathing protection.	First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then take to a doctor.
7. Ingestion	Abdominal pain , sore throat, vomiting (sea inhalation)	Do not eat, drink or smoke during work.	Rinse mouth, rest refer for medical attention.
8. Spoilage disposal	Packaging and labeling Sweep spilled substances into sealable container; if appropriate, moistened first to prevent dusting. Carefully collect remainder then remove to safe place. Do Not let this chemical enter the environment personal protection: P2 filter respirator for harmful particles.		

Operability in situ (ii) permanent elimination of contaminants through either biochemical transformation or cometabolic or mineralization, (iii) avoiding harsh physical and chemical treatment(s), (iv) cost effectiveness and (v) more public acceptability.

3.1 Bacteria

Microbial degradation of *p*-nitrophenol has been described for several genera including *Flavobacterium, Pseudomonas, Moraxella, Arthrobacter* and *Bacillus* [16]. *Bacillus sphaericus*, isolated from an agricultural soil by selective enrichment, transform *p*-nitrophenol [17]. A strain of *Pseudomonas putida* was found to degrade *p*-nitrophenol as a sole source of carbon, nitrogen and energy [18]. *Pseudomonas* sp and *Rhodococcusopacus* can utilize *p*-nitrophenol as a sole source of carbon and energy [19]. *Rhodococcuswratislaviensis* strain capable of utilizing *p*-nitrophenol as the sole source of carbon and energy and release the nitro group from the compound as nitrite. The nitro group of PNP enhances the resistance of the aromatic ring to biodegradation, bacterial strains able to utilize PNP as a sole carbon and nitrogen sources include species of *Bacillus* [19], *Burkholderia*[20] and*Sphingomonas* sp. and *Sphingomonaschlorophenolica* strains can transform *p*-nitrophenol [21].

3.2 Fungi

Fungi have been used from fermentation of foods to production of pharmaceuticals. Fungi thrive well in inhospitable habitats with environmental extremes because of their enzyme system [22]. Fungi are involved in the biodegradation of undesirable materials or compounds and convert them into harmless, tolerable or useful products. Many organisms are involved in the biodegradation of organic waste, which has resulted in the production of novel substances of biotechnological importance.

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Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints [23]. Fungi not only produce various metabolites like citric acid, homogeneous proteins, heterogeneous proteins, peroxidases but have shown their effectiveness for removal, reduction and detoxification of industrial effluents ingredients. Therefore, an attempt has been made to bring out the capabilities of fungi for bioremediationof industrial effluents. [24] have used filamentous soil fungi like *Aspergillus terreus,Cladosporiumcladosporioides, Fusarium oxysporium, Gliocladiumroseum, Penicillium*spp. and *Trichoderma koningii* isolated from industrially polluted sediments for the removal of Cadmium. The fungus *Penicilliumfrequentans* has been found to effectively remove phenanthrene in soil [25].

Fungi especially the white-rot fungi produce enzymes viz. laccase, Manganese peroxidase (MnP) and lignin peroxidase (LiP), which are involved in degradation of lignin in their natural lignocellulosic substrates. This ligninolytic system of white rot fungi is directly involved in the degradation of various xenobiotic compounds and dyes. The ability of the white rot fungi to degrade dye can be directly correlated with its ability to degrade lignin; the dye molecules are degraded along with lignin. Use of white rot fungi is the most unique technology of bioremediation as their ability to degrade structurally diverse xenobiotic organopollutants is more [26].

3.3 Fungi in biodegradation of pollutants

Biodegradation is an application of biological processes to the treatment of pollution. Most research within the field of bioremediation has focused on bacteria, with fungal bioremediation (mycoremediation) attracting interest just within the past two decades. White rot fungi is a physiological grouping of fungi that can degrade lignin (and lignin- like substances). Four main genera of fungi haveshown potential for bioremediation *Phanerochaete, Trametes, Bjerkandera andPleurotus* [27]. These fungi cannot use lignin as a source of energy, and instead requiresubstrates such as cellulose or other carbon sources. Thus, carbon sources such as corncobs, straw, and sawdust can be easily used to enhance degradation rates by these organisms at polluted sites. Also, thebranching, filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil.

3.4 Fungal mechanism of biodegradation

The main mechanism of biodegradation employed by this group of fungi, however, is the lignin degradationsystem of enzymes. These extracellular ligninmodifying enzymes (LMEs) have very low substrate specificity so they are able to mineralize a wide range of highly recalcitrant organopollutants that are structurally similar to lignin [28]. The fact that these fungal enzymes work extracellularly allows them to access many of the non-polar, non-soluble toxic compounds that intracellularprocesses (such as cytochrome P450) cannot [29].

IV. CONCLUSION

This review focuses on various features in bioremediation processes and how the process is acts as biological tool for remediation ofenvironment. It also encompasses the role of bacteria and fungi, their enzymatic activities. Advances in biotechnology, bioremediation has becomes rapidly growing area. Selection of most appropriate strategy to treat hazardous chemicals and contaminated sites by microbial action is still needed.

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Determination of Anthocyanins in Red Grape Juices Made From Different Varieties by HPLC

İlkay Türkmen Özen[1], Aziz Ekşi[2]

[1]Gumushane University, Faculty of Engineering and Natural Sciences, Baglarbasi, 29100, Gumushane,TURKEY
[2]European University of Lefke, Department of Gastronomy,Gemikonagi –Lefke , KKTC Mersin 10, TURKEY

Abstract*—This study was conducted to determine the anthocyanin profiles of red grape juice. As research material, twelve different red grape varieties which were collected from the main producing regions in Turkey and red grape juice samples made from them were analyzed. The anthocyanins peaks on HPLC-chromatograms in red grapes were identified as cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside and petunidin-3-glucoside. According the results, the pre-dominant anthocyanins of red grape juice was malvidin-3-glucoside which was found between 21.77-277.54 mg/L. It was followed by peonidin-3-glucoside which was found between 3.05-74.26 mg/L and then cyanidin-3-glucoside which was found between 3.02-16.94 mg/L. Delphinidin-3-glucoside and petunidin-3-glucoside were not detected in most red grape juices.*

This work is important to chemical description of local grape varieties and selection of suitable raw material for fruit juice industry.

Keywords*— *cyanidin-3-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside.

I. INTRODUCTION

Anthocyanin is a term derived from Greek words "*anthos* (flower)" and "*kyanos* (blue)" (Mazza and Miniati 1994; Castaneda-Ovando 2009).Anthocyanins, which occur in tissues of plants including fruits, flowers, leaves, roots and give those tissues their distinctive coloursat a wide range including pink, red, purple and blue, is a natural water-soluble group of pigments(Gao at al. 1997; Costa et al. 2000; Blando et al. 2004; Cemeroğlu and Karadeniz 2004). Anthocyanins are chemically the glucosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrlium (flavylium cation)(Jackman and Smith 1992) (Fig. 1). They occur in glucoside form in cell cytoplasm and are composed of certain sugars and non-sugar (aglycone) substances. Aglycone part of anthocyanins is called as anthocyanidin(Acar 1998; Koca et al. 2006).

Total anthocyanin amount varies with fruit varieties. Total anthocyanin amount in strawberry is between 450-700 µg/g(Wrolstad et al. 1970), while it is determined as 267-688 mg/L in cherry juice(Erbaş and Cemeroğlu 1992),271-316 mg/L in pomegranate juice(Cemeroğluand Artık 1990) and 285 mg/kg in fresh leaves of Isparta rose(Velioğlu and Mazza 1991).

Anthocyanin distribution reflecting the amount of different fractions such as total anthocyanin amount also varies with fruit varieties. The researches show that dominant anthocyanins are Cy-3-glc and Cy-3-rut in blackberry(Barritt and Torre 1973),Pg-3-glc, Pg-3-gal and Cy-3-glc in strawberry(Belitz and Grosch 1992), Cy-3-glc, Dp-3-glc, Cy-3,5-diglc, Dp-3,5-diglc, Pg-3-glc and Pg-3,5-diglc in pomegranate(Du et al. 1975)andCy-3-rut, Cy-3-glc and Pn-3-rut in cherry (Montmorency)(Dekazos 1970).

One of the main fruits which researches on anthocyanin amount and profile are carried out is black grapes. The reason is that the distinctive and attractive colour of black grapes originates from anthocyanins. In addition, anthocyanins have influence on the taste of black grapes, grapes juice and wine(Mazza and Miniati 1994).

According to Fuleki and Babjak(Fuleki and Babjak 1986), total anthocyanin amount in different grapes varieties varies between 33-603mg/100g, while it is between5.5-105.5 mg/100g according to Lamikanra (1989).

With the implementation of HPLC method, the researches on anthocyanin profile become widespread(Fong et al. 1971; Wulf and Nagel 1978;Pomar et al. 2005). Anthocyanins in grapes are generally in 3-monoglucoside form. The ratio of 3-monoglucoside components including delphinidin,cyanidin, petunidin, peonidinandmalvidinin to total anthocyanins varies between 57.0-84.2% depending on the grapes varieties(Mazza and Miniati 1994). Dominant anthocyanin in grapes is malvidin-3-glucoside.

Many researches on anthocyanins in grapes and food products processed from grapes(Pomar et al. 2005; Bub et al. 2001;Garcia-Beneytez 2002;Bitsch et al. 2004;Revilla et al. 2001)as well as the impacts of anthocyanins on human health (Tsuda et al. 1994;Tamura and Yamagami 1994;Karaivanova et al. 1990;Kamei et al. 1995;Bridle and Timberlake 1997)have been carried out up to date. However, comprehensive anthocyanin profile of black grapes grown in Turkey has not been determined yet. The aim of this research is to determine anthocyanin distribution of grapes juice produced from different black

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grapes varieties in Turkey and to make a contribution to the understanding of the importance of grapes juice in diet.

II. MATERIAL AND METHOD

2.1. Materials

Research material is composed of twelve different grapes varieties. Grapes varieties, regions where they are grown and their processing dates are given in Table 1.

Each grapes varieties is first washed and then grape berries are separated from the stems. Later on, mash is produced by smashing grape berries by hand under laboratory conditions. Mash is first heated to 60 ºC in steam jacketed heater for enzymation application, then it is rapidly cooled to 55 ºC which is the optimum working temperature of mash enzyme and finally a dose of150 mL/ton mashenzyme (Pectinex Ultra Color) is added. Samples to which enzymation is applied are pressed in a laboratory-type press after 1 hour and grapes juice is produced.

2.2. Method

For anthocyanin profile analysis, the HPLC method defined by DrustandWrolstad (2001) is modified and applied.

2.3. Chemicals

Acetonitrile HPLC gradient (Sigma-Aldrich), o-fosforic acid (Sigma-Aldrich), methanol HPLC gradient (Sigma-Aldrich), ciyanidin-3-glucoside, peonidin-3-glucoside, delphinidin-3-glucocide, malvidin-3-glucoside, petunidin-3-glucoside (Sigma-Aldrich), HCl

2.4. Preparation of anthocyanin standards

First of all, 1000 ppm stock solution for each anthocyanin standard is prepared with ultra pure water including 0.1% HCl. For plotting anthocyanin standard curves, solutions at different concentrations are prepared from each stock solution by using 4% phosphoric acid and injected into HPLC device.

2.5. Extraction

50 grams from each grained grapes varieties are taken, homogenised with 100 mL methanol/HCl (98:2) in blender (WARING marka)for 1 minute and retained in the dark under 4 ºC for 24 hours. Samples are then centrifuged at 3500xg for 20 minutes. After liquid phase is separated, 100 mL methanol/HCl (98:2) is added to the residue, homogenised for 1 minute and centrifuged again. This process is repeated till a colourless residue is obtained. All liquid extracts are put together and the final volume is completed to appropriate volume with methanol/HCl (98:2). Sample at certain volume is taken from the extracts and metanol/HCl is evaporated in a rotating vacuum evaporator under 30 ºC. Remaining part is diluted with 4% phosphoric acid.

Samples to be used in analysis are filtered through 0.45 µm membrane filters and 20 µL filtrate is injected into HPLC device.

2.6. Chromatographic conditions

Solvent A : %4 Fosforic acide

Solvent B : %100 Acetonitrile

Flow rate : 1.0 mL/dk

Wavelenght : 520 nm

Linear gradient flow(Table 2):

Coloumn : Reverse phase C_{18} coloumn (250 x 4.6 mm, 5um)

Temperature : 30 ºC

Analysistime : 72 min.

2.7. Identification and Calculation

Acquired chromatograms are evaluated by means of Agilent Chemstationsoftware.

Primary peaks detected in chromatograms (Fig. 2) are identified by comparing them with the incidence time of standard substance of each anthocyanin. Anthocyanin amounts are calculated quantitatively by using equations derived from standard substance curves.

The HPLC chromatograms of anthocyanins in grape juices from grape varieties (only Köhnü, Öküzgözü and Papazkarası varities are given) are shown in Fig. 3 to 5.

III. RESULTS AND DISCUSSION

Anthocyanin profile of grapes juice samples and descriptive values for these data are given in Table 3 and Table 4 respectively.

According to the findings; dominant anthocyanin in grapes juice samples is determined as malvidin-3-glucoside with an average of 114.15±23.21 mg/L. It is also determined that the richest grapes juices in terms of malvidin-3-glucoside amount are produced from Syrah, Cabernet Sauvignon, CiminandÖküzgözüvarieties (277.54 mg/L, 208.32 mg/L, 196.27 mg/L and 170.81 mg/L respectively).

Following malvidin-3-glucoside, the second dominant anthocyanin fraction in grapes juice is determined as peonidin-3-glucoside with 25.63±6.86 mg/L. It is found that the richest grapes juices in terms of peonidin-3-glucoside amount are produced from Merlot, Syrah, Köhnü and Alicante varieties (74.26 mg/L, 66.54 mg/L, 36.08 mg/L and 30.05 mg/L respectively).

Average cyanidin-3-glucoside amount in all varietiesis determined as8.03±1.93 mg/L. Grapes juices produced from Cimin, Köhnüand Merlotvarieties are the richest juices in terms ofcyanidin-3-glucoside (16.94 mg/L, 15.82 mg/L and 9.04 mg/L respectively).

Delphinidin-3-glucoside and petunidin-3-glucoside are not detected in the samples.

Several research (Wulf and Nagel, 1978;Piergiovanni and Volonterio, 1981;Roggero et al., 1984)previously conducted also show that the dominant anthocyanin in grapes is malvidin-3-glucoside.

In accordance with the findings of this research, Nunez et al. (2004) reports in their research carried out for

Graciano, Tempranillo and Cabernet Sauvignon varieties that the dominant anthocyanin is malvidin-3-glucoside and the second dominant anthocyanin in Graciano grapes is peonidin-3-glucoside. Furthermore, according to the research carried out byHmamouchi et.al. (1995), it is determined that malvidin glucosides are the dominant anthocyanins in Alicante Bouschet, Cinsault, Grenache Noir andCarignanevarieties and the amounts ofdelphinidin, cyanidinandpetunidinglucosides are comparatively low.

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Table.1: The growth regions and processing dates of the red grape cultivars

Grape Variety	Growth Region	Processing date
Kalecik karası	Ankara	07.09.2012
Cabernet Sauvignon	İzmir	21.09.2012
Syrah	İzmir	21.09.2012
Alicante	İzmir	21.09.2012
Papazkarası	Tekirdağ	22.09.2012
Isabella	Ordu	25.09.2012
Horozkarası	Kilis	28.09.2012
Köhnü	Ankara	03.10.2012
Öküzgözü	Elazığ	8.10.2012
Boğazkere	Diyarbakır	05.10.2012
Merlot	İzmir	12.10.2012
Cimin	Erzincan	15.10.2012

Table.2: Linear gradiet flow of solvent A and B

Time (min.)	Solvent A (%)	Solvent B (%)
0	94	6
55	80	20
57	30	70
60	5	95
60.1	94	6
70	94	6

Table.3: Anthocyanin profiles of grape juices (mg/L)

Variety	Siyanidin-3-glucoside	Delphinidin-3-glucoside	Peonidin-3-glucoside	Malvidin-3-glucoside	Petunidin-3-glucoside
KalecikKarası	-	-	9.05	65.19	-
Cabernet Sauvignon	-	-	16.01	208.32	-
Syrah	6.17	-	66.54	277.54	-
Alicante	3.02	-	30.05	45.16	-
Papazkarası	-	-	5.52	63.57	-
Isabella	3.86	-	4.48	21.77	-
Horozkarası	-	-	8.17	40.98	-
Köhnü	15.82	-	36.08	110.72	-
Öküzgözü	4.29	-	29.17	170.81	-
Boğazkere	5.10	-	3.05	96.28	-
Merlot	9.04	-	74.26	73.22	-
Cimin	16.94	-	25.15	196.27	-

*calculationswere done accordingto15.9Brix.

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Table.4: Descriptivevaluesoftheanthocyaninfraction ofgrapejuice

Anthocyanin fraction (mg/L)	Minimum	Maximum	Mean	SE[b]	CV[c] (%)
Cyanidin-3-glucoside (N=8)[a]	3.02	16.94	8.03	1.93	68.15
Peonidin-3-glucoside (N=12)	3.05	74.26	25.63	6.86	92.74
Malvidin-3-glucoside (N=12)	21.77	277.54	114.15	23.21	70.44

[a]number of samples
[b]standard error of mean
[c]coefficient of variance

R^1, R^2, R^3 : H, OH, OCH_3
R : saccharine

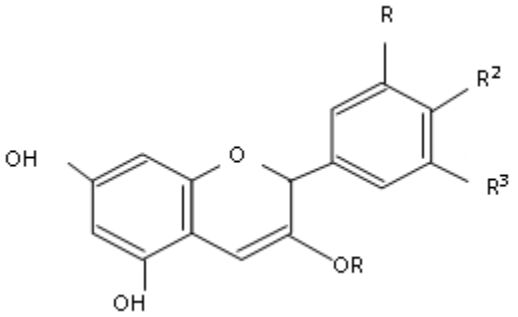

Fig. 1: Chemical structure of flavylium cation

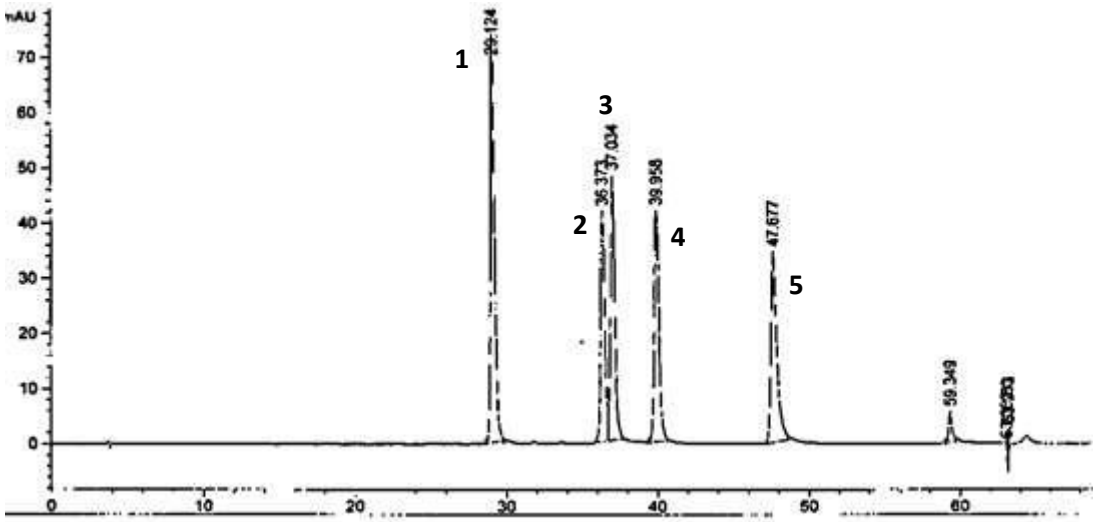

Fig. 2: HPLC chromatogram of standardsubstances of anthocyanin (1: cyanidin-3-glucoside, 2: delphinidin-3-glucoside, 3: peonidin-3-glicoside, 4: malvidin-3-glucoside, 5: petunidin-3-glucoside)

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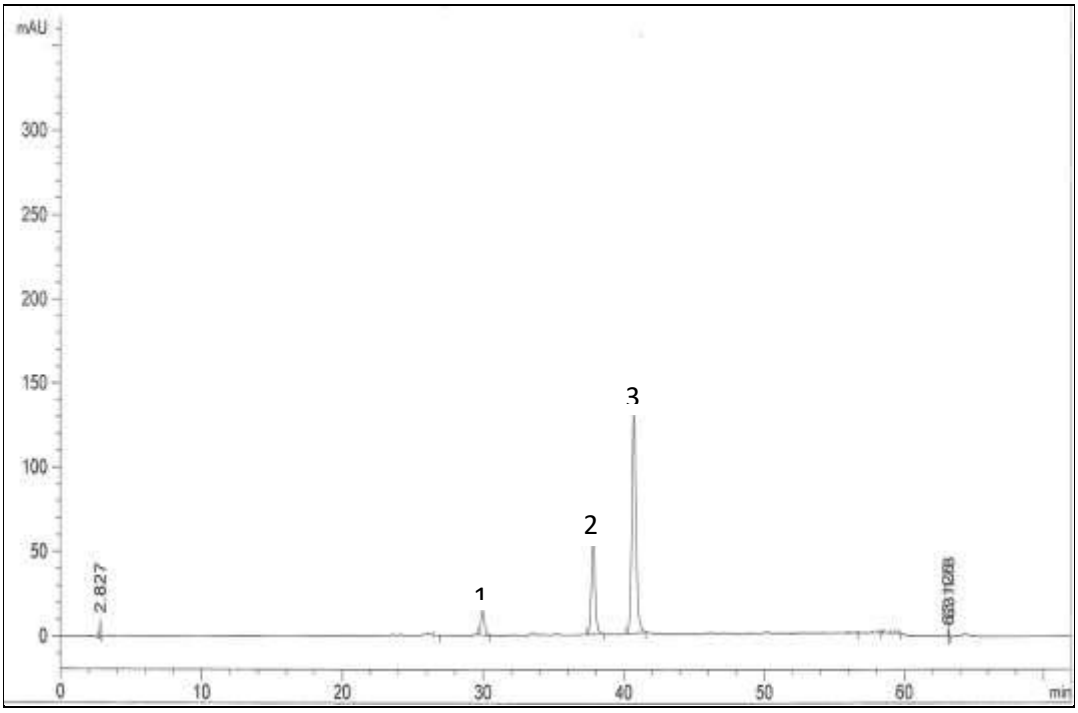
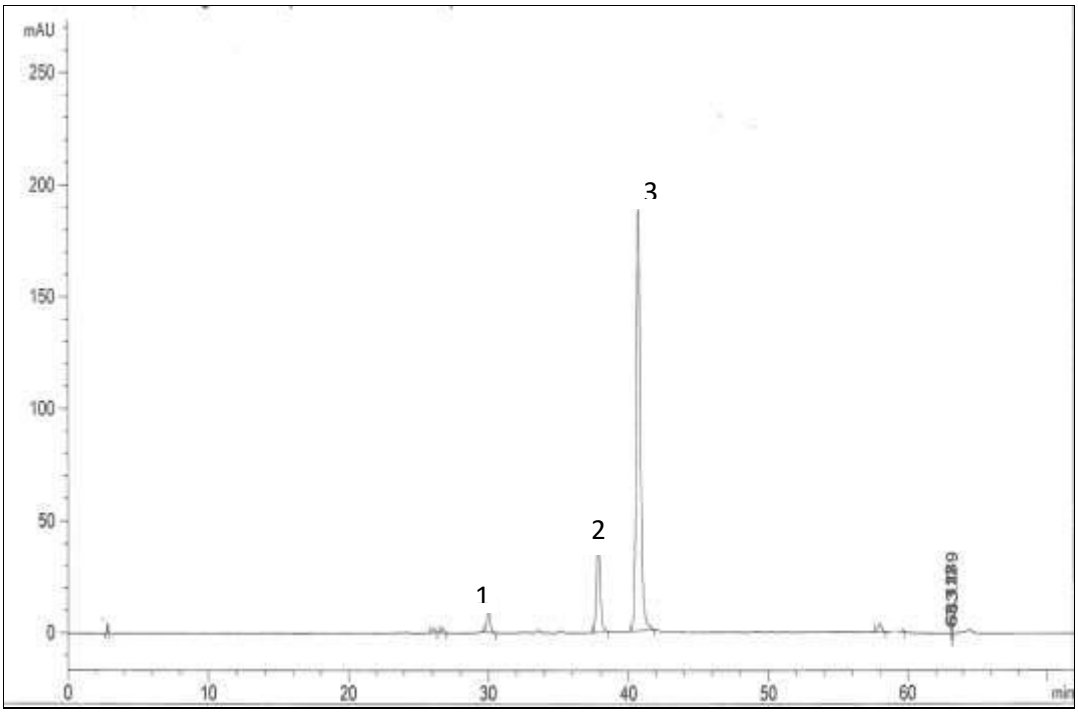


(1) Cyanidin-3-glucoside, (2) Peonidin-3-glucoside, (3) Malvidin-3-glucoside
Fig. 3: Anthocyanin chromatograph of grape juice from Köhnü variety



(1) Cyanidin-3-glucoside, (2) Peonidin-3-glucoside, (3) Malvidin-3-glucoside,
Fig. 4: Anthocyanin chromatograph of grape juice from Öküzgözü variety

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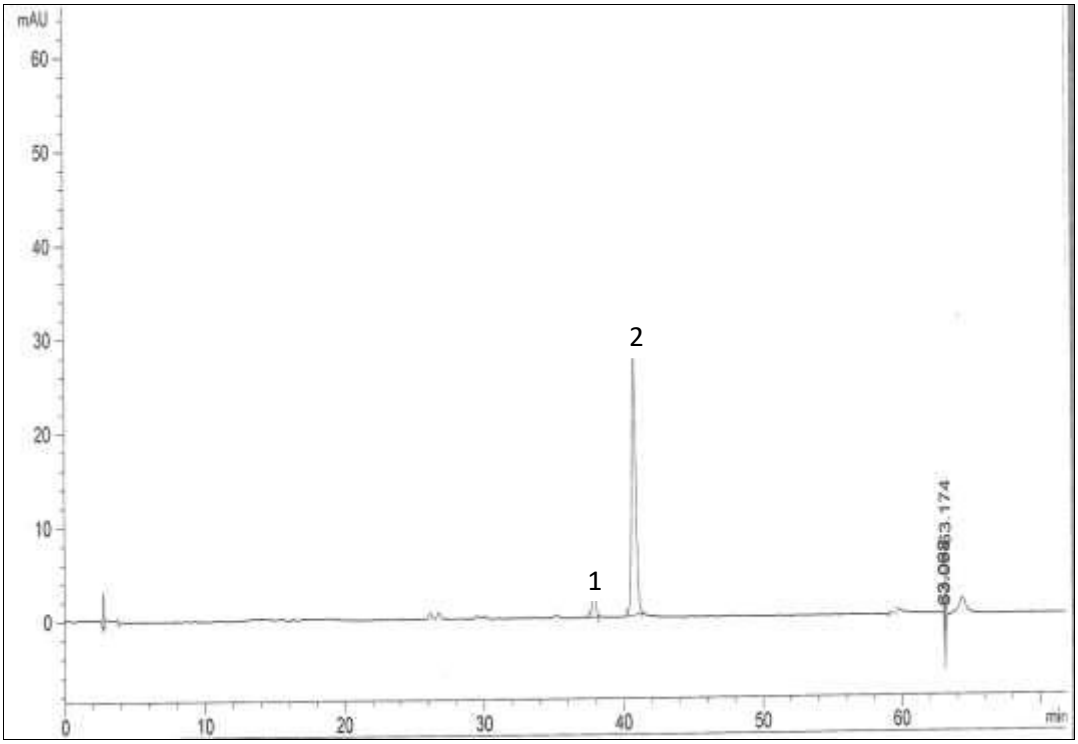

(1) Peonidin-3-glucoside, (2) Malvidin-3-glucoside

Fig. 5: Anthocyanin chromatograph of grape juice from Papazkarası variety

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Productivity and Profitability Assessment of Drought Tolerant Rice Cultivars under Different Crop Management Practices in Central Terai of Nepal

Bishal Dhakal[1*], Samjhana Khanal[2], Lal Prasad Amgain[3]

[1] Regional Agricultural Research Station (RARS), Bhagetada, Dipayal, Doti, Nepal
[2,3] Institute of Agriculture and Animal Science (IAAS), Sundarbazar, Lamjung, Nepal

***Abstract*—** *Reduction in productivity has led to lower profitability of rice production in Nepal. Proper selections of resource conservation technologies and drought tolerant cultivars are being potential strategies determining productivity of rice in drought prone areas. Thus, a field experiment was accomplished in central-terai of Nepal during 2014 to assess the productivity and profitability of drought tolerant rice cultivars under different crop management practices. The experiment was carried out in strip-plot design with three replications consisting four drought tolerant rice cultivars and three crop management practices. The analyzed data revealed that SRI (System of Rice Intensification) produced significantly higher grain yield (5.28 t ha^{-1}) than other management practices. The straw yield of SRI (5.12 t ha^{-1}) was also significantly higher than other management practices. The cultivars had no influence on grain yield, but the straw yield was significantly influenced by cultivars, with the highest straw yield in Sukkha-3 (5.21 t ha^{-1}). Similarly, SRI management practice also had significantly higher gross returns (NRs. 144652 ha^{-1}), net return (NRs. 56647 ha^{-1}) and B:C ratio (1.64:1). Thus, SRI management practice can be adopted as adaptation approach for obtaining higher productivity and profitability in central terai and similar agro-climatic regions of Nepal.*

Keywords*— *B:C ratio, crop management practices, productivity, rice, SRI.

I. INTRODUCTION

Rice is the second most important staple food for more than half of the world's population (Delseny *et al.*, 2001; Feng *et al.*, 2013). Being a most important staple food of Nepalese people, rice ranks first crop for both acreage and production and production amounts to half of the total cereal grains in the country (Ghimire *et al.*, 2013). In Nepal, rice is grown in about 1.42 million hectares with total production about 4.50 million tons, and 3.17 t ha^{-1} productivity (MoAD, 2013). The share of agriculture and forestry for national gross domestic product (GDP) is 33.03%, and therein rice alone contributes 20.75% of the agriculture gross domestic product (AGDP) and 10.2% of total GDP (Poudel, 2011).

In Nepal, more than 70% of the total rice area is grown under rainfed condition (CBS, 2003), whereas only 21 % rice production is under partially or fully irrigated conditions (NARC, 2008). Rice production relies on ample water supply and thus is more vulnerable to drought stress than other crop. The temperature of Nepal has increased by 0.04-0.06 ºC annually on an average during 1977-2005 (MoE, 2010). Increase in temperature due to climate change has resulted an increase in evidences of drought stress in crop production including rice (Karn, 2014). According to statistics, the percentage of drought affected lands areas more than doubled from the 1970s to the early 2000s worldwide (Isendahl and Schmidt, 2006). Further, increased temperature may decrease rice potential yield up to 7.4% per degree increment of temperature (Murdiyarso, 2000). Several other factors like weeds, low factor productivity and reducing resource use-efficiency due to deteriorating soil health are causing the lower productivity of rice in Nepal. Reduction in production has led to lower profitability of rice in Nepal. Among various approaches to climate change adaptation in drought prone areas, proper selections of resource conservation technologies like (SRI, ICM, etc.) (Islam *et al.*, 2014b) and drought tolerant rice cultivars (Basnet, 2015) are potential strategies determining yield of rice. Thus, the present investigation is planned, executed and accomplished with the objective of pursuing the productivity and profitability of various drought tolerant rice cultivars under different crop management practices in central terai of Nepal.

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II. MATERIALS AND METHODS

This study was carried out at Dhauwadi VDC, Nawalparasi (235 masl) from June to October 2014. The experimental site is suitated at 27°48'43" N latitude and 84°4'58" E longitude, where it received 1045 mm of rainfall during the experimental period. The experiment was carried out using a strip plot design, in the fields of three farmers, considering each farmer as a replication. The treatment consists of combination of the column factor (three rice management practices: System of Rice Intensification-SRI, Integrated Crop Management-ICM and Puddled transplanted-conventional) and row factor (four rice cultivars: Sukkha-3, Sukkha-4, Sukkha-5 and Hardinath-2). The size of each plot was 12 m^2, and the net plot was determined after leaving one border row in each side, one destructive sampling row and one guard row. The space between two plots was 0.5 m, and the bund of 0.5 m was made between each management practices to check the flow of water and nutrients between them. The experiment on three management practices were set up considering the production factors (Table 1). Vermicompost was used as a source of organic manure, whereas Urea, DAP and MOP were used as sources of N, P_2O_5 and K_2O, respectively. Full doses of phosphorus and potassium and half dose of nitrogen were applied as basal dose at the time of transplanting. The remaining half dose of nitrogen was applied in two split doses: one-fourth N at 30 DAT and the remaining one-fourth at booting stage. The crop from net plot area was harvested manually with the help of sickles. The whole plant was cut at 2 cm above ground for all varieties, except Hardinath-2 that was harvested by hand picking of panicles due to heavy rainfall during harvesting period. The grains were weighted at their exact moisture content and were adjusted at 14% moisture level. The biometric observations (plant height, tillers number per square meter, LAI, above ground dry matter), yield attributing characters and yields of all the treatments were recorded. These recorded datas were tabulated in MS-Excel which was subjected to ANOVA (Gomez and Gomez, 1984), after analysis through MSTAT-C and mean separation for significant variables were done by Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Table.1: Production factors considered in different management practices

Production factors	SRI	ICM	Conventional
Crop geometry	25 cm × 25 cm	20 cm × 20 cm	20 cm × 15 cm
Seed rate	7.5 kg ha^{-1}	20 kg ha^{-1}	40 kg ha^{-1}
Seedling age	14 days old	21 days old	28 days old
Seedling/hill	1	2	3
Organic manure	10 t ha^{-1}	5 t ha^{-1}	None
NPK	20:15:10 kg ha^{-1}	40:30:20 kg ha^{-1}	80:60:40 kg ha^{-1}
Water management	Alternating wetting and drying	Intermediate condition	Flooded condition

III. RESULTS AND DISCUSSIONS

3.1 Grain yield

The grain yield was significantly influenced by management practices, but the cultivars and its interaction with management practices had no influence on grain yield (Table 2). The grain yield of SRI management practice (5.28 t ha^{-1}) was significantly higher than conventional management practice (4.49 t ha^{-1}), but it was statistically at par with ICM management practice (4.73 t ha^{-1}). The grain yield of ICM was also significantly higher than under conventional (228 m^{-2}) management practice. The higher grain yield of SRI management practice was because of significantly higher number of effective tillers (318 m^{-2}) than ICM (387 m^{-2}) and conventional management practices. Panicle weight, panicle length and filled grains per panicle of SRI management practice were also significantly higher than ICM and conventional management practices. Further, sterility percentage was significantly lower in SRI (14.97%) than ICM (15.13%) and conventional (16.23%) management practices. Higher number of effective tillers, panicle weight and filled grains per panicle were reported in SRI than conventional management practice (Rao *et al.*, 2013; Islam *et al.*, 2014a; Ahmed *et al.*, 2015; Jana *et al.*, 2015). The higher grain yield of SRI was also due to higher LAI as compared to other management practices. The grain yield of rice is also determined by assimilates deposited mainly in vegetative stage, which is directly contributed by leaf area. Carbohydrates produced before heading mainly accumulate in the leaf sheath and stem and translocate to the panicles during grain filling (Fageria, 2007). The contribution of carbohydrates produced before heading to the final grain yield appeared to be in range of 20-40 % (Murata and Matsushima, 1975).

It was revealed that SRI practice produced 17.49% more yield than conventional practice. Although SRI and ICM practices were statistically similar, SRI produced 11.63% more yield than ICM practice. Moreover, ICM produced 5.35 % more grain yield as compared to conventional management practice. The increase in grain yield of 11.8 % was reported under SRI management practice over conventional (Gulshan and Sarao, 2009). Similarly, increase in grain yield under SRI and ICM management practices was 209.9 % and 185.4 % higher, respectively

over conventional management practices (Islam *et al.*, 2014a). Moreover, 100-200 % increase in grain yield was also reported under SRI compared to conventional management practice (Munda *et al.*, 2012).

Table.2: Grain yield, straw yield and harvest index of various cultivars of rice as affected by management practices at Dhauwadi VDC, Nawalparasi, Nepal, 2014

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
Management		
SRI	5.28 ^{a}	5.12 ^{a}
ICM	4.73 ^{ab}	4.73 ^{b}
CON	4.49 ^{b}	4.06 ^{c}
SEm (±)	0.145	0.057
LSD (0.05)	0.57*	0.23**
Cultivars		
Sukkha-3	4.79	5.21 ^{a}
Sukkha-4	4.73	4.43 ^{b}
Sukkha-5	5.16	4.49 ^{b}
Hardinath-2	4.64	4.42 ^{b}
SEm (±)	0.236	0.108
LSD (0.05)	ns	0.37**
CV (%)	10.81	5.1
Grand Mean	4.83	4.64

(Treatment means followed by common letter/letters within column are not significantly different among each other based on DMRT at 0.05; **= significant at 0.01 level, *= significant at 0.05 level and ns= non-significant at 0.05 level)

3.2 Straw yield

The straw yield (5.12 t ha^{-1}) of SRI practice was significantly higher than ICM (4.73 t ha^{-1}) and conventional practices (4.06 t ha^{-1}). The straw yield of ICM practice was also significantly higher than conventional practice. This might be due to longer plant height in SRI and ICM management practices over conventional management practices. Moreover, early vigorous growth due to wider spacing which resulted less competition in space, nutrition and other factors for growth might have resulted higher straw yield in SRI management practice. Further, the higher straw yield in SRI might also be due to higher number of tillers in SRI than other management practices (Wijebandara et al., 2008). The significant higher straw yield in SRI than in conventional management practices was also reported by Wijebandara *et al.* (2008) and Jeyapandiyan and Lakshmanan (2014).

The straw yield of Sukkha-3 (5.21 t ha^{-1}) was significantly higher than other varieties, whereas the straw yield of other cultivars were at par (Table 2). Higher straw yield of Sukkha-3 might be due to longer plant height of this cultivar. Higher straw yield in the cultivars with longer plant height was also reported by Haque and Pervin (2015). Higher dry matter accumulation in Sukkha-3 might also have contributed to its higher straw yield. Further, there was significant influence of interaction of cultivars and management practices in straw yield. The mean straw yield was found highest in Sukkha-5 with SRI (5.66 t ha^{-1}), followed by Sukkha-3 with ICM practices (5.31 t ha^{-1}). The lowest mean straw yield (3.56 t ha^{-1}) was observed in Sukkha-5 with conventional practice.

3.3 Economic Analysis

3.3.1 Cost of cultivation

The data on cost of cultivation is presented in Table 3. The data on cost of cultivation revealed that SRI practice had the lowest cost of production (NRs. 88,005 ha^{-1}), followed by ICM (NRs. 95207 ha^{-1}) and conventional (NRs. 111909 ha^{-1}) practices, respectively. The mean cost of cultivation was NRS. 98374 ha^{-1}.

3.3.2 Gross return

The total monetary value of the economic produce and the byproducts obtained from the crop is called gross return. It is calculated based on the local market price of the products (Reddy and Reddi, 2005). The gross return was significantly influenced by management practices, but the cultivars and interactions of cultivars and management practices had no influence in gross return (Table 3). The gross return of SRI practice (NRs. 144652 ha^{-1}) was significantly higher than ICM (NRs. 129941 ha^{-1}) and conventional (NRs. 121931 ha^{-1}) practices. Higher gross return in SRI practice has also been reported by Islam *et al.* (2014b).

3.3.3 Net return

The ultimate product remained after subtracting the cost of cultivation from the gross return is called net return (Reddy and Reddi, 2005). The net return was significantly influenced by management practices, but the cultivars and interactions of cultivars and management practices had no influence in net return. The net return of SRI practice (NRs. 56647 ha^{-1}) was significantly higher than ICM (NRs. 34733 ha^{-1}) and conventional (NRs. 10022 ha^{-1}) practices (Table 3). Higher net return in SRI practice has also been reported by Islam *et al.* (2014b).

3.3.4 Benefit cost (B: C) ratio

Benefit cost (B: C) ratio is defined as the ratio of the gross returns to the cost of cultivation which can also be expressed as return per rupee invested. For any enterprise relating with agriculture sector to be economically viable, a minimum B: C ratio of 1.5 is fixed. Therefore for any

agriculture enterprise to be sustainable, it should maintain a B: C ratio of 1.5 (Reddy and Reddi, 2005). The benefit cost ratio was significantly influenced by management practices, but the cultivars and interactions of cultivars and management practices had no influence in benefit cost ratio. The benefit cost ratio of SRI practice (1.64:1) was significantly higher than ICM (1.37:1) and conventional (1.09:1) practices (Table 3). Higher benefit cost ratio in SRI practice has also been reported by Wijebandara *et al.* (2008) and Islam *et al.* (2014b).

Table.3: Cost of cultivation (NRs. 000 ha^{-1}), gross return (NRs. 000 ha^{-1}), net return (NRs. 000 ha^{-1}) and B:C ratio of various cultivars of rice as affected by management practices at Dhauwadi VDC, Nawalparasi, Nepal, 2014

Treatment	Cost of production (NRs. 000 ha^{-1})	Gross return (NRs. 000 ha^{-1})	Net return (NRs. 000 ha^{-1})	B:C ratio
Management				
SRI	88.01	144.65 ^{a}	56.65 ^{a}	1.64 ^{a}
ICM	95.21	129.94 ^{b}	34.73 ^{b}	1.37 ^{b}
CON	111.91	121.93 ^{b}	10.02 ^{c}	1.09 ^{c}
SEm (±)		3.387	3.387	0.036
$LSD_{(0.05)}$		13.30*	13.30*	0.14*
Cultivars				
Sukkha-3	98.37	133.30	34.95	1.38
Sukkha-4	98.37	129.03	30.65	1.33
Sukkha-5	98.37	139.55	41.18	1.44
Hardinath-2	98.37	126.82	28.45	1.31
SEm (±)		5.730	5.730	0.061
$LSD_{(0.05)}$		ns	ns	ns
CV (%)		9.39	34.28	9.51
Grand Mean	98.37	132.18	36.20	1.365

(Treatment means followed by common letter/letters within column are not significantly different among each other based on DMRT at 0.05; **= significant at 0.01 level, *= significant at 0.05 level and ns= non-significant at 0.05 level)

IV. CONCLUSION

The results showed that grain yield was significantly influenced by management practices, where SRI management practice recorded the highest grain yield than other management practices. But, the rice cultivars and the interaction of management practices and cultivars had no influence on grain yield and major yield attributing characters. Similarly, SRI management practice had the higher gross return, high net return and B:C ratio. Thus, SRI management practice can be adopted as adaptation approach for obtaining higher productivity and profitability in central terai and similar agro-climatic regions of Nepal.

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Effect of Organic Nutrition in the Nursery Growth and Nutrimental Content of Native Avocados of Ometepec, Guerrero, Mexico

Damián-Nava A.[1], Arellano-Roque L.[1], Hernández-Castro E.[1], Palemón-Alberto F.[1], Cruz-Lagunas B.[2], Vargas-Álvarez D.[1], Díaz-Villaseñor G.[2]., Leiva-Rojo E. I.[3] andRamírez-Pisco R.[3]

[1]Postgraduate in Agricultural Science and Local Management, Autonomous University of Guerrero. Iguala-Tuxpan Hwy, Km. 2.5. Iguala, Guerrero, Mexico. C.P. 40101.

[2]Academic Unit of Agricultural and Environmental Sciences of the Autonomous University of Guerrero. Iguala-Tuxpan Hwy, Km. 2.5. Iguala, Guerrero, Mexico. C.P. 40101.

[3]Faculty of Agricultural Science and [3]Faculty of Sciences of the National University of Colombia, campus Medellín of Antioquia, Colombia.

***Abstract*—** *In Mexico, there are several types of wild and criollo avocados that constitute a genetic heritage of this species; these avocados currently grow in an unordered manner on farmer's lands and in backyards, and they need to be studied as they are being lost because of agricultural activities and edaphoclimatic and phytosanitary factors. On the other hand, in orchards and avocado nurseries, high amounts of chemical fertilizers and pesticides are used affecting the physicochemical and microbiological properties of the soil, modifying the flora and fauna and polluting aquifers and springs that cause health problems among consumers. Therefore, it is very important to have a more friendly agriculture with the nature. The aim of this work was to evaluate under nursery conditions, the effect of organic fertilizers on 12 genotypes (rootstocks) of native avocados of Ometepec, Guerrero, Mexico, under an experimental design of random blocks, with four treatments: T1: sheep manure,T2: Bovine manure, T3: mycorrhizae and T4 (control: water) in four replicates. The variables were: plant height (PH), stem diameter (SD), number of leaves: young (NYL) and mature (NML) per plant; and the content of* NO_3^-, K^+, Ca^{2+} *and* Na^+ *ions obtained by petiole extraction, and the chlorophyll content measured with SPAD, in young (CYL) and mature (CML) leaves. Additionally, an analysis of variance and Tukey mean tests (*$P \leq 0.01$ *and 0.05) and LSD (*$P \leq 0.05$*) were done. It was found that sheep manure was superior to other treatments in PH (76.7 cm), SD (7.2 mm), NYL (6.5 leaves/plant), NML (18.4 leaves/plant), CML (40.2 SPAD) and* Ca^{2+}*(1495ppm). In conclusion, the sheep manure was better than the bovine, mycorrhiza and control (water) as it affected positively the behavior of rootstocks in plant height, stem diameter and number of young and mature leaves. In addition, organic nutrition showed no significant response in the chlorophyll content of young and mature leaves. Young leaves only reached 50% of the chlorophyll content compared to mature leaves.*

***Keywords*— Native Avocados, Vegetative Growth of Rootstocks, Organic Nutrition.**

I. INTRODUCTION

In conventional agriculture, heavy doses of chemical fertilizers and pesticides are often used to correct nutrient deficiencies in soils and to improve crop yields. However, these chemicals cause health problems among consumers (Larios *et al*., 2011; Márquez-Quiroz *et al*., 2014) and to the environment, as in areas where these substances are applied, the leached water draws nitrates, that pollute aquifers and springs in avocado producing regions (Tapia *et al*., 2012). In this sense, it has been reported that higher concentrations than10 mg L^{-1} of $N\text{-}NO_3$ in drinking water can cause serious diseases in humans and young animals (Killpack and Bucholz, 1993); the nitrogen loss in avocado orchards increases during the raining season, July and October, and they pollute the environment as the leachatecarries out the nutrients. In the same way, irrigation contributes to this loss of chemical fertilizers as every year the traditional irrigation produced 80-96 $mgL^{-1}$of $N\text{-}NO_3$ leachate, while the located irrigation only produced from 36-47 mg L^{-1} (Tapia *et al*., 2012). In addition, these

chemical fertilizers affect the physicochemical and microbiological properties of soil, as they modify its pH, structure, aeration and porosity, as well as the flora and fauna (Trinidad *et al*., 2015).In the mutualistic associations, known as arbuscular mycorrhiza, the fungus colonizes the root cortex in an extra and intracellular way, developing an intricate external mycelium that surrounds the root of the colonized plants. This mycelium forms a continuous connection between the solution of the soil and the plant, which allows the uptake of ions from the soil and their transport to the root of the host. In an opposite way, the arbuscular mycorrhizalfungus (AMF) receives carbon compounds from photosynthesis of the plant, which are necessary for its metabolism because it is a symbiont, which requires interaction with the plant to complete its life cycle (Seguel, 2014). These mycorrhizal associations increase access to plant nutrients such as: phosphorus (P), nitrogen (N), copper (Cu) and zinc (Zn). The absorption, transport and transfer of P from the mycelium to the plant is fast and efficient due to the presence of carriers with high affinity to the $H_2PO_4^-$ion, which acts coupled with an H^+ symporter carrier through various H^+ -ATPase. On the other hand, fungal mycelium acts in the release of nutrients from particles and mineral rocks by weathering, and also,they connect to the host plants which are the nutrients required for their growth, allowing the flow of energy-rich compounds required for the mobilization of the nutrient; additionally, they increase the absorbing surface area of the plant system, the mycorrhizal extraradical mycelium provides a direct pathway for the translocation of the carbon derived from photosynthesis to the microsites in soil and a large surface area for the interaction of other microorganisms (Finlay, 2008). In this way, the symbiotic nature of the plants with the arbuscular mycorrhizal fungi have proved to be fundamental for the sustainability of the ecosystems, since they are able to colonize large number of terrestrial plants. This technology represents an alternative to improve the soil biological balance and reduce the use of chemical fertilizers and other agrochemical compounds in the production systems (Jeffries *et al.*, 2003).The avocado root lack of absorbent hairs; however, it has been reported that arbuscular mycorrhizal fungi (AMF) colonize the roots of this fruit tree, and that they favor water absorption and use of soil nutrients by the plant, they also promote growth and they keep the nursery plants healthy (Reyes *et al*., 1997; Bárcenas *et al*., 2007).

Published reports on AMF inoculation are scarce and they have shown a broad range of responses, ranging from zero to clear growth responses (Silveira *et al*., 2002),they also improve nutrition, health, growth, resistance to pathogens and tolerance to adverse conditions in the nursery. In avocado seedlings, the application of Glomus spp. Zac-19 and vermicompost favored stem height and diameter (Reyes *et al.*, 1998); in addition, the application of a 1kg fluid paste of the EcoMic® Biofertilizer and 600ml of water, stimulated the development of avocado rootstocks under nursery conditions, which has been reflected on plants of higher quality, and in turn they are a nutritional alternative for this crop (Rivera-Espinosa *et al*., 2011).Avocadosinoculated with AMFin the nursery,increased their height, diameter, and fresh and dry leaves weight; *Rhizophagus fasciculatum* inoculant (used in sterile soil) showed higher growth; on the contrary, *Pacispora scintillans* and*Acaulospora laevis*(on unsterilized soils), showed a decrease in plant growth (Banuelos *et al.*, 2013). On the other hand, in avocado rootstocksinoculated with AMF in the nursery, *Acaulospora delicata* had better plant height and *Scutellospora pellucida*showed larger stem diameter; while *Rhizophagus intraradices* 28-A and*Scutellospora pellucida*increased twice the stem and root weight in relation to other treatments (Carreón *et al*., 2014).In addition, it has been reported that AMFs influence mineral nutrition and carbohydrate content in 'Carmen' avocado seedlings; in all mycorrhizal types, the inoculated plants had higher contents (mg/plant) than the control ones: *S. heterogama* in N, P, K, Mg, Cu and Zn; with *G. etunicatum* in N, P, K, Ca, Mg, Cu and Zn; with *A. scrobiculata* in P, Cu and Zn; and with *G. clarum* in K, Ca, Cu and Zn. All AMF species increased the amounts of carbohydrates in plants (Silveira *et al.*, 2003).

The State of Michoacan, Mexico, produces millions of avocado plants per year in nursery to meet the demand for new plantations at national and regional level, where their quality is highly appreciated. However, the high amounts of fertilizers and pest control products that pollute the environment have been questioned; which is why there is a need to implement new production technologies that reduce these agricultural products, such as the use of organic fertilizers and the application of arbuscular mycorrhizal fungi that have important functions in plant growth (Rivera-Espinosa *et al.*, 2011).

In avocado plantations and nurseries, high amounts of chemical fertilizers and pesticides are used which affect the physicochemical and microbiological properties of soil, they also alter its flora and fauna and because the leached water drags nitrates, then contaminated aquifers and springs cause health problems among consumers. Therefore, it is necessary to look for alternatives in the agricultural activity, more in line with practices that respect nature, that does not harm the health of the consumers and that allow to obtain

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healthy products; for this reason, the aim of this research was to study the behavior of rootstocks of native avocados in nursery and their response to organic nutrition based on bovine and ovine liquid manures and the application of mycorrhizae, in which the following objective was assessed: to evaluate the effect of organic fertilizers on 12 genotypes of native avocados of Ometepec, Guerrero.

II. MATERIALS AND METHODS

Location of studyarea

The study was conducted in Iguala, Guerrero, Mexico, from August to December 2015. The area is located at 757 m altitude, with following coordinates: 18°20'39"N and 99° 29'53" W (GPS Garmin eTrex 10®). The climate is classified as Awo g (w) (i') (Garcia), the driest among the warm subhumids, with rains in summer (June to October), the average annual rainfall is 977.15 mm and the average annual temperature is 25.7°C (García, 1988).

Methodology

Twelve native avocado genotypes (rootstocks) from Ometepec, Guerrero, Mexico, were studied. They were one month old and grown in nurseries on substrates (85% river mud, 5% peat moss and 10% agrolite) that were fertilized with ground (Crusher mill, CH620model, KOHLER®brand) and disinfected (stainless steel steam cooker at 120°C for 30 minutes)ovine and bovinemanures.Also, the commercial mycorrhiza Glumix Irrigation® Biostimulant, and water as control were used. The preparation of manures was 250 gL^{-1} of water and mycorrhiza of 5 g L^{-1} of water; the mixing and dilution of fertilizers and mycorrhizae was done with an SSP mixer [angle grinder (230 mm) (9"), 127 V-15 A 50/60 Hz 6600 r/min, Makita® brand]. The doses of manures and mycorrhiza were 250 mL/ pot, every 30 days, with additional water irrigations every other day.

Variable recording began 15 days after the first application of treatments, then every 30 days: plant height (cm) from neck to stem apex; diameter of stem (mm)at 10 cm in height with a digital vernier (Digimatic calipbre Model: CD-12'CP, Mitutoyo® brand); number of young leaves (NYL), well-formed andnot 100% grown, and green-yellowish to reddish color; number of mature leaves (NML) with 100% growth and intense green color.In October 2015, the chlorophyll content was determined in sunny, young and mature leaves with a SPAD 502 Plus, Minolta, Model B343, Horiba® Brand. In December 2015, from 7- 10:00 am, the petiole extract was obtained from 4 mature leaves/ replicate/ treatment, from which the petiole was cut into portions that were pressed in a garlic press (Kamp® brand); the extracted sap was deposited in the respective ionometers:NO_3^- (METER, Model B-743), K^+ (METER, Model B-731), Ca^{2+} (METER, Model B-751) and Na^+ (METER, Model B-722).

A randomized complete block design was used, which considered four treatments: T1 (sheep manure), T2 (bovine manure), T3 (mycorrhizae, *Glumix Irrigation® Biostimulant*) and T4 (control,common water tap); each of them with four replicates. A variance analysis, a Tukey mean test ($P \leq 0.01$ and 0.05), LSD ($P \leq 0.05$), and a Pearson correlation between the variableswere carried out with the Statistic Analysis System (SAS), version 9.0.

III. RESULTS AND DISCUSSION

Effect of organic fertilizers on the growth and nutritional content of rootstocks

As for organic fertilizers, significant differences ($P \leq 0.01$) were observed for the following variables: plant height, stem diameter, number of leaves (young and mature) and Ca^{2+}. However, chlorophyll in leaves (young and mature), NO_3^-, K^+ and Na^+ showed no significant differences (Table 1).

Plant height and stem diameter

As for height, sheep manure showed the highest value (76.7 cm) of the rootstocks and it was statistically higher than the bovine manure treatment (73.1 cm), which in turn was better to mycorrhizae (69.9 cm), which surpassed the control (63.1 cm cm) (Figure 1). A similar behavior was observed with the stem diameter, where the sheep manure gave the highest value (7.2 mm), and it exceeded the bovine fertilizer (6.8 mm) and mycorrhiza (6.7 mm), which were statistically better than the control (6.3 mm) (Figure 1).

In this research, the mycorrhizae treatment was surpassed by the ovine and bovine fertilizers, in height and stem diameter of the rootstocks. However, these mycorrhizae values in height of the rootstock, exceeded those reported previously(62.6 and 54.4 cm) with Glomus hoi-like and *Glomus mosseae* (Fundora *et al.,* 2011). Mexican avocado landrace rootstocks(*P. americana* Mill. Var. Drymifolia) inoculated with AMF, showed 32.6- 36 cm height values with the application of *Glomus fasciculatum*, *G. constrictum*, *G. tortuosum*, *G. geosporum* and *Acaulospora scrobiculata* (Castro *et al.,* 2013); whereas as for diameter, in the present study it was observed slightly higher than that reported by Castro *et al.*(2013), who reported an average value of 8.8 cm in diameter with the application of various types of mycorrhizae.

Number of young and mature leaves

The sheep manure compost (6.5 leaves/ plant) showed higher value in young leaves, but it was not statistically superior to the bovine treatments (6.0 leaves/plant) or mycorrhizae (5.9 leaves/plant); it only exceeded the control

(5.2 leaves/ plant). In relation to the number of mature leaves, sheep manure (18.4 leaves/ plant) was statistically superior to the bovine manure (17.1 leaves/ plant), mycorrhizae (17.1 leaves/ plant) and the control (16.4 leaves/ plant) (Figure 2).

However, in this study,the mycorrhizal treatment was on average as for the number of leaves/plant in comparison to other investigations with (*Glomus hoi-like*) 16.0 and (*Glomus mosseae*) 15.6 leaves/plant (Fundora *et al*., 2011); for consortium of *Glomus fasciculatum, G. constrictum, G. tortuosum, G. geosporum* and *Acaulospora scrobiculata* (21.9 leaves/plant); for the mixture of *G. Mosseae* and *G. cubense*(20.3 leaves/plant) (Castro *et al.,* 2013).

Chlorophyll content in leaves (young and mature)

As for the chlorophyll content in young and mature leaves, the treatments did not show significant effects ($P \leq 0.01$) (Figure 3). However, it is important to mention that young leaves only had half of the chlorophyll content in comparison to mature leaves; maybe because they did not have 100% of the size and they had a coloration between yellowish- green and reddish- green. Therefore, they did not reach their maximum photosynthetic rate (Salisbury and Ross, 1994)due to the immaturity of the stomata (Faust, 1989).In coffee plants, values similar to those found in the present research (40 SPAD units) were reported in adult leaves (Torres-Netto *et al*., 2005); as well as in papaya (*Carica papaya* L.) (Torres-Netto *et al*., 2002); coffee (*Coffea canephora* P.) (Torres-Netto *et al*., 2005); cotton (*Gossypium hirsutisms* L.) (Brito *et al.,* 2011); pine nut (*Jatropha curcas* L.) (Gonsiorkiewicz *et al.,* 2013); in rice cv. Bing 9363, at the beginning and at panicle maturation, with 40.4 and 35.5 (SPAD units), respectively (Jinwen *et al.,* 2011). In the Hass and Edrenol avocado varieties on patterns of Allesbeste Nursery, Duiwelkloof, of one year old, the chlorophyll content in mature leaves was slightly higher than those obtained in this work, it ranged from 48-57 SPAD units (Bekker *et al*., 2005).

Nutritional content per extract of petiole in treatments

In avocado plants, the nitrate content was extracted in the petiolate where the mycorrhizal treatment was higher (3778 mg L^{-1}), but it was not statistically different from the other treatments, sheep manure (3200 mg L^{-1}), bovine manure (3099 mg L^{-1}) and control (2144 mg L^{-1}) (Figure 4). The results of this research are not similar to those reported for Hass avocado from "El Rosario", municipality of Nuevo Parangaricutiro, Michoacán, where it was found that the $N\text{-}NO_3$ content in leaves was 24.1, 32.1, 25.3 and 47.1 mg L^{-1}, with the application of a fish derivative, organic compost, microorganisms (*Glomus* sp. and *Azospirillum* sp) and vermicompost, respectively (Tapia *et al.,* 2014).In other plant species,lower nitrate contents have been reported to those found in this research; in poblano chili pepper cv. San Luis in Guanajuato, México, 500 mg L^{-1} (Castellanos-Ramos *et al.,* 2001) and 1050 mg L^{-1} of $N\text{-}NO_3$were reported(Brizuela-Amador *et al.,* 2005), whereas in tomato, 2090 mg L^{-1} (Leyva *et al.,* 2005). This suggests that the photosynthetic rate may be high because the nitrogen content is high (Calderón, 1998) and there is a direct relationship between nitrogen and leaf chlorophyll.

The control treatments (2658 mg L^{-1}), mycorrhizae (2658 mg L^{-1}) and bovine manure (2642 mg L^{-1}) (Figure 4), gave potassium results similar to those reported in avocados with the application of Solupotasse (2329.2 mg L^{-1}), Solupotasse + Foliar Solup (2512.0 mg L^{-1}), Granupotasse (2391.7 mg L^{-1}) and Granupotasse + Foliar Solup (2204.2 mg L^{-1}) (Tapia *et al.,* 2007);but they differ from those reported in avocado Hass from "El Rosario", Nuevo Parangaricutiro municipality, Michoacán, where it was found that the K^+content in the leaves was 30.8, 24.4, 26.7 and 54.6 mg L^{-1}, with the application of fish derivative, organic compost, microorganisms (Glomus sp. and Azospirillum sp) and vermicompost, respectively (Tapia *et al.,* 2014). However, sheep manure (3113 mg L^{-1}) showed values higher than those reported in other studies; the differences observed in potassium can be attributed to the variation throughout the yearof temperature, solar radiation and/or relative humidity, in which high and low potassium contents are found according to the seasons (Aguilera *et al.,* 2005).

The sheep manure treatment gave higher calcium content (1496 mg L^{-1}), which exceeded the control (200 mg L^{-1}), but it was statistically similar to bovine manure (530 mg L^{-1}) and mycorrhizae (495 mg L^{-1}), respectively (Figure 4).

As for sodium, all treatments were statistically similar: mycorrhizae (1783 mg L^{-1}), sheep manure (1475.1 mg L^{-1}), bovine manure (1466.7 mg L^{-1}) and control (1321.7 mg L^{-1}). Castro *et al.* (2000) reported lower values for sodium in Nabal, Duke 7 and UCV 7 avocado varieties: 200 mg L^{-1}, 300 mg L^{-1} and 400 mg L^{-1}, respectively.

Conclusions

- The sheep manure affected positively the behavior of rootstocks in plant height, stem diameter, number of young and mature leaves of native avocados of Ometepec, Guerrero.
- The use of organic nutrition did not show a significant response in the chlorophyll content of young and mature leaves.
- Young leaves only reached 50% of chlorophyll content compared to mature leaves.

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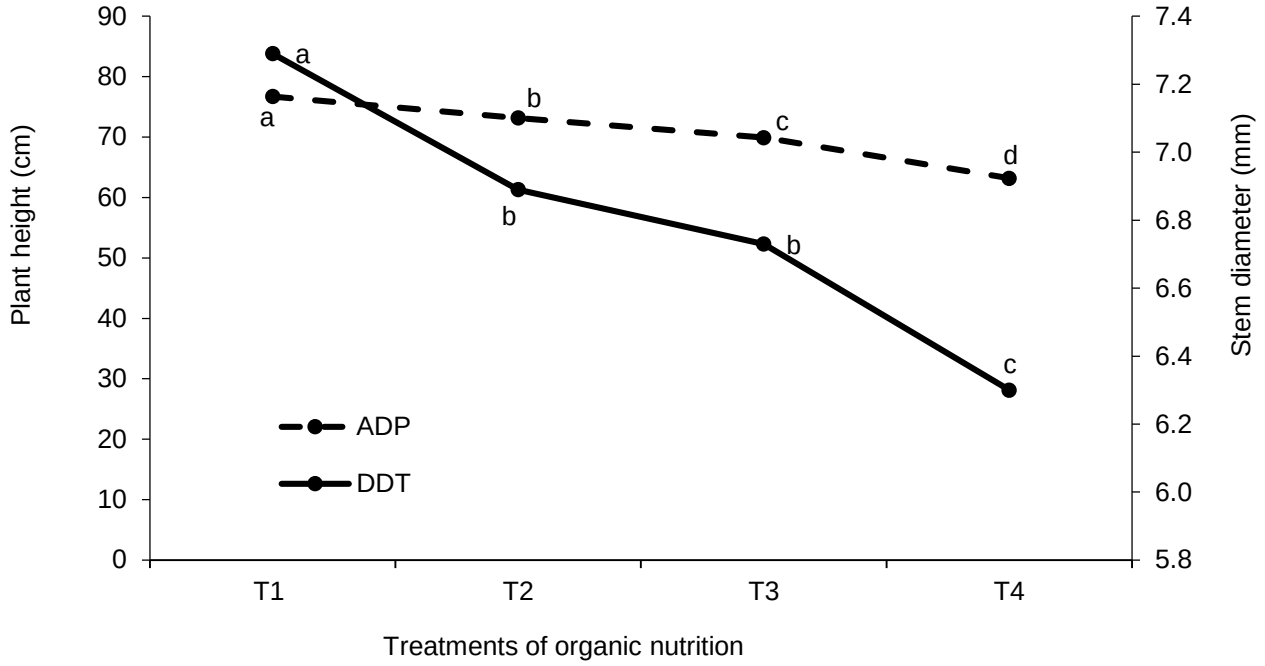

Fig.1: Organic nutrition effect in plant height (PH), stem diameter (SD of genotypes (rootstocks) of native avocados. T1: sheep manure, T2: Bovine manure, T3: mycorrhizae and T4 (control: water) in four replicates, Tukey ($P \leq 0.01$). hojas/planta) (Castro et al., 2013).

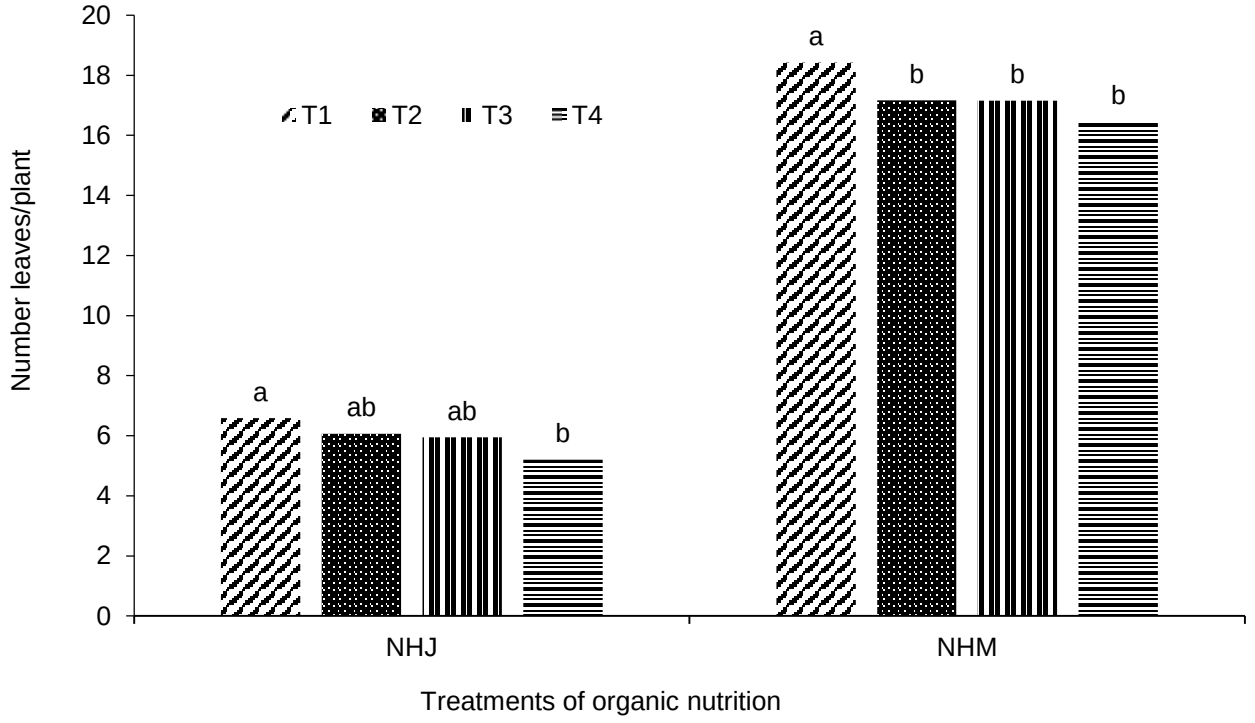

Fig.2: Effect of organic nutrition in number of young leaves (NYL) and number of mature leaves (NML) in native avocados ofOmetepec, Guerrero. T1 (sheep manure), T2 (bovine manure), T3 (mycorrhizae, Glumix Irrigation®Biostimulant) and T4 (control, common water tap), Tukey ($P \leq 0.01$).
SPAD (Bekkeret al., 2005).

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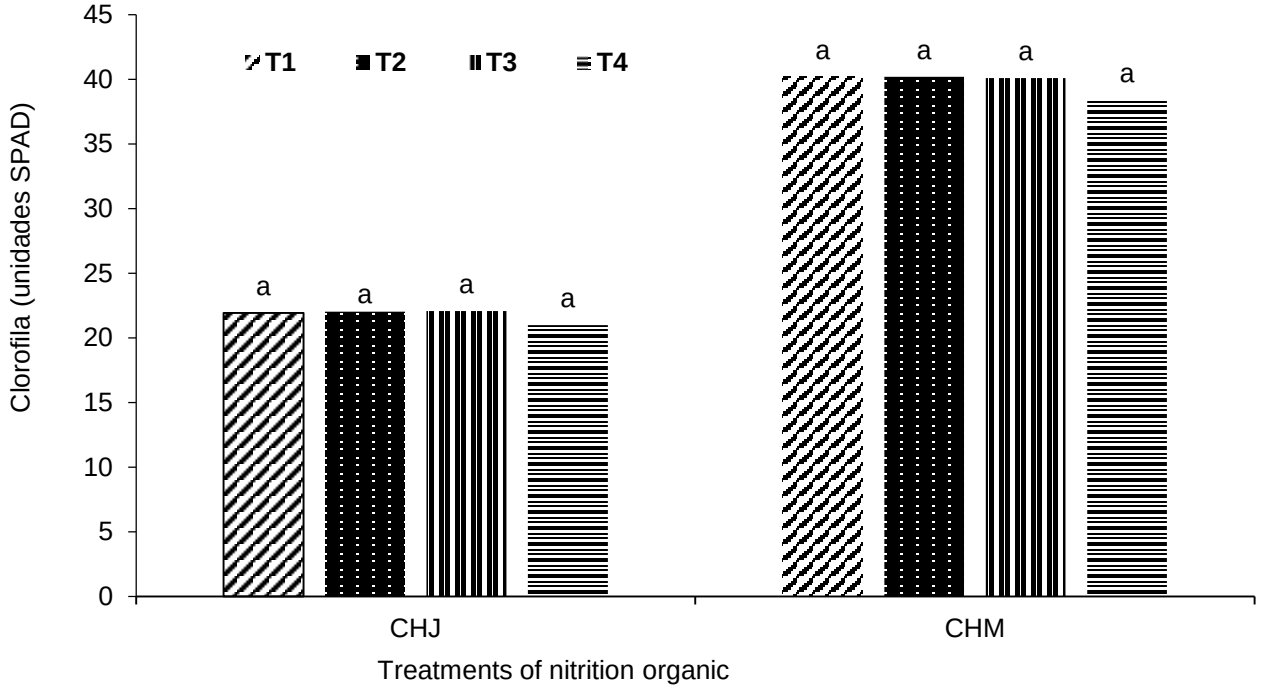

Fig.3: Effect of nitrition organic in content of chlorophyll young leaves (CYL) andchlorophyll of mature leaves (CML) in native avocados of Ometepec, Guerrero. T1 (sheepmanure), T2 (bovinemanure), T3 (mycorrhizae, GlumixIrrigation® Biostimulant) and T4 (control, commonwatertap), Tukey (P ≤ 0.01).en hojas jóvenes (CHJ) y maduras (CHM) de portainjertos de aguacates nativos del municipio de Ometepec, Guerrero. T1 (estiércol ovino), T2 (estiércol bovino), T3 (micorrizas) y T4 (testigo), Tukey (P ≤ 0.01).

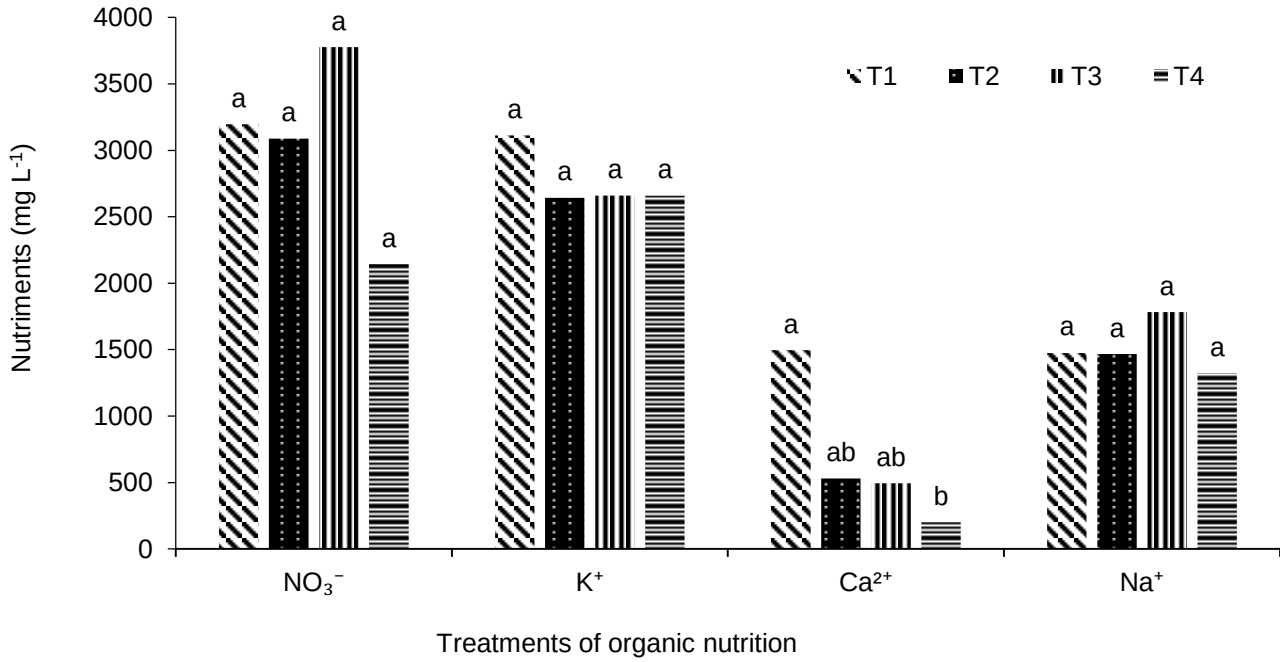

Fig.4: Effect of nutritionorganic in content of ions nitrate, potassium, calcium y sodium in native avocados of Ometepec, Guerrero. T1 (sheep manure), T2 (bovine manure), T3 (mycorrhizae, Glumix Irrigation® Biostimulant) and T4 (control, common water tap), Tukey (P ≤ 0.01).

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Potential of silicon fertilization in the resistance of chestnut plants to ink disease (*Phytophthora cinnamomi*)

Andreia Carneiro-Carvalho[a*], Catarina Pereira[b], Tiago Marques[c], Luís Martins[a,d], Rosário Anjos[a,e], Teresa Pinto[a,e], José Lousada[a,d] and José Gomes – Laranjo[a,e]

[a]CITAB – Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[b] Master student of Agricultural Engineering, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[c] Master student of Biochemistry, Universityof Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[d] CIFAP-ECAV – University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal
[e] DeBA-ECVA – University of Trás-os-Montes and Alto Douro (UTAD), Department of Biology and Environment, 5001- 801 Vila Real, Portugal
*andreiamcc_500@hotmail.com, 910099195

***Abstract*—** *The European chestnut (Castanea sativa Mill.) is a specie with great economic importance in Europe that have been present for thousands of years. In Portugal, the chestnut helps to maintain a positive trade balance, by contributing to the gross national product (GDP). One of the biggest threats for the chestnut is the ink disease caused by Phytophthora cinnamomi, this disease is problematic to chestnut crop with a damaging impact. Silicon (Si) is classified as a beneficial nutrient, having the ability to make plants more resistant to attacks by pathogens. Studies on the effect of silicon on chestnut are practically non-existent, so the aim of this study was to evaluate the impact of silicon in the resistance of chestnut plants to P. cinnamomi. The plants were treated by 0 mM, 5 mM, 7.5mM and 10 mM SiK® with the analyzed mad at 0, 15 and 30 days after inoculation by P. cinnamomi. These findings showed that the Si-treated plants had higher survival rate resulted from the presence of phytoliths in root tissues, that acted as a mechanical barrier reducing the development of pathogenic structures and they arealso associated with the improvement on antioxidant activity through the increase of CAT and SOD, higher values of total phenols compounds and less oxidative damage. The presence of Si in PDA medium reduced the growth of P. cinnamomi all over the time, presenting high PI. This work shows that the Si fertilization in chestnut plants contributes to increase the resistance against P. cinnamomi infection.*

***Keywords*—Biotic stress, Castanea sativa Mill, Fungitoxic, Phytophthora cinnamomi, Resistance, Silicon.**

I. INTRODUCTION

The sweet chestnut (*Castanea sativa* Mill.) is present in all countries of the Mediterranean Sea basin, playing an important role in the economy of these countries (Corredoira *et al*., 2012),covering large areas in France, Greece, Italy, Portugal, Spain, Turkey and the United Kingdom (Fernandez-López and Alia, 2003). This chestnut species has an important historical and cultural value, playing a key role in the economy and environmental sustainability of the mountain areas (Marinoni *et al*., 2013). However, the European chestnut has been strongly threatened by ink disease. *Phytophthora cinnamomi*was detected for the first time in Japan. In Spain and Portugal, it emerged during the 19th Century andwas subsequently reported in other countries of Europe (Italy, Greece, Switzerland, Turkey, France and the United Kingdom) (Vannini and Vettraino, 2001; Vettraino *et al*., 2005; Corredoira *et al*., 2012).

In Portugal, this disease has been responsible for the disappearance of more than 50% of the chestnut-producing areasince the 20th Century (Seabra *et al*., 2001; Martins and Abreu, 2007). This soil oomycete, which has asexual reproduction, attacks the root system and produces a black exudate that stains the surrounding soil leading the collapse of xylem and consequently to the death of the tree (Vannini and Vettraino, 2001). The *P. cinnamomi* representing one of the most devastating root rot pathogens of chestnuts (Balci and Halmschlager, 2003), by these reasons is essential to search for alternative strategies that can help the trees to increase their resistance against this pathogen.

In this context, the fertilization with Si in chestnut plants appear as a possible inducer of resistance against *P. cinnamomi* infection, considering the potential of Si as

an important and promising plant protector against several biotic stresses allowing to decrease the intensity of diseases in different crops in the world (powdery mildew and rice blast). Several authors verified that Si fertilization reduces the infection of angular leaf spot and *Colletotrichum lindemuthianum* in cotton (Oliveira *et al*., 2012) and bean plants (Polanco *et al*., 2014), respectively. On the other side, Côrtes *et al*. (2015) added that the Si is classified as an elicitor with potential through enzymes defense suppress the rice blast.

Several diseases were also suppressed by Si application (Rodrigues and Datnoff 2005). In this context, Seebold *et al*. (2001) have tested the effects of Si on several components of resistance to rice diseases using susceptible, partially resistant and completely resistant rice varietys. They found that the number of sporulation per lesion, lesion size, rate of lesion expansion, number of spores per lesion and diseased leaf area were significantly reduced by Si application. Moreover, the presence of brown spot, stem rot, sheath brown rot on rice, *Fusarium* and *Corynespora* leaf spot on cucumber decreased with the increase of Si supplied. Datnoff *et al*. (2001), suggesting that production inputs can be better managed by using Si, allowing the reduction of pesticide elimination, as well as improved plant resistance. Furthermore, Bakhat *et al*. (2018) note that Si can reduce diseases such as blast to the same level as a fungicide, reducing costs and providing positive environmental benefits.

The objetive of the present study was to investigate the effect of Si fertilization in chestnut plants on the resistance toink disease (*P. cinnamomi*).

II. MATERIAL AND METHODS

Plant material and growing conditions

The experiments used 160 chestnut seeds (*Castanea sativa* Mill var. Sousã) from the same tree growing in the Germobank of University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal (41° 17′ 20″ N, 7° 44′ 0″ W). The seedlings were planted in 2 L filled pots with 3:1 turf and perlite and randomly organized into 4 groups with 40 pots each. The plants with 4 months old were then placed in the growing chamber, with a 12h photoperiod, radiation 1600 µmolfotões.m^{-2}.s^{-1}, 26 ºC, and watered on a daily basis.

Silicon Treatments

Silicon was applied 45 days after the plants were potted, as potassium silicate (SiK®), according to Ma and Takahashi (2002). In this way, four treatments were prepared and evaluated: 0 mM, 5 mM, 7.5 mM and 10mM SiK®. The silicon solutions were adjusted to pH 6.9 using 30 M hydrochloric acid (HCl). Each plant was fertilized with 50 mL of a SiK® solution, which was directly applied to the soil.

Isolation of *P. cinnamomi*

The *P.cinnamomi* isolate (IMI 340340) used in the inoculation was selected due to its virulence in accordance with previous tests (Abreu *et al*., 1999). The high pathogenicity of this isolate in European chestnuts was also confirmed by Dinis *et al*., (2011). The inoculum was prepared for growth in PDA (potato dextrose agar) during 6 days at 25°C in the dark.

Leaf mineral analysis

The samples were analysed using the standard procedures of the University of Trás-os-Montes and Alto Douro Soil Analysis Laboratory. The preparation and analysis of chemical macronutrients (N, P and K) in leaves from Si-treated plants and untreated plants (0 mM SiK®) were done using the methods described by Malavolta *et al*., (1997). The content of Si in chestnut leaves was analyzed by the method described by Korndörfer*et al*., (2004).

Resistance tests to ink disease

Leaf disks inoculation with P. cinnamomi

The inoculation of leaves with *P. cinnamomi* was made according to Gouveia and Abreu (1994), with some changes to verify if there was a correlation between this inoculation form and roots inoculation. Six leaves per treatment were sampled from the non-inoculated plants. In the middle part of each one, 3 disks with 2 cm of diameter, including midrib, were punched. The disks were placed in petri dishes on a damp filter paper to maintain humidity conditions to the development of *P. cinnamomi*. An 8 mm disc of PDA inoculated with *P. cinnamomi* was placed on top of each leaf disc, as described earlier. The time, in hours, between the inoculation and the visible symptoms was evaluated daily over a period of 7 days, recording observations about theappearance of chlorosis in leaf disks was recorded.

Preparation of P. cinnamomi inocolum

The inoculum of *P. cinnamomi* was prepared from a mixture of potatoes, sugar and distilled water until boiling and then drained. The mixture was autoclaved for 20 minutes at 120°C and after the cooling period was inoculated with *P. cinnamomi* mycelium disks of about 8 mm diameter, from colonies with 10 days and posteriorly incubated in the oven at 25°C for 8 days.

Root inoculation with P. cinnamomi

The *P. cinnamomi* inoculum (50 mL) was applied in 20 chestnut plants per treatment (described in the plant material) directly in the soil, 60 days after SiK® fertilization. The plants were then monitored for 4 months, registering the time whenever a plant died.

Histopathology analysis

With a hand microtome, cross sections (1 µm thick) of secondary roots were obtained from untreated (0 mM SiK®) and Si-treated plants (5 mM, 7.5 mM and 10 mM SiK®) at 150 days after inoculation (Monteiro *et al*., 2017). The root samples were collected from three different plants per

treatment, avoiding lignified zones and the root tips. Sections were stained with a solution of 0.1 % toluidine blue-O solution in citrate buffer (pH 0.5) (Ruzin, 1999) to stain vegetative tissues in general and for specific detection of phenolic compounds in the middle lamella of woody species (Ruiz-Gómes *et al.*, 2015) and then sealed with a mounting medium (Entellan; Merck).

Pictures from sections were taken with an Olympus IX 51 inverted microscope (Olympus optical Co., GmbH, Hamburg, Germany) using an Olympus BX50.

The set of images obtained, five per sample, contained different tissue types: outer cortex containing epidermal cells, the central cylinder with medullary parenchyma, the vascular cambium, and the vascular tissue system such as xylem and phloem. The amorphous silicon bodies were analyzed with an optical microscope in the same root samples treated with SiK® mentioned above (Monteiro *et al*., 2017).

The histology analysis was performed to analysis the effect of Si fertilization on the resistance of root tissues against to *P. cinnamomi* infection.

Evaluation of the effect of soluble silicon application in PDA medium on the mycelial growth of *P. cinnamomi*

Filter sterilized solutions with different concentrations of Si (5 mM, 7.5 mM and 10 mM SiK®) were added to autoclaved PDA culture for Si+PDA medium by mixing 200 mL of Si solution with 350 mL of PDA (proportion of 1:2) being this process made for each concentration under study. The Si+PDA solutions were mixed with magnetic stirrers to ensure even distribution of Si and subsequently were decanted into sterilized Petri plates, according to Kaiser (2005). On the other hand, the control (0 mM SiK®) was represented by non-ameliorated PDA medium.

Then a 1 mm square of *P. cinnamomi* from 15 days old culture on PDA were transferred to the center of Petri plates (90 mm in diameter) containing SiK®+PDA and control plates (0 mM SiK®). The present methodology was adapted by Bekker *et al*., (2009) and was performed for each treatment (5 mM, 7.5 mM and 10 mM SiK®) under study.

The Petri plates from all treatments were incubated in a chamber at a temperature of 25ºC in the darkness. The evaluations were carried by measuring the daily diameter of *P. cinnamomi* using a ruler, ending when the control colonies reached the entire surface of the Petri plate. The percentage of inhibition (PI) was used to calculate the colony diameter growth of *P. cinnamomi* according to the formula (Ebrahimi *et al*., 2012):

$$PI = (C-I)/C * 100\%$$

PI – Percentage of mycelial growth inhibition

C – Diameter of mycelial growth in control treatment

I – Diameter mycelial growth of Si treatment

This methodology was made to evaluated the impact directly of Si application on growth of *P. cinnamomi* in PDA medium. Data presented were resulted by twenty replicates for each treatment.

MDA and hydrogen peroxide contents

Lipid peroxidation in the leaves was measured in terms of malondialdehyde (MDA), a product of lipid peroxidation which is formed in a reaction mixture containing thiobarbituric acid. The MDA amount was determined at 532 nm, followed by correction for the non-specific absorbance at 600 nm using an extinction coefficient of 155 $mM^{-1}cm^{-1}$ as described by Farooq *et al*., (2013).

Hydrogen peroxide (H_2O_2) amount was determined according to Schurt *et al*., (2014). The absorbance was measured at 390 nm and the H_2O_2 content was computed by using the extinction coefficient of 0.28 $mmol^{-1}cm^{-1}$.

The MDA and H_2O_2 content quantification was measured to analysis the impact of Si fertilization on oxidative damage. These measurements were held between 0 and 30 days after inoculation and were replicated 6 times per treatment (n=6).

Total phenols compounds determination

The total phenols were determined according to the Folin-Ciocalteu's procedure of Singleton and Rossi (1965) with the remainder alcoholicextractof the photosynthetic pigments. The absorbance of these metabolites was quantified at 795 nm. These measurements were held between 0 and 30 days after inoculation and were replicated 6 times per treatment (n=6).

Antioxidant activity determination

The antioxidant activity determination was made for evaluated the role of Si application in host defense system.The activity of catalase (CAT) was measured by Wu *et al.*, (2014) method. CAT activity was determined as the rate of disappearance of H_2O_2 at 240 nm, for 1 minute. Reaction mixture (3 mL) included 50 mM potassium phosphate buffer (pH 7), and the activity was expressed as µgmol/min/g FW. The activity of superoxide dismutase (SOD) was assayed by Roohizadeh *et al*. (2014) and was expressed as U g^{-1} FW. Reaction mixture containing 50 mM potassium phosphate buffer (pH 7.8), 1.3 µM riboflavin, 0.1 mM EDTA, 13 mM methionine, 63 µM NBT, 0.05 M sodium carbonate (pH 10.2) and enzyme extract, was used. The photoreduction of NBT was measured at 560 nm.

These measurements were held between 0 and 30 days after inoculation and data from all the enzymes corresponded to four replicates per treatment (n=4).

Statistical analysis

Data were expressed as means. For statistical analysis, the Turkey's test ($P< 0.05$) was applied.

III. RESULTS

Leaf mineral analysis

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Table 1 presents the composition in mineral nutrients and Si amount of Si-free plants and Si-treated plants. The Si-treated plants showed a significant increase on Si content, presenting an increase of 298% between 0 mM SiK® and 10 mM SiK® treatments. In addition, data showed that the Si content increases in Si-fertilized chestnuts with the enhance of the Si concentration applied, 1.81, 2.45 and 3.98 mg $Si.g^{-1}$ recordedin 5 mM, 7.5 mM and 10 mM SiK® treatments, respectively while control plants (0 mM SiK®) showed only 1.00 mg $Si.g^{-1}$ (Tab. 1).

Table.1: Amount of mineral nutrients and silicon content in chestnut leaves of all treatments (0 mM, 5 mM, 7.5 mM and 10 mM SiK®) under study (n=3).

Treatment	Si (mg $Si.g^{-1}$)	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})
0 mM SiK®	1.00 ± 0.001	25.3 ± 0.025	3.0 ± 0.004	30.5 ± 0.037
5 mM SiK®	1.81 ± 0.003	30.7 ±0.033	3.5 ± 0.001	40.3 ± 0.056
7.5 mM SiK®	2.45 ± 0.005	34.5 ± 0.019	3.9 ± 0.002	41.6 ± 0.068
10 mM SiK®	3.98 ± 0.001	40.2 ± 0.048	4.8 ± 0.002	42.1 ± 0.026

Additionally, as shown in Tab. 1, the Si application promoted a significant increase in the content of N, P and K, where the percentage increases with the Si concentration applied in chestnut plants, in 10 mM SiK® treatment was 59% N, 60% P and 38% comparatively to control treatment (0 mM SiK®).

Resistance tests for ink disease and survival analysis

Analyzing the resistance tests, the Figure 1a showed clearly that in Si-fertilized leaf disks, the development of chlorosis was significantly delayed and reduced compared to non Si-fertilized plants (0 mM SiK®). The results indicate that the plants behavior differs in relation to *P. cinnamomi* inoculation, on the Si absent plants (0 mM SiK®), the symptoms of oomycete infection appeared after 78h. In contrast, in the Si-fertilized leaf disks with 7.5 mM and 10 mM SiK® (Fig. 1a) it took 156 and 139 h for chlorosis to appear and/or necrosis in leaf disks. These results are consistent with those shown in Figure 1b, where the number of non-affected disks increase with Si concentration applied. On 10 mM SiK® treatment, 72% of the disks did not present chlorosis, while in the control (0 mM SiK®) only 11% of the disks remained free of chlorosis.

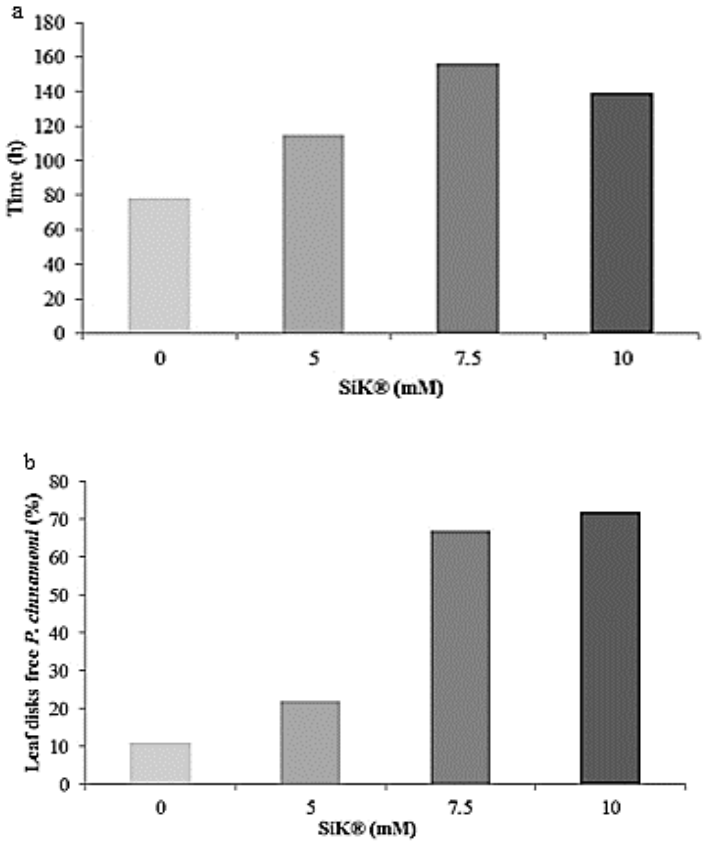

Fig.1: Resistance test to P. cinnamomi. a - Meantime (h) of chlorosis appearance in leaf disks from 0 mM, 5 mM, 7.5 mM and 10 mM SiK®. b - The percentage of leaf disks free from P. cinnamomi (n=6).

The Figure 2 presents the survival rate of chestnut plants from the different treatments under study against to *P. cinnamomi* infection. The higher values of survival rate demonstrated higher resistance to the severity of ink disease.

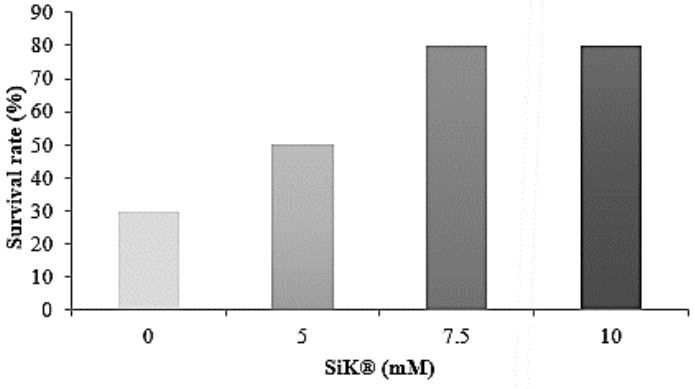

Fig.2: Survival rate of non Si-fertilized (0 mM SiK®) and Si-fertilized (5 mM, 7.5 mM and 10 mM SiK®) plants 150 days after inoculation with P. cinnamomi (n=20).

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Consistently, after 150 days of inoculation, only 40% of control plants (0 mM SiK®) remained alive, unlike Si-fertilized plants, of which 80% survived for the 7.5 mM and 10 mM SiK® (Fig. 2). Therefore, the present findings suggesting that the highest concentrations of Si (7.5 mM and 10 mM SiK®) have more resistance against to *P. cinnamomi* inoculation compared to untreated plants (0 mM SiK®).

Histopathology analysis

Figure 3a illustrates the degree of infection by *P. cinnamomi* in the root cortex and cortical parenchyma from each one of the treatments (0 mM, 5mM, 7.5mM and 10 mM SiK®).

The degree of infection by *P. cinnamomi* was assessed by the amount of the oospores in the cortex and vascular cylinder root cells. Roots from the Si absent plants (0 mM SiK®) showed a high degree of infection in the cortical parenchyma, whichwere fullycolonized by the oospores (Fig. 3b and arrows a), reason why a high number of these pathogenic structures is observed in the parenchyma cells of the vascular cylinder and leading to the disruption and occlusion of xylem vessels (Fig. 3a). In the root cells of cortical parenchyma many oospores were detected, dispersed throughout the cortical tissue. However, as the Si concentration increased in plants a decrease in the infection degree was observed, both in cortical parenchyma and vascular cylinder tissues, (Fig. 3b and arrows a), suggesting that Si fertilization might reduce the incidence of *P. cinnamomi* infection in chestnuts plants.

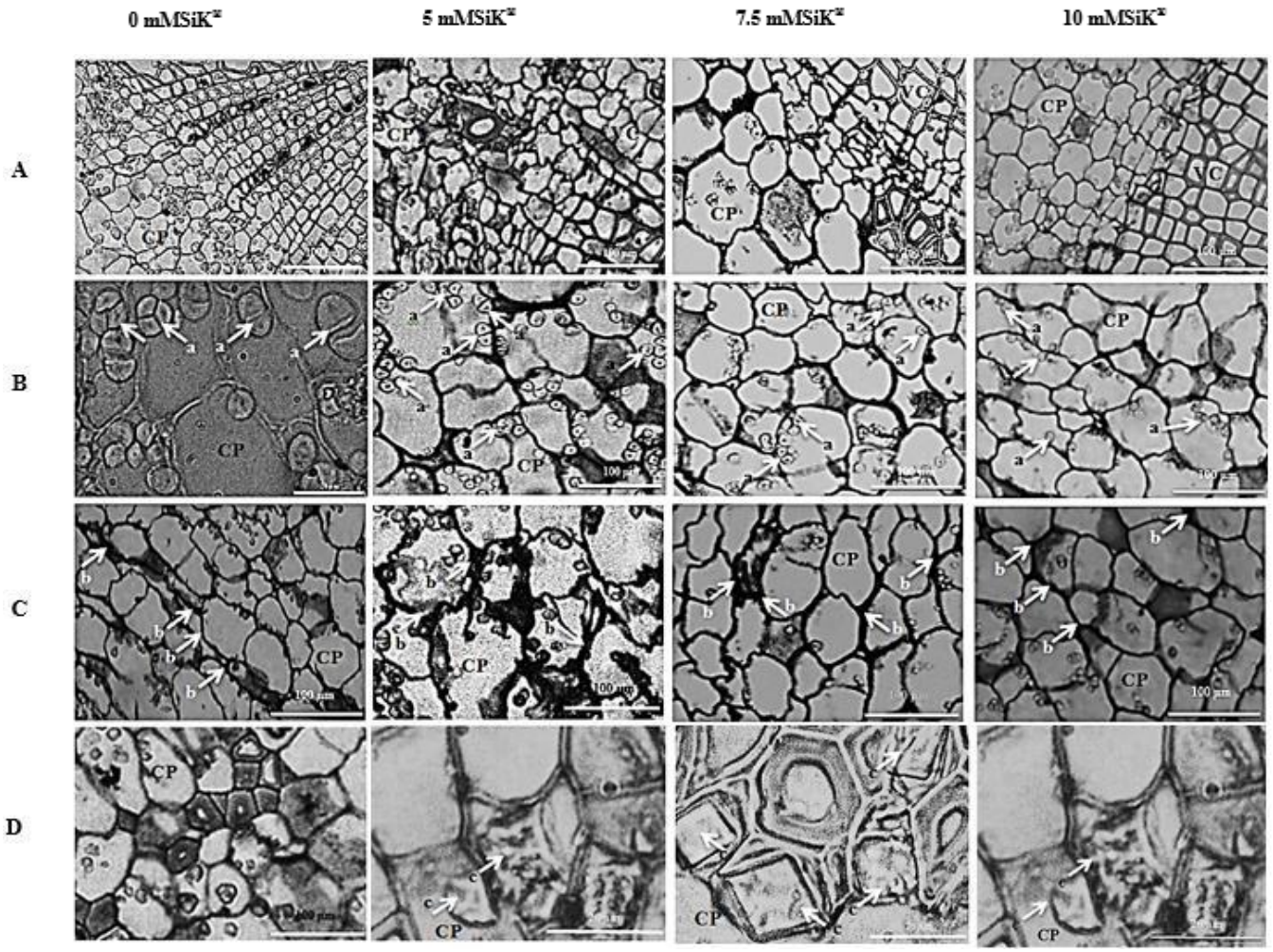

Fig.3: Cross section of 0 mM,5 mM, 7.5 mM and 10 mM SiK® treatment chestnut roots at 150 days after P. cinnamomi infection, analyzing the degree of infection (A), the presence and number of oospores (B), the occurrence of hyphae (C) and the presence of phytoliths (D).CP. Cortical parenchyma, VC. Vascular cylinder, E. Endoderm, arrow a. oospores,arrow b. hyphae,arrow c. phytoliths. Bars = 100 µm in A, B, C and D (0 mM SiK®). Bars = 20 µm *in D of 5 mM, 7.5 mM and 10 mM SiK®* treatments.

Otherwise, multiplication of oospores in the root cortical parenchyma seems to be influenced by SiK® fertilization, since higher rates are visible on untreated plants (0 mM SiK®) and 5 mM SiK® treatment than in 7.5 mM and 10 mM SiK®concentrations (Fig. 3b and arrows a). The presence of these pathogenic structures in the xylem cells was responsible for the reduction of water translocation in the xylem vessels inducing their cavitation. Thus, water stress and, consequently, the death of the plants is the result of the action of this pathogen, as can be observed in the

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results showed in Fig. 2, where the percentage of surviving plants in the control treatment was only 30%.

In addition, a high number of *P. cinnamomi* hyphae (Fig. 3c and arrows b) was detected in the cell walls of root tissues from the Si deprived plants (0 mM SiK®). Their presence was indicated by the strong black color in the cell wall of root tissues (Fig. 3c). In Si-fertilized plants, the number and intensity of these structures (hyphae) in the cortical parenchyma was lower than in the former plants, decreasing with the increase of SiK®concentration (Fig. 3c and arrows b). After 150 days *P. cinnamomi* inoculation, hyphae (Fig. 3c, arrows b) was identified in the cortical parenchyma and the oospores (Fig. 3b and arrows b) in the same tissue and also in the vascular cylinder in non-treated plants (0 mM SiK®), while in SiK® groups they were practically nonexistent inside the vessels. On the other hand, only in Si-treated plants it is possible to observe the presence of the amorphous silicon deposits (phytoliths), crystals with cubic form in cortex tissue next to endoderm, involving all the vascular cylinder (Fig. 3d arrows c).Data suggested a direct correlation between the Si contents (Tab. 1) and the number of phytoliths in chestnuts plants.

The presence of phytoliths in the roots of inoculated plants appears to have a protective effect against pathogen penetration in the vascular system of plants.

Evaluation of the effect of soluble silicon on the radial growth of *P. cinnamomi*

Regarding the effect of different concentrations of Si on the growth of *P. cinnamomi* (Figs. 4, 5 and 6), the results show significantly differences between Petri plates contain Si (5 mM, 7.5 mM and 10 mM SiK®) in PDA medium and control (0 mM SiK®) with only PDA medium. Analyzing the Fig. 4 it can be observed that all Petri plates containing Si+PDAdidn't present any growth of *P. cinnamomi*, demonstrating an evident percentage of inhibition (PI) of 100% at 24h after incubation while the control (0 mM SiK®) showed a faster growth of this pathogen, presenting therefore a PI of 70% (24h, Fig. 4).

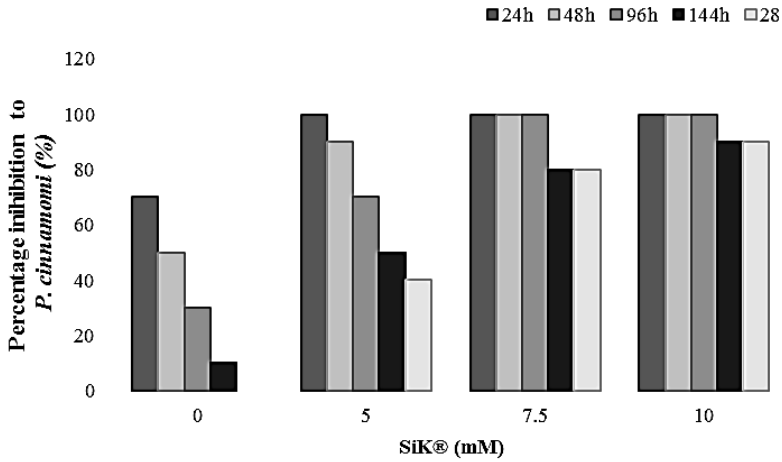

Fig.4: Percentage inhibition (PI) against to P. cinnamomi development in control petri plates (0 mM SiK®) and Si+ PDA petri plates (5 mM, 7.5mM and 10 mM SiK®). The PI were measured at 24, 48, 96, 144 and 288 h after incubation (n=20).

At 48h the 7.5 mM and 10 mM SiK® treatments, still maintain the total inhibitional against *P. cinnamomi*, showing a 100% of PI, followed by 5 mM and 0 mM SiK® treatments with 90 and 50% of PI, respectively (Figs. 4 and 5). As shown in Fig. 5 a great development of *P. cinnamomi* were observed in control and 5 mM SiK® treatments, while the high inhibition capacity were found in the highest concentration of Si applied in PDA medium (7.5 mM and 10 mM SiK®). Between 48h and 144h, the control treatment showed a reduction of 60% on PI comparatively to Si treatments (Figs. 4, 5 and 6).

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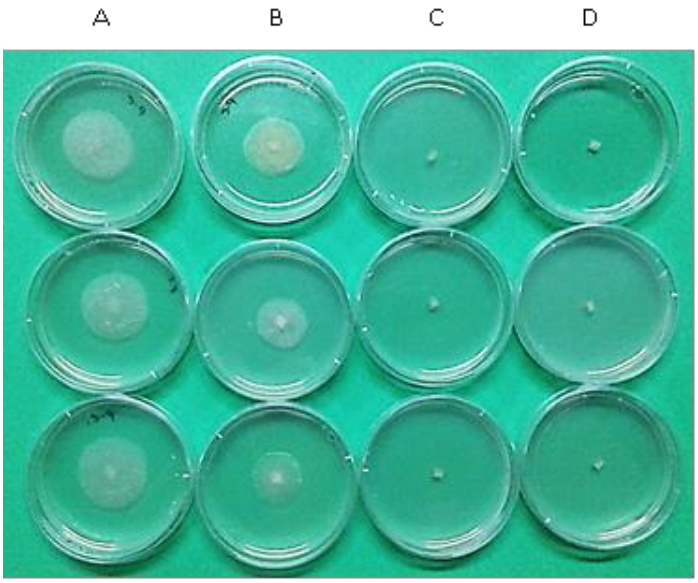

Fig.5: Mycelial grown of P. cinnamomi in response to 0 mM (A), *5 mM* (B), *7.5mM (C) and 10 mM SiK® (D) application on PDA at 48h after incubation.*

In the current study, the soluble Si application was effective in inhibiting the growth of *P. cinnamomi* in vitro which was directly associated to the augment of Si concentrations applied, recording 50%, 80% and 90% of PI in 5, 7.5 and 10 mM SiK® treatments, respectively (Figs. 4 and 6). In these treatments was also noted that from early period of incubation (24h) the inhibition of growth *P. cinnamomi* is quickly manifested by the presence of Si in PDA medium, demonstrating a great ability to reduces the growth and development of this problematic oomycete. Data is consistent with the results obtained previously, indicating that the application of Si to soil can help to reduce their propagation capacity of ink disease in chestnut plants.

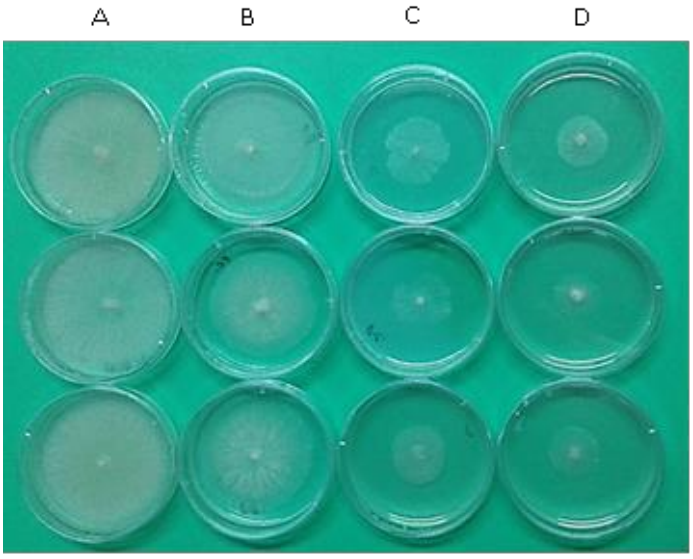

Fig.6: *Mycelial grown of P. cinnamomi in response to 0 mM* (A), *5 mM* (B), *7.5 mM (C) and 10 mM SiK® (D) application on PDA at 288h after incubation.*

MDA and hydrogen peroxide contents

MDA is considered an indicator of lipid peroxidation in the cell wall membrane. In the present study, a significant reduction on MDA amount were observed between 0 mM and 10 mM SiK® treatments, 61% and 68% at 15 and 30 days after inoculation (Fig. 7). Moreover, the 7.5 mM and 10 mM SiK® treatments recorded the lower values of MDA, 0.840 and 0.610 μmol g^{-1} FW (Fig. 7) respectively, compared to control treatment (0 mM SiK®) that achieved the higher value, 1.891 μmol g^{-1} FW at 30 days after

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inoculation (Fig. 7), indicating that Si application can affect the ability of *P. cinnamomi* to infected the plants reducing the damage in cell membrane, while the untreated plants (0 mM SiK®) showed a significative augmented on MDA level in same time.

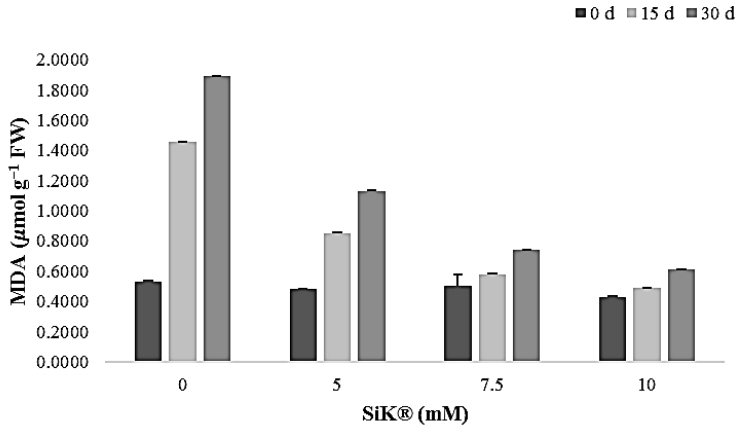

Fig.7: Effect of Si application (0 mM, 5 mM, 7.5mM and 10 mM SiK®) on malondialdehyde (MDA) amount at 0, 15 and 30 days after inoculation by P. cinnamomi (n=6).

A similar trend was observed in H_2O_2 amount, the Si-treated plants (10 mM SiK®) recorded a significant decrease on this parameter, 37% at 15 days and 54% at 30 days after *P. cinnamomi* infection compared to Si-deprived plants (Fig. 8). At 15 days after inoculation the lower values of H_2O_2 amount were 0.410 and 0.390 mmol g^{-1} FW in plants treated with the highest concentrations of Si (Fig. 8). Comparing the results between 0 and 30th day, the H_2O_2 content (Fig. 8) increased 357% in Si-free plants while in Si-supplied plants (10 mM SiK®) recorded an increase of only 14%. These results suggest that Si application contributes to reduce the lipid peroxidation and oxidative stress in chestnut plants inoculated by *P. cinnamomi*.

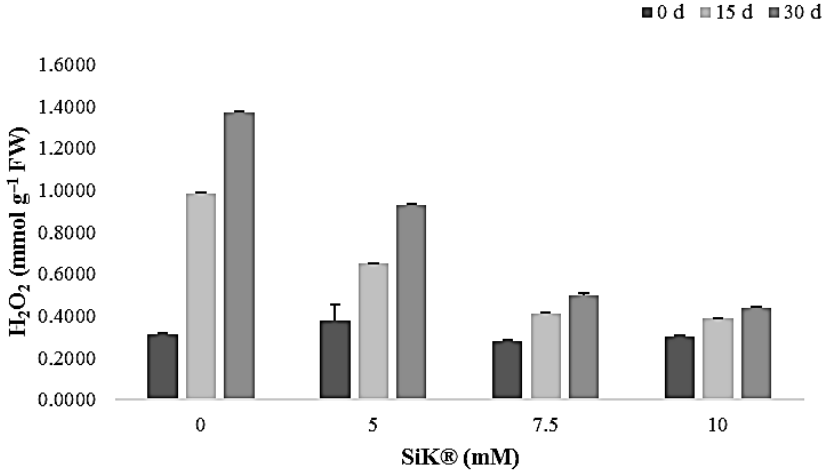

Fig.8: Effect of Si application (0 mM, 5 mM, 7.5mM and 10 mM SiK®) on hydrogen peroxide (H_2O_2) amount at 0, 15 and 30 days after inoculation by P. cinnamomi (n=6).

Total phenols compounds

As shown in Figure 9, the Si fertilization increases the total phenol compounds (TP) content in response to *P. cinnamomi* infection, with an augment of 180% and 194% in 7.5 and 10 mM SiK® treatments, respectively at 15 days after inoculation. At 30 days, the synthesis of TP was more significative, with an increase of 350% and 393% (Fig. 9) recorded by the chestnut plants treated with the highest concentrations of Si (7.5 and 10 mM SiK®). Moreover, it is important highlight that the TP amount increased significative from 0.20 to 0.81 mg g-1 FW^{-1}, between 0 and 10 mM SiK® treatments, representing an increase of about 305% at 30 days after *P. cinnamomi* infection (Fig. 9).

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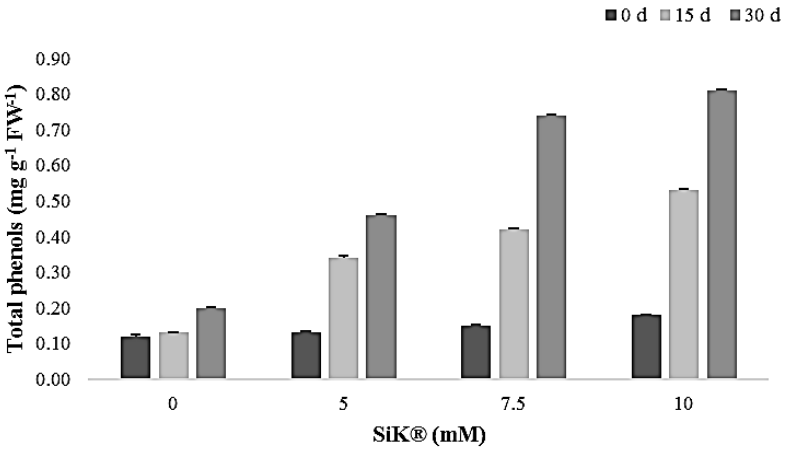

Fig.9: The effect of Si application (0 mM, 5 mM, 7.5mM and 10 mM SiK®)on the total phenol compounds at 0, 15 and 30 days after inoculation by P. cinnamomi (n=6).

Antioxidant activity

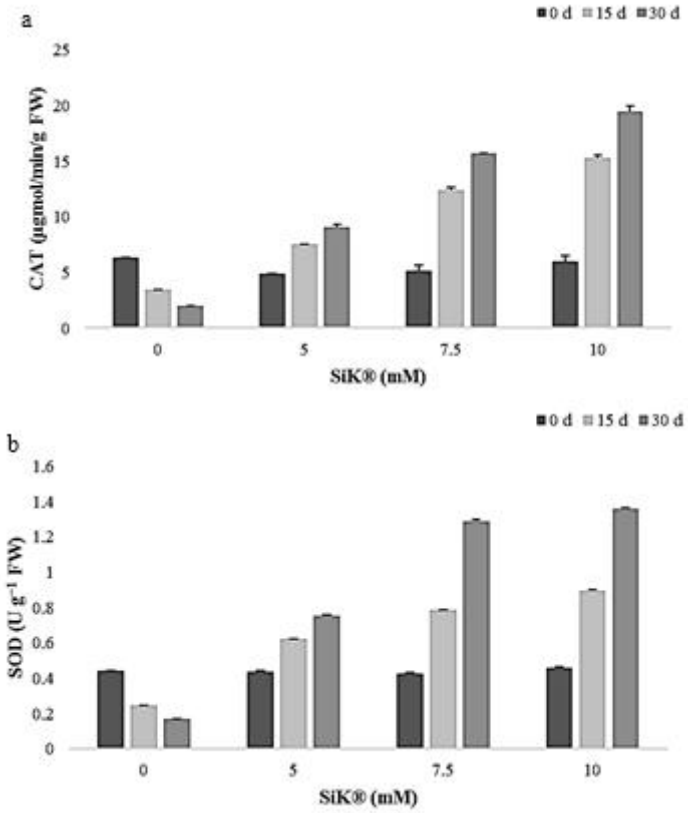

Fig.10: *Activities of catalase (CAT) and superoxide dismutase (SOD) in untreated (0 mM SiK®) and Si-treated plants(5 mM, 7.5 mM and 10 mM SiK®) at 0, 15 and 30 days after P. cinnamomi infection (n=4). a. CAT activity expressed as µgmol/min/g FW. b. SOD activity expressed as U g^{-1} FW.*

The CAT and SOD activity on untreated and Si-treated plants are illustrated in Fig. 10a and 10b, respectively. Data showed that Si application enhanced significantly the CAT activity in response to inoculation, recording an increase of 73% and 102% (10 mM SiK®, Fig. 10a) at 15th and 30th day, while the control (0 mM SiK®) presented a reduction of 46% (15th day, Fig. 10a) and 64% (30th day, Fig. 10a). Similar tendency was observed in SOD activity (Fig. 10b), theSi fertilization influenced significantly the activity of this enzyme, conferring higher protection of the host plants against to this root diseases through improvement their defense system than control plants (0 mM SiK®). The SOD activity increased significantly in 7.5 mM and 10 mM SiK® treatments than control treatment (0 mM SiK®). As shown in Fig. 10b, in Si-treated plants (10 mM SiK®) was recorded a significant increase on SOD activity from 0.456 to

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0.895 U g^{-1} FW between 0 and 15th day after inoculation, however in Si absent plants (0 mM SiK®) is observed an decrease from 0.443 to 0.244 U g^{-1} FW. Furthermore, the values of SOD activity were significantly higher at 30th day than those in 0 day, increasing 198% in 10 mM SiK® treatment, while in untreated plants (0 mM SiK®) suffer a reduction of 62% (Fig. 10b). As shown in Fig. 10b, the SOD activity increased with time in all Si-treated plants by 30%, 50% and 70% for of 5 mM, 7.5 mM and 10 mM SiK® treatments, respectively, reached the highest amount at 30 days (Fig. 10b).

IV. DISCUSSION

The Si have a beneficial role in the protection of agricultural crops against diseases, however the effect of this element in chestnut plants against ink disease, was never approach, reason for why we decided investigated.

The exogenous application of Si enhanced significantly the Si amount and promoted a greater absorption of N, P and K (Tab. 1) in chestnut plants, being this nutrients benefits to the growth and development of crops. Similar results were found by Pati *et al*., (2016) in rice plants. The improvement of mineral nutrition observed in Si-treated plants demonstrates that Si applicationenabled the augment of the solubility and the absorption of the Si, N, P and K, as verified by Epstein, (1999); Bekker *et al*., (2007); Lima Filho and Tsai, (2007).

The increase of Si amount in Si-treated plants can be explainedby the augment of Si availability in the soil and with the enhance of root system, stimulating the chestnuts to absorb more Si from soils. These results are in accordance with Datnoff and Rutherford (2004); Lima Filho and Tsai (2007) who reported a significant increase in the percentage of Si accumulated in bermudagrass, wheat and oat leaves treated with the higher rates of calcium silicate.

The resistance of plants to ink disease is generally determined by the greater or lesser ability of the host plant to limit penetration, development and/or reproduction of this phytopathogen in their tissues after being inoculated by the invading agent (Gouveia and Abreu, 1994).

The beneficial role of Si in plants is associates frequently to the decrease diseases intensity, by their translocation and accumulation in tissues (Pozza *et al*., 2015). Data showed a correlation between the inoculation of leaves (Figs. 1a and b) and roots inoculation method (Fig. 2). In this work, the 7.5 mM and 10 mM SiK®treatments demonstrated a greater resistance against *P. cinnamomi* inoculation by hindering and avoid the appearance of chlorosis in the leaf discs, promoting a larger percentage of free chlorosis disks, as well as a high survival rate (80%) compared to control treatment (30%), suggesting that Si contributes to the augment of resistance in chestnut plants against to ink disease. Consistently, the presence of Si in PDA medium reduces the radial growth of *P. cinnamomi*, presenting a PI of 90% at 288h (Figs. 4 and 6), comparatively to Si-free plants that showed a PI of 0% in the same time. These results can be explained by the phytotoxic effect of Si, which increase with the rise Si concentration applied (Fig. 4 and 6). These findings confirmed previous studies of Ebrahimi *et al*., (2012) and Farahani *et al*., (2012 a, b), who reported that addition of increasing concentrations of Si to PDA medium completely inhibited the mycelial growth of *P. expansum* and *Candida membranifaciens* in apple plants allowing the biocontrol of these pathogens. Additionally, Kaiser *et al*., (2005) and Mahdikhani *et al*., (2008), referred that the application of SiK® in Petri plates has the capacity to reduce the growth of *P. cinnamomi* and *Fusarium oxysporum* in avocado and melon plants, respectively.

The roots histopathology carried out allows to analyze and understand the state of the roots tissues and the degree of colonization of these by *P. cinnamomi* infection in the different treatments under study (Fig. 3). In Si-fertilized plants were observed the accumulation of cubic amorphous Si bodies, denominated phytoliths (Yoshida *et al*., 1962) in the root tissues. The observation of phytoliths with cubic form were also reported by Ma and Yamaji (2006) and Neethirajan *et al*. (2009) in grass plants. Phytoliths act as a mechanical barrier due to the silicified of cells lead to the augment the resistance of the cell wall difficulting the entry of the oomycete, its development and their respective colonization making their walls thicker and more rigid, being one of the explains to the reduced number of hyphae and oospores observed in root cuts from Si-treated plants compared to control, as also verified by Monteiro *et al*. (2017). The presence of oospores in infected root tissues has also been reported by others authors in avocado, soybean and holm oak (Mircetich and Zentmyer; 1967; Ruiz-Goméz *et al*., 2012).These results suggest that the cell wall fortification of chestnut roots induced by Si-addition may be closely associated with the enhance of host resistance to ink disease. Indeed, plants treated with the highest concentration of Si showed a higher total phenols compounds (TP, Fig. 9), a higher minerals content (Tab. 1) and a higher percentage of tolerant plants to ink disease than non-treated plants (Fig. 2). Similar results were also reported by Amaral *et al*., (2008) in coffee plants.

Several authors also observed pathogenic structures (hyphae and oospores) in holm oak, *Quercus suber* (Ruiz-Gómes *et al*., 2015) and *Quercus ilex* (Ebadzad *et al*., 2015) infected with *P. cinnamomi*. These findings are in accordance with the studies of Oh and Hensen (2007) in oregon ceder plants and Monteiro *et al*., (2017) in chestnut plants, who observed the presence of oospores inside phloem and xylem cells in non Si-fertilized plants (0 mM SiK®) and a very lower number in Si-treated plants (10 mM SiK®, Fig. 3). Moreover, the high number of

pathogenic structures (hyphae and oospores, Fig. 3) are justified by oomycete's need for nutrients increased after reaching the parenchymal cells of the central cylinder, allowing for faster growth and, finally, for expansion towards new unexplored root areas through vascular tissues (Ruiz-Gomés *et al*., 2015).

Data also indicate that the augment in concentration of Si applied increase the number of phytoliths in plants tissues and consequently reducethe infection in root tissues by the oomycete responsible for ink disease. Similar results were found by Huang *et al*., (2011), who reported that Si reduce the damages of *Fusarium crown* in root tomato plants, indicating its ability to block the progression of the fungus.

Relatively to the chemical defense proportionated by Si fertilization in chestnut plants, the CAT and SOD are considered important antioxidant enzymes responsible for the defense responses and are frequently associated with the reduction on the reactive oxygen species (ROS) in resistant plants against diseases, according to Sakr, (2016). In the current study, Si applicationenhance significantly CAT and SOD activity (Fig. 10) after infection by *P. cinnamomi*, that suggesting the Si reduce the H_2O_2 amount and restricts the development of this pathogenby inducing synthesis of antioxidant enzymes and phenols compounds in host plants.

CAT is an enzyme that decompose the H_2O_2 into water and oxygen (Song *et al*., 2016). In this context, the results demonstrated high CAT activity in Si-treated plants (Fig. 10a) lead to lower H_2O_2 amount in tissues than control plants (0 mM SiK®, Fig. 10a). The extent of the damage caused by *P. cinnamomi* inoculation can be associated to the oxidative stress, the infection of chestnut plants by ink disease promote an accumulation of H_2O_2(Fig. 8) and consequently, higher MDA amount (Fig. 7), the first product in membrane lipid peroxidation that allow to index the degree of injury to the cells.

The beneficial effect of Si in CAT and SOD activity were also reported by Fortunato *et al*., (2012) and Schurt *et al*., (2014) in banana and rice plants against *Fusarium wilt* and *Rhizoctonia solani*, respectively.

Data showed that the high SOD activity in Si-treated plants (Fig. 10b) reduced MDA level (Fig. 7), while the augment in CAT activity (Fig. 10a) decreased the H_2O_2 accumulation (Fig. 8), demonstrated that Si application as associated with their ability to resist biotic stress. These results are in agreement with Mohaghegh *et al.*, (2011) who reported that the Si addition in cucumber plants inoculation by *P. melonis* increase the CAT and SOD activity, enzymes involved in the plant-pathogen tolerance.

The MDA and H_2O_2 amount (Figs. 7 and 8) in chestnut plants at 30 days after inoculation was significantly higher than 15 days, suggesting that the injury caused by *P. cinnamomi* increased with a prolonged infection period in plants. Our results reinforce that Si addition protects antioxidant enzyme system in chestnut plants helping to increase their resistance caused by ink disease infection. The Si-absent plants recorded a decline in CAT and SOD activity, while Si-treated plants exhibited higher values of this enzymes, indicating that these enzymes reduced the free radical damage and thus improve their resistance, because Si promotes the systemic acquired resistance defense, by plant signaling against pathogens and synthesis of defense compounds, reinforcing the previous studies of Lu *et al*., (2008); Mohaghegh *et al*., (2011) and Fortunato *et al*., (2012) in asparagus, cucumber and banana plants, respectively.

The present results demonstrate that the antioxidant enzyme activities improve with time after *P. cinnamomi* inoculation in 7.5 Mm and 10 Mm SiK® treatments. Additionally, the Si-treated plants recorded the highest levels of TP (Fig. 9), indicating that Si stimulate a quickly and efficient production of this compounds. Phenols in plants are important to their defense against this type of biotic stress by increasing their natural chemical defense. These results are supported by the researches of Chérif et al., (1992, 1994), who suggested that phenols increased in Si-fertilized cucumber plants after infection with *Pythium ultimum*, compared to control plants. Similar results were found by Han *et al*. (2016) in rice plants, who reported that Si interacts with the defense-associated signaling pathways and seems to regulate a range of physiological activities in plant stress defense.

The highest TP content (Fig. 9) recorded by Si-supplied plants are consistently with the highest values of *P. cinnamomi* free leaf discs (67% and 72%) (Fig. 1a) andsurvival rate (80%) (Fig. 2). Furthermore, these metabolites promote defense of the chestnut plants and help to maintain the healthy root tissue resulting in the decrease of pathogenic structures (Fig. 3). The resistance of fertilized plants with SiK® against *P. cinnamomi* may be associated with the physical barrier, composed by phytoliths as mentioned before (Fig. 3c) and with the high phenol amount.

The induction of antioxidant activity by the chestnut fertilization with Si may represent one of the mechanisms of action against the attack of *P. cinnamomi*. Several studies have shown that Si assists plants in defense against phytopathogens by inducing the defense reactions, biosynthesis of phytoalexins, enzymes and PR's proteins (Song *et al*., 2016; Wang *et al*., 2017).

The augment of plants defense by Si application has been associated to the increase of signaling components amount and the defense hormones (such as salicylic acid, jasmonic acid and ethylene), which are important to establish the plant's innate immune system and are associated with resistance. Besides that, after perceiving a pathogen-derived signal, the plant would create a faster and stronger immune

response to the pathogenic agents, the prophylactic effect of silicon is considered to be the result of both passive and active defense (Van Bockhaven *et al*., 2013). In addition, Si also regulates the genes defense as referred by Ye *et al*., (2013), who suggest that silicon application in plants can facilitate the accumulation of inactive cellular proteins involved in signal transduction, such as MAPKs, and lead to the rapid activation of these inert signaling components, thus increasing the host's defensive processes and/or the speed with which they are activated.

The Si-fertilized chestnut plants responded quickly and effectively to *P. cinnamomi* inoculation, rapidly activates the natural defense mechanisms of the host, and provides physical protection and chemical defense that help in increasing the resistance of plants to the attack of pathogen. Data reveal that Si improve the physical and biochemical defense of chestnut plants from 7.5 mM and 10 mM SiK® treatments which demonstrated more resistance against to this oomycete responsible by ink disease.

The present research suggests that SiK® fertilization could be successfully used in control of ink disease and can represent a control method that is efficient, cost-effective and not harmful to the environment. For these reason is necessary the divulgation of the knowledge about Si to farmers in order to helpcontrol chestnut diseases, increase the resistance of their plants and consequently improve the chestnut fruits production and quality.

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The present manuscript doesn't present any conflict of interest.

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Physico-Chemical and Microbial Analysis of Drinking Water of Four Springs of Danyore Gilgit Baltistan Pakistan

Samina Kanwal[1], Saif-Ud-Din[3*], Khalil Ahmed[2], Maisoor Ahmed Nafees[2], Sheheryar Anwar[3]

[1]Elysian Higher Secondary School Chinar Bagh Gilgit, Gilgit-Baltistan, Pakistan.
[2]Department of Biological Sciences, Karakoram International University Gilgit, Gilgit-Baltistan, Pakistan.
[3]Department of Environmental Sciences, Karakoram International University Gilgit, Gilgit-Baltistan, Pakistan.

Abstract— *Drinking water of good quality is essential for human physiology whose continual existence depends on the availability of water and any sort of contamination in water which is above the standard limits set by international water regulating agencies can lead to water related diseases. So, the present investigation was conducted to determine the physico-chemical and bacteriological contents of four springs i.e.Heshi spring 1, Heshi spring 2, Kitaab Roong, and Kooti spring and its distribution system such as water reservoir inlet, outlet, mid and end point of distribution systems, junction where it merge with glacier water. The temperature was in a range of 13ºC - 22ºC. The turbidity of water samples fluctuate from 0.02NTU-1.99NTU. The pH value was in a range of 6.2-7.1. Electrical conductivity range of minimum 122µS/cm to a maximum of 600µS/cm. The TDS of all water samples ranging from minimum of 164-513mg/l. The amount of reactive ortho phosphate was in a range of 26mg/l to 59mg/L. The amount of total phosphorous was in a range of minimum 23m/L to maximum of 120mg/L. The total bacterial count was in a range of 11CFU/100ml to 83 CFU/100ml.The findings showed there should be comprehensive standardization of drinking water of Danyore village according to guidelines of WHO water quality standards and make it safe for human consumption.*

Keywords— Drinking water, springs, Heshi, Kitaab Roong, Kooti, UV Spectrophotometer, Membrane Filtration Technique, Pour Plate Technique.

I. INTRODUCTION

Geographical location dominates the weather conditions of Gilgit-Baltistan, it's a valley in mountainous neighborhood southwest of Karakorum Range region. Winter is the dominant season of this orbit and prevails in the region for eight to nine months a year. Gilgit lacks significant rainfall averaging 120 -140 millimeters annually. While the summertime season is short and warm the piercing sun-ray raise the mercury up to 40 °C[1].

Water covers 71% of earth's surface on the off chance that we gage the level of oceans and seas, it turns out to be 96.5% of planet earth's water. The second and third supplies of water are ground water and ice sheets each contributing 1.7% to add up to globe. New water makes a moment division, which is 2.5% of earth's water and 99% of all crisp water supplies are found in ice sheets. About a fourth of total populace depends on these water sources [2].

One to a few liters of water requires each day to sidestep for dehydration. It is a basic asset to enhance sustenance security, horticulture, condition and biodiversity [3].

Water becomes obviously dirtied when contaminated by anthropogenic exercises. Water contamination has turned into a noteworthy issue overall in view of being cause for more than 14000 passings every day and a few sicknesses [4].

When we consider water contamination, we normally imagine sewage or industrial effluents spilling out of a release pipe and some lethal chemicals acquaint in with water because of human exercises, yet there are regular toxicants that threat as well. It additionally satisfies every single fundamental necessity of living people like their development, upkeep of body and to play out all life supporting exercises [5];[6].

Over the globe, water bodies are essential for survival. A large portion of the water supplies are in the form of streams. Unfortunately, for the most part streams are defiled because of a few human induced components, which dilute and dissolve waste into them. With the expanding of population, urbanization and industrialization, the earth is impressively dirtied even in the developing nations [7].

Massive shortage of edible water is felt in developing nations where requisites of public supply of drinking water is poor owing partially to the lack of enough

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financial obligations towards existing infrastructure. People in rural communities often route to other options for dependable source of water for drinking and to fulfil other domestic needs [8].

The bacteriological contamination of water is major problem in developing countries. According to WHO water pollution in developing countries, like Pakistan estimates about 80% carried out by domestic wastes. Studies have revealed that *E.coli* and *Enterococci* are widely used water quality indicators of fresh water sources, Enterococci particularly is more significant indicator of water quality. The presence of *Enterococci* in water show fecal contamination because of their loads in faeces and subject water is not considered as safe to drink [8].

II. MATERIALS AND METHODS

Study Area

The study was conducted on selected springs of Danyore Nallah "Manu Gah" of district Gilgit. Four springs namely Heshi Spring 1, Kitaab Rong Spring, Heshi Spring 2, Kooti Spring were selected for study they were present at 2637m, 2606m, 2607m, 1990m above sea level respectively. These springs merge with glacier water on its way down to valley and provide drinking water for Danyore village. Samples were collected from the eyes of above mentioned springs, from the junction where the spring water mix with glacier water stream, inlet and outlet of water reservoir and from two different taps of Danyore village.

Fig.1: Map of sampling sites

Sampling Design

Eighteen samples were collected from four springs of Danyore Nallah in the month of March and April four samples from each spring eye, one sample from junction wherespring water mix with glacier water, one sample from inlet and one from outlet of water reservoir, and two samples from distribution were collected for physico-chemical and microbial analysis. Temperature and elevation were measured on the spot using mercury glass in centigrade thermometer and GPS respectively and readings were noted down in data sheet. Samples for microbial assessment were collected in 1000 ml sterilized bottles by autoclaved from 20-30cm deep to avoid to let air bubbles as much as possible avoiding touching the bottom and polluting the samples with hands to prevent any contamination. Samples for physico-chemical assessment were collected in polythene bottles, prior sample collection rinsed thoroughly for at least three times. Each water sample was stored in separate plastic bottle with proper labeling. The watersamples were taken to (EPA certified water quality laboratory in Department of Biological Sciences Karakorum International University Gilgit Baltistan, pakistan) for further analysis. pH of the water samples was done in laboratory using Digital pH meter (AD 1020, ADWA). Prior measurement of pH, meter was calibrated with standard buffers of pH 4.0, 7.0 and 9.0. Turbidity was measured using Electronic Turbidimeter (TB1, VELF SCIENTIFICA) and expressed

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in terms of NTU. Conductivity of water samples was measured using Digital Conductivity Meter (AD3000, ADWA)and stated in terms if µS/cm. Total dissolved solids of the samples were obtained using Digital Conductivity Meter (AD3000, ADWA) and stated in mg/l.

UV Spectrophotometer (UV 23000II, model) was used to determine the concentration of Total phosphorus and reactive Orthophosphate by National research council for Ecosystem Study Verbania Pallanza ItalyWater chemistry laboratory analytical methods [9].

Bacteria were isolated through Membrane Filtration Technique by filtering 100 ml water through Cellulose Nitrate membrane Filter (0.45micro meter). Chromogenic X-Glu agar (Biolife, Itley), Slanetz and Bartley agar (Biolife, Itley) and Bile Esculine Azide agar BEA (Biokar diagnostics) were prepared following the instructions of the product and placed the filtered membrane on Chromogenic Agar for *E. coli* and Slanetz Bartley agar for *Enterococci.* Later on the colonies of *Enterococci* were confirmed by culturing on Bile Esculine Azide Agar.

Total Bacterial Count was done by Pour Plate Technique by culturing on Yeast Extract agar (BIOM lab, Malaysia). Yeast Extract agar was prepared by following the instructions labeled on the product. The procedure for bacteriological analysis was followed as per standard methods for the examination of water and waste water [10].

Statistical Analysis

The observed data was analysed Descriptive statistic and correlation with MS Excel 2007 and Statistix 8.1 software package. The multiple dimensional scaling of various parameters was analyzed using Statica software package (Statsoft Inc. 5.5).

III. RESULTS AND DISCUSSIONS

Correlation for Physico-Chemical Parameters

For all four springs eye, correlation of coefficient among four different springs eye included in the study are given in Table 1. It was found that there were no positive significant correlation among the variables yet a negative correlation was observed in a variable such as between Total Phosphorous and TDS (r=-0.959).

Table.1: Correlation for Physico-Chemical parameter of springs eye at p<.050

	TURBIDIT	**PH**	**TDS**	**REACT_OP**	**TOT_P**	**EC**
TURBIDIT	1					
PH	0.8787	1				
	p=.121					
TDS	0.0806	0.4957	1			
	p=.919	p=.504				
REACT_OP	-0.3116	-0.6551	-0.5049	1		
	p=.688	p=.345	p=.495			
TOT_P	-0.0584	-0.5201	**-0.9596**	0.7094	1	
	p=.942	p=.480	**p=.040**	p=.291		
EC	-0.1451	-0.1676	0.3559	0.6199	-0.087	1
	p=.855	p=.832	p=.644	p=.380	p=.913	

*Marked correlations are significant at $p < 0.05$

Total Dissolved Solids (TDS), Reactive Orthophosphate (REACT_OP), and Total Phosphorous (TP).

Physico-chemical parameters of springs eye by Multidimensional Scaling

The physico-chemical parameters of four spring's eye were based on multidimensional scaling where clear variations among various sources were observed. Kooti spring showed clear difference as compared to other three spring's eye. The main reason of this difference is due to the highest content TDS (513 mg/l) this finding is not in accordance with the WHO standard (<500 mg/l). However other spring eye sources showed less than this range.

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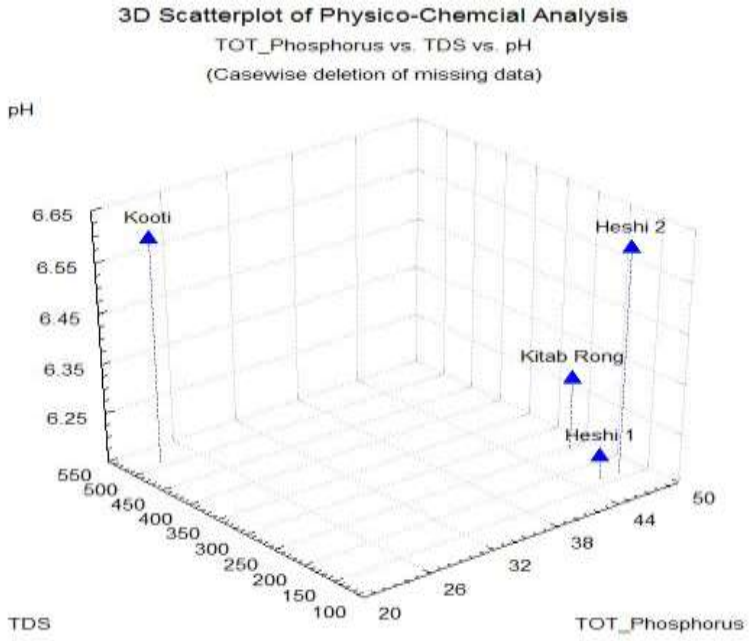

Fig.2: Three dimensional (3D) views of Physico-Chemical parameters of four spring's eye by multidimensional scaling

Correlation of Microbial Parameters

For all four springs eye, correlation of coefficient among four different springs eye included in the study are given in Table II. It was obtained that there were significant correlation among the variables. Like Heshi spring 1 and Kitaab Roong spring have significant correlation. Similarly Heshi spring 2 and Kitaab Roong and Kooti spring and Heshi spring 2 showed significant correlation (r=0.999).

Table.2: Correlation for Microbial parameter of springs eye at p<.050

	Heshi spring 1	**Kitaab Roong spring**	**Heshi spring 2**	**Kooti spring**
Heshi spring 1	1			
Kitaab Roong spring	**0.999**	1.000		
Heshi spring 2	0.997	**0.999**	1.000	
Kooti spring	0.994	0.998	**0.9996**	1.000

Microbiological parameters of springs eye by Multidimensional Scaling

The microbiological parameters of four spring's eye were based on multidimensional scaling where clear variations among various sources were observed. Kooti spring showed clear difference as compared to other three spring's eye. The main reason of this difference is due to the highest level of contamination this finding is not in accordance with the WHO standard (0CFU/100ml). However other spring eye sources showed less than this range.

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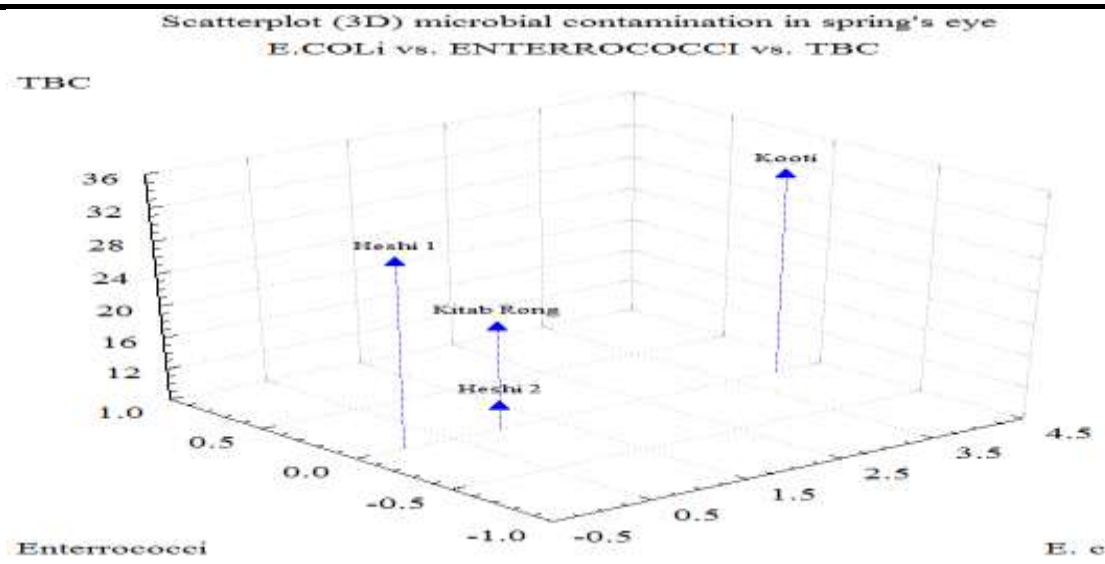

Fig.3: Three dimensional (3D) view of microbial parameters (E.coli vs. Enterococci vs. TBC) of four spring's eye by multidimensional scaling

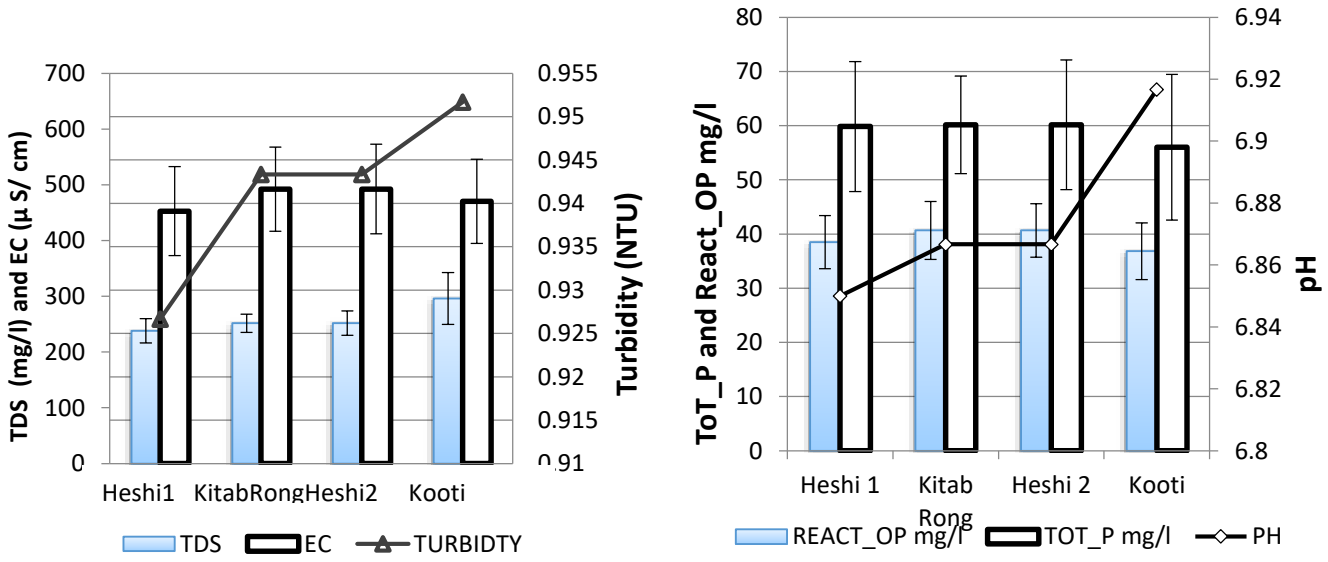

Fig.4: Variation in chemical parameters of all four springs eye

In time period of this study, there were significant variations in temperature measured in all the four springs (Heshi spring 1, Heshi spring 2, Kitaab Rong spring, and Kooti spring) and its various distribution systems. The temperature was in a range of 13ºC - 22ºC. The minimum temperature 13ºC was observed in Heshi spring and the temperature in rest of springs didn't differ much. Maximum temperature 22ºC was observed in distribution system mid (tap). The low temperature may be due to high altitude of spring eye 2637 meter above sea level and high temperature may be due to increased atmospheric temperature at the mid of distribution system. Physico-chemical parameter of four springsTDS was in a range of 164-513 with mean 272±82.681,The remarkable observation of the study was the level of total dissolved solids (TDS) in Kooti spring where it exceeds WHO permissible limit prescribed for drinking water. Excluding Kooti spring all samples fall in the limits set by WHO [11]. Drinking water with high level of TDS may lead to a number of diseases which are not water-borne but as a result of excess salts [12].Electric Conductivitywas in a range of 306-547 with mean 394.75±56.78. The electrical conductivity displayed variation in different water samples. It was in a range of minimum 122µS/cm to a maximum of 600µS/cm. None of the samples cross permissible limit of WHO standards of drinking water. The electric conductivity showed fluctuation possibly because of inorganic fertilizer inputs and from domestic sewage contamination [13] or might be as a result of bicarbonate and calcium ions present in the rocks there.

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Whereas turbiditywas observed in a range 0.02-0.29 with mean value 0.15±0.0561. The minimum value measured was 0.02NTU in the Heshi spring 1 eye. While the maximum value 1.99NTU was observed in the inlet of water reservoir. None of the values exceed permissible limit <5NTU of WHO standards.

Reactive Orthophosphate was in range of 28-51 with mean 38±4.8132. The amount of reactive ortho phosphate was in a range of 26mg/l to 59mg/L. The maximum of 59mg/L was found in end point of distribution network. This value crosses the standard limit of WHO.While total phosphorous was observed in a range of 23-120 with mean 41.25±6.1016. The amount of total phosphorous showed great fluctuation. It was in a range of minimum 23m/L to maximum of 120mg/L. The maximum amount was observed again in end point of distribution system while minimum in Kooti spring. The results showed that all the water samples exceed the prescribed limit of WHO standards 5mg/L of total phosphorous.

pH was observed in a range of 6.2-7.1 with mean 6.425±0.1031. The minimum pH value was observed in the Heshi spring 1 where it was 6.2 while maximum value 7.1 was observed in mid and end point of distribution network. Heshi spring 1 and Kooti spring have pH value below the permissible limit as per WHO drinking water standards rest of the springs and all the samples from distribution system fell within the World Health Organization's limit. These findings were supported by [13] who conducted a study on water used for drinking and swimming purposes in Abeokuta, Nigeria stated that the pH was in a range of 6.8-7.3.while total phosphorous was observed in a range of 23-120 with mean 41.25±6.1016. The amount of total phosphorous showed great fluctuation. It was in a range of minimum 23m/L to maximum of 120mg/L. The maximum amount was observed again in end point of distribution system while minimum in Kooti spring. The results showed that all the water samples exceed the prescribed limit of WHO standards 5mg/L of total phosphorous.

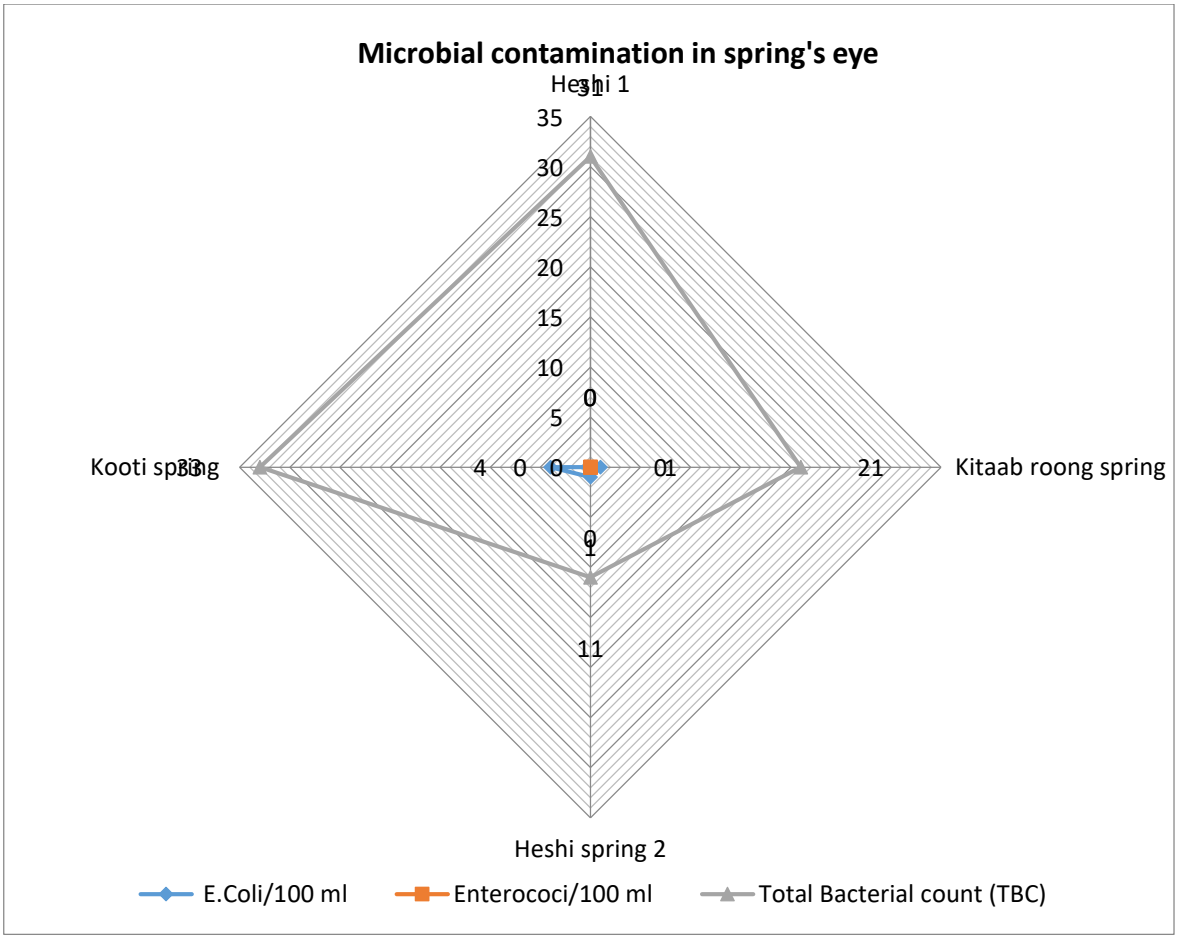

Fig.5: Variation among all microbial parameters in each spring eye

The bacteriological assessment of water establishes the potability of water. The permissible limit of bacteria set by WHO for drinking water quality is 0 CFU/100ml. All the samples of our study were bacterially contaminated.

In the all four spring sources no *enterococci* were observed and three sources were contaminated with *E. coli*. All points of distribution system were highly contaminated with both (*Enterococci* and *E. coli*). The

total bacterial count was in a range of 11CFU/100ml to 83 CFU/100ml. All the findings revealed that all the samples were contaminated with bacterial population and not fit for drinking purpose according to WHO recommendations. Coliform bacteria are prime indicator for faecal contamination of water [15]; [16]. This finding is in accordance with the results of [15] who under took a study on underground water of Gwailor city, India where almost all samples were bacterially contaminated and cross the permissible limit.

IV. CONCLUSION

The present study revealed that the quality of water in all four springs *i.e*. Heshi spring 1, Kitaab Roong spring, Heshi spring 2, and Kooti spring are physically fit for consumption while they exceed the chemical limits (Reactive orthophosphate & Total phosphorous) as per WHO standards. In terms of microbial point of view the springs were contaminated with *E. coli* while no *enterococci* were found in the spring eye. Although springs contain safe edible drinking water but there is no pipeline distribution system that's why they are faecally contaminated. Shepherds take their animals to the meadows so they contaminate water faecally as springs are not protected.

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Extending Shelf Life of Guava Fruits by Mint oil and UVC Treatments

Esameldin Bashir Mohamed Kabbashi*, Islam Kamal Saeed, Mawahib Yagoub Adam

National Food Research Center, Ministry of Higher Education & Scientific Research, Sudan

***Abstract*—** *A lot of quarantine methods have been developed to replace fumigants in the control of arthropods and microorganisms in post–harvest management of fruits and vegetables. That is, guava fruit is infested in Sudan by a number of hexapods which include Ceratitis capitata Weid., Ceratitis quinaria (Bez.), Certatitis cosyra WLK., Bactrocera invadens Drew, Trusta & White and Bactrocera zonata (Saunders).This study aims at using some uncommonly used treatments in improving the storability of guava fruits in Sudan. That is, UVC (ultraviolet rays type C) and coating with mint oil were used to disinfest guava from fruit flies at ambient temperature. The results, after 9 days bench storage, showed an infestation percentage of 20, 33 and 38% for mint, UVC and the control, respectively. The corresponding data for the range of infestation were 18, 20 and 48 and for the mean number of insects in infested fruits were 8.3, 8.8 and 15.2. The quality indexes studied reflected 9.5, 20.5 and 22.6% weight loss, for the mint oil, UVC and the control lots, respectively. The corresponding data for marketable retention (%) were 100, 10 and 13; the fruit firmness, 1.6, 0.3, and 0.1; acidity (%), 0.2 for all; ascorbic acid (mg/ 100 g pulp), 196, 190, and 194; reducing sugar (g/ 100g), 8.2, 7.6, and 7.6; sensory quality includes appearance (%), 84, 42, and 30; taste (%), 79, 41, and 34; flavor (%), 88, 42, and 40, respectively. These results revealed the edge of mint oil coating over UVC and the untreated lots.*

***Keywords*— Guava, fruit flies, mint oil, UVC & quality.**

I. INTRODUCTION

Guava (*P. guajava*) fruit is a lovable international dessert known by its rich nutritional and medicinal values (Kumar, 2012). It's one of the most popular fruits in the tropics and subtropics (Pathak *etal*., 2007) and in Sudan it's counted the fifth fruit in popularity (Ali *etal*, 2014). Guava production in Sudan is witnessed in rather all the states and in good amounts all the year round but this is faced with a lot of constraints related to transport, marketing, suitable storage and processing (Bushara *etal*., 2016). The fruit fly infestation has become a state of concern in Sudan since mid – 1970s when it was very severe and highly pushed the guava farmers to go out of production and some of them uprooted their trees in Shendi area (Bedri, 1978). This is followed by a continuous vigilance and thorough reports of the insect pests of guava fruit. That is about seven species were reported since 1960s which affected the production drastically besides highly reducing the export (Kabbashi, 2014).Roessler (1989) and Bateman (1982) described some management practices against fruit flies, such as getting rid of infested fruits; use of male attractants and lure; use of toxic baits, besides use of hot water dip for eggs and irradiation for male sterilization (Wood, 2000).A number of studies reflect good results of UV treatment in food (López-Rubira *et al*., 2005). That is, the postharvest quality of various crops was improved by exposure to low doses of UVC (Baka *et al*.1999; González-Aguilar *et al*., 2007; Stevens *et al*., 2004).The antimicrobial ability of short wave UVC (200–280 nm) is known as a potent treatment of water and as a disinfectant of package surface in the food processing(Bintsis *et al*., 2000; Keyser *et al* ., 2008; Koutchma *et al*., 2004).The cultivation of guava in Sudan is mainly by seed propagation which results in an uneven and diverse productivity. However, some attempts to improve such trend were taken by the Agricultural Research Corporation (ARC) by importing some cultivars in 1980s but not widely adopted throughout the country (Mahmoud *etal*., 1996). However, a recent work in the Sudanese guava genotypes recommends cultivating 13 out of 100 genotypes tested (Mahmoud and Peter, 2014). Additionally a number of technologies are available for shelf life extension and storage upgrading of horticultural commodities during the last decades, these include the use of anti transpirants (Chahal and Bal, 2003), wax coatings (Mahajan *et al*. 2005), growth retardants (Bisen and Pandey 2008), irradiation (Baghel *et al*. 2005) and other storage facilities that extend life of harvest fruits.

Guava fruit contains 5 times as much as the amount of vitamin C in orange besides oleanolic acid, flavonoids, guaijavarin, quercerlin and essential oils such as nerolidol, limonene and octanol. Its medicinal uses include antispasmodic, anti – inflammatory and antimicrobial effects. These besides its remedial uses against conjunctivitis, coughs, diabetes, malaria and

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rheumatism (Kumar, 2012).Vitamin C in guava was found 67.4% (Waziri and Salih, 2015). Guava also reported to have 5.1 ± 0.85 mg/ 100 g lycopene which is known as a potent immunity helper and carcinogen suppresser (Nwaichi *etal*., 2015). However, a lot of literature speaks about the effectiveness of mint oil as a repellent for fruit flies. That is, pepper mint (*Menthapiperita* Willd.) oil proved a strong repelling effectiveness against all flies that attack a lot of fruits (Renkema *etal*., 2016). This oil also showed a high toxicity for the vine mealy bug [*Planococcusficus* (Signoret)] (Karamaouna et al., 2013).

This study aimed at evaluating two uncommonly used treatments (mint oil coating and UVC) to upgrade the quality of guava fruits in Sudan.

II. MATERIALS AND METHODS

Materials:

Guava fruits, fresh, good looking and uniform, from an orchard in Kadaro, North Khartoum. All of the fruits were of eating quality and were carefully selected to be identical in terms of shape, size, color, ripening stage, and with no blemishes or damage. The fruits were washed and graded according to uniform maturity. Ultra violet (C type, 254 nm) light from a pharmacological company in Khartoum North (Shangahi – Sudan Co. Ltd); mint oil from the Department of Medicinal and Aromatic Plants of the National Center for Research, Khartoum.

Methods:

Guava fruits were randomly selected from an orchard in Kadaro (30 Km North Khartoum Center), any fruit showed a deviation (unfamiliar shape, blemishes, bruises, etc.) from normal was excluded. Three cartoons each had 15 pieces were subjected to 254 nm ultraviolet light type C (UVC light) for one hour. The test fruits were all thoroughly washed.The treated fruits were then stored for 9 days on bench at a laboratory of Postharvest Physiology Department of the National Food Research Center in Khartoum (the average temperature and average RH were 31°C and 21%, respectively). The fruits were then dissected for fruit fly infestation after different storage periods assigned. The same experiment was done for the test of mint oil using a cotton wick to cover the fruit rind with. A corresponding control for each cartoon (15 fruits per cartoon) was used. Readings were taken after five days bench storage. However, fruit characteristics and chemical composition of the treated and untreated lots (at three days interval) were done at the Postharvest Physiology Department of the National food Research Center of the Ministry of Higher Education and Scientific Research, Khartoum.

The physiological weight loss (PWL), physico-chemical composition of fruits were taken after0, 3, 6 and the organoleptical value after 9 days of storage at ambient conditions. Flesh firmness was measured by the Magness and Taylor firmness tester plunger tip. Two readings were taken from opposite sides of each fruit after the peel was removed. The total soluble solids (TSS) of fruits were determined with the help of a hand refractometer of 0–32°Brix range. The acidity, sugar and vitamin C contents were determined as per the method of AOAC (2002).The appearance, taste, and flavor of each sample were evaluated organoleptically by a panel of 10judges, giving scores out of ten.

Statistical analysis:

Analysis of variance (ANOVA), followed by Fisher's protected LSD test with a significance level of $P<0.05$, were performed (Gomez and Gomez 1984).

III. RESULTS AND DISCUSSION

The readings of fruit flies in fruits treated with UVC; mint oil and the control are summarized in Tables 1, 2 and 3, respectively. That is so because any infestation in a fruit may disqualify the whole lot for export and sometimes for local use. However, the statistical analysis of these results are summarized in Table 4. That is, the mean of flies in infested fruits was 8.8, 8.3 and 15.24 for the mentioned three treatments, respectively; the mode of flies in infested fruits was (3, 7 & 8), 3, and 17, respectively; the range of infestation was 20, 18 and 48, respectively; the infestation grand mode was 0, 0 and 0, respectively; the infestation grand mean was 2.93, 1.67 and 5.76, respectively, whereas the infestation percentage is 33.33, 20 and 38%, for the UVC, mint oil and the untreated control, respectively. These results reflect clearly the advantage of using both treatments for the control of fruit flies in guava and the edge effect of mint oil over UVC.

Table.1: UVC (220 NM) for one hour Readings (fruit fly/ fruit)

	Fruit Flies per Fruit														
Rep.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cartoon A	9	0	0	0	0	0	0	0	0	7	7	0	0	0	0
Cartoon B	0	8	0	4	18	0	0	6	0	0	0	8	15	1	21
Cartoon C	0	0	0	0	5	0	0	0	0	0	3	0	3	0	17

Table.2: Mint Readings (fruit fly/ fruit)

	Fruit Flies per Fruit														
Rep.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cartoon A	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0
Cartoon B	0	0	0	0	0	0	0	0	0	20	6	5	3	9	10
Cartoon C	0	0	0	0	0	0	0	0	0	0	0	0	0	25 dead	17

Table.3: Control Readings (fruit fly/ fruit)

	Fruit Flies per Fruit														
Rep.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cartoon A	0	0	0	0	0	0	0	0	0	0	0	17	49	8	36
Cartoon B	0	0	0	0	0	0	11	5	17	0	0	13	19	17	0
Cartoon C	6	0	0	0	0	0	0	5	0	0	1	24	12	11	8

Table.4: Test Statistical Analysis for Infestation

Parameter	Fruit Flies		
	Control	UVC	Mint Oil
Mean of flies in infested fruits	15.24	8.8	8.3
Mode of flies in infested fruits	17	3, 7 & 8	3
Range of infestation	48	20	18
Grand Mode of flies in test fruits	0	0	0
Grand Mean of flies in infested fruits	5.76	2.93	1.67
Infestation Percentage	37.78	33.33	20

Effect of UVC on Hexapods and Fruit Flies:

Irradiation with short wave length UVC was found killing to immature stages of *Drosophilamelanogaster* (Hori *etal*., 2014). The effect of UVC light (254 nm) on two *Tribolium* species (*castaneum* and *confusum*) and *Cardacautella* reflected that all the species eggs hatch was inversely proportional with the exposure and a 24 minutes period exposure effected zero hatchability in 2 and 3 days old *Triboliumcastaneum* eggs. In addition the adult emergence was significantly affected according to the radiation duration which yielded 100% in 2 and 3 days old *T.confusum* eggs exposed for 16 – 24 minutes (Faruki *etal*., 2007). The detrimental ability of UV light was reported in *Drosophilamelanogaster* which suffered eye damage when exposed to intense UV light (Stark *etal*., 1985). It was also reported that the UV light toxicity differs with insect species (Hori *etal*, 2014). The ovicidal effect of UVC was reported to reach total mortality for a dose1.384 kJm^{-2} or higher (Viera *etal*., 2009). However, the eggs of mango fruit fly (*C.cosyra*) and the invader fly (*B. invadens*) were found far more resistant to gamma irradiation than the other developmental stages (Kabbashi *etal*, 2012). This information supports the findings found in this study about UVC irradiation of guava for disinfestation from fruit flies. However, this treatment inspite of being not a choice for disinfesting guava from fruit flies yet it can be considered as a component of an integrated management program.

Effect of mint oil on store product insects:

Mint belongs to the family Lamiaceae (Labiatae) which include members with oils that have potency against insect pests. That is, the LC_{50} and LC_{90}, in order, for *T. castaneum* adults were found 46.8 and 584.3 µl/ l (ppm) (Rastegar *etal*., 2009). The toxicity of some mint species was studied against the house fly (*Muscadomestica*L.). That is, a dose of 225 ppm (LC_{50}) and 270 ppm (LC_{75}) were reported for pepper mint (*Menthapiperita* L.) (Bosly, 2013). In addition mint (*Mentha* spp.) essential oils werereported to have insecticidal activity against the different developmental stages of a number of store insects (Rajendran and Sriranjini, 2008; Kumar *etal*., 2010 & 2011; Michaelakis *etal*., 2012). However, the effectiveness of piper mint (*M. piperita*) essential oils was found distinct among other five tested plant extract considering their insect killing ability (Kumar *etal*., 2011). *M. viridis* analysis revealed its constituents to be dominated by carvone while other components include limonene, terpinen and 1, 8 - cinole(Mkaddem *etal*., 2009). Oil of *Menthalongifolia* L. was found to be mainly composed of pulegone (75%) and other minor constituents that include 1, 8 – cineole, L. menthone and eucarvone. However, its LC_{50} against cowpea beetle [*Callobruchusmasculatus* (F.)] was reported as 4.43 ppm

which exhibited anticholinesterase activity at LC_{50} 1.01 ppm (Al – Sarar *etal*., 2014). Spear mint [*M. viridis* (*spicata*)]at concentration higher than 0.5% produced total kill in *Oryzaephilussurinamensis* (L.) (Al – Jabr, 2006). These studies reflect the killing ability of mint species against an array of insect species which support the findings in this study.

The analyses of the treated and untreated guava fruits (Tables 5 – 8) reflect the success of UVC and mint oil coating treatments in extending the quality life of guava fruits. That is, the study parameters include physical characteristics, total soluble solids (TSS), chemical composition and sensory evaluation. However, the coating with oil mint is superior to UVC treatment according to the findings of this study.

3.1. Effect on weight loss:

Weight loss progressively increased during storage of guava fruits regardless of treatment. Weight loss was followed until the fruits reached the full yellow stage. The control fruits, reached the highest weight loss percentage of 25.2% after 4 days (Table 5). The lowest physiological weight loss of 0, 1.4, 7.0 and 9.5% after 0, 3,6 and 9 days of storage, respectively, were recorded in guava fruits coated with mint oil which were found significantly superior and followed by UVC treatment (Table 5). Mint coating closed the opening of stomata and lenticels thereby, reducing the transpiration and respiration rates and reduced the microbial activity. Multiple oil coating keeps the

fruit value and lessen the ethylene production in pineapple which lead to a lesser weight loss (Thomas *etal*. 2005). Comparable work in guava was also available (Jagadeesh *etal*., 2001). This fact elucidate the effect of mint oil on guava fruits concerning the weight loss parameter. However, on the contrary the energy generated from the UVC treatment may account for an additional difference in weight loss compared to the other treated lot.

3.2. Marketable (Shelf life) period:

Data tabulated in Table (5) showed that the longest marketable period was obtained with fruits treated by mint oil coating *i.e.* all marketable fruits were retained in 100% marketable condition after 0,3,6 and 9 days of storage.The results of the other test fruits are far less compared to the mint treated fruits throughout the storage period. The reason for the extension in shelf life in mint oil treated fruits is attributed to the reduced rate of water loss and lesser availability of oxygen that lead to a slowdownin the rate of ripening of fruits as well as in color change. Similar findings were reported in guava (Singh and Shaffat, 1997), kinnow fruits (Mahajan *etal*., 2005) andin mango fruits (Dhemre and Waskar, 2003).

Table.5: Effect of UVC radiation and mint oil coating on the physical parameters of guava fruits

Treatment	Physiological Loss (%)				Marketable fruit retained *(%)			
	0D	3D	6D	9D	0D	3D	6D	9D
Control	0.00^{j} ±0.0	10.30^{e} ±0.15	25.20^{a} ±0.32	22.60^{b} ±0.28	100.00^{a} ±0.0	86.00^{b} ±0.26	53.40^{d} ±0.19	13.00^{f} ±0.07
Mint oil	0.00^{j} ±0.0	1.40^{i} ±0.09	7.00^{g} ±0.08	9.50^{f} ±0.11	100.00^{a} ±0.00	100.00^{a} ±0.00	100.00^{a} ±0.00	100.00^{a} ±0.00
UVC radiation	0.00^{j} ±0.0	5.20^{h} ±0.07	12.30^{d} ±0.18	20.50^{c} ±0.24	100.00^{a} ±0.0	80.70^{c} ±0.21	40.00^{e} ±0.13	10.00^{g} ±0.05
$LSD_{0.05}$	0.5055**				1.54**			
SE ±	0.1732				0.5276			

Values are mean±SD.

Mean value(s) bearing different superscript(s) are significantly different (P≤0.05).

3.3. Effect on total soluble solids:

The TSS of fruits gradually increased up to 6 days in all treatments and decreased after that irrespective of treatments (Table 6). The maximum (17.6%) TSS was recorded in mint oil coating followed by UV radiation (16.4%) whichwas found also significantly superior to control (14.8%) after 6 days of storage.This corroborates earlier findings that the physico – chemical parameters increase up to 8 days in guava fruits under storage (Chandra, 1995). That is, the drop in TSS in this study was observed after 9 days storage (Table, 6). Heedless, the increase in TSS up to 8 days,in stored guava, may be referred to the decomposition of acids and accumulation of polysaccharides during storage. Additionally, increase in TSS due to coating was reported in pineapple fruits (Das and Medhi, 1990).

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Table.6: Effect of UV radiation and mint oil coating on total soluble solid and flesh firmness of guava fruits

Treatment	TSS (%)				Flesh Firmness* (kg/ cm^2)			
	0D	3D	6D	9D	0D	3D	6D	9D
Control	12.00^{f} ±0.00	14.20^{de} ±0.13	14.80^{cd} ±0.16	13.50^{e} ±0.07	2.30^{a} ±0.00	1.20^{d} ±0.01	0.25^{f} ±0.01	0.10^{g} ±0.01
Mint oil	12.00^{f} ±0.00	16.30^{b} ±0.25	17.60^{a} ±0.28	16.20^{b} ±0.23	2.30^{a} ±0.00	2.30^{a} ±0.00	1.75^{b} ±0.04	1.55^{c} ±0.03
UVC radiation	12.00^{f} ±0.00	15.20^{c} ±0.21	16.40^{b} ±0.26	15.10^{c} ±0.18	2.30^{a} ±0.00	0.75^{e} ±0.01	0.30^{f} ±0.01	0.25^{f} ±0.01
$LSD_{0.05}$	0.8543**				0.1305*			
SE ±	0.2927				0.0447			

Values are mean ± SD.

Mean value(s) bearing different superscript(s) are significantly different (P≤0.05).

3.4. Effect on flesh firmness:

Fruit flesh firmness progressively declined during the storage of guava fruits. Pectin was found the polymer of the firmness in guava fruits. That is, a continuous decrease in this polymer in the cell wall accompanied by its accumulation in the center of the cell was assessed in guava fruit during storage using ruthenium red (De Abreu *etal*., 2012). Mint oil coating significantly delayed the drop in fleshfirmness during the storage of guava fruits and retained maximum texture (67%) up to 9 days of storage, the corresponding results for UVC treated lots and the untreated control were 11% and 4%, respectively (Table 6). The edible oil coatings preserve the quality of fruits, retard ethylene emission and enhance texture(Lin and Zhao, 2007). These results validate the findings of Dashora *etal*. (1999). The UVC treated fruits showed a decline in firmness, and reached the final soft stage (0.25 kg/cm^2) after 9 days storage which is far better compared to the control that reflected a corresponding figure of 0.1 (Table 6). Similar drop in guava fruits have been reported [Bashir and Abu – Goukh (2002) and Abu – Goukh andAbu – Sarra, (1993)].The energy generated from the UVC treatment may account for the more reduction in firmness as compared to the other test lots. However, it is worth reporting that the difference between the readings is significant at 5% level [Table 6 (figures bear different letters)].

3.5. Titratable acidity:

Titratable acidity of guava fruits increased up to the climacteric peak and declined
thereaftertill the end of the storage period (9 days). This as in the fruits of the untreated control. However, the TA increased with time throughout the storage period in the treated lots by both mint oil and the UVC (Table 7). This infers the effect of these treatments in extending the climacteric period, perhaps. Similar results were reported duringripening of banana (Ahmed and Tingwa, 1975; Desai & Deshpande, 1978) and mango (Abu – Goukh and Abu –Sarra, 1993).This sizable decrease in TA could be attributed to its use as a substrate for respiration. Coated fruits, showed the higher flesh acid content value(Table 7).

Table.7: Effect of UV radiation and mint oil coating on the chemical parameters of guava fruits

Treatment	Acidity (%)				Ascorbic Acid *(%)				Reducing sugars (%)			
	0D	3D	6D	9D	0D	3D	6D	9D	0D	3D	6D	9D
Control	0.170^{a} ± 0.00	0.174^{a} ± 0.03	0.200^{a} ± 0.02	0.193^{a} ± 0.01	170.00^{i} ±0.00	188.00^{h} ±0.65	197.0 ±0.76	190.00^{g} ±0.71	5.90^{i} ±0.00	6.11^{g} ±0.03	6.80^{e} ±0.09	7.60^{b} ±0.16
Mint oil	0.170^{a} ± 0.00	0.177^{a} ± 0.01	0.190^{a} ± 0.01	0.210^{a} ± 0.02	170.00^{i} ±0.00	199.00^{c} ±0.81	204.00^{a} ±0.87	196.00^{e} ±0.74	5.90^{i} ±0.00	6.10^{h} ±0.01	7.45^{c} ±0.15	8.20^{a} ±0.19
UVC radiation	0.170^{a} ±0.00	0.175^{a} ±0.01	0.180^{a} ±0.00	0.200^{a} ±0.02	170.00^{i} ±0.00	198.30^{c} ±0.79	201.70^{b} ±0.85	194.00^{f} ±0.72	5.90^{i} ±0.00	6.15^{f} ±0.04	6.85^{d} ±0.11	7.60^{b} ±0.16
$LSD_{0.05}$	0.4706*				1.54**				0.0005*			
SE ±	0.1612				0.5276				0.0002			

Values are mean±SD.

Mean value(s) bearing different superscript(s) are significantly different (P≤0.05)

3.6. Effect on ascorbic acid content:

Vitamin C content of fruits irrespective of treatments increased up to 6 days storage and then declined on day 9 of storage (Table 7). Coated fruits with (mint oil) recorded the highest (204 mg/100 g after 6 days storage) throughout the test period(9 days storage) and was found significantly superior to all other treatments. The increase in vitamin C content in earlier stages of storage may be due to the increasing rate of phenol production whereas, during storage (after 6days), the increase may be due to conversion of L–ascorbic acid into dehydroascorbic acid. Similar results have also been stated in earlier study in guava fruits (Mahajan *et al*., 2005).

3.7. Reducing Sugars:

The reducing sugars in the guava fruits increased up to the climacteric peak and subsequently decreased. Maximum value reached was 8 (g/100g fresh weight) (Table 7). Climacteric fruits, in particular, may show considerable change in sugar content during fruit ripening (Hulme, 1970). Starch and sucrose change into glucose during fruit ripening (Wills *et al*, 1981).Increasing trend of reducing sugars of fruits was observed (Table 7) up to 6 days of storageand then decreased in all test fruits except mint oil the reducing sugars decreased and then increased. This may be due to a rapid conservation of polysaccharides into sugars in the earlier stage and later to utilization of sugars in respiration. These findings are in line with what was found in custard apple (El- Monem *et al*., 2003). Mint oil coating recorded the highest reducing sugars (8.2 g/100 g) after 9 days storage and was found significantly superior to all other treatments.

3.8. Sensory quality:

Maximum acceptability in terms of taste was retained by mint oil coating without any objectionable change up to 9 days of storage followed by UVC treatment (Table 8). Edible oil coating retained good value of taste due to retention of appreciable amount of sugar and a proper TSS/acid ratio up to 9 days of storage. During storage taste scores decreased. Maximum (84%) appearance of fruits was retained undercoating with mint oil after 9 days of storage followed by UVC treatment. This corroborates similar findings in mango fruits(Dhaka *etal*., 2001). Flavor of fruits increased with ripening of fruits and attained its peak at 6 days of storage. Thereafter, during storage up to 9 days, the flavour score decreased. The highest value of 60, 75,91 and 88% for flavour was recorded under mint oil coating at 0,3, 6 and 9 days of storage, respectively. The flavour increased due to enhancement in the chemical attributes of fruits like increase in sugars and TSS/acid ratio where, it decreased at 9 days of storage due to degradative metabolism (Table 8).

Table.8: Effect of UVC radiation and mint oil coating on the sensory scores of guavafruits

Treatment	Appearance (%)				Taste (%)				flavor (%)			
	0D	3D	6D	9D	0D	3D	6D	9D	0D	3D	6D	9D
Control	100.00^{a} ±0.00	85.00^{e} ±0.55	49.00^{g} ±0.39	30.00^{i} ±0.22	60.00^{g} ±0.00	71.00^{f} ±0.43	45.00^{h} ±0.36	34.00^{j} ±0.26	60.00^{g} ±0.00	74.00^{d} ±0.48	65.00^{e} ±0.47	40.00^{h} ±0.31
Mint oil	100.00^{a} ±0.00	97.00^{b} ±0.61	93.00^{c} ±0.59	84.00^{e} ±0.54	60.00^{g} ±0.00	91.00^{b} ±0.58	96.00^{a} ±0.63	79.00^{d} ±0.51	60.00^{g} ±0.00	75.00^{d} ±0.49	91.00^{a} ±0.58	88.00^{b} ±0.54
UVC radiation	100.00^{a} ±0.00	89.00^{d} ±0.56	79.00^{f} ±0.53	42.00^{h} ±0.34	60.00^{g} ±0.00	87.00^{c} ±0.55	73.00^{e} ±0.46	41.00^{i} ±0.32	60.00^{g} ±0.00	75.00^{d} ±0.49	83.00^{c} ±0.52	42.00^{g} ±0.33
$LSD_{0.05}$	1.685				1.685				1.685			
SE ±	0.5774				0.5774				0.5774			

Values are mean±SD.

Mean value(s) bearing different superscript(s) are significantly different (P≤0.05)

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